CHAPTER 4
RESULTS AND DISCUSSION

4.1 Results of validation method

4.1.1 Development and optimization of the HPLC method

Early method development highlighted limitations placed on the chromatography due to the physico-chemical properties of glucosamine, citrinin and mevinolin. The UV absorbance for glucosamine, citrinin and mevinolin were highest at 195 nm, 330 nm and 238 nm, respectively (Appendix C). It is necessary to use mass spectrometer as detector to analyse both of substances because it is more sensitive than diode array detector (Watson, 1997). An 250X4.6 mm, i.d.; 5 μm Nucleosil column with a 55:35:10 (v/v/v) water/acetonitrile/isopropanol (added with concentrated formic acid to 0.1% of mixed solution) as solution A and 100% acetonitrile as solution B. Both solution A and B were used to run gradiently, starting from 0 to 4 min with 100% of solution A with flow rate of 1.0 ml/min followed by decreasing the ratio of solution A from 100% at 4.1 min to 50% at 12 min .The volume of 20 μl. either of samples or standards were injected into the column under room temperature (25 ^{O}C). This was the optimum condition of HPLC to detect glucosamine and citrinin with retention time of 1.30 min, 4.45 min, respectively.

Mass spectrometer chromatograms of glucosamine and citrinin standard were shown in Appendix C (Figs C4 and C5).

The optimum condition of HPLC/DAD for analysing mevinolin substance was using an 250X4.0 mm, i.d.; 5 μm Hypersil ODS column with water (pH 2.5)/acetonitrile/isopropanol (55:35:10 v:v:v) as solution A and 100% acetonitrile as solution B. Both solution A and B were run isocratically at ratio of 50:50 (v:v) for 20 min. The injection volumn was 20 μl. Diode array chromatogram of mevinolin standard at 238 nm with retention time of 11.14 min was shown in Fig C6 (Appendix C).

4.1.2 Precision and Accuracy determination

The results in Table 4.1 showed %recovery at each analysed standard amount of glucosamine, citrinin and mevinolin in samples. The range of acceptability of %recovery for 100 ppb-10 ppm substances in detected sample must be 80-110% (100 ppb-10 ppm). The results showed the accuracy of method as %recovery of glucosamine, citrinin and mevinolin were 97.86%, 106.32% and 98.74%, respectively (Table 4.1).

Relative standard deviation (%RSD) is demonstrated as the precision of analysis method for detection of the substance in samples. For checking the precision of the method, it was started from extraction and running through HPLC/DAD/MSD to detect for Horrat value which represents repeatability acception of the method. AOAC (1995) recommended that horrat value must be less than or equal to 2. Horrat value of glucosamine, citrinin and mevinolin were 0.0058, 0.3214 and 1.1482, respectively (Appendix D). Moreover, %RSD of glucosamine, citrinin and mevinolin were 0.0153%, 0.8486% and 2.1425%, respectively (Table 4.1).

4.1.3 Linearity

A set of seven standards at the following concentrations were prepared: 0, 0.5, 1.0, 2.0, 5.0, 8.0 and 10.0 ppm glucosamine. The calibration curve was constructed by plotting the peak area against the concentration using linear regression analysis. It showed that the slope was 80030 and correlation coefficient of 0.9984, indicating an excellent linearity (Fig 4.1).

Eight citrinin standards at 0, 0.2, 0.5, 0.8, 1.0, 1.5, 2.0 and 5.0 ppm were prepared for constructing the calibration curve. The slope of this curve was 355976 and correlation coefficient was 0.9989 (Fig 4.2).

Mevinolin standard at concentrations of 0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 were prepared for constructing the calibration curve. The slope of this curve was 62.33 and and correlation coefficient was 0.9984 (Fig 4.3).

4.1.4 Limit of detection and limit of quantitation

The LOD and LOQ were measured as the concentrations corresponding to signal-to-noise ratio of 3:1 and 10:1, respectively. The LOD values for 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 (Table 4.1).

Table 4.1 Validation of Glucosamine, Citrinin and Mevinolin

Substances	LOD	LOQ	%RSD	%Recovery
Glucosamine	0.0153 ppm	0.0510 ppm	0.0153 %	97.86%
Citrinin	0.0035 ppm	0.0112 ppm	0.8486 %	106.32 %
Mevinolin	0.1023 ppm	0.3411 ppm	2.1425 %	98.74%

Calculation was shown in Appendix D

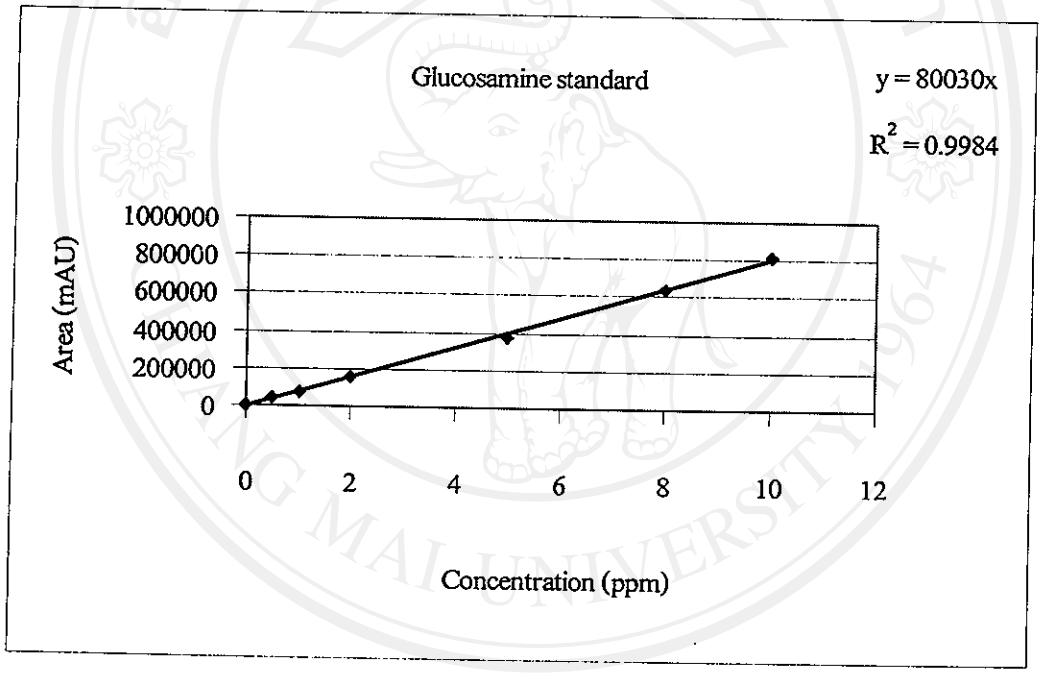

Fig 4.1 Standard curve of glucosamine (by MSD detection)

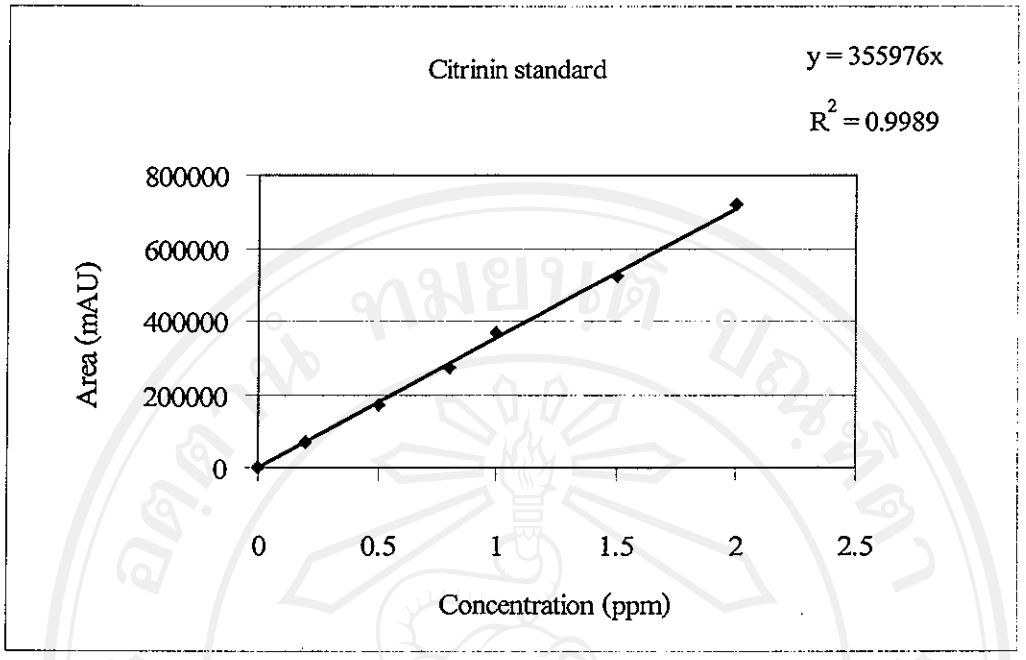

Fig 4.2 Standard curve of citrinin (by MSD detection)

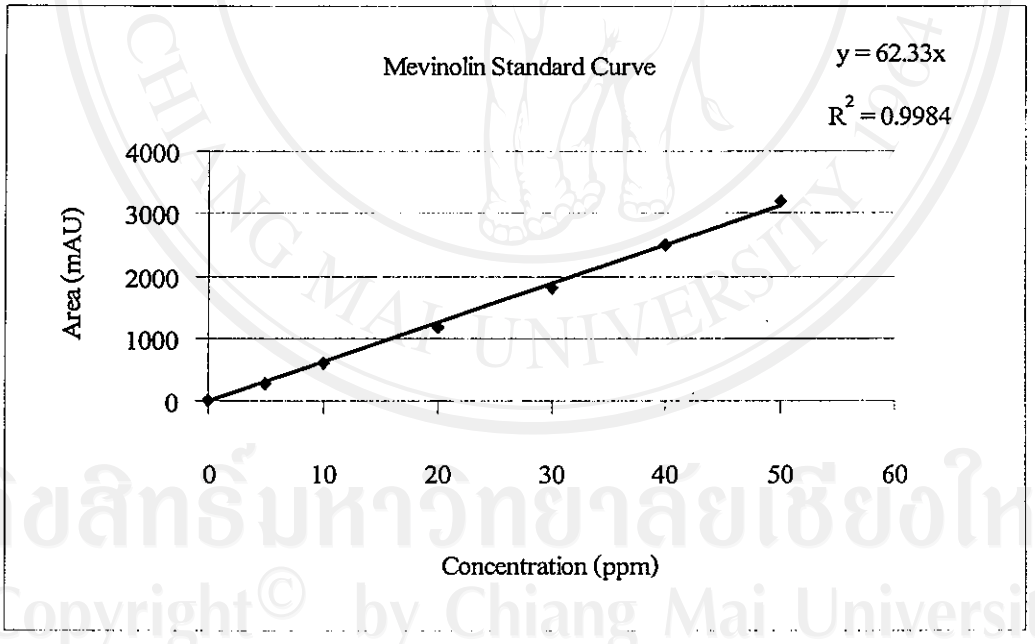

Fig 4.3 Standard curve of mevinolin (by DAD detection)

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

4.2.1 Metabolites produced from Monascus strains in adlay angkak

The contents of mevinolin and citrinin in adlay angkak produced from strains of *Monascus* were investigated after fermentation for 28 days using sterilized adlay as a substrate. It was found that all the *Monascus* strains produced both mevinolin and citrinin. However, *Monascus ruber* TISTR 3006 produced the highest amount of citrinin of 14.64 ppm while other strains produced this metabolite at lower level (0.26-0.55 ppm) (Table 4.2). *M. ruber* TISTR 3006 grew on adlay substrate faster than other strains as indicated by the glucosamine content of 54.50 ppm. Glucosamine is a monomer of chitin which is the main component in fungi cell wall (Vignon *et al.*, 1986). Therefore, the content of this component can be used for representation of its growth. The growth rate of *M. ruber* TISTR 3006 resulted in higher amount of citrinin accumulated during cultivation period. This result was well agree with Blanc *et al.* (1995) who reported that *Monascus ruber* produced citrinin of 300 ppm on rice substrate while *Monascus purpureus* CBS 109.07 produced the citrinin of 100 ppm on the same substrate. However, as compared with rice substrate, using adlay to replace rice for producing angkak could reduce citrinin production during fermentation. Interestingly, *M. purpureus* DMKU produced highest content of mevinolin and lowest citrinin concentration.

