CHAPTER 2

Literature review

2.1 *Monascus* strains

Monascus spp. (Alexopoulos *et al.,* 1996) classified to

Phylum : Ascomycota
Class : Ascomycetes
Subclass : Plectomycetidae
Order : Eurotiales
Family : Monascaceae
Genus : *Monascus*

The genus *Monascus* consist of 9 species; *M. floridanus, M. pallens, M. pilosus, M. purpureus, M. ruber, M. sanguineus, M. eremophilus, M. lunisporas* and *M. argentinensis* that can reproduce either vegetatively with filaments and conidia or sexually by the formation of ascospores (Figure 2.1).

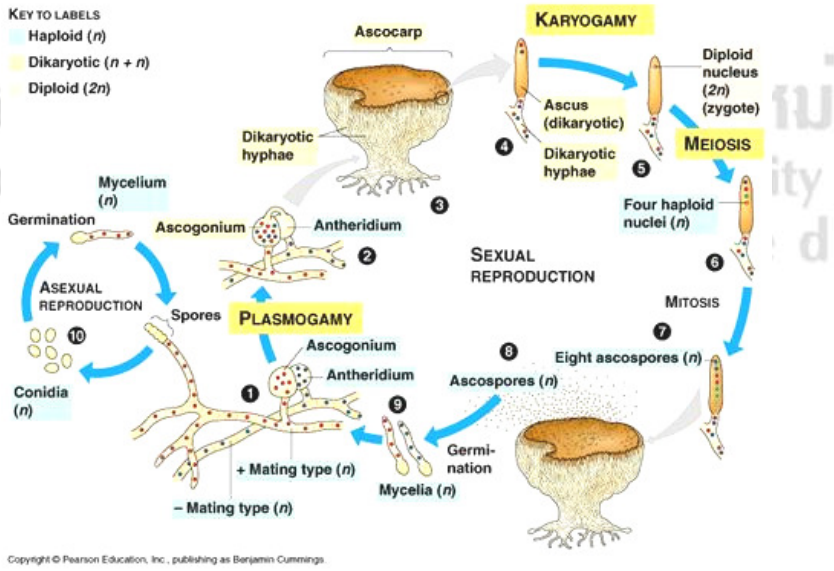


Figure 2.1 Life cycle of *Ascomycetes* (Shao *et al.,* 2011)

Monascus purpureus (Figure 2.2), *M. ruber* and *M. pilosus* are often used for RMR production (Patakova, 2013).

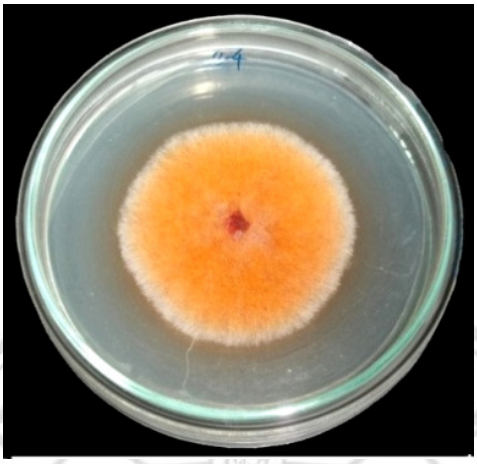


Figure 2.2 *Monascus purpureus*

2.2 Red mold rice

RMR is a product of rice fermented by fungus known as *Monascus* spp., this fungus grown and fermented rice while also produced red pigments that made rice had dark red color and odor (Figure 2.3) (Chen and Hu, 2005). The product is called differently depending on local language. Chinese calls the product as “Ankak” or “Anka” or “Ang Khak” or “Hong Qu” while Japanese calls “Beni-Koji” or “Anka-Koji” and general calls as Red Yeast Rice (Hesseltine, 1965).

Figure 2.3 Red mold rice

Using of RMR in China was first recorded in the Tang Dynasty in 800 AD. It has been used to make a food preservative and food colorant (Figure 2.4).

Figure 2.4 RMR used as a food preservative for maintaining the color, including pickled tofu, Chinese wine and Japanese sake (akai-sake) (Stuart, 1979; Ma *et al.,* 2000)

During the Ming Dynasty (1368–1644), RMR was used for improving the blood circulation and recorded on the books of Chinese medicine named *Ben Cao Gang Mu-Dan Shi Bu Yi* (Heber *et al.,* 2001). In 1998 Pharmanex had sold the "Cholestin" dietary supplement, the result showed that consumption 4 capsules for 8 weeks can reduce the cholesterol in the body but it may affect to the liver and muscle (Havel, 1999). The composition of RMR was shown in Table 2.1 and many metabolites in RMR has medicinal properties that showed in Table 2.2

Table 2.1 Composition of Chinese RMR dietary supplement

Component	Percentage by weight
Rice starch	73.4
Fiber	0.8
Protein	5.8
Moisture	3–6
Total natural pigment	< 0.33
Ash	< 3
Phosphorus (organic phosphorus 0.02%)	0.44
Trace elements (Ca, Al, Fe, Mn, Mg, Cu and Ag)	Trace
Total HMG-CoA reductase inhibitors	0.4
Fatty acids	
Saturated	< 0.5
Mono- and polyunsaturated	< 1.5

Table 2.2 Medicinal properties of RMR (Modified from Hsu and Pan, 2012)

Properties	Reference
Hypolipidemic effect	Lee *et al.,* 2006
Blood pressure lowering	Wu *et al.,* 2009
Anti-fatigue	Wang *et al.,* 2006b
Alzheimer's disease prevention	Lee *et al.,* 2011
Obesity prevention	Chen *et al.,* 2008
Anti-diabetic effect	Shi and Pan, 2010
Anti-cancer	
- Colon	Hong *et al.,* 2008
- Lung	Chen *et al.,* 2010
- Oral	Tsai *et al.,* 2009
- Breast	Lee *et al.,* 2013

2.3 Secondary metabolites in RMR

2.3.1 *Monascus* pigments

Monascus pigments are a group of azaphilones that is the metabolically synthesized from polyketide chromophores and beta-keto acids by esterification. *Monascus* species could produce at least 6 molecular structures of pigment which can be classified into 3 groups depending on their color (Figure 2.5) including yellow pigments monascin ($C_{21}H_{26}O_5$) and ankaflavin ($C_{23}H_{30}O_5$), the orange pigments monascorubrin ($C_{23}H_{26}O_5$) and rubropunctatin ($C_{21}H_{22}O_5$), and finally the red pigments monascorubramine ($C_{23}H_{27}NO_4$) and rubropuntamine ($C_{21}H_{23}NO_4$) (Campoy *et al.,* 2006).

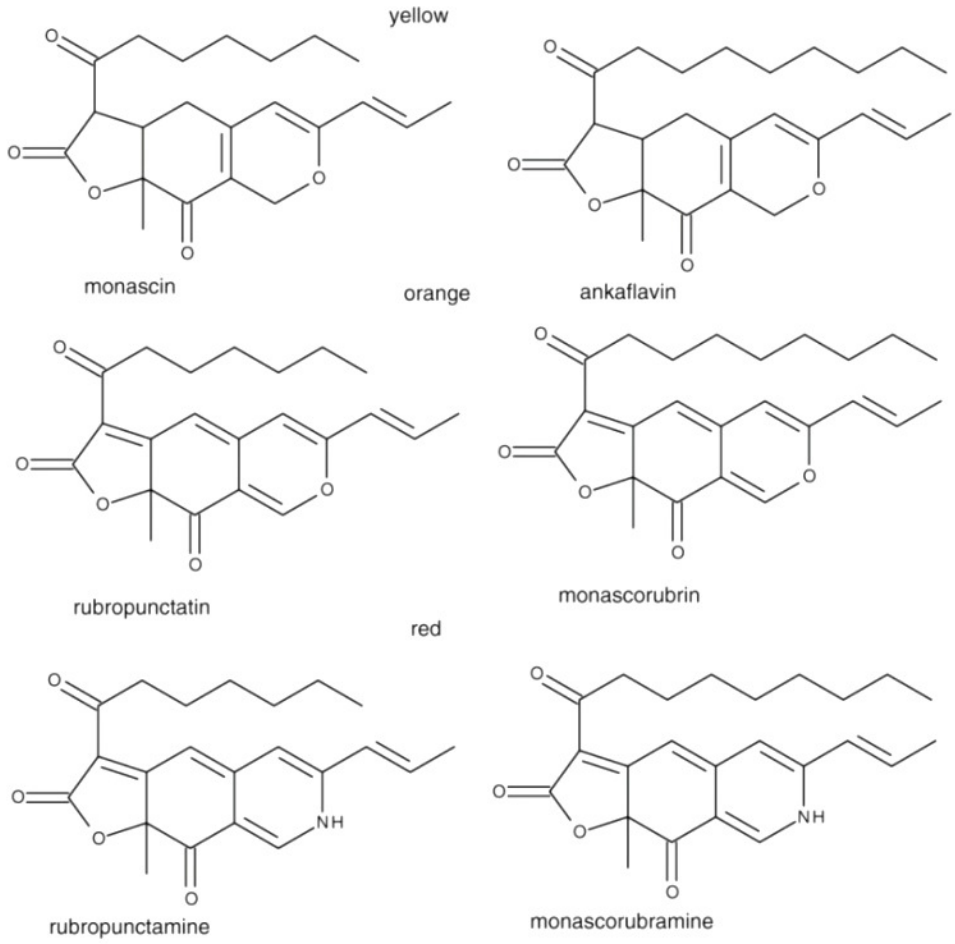


Figure 2.5 *Monascus* pigments

Monascus pigments have biological functions to inhibit the enzymatic activities, leading to antimicrobial, antiviral, antioxidant, anti-inflammatory or other characteristic activities (Yang *et al.,* 2014). In addition, novel azaphilonoid derivatives from RMR have been reported to be anti-inflammatory and anticancer effects on human laryngeal carcinoma and colon adenocarcinoma cell lines (Hsu *et al.,* 2010a; Hsu *et al.,* 2010b; Hsu *et al.,* 2013; Li *et al.,* 2010). Yellow pigment was produced by *Monascus* sp. has been proven to be beneficial compounds as antihypercholesterolemic and anti-inflammation agents, respectively (Sani *et al.,* 2013). In recent years, raw materials and agriculture wastes from peels, seeds, whey, molasses and bagasses are rich in nutrient component for fermentation. These have been proposed as sources for microbial pigment production (Panesar *et al.,* 2015).

