CHAPTER 2

LITERATURE REVIEWS

2.1 Red yeast rice (RYR)

Red yeast rice (Figure 2.1) is a fermented product of steamed rice using a fungus, *Monascus purpureus*, that has been used in China and other Asian countries for centuries as a traditional medicine. It's also used as a food coloring, additive, and preservative. The fungus is capable of producing red pigment and giving a typical odor red rice after drying. Red yeast rice is well known among oriental people such as in China, Philippine, Taiwan, Malaysia, Hong Kong, Thailand, Cambodia and Japan (Johns and Stuart, 1991). It has been used in China for over 1,000 year for medical purposes. It was described in an ancient Chinese list of drugs useful for improving blood circulation. Chinese and American scientists developed it to be a product to lower blood lipids, including cholesterol and triglycerides. In China the rice is called Ankak or Anka. While in Japan the rice is called Beni-koji or Anka-koji. In The United State, Singapore and Canada the rice is called Cholestin, Hypocal and red yeast rice respectively.

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Figure 2.1 Red yeast rice

The rice is also used as food or food additives. It is a kind of an Asian dietary staple made by fermenting fungus (*Monascus purpureus*) on rice. It is rapidly gaining recognition as a cholesterol-lowering agent in United States, Indonesia, Japan, Taiwan, and Philippine. It is also used as coloring and flavoring agent and reduces total cholesterol as well as hyperlipidemia. Other exiting applications for red yeast rice are suggested by recent discoveries that lovastatin and other compounds may be useful for treating or preventing cancer, osteoporosis, stroke, Alzhemer's disease and other dementias and muscular degeneration (Erdoğrul and Azirak, 2004). Lee *et al*. (2002) studied red pigment production as well as monacolins, the cholesterol-lowering compounds, which had been produced by a fungus, *Monascus purpureus* 72 on solid state (agar medium) under suitable humidity and aeration. The monacolin K content in fermented rice and liquid medium using *Monascus purpureus* was found to be varied from 0.28-0.35 mg/g in rice and only 0.108 mg/g in liquid medium (Hai, 1998). The addition of sodium nitrate increased the amount of monacolin K and γ-aminobutyric acid (GABA) (Su *et al*., 2003). A part from studying on useful compounds from red yeast rice mycotoxin affecting the function of kidney, citrinin, has also been reported (Blanc *et al.*, 1995a, 1998). The amount over 200 ng/ g of citrinin uptake can cause kidney failure (Chen and Hu, 2005). Therefore, the best condition for making red yeast rice is the low content of citrinin. Consumption of 14-55 g/day/individual decrease 11-32 % of cholesterol and 12-19% of triacylglycerol (Heber *et al*., 1999).

In Thailand there are many reports concerning red pigment, citrinin and monacolin K. Comparison of different rice cultivars and *Monascus* spp. for preparation of red yeast rice was done, for axample adlay ankak (Pattanagul *et al*.,

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2008). Chainat rice variety gave the most intense red color. Pinthong *et al*. (2004) found that addition of monosodium glutamate or histidine decreased the citrinin content. Khaw Hom Dok Mali which contains lower amylase has less citrinin (Wongpiyachon, 1997). Boonsangsom *et al*. (2004) reported that fermentation of coarse rice, non-glutinous rice and Khaw Hom Dok Mali using different strain of *Monascus purpureus* gave various amount of red pigment and citrinin. Non-glutinous rice variety, Pichit rice had more intense red color than coarse rice while Khaw Hom Dok Mali had less citrinin content.

2.2 Manufacturing process

Red yeast rice is produced by cultivating *Monascus purpureus* on polished rice. The rice is first soaked in water until the grains are fully saturated. The raw soaked rice can then either be directly inoculated, or steamed for the purpose of sterilizing and cooking the grains prior to inoculation. Inoculation is done by mixing *M. purpureus* spores or powdered red yeast rice together with the processed rice. The mix is then incubated in an environment around room temperature (30° C) for 2,3 weeks. During this period of time, the rice should be fully cultured with *M. purpureus*, with each rice grain turning bright red in its core and reddish purple on the outside. The fully cultured rice is then either sold as the dried grain, or cooked and pasteurized to be sold as a wet paste, or dried and pulverized to be sold as a fine powder. China is the world's largest producer of red yeast rice.

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In Taiwan, red yeast rice production is developed to industrial scale. The 1,450 kg of rice is washed and steamed for 60 minutes before spraying with 180 liters of water. The rice is steamed again for 30 minutes and cooled down. Starter is

prepared using 60 minutes steamed rice at pH 3.3 for 4 days with uniformly stirring. The 32 liters of starter are added into the rice prepared and left at 42°C. During 8 days of fermentation adequate amount of water is added 3 times to maintain enough moisture. The product is finally dried at 45°C to give 700 kg of red yeast rice (Hanpongkittikul *et al*., 1988).

2.3 Rice (*Oryza sativa* L.)

Figure 2.2 Rice plants

(http://en.wikipedia.org/wiki/Rice)

Rice is considered as an economical important plant of Thailand. Most of Thai farmers grow rice as their main products because of the demand from domestic and international markets. Rice is belonged to the family Gramineae with the scientific name as *Oryza sativa* L. Rice is the main source of carbohydrate for Thai people. A part of being the main food, it is also used in beverage industry for production of alcohol which is the main component in whisky. Scientifically, there are 2 kinds of rice; Asian rice and African rice (*Oryza sativa* L., *Oryza glaberrima*

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Steud.). Asian rice is very popular and cultured in the United States, Australia, Europe and even in Africa. The African rice is known only around West Africa.

Scientific study on *Oryza sativa* in most rice growing country shows that there are 3 varieties; Japonica, Indica and Javanica corresponding to profile of stalk, grain and the percentage of incomplete grains of hybrid rice. Japonica is the variety grown in the northern part of China, Japan and Korea. Indica is the variety grown in warmer are such as Sri Lang Ka, Southern China, Central India, Indonesia, Bangladesh, Thailand and Philippine. Javanica is the rice variety only grown in Indonesia. However, other rice can grow between latitude of 50° north and 35° south.

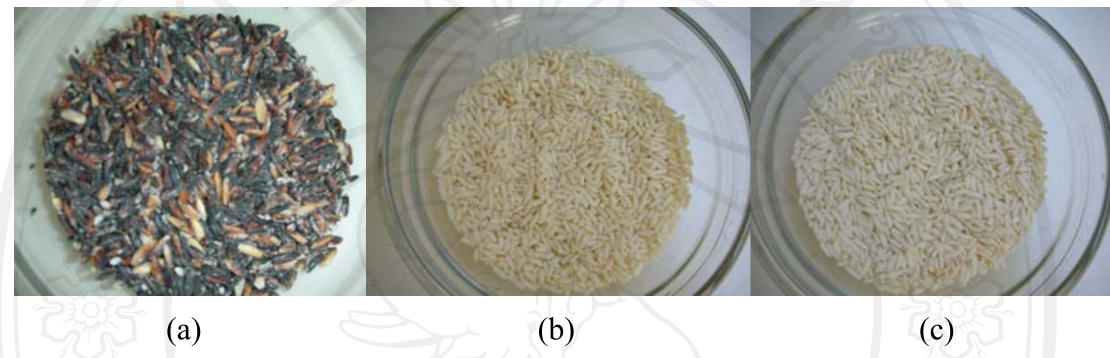
In Thailand, rice (*Oryza sativa* L.) classification is based on the properties of rice starch and chemical properties of rice grain. It can be divided into 2 categories (Insomphun, 2003) as follows;

1. Non-glutinous rice containing 90 % of starch endosperm with 70-90 % of amylopectin. The grain is transparent but becomes opaque white rice and loose after streaming. Figure 2.3 shows Mali rice, one of non-glutinous rice used for this works.

(a) (b)

Figure 2.3 Khaw Hom Dok Mali 105, (a) Non-glutinous rice grains, (b) Non-glutinous streamed rice

2. Glutinous rice contains soluble starch endosperm including dextrin. The main composition in glutinous rice is amylopectin (95 %). The content of amylose is very few or may be none. The rice grain is opaque white and become stick together after streaming. Figure 2.4 shows 3 varieties; Purple rice (Kam), Kor Kho 6 (RD6) and Sanpathong 1(SPT1) used in this work.



(a) (b) (c)

Figure 2.4 Glutinous rice, (a) Purple rice (Kam), (b) Kor Kho 6 (RD6), (c) Sanpathong1(SPT1)

(a) (b)

Figure 2.5 (a) Glutinous rice grains, (b) Steamed glutinous rice

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Amylose content is also used to classify the rice variety. High content of amylose give stream rice with high water content. Amylose is able to retrograde as an insoluble solid, The property supports why non-glutinous rice cannot stick together after streaming. The porosity of streamed non-glutinous rice due to insoluble property give larger volume, classified rice using amylose content as shown in Table 2.1.

Table 2.1 Rice classification based on amylose content

Type	Amylose (%)	Appearance	Sample
Glutinous rice	0-2	most sticky and yummy	General glutinous rice
low amylose	10-20	sticky and soft	Kaw Hom Mali (Non-glutinous rice)
medium amylose	20-25	Rather not sticky	Kaw Kao Ta Hang (Non-glutinous rice)
high amylose	25-34	not sticky and hard	Kaw Sao Hai (Non-glutinous rice)

Source: Insomphun (2003)

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2.4 *Monascus* species

Filamentous *Monascus* fungi are well known as producers of a family of structurally related hexaketide pigments which are yellow and red in color. They belong to the class *Ascomycetes* and the family *Monascaceae* and reproduce both sexually and asexually.

Monascus spp. are the fungi taxonomically classified by Alexopoulos and Mims (1979) as follows:

Division Amastigomycota

Subdivision Ascomycotina

Class Ascomycetes

Subclass Plectomycetidae

Oder Eurotiales

Family Monascaceae

Genus *Monascus*

The colony of *Monascus purpureus* is shown in Figure 2.6

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Figure 2.6 *Monascus purpureus* colony

7 days

Taxonomy and identification of *Monascus* spp. Can be based on the key to the *Ascomycetes* (Samson *et al*., 1995) and the key to the species of *Monascus* (Stchigel *et al.,* 2004).

Key to the Genera Treated

1a Ascomata distinctly stalked; stalk composed of a single
Hypha. Anamorp *Basipetospora*…………………………………*Monascus*

1b Ascomata not stalked. Anamorph *Penicillium*,
Paecilomyces or *Aspergillus*………………………………………2

2a Ascomata without distinct wall. Anamorph
Paecilomyces……………………………………………………...*Byssochlamys*

2b Ascomata with a wall. Anamorph *Aspergillus* or
Penicillium……………………………………………………..3

3a Anamorp *Penicillium*, ascomata with distinct covering,
Yellow, sometimes becoming reddish……………………………*Talaromyces*

3b Anamorph *Aspergillus,* ascomata with Hülle cells, or
With one- to several layered wall…………………………………4

4a Wall of the ascomata surrounded by thick-walled
"Hülle cells" . Anamorph *Aspergillus nidulans* group……………..*Emericella*

4b Wall of the ascomata not surrounded by "Hülle cells"……………5

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5a Ascomata yellow, wall concisting of one layer of

Flattened cells. Anamorph *Aspergillus glaucus* group…………….*Eurotium*

5b Ascomata white to creamish, wall concisting of several

Layers of flattened cells. Anamorph *Aspergillus*

fumigates group……………………………………………..*Neosartoya*

Key to the species of *Monascus* (modified from Udagawa and Baba 1998)

1. Ascomata sessile; asci 2-spored ....*M. bisporus* (L.R. Fraser) Arx (1970)
1. Ascomata stalked; asci 8-spored or undetermined ……......................2

2. Colonies not growing on CYA or MEA……..*M. eremophilus* A.D. Hocking & J. Pitt (1988)
2. Colonies growing on CYA or MEA..3

3. Ascomata remaining hyaline or pale colored.......................................4
3. Ascomata significantly pigmented. 7
4. Ascospores 3.5 x 2.5-3 µm...*M. pallens* P.F. Canon, Abdullah & B.A Abbas (1995)
4. Ascospores ≥5 µm in length……..5

5. Soluble pigment not formed............................. *M. aurantiacus* Zhong Q. Li (1982)
5. Soluble pigment formed..6

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6. Ascospores 5-7(-8.5) x 3-3.5(-4) µm. ....*M. pilosus* K. Saitô ex D. Hawksw. & J. Pitt (1983)
6. Ascospores (5.5-)6-7 x (4-)4.5-5µm. ....*M. purpureus* Went (1895)

7. Ascomatal wall with dark-coloured patches…..8
7. Ascomatal wall without these patches 9

8. Ascospores reniform to allantoid, 6-7 x 2-2.5 µm...........................*M. lunisporas* Udagawa & H. Baba (1998)
8. Ascospores broadly ellipsoidal to subglobose, 3-4 x 2.5-3 µm........*M. argentinensis*

9. Ascospores 3.5-4.5 x 2-3 µm……………………………………*M. floridanus* P.F. Canon & E.L. Barnard (1987)
9. Ascospores ≥ 5 µm in length...10

10. Red soluble pigment produced... ....*M. sanguineus* P.F. Canon, Abdullah & B.A Abbas (1995)
10. Soluble pigment usually absent, brown when present ....*M. ruber* Tiegh. (1884)

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The mycelia of the *Monascus* are in the form of septation and branching able to stick tightly on solid agar medium. The young colonies are white and become red or purple when getting older. Both sexual and asexual reproductions occur in the reproduction system. Asexual reproductive states, the anamorph, forms conidia which

are developed from conidiophores. The conidia are oval shape with separated conidia or a chain of conidia. Conidia are colorless and may become red later. Conidiophores may have septation. The growth of the conidiophores varies with the nutrient formulation such as the amount of C-source.