The strain that produced the highest mevinolin content was *M. purpureus* DMKU. It produced mevinolin up to 25.03 ppm during 28 days of fermentation. Futhermore, DMKU strain produced citrinin (0.26 ppm) lower than *M. ruber* TISTR 3006 (Table 4.2).

Table 4.2 Mevinolin, citrinin and glucosamine content of adlay angkak during fermentation at room temperature for 28 days

Monascus strains	Mevinolin (ppm)	Citrinin (ppm)	Glucosamine (ppm)
M. purpureus ATCC16365	14.97±0.29^{d}	0.53±0.09^{b}	6.11±0.47^{d}
M. purpureus BCC 6131	19.84±0.40^{b}	0.44±0.09^{b}	20.17±0.58^{b}
M. purpureus DMKU	25.03±0.42^{a}	0.26±0.05^{b}	22.05±1.61^{b}
M. purpureus FTCMU	15.56±0.22^{c}	0.55±0.07^{b}	13.40±0.39^{c}
M. ruber TISTR3006	15.33±0.07^{cd}	14.64±2.46^{a}	54.50±4.41^{a}

Means within columns with different superscripts were significantly different ($P<0.05$)

4.2.2 Pigments in adlay angkak produced from Monascus strains

The *Monascus* pigments are a complex mixture of at least six compounds. The orange monascorubrin and rubropunctatin are believed to be initially biosynthesized while the yellow pigments (monascin and ankaflavin) and red pigments (monascorubramine and rubropunctamine) are derived from the orange ones (Wong *et al.*, 1981). The color of pigment production varied by different strains of *Monascus*. *M. purpureus* ATCC 16365 and TISTR 3006 strains produced more pigment than other strains. The yellow pigment produced from both strains was not significantly different ($P>0.05$) but the orange and red pigment produced from *M. ruber* TISTR 3006 strain were higher than those of *M. purpureus* ATCC 16365. Although, *M. purpureus* DMKU produced all pigments less than those synthesized by *M. purpueus* ATCC 16365 and *M. ruber* TISTR 3006, it produced all pigments relatively higher than *M. purpureus* BCC 6131 and FTCMU (Table 4.3).

Table 4.3 Pigments concentration in adlay angkak produced from difference *Monascus* strains

Monascus strains	Absorbance*		
	400 nm	470 nm	500 nm
M. purpureus ATCC16365	13.45±0.30^{a}	5.53±0.67^{b}	5.91±0.92^{b}
M. purpureus BCC 6131	2.93±0.36^{c}	0.88±0.12^{d}	1.03±0.13^{d}
M .purpureus DMKU	9.76±3.22^{b}	3.03±1.31^{c}	3.43±1.61^{c}
M. purpureus FTCMU	2.54±0.16^{c}	1.12±0.15^{d}	1.37±0.19^{d}
M. ruber TISTR3006	13.14±0.00^{a}	10.82±1.62^{a}	9.56±1.34^{a}

* Absorbance at 400, 470 and 500 nm is indicative of yellow, orange and red pigment concentrations respectively

Means within columns with different superscripts were significantly different ($P<0.05$)

4.2.3 Color in adlay angkak produced from Monascus strains

Results from colorimeter indicated that, *M. ruber* TISTR 3006 presented the highest ranges of a and b values in HunterLab color system. These values showed the relative intensity of red and yellow color, respectively (Table 4.4). This may be due to *M. ruber* TISTR 3006 which had grown faster than the other strains. The maximum

lightness of adlay angkak was the one, which produced from *M. purpureus* FTCMU (L = 48.36) while *M. purpureus* ATCC 16365 was the lowest (L = 39.62) (Table 4). However, this result was not correlated with extractd pigments analysis because colorimetric results are measurements of both cellmass and pigments while ethanol extraction showed only pigments extractd (Lin and Iizuka, 1982).

Table 4.4 L, a and b values in adlay angkak produced from difference *Monascus* strains

Monascus strains	L value	a value	b value
M. purpureus ATCC16365	39.62 ± 0.85^{d}	10.41 ± 2.60^{b}	3.22 ± 1.10^{c}
M. purpureus BCC6131	43.22 ± 0.65^{c}	10.83 ± 0.54^{b}	4.49 ± 0.29^{bc}
M. purpureus DMKU	43.78 ± 0.88^{c}	12.59 ± 1.18^{b}	4.62 ± 1.04^{bc}
M. purpureus FTCMU	48.36 ± 0.52^{a}	12.65 ± 0.90^{b}	7.15 ± 0.99^{a}
M. ruber TISTR3006	45.65 ± 0.45^{b}	16.61 ± 0.37^{a}	6.10 ± 0.65^{ab}

L, a and b value in HunterLab color system

Means within columns with different superscripts were significantly different ($P<0.05$)

4.2.4 Moisture content and pH in adlay angkak produced from Monascus strains

The ranges of moisture content of adlay angkak produced by *Monascus* strains was 78.17-84.38% (Table 3.5). *M. ruber* TISTR 3006 adlay angkak showed the lowest moisture content because this strain used up more water during cultivation for growth and metabolite production. Ganrong *et al.* (2000) reported that moisture content of wet angkak produced from rice substrate was more than 70% during 20-24 fermentation days and that moisture was a key parameter in controlling the fermentation of *Monascus* fungi. Therefore, this could be the reason that *M. ruber* TISTR 3006 could produce the highest citrinin content, with yellow, orange and red pigments. This strain possessed high growth rate as indicated by its glucosamine content thus the pigment accumulated during fermentation period was high.

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.

Table 4.5 Moisture content and pH of adlay angkak produced from different strains of *Monascus*

Monascus strains	Moisture content (%)	pH
M. purpureus ATCC 16365	84.38±0.19^{a}	6.28±0.05^{ab}
M. purpureus BCC 6131	84.09±0.34^{ab}	6.02±0.07^{bc}
M. purpureus DMKU	83.51±0.26^{c}	6.54±0.37^{a}
M. purpureus FTCMU	83.71±0.26^{bc}	5.88±0.17^{c}
M. ruber TISTR3006	78.17±0.35^{d}	5.74±0.05^{c}

Means within columns with different superscripts were significantly different ($P<0.05$)

4.3 Kinetic behavior of *Monascus purpureus* DMKU on adlay angkak

4.3.1 Effects of cultivation time on mevinolin production

Fig 4.4 represented the mevinolin production kinetics. It showed that 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. In general, as a secondary metabolite, mevinolin started to produce increasingly after the initial of cell growth period had pass (Yongsmith, 1999) (Fig 4.5) which illustrated by glucosamine synthesis (Fig 4.7). Highest mevinolin content was produced on 24th day and after that it might be converted to another form of mevinolin (e.g. acid form) (Ganrong *et al.*, 2000) or used as a substrate for producing other substances in fermentation process (Yongsmith, 1999).

