CHAPTER 2

REVIEW OF LITERATURE
Microorganisms are capable of producing natural products with widely divergent chemical structures. Greatest attention in the past has been paid to natural products that have antibiotic properties and accumulate in the fermentation broth during secondary metabolism, a characteristic of the incomplete metabolic control operative in growth-inhibited microorganisms. With this general mechanism of biosynthesis, the natural products synthesized by microorganisms would be expected to have a broad range of pharmacological activities (Demain, 1999). The directed screening for non-antibiotic natural products has been of limited scope. The expectation of new compounds of interest has been validated. The pharmacologically active natural products provide previously unrecognized structures as tools for fundamental research programs, as well as offering the possibility of direct use in medicine or in industrial processes.

Campbell-Platt (1987) has defined fermented foods as those foods, which have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification to the food. The use of microorganisms to process foods goes back to ancient times. Fermented foods are essential parts of diets in all parts of the world. A number of food fermentation processes-including those that yield dairy products. Sausages, pickles, sauerkraut, and bread have been extensively investigated and accurately documented (Campbell-Platt, 1987). But many other foods prepared by the action of diverse species of fungi, bacteria, and yeasts on plant materials have been neglected. Mainly because they are little known outside their native countries.

Growth of microbial cells on hydrocarbons or cellulose involves the use of inedible substrates. Another body of microbial technology involves the growth of microbial cells on edible substrates (e.g., Tempeh). This is based on the indigenous fermented foods, which serve as staples in the diets of the lower income groups-the villagers and peasants
In many developing countries. In these foods, human beings consume both the microbes and substrates.

In the production of tempeh, the Indonesians convert food by-products such as peanut and coconut presscakes into meat substitutes suitable for human consumption, although they are generally fed to animals in the western world. In Indonesia, the presscakes are soaked, ground, formed into rectangular cakes, steamed, cooled, and inoculated with either the tempeh mold or Neurospora intermedia. As the cakes are overgrown with the mold, the mycelium knits the particles into pieces that can be sliced and fried or used as chunks in soups. It is basically the tempeh process applied to another type of substrate. Because the substrate is a by-product of the food industry, this oncom (or onijom) generally costs less than tempeh. As with tempeh, the microbial protein is consumed with the substrate, and the product contains vitamin B-12 activity (Steinkraus, 2002).

The Orientals developed the soy sauce and miso processes centuries ago (Yokotsuka, 1982). Soy sauce provides essential amino acids and peptides important in nutrition. In the soy sauce process, soybeans are soaked, cooked, coated with ground roasted wheat, and then overgrown with the mold Aspergillus oryzae. Subsequently, the soybean and wheat mixture is covered with 18% salt brine, and fungal enzymes solubilize the proteins, lipids, and other soybean components to produce the typical meat-like flavor of filtered soy sauce (Hesseltine, 1965).

Cereal proteins generally do not contain enough of the essential amino acid lysine. Thus, people consuming principally polished rice are likely to be short of lysine as well as the essential vitamin thiamine, which is removed with the bran. The Chinese and the Indonesians centuries ago developed processes in which rice is soaked, steamed, and inoculated with certain molds and yeasts of the Amylomyces rouxii and Endomycopsis burtonii types, which transform the rice to sweet / sour alcoholic pastes. These products, called tape-have, have an attractive flavor and aroma. The microorganisms selectively synthesize lysine, improving the rice protein quality, and thiamine, increasing the thiamine content threefold-to its initial value in the unpolished rice. Because the
microorganisms also use a portion of the rice starch, the total protein doubles on a dry-solid basis (Cronk et al., 1977; Hesseltine, 1983).

Fermented foods made with known microorganisms by "Traditional" fermentation processes are those that have been used for centuries and even predate written historical records. In the oriental food fermentations, soybeans and filamentous fungi are predominant, although the fermentation may involve a substrate consisting of soybeans and cereals plus an inoculum of bacteria, yeasts, or fungi. Many of these products have been industrialized and are prepared using commercial inocula. Most oriental fermented foods are used mainly as flavoring agents and protein sources.

The fermented foods of mid-Asia, The Middle East, and Africa are acid products prepared by bacterial and yeast fermentation of cereals such as millet, sorghum, maize, and wheat. The cereals are supplemented with a protein source of either animal or plant origin. In the Middle East, this is usually a milk product, whereas in India and Pakistan, it is locally grown legumes, typically, the microorganisms used are those present in or on the ingredients and are selected by adjusting the fermentation conditions. Except for the industrialized bantu beer and mahewu processes, all the fermentations are self-inoculated. Most of the foods are liquid fermentations, whereas Far East fermentations are generally moist solid substrates.