Sexual reproduction is similar to other fungi in the class Ascomycetes. The ascigerous state or teleomoph is developed. The globose ascocarps or ascomata are formed on the stalks with or without septa. Ascocarp grows on homothallic mycelia. Asci enclosed in ascocarps are formed by karyogamy of two nuclei from different gametangia(male: antheridium, female: ascogonium). Cell division continues both meiosis and mitosis with daughter nuclei and enlargement of cell wall, ascocarp. There are 2-8 oval ascospores in an ascus formed in perithecium. A lot of ascospores in perithecium will disperse when the membranes are broken. The life cycle and morphological structures of ascomycetes are shown in Figure 2.7

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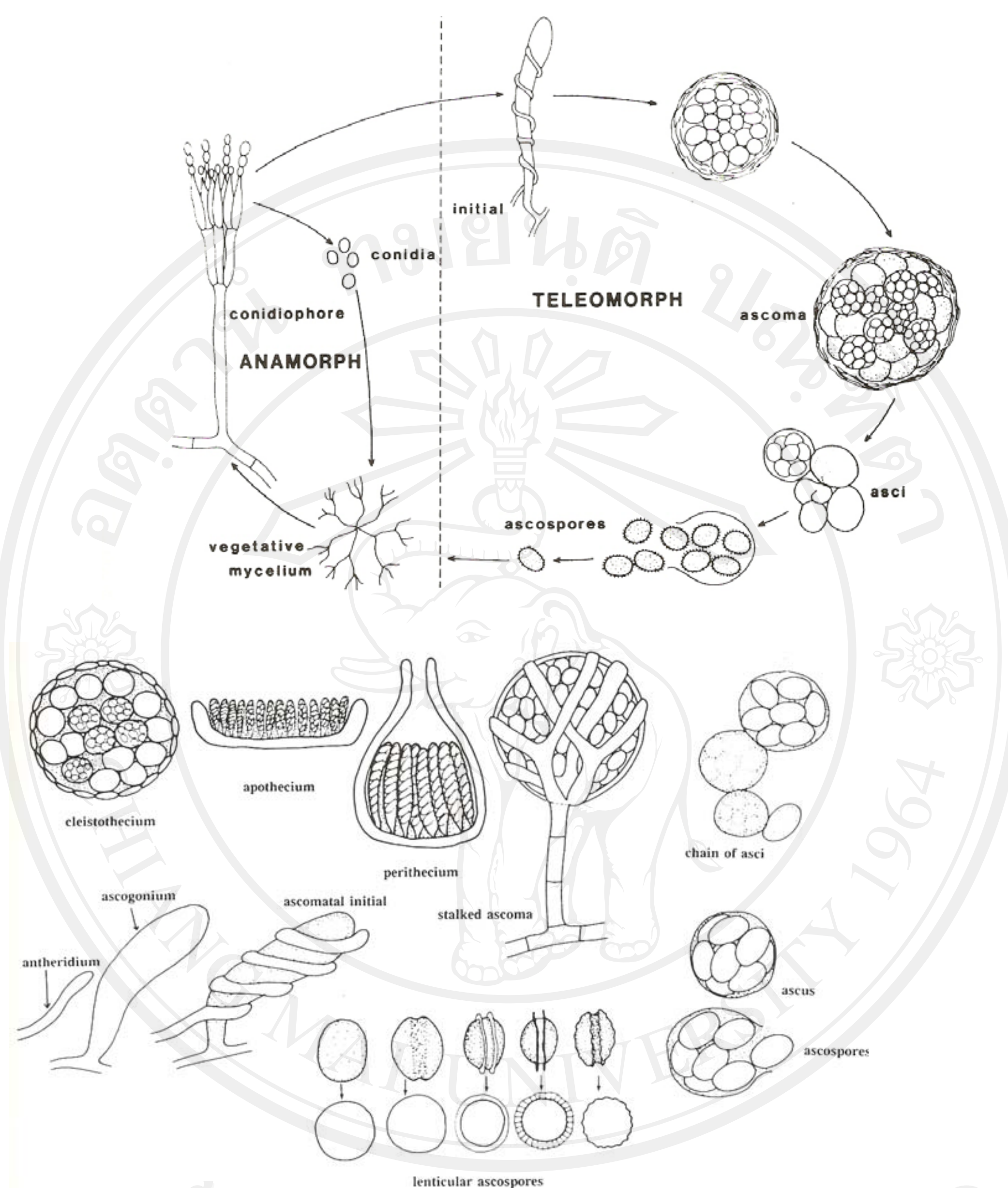

Figure 2.7 Life cycle and morphological structure of *Ascomycetes* (Samson *et al.*, 1995)

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The most suitable medium to produce conidia is 10g sucrose 0.3g yeast extract 0.5g amino acids, 0.1g potassium dihydrogenphosphate (KH_2PO_4), 0.2g sodium nitrate ($NaNO_3$), 0.05g magnesium sulfate mono hydrate ($MgSO_4.H_2O$), 0.05g potassium chloride (KCl) and 2.0g agar in 100 ml water. Others parameter such as spores age, spore density, pH, light and temperature may affect the conidia forming. The optimum pH is around 35 ^{o} C. Generally, conidia grow within 4 hours with 2-6 germ-tubes and can be stimulated by several carbohydrates (Yongsmith,1999).

There were two strains of *Monascus* discovered at the beginning, *M. mucoroides* and *M. ruber*. Then *M. purpureus* was isolated from red yeast rice in 1895 by Went. At present, about 20 strains of *Monascus* are known. The classification of *Monascus* strain based on morphology and chemical properties by Iizuka and Lin (1981); Hawksworth and Pitt (1983) and Nishikawa *et al*. (1988) is shown in Table 2.2. The five groups of *Monascus* were differentiated. Some of the *Monascus* properties used for classification are in Table 2.2 such as salt tolerance, ethanol tolerance, red pigment production, mycelium shape and colony shape. Enzymology and enzyme properties are also be used (Bridge and Hawksworth, 1985).

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Table 2.2 Properties of *Monascus*

<table>
<tr><th></th><th>6% NaCl tolerance</th><th>30% ethanol tolerance</th><th>Production of red pigment</th><th>Mycelium shape</th><th>Colony shape</th></tr>
<tr><td>M. anka Nakazawa et Sato</td><td>-</td><td>+</td><td>+</td><td rowspan="2">Helix</td><td rowspan="3">Lava</td></tr>
<tr><td>M. anka var. Rubellus Sato</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>M. kaoliang nov. sp.</td><td>+</td><td>+</td><td>+</td><td>Linear</td></tr>
<tr><td>M. barkeri Dangeard</td><td>+</td><td>-</td><td>+</td><td rowspan="2">Helix</td><td rowspan="3">Turf</td></tr>
<tr><td>M. ruber van Tieghem</td><td>-</td><td>-</td><td>+</td></tr>
<tr><td>M. araneosus Sato</td><td>+</td><td>-</td><td>+</td><td>Linear</td></tr>
<tr><td>M. major Sato</td><td>-</td><td>+</td><td>+</td><td rowspan="6">Helix</td><td rowspan="2">Lava</td></tr>
<tr><td>M. purpureus Went</td><td>-</td><td>-</td><td>+</td></tr>
<tr><td>M. pubigerus Sato</td><td>+</td><td>+</td><td>+</td><td rowspan="6">Turf</td></tr>
<tr><td>M. pilosus Sato</td><td>+</td><td>+</td><td>-</td></tr>
<tr><td>M. vitreus Sato</td><td>+</td><td>-</td><td>-</td></tr>
<tr><td>M. vitreus Sato var. serorubescens nov. var.</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>M. rubiginosus Sato</td><td>-</td><td>-</td><td>+</td><td rowspan="2">Linear</td></tr>
<tr><td>M. albidus Sato</td><td>-</td><td>-</td><td>-</td></tr>
</table>

Source: Modified from Iizuka and Lin (1981)

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Table 2.3 Different some enzyme activities for *Monascus* strains

Enzyme Activity	*M. floridanus*	*M. pilosus*	*M. purpureus*	*M. ruber*
Valine arylamidase*	-	+	+	+
Cystine arylamidase*	-	-	+	-
Trypsinase*	+	-	-	-
α-galactosidase*	-	+	-	+
β- galactosidase*	-	+	-	-
α-glucosidase*	-	+	-	-
Polypectase pH 6.0	-	-	+	-
Cellulose hydrolysis	-	-	-	+

* API ZYM strip tests with fungi 14 days old.

Source: Bridge and Hawksworth (1985)

Table 2.3 shows some enzyme activity expressed by *M. floridanus, M. pilosus*, *M. purpureus* and *M. ruber*.

The different proteinase activity of *Monascus* strains are also shown in Table 2.4. The different proteinases; acid proteinase, alkali proteinase and acid and alkali proteinase were used to study *Monascus* strains. Most of *Monascus* strains shows alkali proteinase activity with *M. kaoliang* strain is the most numerous strains.

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Table 2.4 The different proteinase activity of *Monascus* strains

Strains	Enzymes**			Total of strains
	A	B	C	
M. albidus		1		1
M. albidus var.glaber		1		1
M. anka		2		2
M. anka var. rubellus		1		1
M. araneosus	1	4		5
M. fuliginosus	1			1
M. kaoliang	3	9	3	15
M. major		1		1
M. pilosus	1			1
M. pubigerus	1			1
M. purpureus		1		1
M. vitreus	1			1
M. ruber		2		2
M. rubiginosus		1		1
M. serorubescens		1		1

** A = Acid proteinase (pH 3),

B = Alkali proteinase (pH 8-9)

C = A and B

Source: Nishikawa *et al.* (1988)

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2.5 Metabolites of *Monascus* spp.

Many kinds of metabolites were studied. These metabolite are economically important. Table 2.5 shows some of metabolites from *Monascus.*

Table 2.5 Metabolites from *Monascus.*

Enzymes	Primary metabolites	Secondary metabolites
1. Glucoamylase 2. Protease 3. α-Galactosidase 4. α-Amylase 5. Ribonuclease	1. Ethyl alcohol 2. Organic acids 3. Vitamin B_2 4. Fat and Oil 5. Fatty acids	1. Pigment (red, yellow and orange) 2. Antibiotics 3. Monacolins, Cholesterol lowering 4. compounds 5. Flocculants 6. antihypertensives 7. Traditional medicine for digestive aid and woned muscle 8. Coumarin 9. Preservatives (for meat) 10. Co Q_{10} 11. Aroma (e.g.methyl ketones) 12. Antimutation substance

Source: Modified fromYongsmith (1999)

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2.5.1 Pigments

There are six major pigments (Lin *et al*., 2008). The red colorants named rubropunctamine (Fig. 2.8e) and monascorubramine (Fig. 2.8f) are most abundant. The orange colorants are rubropunctatin (Fig. 2.8a) and monascorubrin (Fig. 2.8b). The yellowish colorants are monascin (Fig. 2.8c) and ankaflavin (Fig. 2.8d). Moreover, a yellowish colorant named Xanthomonasin A (Fig. 2.8g) in the mutant of *Monascus* anka was identified (Martinkova *et al*., 1999). The pigments extracted from *M.* anka inhibited the 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced carcinogenesis in mice (Yasukawa *et al*., 1996). Among the pigments, monascorubrin was the most effective one, and its function was assumed through its anti-inflammatory activity (Yasukawa *et al.,* 1994). In the mouse model, oral administration of monascin inhibited the carcinogenesis of skin cancer initiated by peroxynitrite or ultraviolet light and after the promotion of TPA (Akihisa *et al*., 2005a). Ankaflavin showed selective cytotoxicity to cancer cell lines by an apoptosis-related mechanism and showed relatively low toxicity to normal fibroblasts. The structure analog monascin, however, showed no cytotoxicity to all cell lines tested (Su *et al*., 2005). The orange pigments, rubropunctatin and monascorubrin, had been found to possess antibiotic activity against bacteria, yeast, and filamentous fungi (Martinkova *et al*., 1995). Rubropunctatin and monascorubrin could inhibit the growth of *Bacillus subtilis* and *Candida pseudotropicalis*. Yellow pigments, monascin and ankaflavin, showed immunosuppressive activity on mouse T splenocytes (Martinkova *et al*., 1999). The stability of *Monascus* pigments had been studied (Fabre *et al*., 1993). The pigments were prepared by methanol/chloroform (1:1) extraction on freeze-dried culture broth of *M. ruber* van Tieghem. Results showed that

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the stabilities of these pigments were seriously affected by light exposure, surrounding temperature, and pH value. To the light exposure for 50 days, the red pigment decayed to 20%. After 100°C treatment for 8 h, red colorants decreased to 30%. The red pigment was found more stable in neutral (pH 7) or alkaline (pH 9.5) than in acidic condition (pH 3) for 5 h. The author further investigated the applicability of these pigments for replacing traditional colorants in meat products (such as nitrite salts or cochineal). The added concentrations of *Monascus* pigment extracts were in the range of 0.25–1.2 g per kilogram of different meat products (sausage or pâté). The *Monascus* colorants incorporated meat products exhibited stable color (95% stability after 3 months at 4 °C under vacuum) and enhanced flavor and texture by sensory evaluation. The authors concluded that *Monascus* pigments were superior to nitrite salts and could serve as a suitable substitute for food additives in meat products.

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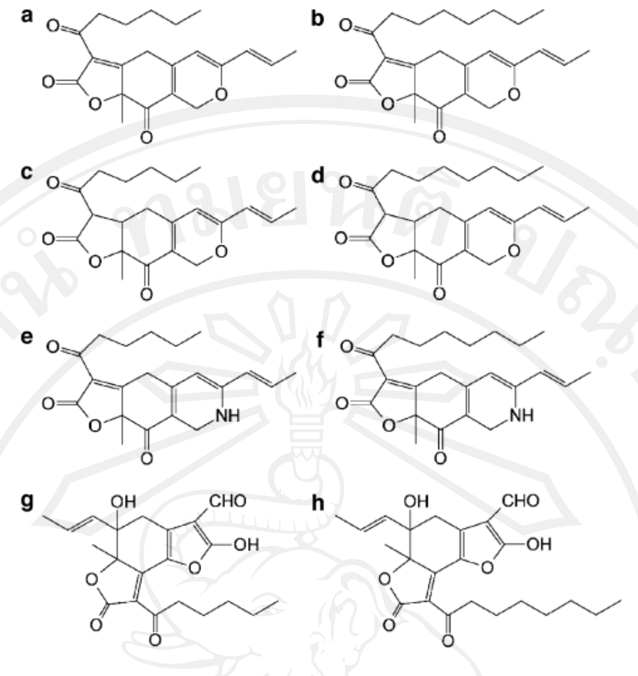

Figure 2.8 Chemical structure of pigments from *Monascus*: rubropunctatin a, monascorubrin b, monascin c, ankaflavin d, rubropunctamine e, monascorubramine f, xanthomonasin A g, and xanthomonasin B h.

source: Lin *et al*. (2008)

Carels and Shepherd (1977) proposed that firstly, the orange pigments are produced from which the yellow and red pigments are developed. The mechanism of pigment synthesis in *Monascus* starts with the condensation of 1 mole of acetate and 5 moles of malonate to form hexaketide chromophore. The trans-esterification reaction between the chromophore and medium chain fatty acids (C_6-C_{18}) such as octanoic acid forms orange pigments. The amination reaction of the orange pigments gives rise to the formation of monascoburin or rubopunctatin. Intracellular pigments are hydrophobic except when secreted out and reacted with amino group of amino acids

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that are water soluble. An example is the formation of N-glutarylmonascorubramine (Figure 2.9).