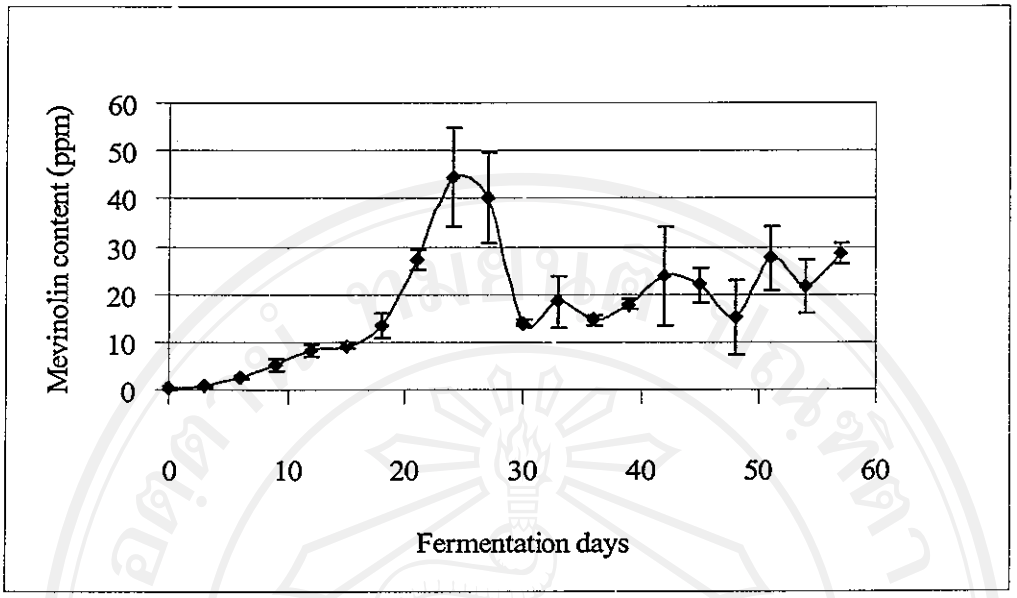

Fig 4.4 Kinetic behavior of mevinolin production by *Monascus purpureus* DMKU

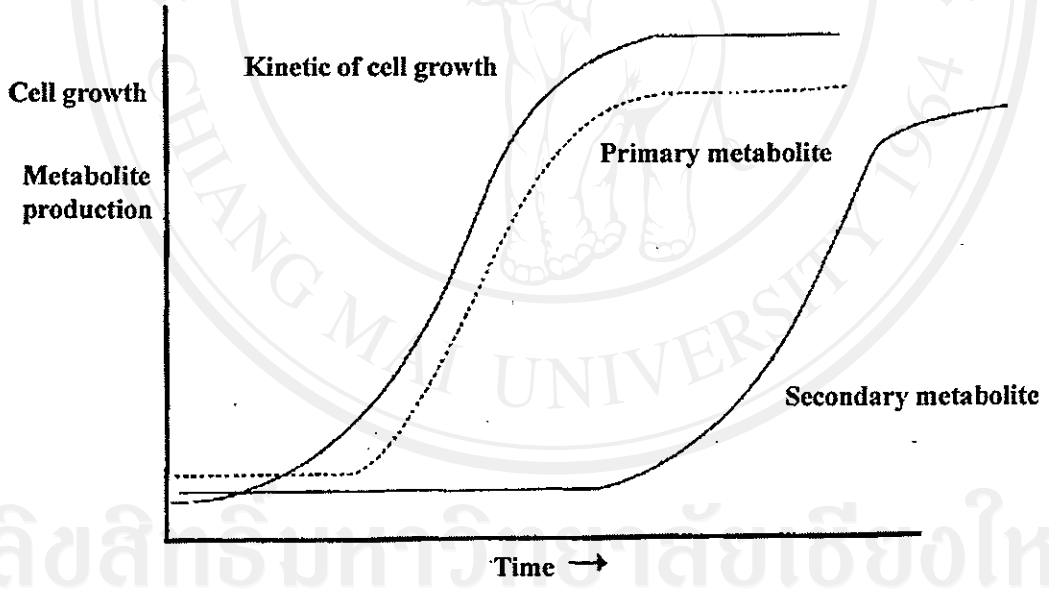

Fig 4.5 Kinetic behavior of microorganism
Source: Yongsmith (1999)

4.3.2 Effects of cultivation time on citrinin production

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. From the results it was evident that citrinin produced on adlay as substrate was less than 1 ppm (Yang *et al.*, 2004) while it was produced about 100-300 ppm on rice substrate by various species of *Monascus* (Blanc *et al.*, 1995). The citrinin content detected from 12 different commercial *Monascus* rice angkak samples was varying between 0.2 and 17.1 ppm (Sabater-Vilar *et al.*, 1999). Therefore, it is possible to use adlay as a new substrate for making adlay angkak with less or free-citrinin production by optimizing their growth condition. Moreover, citrinin is a secondary metabolite which showed the result similar to mevinolin on the period of production. It could be detected after the first stage of *Monascus* growth had passed.

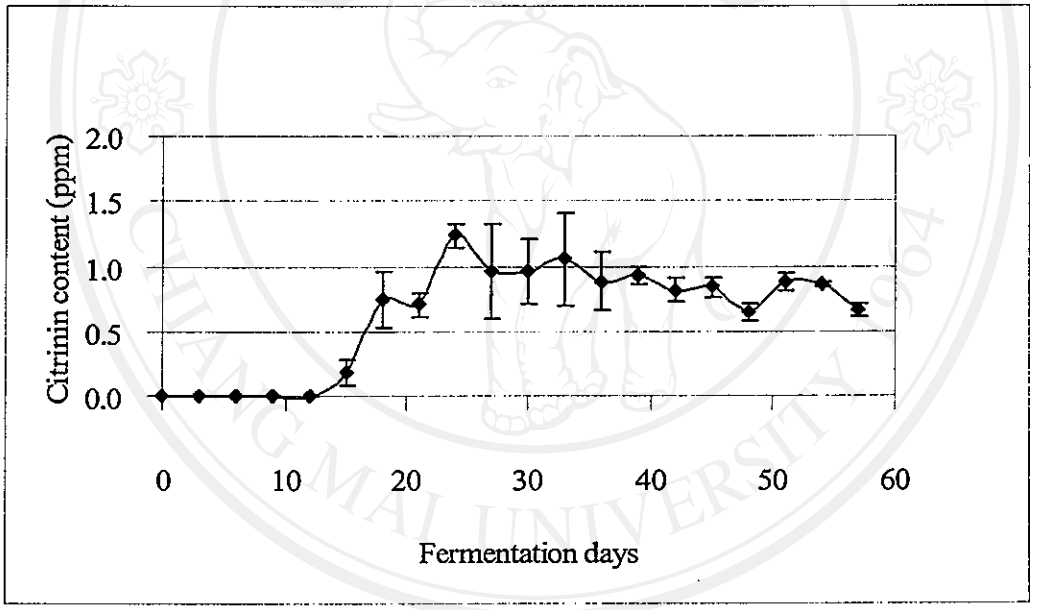

Fig 4.6 Kinetic behavior of citrinin prodution

4.3.3 Effects of cultivation time on glucosamine content of adlay angkak

Fig 4.7 represented effects of cultivation time on glucosamine content of adlay angkak cultivated by *M. purpureus* DMKU. It was found that the trend of growth curve increased from 0-18th day. At 18th day, *Monascus* showed the maximum growth represented by the highest glucosamine content (10.87±0.86 ppm). The specific growth rate related to glucosamine content during 0-18th day was 0.16±0.07 d^{-1}. The initial moisture content of adlay angkak was 55.54±0.51 % which was in the range of

required moisture content of fungi cultures (20-70%) for fermentation in solid substrate (Babitha *et al.*, 2007). Moisture content in adlay angkak had increased over the time (Fig 4.8). This result explained that during the first stage of fermentation period, the fungi utilized the nutrients from substrate to produce its primary metabolites, bioconversion, energy, carbondioxide and water. Therefore, moisture content showed increasing trend during the first stage of fermentation (Yongsmith, 1999). Increasing of moisture content resulted in increasing the solubility of substrate and minimizing heat exchange, high oxygen transfer and availability of nutrients to the culture (Babitha *et al.*, 2007). The moisture content in adlay angkak at stationary stage became almost stable which resulted from their utilizing of water for producing pigment (Babitha *et al.*, 2007) and citrinin including hydrolyzing enzymes (e.g. alpha amylase and glucoamylase (Wang *et al.*, 2003). And, incorperating of fungi respiration, it was showed a little changing of moisture content in range 65.44-75.33% during 21^{th} to 57^{th} day.

The pH of adlay angkak at the first stage of fermentation decreased slightly because *Monascus* produced fatty acids as primary metabolites resulting in the reduction of pH. In the last stage, pH increased and was stable to 5.92-6.22 during 24^{th}–57^{th} day because the fungal cells could act autolysis on themselves after the nutrients in substrate were depleted. So, it could produce the waste as ammonium compounds and effect on increasing of pH (Alexopoulas and Bold, 1969).

