CHAPTER 1

INTRODUCTION

1.1 Introduction of Red Yeast Rice

Monascus species belong to the group of Ascomycetes and particularly to the family of Monascaceae (Hawksworth and Pitt, 1983). Red yeast rice, usually obtained by cultivation of *Monascus* species on the rice grains, has been used in Asian nations for several hundreds years to make rice wine, as a food preservative for maintaining the color and taste of fish and meat, and as well as for its medicinal properties. Red Yeast Rice, also known as Chinese Red Rice, *Monascus* Fermented Rice, Red Rice, Red Mold Rice, Red Fermented Rice, anka, ankak, ang-kak or Hongquor, becomes an important topic in the field of functional food because of its multifunctional compounds. The types of secondary metabolites produced from the *Monascus* species include (1) a group of pigments : yellow pigment (ankaflavin and monascin), orange pigment (monascorubrin and rubropunctanin), red pigment (monascorubramine and rubropuctamine), (2) a group of antihypercholesterolemic agents including monacolin K, (3) hypotensive agent : γ -aminobutyric acid (GABA), (4) antioxidant compounds including dimerumic acid and 3-hydoxy-4-methoxy-benzoic acid , and (5) an antibacterial activity compound including pigment and citrinin (also known as monascidin)

There are many reports on the medical effects of secondary metabolites found in red yeast rice both as a folk remedy and in scientific publications, such as antimicrobial (Wang *et al*., 2002), antihypercholesterolemic (Alberts *et al*., 1980; Lee

et al., 2005), antioxidant (Aniya *et al*., 2000; Dhale *et al*., 2007; Taira *et al*., 2002) and hypotensive (Su *et al*., 2003).

The modern research has revealed that the main components of red yeast rice contributing to the antihypercholesterolemic effect involves monacolin-type compounds, such as compactin or ML-236B (Endo *et al*., 1976), monacolin J, monacolin L (Endo *et al*., 1985), dihydromonacolin L, monacolin X (Endo and Hasumi, 1985). In 1979, Prof. Akira Endo of Japan isolated a more active methylated form of compactin, monakolin K (Fig 1.1), from the cultures of *Monascus purpureus* and showed that monacolin K was capable of inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), an enzyme for the biosynthesis of cholesterol. Without this enzyme, the entire sequence of biosynthesis of cholesterol is blocked. Consequently, monacolin K represents an elective target in regulating the levels of cholesterol in blood. Endo also subsequently established the identity between monakolin K and lovastatin, the precursor of a whole class of hypocholesterolemic drugs statins, which are still considered today the treatment of choice for hyperlipoproteinemia and one of the most important categories of available drugs.

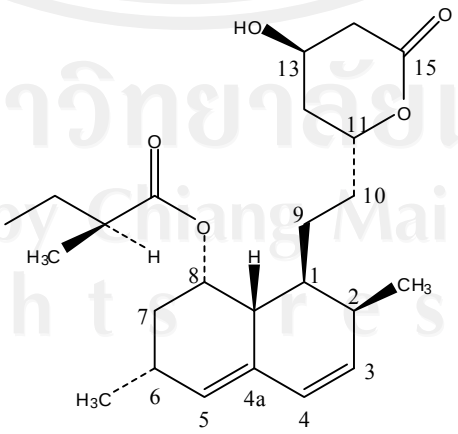

Fig 1.1 Structure of monacolin K (Source : Endo, 1980)

Monacolin K, known as a statin compound, has been regarded as a cholesterol-lowering agent because it was proven to be active not only *in vitro* to inhibit cholesterol biosynthesis (Alberts *et al*., 1980; Endo, 1980) but also *in vivo* to lower plasma cholesterol level in human (Wang *et al*., 1997) and animals (Alberts *et al*., 1980; Lee *et al*., 2005).