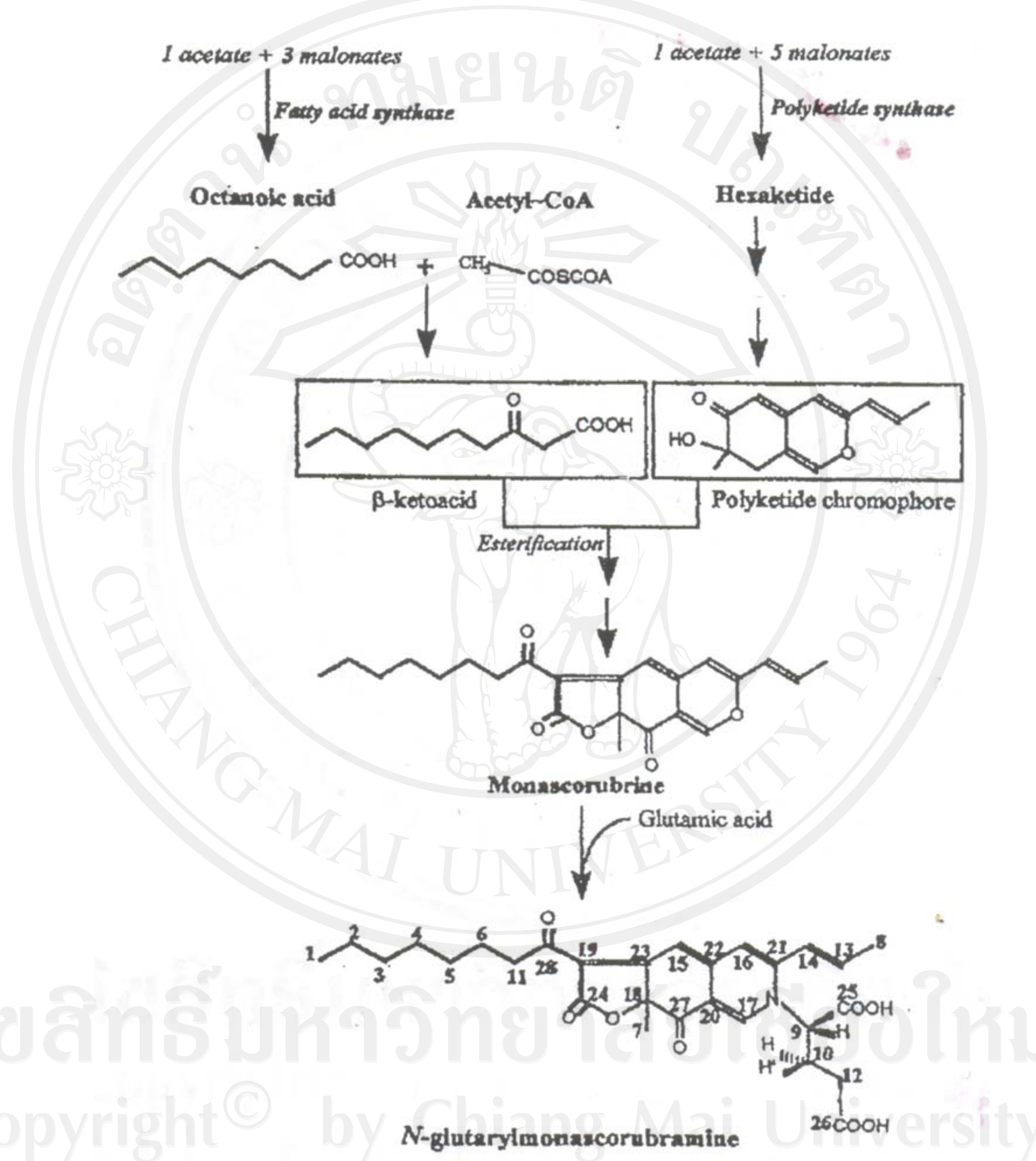

Figure.2.9 Scheme of the hypothetic metabolic routes leading to the final structure of the water-soluble red pigment *N*-glutarylmonascorubramine in *M. ruber*.

Source: Hajjaj *et al*. (2000a)

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2.5.2 Pigment isolation

The isolations are different depending on the pigments. The solvents and their concentration may be varied. Broder and Koehler (1980) used methanol, chloroform, ethanol and acetone to extract the pigments from fibrous samples. It was found that methanol gave the best result with absorbance at 390 and 500 nm due to the yellow and red pigment respectively. Lin (1973) used ethanol to extract the pigments from fiber and determined the red pigment concentration using spectrometry at 500 nm. Lin and Iizuka (1982) included the determination of water soluble pigments in aqueous sample and 95% ethanol solution measured the absorbance at 400 and 500 nm.

The schematic illustration in Figure 2.10 shows the isolation steps of colored pigments from red rice powder.

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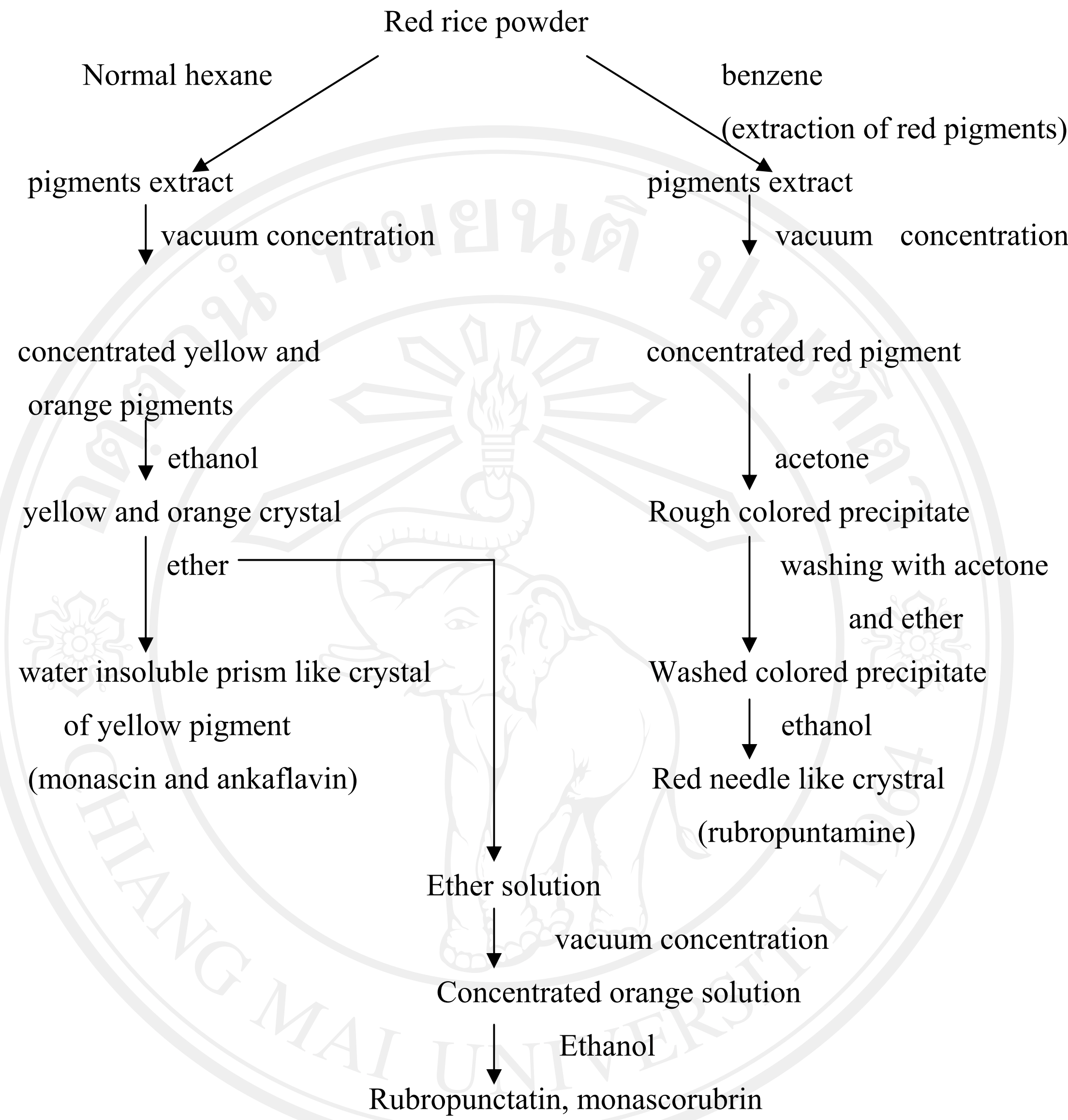

Figure 2.10 Isolation of pure pigments from red yeast rice
Source: Modified from Sweeny *et al*. (1981)

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2.5.2 Monacolin K

2.5.2.1 General characteristics of monacolin K

Monacolin K also known as mevinolin or lovastatin, an inhibitor for cholesterol synthesis, is the secondary metabolite of *Monascus* spp; *M. ruber*, *M. purpureus* (Lee *et al.,* 2006). The formation of secondary metabolite of the *Monascus* species is affected by cultivation environment. Red mold rice fermented by *M. purpureus* or red yeast rice has higher content of monacolin than pure strain fermentation, according to Chen and Hu (2005). Monacolin contents in red yeast rice and in pure *M. purpureus* was found to be 2.52 and 0.22 mg/g respectively.

M. purpureus-fermented rice (red yeast rice) was one of food supplements that had the ability of lowering the blood lipid levels, and monacolins have been proved to be main active constituents. In total 10 monacolin compounds such as monacolin K(Mevinolin), J, L, M, X and their hydroxyl acid form, as well as dehydromonacolin K, dihydromonacolin L, compactin, and 3-α-hydroxy-3, 5-dihydromonacolin L as shown in Figure 2.11 and 2.12 (Li *et al*., 2004).

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Structure	Name	R	MW	UV (λ_{max})
	1. Monacolin K (MK)		404	230, 237, 246
	2. Monacolin J (MJ)	OH	320	230, 237, 247
	3. Monacolin L (ML)	H	304	230, 237, 247
	4. Monacolin X (MX)		418	230, 237, 247
	5. Monacolin M (MM)		406	
	1a. MK acid form (MKA)		422	
	2a. MJ acid form (MJA)	OH	338	
	3a. ML acid form (MLA)	H	322	
	4a. MX acid form (MXA)		436	
	5a. MM acid form (MMA)		424	
	6. Compactin (P1)		390	230, 237, 247
	7. Dehydromonacolin K (DMK)		386	
	8. Dihydromonacolin L (DML)	H	306	
	9. 3α-hydroxy-3,5-dihydromonacolin L (HDML)	H	340	

Figure 2.11 Structural data of monacolins in fermented rice

Source: Li *et al*. (2004)

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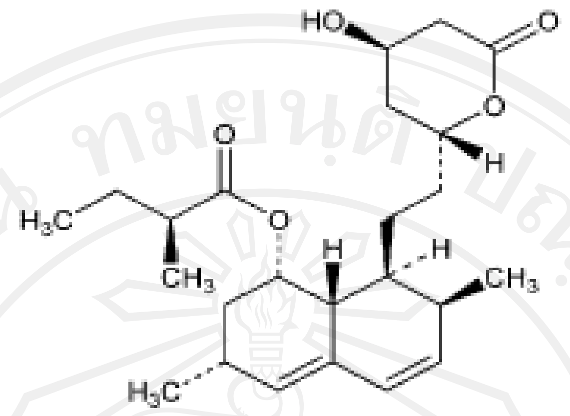

Figure 2.12 Structure of monacolin K (Helen, 2002)

Common name : Monacolin K, Lovastatin, Mevinolin (Feiedrich *et al*., 1995)

Commercial name : Mevacor, Lipivas, Lovalip Mevinacor, Nergadan, Rovacor and Taucor (Helen, 2002)

Molecular formula : $C_{24}H_{36}O_5$ Molecular weight : 404

Melting point : 157-159 ^{o} C (Endo, 1979)

Physical properties : insoluble in water and slightly soluble in methanol, ethanol and acetonitrile (Helen, 2002)

IUPAC name : (1S-(1 alpha(R), 3alpha, 7-beta, 8-beta(2S, 4S), 8a-beta-(1, 2, 3, 7, 8, 8a-hexahydro-3, 7-dimethyl-8-(2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl) ethyl)-1-naphthyl) 2-methylbutanoate (Helen, 2002)

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2.5.2.2 Mechanism of monacolin K synthesis

Aspergillus terreus is able to produce monacolin K by polyketide pathway as shown in Figure 2.13. Two possible pathways are proposed. The first one is the action of lovastatin nonaketide synthase (LNKS). One mole of acetate and eight moles of malonate form dihydromonacolin L, genetically encoded by lov B and lov C genes. lov B Only causes heptaketide pyrone and hexaketide pyrone instead. Dihydromonacolin L can be changed to α-hydroxy-3, 5-dihydromonacolin L by lov A, forming Monacolin L and Monacolin J subsequently. The second concurrent pathway is the actiun of lovastatin diketide synthase(LDKS). Starting with 1 mole of acetate and 1 mole of malonate with the presence of SAM (S-adenosylmethaionine) encoded enzyme by lov F. The product reacts with Monacolin J of the first pathway with lov D encoded and causes lovastatin or monacolin K or Mevinolin (Sorensen *et al*., 2003)