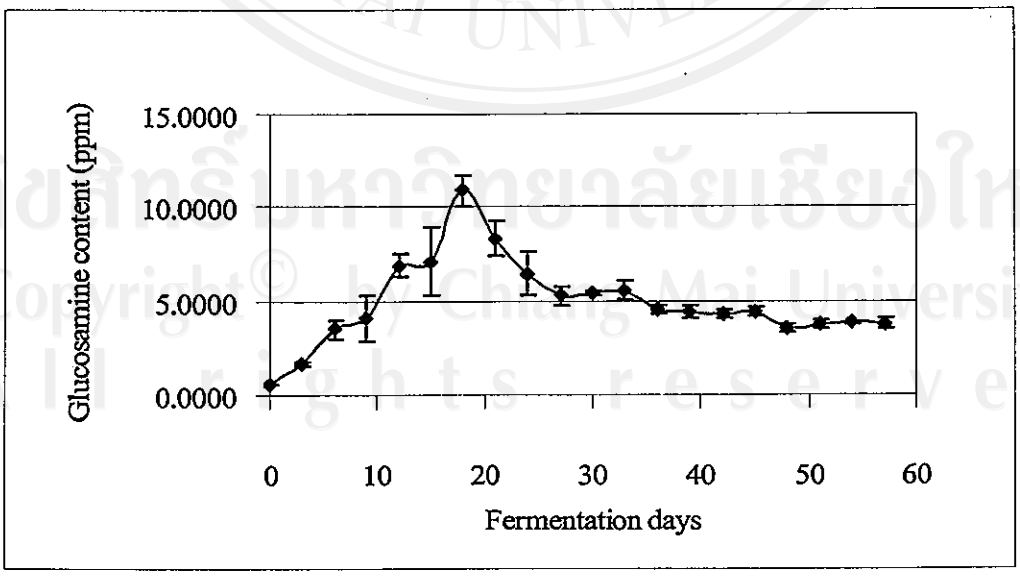

Fig 4.7 Kinetic behavior of glucosamine production

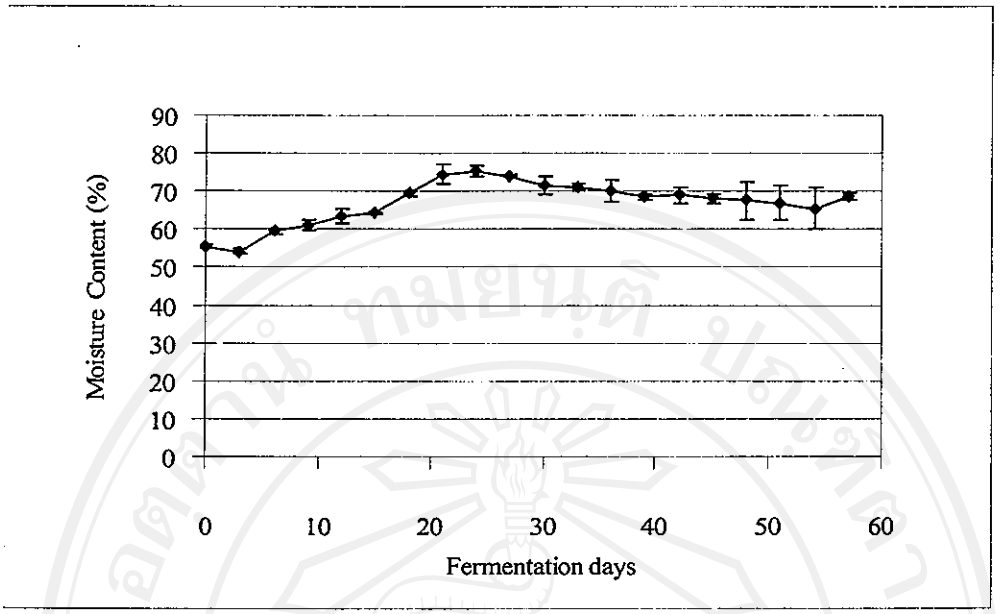

Fig 4.8 Moisture content of adlay angkak (wet weight) varied on cultivation time

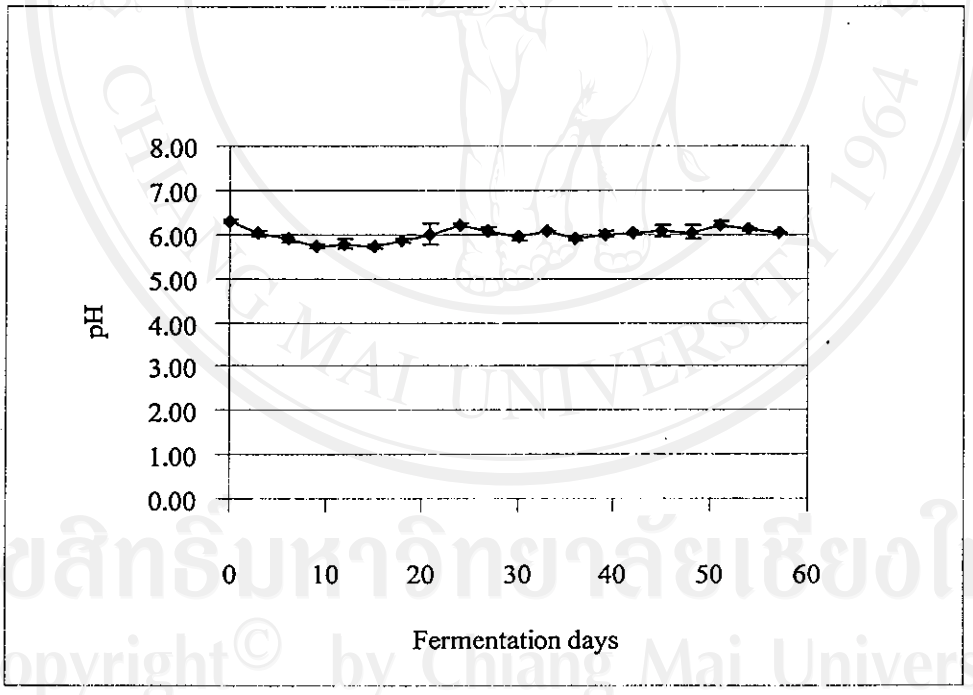

Fig 4.9 pH of adlay angkak varied on cultivation time

4.3.4 Effects of cultivation time on pigment production and Hunter Lab color system

As commercial red rice is used as a natural food colorant and dietary supplement for reducing blood cholesterol, therefore, several form such as purified

extract, red rice or powder are sold wildspread in the market (Carvalho *et al.*, 2005). Purified extract form is expressed as the shade of color by determining alcohol extract of angkak and rice or powder form, which is indicated by Hunter Lab color system as the eye apperance.

Monascus can produce three pigment groups; yellow (400 nm), orange (470 nm) and red (500 nm) during cultivation time in suitable condition. All of pigments were increased during 9^{th}-18^{th} day and after that it maintained at the same level unit 33^{th} day. Therefore, between 18^{th}-33^{th} cultivation days were the suitable range for fermentation adlay angkak in which produced the highest pigment concentration. 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 (Fig 4.10).

Redness and yellowness of angkak are express as a and b values (respectively) of Hunter color system. The lightness of samples is indicated by L value. Eye apperance is an important parameter to use adlay angkak in powder form as food colorant which L, a and b values are describe the color shade of angkak related with the eye appearance. Fig 4.11 showed the results of cultivation time affected on L, a and b values. L value of angkak in the early stage of fermentation decreased quickly decrease from 72.04±0.60 to 48.87±1.89 (0-18^{th} day) while a value increased in the same time. After 18^{th} day, both values were stable in which L value ranged from 44.15-51.76 and a value ranged from 14.90-19.35. The yellowness of angkak remained unchanged during start to finish (57 days) of fermentation. The range of b value was 6.13-9.86.

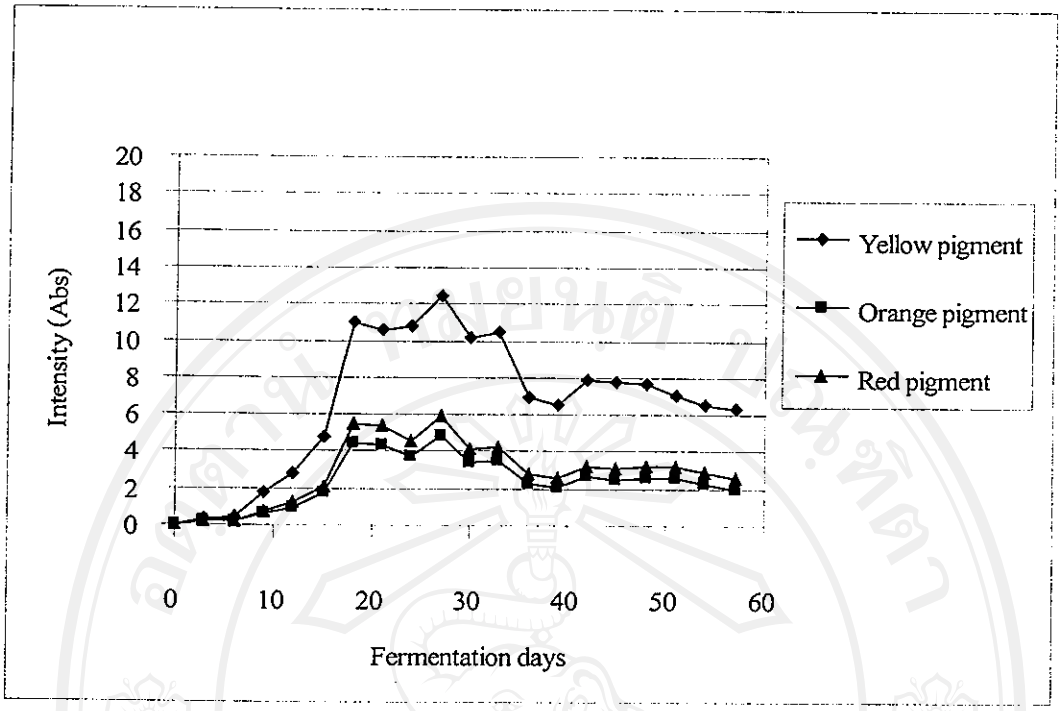

Fig 4.10 *Monascus* pigments varied on cultivation day

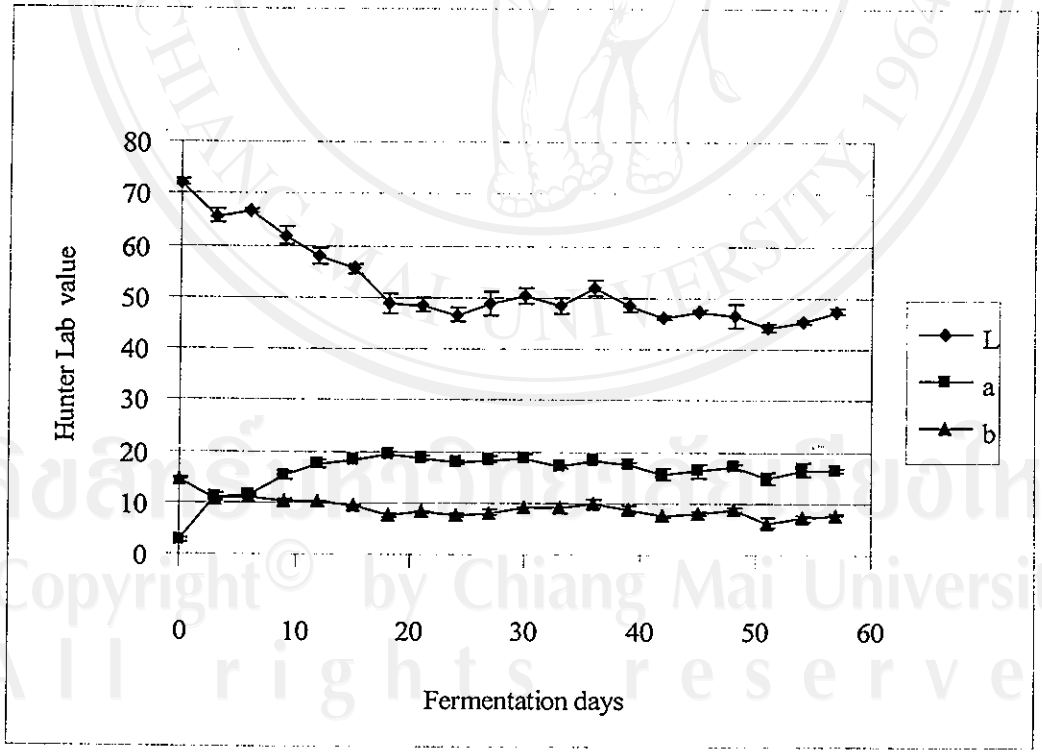

Fig 4.11 Hunter Lab value of powdered adlay angkak varied on cultivation day

4.4 Optimization of carbon and nitrogen source for adlay angkak

4.4.1 Effects of glucose and peptone on properties of adlay angkak

This research used response surface methodology to investigate optimum nutrient supplement of combination of 1-5% glucose and 0.1-0.5% peptone. These factors were added in cooked adlay and cultivated with *M. purpureus* DMKU and *M. ruber* TISTR 3006. The regression equations by Design Expert program were created on properties of a value, orange pigment and red pigment dependent on both supplements by *M. ruber* TISTR 3006 as indicated by below Equation 4.1, 4.2 and 4.3.

$$\text{a value} = 22.5835+(0.2180*\text{glucose})-(13.8545*\text{peptone})-(0.1219*\text{glucose}^2)+(11.4375*\text{peptone}^2)+(1.7625*\text{glucose}*\text{peptone}) \quad \text{Equation 4.1}$$

$$\text{orange pigment} = 10.2112-(3.3009*\text{glucose})-(9.4268*\text{peptone})+(0.5787*\text{glucose}^2)+(15.07*\text{peptone}^2)+(0.7*\text{glucose}*\text{peptone}) \quad \text{Equation 4.2}$$

$$\text{red pigment} = 8.6076-(2.9578*\text{glucose})-(5.3835*\text{peptone})+(0.5384*\text{glucose}^2)+(11.04*\text{peptone}^2)+(0.32*\text{glucose}*\text{peptone}) \quad \text{Equation 4.3}$$

The percentages of variability in the responses accounted by the factors for the models were shown with R^2 value. The R^2 value of a value, orange pigment and red pigment were 74.54%, 71.09% and 70.57%, respectively (Equation 4.1, 4.2 and 4.3). Fig 4.12 showed three-dimensional (3-D) response surface plots of effect of glucose and peptone on a value of color system. The a value of adlay angkak gradually increased along with decreasing of glucose and peptone. However, when the addition higher peptone level at lower glucose level resulted in lower a value of adlay angkak.

Fig 4.13 and 4.14 showed 3-D response surface plots of the effect of glucose and peptone added on the production of orange and red pigments, respectively. It was obvious that orange and red pigments had similar trends under the same nutrient condition. At the range of 2.5-4.5% glucose, orange and red pigments became the lowest. At higher level than 4% of glucose addition, the orange pigment was produced increasingly along with glucose level. The red pigment production had similar result at higher level than 3.5% of glucose addition, it gradually increased to the highest level.

