CHAPTER 5
CONCLUSIONS

5.1 Validation of HPLC/DAD/MSD for analysis *Monascus* metabolites

Agilent 1100 high-performance liquid chromatograph equipped with a photodiode array detector was applied to detect mevinolin. Analysis was perfomed using a Hypersil ODS column (Agilent technologies 250×4.0 mm i.d.; 5 μm) connected to two high precision pumps set at a flow rate of 1.0 ml/min. The photodiode array detector set at 238 nm. The mobile phase consisted of water (pH is adjusted to 2.5 with H_3PO_4)/acetonitrile/isopropanol (55:35:10 v:v:v) (solution A) and 100% acetonitrile (solution B), running isocratically at ratio of 50:50 (v:v) for 20 min after 20 μl of extracted solution was directly injected into the HPLC system.

The concentration of glucosamine and citrinin solutions were analysed using Agilent 1100 high-performance liquid chromatograph (HPLC) equipped with a Mass spectrometer detector. Analysis was perfomed using a Nucleosil column (250×4.6 mm i.d.; C-18; 5 μm) connected to two high precision pumps (Agilent 1100) set at a flow rate of 1.0 ml/min. The mobile phase consisted of water/acetonitrile/isopropanol (55:35:10 v:v:v) added with formic acid to 0.1% of solution A and 100% acetonitrile (solution B), running at ratio of 100% of (solution A) from 0 to 4 min and the ratio of solution A was decreased gradiently to 50% from 4.1 to 12 min. The column was then reconditioned by using the initial solvent composition after 20 μl of extracted solution was directly injected into the HPLC system.

Limit of detection (LOD) of glucosamine, citrinin and mevinolin were found to be 0.0153, 0.0035 and 0.1023 ppm, respectively. The LOQ values for glucosamine, citrinin and mevinolin were found to be 0.0510, 0.0112 and 0.3411 ppm, respectively. Horrat value of glucosamine, citrinin and mevinolin were 0.0058, 0.3214 and 1.1482, respectively. In addtion, %RSD of glucosamine, citrinin and mevinolin were 0.0153%, 0.8486% and 2.1425%, respectively.

5.2 Effects of *Monascus* strains on properties of adlay angkak

M. purpureus DMKU produced highest content of mevinolin (25.03 ppm). It produced citrinin (0.26 ppm) as low as other strains but lower than *M. ruber* TISTR 3006 (14.64 ppm). *M. ruber* TISTR 3006 grew on adlay substrate faster than other strains as indicated by the glucosamine content of 54.50 ppm. The high growth rate of *M. ruber* TISTR 3006 resulted in the highest amount of citrinin, yellow, orange and red pigments accumulated during cultivation period. However *M. purpureus* ATCC16365 produced yellow pigment as much as *M. ruber* TISTR 3006.

For red and yellow colors of adlay angkak investigated by a and b value (respectively) in Hunter color system, *M. ruber* TISTR 3006 showed the highest level of a value (16.61) and b value (6.10). The range of moisture content of adlay angkak produced by *Monascus* strains was 78.17-84.38%. *M. ruber* TISTR 3006 adlay angkak showed the lowest moisture content. The pH of adlay angkak was in the range of 5.74-6.28. There was a little variation of pH values among the strains of *Monascus* studied. Adlay angkak produced by *M. purpureus* DMKU and ATCC 16365 strains showed the highest pH value of 6.54 and 6.28, respectively.

5.3 Kinetic behavior of adlay angkak fermented by *Monascus purpureus* DMKU

Mevinolin started to slightly increase from 0-18th cultivation days and the maximum mevinolin production was obtained after 24 days of cultivation (44.30±10.42 ppm). After that, there was a decrease in mevinolin content until 30th day and maintained at almost the same level.

Citrinin started to be produced at 12th day and the maximum productivity in the period from 15th-24th day was about 0.12 ppm d^{-1}. The highest concentration of citrinin was 1.3 ppm at 24th day. After that, it showed that citrinin production remained stable until 57th day.

The trend of growth curve increased from 0-18th day. At 18th day, *M. purpureus* DMKU showed the maximum growth represented by the highest glucosamine content (10.87 ppm). The specific growth rate related to glucosamine content during 0-18th day was 0.16 d^{-1}. The initial moisture content of adlay angkak was 55.54%. Moisture content of *M. purpureus* DMKU adlay angkak in range 65.44-75.33% during 21th to 57th day. The pH of adlay angkak at the first stage of

fermentation decreased slightly, in the last stage, pH increased and was stable to 5.92-6.22 during 24^{th}–57^{th} day.

All of pigments were increased during 9^{th}-18^{th} day and after that it maintained at the same level unit 33^{th} day. After 33^{th} day, it decreased slightly but was not completely disappeared. The highest optical density of yellow, orange and red pigment were 12.45±2.81, 4.87±1.31 and 5.92±1.51, respectively.

L value of angkak in the early stage of fermentation decreased quickly from 72.04±0.60 to 48.87±1.89 (0-18^{th} day) while a value increased simultaneously. The yellowness of angkak remained unchanged from start to finish (57 days) of fermentation. The range of b value was 6.13-9.86.

