

CHAPTER 7

General discussion and conclusion

Red mold rice (RMR) or red yeast rice is a traditional food and medicine that has been used for long time in Asia (Lee *et al.*, 2013). Many countries included China, Japan, Philippines and Taiwan used RMR as food coloring and food preservative as well as commonly for alternative of statin therapies and available as a dietary supplement for the treatment of dyslipidemia (Erdogrul and Azirak, 2005; Becker *et al.*, 2009). Statins are class of drugs widely used and have been proven to be effective in the prevention of atherosclerotic vascular disease and reducing plasma low-density lipoprotein (LDL) cholesterol concentrations (Hu *et al.*, 2012). Many publications were focused on the properties of active component in RMR especially monacolin K. Monacolin K can inhibit the mevalonate synthesis by inhibiting HMG CoA (3-hydroxy-3-methyl-glutaryl-Coenzyme A) reductase which is involved in the cholesterol biosynthesis (Lin *et al.*, 2008).

RMR was prepared by solid state fermentation on rice by *Monascus* species (Wang and Lin, 2007). The suitable conditions for RMR production were obtained from the selection of *Monascus purpureus* strain with high monacolin K and low citrinin production. Many studies mostly focused on using of UV irradiation for improve the metabolites production included high yield of pigment or removal citrinin (Wang *et al.*, 2004; Fang *et al.*, 2008). *M. purpureus* CMU002U, 002N, 003U, 004U and 004N are the mutant strains from induced mutation by UV irradiation and NTG treatment that showed the low citrinin production than wild type strains. The selection of suitable substrates and fungal strains for high monacolin K production were evaluated under solid state fermentation at 30°C over 14 days. After the selection, a high yield of monacolin K was obtained from Sanpatong glutinous rice fermented by *Monascus purpureus* CMU002U. Plackett-Burman design and response surface methodology (Central Composite Design) were found to be effective in optimizing the significant

factors that would improve the monacolin K yield. The optimized level of the two factors including soaking time and fermentation time were found to be 3.54 hours and 35 days, respectively under SSF conditions at 30°C by *M. purpureus* CMU002U. The maximum yield of monacolin K led to a 2.83 fold increase after optimization and citrinin was decreased 2.73%. The method to obtain high monacolin K production was similar to Suh *et al.* (2007) that used the *Monascus* KU609 mutant strain and optimized by response surface methodology and the final monacolin K production was increased 100-fold compared to the parental strain.

Moreover, the other main purpose of this study was to increase the value of rice to high quality of RMR product. RMR is the product with high commercial value compared with non-processed rice. Solid state fermentation of RMR by temperature shifting was the alternative method for obtaining high monacolin K yield. Temperature shifting was a simple technique that has been used to improve monacolin K yield. Feng *et al.* (2014) who stated that solid state fermentation by temperature shifting between 30°C and 23°C could increase monacolin K yield. Six varieties of purple glutinous rice (Doi Muser, Doi Saked, Na, Nan Phayao and Hom CMU) were used as substrate. Our study showed that the production of monacolin K, citrinin, red pigments and antioxidant properties in solid state fermentation by *M. purpureus* CMU002U was dependent on the purple rice varieties. The solid state fermentation of the Doi Muser variety showed a high level of monacolin K and red pigment production. Interestingly, the fermented product of the Na variety showed the lowest citrinin concentration (132 ppb), which passed the standards of Japan, Taiwan and the EU (Chung *et al.* 2009). The extracts of the fermented products acquired from Doi Saked revealed the highest DPPH scavenging activity. Moreover, the temperature shifting treatment by fermentation for 5 days at 30°C followed by 25 days at 25°C on the solid state fermentation of the Doi Muser variety yielded higher values of monacolin K and citrinin.

Monacolin K present in RMR was identical to lovastatin (Endo, 1979). The concern over statin safety was first raised with the revelation of myopathy and rhabdomyolysis as well as its cause nephrotoxic and hepatotoxic in mammals (Maji *et al.* 2013). However, the occurrence of myopathy and rhabdomyolysis are rare 1/100000 patient-years. RMR is safe for renal and liver function when compared with other statins as the low adverse effects and no reported between food and drug interactions