Medium composition may affect the growth of filamentous fungi and alter secondary metabolite production such as pigments. Chen and Johns (1994) reported

glucose and peptone concentration influenced the synthesis of *Monascus* pigments. Low glucose concentrations favored the synthesis of relatively large quantities of monascorubrin, the orange analogue of monascorubramine, whereas high glucose concentrations favored the synthesis of ankaflavin, the yellow analogue. The use of peptone increased the yield of monascorubramine, the red analogue. Additional nutrients in adlay substrate such as glucose and peptone, could result in *Monascus* pigment production, as proposed by Chen and John (1994).

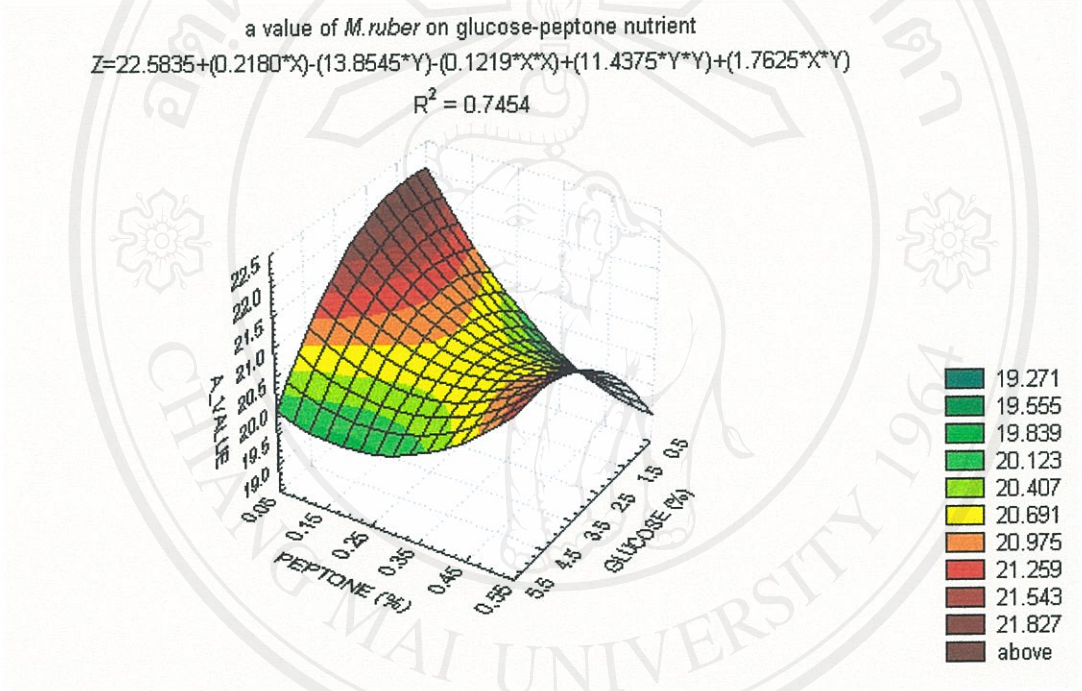

Fig 4.12 a value of Hunter color system of *Monascus ruber* TISTR3006 on adlay added with glucose and peptone

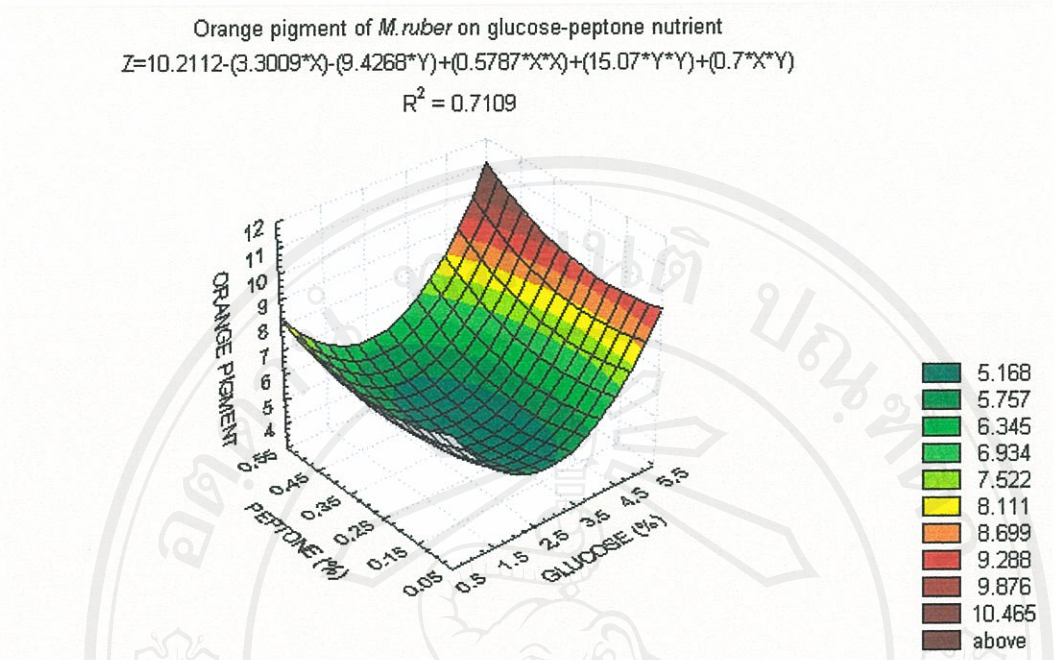

Fig 4.13 Orange pigment of *Monascus ruber* TISTR3006 on adlay added with glucose and peptone

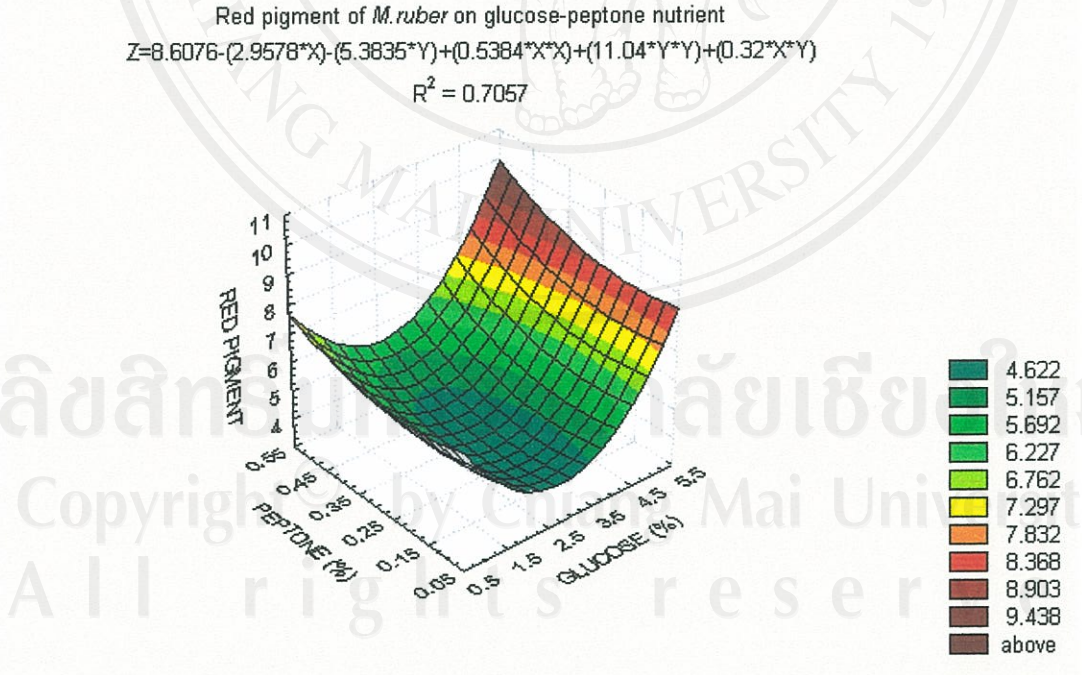

Fig 4.14 Red pigment of *Monascus ruber* TISTR3006 on adlay added with glucose and peptone

In case of other properties related to *Monascus ruber* TISTR3006 for examples: glucosamine, citrinin, mevinolin, yellow pigment, L value, b value, moisture content and pH did not fit in second-order model under this experiment (R^2<0.7). There was possibility that optimization of these characteristic was beyond the ranges of glucose and peptone levels under this study. The ranges of these properties were shown in Appendix E.

With regard to *M. purpureus* DMKU, production of glucosamine, citrinin, mevinolin, yellow pigment, orange pigment, red pigment, L value, a value, b value, moisture content and pH responsed from combined glucose and peptone supplements did not fit in second-order model under this experiment (R^2<0.7). There was possibility that optimization of these characteristic was beyond the ranges of glucose and peptone levels under this study. The ranges of these properties were shown in Appendix E.

4.4.2 Effects of lactose and yeast extract on mevinolin production

A response surface plot in Fig 4.15 showed effects of lactose and yeast extract on mevinolin production. Addition of lactose and yeast extract near 0.5% and 0.50% encouraged the higher mevinolin production while the other concentration of lactose and yeast extract resulted in a reduction of the production of mevinolin according to following equation.

$$\text{Mevinolin} = -8.1252-(8.8332*\text{lactose})+(202.5713*\text{yeast extract})+(2.5688*\text{lactose}^2) -(71.9060*\text{yeast extract}^2)-(43.0404*\text{lactose}*\text{yeast extract}) \quad \text{Equation 4.4}$$

R^2 of mevinolin production related to lactose and yeast extract nutrient was 0.8564. The optimum concentration of lactose and yeast extract were 1.00% and 0.50%, respectively which should be added in adlay as substrate for producing the highest level of mevinolin. The highest mevinolin concentration could be forecased by Design Expert program indicated by the quadratic equation 4.4 to be 47.3993 ppm. The results of this study was coresponsed to López *et al.* (2003) which reported the maximum value of mevinolin yield (29.196 mg/g) by using 20g/l lactose and 1.33 g/l yeast extract in medium. Lactose and yeast extract were the suitable nutrients for producing the highest mevinolin when compared to fructose or glycerol (carbon

source) combined with corn steep liquor or soybean meal additionally supplemented with the following components (per liter of medium): 1.51 g KH_2PO_4, 0.52 g $MgSO_4.7H_2O$, 0.40 g NaCl, 2 mg $Fe(NO_3)_3.9H_2O$, 1 mg $ZnSO_4.H_2O$, 0.04 mg biotin and 1 ml of the trace element solution. It is possible that these supplements might encourage the production of more mevinolin while in our experiment just had the combination of carbon and nitrogen source in adlay in which did not supply as complete supplement as the medium tested by López *et al.* (2003).