The red pigment has been of increasing interest in the food industry as color enhancing agent because the pigment is extracellular and water soluble making it easy to use (Fabre *et al.,* 1993). There are various factors influencing the pigments production including pH, temperature and carbon source.

The effect of variation of medium for red pigments synthesis by *M. purpureus* CMU 001 found that corn meal was the best medium that could produce 19.4 unit/gram dried weight when compared with peanut meal and soybean meal (Nimnoi and Lumyong, 2009). Maximum red pigment production from *M. purpureus* MTCC 369 was optimized by central composite design of response surface methodology under SSF. The solid medium was supplemented with dextrose, peptone, NH_4Cl, $MnSO_4 \cdot H_2O$ and malt extract (Ahmad, 2014). Extremely low-frequency magnetic fields is a novel method to increase the red and yellow pigment production by *M. purpureus* SKY219, The medium that containing the cellular suspension was exposure 0.4 mT magnetic field for 8 days could increase red and yellow pigment production (Zhang *et al.,* 2014b; Zhang *et al.,* 2015).

The influence of carbon and nitrogen ratio on red pigments production by *M. ruber* has been studied on submerged culture. The optimal glucose and glutamate ratio was about 10 and could increase red pigment production (Hajjaj *et al.,* 2015). The influence of various carbon sources on pigment production by

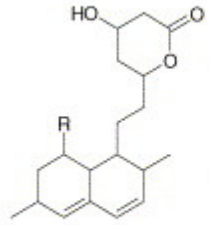
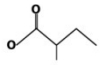
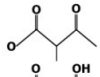
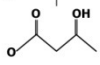
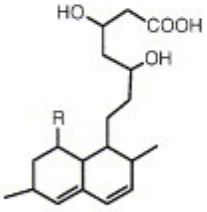
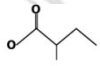
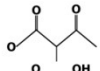
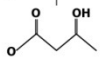
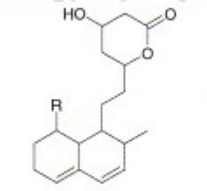
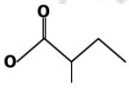
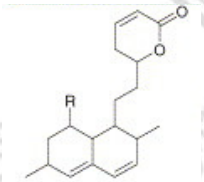
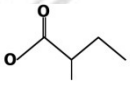
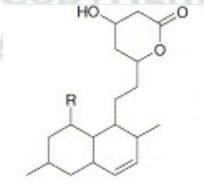
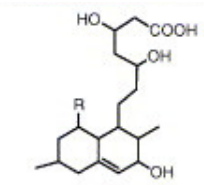
Monascus FJ46 showed that naked oats flour, millet flour, sorghum flour, corn flour, *Cordyceps sinensis* residues, and sweet potato can be used substrates. Additionally, this is the first report on pigments production using *Cordyceps sinensis* residues as substrate (Mu *et al.,* 2015). There are reported that corn and sugarcane bagasses were the best substrate for red pigment production. The maximum pigment was obtained from SSF than in submerged fermentation (Rajeswari *et al.,* 2014). The optimum condition of liquid culture by cultivation of *M. purpureus* ATCC1603 was studied from Response surface design. The highest pigments yield was achieved with a pH 3, supplemented with maltose and shaken at 150 rpm 25°C for 14 days (Baneshi *et al.,* 2014). Other studies showed that glucose and tryptone addition could achieve the highest red pigment production (Prajapati, 2014).

The influence of oxygen supply on *Monascus* pigments and citrinin production by *M. ruber* HS.4000 in submerged fermentation was studied by Yang *et al.,* (2015). The initial growth phase, mid-stage phase, and later-stage production phase were separated by shifting oxygen supply. The optimal condition had 3 stage rotating rate as follow 0-48 hour at 150 rpm, 48-108 hour at 250 rpm and 108-120 hour at 200 rpm) with 100 mL medium added to 250 mL Erlenmeyer flasks at 30°C for 120 hour cultivation. The pigments were reduced by 40%, but citrinin was reduced by 64% compared to constant one-stage cultivation (rotating rate at 250 rpm).

2.3.2 Monacolins

Monascus fermented products (MFP) contain many major chemical constituents which have chemical similarity to statin. At least 14 monacolin compounds such as monacolin K, J, L, M, X both lactone and acid form as well as dehydromonacolin K, dihydromonacolin L, compactin, and 3-α-hydroxy-3,5-dihydromonacolin L. The structure and molecular weight of these compounds are shown in Table 2.3.

Table 2.3 The structural data of monacolins in RMR (Li *et al.,* 2004)

Structure	Name	R	MW
Lactone form 	Monacolin K		404
	Monacolin J		320
	Monacolin L		304
	Monacolin X		418
	Monacolin M		406
Acid form 	Monacolin K		422
	Monacolin J		338
	Monacolin L		322
	Monacolin X		436
	Monacolin M		424
	Compactin		390
	Dehydomonacolin K		386
	Dihydromonacolin L	H	306
	3α-hydroxy-3,5-dihydromonacolin L	H	340

Monacolin K is a secondary metabolite can inhibit the activity of HMG-CoA reductase in cholesterol synthesis by conversion of HMG-CoA to mevalonate (Alberts *et al.,* 1980).

Monacolin K is a member of the drug class of statins used for lowering cholesterol (Endo, 1979), tumor progression and metastasis of Lewis lung carcinoma cells (Ho and Pan, 2009). Monacolin K was also produced from *Aspergillus terreus*, *Monascus purpureus*, and other species of *Monascus* (Ahmad *et al.,* 2009; Jaivel and Marimuthu, 2010).

Monacolin K has two forms (Figure 2.6), lactone (also known as lovastatin or mevinolin) and hydroxyl acid forms. Monacolin K is the main monacolin in *Monascus purpureus*-fermented rice (75-90% of total monacolin content) (Heber *et al.,* 1999; Li *et al.,* 2004).

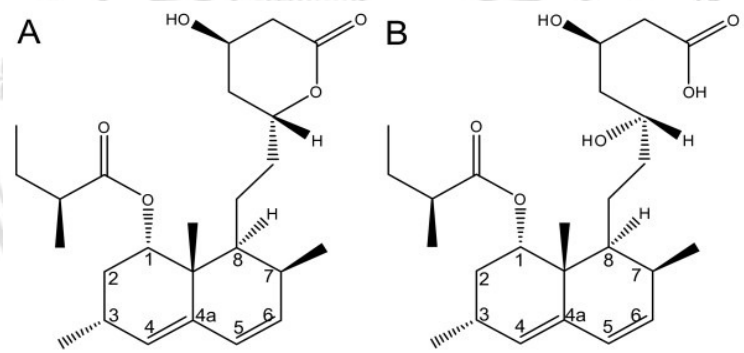


Figure 2.6 Monacolin K in RMR as lactone (A) or hydroxyl acid (B) (https://www.researchgate.net/figure/221968475_fig2_Lovastatin-monacolin-K-may-occur-in-red-yeast-rice-as-lactone-A-or-hydroxy-acid-B)

Monacolin K ($C_{24}H_{36}O_5$, IUPAC Name,1S,3R,7S,8S,8aR)-8-{2-[(2R,4R 4-hydroxy-6–oxooxan-2-yl]ethyl}3,7dimethyl1,2,3,7,8,8ahexahydronaphtha-len-1-yl(2S)-2methylbutanoate), molecular weight 404.55, white crystal, dissolved in ethanol, methanol and benzene, chloroform, acetone, but insoluble in hexane and petroleum ether (Endo, 1979).

There are many substrates have been used for monacolin K production such as glutinous rice, non-glutinous rice (Chairote *et al.,* 2008; Chairote *et al.,* 2009), durian seed (Srianta, 2012), coconut oil, grape waste, jackfruit seed powder, palm kernel cake, sesame oil cake and wheat bran (Babitha *et al.,* 2006; Silveira *et al.,*

2008). Agricultural waste including coconut residue, corn meal, peanut meal, soybean meal, corn hull, gram bran, orange peel, orange pulp, sugar cane bagasses and wheat bran were also reported as substrates (Pansuriya and Singhal 2010).