FERMENTED FUNCTIONAL FOOD

"Nutrition" or "nutritional" is the supplying of calories/energy. Protein, essential amino acids/peptides, essential fatty acids, vitamins and mineral requirements are essential to satisfy metabolic needs of the consumer. Two major food problems exist in the world, starvation (or under-nutrition where there is insufficient food or insufficient economic means to provide the necessary food) and obesity (or over-consumption of food in the wealthy, developed world). "Functional foods," and "nutraceuticals," have been used to describe foods, or parts of foods that offer a health benefit that goes beyond meeting traditional nutrient needs. Understanding the potential health benefits that have been linked to functional foods and nutraceuticals is another step you can take toward
enhancing your health and preventing disease. The term "functional foods" refers to foods and their components that may provide unique health benefits that go beyond simply meeting basic nutrient needs. Functional foods do more than meet minimum daily nutrient requirements—they also can play a role in reducing the risk of disease and promoting good health. Functional foods contain what nutrition researchers call “bioactive compounds,” or naturally occurring chemicals that act on our bodies. It is these bioactive compounds that offer the health and wellness benefits and imparts health benefits or desirable physiological effects that have been linked to functional foods.

Nutraceuticals are often defined synonymously with functional foods in the media and literature. In fact, the term nutraceutical, as coined by Stephen De Felice, M.D., founder of the Foundation for Innovation in Medicine in Cranford, N.J., covers the gamut—including dietary supplements, those fortified foods that are enriched with nutrients not natural to the food, functional foods and medical foods. Thus, nutraceuticals are more correctly defined as parts of a food or a whole food that have a medical or health benefit, including the prevention and treatment of disease. Such products may range from isolated nutrients, dietary supplements and specific diets to genetically engineered designer foods, herbal products, and processed (chemical/fermentation) foods such as cereals, soups, and beverages (De Felice, 2002).

The nutritional impact of fermented foods on nutritional diseases can be direct or indirect. Food fermentations that raise the protein content or improve the balance of essential amino acids or their availability will have a direct curative effect. Similarly, fermentations that increase the content or availability of vitamins such as thiamine, riboflavin, niacin or folic acid can have profound direct effects on the health of the consumers of such foods. This is particularly true of people subsisting largely on maize where niacin or nicotinic acid is limited and pellagra is incipient and in people subsisting principally on polished rice, which contains limited amounts of thiamine and beri-beri, is incipient (Jelliffe, 1968). Biological enrichment of foods via fermentation can prevent this.
Chinese Fermented Foods

Sufu (Chinese cheese) is another Oriental food fermentation using *mucoraceous* fungi *Actinomucor elegans* and *Mucor dispersus*. Sufu is made from soybeans; initially soybeans are washed, soaked overnight, and then ground in a mill. The milk is pressed from the mash and cooked in excess water. Often the milk is precipitated with calcium sulfate, the curd is pressed into a mold (typically a wooden box) to remove much of the water and the solids then form a cake. The cake is carefully removed from the mold or box under water. This unfermented material can then be sliced into small cubes and is used widely in the Orient as *tofu* (soybean curd). *Tofu* is cut into cubes of 2 X 2 X 4 cm and partially sterilized in a hot air oven at 100 °C for about 10-15 minutes. To aid in the prevention of bacterial growth, the cubes before sterilization should be sprayed with an acid saline solution of 2% sodium chloride and 0.8% citric acid. The cubes should be separated from one another and should be placed in a tray that has an opening in the bottom to allow circulation of air. The cubes after cooling are inoculated on their surface with a pure culture of an appropriate fungus. The inoculated *tofu* is then placed in an incubator at below 20 °C and in some cases as low as 12 °C. The inoculated cubes are then incubated for 3 to 7 days, depending on the culture used. The fresh molded cubes are then known as *pehtzes*. The *pehtzes* are then placed in a solution of 12% sodium chloride and 10% ethanol (sometimes added as rice wine or distilled liquor). In other cases, only the salt brine is used. It is then allowed to age for varying periods of 2 months. The cheese and liquid brine are bottled, sterilized, and marketed as *sufu* (Hesseltine, 1965).

*Lao-Chao* (chiu-niang, Tape ketan) is an alcoholic rice paste from China; the substrate used for production of *Lao-Chao* is glutinous rice (waxy variety of rice) and fungus *Rhizopus oryzae*, *Chlamydomucor oryzae*, *Aspergillus rouxii*, *Amylomyces rouxii*, *Rhizopus chinensis*, *Saccharomycopsis fibuligera* and *Saccharomycopsis malanga* carry out fermentation process (Hesseltine, 1965).