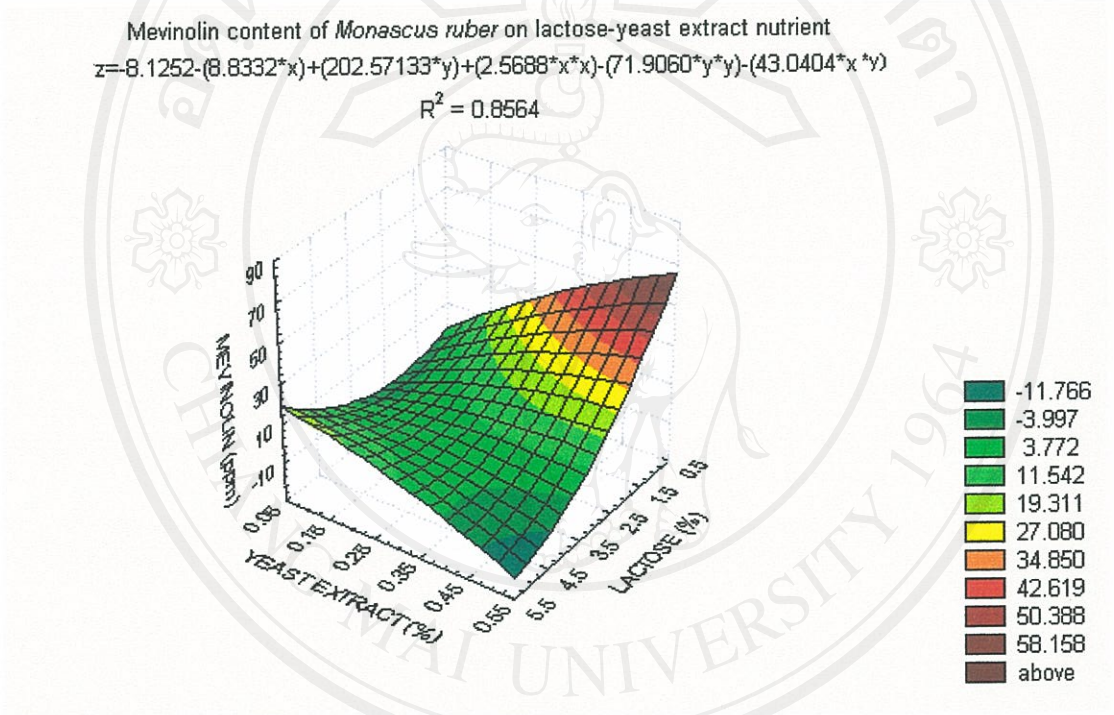

Fig. 4.15 Mevinolin content of *Monascus ruber* TISTR3006 on adlay added with lactose and yeast extract

4.4.3 Effects of lactose and yeast extract on properties of adlay angkak

Addition of 1-5% lactose and 0.1-0.5% yeast extract in adlay as substrate supplement. These carbon and nitrogen sources had an effect on L and a value of angkak color after fermented by *M. ruber* TISTR3006. L and a values were represented by following equation.

$$L\ value = 48.5985+(0.5585*lactose)-(16.0963*yeast\ extract) -(0.2244*lactose^2)+(14.4313*yeast\ extract^2)+(1.9*lactose*yeast\ extract) \quad \text{Equation 4.5}$$

a value = 20.4851-(0.414*lactose)+(6.9937*yeast extract)

-(0.1944*lactose^2)-(24.6875*yeast extract^2)+(3.4*lactose*yeast extract) Equation 4.6

The R^2 of L value and a value were 0.8401 and 0.8103, respectively. Fig 4.16 and 4.17 showed 3-D response surface plots of the effect of lactose and yeast extract added on L and a values in color system. 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.

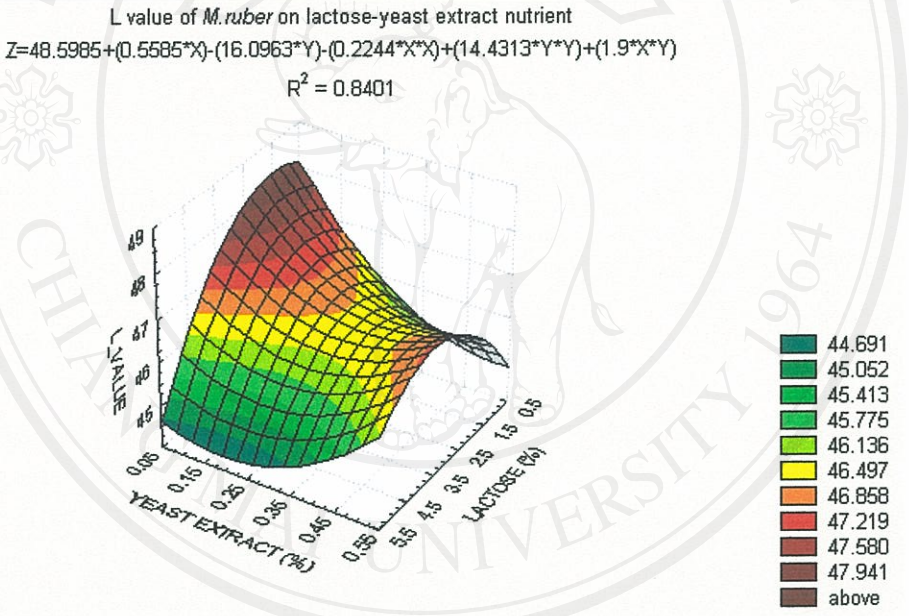

Fig 4.16 L value by Hunter color system of *Monascus ruber* TISTR3006 on adlay added with lactose and yeast extract.

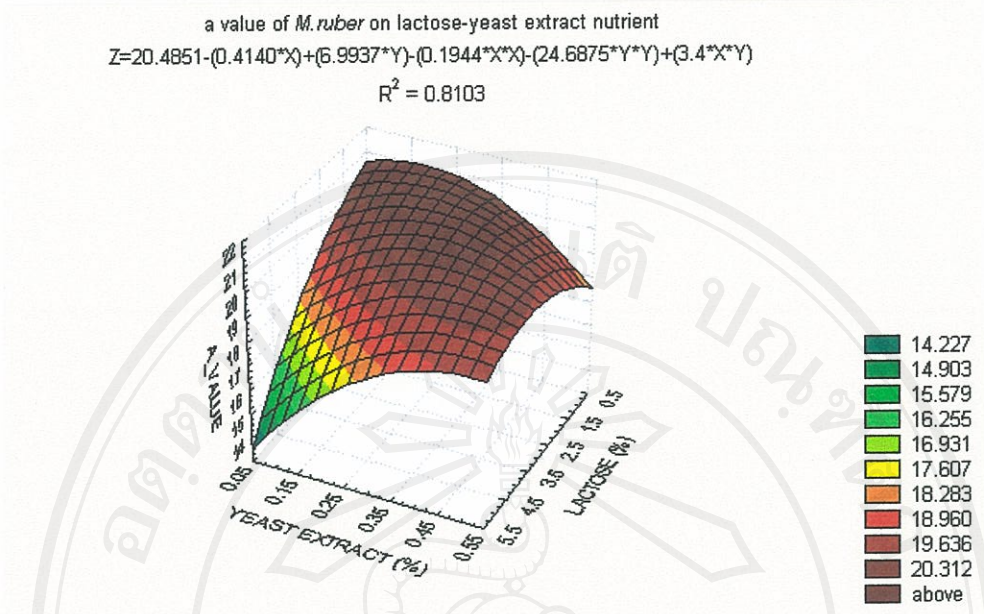

Fig 4.17 a value of Hunter color system of *Monascus ruber* TISTR3006 on adlay added with lactose and yeast extract

In case of other properties related to *Monascus ruber* TISTR3006 for examples: glucosamine, citrinin, yellow pigment, orange pigment, red pigment, b value, moisture content and pH did not fit in second-order model under this experiment (R^2<0.7). There was possibility that optimization of these characteristic was beyond the ranges of lactose and yeast extract levels under this study. The ranges of these properties were shown in Appendix E.

With regard to *M. purpureus* DMKU, production of glucosamine, citrinin, mevinolin, yellow pigment, orange pigment, red pigment, L value, a value, b value, moisture content and pH responsed from combined lactose and yeast extract supplements did not fit in second-order model under this experiment (R^2<0.7). There was possibility that optimization of these characteristic was beyond the ranges of lactose and yeast extract levels under this study. The ranges of these properties were shown in Appendix E.

4.5 Effects of carbon and nitrogen source on adlay angkak production

***4.5.1 Effects of glucose-peptone or lactose-yeast extract on M. purpureus* DMKU**

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.

Combinations of glucose-peptone or lactose-yeast extract had no effect on *Monascus* pigments. 5% lactose and 0.3% yeast extract lowered of the level of L value and b value. The ranges of moisture content of adlay angkak added with glucose-peptone or lactose-yeast extract was 59.06-76.32% and the range of pH was 5.43-5.90. However, the alternative way to develop adlay angkak cultivated by *M. purpureus* DMKU for increasing mevinolin content should use 1% glucose and 0.3% peptone, 3% glucose and 0.1% peptone or 3% lactose and 0.5% yeast extract as supplement and optimize the physical factors such as initial moisture content, pH, incubation temperature and oxygen content (Palo *et al.*, 1960).

Properties of pigments, Hunter Lab, moisture content and pH did not have direct correlation with the production of both citrinin and mevinolin of *M. purpureus* DMKU.