Yu *et al.,* (2013) concluded that glutinous rice was suitable substrate for SSF by *M. purpureus* to produce monacolin K Under optimal condition, 50% of initial moisture content, 4 cm of material thickness and fermentation time for 13 days could increase monacolin K to 2710 ppm.

RMR is commonly used in the cuisine of Fujian regions of China. The study of monacolin K in RMR from Fujian and the other products by HPLC method by Huang *et al.,* (2006) found that RMR from Fujian had monacolin K 2100-3800 ppm more than commercial product that had 310-624 ppm. Huang *et al.,* (2011) who isolated the high monacolin K-producing *Monascus* strains from Xiangxi natural savory vinegar. The result showed that *Monascus* M3 could produce the highest monacolin K 157 ppm in broth. This strain was optimized based on Box-Behnken central composite design. The optimal conditions were found to be: initial pH of 5.3, 25°C at 112 rpm for 10.5 days, the yield of monacolin K reached up to 355 ppm. Cultural parameters like carbon, nitrogen, pH and temperature had a significant influence on monacolin K production in SMF by *M. purpureus*. The effect of different carbon sources (glucose, fructose, maltose, sucrose and lactose) on monacolin K production indicated that maltose could increase monacolin K yield to 71 ppm

Among different nitrogen sources (ammonium nitrate, ammonium sulphate, yeast extract, peptone and ammonium chloride), peptone produces highest concentration of monacolin K (62 ppm). The optimum temperature was found to be 28°C (72 ppm of monacolin K) and pH 5 was found optimum (56 ppm) that played a major role on the synthesis of monacolin K. The maximum of monacolin K yield (81 ppm) was achieved with the above optimum parameters (Dhale and Govindaswamy, 2012).

Similarity, monacolin K yield could increase by proper optimization of fermentation parameters using a Box-Behnken experimental design. The optimal conditions for were found to be supplement with fructose, sodium nitrate, and acetic acid at pH 5 and incubated at 30°C. A maximum yield of monacolin K

increased to 37 ppm (Rajasekaran and Kalaivani, 2012). Liquid state fermentation (LSF) of *Monascus* strains could produce red pigment highly that corresponded to monacolin K production as pH of media is changed (Seo *et al.,* 2012). However, Kim *et al.,* (2012) stated that high pigment-producing strains produced less monacolin K while low pigment-producing strains produced much more monacolin K. The growth rates of most of *Monascus* strains were highest on PDYA medium (PDA medium with yeast extract.), and hyphal growth was faster at 30℃ than at 25℃.

SSF of *M. purpureus* for monacolin K production was carried out by using agar as carrier. The results showed that the optimal particle size, agar and glycerol concentration were 4×4×4 mm, 4% and 18%, respectively The monacolin K yield reached to 2047 ppm, 4.47 times higher than submerged fermentation (SMF) (Zhang *et al.,* 2013). There are reports that surfactant and precursor could enhance monacolin K production using *M. purpureus* 9901 in SMF. Sodium citrate and triton X-100 addition could increase monacolin K by 53 and 85%, respectively (Zhang *et al.,* 2014a).

Apart from that monacolin K could produced by other strain of fungal. Cultivation of *Aspergillus terreus* ATCC 74135 in synthetic media that supplemented with inorganic nitrogen could increase the biomass but monacolin K yield was decreased. The higher monacolin K was obtained by adding glucose and lactose. On the other hand, the addition of ethanol and glycerol leaded to low monacolin K production because these substances reduced NADH synthesis via polyketide pathway which is a precursor of monacolin K synthesis (Hajjaj *et al.,* 2001).

There are many studies reported that monacolin could decrease significantly under the conditions of high humidity at high temperature (75% RH, 60°C) and sunlight (Li *et al.,* 2005). Ou *et al.,* (2009) suggested that monacolin K was easily degraded when the products exposure heat. However, more than 50% of monacolin K could be remained in products when heated under the pasteurization temperature that used for food processing. The degradation of monacolin K in products depended on storage conditions. At 4°C under vacuum package was found to enhance retention of monacolin K (Jirasatid *et al.,* 2013).

2.3.3 Citrinin

Citrinin is one of the toxic secondary metabolite, first isolated from filamentous fungus *Penicillium citrinum* (Hetherington and Raistrick, 1931). It is also produced by other species of *Penicillium*, *Aspergillus* and *Monascus* (Kurata, 1990; Li *et al.,* 2003). On account of its antibacterial effects, citrinin was investigated as an antibiotic (Wong and Koehler, 1981).

Citrinin (Figure 2.7) is an acidic lemon yellow crystal with a maximal absorption at 250 and 331 nm (Magan and Olsen, 2004).

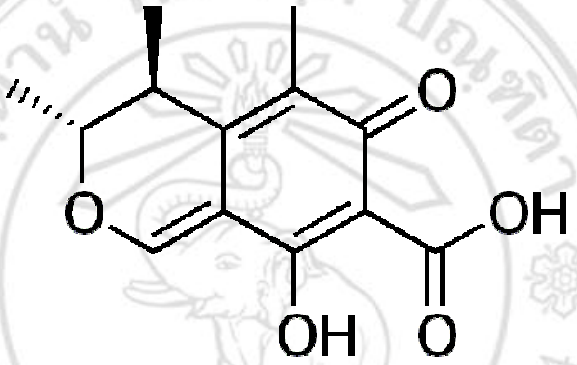


Figure 2.7 Citrinin structure ($C_{13}H_{14}O_5$)
(https://en.wikipedia.org/wiki/Citrinin)

It could sparingly soluble in water but soluble in dilute sodium hydroxide, sodium carbonate, or sodium acetate; in methanol, acetonitrile, ethanol, and most of other polar organic solvents. It can be degraded in acid, alkaline solution, or heat (Deshpande, 2002). Contaminations of citrinin were reported in agricultural commodities, foods, feedstuffs as well as biological fluids Citrinin may occur naturally in maize, cheese, wheat, barley, silage, fruit excluding irradiated fruits and RMR (Xu *et al.,* 2006). From 200 samples of wheat grain in Tunisia found that 50% of sample was contaminated with citrinin average of 28 ppb (Zaied *et al.,* 2012). Sampling of RMR in Malaysia, citrinin was detected in all samples. The highest citrinin level was about 20,700 ppb (Samsudin and Abdullah, 2013). Citrinin is the secondary metabolites, had a negative impact on the acceptance of RMR product. The toxicity studies showed that this secondary metabolite acted in

animals as a nephrotoxin (Betina, 1989), damaged the proximal tubules of the kidney, and was implicated as a potential causative.

Fermentation conditions and different fungal strains may lead to difference in the citrinin level. SSF on rice by *Monascus* sp. could produce citrinin levels up to 2,500,000 ppb whereas in LSF culture up to 56,000 ppb (Eisenbrand, 2006). Addition of fatty acid including hexanoic and octanoic in the medium, the pigment and citrinin were increased while malic acid addition, pigments were decreased but did not affect to the citrinin synthesis. The culture conditions were improved by histidine addition. Red pigments increased to 6-fold and no citrinin because histidine was taken and hydrogen peroxide was presented and damage the citrinin structure during fermentation (Blanc *et al.,* 1998). These results according to Fouler *et al.,* (1993) who concluded that hydrogen peroxide could detoxify the citrinin.

The red pigments were increased to 30-40% and citrinin was decreased when octanoic acid addition in liquid medium for cultivation of *M. ruber* (Hajjaj *et al.,* 2000a). However, some organic acids such as L-malate and fumarate could inhibit the red pigments synthesis (Hajjaj *et al.,* 2000b). Moreover, histidine addition into rice substrate, the citrinin content was reduced by 62% (Baipong, 2003).

Cultivation of *M. purpureus* NTU 568 in liquid medium that contained 1% dioscorea concentration, 0.5% ethanol concentration and pH 5.7 could increase monacolin K levels by 47% and decrease citrinin level by 54% (Lee *et al.,* 2007a). Furthermore, phosphate ethanol extraction was the effective method for the citrinin removal. Under optimal condition, 91.6% citrinin was removed and 79.5% monacolin K was retained in the RMR (Lee *et al.,* 2007b). Cultivation of *Monascus* sp. under blue illumination in submerged culture found that red pigments were increased 28.5% and citrinin was decreased by 79% (Wang *et al.,* 2009). Apart from that, the genetic recombination of *pks*1 gene which associated pigments synthesis was replaced *ctn*A gene. The results showed that pigments were increased 33.9% and citrinin was decreased 42% (Xu *et al.,* 2009). Finally, citrinin and monacolin K production were similar trends under the same conditions when monacolin K increased, tended to result in high citrinin. In

addition, the synthetic pathways of monacolin K and red pigment from *Monascus* species were closely related as low monacolin K production is the cause to decreased red pigment (Juzlova *et al.,* 1996). However, both red pigment and citrinin are produced from a polyketide derivative (Figure 2.8). Therefore, the synthetic pathways of monacolin K and citrinin from *Monascus* are closely related (Hajjaj *et al.,* 1999).