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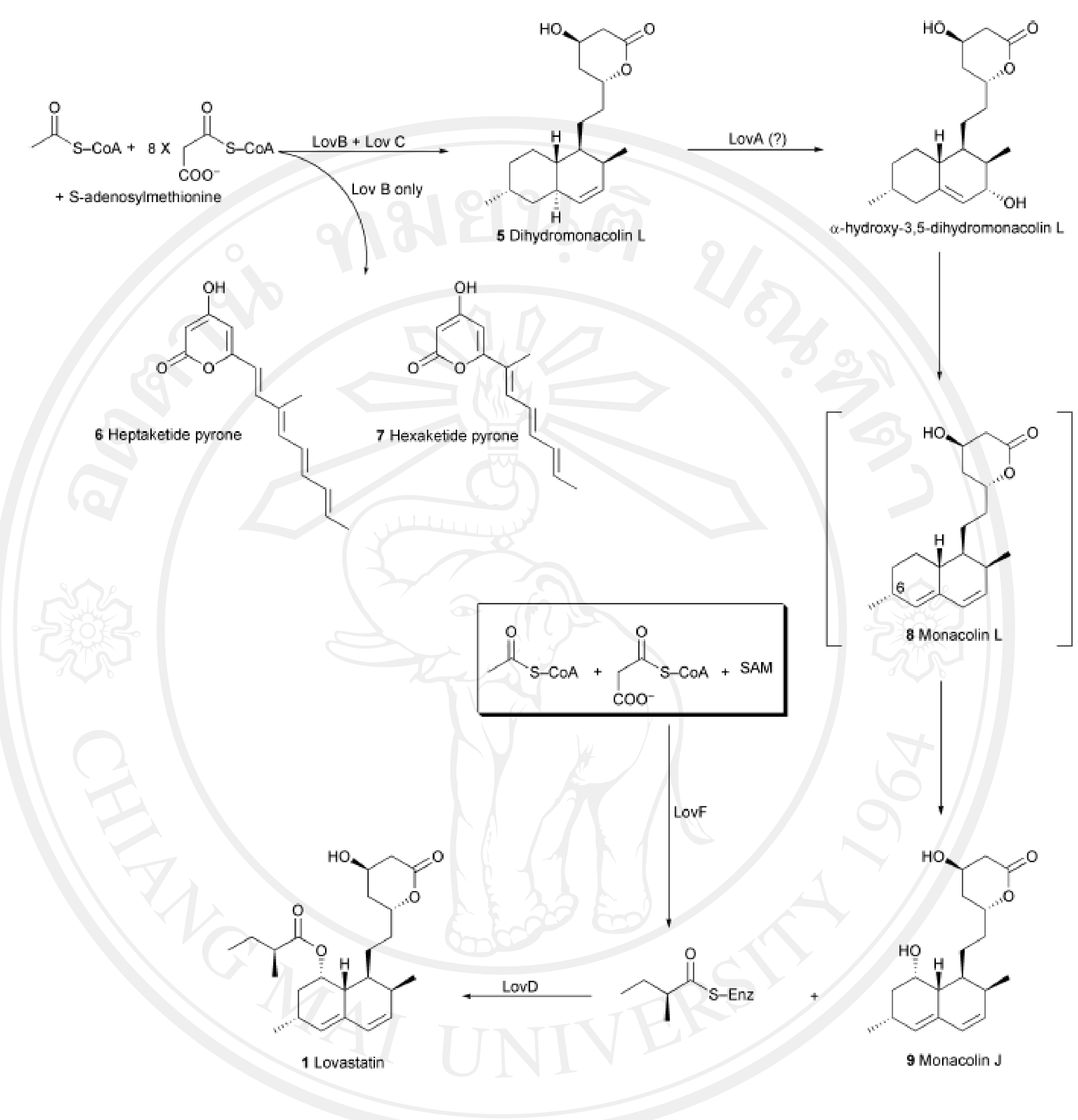

Figure 2.13 Mechanism of monacolin K synthesis

Source: Sorensen *et al*. (2003)

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Hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase or Mevalonate-NADP$^+$ oxidoreductase [EC 1-1-1-34] is an enzyme that catalyze HMG-CoA to mevalonate in the cholesterol synthesis pathway (Hajjaj *et al*., 2001; Ganong, 1999) The enzyme is inhibited by monacolin K at the step shown in Figure 2.14.

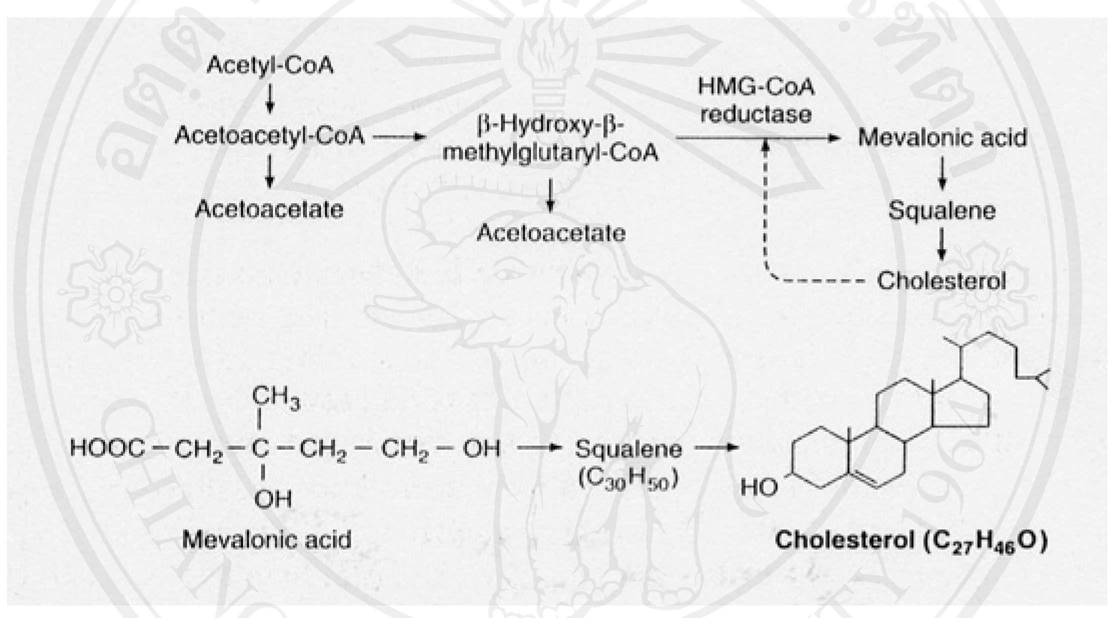

Figure 2.14 Pathway of cholesterol synthesis

Source: Ganong (1999)

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2.5.3 Antioxidant properties

2.5.3.1 Antioxidants

Definition of antioxidant

Historically, the term "antioxidant" referred to any substance that hindered the reaction of a substance with dioxygen. Since such reactions frequently involve radicals, now "antioxidant" generally refers to any substance which inhibits a free radical reaction. In living systems this often involves oxygen in one manner or another (Baskin and Salem, 1997). Hence a broader definition of an antioxidant is "any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate". The term 'oxidizable substrate' includes almost everything found in foods and in living tissues including proteins, lipids, carbohydrates and DNA. This definition emphasizes the importance of the target chosen and the source of oxidative damage in characterizing an antioxidant (Halliwell *et al*., 1995). Antioxidants are divided into two groups as described below.

1) Natural enzymatic antioxidants

Superoxide dismutase (EC 1.15.1.1)

Natural superoxide dismutase (SOD) has been utilized in a wide variety of pathological states as a protective agent. Such a list of diseases and abnormalities related to reactive oxygen species is by no means exhaustive, but it includes protective effects of SOD in radiation injuries, inflammatory processes, postischemic tissue injuries, and experimental skin chemical carcinogenesis.

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Catalase (E.C. 1.11.1.6)

Catalase, an enzyme which is located mostly in peroxisomes, protects cells from the accumulation of H_2O_2 by catalysing it to form H_2O and O_2, or by using it as an oxidant when it works as a peroxidase.

Catalase has been utilized to treat processes in which H_2O_2 has been involved as a major deleterious agent, such as inflammatory injuries and xeroderma pigmentasum (Roberfroid and Calderon, 1995).

2) Natural and synthetic no enzymatic antioxidant molecules

Vitamin E and related antioxidants

Vitamin E is the generic name of a homogenous family of compounds having a more or less methylated hydroquinone moiety and an isoprenoid chain (Figure 2.15). α-Tocopherol, a major component of vitamin E, is well known to represent the last possibility of preventing membrane peroxidation by scavenging the radicals involved in the peroxidation chains.

Most important is the fact that vitamin E can react with lipid peroxyl radicals to from vitamin E radicals which are insufficiently reactive to abstract $H^{\cdot}$ from the methylene groups of the unsaturated fatty acid moieties of membrane phospholipids. Thus, it stops the radical reaction chain of lipid peroxidation by acting as a chain terminator (Roberfroid and Calderon, 1995).

Class of tocopherols	R_1	R_2	R_3	Source
α	CH_3	CH_3	CH_3	Sunflower and olive oil
β	CH_3	H	CH_3	Corn oil
γ	H	CH_3	CH_3	Soya, rape and maize oil
δ	H	H	CH_3	Maize oil

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Figure 2.15 Structure of vitamin E.
(Roberfroid and Calderon, 1995)

Vitamin C (ascorbic acid)

Plants and animals synthesize ascorbic acid from glucose; however, humans are unable to synthesize it and require ascorbic acid in the diet. Ascorbic acid is required *in vivo* as a cofactor for enzyme activity, and its dietary deficiency causes scurvy.

It seems likely that in this way dietary ascorbate inhibits the carcinogenic action of several nitroso-compounds by blocking the nitrosating reaction, thus preventing *in vivo* formation of N-nitrosamines.

Ascorbate is the only endogenous antioxidant in plasma that can completely protect against peroxidative damage induced by aqueous peroxyl radicals and the oxidants released from activated neutrophils.

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Carotenoids

β-Carotene has been referred to as pro-vitamin A The function of β-carotene may be summarized as follows

- It is metabolized to form retinol and retinoic acid.

- It prevents photosensitized damage in bacteria, animals, and humans.
- It prevents psoraten-induced ultraviolet A light phototoxicity.
- It decreases sister chromatid exchanges and malignant transformation in cultured cells.
- It inhibits UV- and chemically induced tumors in rodents.
- It decreases premalignant lesions in humans.
- It modifies the immune response.

Glutathione and derivatives

Reduced glutathione (GSH), the γ-Glu-Cys-Gly tripeptide, is known to play a pivotal role in the cellular defense system and is necessary for preventing lipid peroxidation by radicals and oxygen-derived reaction species.

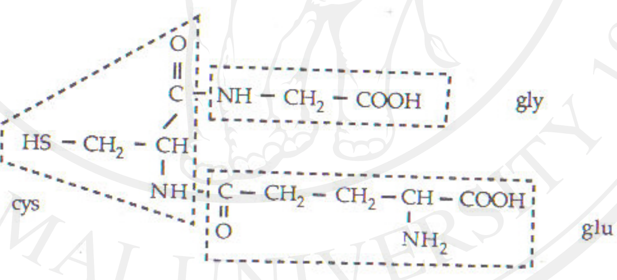

Figure 2.16 Reduced glutathione (GSH).

(Roberfroid and Calderon, 1995)

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Glutathione plays a role in the inactivation of a number of drugs and in the metabolic processing of certain endogenous compounds, such as estrogen, prostaglandins, and leukotrienes

Phenolic compounds

Phenolic compounds exhibit a wide diversity of structures. All of these phenolic classes have a large number of structures that differ in the number and position of hydroxy (-OH) and methoxy (-OCH_3) groups on the basic skeleton. The various acids are differentiated by substitution of their benzene ring.

Plant phenolics (Figure 2.17) constitute a group of natural products of structural diversity and wide phylogenetic distribution, of which the precise physiological function remains largely unknown but they have a beneficial effect on the health of human beings. Their high antioxidant capacities are thought to have links with the inhibition of oxidative damage diseases, such as coronary heart disease, stroke, and cancers (Theunissen, 1995).

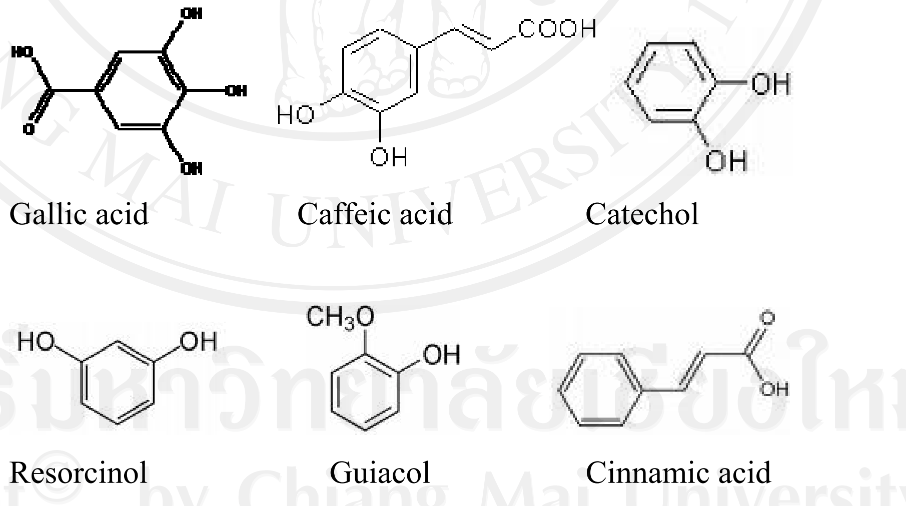

Figure 2.17 Phenolic compounds.
(Stafford and Ibrahim, 1992)

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Flavonoids

Flavonoids, derivatives of phenylchromone (Figure 2.18), constitute one of the largest groups of naturally occurring phenols belonging to a large group of compounds broadly distributed in plants. The variously substituted (e.g. glycosylated, acylated, esterified) derivatives of phenolic compound can divide phenolic compounds into flavonoids (Figure 2.19). They have been shown to affect various biological functions: capillary permeability, cellular secretory processes involved in the inflammatory response, and inhibition in the activity of enzymes, receptors, and carriers.

Recently, flavonoids have been proposed to act as antioxidants, most probably because of their radical-scavenging abilities. Concerning scavenging of $O_2^{\cdot -}$, the presence of hydroxyl groups in the B-ring is essential for activity. Moreover, the presence of a hydroxyl, at C-3, enhances the scavenging ability of flavonoids. Some flavonoids are strong oxygen radical scavengers and good metal chelators, effective in preventing lipid peroxidation. For instance, polyphenolic flavonoids inhibit the peroxidation of low-density lipoprotein (LDL) and their subsequent cytotoxicity.