*Shoyu*, it is a dark brownish liquid with a distinct pleasant aroma. It is used widely for adding flavor to many foods, such as meat, poultry, and fish and for barbecue and other sauces, for flavoring cooked vegetables, and for seasoning in general. In the preparation
of shoyu first soybeans are washed, soaked for 15 h at room temperature, and autoclaved. At the same time as the soybeans are being prepared, wheat is cleaned, roasted, then crushed into approximately five pieces. Wheat is believed to contribute flavor and aroma to shoyu. If wheat bran is used, it is steamed rather than roasted. The treated wheat and soybeans are mixed together at the ratio of 45:55 by volume. After the material is cooled it is inoculated with tane koji composed of either *Aspergillus oryzae* or *A. sojae*. The mold is mixed at the rate of about 0.1 to 0.2%, to these 1.2 liters of salt brine of 22.6% is added to each kilo of inoculated substrate. The mixture to be made into koji is then placed in about 2 liter amounts in boxes. The molding wheat and soybeans are placed in special rooms with the temperature controlled between 25 and 35°C. As the fermentation proceeds cooling must be done to prevent the material from reaching a temperature of 40°C or higher. Incubation is carried on for 3 or 1 clays and there are at least two turnings of the fermenting substrate. At the end of the koji fermentation it is placed in tanks and about an equal amount of brine is added. The material is then left in the tanks from 3 or 1 month if it is warmed or to 1 year if no heat is supplied (Hesseltine, 1965).

Tamari, in China, shoyu is more of the tamari type, in which more soybeans are used and less wheat and the process of manufacturing tamari is same as that of shoyu (Yokotsuka, 1960). Yen-Tsai, this is "vegetables preserved in brine" and is a typical lactic fermentation of various vegetables in China, similar to the Indian *achar* (Hesseltine, 1965). Miso, it is a food prepared by a two-step fermentation process. The first involves the fermentation of rice to produce enzymes followed by a second fermentation of the mold rice or koji along with soybeans, salt, and suitable inoculum and it is used as a flavoring agent with various foods including fish, vegetables, and meats (Steinkraus, 2002). Angkak or red rice is a product made by fermenting rice with certain strains of *Monascus purpureus* (Journoud and Jones, 2004)

ANGKAK (Chinese Functional Food)

Angkak, red fermented rice (RFR), Chinese red rice, also known as Hung-ch'u or Hongqu, red yeast rice, red koji, anka, red mold rice, red fermented rice, is an Asian
traditional fermentation nutraceutical / functional food, which contains numbers of functional materials such as monacolin K, \( \gamma \)-amino butyric acid (GABA), natural red pigment, and other unidentified active components (Blanc et al., 1995a; 1995b; Aniya et al., 1999; Su et al., 2003). These components are the secondary metabolites of fermentation and are medically proven to possess anticholesterol, anticarcinogenic activities (Endo, 1979; 1980; Lee et al., 2006a), and antifatigue activity (Wang et al., 2005). The, *Pen Tsao Gum Mu* (ancient Chinese pharmacopoeia), indicates the use of red mold rice to promote the health of the cardiovascular systems (Kao, 1997).

**Historical Background**

In China, angkak was extensively used in foods and folk medicine, prior to the Zhou Dynasty (770–221 B.C.) and in the time of the Han Dynasty (206 B.C.–220 A.D.). It was apparently first noted as a medicine during the Tang Dynasty (A.D. 618-917) and employed for treating indigestion, diarrhoea, congestion of the spleen and for improving blood circulation. Tao Gu, after the Tang Dynasty, recorded "Red Yeast Rice Cooked with Meat," in *Qing Yi Lu*.

During Ming Dynasty, *Tien Kyng Kai Wu* by Sung Ying-Hsing, (1637 A.D.) described red rice as useful for preserving the color and taste of fish or meat. The method of making angkak was originally recorded in *Tien Kyng Kai Wu* and *Pen Tsao Kang Mu* [Chinese ancient pharmacopoeia, which was published during the Ming Dynasty (A.D. 1368-1644)] contains detailed description of the medical applications of angkak.

In *Pen Tsao Kang Mu*, angkak was described as mild, non poisonous, and useful for treating indigestion and diarrhoea. It was also described as useful for improving blood circulation and promoting the health of the spleen and stomach for treating ailments, such as indigestion, diarrhoea, and heart and abdominal pains. Red rice, as described in *Pen Tsao Kang Mu*, was subsequently recognized to be the fungal species known as *Monascus purpureus* Went (Journoud and Jones, 2004).
Fermentative Production