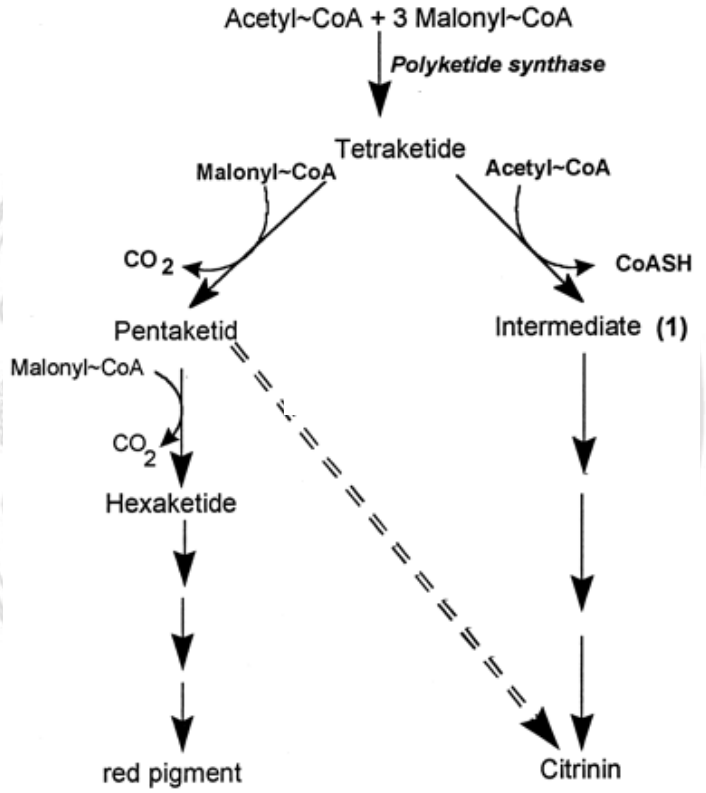


Figure 2.8 Biosynthesis of citrinin and red pigment in *M. ruber*

2.4 Solid state fermentation

Solid state fermentation (SSF) denoted that the cultivation of microorganisms on solid, moist substrates in the absence of a free aqueous phase. However, substrate must have enough moisture to support growth and metabolism of microorganism. It has been developed as a potential technology for the production of microbial products including feed, fuel, food, industrial chemicals and pharmaceutical products (Pandey, 2003). The rates of utilization of various nutrients differ in each substrate. In SSF technique, the substrates were utilized very slowly and can be used for longer periods during

fermentation. This fermentation was suited for fungi and microorganisms that require less moisture content (Joshi, 2006).

2.5 Applications of RMR

In 1998, Li *et al.* has been studied the effect of RMR and monacolin K on the serum cholesterol of rabbits and quails. The rabbits were fed with 25% casein of diet to induce hypercholesterolemia. The total cholesterol was increased from 70 to 290 mg/dl within 60 days and the total cholesterol showed a 4-fold increase above normal rabbits. After that, hyperlipidemic rabbits were treated daily with RMR 0.2, 0.4, 0.8 g and monacolin K 8 mg/kg (body weight) also reduced total cholesterol by 45, 43, 59 and 52%, respectively that compared to the cholesterol level on day 60 and 90. In addition, quails were fed with high saturated fat to induce high cholesterol level in blood. Total cholesterol increased 5 fold after 2 weeks. After that, quails were treated with RMR 0.1, 0.2, 0.4 g and mevinolin 4 mg/kg (body weight), the total cholesterol reduced by 29, 28, 39 and 43%, respectively. Triglyceride and LDL-cholesterol concentration also reduced. In USA, 83 subjects both man and woman with hyperlidemia were treated with 2.4 g RMR/day for 8 weeks. Total cholesterol, LDL and triglyceride concentration were decreased by 17, 22 and 11%, respectively (Heber *et al.,* 1999). The effect of RMR was studied in rabbit for long term (Wei *et al.,* 2003). The rabbits were treated with 1.35 g RMR/kg/day throughout experiment for 200 day. Total cholesterol, LDL and triglyceride level were decreased from control group by 40, 24 and 60%, respectively RMR could reduce serum cholesterol, triglyceride and low density lipoprotein (LDL) in laying hen egg when mixed the RMR in feed. The results showed that 0.5% and 2.5% of RMR in feed could reduce serum cholesterol level in egg yolk by 20% and 40%, respectively (Wang and Pan, 2003; Wang *et al.,* 2006a). In addition, the pigments RMR also accelerated the egg yolk color (Chayawat *et al.,* 2008). In 2009, Wu *et al.* studied the effect of RMR and red mold dioscorea (RMD) on blood pressure in rat. The result showed that only 0.5 dose of RMD could decrease both systolic blood pressure and diastolic blood pressure. In equal dose, RMD had more effective than RMR. One study also showed the ability of an aqueous extract of RMR to induce vascular relaxation in rat aortic tissue by stimulating endothelial enzyme nitric oxide synthase to release nitric oxide (Rhyu *et al.,* 2000). RMR had ability to decrease serum total cholesterol, LDL

cholesterol and reduced the accumulation of fat in the liver in Wistar rat. In addition RMR extracts could not affect to growth, organ weight, feed intake, the enzymes which indicate liver and kidney function, blood circulation including the liver and kidneys tissue (Bunnoy *et al.,* 2015).

2.6 Status of RMR

In 1987, the lovastatin was approved as drug by FDA then RMR was presented on the USA market as dietary supplements. In 2007, the FDA considered that RMR was not a supplement because it contained unauthorized drug and issued a consumer warning to avoid RMR. However, RMR widely available to the public as drug store or on the internet. The status and using of monacolin K from RMR is limited in some countries that showed in Table 2.4

Table 2.4 The status and using of monacolin K from RMR in each country (Le Bloc'h *et al.*, 2015).

Country	Status and limitation of monacolin K using
Japan	Monacolin K in the product is an unauthorized drug.
Korea	The monacolin K concentration in product is restricted between 4 and 8 mg.
China	RMR has been used as food additive for centuries but RMR was certified as a new drug in 2005 by the Chinese department of health.
Singapore	Less than 1% of RMR is permitted for using.
Canada	The initial dose of monacolin K for treat high cholesterol is 10-20 mg/day. In the case of RMR, less than 1 mg/day of RMR extracts may be allowed to use as natural health product.
European	The regulatory status depends on the dosage of monacolin K in the product. The products providing 10 mg/day of monacolin K is considered as drug. The limit of monacolin K of each country in Europe is shown as below. - France < 10 mg/day - Finland = 3.99 mg/day - Hungary ≤ 3 mg/day - Slovenia ≤ 4 mg/day - Estonia ≤ 10 mg/day - Other Belgium, Denmark, Ireland, United Kingdom, etc. using without limitation

Many countries concerned about citrinin contamination in the agricultural products or RMR, the limit of citrinin concentration were shown in Table 2.5.

Table 2.5 Limitation of citrinin in RMR

Country	Limitation of citrinin (ppb)
US Food and Drug Administration (FDA)	20
Korea	50
Japan	200
European union (EU)	2000
Taiwan	2000

(Korean Ministry of Food and Drug Safety, 2010; Shi and Pan, 2011)

A total of 302 samples were collected from 25 cities of Taiwan from 2009 to 2012. The result was shown in Table 2.6 (Liao *et al.,* 2014).

Table 2.6 Incidence of citrinin in RMR and MFP

Samples	Citrinin contamination	Range of contamination (ppb)
84 RMR (Raw material)	58 (69%)	400 – 9350
77 Dietary supplements	27 (35.1%)	100-15200
141 Processed products*	8 (5.7%)	100-1300

** including RMR sauce, crackers, oatmeal, soy sauce and RMR wine

2.7 Improvement the secondary metabolites of *Monascus* strains

Microbes that isolated from the nature have highly regulated metabolic system for prevent the overproduction of biochemical. The important step in industrial microbiology for increasing of biochemical production is decreasing of metabolic regulation. The traditional method to improve the secondary metabolites yield was mutagenesis and fermentation analysis for obtain optimum condition (Baltz, 1998). In the present, metabolic engineering has been used for improve the secondary metabolites in many organism both plants and microorganisms (Olano *et al.,* 2008; Chen *et al.,*

2010). However, there are many countries including European, Asia especially Japan debated the genetic modified organism (GMOs) food. Japanese consumer concerned about the food safety issue including effect on human health and environmental despite the efforts of Japanese ministry of agriculture to persuade consumer that GMOs food are safe for consumption (Hino, 2002; McCluskey *et al.,* 2003). For example Asahi and Kirin, 2 leading Japanese beer factory have switched to non-GMOs ingredients and seeds (Tolbert, 2000). A mutation is a change in a specific DNA sequence when compared with the reference sequence (Cooper and Krawczak, 1993). Mutations can lead to changes in the structure of an encoded protein or to a decrease or complete loss in its expression and mutation is mentioned in this study (Lodish *et al.,* 2003). Mutagenic treatments that are most widely used for selected the mutant and the examples of *M. purpureus* strain improving are shown in Table 2.7 and 2.8.

Table 2.7 Mutagenic treatment

Treatment	Results
1. Physical agents	
1.1 Non-ionizing Radiations	These are low energy radiations and do not cause any ionization.
1.1.1 UV radiation	UV induces the formation of pyrimidine dimers (mainly thymine dimers) in the DNA. Transitions from G/C to A/T and deletions mainly occur in A/T rich regions (Tillich, 2012). It is a simple method and non toxicity.
1.2 Ionizing Radiations	The major effect is due to breaking of DNA strand.
1.2.1 X-rays	X-ray induce mutations is a single base change, a C to G transversion (Mahmoud *et al.,* 1991). Ionizing radiation increases the rate of mutation
1.2.2 Gamma-rays	^{60}Co-gamma rays were preferentially GC to AT and AT to GC transitions (Chuan *et al.,* 2004). It has long half-life and cheap.
1.2.3 High-energy-pulse-electron (HEPE) beam radiation	HEPE induce the damage of single strand and double strand for gene mutation (Zhu *et al.,* 2008).