The cellular mechanisms involved in the protection, by flavonoids, of LDL oxidation are not known, but, as mentioned, such mechanisms may be related to the following:

- Their antioxidant properties (by sparing vitamin E or by regenerating vitamin C).
- Their inhibitory activity toward lipoxygenases.
- Their inhibition of cellular enzymes involved in signal transduction (Roberfroid and Calderon, 1995).

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Figure 2.18 Basic structure and numbering system for flavonoids.

(Packer *et al*., 1999)

Kaemopherol Myricetin Naringenin

Catechin Quercetin Apigenin

Figure 2.19 Flavonoids structure.

(Stafford and Ibrahim, 1992)

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Alkaloids

Alkaloids are a chemically heterogeneous group of basic nitrogen containing substances found predominantly in higher plants. However, such basic substances also occur in lower plants, animals, microorganisms and marine

organisms. Alkaloids usually contain one or two nitrogen atoms although some, like ergotamine may contain up to five nitrogen atoms. True alkaloids are defined as a compound meeting the additional four qualifications namely:

i Nitrogen is a part of the heterocyclic ring.

ii The occurrence of the compound is restricted to the plant kingdom.

iii The compound has a complex molecular structure.

iv The compound manifests significant physiological activity.

Alkaloids (Figure 2.20) are found in about 15% of vascular plants belonging to more than 150 families. Usually the occurrence of particular alkaloids is restricted to the seeds, leaves, barks or roots of the plants and each site may contain closely related alkaloids. In plants, basic nature alkaloids occur largely as salts of organic acids like acetic, oxalic, citric acid etc. Some basic pyridine alkaloids like nicotine occur in free stage. A few alkaloids are present as glycosides of common sugars such as glucose, rhamnose, galactose or as esters of organic acids.

Alkaloids have a bitter taste but it occurs in plants because of some function, such as: poisonous agents protecting the plant against insects and herbivores; reserve substances capable of supplying nitrogen or other necessary fragments of the plant development; regulatory growth factors similar to hormones; and end or byproducts of plant metabolism.

Today alkaloids are extremely important in the pharmaceutical industry and they display a variety of pharmacological activities such as analgestic potenttiator, antiamebia, anticholinergic, scopolamine etc (Bhat *et al*., 2005). Moreover, alkaloids were studied for their antioxidant and antimutagenic properties as a phytochemical in plants (Bhat *et al*., 2005; Fragoso *et al*., 2008; McGawa *et al*., 2007).

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Capsaicin Hordenin Cocaine

Quinine Caffeine Ergonovine

Figure 2.20 Some examples of alkaloids.

(Bhat *et al*., 2005)

2.5.3.2 Radicals

Definition of radicals

A radical, or free radical, is any chemical species that has an odd orsingle number of electrons, because it contains one or more unpaired electrons; that is, an electron that occupies an atomic or molecular orbital by itself. The radical may be positively charged (cation radical $(R)^{+\cdot}$), like the pyridinyl cation radical that forms on $(NAD)^{+\cdot}$; negatively charged (anion radical $(R)^{-\cdot}$), like the superoxide anion radical$(O_2)^{-\cdot}$; or neutral (neutral radical $(R)^{\cdot}$), like the hydroxyl radical $(OH)^{\cdot}$, the alkoxyl radical $(alkO)^{\cdot}$, the alkylperoxyl radical $(alkOO)^{\cdot}$, and alkylthiyl radical $(alkS)^{\cdot}$. Most organic radicals have very short lifetimes. Without stabilizing features, such as steric hindrance at the odd-electron site and/or extensive delocalization of the

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odd-electron, they decompose rapidly, even in the absence of external agents (Baskin and Salem, 1997; Roberfroid and Calderon, 1995).

2.5.3.3 Antioxidant activity

Total antioxidant capacity

A spectrophotometric method has been developed for the quantitative determination of antioxidant capacity. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH. The formation of a green-colored complex of phosphate and Mo (V) was presented as the basis of a spectrophotometric method to determine inorganic phosphate. The requirement of a reducing agent to produce Mo (V) from the Mo (VI) supplied with the reagent mixture suggested to us the modification of this method for the determination of any reducing species. The method has been optimized and characterized with respect to linearity interval, repetitivity and reproducibility, and molar absorption coefficients for the quantification of several antioxidants, including vitamin E. The phosphomolybdenum method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts and to determine vitamin E in a variety of grains and seeds, including corn and soybean (Prieto *et al.,* 1999).

β-carotene bleaching activity

1) Lipid peroxidation

Lipid peroxidation is the introduction of a functional group containing two catenated oxygen atoms, O-O, into unsaturated fatty acids in a free radical chain reaction. There are three stages to the reaction as described below.

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Initiation

The chain reaction is initiated by the formation of a carbon-centre radical by hydrogen abstraction from the lipid:

$$\text{--CH}_2\text{--CH=CH--CH}_2\text{--} \xrightarrow{-\,H^{\bullet}} \text{--CH=CH--}^{\bullet}\text{CH--CH}_2\text{--}$$

Different initiation processes (e.g., photolysis, Q-radiation, singlet oxygen) produce different initial radicals.

Propagation

The radicals produced in the initiation process undergo rearrangements from 1, 4-pentadiene (methylene-interrupted) structures into 1, 3-pentadiene (conjugated) systems and also react with atmospheric triplet oxygen to produce lipid peroxide radicals, which can abstract more hydrogen, thus propagating the chain.

$$\text{HC}^{\bullet} \xrightarrow{^{3}O_2} \text{HC--O--O}^{\bullet} \xrightarrow{RH \;\to\; R^{\bullet}} \text{HC--O--OH}$$

Enzymic lipid peroxidation involves hydrogen abstraction and dioxygen addition at specific positions. Non-enzymic auto-oxidation is not region specific; peroxidation occurs predominantly at two `outer' positions, which are the last carbon atoms before the first double bond from each end of the molecule.

$$\text{--CH=CH--CH(O--OH)--CH}_2\text{--} \xrightarrow{-\,H^{\bullet}} \text{--CH=CH--CH(O--C}^{\bullet}\text{)--CH}_2\text{--}$$

$$\text{--CH=CH--CH(O--OH)--CH}_2\text{--} \xrightarrow{-\,OH^{\bullet}} \text{--CH=CH--CH(C}^{\bullet}\text{)--CH}_2\text{--}$$

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The transition of metal ion-catalysed hydroperoxide breakdown to alkylperoxy and alkoxy radicals is chain-propagating but also leads to the formation of secondary alcohols, ketones and aldehydes, including (by chain cleavage on either side of the alkoxy-radical) short-chain aldehydes. Moreover, aldehydic products can form adducts with proteins and also form adducts with DNA.

Termination

The chain reaction is terminated by reactions between radicals producing dimers and higher polymers. As is shown in equations below the process of lipid peroxidation causes the double bonds in fatty acid molecules to move; methylene-interrupted dienes become conjugated. In addition, new functional groups are introduced.

$$2\ \text{--CH=CH--}^{\bullet}\text{CH--CH}_2\text{--} \longrightarrow \begin{array}{c}\text{--CH=CH--CH--CH}_2\text{--} \\ | \\ \text{--CH=CH--CH--CH}_2\text{--}\end{array}$$

$$2\ \begin{array}{c}\text{--CH=CH--CH--CH}_2\text{--} \\ | \\ \text{O--O}^{\bullet}\end{array} \longrightarrow O_2 + \begin{array}{c}\text{--CH=CH--CH--CH}_2\text{--} \\ | \\ \text{O} \\ | \\ \text{O} \\ | \\ \text{--CH=CH--CH--CH}_2\text{--}\end{array}$$

Lipid peroxidation is a major cause of food deterioration (rancidity and off-flavors); its measurement is a leading objective of food analysis. Current practice is seen in a variety of ways, though some common ground emerges among the opinions of different researchers such as the determination of peroxide value, hexanal and malondialdehyde, as the most important assays. The thiobarbituric-acid-reactive substances (TBARS), lipid peroxide, fluorescence and volatiles (e.g., hexanal) are the most widely used lipid peroxidation determinations but they do not measure initial processes that are highly relevant to shelf-life. Oxygen consumption has been

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extensively used but other processes interfere. The determination of peroxide value is the current method of choice. Lipid peroxidation is also a pathological phenomenon of wide-ranging consequence and, as such, is frequently investigated in biomedical research. But the assays for TBARS are much more widely used than any other index of lipid peroxidation (Wheatley, 2000). β-carotene bleaching method is one common assay used for lipid peroxidation. This method is based on the losing of the yellow color of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. This approach has been widely used to establish the rate of reaction for various radical molecules. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants. However, some essential controls must not be forgotten, particularly that the reaction must be checked to ensure that the substance under test does not inhibit $O_2^{\cdot -}$ generation and the reaction must be maintained under ambient conditions with oxygen (Halliwell *et al*., 1995; Kulisica *et al*., 2004)

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Figure 2.21 A peroxidation pathway from linoleic acid to hexanal.

(Wheatley, 2000)

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2.5.4 Flavor and aroma of red yeast rice (Berger, 1995)

Flavor is the sensation produced by a material taken in the mouth, perceived principally by the chemical senses of taste and smell. Aroma is a desirable olfactory sensation and may be considered as some chemicals causing a good smell of food, drinks or other product. Flavor and aroma contribute to acceptable foods qualities for

consumption. Volatile flavor and odorous compounds can be used as synonyms in some published articles. Fragrance substances used in perfumes, cosmetics, and toiletries, are distinguished from flavor and aroma by the difference range of applications.

Aroma can be classified into natural aroma and artificial aroma. Natural aroma compounds may originate from microbial or plant metabolism or, to a much lesser extent, from animal metabolism. Flavor or aroma separated from food source or generated during cooking or processing by enzymatic activities, or fermentation are all regarded as natural in USA. Artificial aroma compounds may be classified as nature-identical in EU.

The current compilation of identified aroma compounds contains more than 6200 entries from about 400 different food sources. Extrapolating from the number of facultative aroma precursors, from confidential industrial results, and from recently published progress it has been estimated that up to 10,000 volatiles may be present in food.

The character impact compounds are defined as the compounds responsible for the characteristic odor of a product. By means of coupled GC-O or gas chromatography-olfactory also called sniffing-gas chromatography, the character impact compounds are identified. The technique is the used of gas chromatography to separate and identify qualitatively the aroma constituents. The olfactory which is the use of sniffing each compound separated of which let us know characteristic odor. Some impact compounds, such as the carboxylic acid esters or typical products of lipid oxidation, such as (cis-3) - hexenol, (cis-3) - hexenal, 1-oclen-3-one, 1-(Z5)-octadien-3-one, trans-2-nonenal, or cis-2-trans-6-nonadienal contribute to the aroma

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of many different foods. Other impact components, such as 2- furfurylthiol(coffee), bis(2-methyl-3-furyl) disulfide (cooked meat, rice) or 1, 3- tran-5-cis-8-cis undecatetraene (some plant foods) depend on more specific precursors or pathways and determine the odor of only a few food products.

There are a number of works and reports according to aroma compounds present in rice grain and fermented rice product, especially red mold rice.

Aromatic rice popular in South East Asia were studied on the composition of aroma and volatile compounds (Apintanapong, 2003). Cook rice were used for determination of 2-acetyl-1-pyrroline (ACPY). The result is shown in Table 2.6

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Table 2.6 Concentration of ACPY found in different varieties of cooked rice

Variety	Concentration of ACPY (ppm, base on dry weight of rice)	
	Milled rice	Brown rice
Malagsit Sungsong	0.09	0.2
IR 841-76-1	0.07	0.2
Khao Hom Dok Mali 105	0.07	0.2
Milagrosa	0.07	
Basmati 370	0.06	0.17
Seratus Malam	0.06	
Azucena	0.04	0.16
Hieri	0.04	0.1
Texas Long Grain	<0.008	
Calrose	<0.006	

Source: Buttery *et al.* (1982)

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Buttery *et al.* (1988) compared the odor thresholds of 64 of the known rice volatiles for their contribution to the aroma of cooked rice. Their studies indicated that the probable major contributors to the cooked rice odor included ACPY, (EE)-2, 4-decadienal, nonanal, hexanal, (E)-2-nonenal, octanal, decamal, 4-vinyl-guaiacol and 4-vinylphenol.

Paule and Power (1989) studied the separation of rice volatiles by gas chromatography equipped with a packed column and a sniffing port. Two groups of judges (Orientals and non-Orientals) were asked to describe the odor of the effluent as the peak eluted. The ACPY showed highly significant positive correction with the descriptive terms: “pandan-like” (by Orientals) and :popcorn-like” (by non-Orientals). These findings support the conclusion of Buttery *et al.*(1983) that ACPY is chiefly responsible for the characteristic odor of aromatic rice. In addition, they indicated that the intensity of the aromatic principle was related to the amount of ACPY.

A study was conducted by Lin *et al*.(1990) to identify and quantify the flavor compound responsible for the characteristic “popcorn-like” aroma in Della and Lemont white rice. The results indicated that cooked Della white rice contained almost 300 ng ACPY per g (dry weight) rice, while cooked Lemont white rice (non-aromatic cultivar) contained only 4 ng/g (dry weight).

Laksanalamai and Ilangantieke (1993) identified ACPY in KDML-105 rice using a similar method used by Buttery *et al*. (1986) and compared it with the ACPY found in pandan leaves. The study confirms the importance of ACPY as a key compound contributing to the pandan-like aroma in KDML-105 and indicates the need to determine measure to properly store the rice to ensure aroma stability.

Mahatheeranont *et al.* (1995) suggest that the compounds assumed to play an important role in aroma of KDML-105 were ACPY as the major component, butyl acetate, diethyl carbonate, butyl cyclopropane, 1,4-dimethylbenzene, isocyanatomethylbenzene, hexanal, nonanal, 7-octane-4-ol, 2-(2-propoxyethoxy) ethanol and 2,6-bis (1,1-dimethyl-ethyl)-4-methylphenol. They indicated that the odor

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of synthetic ACPY was shown to be most match with the cooked pandan leaves aroma.