Many people in the developed countries suffer from elevated plasma cholesterol, and it is now generally accepted that a major risk factor for the development of coronary heart disease is an elevated concentration of plasma cholesterol, especially low density lipoprotein (LDL) cholesterol. Since hypercholesterolemia is a major cause of coronary heart disease and potential cause of death, an effective cholesterol-lowering agent has long been sought. Agents used in treating hypercholesterolemia includes (1) Bile acid–binding Resin : Examples are colestipol and cholestyramine. They are not absorbed from the intestine and bind bile acid there, carrying them out with the stool. This results in increased bile acid synthesis in the liver with increased expression of LDL receptors. (2) Niacin : decreases level of LDL and VLDL (very low density lipoprotein). This water-soluble B vitamin primarily inhibits secretion of the LDL precursor, VLDL. (3) Clofibrate and Gemfibrozil : decrease level of VLDL in plasma by assisting in hydrolysis of VLDL by lipoprotein lipase. Clearance of triglyceride-rich proteins may be increased by increasing the activity of lipoprotein lipase. (4) Probucol : increases uptake of LDL via receptor-independent pathways. (5) Competitive inhibitors of HMG-CoA Reductase : inhibit the rate-limiting enzyme of cholesterol biosynthesis, 3-hydroxy-3-methyglutaryl coenzyme A reductase (HMG CoA reductase) (Kane and Malloy, 1990; Well, 2006).

In general, monacolin K biosynthesis from *Monascus* species can be influenced by various factors, such as *Monascus* strains, the medium composition especially by the type of carbon and nitrogen source which are significant for development of the fungi. In addition, other components (eg. ammonium chloride, sodium nitrate and monosodium glutamate) and environmental parameters, such as cultivation method, agitation, temperature and moisture content also affect the production (Wang *et al*., 2003).

Although many *Monascus* species could synthesize monacolin K, the production of monacolin K is still less than 500 mg/kg. However, the recommended daily allowance of monacolin K for adults was suggested to be at least 10 mg per day. Consequently, a dosage of general red yeast rice powder against cholesterol biosynthesis for a human adult has to be at least 10 g per day. Therefore, *Monascus* species with low monacolin K productivity would become more and more difficult to develop and apply on functional food with cholesterol-lowering effect, so it is important to increase the monacolin K production of the *Monascus* species.

Stability of monacolins is also crucial and requires attention due to the fact that monacolins will be transformed from their lactone forms into hydroxyl acid forms in high pH (Li *et al*., 2004) which reduces their pharmacological properties. In addition, monacolins also decreased significantly under the conditions of high humidity at high temperature and sunlight. Monacolin K and its hydroxyl acid form would be dehydrolyzed and turned to dehydromonacolin K at high temperature while the monacolin K, J and L would be transformed into their corresponding hydroxyl acid forms under the condition of high humidity (Li *et al*., 2005).

However, Red yeast rice is still restricted and not accepted in many countries, and causes much controversy because of mycotoxin–citrinin (fig 1.2) which is often secreted by *Monascus* species. Citrinin is a potent renal toxin and hepatotoxin, which causes functional and structural kidney damage and alterations in liver metabolism. It also inhibits several enzymes linked to the respiratory chain of the kidney cortex and liver mitochondria, as well as malate and glutamate dehydrogenase and the ATP-synthase complex. Nevertheless, many researchers suggest that Red yeast rice has been proven to contain multifunctional compounds and that, therefore, the function and application of *Monascus* species should not be annulled because it possesses citrinin. In addition, red yeast rice would be safe and harmless for the daily diet if the content of citrinin is less than the level that induces in vivo damage.

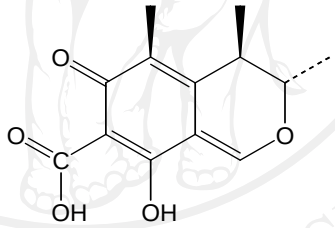

Fig 1.2 Structure of citrinin (Source : Xu *et al*., 2006)

Since there is no universal and excellent method for stimulating the formation and isolation of monacolin K by *Monascus* species regarding stability, an effective means to increase productivity of monacolin K and quality control are imperative in order to develop red yeast rice as a drug or food supplement.

Objectives of this study

1. To investigate the optimal conditions for production of monacolin K biosynthesized by *Monascus* species in Thai rice.

2. To investigate the optimal conditions for extraction and isolation of

monacolin K biosynthesized by *Monascus* species in Thai rice with respect to stability of obtained substances.

3. To control quality of the production of monacolin K biosynthesized by *Monascus* species in Thai rice in order to develop into industrial level.

Application advantages of this study

1. To be able to find out the environmental factors that influence production of monacolin K biosynthesized by *Monascus* species in Thai rice in order to increase the production of mentioned substances.

2. To be ale to extract and isolate monacolin K biosynthesized by *Monascus* species in Thai rice from other constituents in Red Yeast Rice with respect to stability.

3. To be able to produce monacolin K biosynthesized by *Monascus* species in Thai rice which are safe and can be developed into industrial level.