Table 2.7 Mutagenic treatment (continued)

Treatment	Results
2. Chemical agents	
2.1 EMS (Ethyl methanesulfonate)	EMS induces C-to-T changes resulting in C/G to T/A substitutions. It effectiveness and ease of handling, especially its detoxification through hydrolysis for disposal.
2.2 MMS (Methyl methanesulfonate)	MMS induces T/A to G/C transversion and A/T to G/C transitions (Kim *et al.,* 2006)
2.3 NTG (N-methyl-N'-nitro-N-nitrosoguanidine)	NTG act like EMS by inducing lesions such o-methylguanine that cause mutation by directly mispairing, bypassing the SOS repair system (Del Gallo *et al.,* 2012). It induces mutation with much less lethality than other mutation.

Table 2.8 Example of method for improvement of *M. purpureus* strain

Methods	Results
1. Neutron and X-ray	Three mutants and 4 mutants were isolated from fast neutron and X-ray treatments, respectively. The total amount of yellow and red pigments from N4S and N11S were 2 times than wild type (Wong and Bau, 1977).
2.Gamma and electron beam radiation	Four from five mutants produced red pigment than the parental strain (Ungureanu *et al.,* 2004).
3. UV ray and chemical mutagen (EMS and NTG)	The mutant strain *M. purpureus* N 301 produced citrinin, which was 50% less than the parent strain and maintained 91% of monacolin K production (Wang *et al.,* 2004).
4. Ultraviolet and gamma-ray	Highest pigment production (48.4 U/ml) was obtained from mutant strain and had 3.67 times higher than original strain (Zhang and Huang, 2007).
5. UV ray and ultrasonic	The pigment production from mutants was 922 U/ml, which was 3 times higher than original strain (Fang *et al.,* 2008).

Table 2.8 Example of method for improvement of *M. purpureus* strain (continued)

Methods	Results
6. Microwave, ultrasonic and LiCl	Combined method on pigments production was more effective than using single method. Higher yield of pigment obtained by ultrasonic for 35 min, microwave for 20 s with 1.4% lithium chloride (Wu *et al.,* 2010).
7. UV ray and LiCl	The highest monacolin K production by the mutated strain was 3 times greater than the control (Sun *et al.,* 2011).
	Mutant strains of *Monascus* that treated by UV irradiation and incubated in media containing LiCl to could increase monacolin K yield reached up to 5,330 ppm (Yang and Hu, 2012).
8. UV ray	254 isolates of free citrinin were obtained from random mutagenesis using UV irradiation. Under optimal condition could increase monacolin K by 74% (Kalaivani and Rajeskaran 2014).

2.8 Substrates

2.8.1 Rice

Rice (*Oryza sativa* L.*)* is one of the leading food crops of the world and is the staple food of more than half of the world's population. Glutinous rice is a major type of cultivated rice with long standing cultural importance in Asia (Olsen and Purugganan, 2002). Glutinous rice (*Oryza sativa* var. glutinosa; also called sticky rice) is a type of short- or long-grained rice that grown mainly in Southeast and East Asia. It is used in preparing many kinds of traditional Asian desserts (Wittenberg, 2007; Kang *et al.,* 2010). It differs from other types of rice which the grain contains high amount of amylopectin (Juliano, 1979). However, sticky rice becomes translucent, while regular rice turns opaque white, when cooked.

Glutinous black rice (also known as purple rice) has a deep black color and turn to deep purple when cooked. Its dark purple color is due to anthocyanin content which is higher by weight than that of other colored grains (Ichikawa *et al.,* 2001; Abdel-Aal *et al.,* 2006).

Any rice, including long-grain, short-grain, or glutinous rice, may be eaten as brown rice. Brown rice and white rice have similar amounts of calories and carbohydrates. However, the main differences between two types of rice are processing and nutritional content. Brown rice is unpolished whole grain rice that is produced by removing only the hull or husk. To produce white rice, the next layers underneath the husk (the bran layer and the germ) are removed (Babu *et al.,* 2009). Brown rice has high dietary fiber, a good source of B vitamins, minerals and fat. Brown rice also contains phytic acid which is antioxidant and anti-cancer that could decrease serum cholesterol for prevent CVDs.

Many cultivars of rice and glutinous rice have been used as substrate to cultivate the mold and yeast for metabolite production. Many countries in East and Southeast Asia used the rice as substrate for winemaking. Korean traditional wine from glutinous rice and non-glutinous rice by *Ganoderma lucidum* become a new functional beverage with antihypertensive properties (Kim *et al.,* 2004b). Vietnamese rice wine made from purple glutinous rice using *Amylomyces rouxii*

and *Saccharomyces cerevisae* in mix culture had difference from regular rice wine by its sherry-like taste and flavor (Dung *et al.,* 2005; Dung *et al.,* 2006).

Leung11 rice is a variety of Thai non-glutinous rice that suitable for koji preparation using *Aspergillus Oryzae* for mirin production (Kanlayakrit and Maweang, 2006). Moreover, Hong Qu glutinous rice wine is one of the most popular traditional rice wines in China that produced from glutinous rice with the addition of RMR (Lv *et al.,* 2012).

2.8.2 Sweet potato

Sweet potato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the family *Convolvulaceae*. Its large, starchy, sweet-tasting, tuberous roots are a root vegetable (Purseglove, 1968). Sweet potato is a staple food source for many indigenous populations in Central and South Americas, Ryukyu Island, Africa, the Caribbean, the Maori people, Hawaiians, Papua New Guineans and Asia (Nabias *et al.,* 2007). It could be considered as an excellent source of natural health promoting compounds, including β-carotene and anthocyanin (Bovell-Benjamin, 2007; Johnson and Pace, 2010).

Many reports showed that sweet potato could be used as substrate for fungal growth. Citric acid is widely used in the pharmaceutical, food, beverage, chemical and other industries as an acidifying and flavor-enhancing agent (Lu *et al.,* 1998). Citric acid could be obtained from SSF of *Aspergillus niger* using dried sweet potato (Yuguo *et al.,* 1999). Sweet potato was used as a solid support and carbon source for growth of *Gongronella butleri* to chitosan production (Nwe and Stevens 2002; Nwe *et al.,* 2002). Moreover, sweet potato pickles prepared by lactic fermentation of sweet potato using *Lactobacillus plantarum* as a starter culture would be a good prospect for commercialization in small-scale industries (Panda *et al.,* 2007).

2.8.3 Lesser yam

Lesser Yam is a tuberous vegetable widely grown in tropical region. Its botanical name is *Dioscorea esculenta* and it belongs to the family of

Dioscoreaceae. It has a smaller corm than most other yams and closer in size to a potato or sweet potato (Thompson, 2003).

Lesser Yam is the most ancient species of this genus. It has been domesticated and is documented as a staple food in southern China and Southeast Asia. The tubers are cooked and consumed as a carbohydrate foodstuff (Bourke *et al.,* 1982). Lesser yam could convert to flour and used in bread making (Ukpabi, 2010).

2.8.4 Taro

Taro or cocoyam is a tropical plant grown for its edible corms and root. It belongs to the genus *Colocasia*, within the monocotyledonous family Araceae (Onwueme, 1999). The corms, which have a light purple color due to phenolic pigments when roasted, baked or boiled (Sai, 2007). The starch is easily digestible and natural sugars give a sweet nutty flavor. Furthermore, agricultural including cocoyam peel could be used as substrate for the mushroom cultivation (Obodai *et al.,* 2003) and diets for growing snails (Omole *et al.,* 2004). A comparison of the nutritional value of substrates as mentioned above is shown in Table 2.9

Table 2.9 Nutrition value in glutinous rice, non -glutinous rice, sweet potato, lesser yam and taro

Nutrient	Glutinous rice		Non -glutinous rice		Sweet potato	Lesser Yam	Taro
	Purple	White	Brown	White			
Calorie Information (kcal)	362	370	370	365	86	118	112
Total Carbohydrate (g)	76.17	81.70	77.2	79.9	20.1	27.9	26.5
* Dietary Fiber	3.40	2.8	3.5	1.3	3	4.1	4.1
* Sugar			0.9	0.1	4.2	0.5	0.4
* Starch					12.7		
Total Fat (g)	2.68	0.5	2.9	0.7	0.1	0.2	0.2
* Monounsaturated Fat	0.97	0.2	1.1	0.2			
* Polyunsaturated Fat	0.96	0.2	1	0.2		0.1	0.1
* Total saturated Fat	0.54	0.1	0.6	0.2			
Protein (g)	7.5	6.8	7.9	7.1	1.6	1.5	1.5
* Amino acid (g)							
Alanine	0.437	0.395	0.463	0.413	0.077	0.063	0.073
Arginine	0.569	0.586	0.602	0.594	0.055	0.127	0.103
Aspartic acid	0.702	0.640	0.743	0.67	0.382	0.155	0.192
Cystine	0.091	0.140	0.096	0.146	0.022	0.019	0.032

Table 2.9 Nutrition value in glutinous rice, non -glutinous rice, sweet potato, lesser yam and taro (continued)