In 1996, Petrov *et al.* and Widjaja et al. reported about rice aroma analysis, their studies aimed to compare the volatile components between a aromatic and non-aromatic rice. Petrov *et al.* (1996) observed that the major difference between Azucena (aromatic rice) and IR -64 (non-aromatic rice) concerned ACPY. They also found that ACPY is the key-compound of aromatic rice aroma in Basmati, differentiation between these three aromatic rice varieties could be based on the concentrations of five compounds: pentanol, heptan-2-one, benzaldehyde, octanal and 6, 10, 14-trimethyl-pentadecan-2-0ne (Table2.7)

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Table 2.7 Compound involved in the discrimination between Azucena, Basmati, and Thai rice: results of canonical variate analysis

Variables	Azucena		Basmati		Thai	
	C[a]	var[b]	C	var	C	var
Pentanol	8.5	17	4.7	12	14.7	6
Hexanal	31.2	20	23	23	44	13
Hexanol	4.3	19	4.3	15	5.7	4
Heptan-2-one	2.2	13	1.3	15	4.8	3
Heptanal	1.4	19	1.3	12	2.3	10
2-acetyl-1-pyrroline	24.2	16	26	20	19	17
(E)-hept-2-enal	4	13	3.7	21	3.7	6
Benzaldehyde	10	15	5.3	13	11	7.6
Oct-1-en-3-ol	2.5	12	2.5	20	4	3.4
Octanal	1.3	19	1.9	10	3.2	9.7
(E)-oct-2-enal	2.8	18	2.4	12.5	4.3	1
(E,E)-deca-2,4-dienal	13.2	16	11.6	16	15.6	15
Pentadecan-2-one	5.1	23	6.7	12	6.2	14
6,10,14-trimethylpentadecan-2-one	2.8	22	5.8	8	12.5	6.8
Hexadecanol	4.2	26	2.4	20	3.7	25

[a]Concentration in rice cooking-water, mean of 12 extractions for Azucena, and 3 for Basmati and Thai, expressed in $\mu g.kg^{-1}$

[b]Coefficient of variation in %

Source: Petrov *et al*. (1996)

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Widijaja *et al.* (1996) suggested that non-aromatic rice (Pelde) contained much more n-hexanal, (E)-2-heptanal, 1-octen-3-ol, n-nonanal, (E0-2-, (E)-4-decadienal, 2-pentylfuran, 4-vinylguaical and 4-vinylphenal (the last two compounds possess unpleasant odor) than the aromatic rice (Basmati, Jasmine, Goolarah and YRF9). Jasmine and Goolarah had much more in dole, Goolarah a YRF9 had higher amounts of ACPY compared with those of Pelde, whilst Basmati had the highest amount of 2-phenylethanol and the lowest content of n-hexanal among all the rice types examined (Table 2.8). Sensory evaluation showed that YRF9 and Goolarah had the highest pandan-like aroma whilst Basmati had the highest popcorn-like aroma. Odor characteristics of various volatile components identified from these rice varieties, assessed using a sniffing port on the GC, are also shown in Table 2.8.

ACPY was not only identified as a key component contributing to the aromatic rice flavors but also shown to be a major component of the volatile oil of pandan leaves (*Pandanus amaryllifolius*) (Buttery *et al.*, 1982, 1983; Laksanalamai and Ilangantieke, 1993) and the sweet corn flavors (Buttery *et al.*, 1984). ACPY cab be isolated and identified from many other sources. It was also found to be an important component in the production of aroma in wheat bread and rye breads (Schieberle and Grosch, 1985, 1987; Schieberle, 1991) and was reported as a major odorant in popcorn (Schieberle, 1991; Karahadian and Johnson, 1993)

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Table 2.8 Concentrations ($\mu g.kg^{-1}$, wet wt) and odor description of major volatile compounds in various types of cooked rice[a]

Compound	Jasmine	Basmati	Goolarah	YRF9	Pelde	Odor description
n-Hexanal	1818	829	1498	1396	2038	Green, grass-like
2-Hexanone	7	13	7	18	7	Fruity
Pyridine	tr	tr	11	9	28	Pungent
n-Pentanol	152	130	82	104	160	Fusel oil-like
2-Pentylfuran	98	65	118	121	274	Nutty, bean
n-Heptanal	78	111	94	102	132	Fruity, fatty
2-Heptanone	94	204	70	92	117	Fruity, floral
(E)-2-Hexenal	161	598	193	233	294	Green
n-Hexanol	78	41	23	60	48	Herbaceous
ethyl heptanoate	tr	tr	18	21	26	Green, fruity
2-Acetyl-1-pyrroline[b]	d	d	691	670	15	Sweet, pleasant
Collidine(TMP)[c]						
n-Octanal	d	d	58	83	105	Slightly fruity
6-Methyl-5-hepten-2-one	28	10	32	34	58	Banana-like
(E)-2-Heptenal	99	58	108	97	208	Herbaceous
1-Octen-3-ol	87	46	57	75	111	Raw mushroom
n-Heptanol	32	54	17	30	32	Woody, sweet
n-Nonanal	158	125	210	244	429	Floral, fruity
2-Nonanone	6	11	4	tr	5	Fruity, herbaceous
Benzaldehyde	136	142	78	52	126	Nutty, bitter
(E)-2-Octenal	113	55	91	98	192	Green, fatty
n-Octanol	49	41	33	52	56	Fruity, floral
(E)-2,(E)-4-Heptadienal	d	d	13	14	26	Hay-like
n-Decanal	26	16	24	36	45	Sweet, waxy, floral
(E)-2-Nonenal	40	24	36	41	67	Fatty, woody
n-Nonanol	19	d	14	16	20	Floral, citrus
Acetophenone	33	20[d]	24	48	44	Sweet, floral
Phenylacetaldehyde	76	25	17	23	21	Sweet (dilute)
n-Undecanal	20	23	5	6	6	
2-Undecanone	11	d	8	9	14	Fruity, floral
(E)-2-Decenal	20	19	20	30	34	Waxy
(E)-2,(E)-4-Nonadienal	18	8	9	6	21	Waxy
2-Phenylethanol	195	703	318	217	353	Sweet, floral
(E)-2,(E)-4-Decadienal	50	16	77	97	150	Fatty, waxy
2-Tridecanone	tr	tr	tr	tr	2	Oily, nutty
2-Pentadecanone	67	67	58	95	99	
4-Vinylguaiacol[b]	29	109	81	84	119	Unpleasant
4-Vinylphenol[b]	67	47	99	72	108	Unpleasant
Indole	253	91	168	88	102	Floral

[a] Calculations have been divided by the compounds retative recovery factor

[b] Tentatively identified, [c] Internal standard, [d] Coeluted peaks

Source: Widjaja *et al*. (1996)

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Aroma constituents of fermented rice products are also reported. Good flavor and aroma in Sato (Thai rice wine) (Trinetra *et al.,* 2005) comes from several factor various rice strain gave different flavor when used to make Sato. Sato produced from black sticky rice, Hom-nin rice and white sticky rice from Bangkok, Kalasin and Chaiyapoom province were used to compare their aroma. Sato produced from black sticky rice was the best acceptable in the sensory test. Black sticky rice Sato contains more ethyl propionate, ethyl butyrate which gave fruity aroma and pineapple-like aroma respectively. More over, these compounds were detected only in black sticky rice and Hom-nin rice but not in white sticky rice.

There is some rarely available study on the aroma of rice fermented by using *M. purpureus*. Patakova-Juzlova *et al*. (1998) studied the identification of volatile metabolites from fermented rice by the fungus *M. purpureus* (Ang-kak). They found that 80 volatile compounds create typical ang-kak aroma. These volatile metabolites include alcohols, aldehydes, ketones, esters and terpenoid compounds. The identification has been performed by means of GC-MS after sample distillation and extraction with dichloromethane.

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2.5.5 Mycotoxin (Citrinin)

Mycotoxins are a group of structurally diverse secondary metabolites produced by various fungal species. These toxic compounds can contaminate foodstuffs, crops or human foods. Theingestion of these contaminated materials may be pathogenic in animals and humans as they may lead to serious health problems, such as liver, kidney or nervous system damage, immunosupression and carcinogenesis (Bennett and Klich, 2003) Due to the widespread nature of fungi in the environment, mycotoxins are considered unavoidable contaminants in foods and feeds; therefore, one of the most effective measures to protect the public health is to establish reasonable regulatory levels of these toxins. It is important to develop rapid, sensitive and reproducible assays to detect the presence of mycotoxin. Numerous studied were performed on the occurrence of mycotoxins in raw commodities and foodstuffs. However, due to trace property of mycotoxin and co-occurrence of diverse mycotoxins, their accurate and rapid qualitative and quatitative analysis remains still a challenging task.

The mycotoxin citrinin is a toxic secondary metabolite, first isolated from filamentous fungus *Penicillium citrinum* (Hetherington and Raistrick, 1931). It is also produced by other species of *Penicillium* (Ei-Banna, Pitt and Leistner, 1987), *Aspergillus* (Kurata, 1990) and *Monascus* (Blanc *et al.,* 1995a, 1995b; Li *et al.,* 2003). On account of its antibacterial effects, citrinin was investigated as an antibiotic (Wong and Koehler, 1981), but rerative toxicity studies showed that this secondary metabolite acted in animals as a nephrotoxin (Betina, 1989a), damaged the proximal tubules of the kidney (Phillips *et al.*, 1980b), and was implicated as a potential causative agent in human endemica Balkan nephropathy (Frank, 1992; IARC, 1986).

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Contaminations of citrinin were reported in a number of agricultural commodities, foods, feedstuffs as well as biological fluids at geographically diverse locations (Abramson *et al*., 1999; CAST, 2003; Comerio *et al*., 1998; Gimeno and Martins, 1983; Heber *et al*., 2001; Kpodo *et al*., 1995; Meister, 2004; Phillips *et al*., 1980a) (Table 2.9). However, these relative limited analytical data were based on diverse analytical methods with different sensitivity and accuracy. It was difficult to establish widely acceptable limits for citrinin. Presently, there is no specific registration for citrinin worldwide. The main reason was either rack of suitable analytical routine methods (Pohland and Wood, 1987), or its instability in food stuffs. So far, commonly used methods to analyze citrinin are thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) with UV or fluorescence detection (FD), and enzyme immunoassays (EIA). Recently, LC-MS and GC-MS technique have become available for qualitative and quantitative determination of citrinin. In order to protect public health and prompt international trade, more sensitive and accurate analytical methods for citrinin should be developed, and international criteria should be established for quality control of products contaminated with citrinin. Hence, we summarized physicochemical properties and quantitative analytical methods of citrinin, compared their chromatographic properties, sample pre-treatment, recovery rate and detective limit among various analytical methods.

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Table 2.9 Natural occurrence of citrinin in commoditics

Commodity Contaminated	Citrinin contents (µg/kg)	Reporting country	References
Fermented maize	548 g/kg	Ghana	Kpodo *et al*. (1995)
Cheese	600 mg/kg	France	Franco *et al*. (1996). Vazques *et al*. (1996) and Bailly *et al*. (2002)
Wheat	65 µg/kg at a_w 0.810 460 µg/kg at a_w 0.825 25,000 µg/kg at a_w 0.885	Argentina	Comerio *et al*. (1998)
Barley	38 g/kg in 19% moisture absent in the 15% moisture	Canada	Abramson *et al*. (1999)
Maize	12 µg/kg	India	Janardhana *et al*. (1999)
Red yeast rice	0.47-11.82 µg/capsule 0.2-140 mg/kg 4.2-25.51 mg2kg	USA China Taiwan, China	Heber *et al*. (2001) Xu *et al*. (1999) Shu and Lin. (2002)

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Table 2.9 (continued)

Commodity contaminated	Citrinin contents (µg/kg)	Reporting country	References
Silages	2.4-64.2 µg/kg	Germany	Schneweis *et al*. (2001)
Fruits	280-400 µg/kg for un-irradiated grape, fig, pear, absent for all irradiated fruits	Egypt	Aziz and Moussa. (2002)
Apples	320-920 µg/kg	Portugal	Gimeno and Martins. (1983) and Matins *et al*. (2002)
Brewed beers	Not detected	South Africa	Odhav and Naicker. (2002)
Cereal products	Rye wholemeal: 1.1 µg/kg Wheat wholemeal: 0.5 µg/kg Weat bran: 1.9-2.0 µg/kg Cocoa shells: 2.4 µg/kg Red yeast rice: 2.9 µg/kg	Germany	Meister, (2004)

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2.5.5.1 General characteristics of citrinin

Physicochemical properties

Citrinin [$C_{13}H_{14}O_5$, IUPAC: (3R, 4S)-4, 6-dihydro-8-8hydroxy-3, 4, 5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid; CAS No.: 518-75-2] (Figure 2.22), is an acidic lemon-yellow crystal with maximal UV absorption at 250 nm and 333 nm (in methanol), melting at 172 ^{o} C. It is sparingly soluble in water but soluble in dilute sodium hydroxide, sodium carbonate, or sodium acetate; in methanol, acetronitrile, ethanol, and most of other polar organic solvents (Deshpande, 2002). It is capable of forming chelate complexes, and can be degraded in acidic or alkaline solution, or by heating. It is a quinine methide with two intramolecular hydrogen bonds. Citrinin crystallizes in a disordered structure, with the *p*-quinone and *o*-quinone two tautomeric forms in a dynamic equilibrium in the solid state. In methanol/methylene chloride mixtures, citrinin undergoes a Michael-type nucleophilic addition reaction. This reaction is reversible, and the equilibrium shifts toward the nolmal citrinin if temperature is increased in methylene chloride (Poupko *et al*., 1997).