According to the earliest reported method as recorded in ancient Chinese pharmacopoeia, *Tien Kyng Kai Wu*, rice can be prepared by the fermentation of washed and cooked nonglutinous rice using red wine mash, natural juice of *Polygonum* grass, and alum water. The rice is fermented in open air for 7 days on bamboo trays under very clean conditions (Figure 2.1). The rice changes its color from white to black, black to brown, brown to red and then red to yellow, which is then harvested as red rice. According to an alternative traditional method, non-glutinous rice can be fermented in a hole in the ground lined by bamboo mats, which is securely covered. Fermentation is allowed to take place underground for one year or more, up to four years.

| Day 1 | 1st day, inoculated with Monascus koji, temperature controlled at 33 to 35°C |
| Day 2 | 2nd day, stirring and mixing of koji, temperature controlled at 34°C |
| Day 3 | 3rd day, 1st water soaking of koji for 30 min, moisture controlled at about 50% |
| Day 4 | 4th day, 2nd water soaking of koji, moisture controlled at about 47% |
| Day 5 | 5th day, last time water soaking of koji, moisture controlled at about 48% |
| Day 6 | 6th day, post maturing, stirring every 10 hours, temperature controlled at 30°C |
| Day 7 | 7th day, drying at 45°C for 22 hours |
| Day 8 | 8th day, harvesting of dried red mold rice |

Figure 2.1. Production of red yeast rice in ancient China According to *Tien Kyng Kai Wu*
In the traditional process, for easy control of aeration and removal of fermentation heat, the inoculated cooked rice is put in a round shallow bamboo tray about 5–6 cm in depth. Trays are stacked in shelves in a fermentation room. Agitation with hands is needed to flip over the bottom part of the rice koji and removing fermentation heat. During fermentation, each tray is taken out at least three times from the room and soaked in water to maintain the proper moisture content of the rice koji. However, the traditional method needs a large space for aerobic solid-state fermentation, high labor costs for koji agitation by hands and water soaking, and a long process time. Fermentation is easily contaminated by the open environmental factors, which always results in inconsistent and unsatisfactory quality (Bau, 1996).

The traditional method has been improved by use of modem fermentation techniques and equipment to more precisely control temperature, pH, pressure and other fermentation parameters, which, inter alia, reduces the time of fermentation. The key feature of the improved red rice preparation is that it contains active ingredients that can prevent or treat hyperlipidemia and related cardiovascular diseases (Chiu et al., 2006a).

**Micro Flora In Angkak**

Microbiological studies of angkak were first conducted in 1884 by van Tieghem, a French microbiologist, and were categorized as the genus *Monascaceae* (Su, 2001). *Monascus* species is traditionally used for fermentation in East Asia. Its medicinal values and food applications were documented in ancient Chinese records. (Kohama et al., 1987; Wang et al., 2004). In 1885, Indonesian researcher Went named the fungus isolated from red yeast rice as *Monascus purpureus* (Ma et al., 2000). The filamentous fungus *Monascus* is an ascomycete traditionally used to produce fermented food. The fungi as a group exhibit a great variety of life cycles that may categorized into three major types: haploid, diploid, and dikaryon. The dikaryon is found in transient phase in the sac fungi (ascomycetes) (Elander et al., 1999). *M. purpureus* produces cleistothecia with oval ascopores and aleuroconidia. Many species with similar red fungal filamentous appearance and physiological characteristics were isolated and named after different
kinds of products since then. The most widely used red mold species in Taiwan was first named *Monascus anka* in 1931 by two Japanese, Misawa and Sato (Su, 2001). This finding led to the use of pure culture in commercial production of angkak.


However, modern day manufacture of angkak is done by fermenting rice or rice powder under solid state fermentation by with strains of *Monascus* such as *Monascus pilosus* IFO 4520, (Kohama et al., 1987) *Monascus purpureus* DSM1379 (Wild et al., 2002), *M. purpureus* NTU 601 (Wang et al., 2003) *M. ruber* CCRC 31538, *M. purpureus* CCRC
Production of Angkak by Solid-State Fermentation

Fermented red rice is produced traditionally by fermenting the washed and cooked rice with red wine mash, Polygonum grass juice, and alum water (Li et al., 1998). In contrast, the commercially prepared red yeast rice extract is fermented during 9 days with a specific strain of red yeast called Monascus purpureus Went at a temperature of 25 °C, and at a pH range of 5 to 6 (Ma et al., 2000). The rice is then air-dried, pulverised and encapsulated into gel capsules (Ma et al., 2000; Heber et al., 1999). This procedure is assumed to be the standard preparation method used in almost all animal and human trials reviewed (Li et al., 1998; Ma et al., 2000; Wang et al., 1997; Heber et al., 1999; Qin et al., 1999; Rippe et al., 1999; Keithley et al., 2002). The red yeast rice material produced in the traditional way has yielded different amounts of active compounds compared with the commercial preparation, due to the fact that the home process does not involve selecting a specific strain of yeast compared to the commercial process. As a result, the home-processed red yeast rice may not exhibit the same hypolipidemic effect as a commercial extract (Havel, 1999; Physicians’ Desk Reference, 2003).