Nutrient	Glutinous rice		Non -glutinous rice		Sweet potato	Lesser Yam	Taro
	Purple	White	Brown	White			
Glutamic acid	1.528	1.328	1.618	1.389	0.156	0.181	0.174
Glycine	0.369	0.310	0.391	0.325	0.063	0.053	0.074
Histidine	0.190	0.160	0.202	0.168	0.031	0.034	0.034
Isoleucine	0.318	0.294	0.336	0.308	0.055	0.052	0.054
Leucine	0.620	0.563	0.657	0.589	0.092	0.096	0.111
Lysine	0.286	0.246	0.303	0.258	0.066	0.059	0.067
Methionine	0.169	0.160	0.179	0.168	0.029	0.021	0.02
Phenylalanine	0.387	0.364	0.41	0.381	0.089	0.071	0.082
Proline	0.352	0.321	0.372	0.335	0.052	0.054	0.06
Serine	0.388	0.358	0.411	0.375	0.088	0.081	0.092
Threonine	0.275	0.244	0.291	0.255	0.083	0.054	0.069
Tryptophan	0.096	0.079	0.101	0.083	0.031	0.012	0.023
Tyrosine	0.281	0.228	0.298	0.238	0.034	0.04	0.055
Valine	0.440	0.416	0.466	0.435	0.086	0.062	0.082

Table 2.9 Nutrition value in glutinous rice, non -glutinous rice, sweet potato, lesser yam and taro (continued)

Nutrient	Glutinous rice		Non -glutinous rice		Sweet potato	Lesser Yam	Taro
	Purple	White	Brown	White			
Vitamins (mg)							
Thiamin	0.41	0.2	0.4	0.6	0.1	0.1	0.1
Riboflavin	0.04	0.1	0.1		0.1		
Niacin	4.31	2.1	5.1	4.2	0.6	0.6	0.6
Vitamin B6	0.51	0.1	0.5	0.2	0.2	0.3	0.3
Pantothenic Acid	1.49	0.8	1.5	1	0.8	0.3	0.3
Vitamin E			1.2	0.1	0.3	0.4	2.4
Choline			30.7	5.8	12.3	16.5	17.3
Vitamin C					2.4	17.1	4.5
Vitamin A (IU)					14185	138	76
Minerals (mg)							
Calcium	33	11	23	28	30	17	43
Iron	1.8	1.6	1.5	4.3	0.6	0.5	0.5
Magnesium	143	23	143	25	25	21	33
Phosphorus	264	71	33	115	47	55	84

Table 2.9 Nutrition value in glutinous rice, non -glutinous rice, sweet potato, lesser yam and taro (continued)

Nutrient	Glutinous rice		Non -glutinous rice		Sweet potato	Lesser Yam	Taro
	Purple	White	Brown	White			
Potassium	268	77	223	115	337	816	591
Sodium	4	7	7	5	55	9	11
Zinc	2.02	1.2	2	1.1	0.3	0.2	0.2
Copper	0.28	0.2	0.3	0.2	0.2	0.2	0.2
Manganese	3.74	1	3.7	1.1	0.3	0.4	0.4
PhytoSterol (mg)					12	10	19
Ash (g)	1.27	0.5	1.5	0.6	1	0.8	1.2
Water (g)	12.37	10.5	10.4	11.6	77.3	69.6	70.6

(SELF Nutrition Data, 2014)

2.9 Cardiovascular diseases

The American Heart Association (AHA) uses the term of cardiovascular disease (CVDs) for describe the various diseases that affect the heart and circulatory system (Mozaffarian *et al.,* 2015). CVDs are chronic disease that including coronary heart disease, hypertension, congestive heart failure, congenital cardiovascular defects, and cerebrovascular disease (Mann, 2014). In 2012, about 17.5 million people died from CVDs more than other cause, representing 31% of all global deaths. An estimated 7.4 million were due to coronary heart disease and 6.7 million related to stroke (Rastogi *et al.,* 2016).

In Thailand during 2011−2013, the death rate of CVDs per 100,000 people has increased. In 2013, a total death was 54,530 people (Treesak *et al.,* 2015). Along with high blood pressure, high blood cholesterol levels and obesity (Grundy *et al.,* 2004) that the results from personal behavior including unhealthy diet, physical inactivity, smoking and harmful use of alcohol are the intermediate risk factors for CVDs like coronary heart disease and strokes (World Health Organization, 2009).

2.10 Cholesterol

Cholesterol (Figure 2.9) is a waxy fat-like substance that is one of three major classes of lipids which all animal cells utilize to construct their membranes and is thus manufactured by all animal cells (Bellows and Moore, 2012). It can be found in large concentrations within the liver, spinal cord, and brain. It is the major precursor for the vitamin D synthesis, the various steroid hormones and sex hormones. Cholesterol also has an important role for the brain synapses as well as in the immune system (Mahley, 1988).

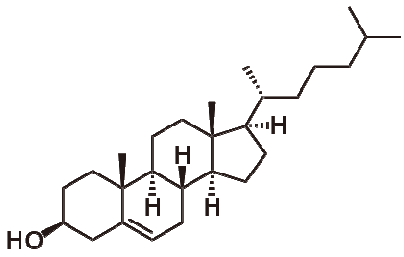


Figure 2.9 The structure of Cholesterol
(https://en.wikipedia.org/wiki/Cholesterol)

Cholesterol is the primary lipids synthesized in liver (Dietschy, 1997), and necessary for nerve cell function, sex hormone production and vitamin D production from sunlight exposure to the skin (Shekelle *et al.,* 2001). It found in certain foods, such as food from animals, like dairy products, eggs, and meat (Figure 2.10). In subjects with a regular lipid metabolism, only 1/3 of the total cholesterol is diet derived, 2/3 being synthesized directly from intracellular precursors by various organs of the body (Alberts *et al.,* 1980; Demain, 1999; Furberg, 1999). This compound is insoluble in water; it is transported in the blood plasma within protein particles that called lipoproteins, which have fat (lipid) inside and protein outside (Greenberg, 2014). There are 2 main kinds of lipoproteins (Figure 2.11) carry cholesterol in the blood.

Low density lipoprotein (LDL) also called the "bad cholesterol" because it carries cholesterol to tissues and arteries. Excessive consumption of saturated fat is the major cause of elevated LDL levels (Ascherio, 2002). LDL cholesterol often forms plaque deposits in the walls of arteries. The plaque can narrow vessels and make them less flexible, a condition known as atherosclerosis (Figure 2.12) which is a major contributor to coronary heart disease and other forms of cardiovascular disease (Goldstein and Brown, 1977). High density lipoprotein (HDL) called the "good cholesterol" because it takes cholesterol from tissues to the liver, which removes it from the body (Krieger, 1999).

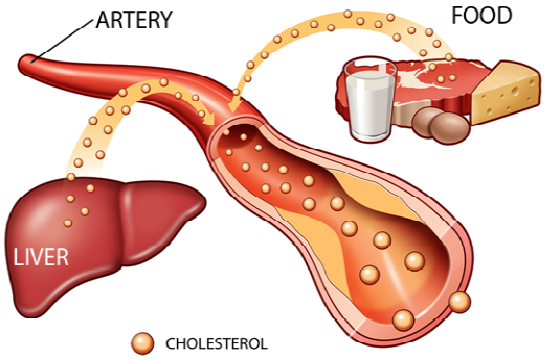


Figure 2.10 Cholesterol sources
(http://www.primecareinternalmed.com/have-you-checked-your-cholesterol-level/)

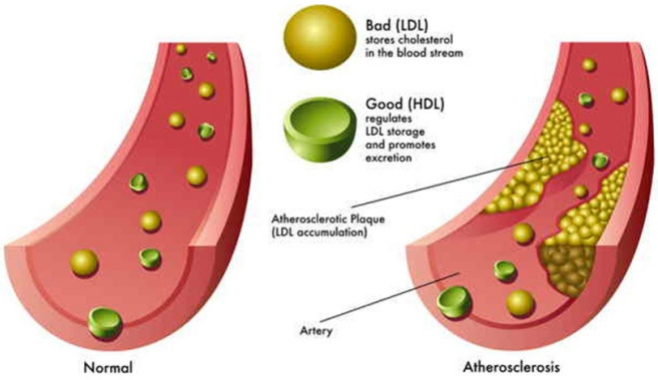


Figure 2.11 Two kinds of lipoproteins: LDL and HDL
(http://www.balancinghealth.net/4-tips-for-lowering-bad-cholesterol/)

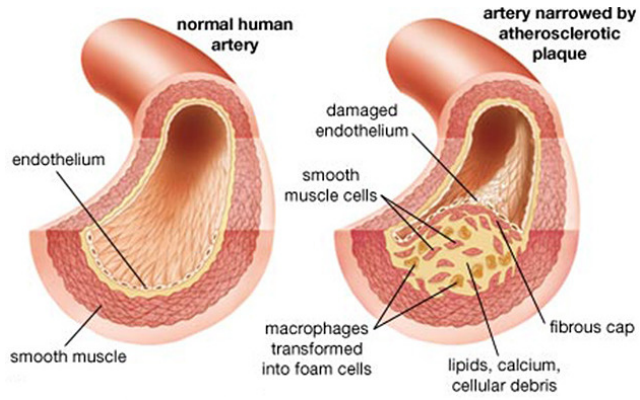


Figure 2.12 Atherosclerosis
(https://global.britannica.com/science/atherosclerosis)

Hypercholesterolemia is the presence of high cholesterol level in blood. Cholesterol levels are measured as milligrams/deciliter of blood (mg/dL) or mmol/L. To convert from mmol/L to mg/dL, for total cholesterol, HDL and LDL cholesterol multiply mmol/L by 38.67, for triglyceride multiplies mmol/L by 88.57 (Rugge *et al.,* 2011). Most people do not have any symptoms of high cholesterol. A blood test is the only way to check levels of cholesterol in your blood. In Table 2.10 gives the classifications for total, LDL and HDL cholesterol.