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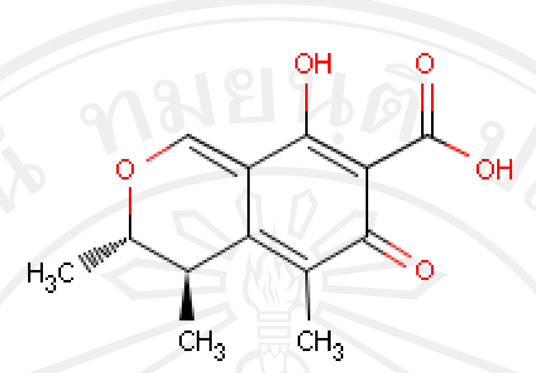

Figure 2.22 Structural formular of citrinin isomers

IUPAC name : (*3R, 4S*)-4, 6-dihydro-8-hydroxy-3, 4, 5-trimethyl-6-oxo-3*H*-2-benzopyran-7-carboxylic acid

Other names : Citrinin, Antimycin

Molecular formula : $C_{13}H_{14}O_5$

Molar mass : 250.24

Appearance : Lemon-yellow needles

Melting point : 172 ^{o} C

Solubility in water : Insoluble

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2.5.5.2 Toxicity and stability of citrinin

As one of mycotoxins, citrinin possesses antibiotic, bacteriostatic, antifungal and antiprotozoal properties. While it is also known as a hepato-nephrotoxin in a wide range of species (Berndt, 1990; Bilgrami *et al*., 1988; Hanika *et al*., 1983), in vitro studies have demonstrated that citrinin produced multiple effects on renal mitrocondrial function and macromolecule biosynthesis that ultimately resulted in cell death (Chagas *et al.,* 1992a, 1992b, 1995). In addition, citrinin occurred frequently together with another nephrotoxin-ochratoxin A in foodstuffs such as cereals, fruits, meat (Nishijima, 1984) and cheese (Lepom, 1986; Vazques *et al.,* 1996; Vrabcheva *et al*., 2000) and acted synergistically (Glahn *et al.,* 1989). To avoid the direct/indirect intake of citrinin, it is important to develop detoxification methods for citrinin during food processing. So far, there have been several reports on the detoxification of citrinin. The investigation on thermal decomposition and detoxification showed that, in the presence of a small amount of water, heating citrinin at 130 ^{o} C caused a significant decrease in its toxicity to Hela cells (Kitabatake *et al*., 1991); whereas heating at 140 ^{o} C or 150 ^{o} C in water caused formation of highly toxic compound (Trivedi *et al.,* 1993). Citrinin was also Known to form a novel toxin citrinin H_1 (Figure 2.28). Which is made up of two citrinin molecules at temperature above 100 ^{o} C. Further examination of the composition products from heated citrinin at 140 ^{o} C in water led to the isolation of another compound, citrinin H_2 [3-(3, 5-dihydroxy-2-methylphenyl)-2-formyloxy-butane] (Figure 2.23), which showed much weaker cytotoxicity than that of citrinin (Hirota *et al*., 2002). It was found that after boiling in water, concentration of citrinin in *Monascus* was dramatically decreased; 20 minutes of heating could decreased the concentration of citrinin by 50% (Shu and Lin, 2002).

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These facts indicated that citrinin was unstable and thermolabile in aqueous solution. Our previous study also verified that its stability was effected by temperature, solvent standard citrinin solution (Xu *et al.,* 2003). These facts explained the necessity for a sensitive analytical procedure for determination of citrinin in various foodstuffs.

Figure 2.23 Structural of citrinin H_1 and H_2

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Ober and Kunz (1989) studied the antibacterial fungi. Monascidin A was isolated from various strains of *Monascus.* The compound was identified and the structure was the same as citrinin (Blanc *et al*., 1995b). Citrinin is the substance causing renal failure and is formed in most of fungi (Sabater-Vilar *et al*., 1999). Monascidin A or citrinin is also studied in *M. purpureus* which is an antibacterial fungus by Wong and Koehler (1981). The structure of citrinin was studied by Blanc

et al. (1995b) using mass spectrometric method. Hajjaj *et al*. (1999) studied the mechanism of citrinin biosynthesis in *M. ruber* ATCC 96218 using ^{13}C nuclear magnetic resonance method. The experiment confirmed that citrinin formed tetraketide instead of pentaketide as in *Penicilium* and *Aspergillus.*The mechanic of citrinin biosynthesis in *Monascus* is shown in Figure 2.24 and 2.25

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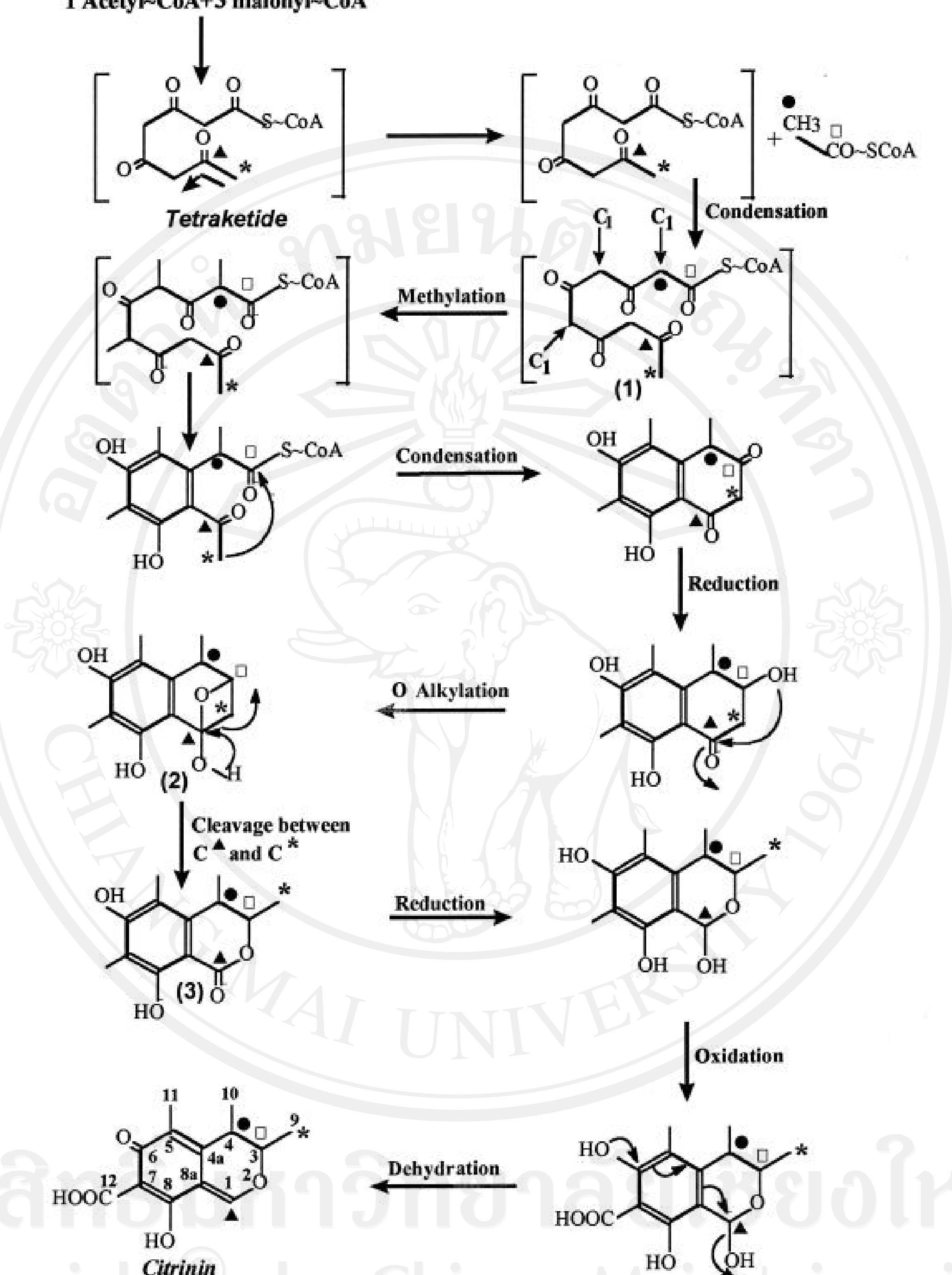

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Figure 2.24 Scheme of the biosynthesis of citrinin by *M. ruber*. The start of the condensing reaction is indicated by the bent arrow in the upper left panel. Intermediates are numbered. Enrichment of C-1 (▲), C-3 (□), C-9 (*), and C-4 (•)

Source : Hajjaj *et al*. (1999)

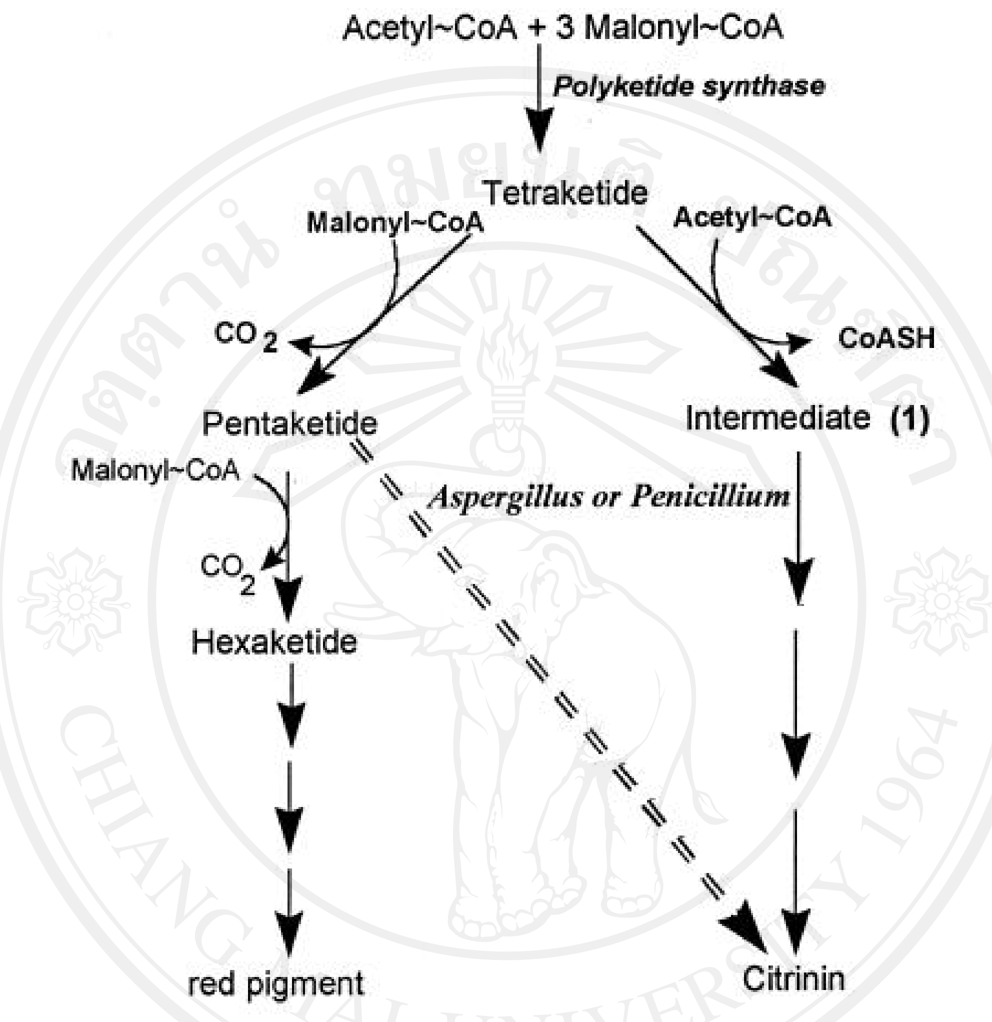

Figure 2.25 Biosynthesis of citrinin and red pigment in *M. ruber*. The toxin pathway in *Aspergillus* and *Penicillium* is indicated by the dashed arrow.

Source : Hajjaj *et al.* (1999)

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Blanc *et al.* (1995b) studied characterization of monascidin A from *Monascus* as citrnin. Following our investigations on red pigments and monascidin co-production by *Monascus* spp, the antibiotic called monascidin A was characterized as citrinin. Evidence was given by qualitative methods, mass spectra and NMR. Citrinin, a nephrotoxic agent was produced both by *M. purpureus* and *M. ruber*, either in submerged culture of concentrations of 270 and 340 mg/l, respectively, or in solid state culture of concentration of 100 and 300 mg/kg dried matter, respectively. Since citrinin is a toxic product, it is essential that the production of red pigments as food additives from *Monascus* spp. avoid the occurrence of citrinin. Regulation and control of the condition of fermentation is also studied in order to decrease citrinin. The presence of fatty acids, such as hexanoic and octanoic acid favors the production of pigments as well as citrinin. The citrinin content becomes higher when using aeration. Malic acid addition decrease pigments concentration but has no effect to citrinin content. Incase of amino acid addition, it was found that histidine increase the red pigment for 6 times, while citrinin was not increased. The fermentation of hydrogen peroxide caused by histidine addition may break down the citrinin structure. Apart form controlling of the cultivation condition, the amount of citrinin can be blocked by using peroxidase enzyme (Blanc *et al*., 1998). This result agree with the work of Hajjaj *et al*. (2000a) who studied the effect of 6-8 carbons fatty acids to the formation of citrinin in *Monascus*. The studied was carried out by liquid fermentation with 5.20 g/l of glucose, 5 g/l glutamate adding with octanoic acid which was the precursor of red pigment synthesis via polyketide synthesis pathway. The fatty acid increased the red pigment for 30-40 % while the amount of citrinin was decreased.

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Studying the condition for yellow pigment production with the control of citrinin concentration. It was found that fermentation from in 5 litter fermenter at 30 ^{o} C for 5 days using rice powder and glutamate as nutrients, the genetically, modified *M. purpureus* gave 40 units of yellow pigment with citrinin less than 5 mg/l. In 2004, the study on modified mutation method for screening low citrinin producing strains of *M. purpureus* on rice culture was done. It was found that *M. purpureus* NTU 601 is a strain that produces monacolin K, γ-aminobutyric acid (GABA), and citrinin under solid culture condition. Because citrinin is a mycotoxin and possesses nephrotoxic and hepatotoxic effects, it has a negative impact on the acceptance of red mold rice by people. In this research, a simple and quick selection method for mutant strains with low citrinin production was designed based on the fact that citrinin possesses antibacterial activity for *Bacillus subtilis* and will form an inhibition zone around the colony of *Monascus* strain. The mutant strain *M. purpureus* N 301 only produced 0.23 ± 0.01 ppm citrinin, which was 50% less than that of the parent strain, and the monacolin K production was 481.29 ± 7.98 ppm and maintained 91% productivity. *M. purpureus* N301, the other mutant strain, produced 0.27 ± 0.01 ppm citrinin, which was 41% less than that the percent strain, and the monacolin K production was 526.29 ± 5.54 ppm, which showed no significant changes when compared with the parent strain. The GABA content of the two strains was 5000 ppm, which is similar to that of the parent strain. The results showed that the method could be used to select red mold rice with low citrinin production (Wang *et al*., 2004). And Chen and Hu, (2005) study on red fermented rice with high concentration of monacolin K and low concentration of citrinin, found that, a mutation strain, *Monascus* spp. M12-69, was acquired by treatment with mutagenic agents from a wild

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strain M12 of *Monascus* screened from RFR samples gathered around China. According to the classification guide of Hawksworth and Pitt on *Monascus* genus, they belong to *M. pilosus* Sato. The conditions of the solid state fermentation of M12-69 were optimized. At the optimum conditions, the concentrations of monacolin K and citrinin in RFR, which was dried at 50 ^{o}C to a constant weight, were 2.52 mg/g and 0.13 ng/g, espectively. These results reveal that Strain M12-69 is a potential strain, which can be used to produce RFR with high concentration of monacolin K and low concentration of citrinin. From the reviews of Xu *et al*. (2006) about the analysis of qualitative and quanyitative. There are many methods used such as colorimetric technique, chromatographic technique (TLC, HPLC; HPLC-UV, HPLC-fluorescence), chromatography and mass spectrum combination technique (LC-MS, GC-MS), bioassay technique and enzyme immunoassay technique (EIA).