(Wang *et al.*, 1997; Heber *et al.*, 1999). Meta-analysis of the efficacy of 3 brands of RMR preparations (Cholestin, Xuezhikang, and Zhibituo) in 9,625 patients documented no serious adverse reactions. The most common adverse reactions included dizziness, decreased appetite, abdominal discomfort, and heartburn. However, RMR was safe when compared with other available statins, as the incidence of adverse side effects is fairly low (Journoud and Jones, 2004). US FDA issued a consumer warning to avoid RMR products because some products contained unauthorized drug (lovastatin) and citrinin (Childress *et al.*, 2013). Using of monacolin K from RMR is limited in some countries of European. The products providing 10 mg per day of monacolin K is considered as drug (Le Bloc'h *et al.*, 2005). Many publications supported that intake of RMR could lead to reduced the cholesterol levels compared to more efficient statins, and less several adverse side effects. Kumari *et al.* (2007) studied the safety of 2, 4, 8 and 12% of RMR for 14 weeks in albino rats. The results showed that no significant differences in the hematological parameters, macroscopic and microscopic changes in vital organs and serum clinical enzyme levels between the experimental and control groups. Moreover, the extract of RMR could decrease the concentrations of serum total cholesterol and low density lipoprotein cholesterol in induced hypocholesterolemic rats (Bunnoy *et al.* 2015). Furthermore, Lee *et al.* (2010) confirmed that 200 ppm (200,000 ppb) of citrinin in *Monascus*-fermented products did not affect the functions of liver and kidney or cause any nephrotoxicity and hepatotoxicity.

Eggs are major source of many nutrients including minerals, proteins, and unsaturated fatty acids, which could lower the risk of cardiovascular disease (Song and Kerver 2000). One large egg contained almost 210 mg of cholesterol (Krauss *et al.*, 2000). For many years, people believed that consumption of eggs would raise cholesterol in the blood and lead to heart disease. Many people have been advised to limit their consumption of eggs included one yolk per day. There isn't much scientific support for these limitations (Kritchevsky, 2004). Consumption of eggs up to one egg per day is not related to increase the risk of coronary heart disease (Rong *et al.*, 2013). Some organizations recommended that reducing of egg consumption is not important for healthy people with normal levels of cholesterol (Better Health Channel, 2009). Some studies showed that participants who became diabetic during observation, consumption of one egg a day markedly increased cardiovascular risk compared with

less than one per week (Spence *et al.*, 2010). However, general population has been suggested to limit cholesterol intake to less than 300 mg/day (Krauss *et al.*, 2009).

RMR is one of the dietary supplements that used for improvement of egg qualities in poultry industry. Wang and Pan (2003) demonstrated that RMR could decrease the egg yolk cholesterol in hens as well as the level of cholesterol in serum. Japanese quail eggs are becoming popular due to their high nutritional value. The egg taste is no different from chicken or duck eggs. However, their eggs have higher cholesterol levels than other poultry. The cholesterol concentration of egg yolk from chicken was 7.65 mg/g whereas that of ducks and quails was 10.36 and 16.05 mg/g (Aziz *et al.*, 2012).

Japanese quail was used as a model for studying the properties of RMR. Plants included fruits, vegetable and herbs in a dried form or as extracts were used as dietary supplement as well as mineral, vitamin and probiotic. There are containing numerous biologically active compounds to improve the growth performance, egg production and egg qualities (Szczerbinska *et al.*, 2015). The properties of RMR and lovastatin drug were evaluated in 35-days old female Japanese quails for 8 weeks. RMR and lovastatin could decrease level of egg yolk cholesterol as well as total cholesterol in blood. In addition RMR could increase egg production whereas did not affect to egg qualities included yolk colour and composition in eggs. The recommended of RMR doses to decrease the cholesterol in egg yolk is 6 or 12 mg per day. In addition, throughout the experiments had no mortalities and Japanese quail that treated with RMR and lovastatin drug did not have any significant effect on BUN, creatinine, globulin and albumin. Finally, for practical application, RMR should be mixed to diet that is easy to manage than oral gavage.

In conclusion, RMR has a high potential to be developed as a dietary supplement that is of the way to increase value of rice to high value. High yield of monacolin K and low citrinin content in RMR were obtained from solid state fermentation of glutinous rice by using mutant strain under suitable condition. Due to properties of monacolin K in RMR on performance of Japanese quail can increase their egg production and decrease egg yolk cholesterol level as well as total cholesterol and LDL in blood serum. Supplementation of RMR did not affect on egg qualities. The egg production with low content of cholesterol is alternative diet food for people that are of concern about high cholesterol.