Table 2.10 Cholesterol Classifications

Level of cholesterol	Result
Total Cholesterol	
- Less than 200 mg/dL	Desirable
- 200–239 mg/dL	Borderline high
- 240 mg/dL and above	High
LDL (Bad) Cholesterol	
- Less than 100 mg/dL	Optimal
- 100–129 mg/dL	Near optimal/above optimal
- 130–159 mg/dL	Borderline high
- 160–189 mg/dL	High
- 190 mg/dL and above	Very High
HDL (Good) Cholesterol Level	
- Less than 40 mg/dL	A major risk factor for heart disease
- 40–59 mg/dL	The higher, the better
- 60 mg/dL and higher	Considered protective against heart disease

(US Department of Health and Human Services, 2005)

In some cases, high cholesterol level is the factor that cannot change; it depends on inheritance, age and gender (Austin *et al.,* 1990). The amount of LDL cholesterol your body makes and how fast it is removed from your body is determined partly by genes (Lawn *et al.,* 1999). Aging is associated with increase concentrations of total cholesterol and LDL cholesterol (Stevenson *et al.,* 1993). Blood cholesterol level begins to increase at age 20 and continues to go up until age 60 or 65. Before age 50, total cholesterol levels in men tend to be higher than those of women at the same age. After age 50, women have higher concentrations of total cholesterol and LDL cholesterol more than men because of postmenopausal (Carr, 2003).

Nevertheless, most often is caused by high consumption of saturated fat include animal fat products such as cream, cheese, butter, ghee, suet, tallow, lard, and fatty meats (Mahan and Escott-Stump, 2004). Some vegetable products have high saturated fat content, such as coconut oil and palm kernel oil (Chong and Ng, 1991). Many

prepared foods are high in saturated fat content such as pizza, dairy desserts, and sausage (Astrup, 2011; De Oliveira Otto *et al.,* 2012).

2.11 Prevention and treatment for lower cholesterol

Most people can lower cholesterol levels by weight management and regular exercise that reduces the risk of death from CVDs and lower LDL cholesterol levels especially when combined with a healthy diet include high consumption of fruits and vegetables (Wang *et al.,* 2014). After changing behavior and LDL cholesterol level remains high, it necessary to start drug therapy under a doctor's supervision. There are several types of cholesterol lowering drugs available that show in Table 2.11. Statins are the drug of first choice for aggressive lipid lowering actions (Tenenbaum *et al.,* 2006) and reducing risk of coronary artery disease in these patients with combined atherogenic dyslipidemia that refers to elevated levels of triglycerides and small-dense low-density lipoprotein and low levels of high-density lipoprotein (Manjunath *et al.,* 2013).

Table 2.11 Cholesterol-lowering drugs (modified from US Department of Health and Human Services, 2005)

Drugs	Process	Results
Statins	stop an enzyme that controls the rate of cholesterol production	decrease LDL levels about 20–55 % (more than other types of drugs) and moderately decrease triglycerides and raise HDL
Ezetimibe	reduces the amount of cholesterol absorbed by the body can be combined with a statin to get more lowering of LDL	lower LDL by about 18–25%
Bile acid resins	bind with cholesterol-containing bile acids in the intestines and then eliminated from the body in the stool	lower LDL cholesterol by about 15–30%
Nicotinic acid (niacin)	improves all lipoproteins; total cholesterol, LDL, triglycerides and HDL	LDL levels are usually reduced by about 5–15 %
Fibrates	mostly lower triglycerides and raise HDL levels	less effective in lowering LDL levels

The principal way to reduce the risk of developing CVDs are to lower serum LDL cholesterol levels by reducing saturated fat intake and consume a healthy diet with regular exercise. Furthermore, some specific foods and supplements can decrease cholesterol level (Niinikoski *et al.,* 2014). Several studies show that the dietary fibers include barley, beans, flaxseed, glucomannan, oat bran, and psyllium can lower cholesterol (Anderson *et al.,* 1994; Anderson, 2000; Gallaher *et al.,* 2003; Kristensen *et al.,* 2012).

Plant sterols are supplementary foods for prevention of cardiovascular disease by inhibit the cholesterol absorption at intestine (Figure 2.13) (Gylling *et al.,* 2014; Carden *et al.,* 2015). The results showed 2 g of plant sterols per day can reduce cholesterol level by about 10% (Weingartner *et al.,* 2014).

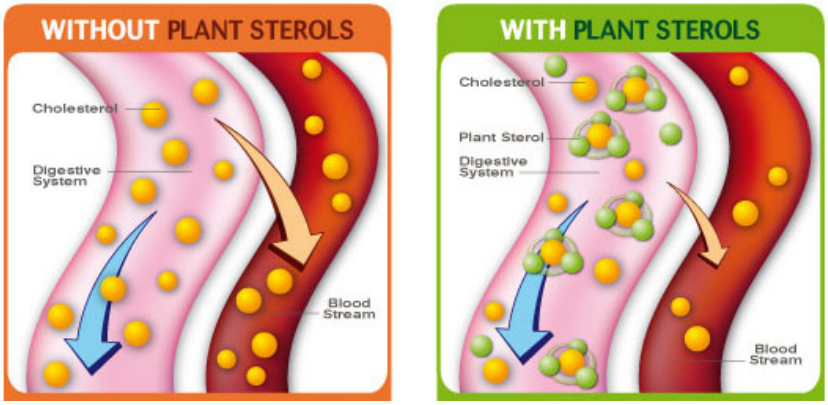


Figure 2.13 Cholesterol absorption between without and with plant sterols (http://www.gojavita.com/the-power-behind-fibers-plant-sterols/)

Several studies indicate that RMR or red yeast rice (Cholestin) can lower cholesterol levels. In China, consumption of RMR has been studied in animals and humans and has been found to reduce cholesterol concentrations by 11–32% and triacylglycerol concentrations by 12–19% (Heber *et al.,* 1999). Statins therapy is the most effective way to lower the LDL-cholesterol level in the body. High level of LDL-cholesterol has been associated with CVDs (Faltaos *et al.,* 2006; Kaptoge *et al.,* 2007). A variety of statins are produced by synthetic or fungi as secondary metabolites. These statins have a role (Figure 2.14) to inhibit the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase enzymes (Manzoni and Rollini, 2002).

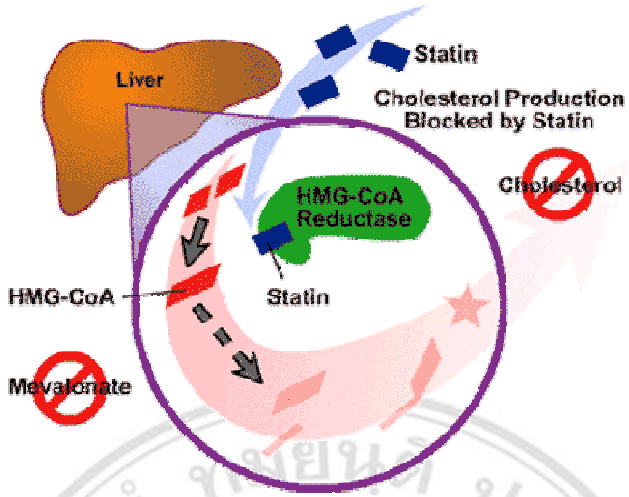


Figure 2.14 The function of statin to inhibit the cholesterol biosynthesis (http://www.medscape.org/viewarticle/416521_13)

HMG-CoA reductase has a role involve with cholesterol production in the liver, which produces about 70 % of total cholesterol in the body. Statins structure is similar to HMG-CoA and act as competitive inhibitor of HMG-CoA reductase to reduce the rate of mevalonate production. Mevalonate is a precursor of many substances required by organisms for the maintenance of their cell walls or cytoskeleton (Endo, 1992). Nevertheless, statins have the side effects including inflammation of muscles (myositis) that related to high level of creatine kinase (CK) (Staffa *et al.,* 2002; Abd and Jacobson, 2011; Rosenbaum *et al.,* 2013). Moreover, statins also increased the risk of diabetes mellitus, and abnormalities in liver enzyme tests (Naci *et al.,* 2013). There are several types of statins available on the market that showed in Table 2.8 and Figure 2.15–2.21.