Table 4.6 Glucosamine, citrinin and mevinolin content of adlay angkak cultivated with *M. purpureus* DMKU

Nutrients	Glucosamine (ppm)	Citrinin (ppm)	Mevinolin (ppm)
1. 1%Glucose+0.3%Peptone	41.91 ± 49.44^{ns}	0.89 ± 0.15^{ab}	22.21 ± 7.60^{a}
2. 3%Glucose+0.1%Peptone	3.16 ± 4.46^{ns}	0.62 ± 0.36^{ab}	16.33 ± 2.17^{ab}
3. 3%Glucose+0.5%Peptone	3.17 ± 0.72^{ns}	0.00 ± 0.00^{b}	0.48 ± 0.45^{c}
4. 5%Glucose+0.3%Peptone	3.38 ± 0.26^{ns}	0.00 ± 0.00^{b}	0.40 ± 0.09^{c}
5. 1%Lactose+0.3%Yeast Extract	7.88 ± 4.12^{ns}	0.63 ± 0.89^{ab}	0.00 ± 0.00^{c}
6. 3%Lactose+0.1%Yeast Extract	12.13 ± 16.86^{ns}	0.10 ± 0.15^{ab}	0.22 ± 0.00^{c}
7. 3%Lactose+0.5%Yeast Extract	6.70 ± 0.51^{ns}	0.96 ± 0.23^{a}	10.00 ± 4.73^{b}
8. 5%Lactose+0.3%Yeast Extract	11.46 ± 14.10^{ns}	0.00 ± 0.00^{b}	0.18 ± 0.01^{c}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.7 Pigments of adlay angkak cultivated with *M. purpureus* DMKU

Nutrients	Absorbance		
	400 nm	470 nm	500 nm
1. 1%Glucose+0.3%Peptone	5.08±1.01^{ns}	1.73±0.00^{ns}	1.94±0.01^{ns}
2. 3%Glucose+0.1%Peptone	5.94±1.84^{ns}	1.78±0.54^{ns}	2.02±0.64^{ns}
3. 3%Glucose+0.5%Peptone	5.90±1.15^{ns}	1.85±0.44^{ns}	2.10±0.49^{ns}
4. 5%Glucose+0.3%Peptone	7.59±0.42^{ns}	2.29±0.27^{ns}	2.57±0.30^{ns}
5. 1%Lactose+0.3%Yeast Extract	5.17±1.13^{ns}	1.64±0.40^{ns}	1.86±0.46^{ns}
6. 3%Lactose+0.1%Yeast Extract	6.34±0.29^{ns}	2.01±0.12^{ns}	2.30±0.17^{ns}
7. 3%Lactose+0.5%Yeast Extract	5.93±0.67^{ns}	1.95±0.20^{ns}	2.24±0.23^{ns}
8. 5%Lactose+0.3%Yeast Extract	6.67±1.74^{ns}	2.31±0.87^{ns}	2.70±1.09^{ns}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.8 L, a and b values of adlay angkak cultivated with *M. purpureus* DMKU

Nutrients	Hunter Lab		
	L value	a value	b value
1. 1%Glucose+0.3%Peptone	48.67±3.73^{ab}	18.01±1.83^{ns}	7.76±2.00^{ab}
2. 3%Glucose+0.1%Peptone	48.40±1.56^{ab}	17.93±0.88^{ns}	7.50±0.75^{ab}
3. 3%Glucose+0.5%Peptone	49.98±1.32^{a}	18.96±0.08^{ns}	8.71±0.48^{a}
4. 5%Glucose+0.3%Peptone	49.76±0.96^{ab}	19.41±0.78^{ns}	8.22±0.71^{a}
5. 1%Lactose+0.3%Yeast Extract	47.94±0.80^{ab}	19.25±0.59^{ns}	7.67±0.37^{ab}
6. 3%Lactose+0.1%Yeast Extract	46.71±0.23^{ab}	18.96±0.15^{ns}	6.73±0.37^{ab}
7. 3%Lactose+0.5%Yeast Extract	48.54±0.36^{ab}	19.52±0.56^{ns}	7.70±0.60^{ab}
8. 5%Lactose+0.3%Yeast Extract	45.92±0.59^{b}	18.34±0.00^{ns}	5.59±0.35^{b}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.9 Moisture content and pH of adlay angkak cultivated with *M. purpureus* DMKU

Nutrients	Moisture content (%)	pH
1. 1%Glucose+0.3%Peptone	67.01±3.10^{abc}	5.63±0.13^{ab}
2. 3%Glucose+0.1%Peptone	73.92±4.57^{a}	5.77±0.20^{ab}
3. 3%Glucose+0.5%Peptone	59.06±7.42^{c}	5.43±0.18^{b}
4. 5%Glucose+0.3%Peptone	76.32±1.23^{a}	5.80±0.30^{ab}
5. 1%Lactose+0.3%Yeast Extract	71.88±2.41^{ab}	5.80±0.23^{ab}
6. 3%Lactose+0.1%Yeast Extract	65.81±3.58^{abc}	5.72±0.14^{ab}
7. 3%Lactose+0.5%Yeast Extract	68.05±6.11^{abc}	5.90±0.07^{a}
8. 5%Lactose+0.3%Yeast Extract	61.82±4.16^{bc}	5.70±0.12^{ab}

Means within columns with different superscripts were significantly different ($P<0.05$)

4.5.2 Effects of glucose-peptone or lactose-yeast extract on M. ruber TISTR3006

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.

Combinations of 3% lactose and 0.1% yeast extract or 5% lactose and 0.3% yeast extract encouraged the production of orange and red pigments. Therefore, these supplement suitable for the production of adlay angkak with high orange and red pigments. This is a specific characteristic of each strain and species of microorganism which consumed carbon and nitrogen source to generate different metabolites (Yongsmith, 1999).

With regard to visual determination of color by L, a and b values, this experiment showed that all formulae of supplement except 3% lactose and 0.1% yeast extract produced highest a value. While 1% glucose and 0.3% peptone, 3% glucose and 0.5% peptone or 5% glucose and 0.3% peptone favored highest b value. The range of moisture content of adlay angkak added with glucose-peptone or lactose-yeast extract was 58.92-65.89% and the range of pH was 5.20-6.00. Moisture content of adlay angkak from *M. purpureus* DMKU was higher than *M. ruber* TISTR3006 in most samples.

Properties of pigments, Hunter Lab, moisture content and pH did not have direct correlation with the production of both citrinin and mevinolin of *M. ruber* TISTR3006.

Table 4.10 Glucosamine, citrinin and mevinolin content of adlay angkak cultivated with *M. ruber* TISTR3006

Nutrients	Glucosamine (ppm)	Citrinin (ppm)	Mevinolin (ppm)
1. 1%Glucose+0.3%Peptone	64.52±78.91^{ab}	11.59±4.99^{a}	28.63±7.82^{ns}
2. 3%Glucose+0.1%Peptone	58.84±48.91^{ab}	2.03±2.87^{b}	18.11±5.66^{ns}
3. 3%Glucose+0.5%Peptone	96.43±3.52^{ab}	0.00±0.00^{b}	0.00±0.00^{ns}
4. 5%Glucose+0.3%Peptone	11.65±4.21^{b}	0.00±0.00^{b}	0.20±0.03^{ns}
5. 1%Lactose+0.3%Yeast Extract	149.20±45.18^{a}	9.95±1.40^{a}	13.20±18.67^{ns}
6. 3%Lactose+0.1%Yeast Extract	7.00±0.53^{b}	0.00±0.00^{b}	0.11±0.15^{ns}
7. 3%Lactose+0.5%Yeast Extract	72.79±49.54^{ab}	3.14±4.44^{b}	20.27±28.67^{ns}
8. 5%Lactose+0.3%Yeast Extract	35.61±1.43^{b}	0.00±0.00^{b}	0.15±0.06^{ns}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.11 Pigments of adlay angkak cultivated with *M. ruber* TISTR3006

Nutrients	Absorbance		
	400 nm	470 nm	500 nm
1. 1%Glucose+0.3%Peptone	31.31±0.09^{ns}	5.62±0.17^{c}	5.01±0.34^{b}
2. 3%Glucose+0.1%Peptone	36.38±4.39^{ns}	7.02±2.36^{bc}	6.22±2.38^{b}
3. 3%Glucose+0.5%Peptone	29.70±4.05^{ns}	5.18±0.68^{c}	4.74±0.36^{b}
4. 5%Glucose+0.3%Peptone	32.70±11.04^{ns}	6.52±3.40^{bc}	6.00±3.10^{b}
5. 1%Lactose+0.3%Yeast Extract	34.05±3.10^{ns}	8.24±0.52^{bc}	7.10±0.37^{b}
6. 3%Lactose+0.1%Yeast Extract	34.32±7.26^{ns}	10.12±2.21^{ab}	8.31±1.89^{ab}
7. 3%Lactose+0.5%Yeast Extract	33.51±0.96^{ns}	7.74±0.06^{bc}	6.86±0.06^{b}
8. 5%Lactose+0.3%Yeast Extract	38.93±0.14^{ns}	13.11±1.57^{a}	11.41±1.15^{a}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.12 L, a and b values of adlay angkak cultivated with *M. ruber* TISTR3006

Nutrients	Hunter Lab		
	L value	a value	b value
1. 1%Glucose+0.3%Peptone	49.74±1.07^{a}	20.44±0.85^{a}	7.71±0.44^{a}
2. 3%Glucose+0.1%Peptone	47.10±0.98^{b}	20.70±0.62^{a}	6.76±0.06^{b}
3. 3%Glucose+0.5%Peptone	51.12±0.88^{a}	20.48±0.49^{a}	7.99±0.49^{a}
4. 5%Glucose+0.3%Peptone	49.67±2.48^{a}	20.45±0.30^{a}	7.95±0.69^{a}
5. 1%Lactose+0.3%Yeast Extract	46.20±0.25^{b}	20.51±0.17^{a}	5.99±0.04^{bc}
6. 3%Lactose+0.1%Yeast Extract	46.72±1.50^{b}	19.07±0.69^{b}	6.39±0.41^{bc}
7. 3%Lactose+0.5%Yeast Extract	46.28±0.15^{b}	19.99±0.01^{ab}	5.59±0.00^{c}
8. 5%Lactose+0.3%Yeast Extract	45.69±0.71^{b}	19.64±0.45^{ab}	5.73±0.40^{c}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.13 Moisture content and pH of adlay angkak cultivated with *M. ruber* TISTR3006

Nutrients	Moisture content (%)	pH
1. 1%Glucose+0.3%Peptone	65.75±1.33^{ab}	5.81±0.07^{ab}
2. 3%Glucose+0.1%Peptone	65.89±0.86^{a}	5.56±0.01^{bcd}
3. 3%Glucose+0.5%Peptone	63.31±3.43^{ab}	6.00±0.05^{a}
4. 5%Glucose+0.3%Peptone	63.08±0.52^{ab}	5.70±0.17^{bc}
5. 1%Lactose+0.3%Yeast Extract	62.19±1.32^{ab}	5.46±0.11^{cde}
6. 3%Lactose+0.1%Yeast Extract	59.32±4.71^{ab}	5.20±0.06^{e}
7. 3%Lactose+0.5%Yeast Extract	62.56±3.71^{ab}	5.56±0.05^{bcd}
8. 5%Lactose+0.3%Yeast Extract	58.92±2.88^{b}	5.38±0.21^{de}

Means within columns with different superscripts were significantly different ($P<0.05$)

4.6 Comparison of properties of adlay angkak added carbon-nitrogen source with commercial red rice

4.6.1 Glucosamine, citrinin and mevinolin content in adlay angkak added carbon-nitrogen source and commercial red rice

Glucosamine, citrinin and mevinolin were investigated from adlay angkak cultivated by *M. purpureus* DMKU and *M. ruber* TISTR3006 and these also were detected in commercial red rice (Table 4.14 and 4.15). The results showed that Xeuzhikang Jiaonang (drug) contained highest concentration of mevinolin (259.8516 ppm) but citrinin and glucosamine contents were not detected. It was possible to explain that Xeuzhikang Jiaonang was a kind of drug, in which pure mevinolin was added. Therefore, this drug was not contaminated with citrinin as a mycotoxin and glucosamine derived from chitin, fungal cell wall component. Mevinolin content in original adlay angkak sample (without carbon and nitrogen supplement) cultivated by *M. ruber* TISTR3006 was lower (6.04 ppm) than original adlay angkak sample (without carbon and nitrogen supplement) cultivated by *M. purpureus* DMKU (12.77 ppm). *M. purpureus* DMKU confirmed red production of citrinin (0.01 ppm or 10 ppb) than *M. ruber* TISTR3006 (0.67 ppm or 670 ppb). Although, *M. ruber* TISTR3006 produced 30.82 ppm glucosamine twice more than *M. purpureus* DMKU (15.03 ppm). However, addition of 1% glucose and 0.3% peptone as carbon-nitrogen source encouraged on the mevinolin production by *M. ruber* TISTR3006 (28.63 ppm).