Angkak production was carried out using Monascus ruber CCRC 31535 (ATCC 18199), Monascus cultured on potato dextrose agar (PDA) containing agar (1.5%), diced potatoes (30%) and glucose (2%) in order to induce spore formation. After cultivation at 30 °C for 7 d, colonies of spores that appeared on the plates were transferred and inoculated into 100 ml of potato dextrose broth (PDB), and incubated at 30 °C for 4 d with shaking at 150 rpm and finally inoculum was then transferred to rice substrate for production under solid state fermentation (Chang et al., 2002). Seed cultures of M. ruber CCRC 31538, M. purpureus CCRC 31497, 31498, 31499, 31501, 31504, 31530, 31540, 31542, 31615, 32966 and M. anka M-13 were prepared by transferring a loopful of spores from a PDA agar slant into a 500 ml Hinton flask containing 100 ml basal medium (100 g dextrose, 10 g peptone, 2 g KNO₃, 2 g NH₄H₂PO₄, 0.5 g MgSO₄·7H₂O, 0.1 g CaCl₂ in 1,000 ml
distilled water; pH adjusted to 6.0). Cultures were incubated at 30 °C for 48 h at 110 rpm and 5% Monascus inoculum was then transferred to rice substrate (solid-state fermentation medium, was prepared as follows: 500 g rice was soaked in distilled water for 8 h. Water was then removed using a sieve. The soaked rice was autoclaved for 20 min at 121°C in a "koji-dish" and cooled). The inoculated substrate was cultivated at 30°C for 14 days for production of angkak (Su et al., 2003). Zhang in the year 2003 described a procedure for large-scale production of angkak, in which rice (500 kg) was placed in several layers of baskets. The chaff was cleaned in water, and the rice soaked in water for 16-24 hours. The rice was dredged from the water and dried (the content of water was approximately 22-24%). Then dried rice was poured into a rice steamer and steamed for 50-70 minutes. The steamed rice was spread out on a bamboo mat or in baskets, dispersed, and cooled to a temperature below 40 °C. The rice was then inoculated with approximately 20 kg of solid Monascus strain and 2.5-3 kg of acetic acid and stirred. For the first 3 days, the rice was turned over several times per day. The temperature was controlled between 30 °C to 34 °C. After 3 days, the temperature was reduced to 24 ±1 °C. The rice was turned over once daily, during which water (pH value adjusted to 3.5 using acetic acid) was added at quantity depending on the humidity of the fermenting mixture. The mixture was fermented for over 15 days. After the fermentation process, the mixture was sterilized, dried and preserved (Zhang et al., 2003). Angkak production by using Monascus serorubescens for first time was reported by Chung et al. (2004). The Monascus serorubescens was initially cultured in a complete medium agar plate at 37 °C for 2 weeks until mycelium and red pigment developed. The composition of the complete medium agar prepared in 1 l of water included the following compounds: MgSO4 (0.5 g), KH2PO4 (0.46 g), K2HPO4 (1 g), peptone (2 g), dextrose (20 g), agar (15 g), yeast extract (2 g), and thiamin-HCl (0.5 mg). To produce a liquid fungal stock, two pieces of Monascus-infested agar (1 cm X 1 cm) were aseptically removed from the agar plate and transferred to a liquid medium. They were incubated at 37 °C for 2 weeks with continuous shaking. To prepare rice samples for the fungal inoculation, 300 g of rice was steamed with 800 ml of water and then cooled. The steamed rice (60 g) was transferred to 150 ml conical flasks, which were covered with aluminum foil and rice-containing flasks were autoclaved at 121 °C for 15 min. Then, 1 ml of Monascus seed
culture cultivated in liquid culture media was aseptically transferred to conical flasks (Chung et al., 2004).