Table 2.12 Available forms of statin

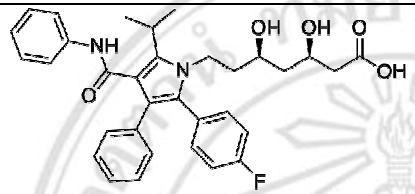
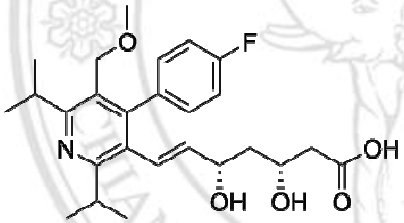
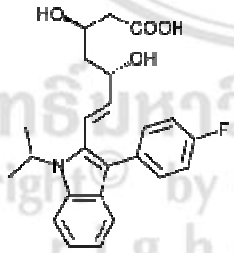
Statin	Image	Brand name	Derivation
Atorvastatin (**Figure 2.15**) 		Lipitor Torvast	Synthetic (CYP3A4)*
Cerivastatin (**Figure 2.16**) 		Lipobay Baycol	Synthetic (Various CYP3A)
Fluvastatin (**Figure 2.17**) 		Lescol Lescol XL Luvinsta	Synthetic (CYP2C9)**

Table 2.12 Available forms of statin (continued)

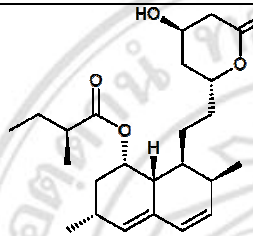
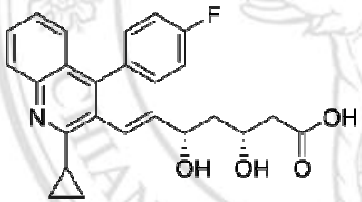
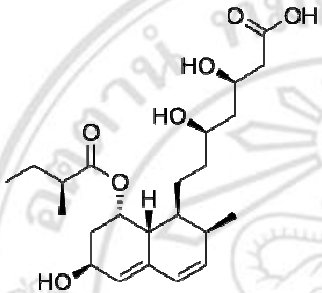
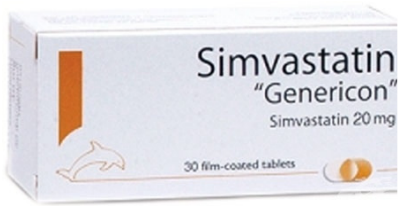
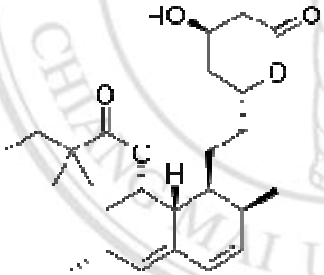
Statin	Image	Brand name	Derivation
Lovastatin (**Figure 2.18**) 		Mevacor Altocor Altoprev	Naturally (CYP3A4) - Oyster mushrooms - Red mold rice (RMR)
Pitavastatin (**Figure 2.19**) 		Livalo Pitava	Synthetic

Table 2.12 Available forms of statin (continued)

Statin	Image	Brand name	Derivation
Pravastatin (**Figure 2.20**) 		Pravachol Selektine Lipostat	A fermentation product of *Nocardia autotrophic* (Non CYP)
Simvastatin (**Figure 2.21**) 		Zocor Lipex	synthetic derivate of a fermentation product of *Aspergillus terreus* (CYP3A4)

* CYP3A4 = Cytochrome P450 3A4

** CYP2C9 = Cytochrome P450 2C9

2.12 Japanese quail

The Japanese quail belongs to the order *Galformes*, family *Phasidae* and genus *Coturnix.* The scientific name for Japanese quail is *Coturnix japonica*, different from the common quail "*Coturnix coturnix*" (Thear, 1998).

Figure 2.22 Japanese quail (Left: female, Right: male)

Adult females have pale breast feathers that are speckled with dark colored spots as well as the males have uniform dark rustred feathers on the breast and cheek (Figure 2.22). Males also have a cloacal gland, a bulbous structure on the upper edge of the vent that secretes white material. This unique gland can be used to assess the reproductive fitness of the males (Mizutani, 2003). Japanese quails are hardy birds that easy to management. They can grow in small cages and are inexpensive to keep (Figure 2.23). Japanese quail mature in about 6 weeks and are usually in full egg production by 7 weeks (Randall and Bolla, 2008).

Figure 2.30 Japanese quails in cage

Japanese quail are now farmed mainly for egg production in Japan and Southeast Asia while meat is the main product in Europe (Minvielle, 1998). As mentioned above, several aspects for the utility of this bird. First, theirs have economic importance as an

agricultural species to eggs and meat production. Second, the low maintenance cost associated with theirs small body size, resistance to diseases (Woodard *et al.,* 1973; Yalcin *et al.,* 1995; Oguz and Minvielle, 2001). Third, Japanese quail also is the smallest avian species farmed for meat and egg production (Baumgartner, 1994). It becomes popular as a laboratory animal, because of its small body size, little consumption and rapid maturation (Kayang *et al.,* 2004). Some standard biological data are shown in Table 2.13.

Table 2.13 Japanese quail information

Trait	Range
Body weight at 1-day-old	6–8 g
Adult male	100–130 g
Adult female	120–160 g
Egg weight	9–10 g
Egg number/100 days	80–90
Age at sexual maturity	38–42 days of age
Life span	Max: 7 years in male, Mean: 3–4 years

The most popular choice for egg consumption is chicken eggs. Other popular choices for egg consumption are duck quail, and caviar (Figure 2.24). Egg yolks and whole eggs store significant amounts of protein and choline. Due to their protein content, the United States Department of Agriculture categorizes eggs as meats within the Food Guide Pyramid (Williams *et al.,* 2004).

Figure 2.24 Varieties of eggs
(https://quarteramish.com/2011/09/05/the-chicken-or-the-egg/)

Smaller eggs, such as quail eggs (Figure 2.25), are common consume everyday in many parts of East Asia including China, Japanese, Philippine and Vietnam. Japanese quail eggs are a mottled brown colour and are often covered with a light blue, chalky material. The average egg weighs about 10 g, about 8% of the body weight of the quail hen (Randall and Bolla, 2008).

Figure 2.25 Japanese quail egg

Quail eggs are rich in vitamins and minerals. Even with their small size, their nutritional value is three to four times greater than chicken eggs (Tunsaringkarn *et al.,* 2012). Quail eggs contain 13.1% proteins compared to 12.6% in chicken eggs. In addition, quail eggs provide high calcium and iron and more nutrition details are showed in Table 2.14.

Table 2.14 Comparison of nutrient between quail, chicken and duck egg

Nutrient	Egg whole, fresh, raw (100 g)	
	Quail	Chicken
Calorie Information (kcal)	158	143
* From Carbohydrate	1.1	3.2
* From Fat	100	89.5
* From Protein	56.9	50.3
Total Carbohydrate (g)	0.4	0.8
* Sugar	0.4	0.8
Total Fat (g)	11.1	9.9
* Monounsaturated Fat	4.3	3.8
* Polyunsaturated Fat	1.3	1.4
* Total saturated Fat	3.6	3.1
Protein (g)		
* Amino acid (g)	13.1	12.60
Alanine	0.762	0.736
Arginine	0.835	0.821
Aspartic acid	1.294	1.330
Cystine	0.311	0.272
Glutamic acid	1.662	1.676
Glycine	0.434	0.432
Histidine	0.315	0.309
Isoleucine	0.816	0.672
Leucine	1.146	1.088
Lysine	0.881	0.914
Methionine	0.421	0.380
Phenylalanine	0.737	0.681
Proline	0.518	0.513
Serine	0.992	0.973
Threonine	0.641	0.556
Tryptophan	0.209	0.167

Table 2.14 Comparison of nutrient between quail, chicken and duck egg (continued)

Nutrient	Egg whole, fresh, raw (100 g)	
	Quail	Chicken
Tyrosine	0.543	0.500
Valine	0.940	0.859
Vitamins (mg)		
Choline	263.0	251.0
Niacin	0.2	0.1
Pantothenic Acid	1.8	1.4
Riboflavin	0.8	0.5
Thiamin	0.1	0.1
Vitamin A (IU)	543.0	487.0
Vitamin B12	0.0016	0.0
Vitamin B6	0.2	0.1
Vitamin D (IU)		35.0
Vitamin E	1.1	1.0
Minerals (mg)		
Calcium	64.0	53
Iron	3.6	1.8
Magnesium	13.0	12
Phosphorus	226.0	191
Potassium	132.0	134
Sodium	141.0	140
Zinc	1.5	1.1
Copper	0.1	0.1
Cholesterol (mg)	844	423
Ash (g)	1.1	0.9
Water (g)	74.3	75.8

(SELF Nutrition Data, 2014)

Numerous reports showed the study about Japanese quail for egg quality improvement. High temperature changed some blood parameters as well as to affect on value of the egg productivity, egg weight and eggshell thickness (Ozcelik *et al.,* 2004). L-carnitine and humates are common used as feed additives in poultry diets to improve nutrient utilization and increase laying performance. However, the combination of L-carnitine and humate didn't have significant effects on the parameter (Yalcin *et al.,* 2005). Dried baker's yeast can be used in the diets as a protein source without effects on body weight, egg production, feed efficiency, egg quality characteristics and blood serum parameters (Yalcin *et al.,* 2009). The supplementation of fish and flax oils diet of quails resulted in blood profile improvement (Al-Daraji *et al.,* 2010). In addition, Turkish propolis could increase the Japanese quail growth performance and improved the serum lipid including HDL and LDL (Denli *et al.,* 2012). Some experiments showed that *Wolffia arrhiza* could be instead of soy bean meal for increased the color intensity of yolk (Suppadit *et al.,* 2012).