Shu and Lin (2002) proposed a method for citrinin determination in *Monascus* by GC-Selected ion monitoring mass spectrometry. This method (GC-SIM) mass spectrometry has been developed. GC separation of citrinin in *Monascu*s extract was achieved without the need for chemical derivatization, and could be detected as a single peak when the SIM mode selected 5 prominent fragmentations (m/z of 200, 205, 177, 105 and 91). The quantitative detection limit for citrinin was ~1 ppb. Finally, the GC-separated analyte from *Monascus* extract, at a retention time of 10.89 min, was examined by the method of pattern recognition by comparison with a citrinin standard. The results show that the 2 compounds had a 94% similarity when the SIM mode was used.

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2.6 Application of *Monascus* metabolites

The application of natural colorants has been developed for more than thousand years in China. The application in various purposes is listed in Table 2.10. More than 50 patents of the colorant from *Monascus* are registered in Japan, USA, France and Germany (Lin and Demain, 1991).

Table 2.10 Application of *Monascus* pigments

Application	Products
1. Alcoholic beverages	-Sake or rice wine), red wine, Chinese whisky
2. Drinks	- Sweet juice, Milk, Milk product and juice
3. Foods	- Bean jam, Chinese cheese or sufu, bean sweet -artificial meat product, Sausages, Hams -coconut milk, ice cream -Ketchup
4. Miscillaneous	- Shoyu, -Phamacueticals, Chinese medicine -Pulp and cosmetic - aperififs - silk - paper products

Source: Modified from Yongsmith (1999)

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Lee and Chen (1998a) applied *Monascus* to prepare Chinese sausage and dumpling (Bao). The meat had best color than the fat part. Using the pigments as instant did not interfere the flavor. Milk and milk product such as yoghurt gives the product similar to strawberry when use the pigments. The color could be maintained for more than one month. In case of candy the color may not be stable due to heating process at the temperature exceeding 150 ^{o} C. The factors affecting color production is also studied on agar medium by varying the fungal strains, rice varieties, pH, temperature and moisture. There are some reports using different strains of *Monascus* in Thailand. *M. purpureus* K001 has the darkest red color than other four strains. The same research later found that *M.* CMU.KU produced darker red color than other 9 strains. The strain *M. purpureus* TISTR 3090 was the second dark red strain (Hanpongkittikul, 1988)

Lee *et al.* (2002) studied 72 strains of *Monascus* cultured in liquid medium. *M. purpureus* M15 which is the most efficient strain had the red color value of 12. Xu *et al.(*2002) used *Monascus* spp. for the red color production. The strain XFP-1, when grown on agar medium for 1 week, gave the absorbance of 15000U at 505 nm and 2000 U at 410 nm.

Concerning rice grains used to prepare red yeast rice, there are two main kinds of rice grown in Thailand, non-glutinous rice and glutinous rice. Both kinds of rice compose of various cultivars. Many works use non-glutinous rice for making red yeast rice prepared red yeast rice from non-glutinous rice and glutinous rice Comparing of the color between different cultivars, using the condition reported by Palo *et al*. (1960), it was found that glutinous rice; Keaw Ngoo and non-glutinous

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rice; Mali, had the same red color which was similar to Japanese rice. The color of glutinous red yeast rice was more pleasant than Mali rice. Hanpongkittikul (1988) claimed that the amylose content affected the production of red color. He reported that rice cultivars with more than 24 % amylose such as Luang 148, Kor Kho 23 and Kor Kho 25 were better than Kor Kho 7 and Mali 105 concidering red color yield. In the year 2002, red yeast rice used as red colorant in sausage was studied (Pattanagul, 2002). Red yeast rice using Chai Nat rice was the most suitable for the sausage. Recently, Boonsangsom *et al.* (2004) reported that Mali rice fermented by *M. purpureus* ATCC 16365 could reach 623 unit/g of red color value. On the other hand, in other countries, it was found that Punpo rice in Korea gave a good result in preparing red yeast rice.

Considering the use of alternative nutrient sources, the color production by *Monascus* of bread had better red color than potato (Lin and Iizuka, 1982). Other nutrient sources such as maize grain, green bean and cassava gave unsatisfied products. Rashbaum and Yueh (1983) found that oat grains, wheat and barley could be used for red yeast rice preparation. Xu *et al. (*1998) reported that the difficulties of using corn grains was due to the bad penetration of micelle through the grain coat.

According to the condition of culture, Palo *et al.* (1960) found that the optimum pH was 3.0-7.5. John and Stuart (1991) determined the most effective pH to be 6.0. Later, the optimum temperature at 27-30 ^{o} C was proposed by Yongsmith (1999).

Moisture is also one of the important factors. Palo *et al.*(1960) also mentioned that the moisture below 50 % was the good condition. Han (1990) and John and Stuart (1991) who used the higher initial moisture (50-56 %) observed the intense color in

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with 8 days. The moisture of 95 % also gave colored product when the effective strains were used in fermentation for 7 days (Lee *et al*., 2002).

Nitrogen source may affect the production of the pigments. Dussa *et al*.(1998) found that the increases of monascorubamin and rubropunctatin correlated to the decrease of valine, methionine, isoleucine, glycine, glutamic acid and alanine.

Researches and studies on the production of monacolin K to improve the application consist of studies on various factors affecting the production as well as the properties of the compounds. Lee *et al*. (2002) studied the cultivation of 72 strains using both solid and liquid medium. The results showed the amount of monacolin were 4 mg/g and 5 mg/g solid and liquid medium respectively. Xu *et al.* (2002) cultivated the strains XFP-1 on solid medium for 1 week. The HPLC analysis showed the monacolin content of 0.71 mg/g. The same result was obtained by Hai (2002) who found that solid state culture produced higher amount of monacolin K. The researcher also applied the product to improve hypercholesterol suffering patients. The addition of sodium nitrate in the fermentation using *M. purpureus* CCRC 31651 obtained 378 mg/kg of monacolin K (Su *et al.,* 2003). Lee *et al*. (2006) used sweet potato (Ipomoeabatatas), Potato (Solanum tuberosum), cassava (Manihotesculenta), and diascorea (Dioscoreabatatus) as the substrates to find the best one to produce monacolin K. The results show that *M. purpureus* NTU 301, with dioscorea as the substrate, can produce monacolin K, at 2584 mg/kg, which is 5.37 times the result obtained when rice was used as the substrate. In addition, more amount of yellow pigment can be found in *Monascus*-fermented dioscorea than in *Monascus*-fermented rice. The certain composition of yellow pigment is identified as monascin,

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which has been shown as an anti-inflammation agent exhibiting potent inhibitory effects on 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-induced inflammation in mice in previous studies. Company, WBL Peking University Biotechnology, in China manufactured encapsulated medicine for anti - hyperlipidaemia. The capsule were filled with red yeast rice from *M. purpureus* Went M5801 fermented using solid culture. The strain was obtained by mutation of *M. purpureus* 1003 with UV, neutron and X-ray irradiation. The amount of 6-10 mg/g were obtained (Li, 2002). Anticancer effects of Chinese red yeast rice versus monacolin K alone on colon cancer cells were compared (Hong *et al*., 2008), Chinese red yeast rice (RYR) is a food herb made by fermenting while rice with *M. purpureus* Went. RYR contains a mixture of monacolins, one of which monacolin K (MK) is identical to lovastatin (LV). Epidemiological studies show that individuals taking strains have a reduced risk of colon cancer. In the present study, LV decreased cellular proliferation (p<.001) and induced apoptosis (p<.05) in HCT-116 and HT-29 human colon cancer cells(p<.001) and enhanced apoptosis (p<.05) in HCT-116 cells. Inhibition of proliferation was reversed by mevalonate (MV) in LV-treated cells, since LV is a 3-hydroxy-3-methyl-glutaryl Co A reductase (HMGCR) inhibitor. However, RYR with MV did not reverse the observed inhibition of growth. MK-free RYR did not reverse the observed LV-mediated inhibition of cancer cell growth. These observations suggest that other components in RYR, including other monacolins, pigments or the combined matrix effects of multiple constituents, may effect intracellular signaling pathways differently from purified crystallized LV in colon cancer cells. RYR was purified into two fractions: pigment-rich fraction of Chinese red yeast rice (PF-RYR) and monacolin-rich fraction of Chinese red yeast rice (MF-RYR). The effect of MF-RYR was similar

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to that of LV, while the effect of PF-RYR was similar to the effect of the whole RYR extract on the proliferation, apoptosis and mRNA level of HMGCR and sterol response element binding protein-2. These results suggest that the matrix effects of RYR beyond MK alone may be active in inhibiting colon cancer growth. RYR with or without MK may be a botanical approach to colon cancer chemoprevention worthy of further investigation. Li *et al*, (1998) studied the effects of red yeast rice (fermented by *M. purpureus*) on blood lipids and lipoprotein concentrations in three animals models. In rabbits fed on a diet of 25% casein, which induced endogenous hypercholesterolemia, serum cholesterol concentration increased from approximately 1.81 to 7.51 mmol/L with in 60 days. Treatment with red yeast rice for 30 days at doses of 0.4 and 0.8 g/kg/day significantly lowered serum total cholesterol (TC) concentration and TC:HDL-c ratio ($p<0.05$). In a second rabbit model where hyperlipidemia was induced exogenously by an atherogenic diet which included 0.5% cholesterol, 15% yolk powder, and 5% lard, oral red yeast rice (0.8g/kg/day for days) prevented increases of serum total cholesterol (TC), triglyceride (TG) concentration and TC:HDL-c ratio ($p<0.05$). Importantly, lesions in the aorta and lipidosis in the livers of red yeast rice –treated rabbits were less severe than those of the control model rabbits. In quail where hyperlipidemia was induced exogenously by an atherogenic diet which included 1% cholesterol, 14% lard, 6% Soya-bean oil, oral red yeast rice (0.1, 0.2 and 0.4 g/kg/day for 2weeks) largely prevented increases of serum TC and TG concentrations ($p<0.05$ or $p<0.01$). This study demonstrated that red yeast rice reduced serum TC and TG in rabbits and quail with experimental hyperlipidemia and suppressed atherosclerosis by an atherogenic diet. Apart from monacolin K, there are other monacolin compounds similarly active. Endo *et al,* (1986) found a new

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inhibitor of cholesterol biosynthesis, Monacolin M, having a structure related to monacolin K (mevinolin). It was isolated from culture of a *M. ruber* strain. The structure of monacolin M elucidated by a combination of physical techniques was determined to be β-hydroxybutyryl ester of monacolin J. It was suggested that monacolin M is derived from monacolin J *via* a synthetic pathway distinct from that for the synthesis of monaclin K, α-methylbutyryl ester of monacolin J. The inhibitory effect of monacolin M on β-hydroxy-β-methylglutaryl-Co A reductase was slightly lower than of monacolin K. Monacolin exist in 3 categories when used *M. ruber* of *Aspergillus terreus*; lactone, β-hydroxy acid and methyl ester (Friedrich *et al.,* 1995) The study of 70 strains of *Aspergillus terreus* in liquid medium found that TUB F-514 produced 140 mg/ml in 1 week (Morovjan *et al*., 1997). Moreover, nitrogen and carbon sources which affected the monacolin production using *Aspergillus Thom* ATCC74135 in liquid medium were also studied (Hajjaj *et al*., 2001). Using lactose, glycerol, ethanol or glucose as carbon source and 12.5 g/l sodium glutamate as nitrogen source with 160 hours of cultivation, glucose concentration of 20 and 45 g /l gave 37 and 35 mg/l of monacolin respectively. Lactose concentration of 20 g/l gave highest amount of monacolin K at 54 mg/l. The addition of glucose, therefore, did not affect the monacolin K production while lactose increased the concentration of monacolin K. In case of ethanol and glycerol, very small amount of monacolin K was obtained. Biomass production was enhanced when using sodium glutamate, histidine, glycine, arginine, isoleucine as organic nitrogen source and ammonium tartrate, ammonium acetate, sodium nitrate and urea as inorganic nitrogen source while monacolin K is still not changed. The addition of 12.5 g/l sodium glutamate and 12.5 g/l histidine gave 47 and 46 mg/l of monacolin K respectively. Monacolin K from

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Aspergillus terreus was also applied for cholesterol lowering in mouse and dog (Alberts *et al*., 1980), the structure and absolute configuration of mevinolin and its open acid from, mevinolinic acid, were determined by a combination of physical techniques. Mevinolin was shown to be 1, 2, 6, 7, 8a-hexahydro-β, δ-dihydroxy-2, 6-dimethyl-8- (2-methyl-1-oxobutoxy)-1-napthalene-heptanoic acid δ-lactone. Mevinolin in the hydroxyl acid form, mevinolinic acid, is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase [mevalonate: $NADP^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34]; its K_i of 0.6 nM can be compared to 1.4 nM for the hydroxyl acid form of the previously described related inhibitor, ML-236B (compactin, 6-demethylmevinolin). In the rat, orally administered sodium mevinolinate was an active inhibitor of cholesterol synthesis in an acute assay (50% inhibitory dose = 46 μg/kg. Further more, it was shown that mevinolin was an orally active cholesterol-lowering agent in the dog. Treatment of dogs for 3 weeks with mevinolin at 8 mg/kg per day resulted in a 29.3 ± 2.5 % lowering of plasma cholesterol.

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