5.4 Optimization of carbon and nitrogen source for adlay angkak

Combinations of 1-5% glucose and 0.1-0.5% peptone influented on properties of adlay angkak cultivated by *M. ruber* TISTR3006; a value, orange and red pigments. The a value of adlay angkak gradually increased along with decreasing of glucose and peptone addition. However, when the addition of lower glucose and higher peptone level, a value of adlay angkak was lower. At higher level than 3.5% of glucose addition, the orange and red pigments was produced increasingly along with glucose level.

Combinations of 1-5% lactose and 0.1-0.5% yeast extract influented on properties of adlay angkak cultivated by *M. ruber* TISTR3006; mevinolin, L and a values. The optimum concentration of lactose and yeast extract which should be added in adlay as substrate for producing the highest of mevinolin was 1.00% and 0.50%, respectively. This concentration could forcase the highest of mevinolin content in adlay angkak of 47.3993 ppm. The highest level of L value would be expected when the lactose concentration was in the range of 0.5-3.5% and yeast extract concentration at 0.1-0.15%. The highest level of a value would be expected at 0.5% lactose and 0.1-0.25% yeast extract.

5.5 Effects of carbon and nitrogen source on adlay angkak production

Combined effects of 1% glucose and 0.3% peptone, 3% glucose and 0.1% peptone or 3% lactose and 0.5% yeast extract influenced the growth of *M. purpureus* DMKU in adlay to produce 22.21, 16.33 and 10.00 ppm mevinolin, together with 0.89, 0.62 and 0.96 ppm citrinin respectively (Table 4.6). However, addition of 3%

glucose and 0.5% peptone, 5% glucose and 0.3% peptone, 5% lactose and 0.3% yeast extract or 1% lactose and 0.3% yeast extract inhibited the production of both mevinolin and citrinin. Properties of pigments, Hunter Lab, moisture content and pH did not have direct correlation to the production of both citrinin and mevinolin of *M. purpureus* DMKU.

Combined effects of 1% glucose and 0.3% peptone, 3% glucose and 0.1% peptone, 1% lactose and 0.3% yeast extract or 3% lactose and 0.5% yeast extract influenced on the growth of *M. ruber* TISTR3006 in adlay to produce 28.63, 18.11, 13.20 and 20.27 ppm mevinolin, together with 11.59, 2.03, 9.95 and 3.14 ppm citrinin respectively (Table 4.10). This result indicated that the suitable combinations of carbon and nitrogen source encouraging mevinolin as well as citrinin production. Whereas, the unsuitable combination of both carbon and nitrogen source inhibited both citrinin and mevinolin. Combinations of 3% glucose and 0.5% peptone, 5% glucose and 0.3% peptone, 3% lactose and 0.1% yeast extract or 5% lactose and 0.3% yeast extract inhibited the production of both citrinin and mevinolin. Whereas, glucose-peptone or lactose-yeast extract had no effect on yellow pigment.

The main effect of three combination between of 1% glucose and 0.3% peptone, 3% glucose and 0.1% peptone or 3% lactose and 0.5% yeast extract encouraged mevinolin production by both *M. purpureus* DMKU and *M. ruber* TISTR3006 in the range of 10-28 ppm. Whereas, *M. ruber* TISTR3006 produced citrinin in the ranges of 2-11 ppm higher than *M. purpureus* DMKU with 0.62-0.96 ppm citrinin only. This result confirmed that *M. ruber* TISTR3006 produced much higher citrinin than *M. purpureus* DMKU.

5.6 Comparison of adlay angkak added carbon-nitrogen source and commercial red yeast rice

Xeuzhikang Jiaonang (drug) was the highest concentration of mevinolin (259.8516 ppm) but it was not detected citrinin and glucosamine contents. Addition of glucose-peptone or lactose-yeast extract as carbon-nitrogen source encouraged on the mevinolin production of *M. ruber* TISTR3006. It showed higher concentration of mevinolin than adlay angkak cultivated by *M. purpureus* DMKU (28.6264 ppm). Most adlay angkak samples cultivated by *M. ruber* TISTR3006 were detected more glucosamine content than most samples cultivated by *M. purpureus* DMKU.

Using 3% glucose and 0.5% peptone or 1% lactose and 0.3% yeast extract influented to the highest growth of *M. ruber* TISTR3006 of 96.4298 or 149.2013 ppm, respectively. While using 1% glucose and 0.3% peptone or 1% lactose and 0.3% yeast extract influented with the production of citrinin by *M. ruber* TISTR3006 (11.5890 and 9.9511 ppm, respectively). Adlay angkak without carbon and nitrogen soured rather high variation of mevinolin content 12-44 ppm.

Orange and red pigments of commercial red yeast rice and Xuezhikang Jiaonang were higher concentration than all of adlay angkak supplemented with carbon and nitrogent medium as well as that of no added medium (blank). However, yellow pigment of commercial red yeast rices from Thailand and U.S.A. and drug as Xuezhikang Jiaonang were higher concentration than adlay angkak (*M. purpureus* DMKU) supplemented carbon-nitrogen source but it showed lower concentration when compared with adlay angkak cultivated by *M. ruber* TISTR3006.

L, a and b values in color system of Xuezhikang Jiaonang were lower value than adlay angkak produced by *M. purpureus* DMKU and *M. ruber* TISTR3006. Adlay angkak produced by *M. purpureus* DMKU using 3%glucose and 0.5%peptone was detected as the lowest moisture content of 59.06% while other treatments were seemed not significantly different. pH of angkak produced by rice (commercial red yeast rice and Xuezhikang Jiaonang) were lower level than angkak produced by adlay.