Commercial red rice sample from U.S.A. had the higher concentration of mevinolin than commercial sample from Thailand. However, both samples were detected for citrinin concentration 0.3862 and 1.1812, respectively. Sabater-Vilar *et al.* (1999) reported the citrinin could be detected in all the commercial *Monascus* samples at concentrations varying between 0.2-17.1 ppm. It showed that the citrinin concentration of red rice from Thailand and from U.S.A. detected by our method of HPLC/MSD were presented in the range, which detected by the other research. Mevinolin content of red rice from U.S.A. was detected with higher concentration than commercial sample from Thailand, 38.5129 and 14.1255 ppm, respectively. Therefore, commercial red rice had been used as dietary supplement for treatment blood cholesterol for a long time because they had mevinolin substrance for reducing blood cholesterol and low mycotoxin or citrinin.

Table 4.14 Glucosamine, citrinin and mevinolin content of adlay angkak (*M. purpureus* DMKU) and commercial red rice

Products	Glucosamine (ppm)	Citrinin (ppm)	Mevinolin (ppm)
1. Adlay angkak without supplement	15.03±0.98^{ns}	0.01±0.00^{c}	12.77±3.60^{c}
2. Adlay angkak (1%Glucose+0.3%Peptone)	41.91±49.44^{ns}	0.89±0.15^{ab}	22.21±7.60^{bc}
3. Adlay angkak (3%Lactose+0.5%Yeast Extract)	6.70±0.51^{ns}	0.96±0.23^{ab}	10.00±4.73^{c}
4. Red rice (Thai)	44.85±12.70^{ns}	1.18±0.15^{a}	14.13±2.59^{c}
5. Red rice (U.S.A.)	36.81±0.26^{ns}	0.39±0.55^{bc}	38.51±1.50^{b}
6. Xuezhikang Jiaonang (drug)	0.00±0.00^{ns}	0.00±0.00^{c}	259.85±20.75^{a}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.15 Glucosamine, citrinin and mevinolin content of adlay angkak (*M. ruber* TISTR3006) and commercial red rice

Products	Glucosamine (ppm)	Citrinin (ppm)	Mevinolin (ppm)
1. Adlay angkak without supplement	30.82±3.28^{ns}	0.67±0.15^{b}	6.04±0.90^{b}
2. Adlay angkak (1%Glucose+0.3%Peptone)	64.52±78.91^{ns}	11.59±4.99^{a}	28.63±7.82^{b}
3. Adlay angkak (3%Lactose+0.5%Yeast Extract)	72.79±49.54^{ns}	3.14±4.44^{b}	20.27±28.67^{b}
4. Red rice (Thai)	44.85±12.70^{ns}	1.18±0.15^{b}	14.13±2.59^{b}
5. Red rice (U.S.A.)	36.81±0.26^{ns}	0.39±0.55^{b}	38.51±1.50^{b}
6. Xuezhikang Jiaonang (drug)	0.00±0.00^{ns}	0.00±0.00^{b}	259.85±20.75^{a}

Means within columns with different superscripts were significantly different ($P<0.05$)

4.6.2 Physical and chemical properties of adlay angkak added carbon-nitrogen source and commercial red rice

Intensity of pigments of adlay angkak cultivated by *M. purpureus* DMKU and *M. ruber* TISTR3006 were showed in Table 4.16 and 4.17, respectively. Orange and red pigments of commercial red rice and Xuezhikang Jiaonang were higher concentration than all of adlay angkak supplemented with carbon and nitrogent medium as well as that of original adlay angkak without supplement (blank). However, yellow pigment of commercial red rice from Thailand and from U.S.A. and drug as Xuezhikang Jiaonang from Beijing, China were higher concentration than adlay angkak (*M. purpureus* DMKU) supplemented carbon-nitrogen source but it showed lower concentration than 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 (Table 4.18 and 4.19). It demonstrated that this drug was darker, less red and yellow color than other samples of angkak.

Adlay angkak produced by *M. purpureus* DMKU had moisture content of 68.05-69.25% more than adlay angkak produced by *M. ruber* TISTR3006 (62.37-65.75%). However, treatments between the same strain were not significantly different. On the other hand, glucose-peptone medium and lactose-yeast extract medium had no effect on moisture content of adlay angkak produced by *M. ruber* TISTR3006 and *M. purpureus* DMKU.

The pH of commercial red rice angkak and Xuezhikang Jiaonang were lower than adlay angkak (Table 4.20 and 4.21). The fatty acids originally present in the raw adlay might have been consumed by the growing fungus (Yang, *et al.*, 2004). In addition, Ma *et al.* (2000) reported that *Monascus*-fermented rice contained 2.8% of fatty acids while Yang *et al.* (2004) found that the acid values of adlay angkak products were 0.635-0.708 %. Therefore, fatty acids might affect the level of pH resulted in lower pH in rice angkak than adlay angkak.

Table 4.16 Pigments of adlay angkak (*M. purpureus* DMKU) and commercial red rice

Products	Absorbance		
	400 nm	470 nm	500 nm
1. Adlay angkak without supplement	8.93±1.70^{d}	3.24±1.07^{d}	3.76±1.36^{d}
2. Adlay angkak (1%Glucose+0.3%Peptone)	5.08±1.01^{e}	1.73±0.00^{e}	1.94±0.01^{e}
3. Adlay angkak (3%Lactose+0.5%Yeast Extract)	5.93±0.67^{e}	1.95±0.20^{e}	2.24±0.23^{de}
4. Red rice (Thai)	16.66±0.26^{b}	12.22±0.26^{b}	18.21±0.45^{b}
5. Red rice (U.S.A.)	26.93±0.20^{a}	20.20±0.03^{a}	28.42±0.20^{a}
6. Xuezhikang Jiaonang (drug)	13.26±0.64^{c}	8.44±0.44^{c}	11.23±0.56^{c}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.17 Pigments of adlay angkak (*M. ruber* TISTR3006) and commercial red rice

Products	Absorbance		
	400 nm	470 nm	500 nm
1. Adlay angkak without supplement	34.08±5.63^{a}	8.37±1.74^{c}	7.42±1.79^{d}
2. Adlay angkak (1%Glucose+0.3%Peptone)	31.31±0.09^{ab}	5.62±0.17^{d}	5.01±0.34^{e}
3. Adlay angkak (3%Lactose+0.5%Yeast Extract)	33.51±0.96^{a}	7.74±0.06^{c}	6.86±0.06^{de}
4. Red rice (Thai)	16.66±0.26^{c}	12.22±0.26^{b}	18.21±0.45^{b}
5. Red rice (U.S.A.)	26.93±0.20^{b}	20.20±0.03^{a}	28.42±0.20^{a}
6. Xuezhikang Jiaonang (drug)	13.26±0.64^{c}	8.44±0.44^{c}	11.23±0.57^{c}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.18 L, a and b values of adlay angkak (*M. purpureus* DMKU) and commercial red rice

Products	Hunter Lab		
	L value	a value	b value
1. Adlay angkak without supplement	48.45±0.95^{a}	18.00±0.16^{b}	6.70±0.66^{a}
2. Adlay angkak (1%Glucose+0.3%Peptone)	48.67±3.73^{a}	18.01±1.83^{b}	7.76±2.00^{a}
3. Adlay angkak (3%Lactose+0.5%Yeast Extract)	48.54±0.36^{a}	19.52±0.56^{ab}	7.70±0.60^{a}
4. Red rice (Thai)	47.06±0.29^{a}	20.76±0.11^{a}	4.08±0.07^{b}
5. Red rice (U.S.A.)	46.28±0.04^{a}	20.33±0.28^{a}	4.23±0.24^{b}
6. Xuezhikang Jiaonang (drug)	41.92±0.59^{b}	11.08±0.27^{c}	0.60±0.18^{c}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.19 L, a and b values of adlay angkak (*M. ruber* TISTR3006) and commercial red rice

Products	Hunter Lab		
	L value	a value	b value
1. Adlay angkak without supplement	47.19±0.89^{b}	20.03±0.88^{a}	6.04±0.28^{b}
2. Adlay angkak (1%Glucose+0.3%Peptone)	49.74±1.07^{a}	20.44±0.85^{a}	7.71±0.44^{a}
3. Adlay angkak (3%Lactose+0.5%Yeast Extract)	46.28±0.15^{b}	19.99±0.01^{a}	5.59±0.00^{b}
4. Red rice (Thai)	47.06±0.29^{b}	20.76±0.11^{a}	4.08±0.07^{c}
5. Red rice (U.S.A.)	46.28±0.04^{b}	20.33±0.28^{a}	4.23±0.24^{c}
6. Xuezhikang Jiaonang (drug)	41.92±0.59^{c}	11.08±0.27^{b}	0.60±0.18^{d}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.20 Moisture content and pH of adlay angkak (*M. purpureus* DMKU) and commercial red rice

Products	Moisture content (%)	pH
1. Adlay angkak without supplement	69.25±0.40^{ns}	5.93±0.13^{a}
2. Adlay angkak (1%Glucose+0.3%Peptone)	67.01±3.10^{ns}	5.63±0.13^{b}
3. Adlay angkak (3%Lactose+0.5%Yeast Extract)	68.05±6.11^{ns}	5.90±0.07^{a}
4. Red rice (Thai)	-	4.89±0.09^{c}
5. Red rice (U.S.A.)	-	4.69±0.05^{c}
6. Xuezhikang Jiaonang (drug)	-	4.66±0.01^{c}

Means within columns with different superscripts were significantly different ($P<0.05$)

Table 4.21 Moisture content and pH of adlay angkak (*M. ruber* TISTR3006) and commercial red rice

Products	Moisture content (%)	pH
1. Adlay angkak without supplement	62.37±3.79^{ns}	5.54±0.13^{b}
2. Adlay angkak (1%Glucose+0.3%Peptone)	65.75±1.33^{ns}	5.81±0.07^{a}
3. Adlay angkak (3%Lactose+0.5%Yeast Extract)	62.56±3.71^{ns}	5.56±0.05^{b}
4. Red rice (Thai)	-	4.89±0.09^{c}
5. Red rice (U.S.A.)	-	4.69±0.05^{d}
6. Xuezhikang Jiaonang (drug)	-	4.69±0.04^{d}

Means within columns with different superscripts were significantly different ($P<0.05$)