Different substrates such as long-grain rice, sweet potato (*Ipomoea batatas*), potato (*Solanum tuberosum*), cassava (*Manihot tesculenta*) and dioscorea (*Dioscorea batatas*) are used for red mold metabolite production under solid-state cultivation. Five hundred grams of rice and the other substrates are, respectively, soaked in distilled water for 8 and 1 h. After that, excess water is removed with a sieve. The substrate is autoclaved for 20 min at 121 °C in a wood koji-dish (30×20×5 cm). After being cooled, the substrate is inoculated with a 5% (v/w) spore suspension (10^7 spores ml^-1) and 0.3% (v/w) ethanol. The inoculated substrate is cultivated at 30 °C for 10 days for angkak production and reported that changing the substrate affects the secondary metabolites production in term of concentration and quality (Lee et al., 2006b). A commercial Nagata type koji maker with a rotary perforated bed of 5-m diameter was modified for red mold rice production. *Monascus purpureus* BCRC 31499 was selected for its high production capacities of monacolin K and red pigment. The selected strain was first cultivated in a 120-l submerged type fermentor at 34 °C and 2 vvm aeration rate with 60 rpm agitation for 5 days using 20% liquefied rice porridge as carbon source. The high concentration red mold rice broth (>3.5 g/ml) was harvested for inocula and well mixed with cooked rice to an initial concentration of 2% v/w. The inoculated cooked rice then was directed into the Nagata type koji maker, and temperature of 37–38°C for 86 h and then temperature was reduced to 34 °C to direct red pigment production, fermentation was carried out for 7d and after that high quality angkak was harvested (Chiu et al., 2006a).

**Chemical Constituents of Angkak**

Red yeast rice consists mainly of rice and byproducts of the fermentation. The most abundant ingredient is starch shown as total sugar, accounting for more than 73% of the bulk. The crude protein content is approximately 15%, and other ingredients are found in lesser quantities. Of the trace elements, magnesium and sodium are the most abundant metal elements in the rice. The analysis of the metabolic byproducts of the fermentation was achieved by separating the components of the aqueous methanol extract of cholestin
(cholestin is a commercial preparation of red yeast rice) into various chemical classes. Further separation led to the isolation of individual components for further study. The various groups that were analyzed are polyketides, fatty acids, and pigments (Journoud and Jones, 2004). When untreated rice was analyzed, no pigments or polyketides were detected, leading to the conclusion that these compounds are indeed secondary metabolites of Monascus purpureus.

Major components found in red yeast rice by weight (%) are given in Table 2.1

<table>
<thead>
<tr>
<th>Components</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>73.4</td>
</tr>
<tr>
<td>Fiber</td>
<td>00.8</td>
</tr>
<tr>
<td>Protein</td>
<td>05.8</td>
</tr>
<tr>
<td>HMG-CoA reductase inhibitors (monacolins)</td>
<td>00.4</td>
</tr>
<tr>
<td>Fatty acids (mono unsaturated)</td>
<td>&lt;01.5</td>
</tr>
<tr>
<td>Fatty acids (saturated)</td>
<td>&lt;00.5</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>00.44</td>
</tr>
<tr>
<td>Trace elements (Na, Ca, Al, Fe, Mg, Mn, Cu, Ag)</td>
<td>Trace</td>
</tr>
</tbody>
</table>

Table: 2.1. Major components of angkak

Monascus purpureus, Monascus ruber, M. anka and other fungus belong to Monascus species produces monacolins (Kimura et al., 1990). Endo in the year 1979 first reported that Monascus ruber No.1005 produces monacolin K (an active methylated form of compactin) when grown aerobically at 28 °C in a medium containing 6% glucose, 2.5% peptone 0.5% corn steep liquor and 0.5% ammonium chloride for 10 days. Monacolin K functions as an inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A reductase, which is the regulatory and rate-limiting enzyme of cholesterol biosynthesis. Apart from monacolin K six other monacolins were isolated from methanol extracts of red yeast rice, such as dehydromonacolin K; methyl ester of monacolin K hydroxy-acid form; hydroxy acid form of monacolin K (lactone ring-opened form); monacolin L; methyl ester of monacolin L hydroxy acid form and dihydromonacolin K from angkak. (Ma et al., 2000). Two hypotensive compounds, acetylcholine and γ- amino butyric acid (GABA) from red
mold rice were prepared with cultures of *Monascus pilosus* IFO 4520 (Kohama *et al.*, 1987). GABA is produced by the decarboxylation of glutamic acid by a glutamate decarboxylase. In the process of making red mold rice, glutamic acid is produced from steamed rice by an acid protease and an acid carboxypeptidase that are secreted upon growth of koji mold (Narahara, 1994).

Antibacterial compound citrinin, monascidin A (Blanc *et al.*, 1994; Wong and Koehler, 1981) are isolated from angkak. Pigments of polyketide origin are respectively the orange pigments monascorubrin and rubropunctatin, the red pigments monascorubramine and rubropunctamine, as well as the yellow pigments ankaflavin and monascin. These pigments are linked to proteins, peptides and amino acids (Blanc *et al.*, 1994; Hajjaj *et al.*, 1999; Wang *et al.*, 2004). Pigments of *M. purpureus* are authorized for food in Japan (Blanc *et al.*, 1994). *M. purpureus* also reported for production of two enantiomeric azetidine-type amino acids, (+)-monascumic acid and (-)-monascumic acid (Su *et al.*, 2003) along with two furanoisophthalides xanthomonasin A and xanthomonasin B (Akihisa *et al.*, 2004; 2005) and metabolites like monascodilone, monascopyridin A and monascopyridin B (Wild *et al.*, 2002; 2003) and antioxidant compounds as dimerumic acid and 3-hydroxy-4-methoxy benzoic acid (Wang *et al.*, 2004). Several species of *Monascus* have also been used in making red wines and red soya cheese (Ma *et al.*, 2000). This mold is still used in food industries for processing of poultry, fish and meat products. Unsaturated fatty acids in red yeast rice extract are also believed to help possibly in lowering serum lipids especially triglycerides (Erdogrul and Azirak, 2004). However, a greater number of *Monascus* metabolites have not been characterized chemically.

**Mycotoxin of Monascus**

Citrinin \([\text{C}_{13}\text{H}_{14}\text{O}_{5}, \text{IUPAC (3R, 4S-trans)-4,6-dihydro-8-hydroxy-3, 4,5-trimethyl-6-oxo-3H-2-benzopyrane-7-carboxylic acid]}\) is a fungal metabolite that has been known since 1931, when it was isolated from *Penicillium citrinum*. Ten-year later it was characterized as an anti bacterial and anti fungal compound. It is acidic lemon-yellow needle with maximal absorption at 250 and 311 nm, a specific rotation at +217.1°, and a melting
point of 175 °C. Citrinin has nephrotoxic and hepatotoxic properties with LD$_{50}$ values in mice and in rabbits were 35 and 19 mg/kg, respectively. Citrinin was also implicated in porcine nephropathy and it has been found as a natural contaminant of corn, rice, wheat, rye, barley and decaying tomato fruit (Betina, 1984).

**Bioactivities of Angkak**

In China, *Monascus* has been widely used as a natural food-coloring agent for many kinds of foods. The metabolites of *Monascus* species, specifically, monacolin K, γ-aminobutyric acid, and dimerumic acid, have been proven to have cholesterol lowering, blood pressure lowering, and antioxidant effects. Currently, the public has recognized the importance of *Monascus* products for its many health benefits.

It is generally recognized for its health benefits because of the proven benefits of the secondary metabolites such as the monacolins (Budavari et al., 1989; Endo, 1980; Su et al., 2003), γ-aminobutyric acid (GABA), and dimerumic acid. One of the well-documented metabolites of *Monascus* is monacolin K, which has been identified for its cholesterol-lowering properties due to the competitive inhibitory affect on HMG-Co A reductase (Endo, 1980). In addition, the blood pressure-lowering effects of GABA (Kohama, et al., 1987; Kushiro et al., 1996) and the antioxidant effects of dimerumic acid (Aniya et al., 1999) are also well known. Although *Monascus* is capable of producing these bioactive compounds, there is a possibility of synthesizing citrinin, a hepatonephrotoxic mycotoxin, during fermentation (Blanc et al., 1995b).

*Monascus* species have been proven to produce many functional secondary metabolites. These pigments (yellow pigment: ankaflavin and monascin; orange pigment: monascorubrin and rubropunctatin; red pigment: monascorubramine and rubropuctamine) were investigated and applied to the food colorant in early study (Wong and Koehler, 1981). In present day study, *Monascus*-fermented product was gradually regarded as the functional food because the monacolin K (antihypercholesterolemic agents), γ-aminobutyric acid (GABA) (hypotensive agent), and dimerumic acid (antioxidant compound) (Su et al., 2003; Aniya et al., 1999). A more active methylated
form of compactin, known as monacolin K, would be formed in the broths of *Monascus ruber* (Endo, 1979). Monacolin K, known as a statin compound, has been regarded as a cholesterol-lowering agent because it was proven to be a potent competitive inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-Co A reductase) (Albert *et al.*, 1980). *Monascus*-fermented rice has been demonstrated to perform significant hypolipidemic effects in hyperlipidemia hamster model (Lee *et al.*, 2006a). Therefore, it was proven to have special effect when used as a cholesterol-lowering drug.

Apart from cholesterol-lowering properties, blood pressure lowering and the antioxidant effects of angkak, monasecin possessed mild antibiotic activity against *Bacillus subtilis* and *Candida pseudotropicalis*, and possess immunosuppressive activity (Martinkova *et al.*, 1995). Angkak significantly decreased high-sensitivity C-reactive protein (hs-CRP) levels in addition to its lipid-lowering effects. Furthermore, angkak significantly improved flow-mediated vasodilation (FMD) during preprandial and postprandial states and protected endothelial function through its potent systemic anti-inflammatory. Lipid-lowering effects, moreover, lowering postprandial hypertriglyceridemia may lead to a reduction in coronary heart diseases (CHD) risk. (Zhao *et al.*, 2004). Ankaflavin from *Monascus* metabolites possessed cytotoxicity to human cancer cell lines HepG2 and A549. However, monasecin, an analogue of ankaflavin, showed no cytotoxicity (Su *et al.*, 2005). Moreover *Monascus* extract showed strong anti-oxidation capacity to inhibit lipid peroxidation and enhanced expression of the tumor suppressor gene p53 (Chiu, 2006b).

The secondary metabolites of *Monascus* species, the biomass growth and the types and production of metabolites would be directly or indirectly affected by the environment and cultivation methods. As far as the cultivation methods were concerned, solid state cultivation resulted in higher pigment yield than cultivation in shaken culture and concluded that this phenomenon was due to the fact that pigments are released into grains under solid state culture, and the pigments were accumulated in the mycelium under submerged cultivation (Lin, 1973). Under solid-state cultivation, production conditions of lovastatin by *Monascus ruber* were different from those of other pigment formation. The report also pointed out the accumulated depth of substrate and the water content would
affect lovastatin production, and the lovastatin production quantity from solid-state cultivation was 20 times more than that of liquid state cultivation (Wang et al., 1999). Furthermore, different fermentation methods would affect monacolin K and GABA production (Su et al., 2003; Wang et al., 2003).

Carbon and nitrogen sources are nutritional sources required for microbial growth. Generally, glucose is considered as the best carbon source for pigment formation (Broder and Koehler, 1980; Lin and Demain, 1991), but various strains also lead to different results. The difference of pigment formations when glucose and ethanol are used as carbon sources, and found out that ethanol has better pigment formation ability when it is used as carbon source (Santerre et al., 1995). Moreover, others like starch, maltose, sucrose, and galactose (Lin, 1973; Panitz et al., 1991; Yoshimura et al., 1975) are also good carbon sources. The effect of different nitrogen sources on Monascus species growth, by adding yeast extract to broth medium are helpful for the biomass production, but they have adverse effect on pigment formation (Carel and Shepherd, 1977). In addition, ammonium chloride, sodium nitrate, peptone, and monosodium glutamate have different effects on metabolite formation (Blanc et al., 1995b; Lin and Demain, 1991; Su et al., 2003). As far as substrate was concerned, Lin and Lizuka (1982) compared the effect of different substrates on pigment production and found that steamed bread was a good substrate. Besides, rice, bread, oat (Rashbaum and Barrington, 1983), corn, or wheat grain (Hesseltine, 1965; Lin and Lizuka, 1982), all can be used as the substrate for Monascus species to produce large amount of pigment. Previous study on red mold rice production by Monascus species under monoculture conditions shows that secondary metabolites production is greatly effected by fermentation medium, cultivation conditions, and types of Monascus species used in fermentation process (Su et al., 2003; Wang et al., 2004; Chiu et al., 2006a; Miyake et al., 2005; 2006; Babitha et al., 2007). Increasing concentration of fungal secondary metabolites in angkak is a prime area of research in order to develop it into high quality functional food by newer fermentation methods using optimized medium parameters, fermentation parameters and development of mutated Monascus species.
Angkak has empirically recognized as safe in Asia for centuries (Hu, 1997). However, during the past 10 years, some researchers have discovered and demonstrated that few strains of Monascus could produce citrinin, a nephrotoxin, which was previously found mainly in Aspergillus and Penicillium genera (Deruiter et al., 1992), and might contaminate angkak (Blanc et al., 1995b; Hajjaj et al., 1999; Sabater-Vilar et al., 1999; Heber et al., 2001). This triggered a controversy about the safety of angkak. Although recent research has confirmed that angkak implies no threat to health at all (Hu, 1997; Allok, 2004). Most researchers consider that some actions should be taken to control citrinin concentration in angkak (Mandt, 1998; Hu and Chen, 2003). It was reported that medium chain fatty acid when incorporated into fermentation medium, reduces the citrinin production by Monascus ruber (Hajjaj et al., 2000) In Japan, the maximum allowed level of citrinin in angkak is authorized to be 200 ng/g (The Ministry of Health and Welfare of Japan, 2000). In China and the European Economic Community, the similar citrinin level in angkak is still under debate (Mandt, 1998). So, to avoid citrinin-contaminating RFR, or to keep citrinin concentration low, screening some strains of Monascus with non-producing/low-producing citrinin is very important.

Therefore, the objective of this research is to seek for the substrate or fermentation medium that could result in higher quantity of monacolin K (lovastatin) production and lower citrinin level in angkak, through novel solid-state fermentation process.
REFERENCES


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