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Contributed Paper

Review of Angkak Production (Monascus purpureus)

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ABSTRACT

Angkak has been used for a long time as food colorant and blood circulation treatment agent. There are many of their metabolites such as pigment, mevinolin, citrinin, vitamin and enzyme which are produced during fermentation. Controlling or selecting the factors of fermentation is the important objective to produce the good quality angkak. This paper reviews the process of angkak production for safe consumption including its microbial producing strains, history, traditional uses, pigments, blood cholesterol reduction and toxicity effect.

Keywords: Angkak, *Monascus* spp., metabolites, fermentation condition.

1. INTRODUCTION

Angkak or red yeast rice is a product resulting from rice fermentation using *Monascus* spp. It has been used extensively in Asia as a natural food colorant in fish, Chinese cheese, red wine and sausages [1-4]. *M. purpureus* red yeast rice is an effective natural dietary supplement for controlling serum cholesterol. *Monascus* fungi, organisms produce angkak can convert starchy substrates into several metabolites such as alcohols, antibiotic agents, antihypertensives, enzymes, fatty acids, flavor compounds, flocculants, ketones, organic acids, pigments and vitamins [8]. Thus, the implementation of *Monascus* pigment as a coloring agent in food provided an additional advantage of specific flavor in the products. This product is supported by long-standing traditional use by millions of people [5]. The applications of some synthetic colorants in food such as azorubin or tartrazin have been limited due to their possible allergeric effects [6]. It is possible to use angkak as food colorant in order to avoid allergic problem. However, some researchers demonstrated that *Monascus* toxin, known as citrinin, could contaminate angkak during the production process. Therefore, many factors in *Monascus* production must be considered to ensure that angkak production could be carried out safely while maintaining its functional characteristics.

2. HISTORY AND TRADITIONAL USES

In ancient time, angkak production was originated in China and kept as a secret. It had been used for Chinese cheese preparation and Chinese beverage known as *anchu*. Later, one report suggested that angkak was used in the Philippines for coloring of *bagoong, atsike*-salted fish, and in the preparation of alcoholic

beverages such as *anchu* and *somsu*. It was believed that angkak in the Philippines came from China by the way through the internation port of the Philippines since the long distance past and not manufactured locally. The reason supported this that it had trademark related with the mainland of China. In addition, angkak in the form of cake or ground red powder was exported from China to Eastern Asia including Thailand. At present, several countries produce angkak both for internal use and export as food additive and dietary supplement [7].

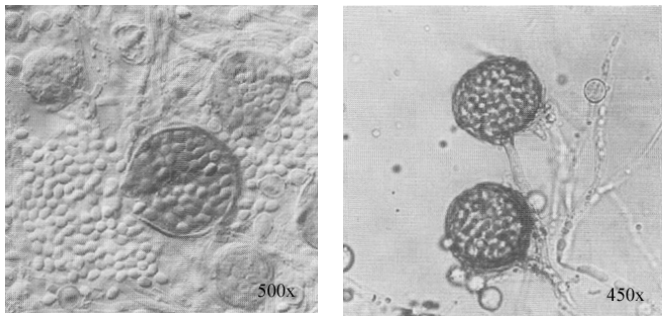
Angkak also has a pharmaceutical characteristic. The ancient Chinese pharmacopoeia, *Ben Cao Gang Mu-Dan Shu Bin Yi*, published during the Ming Dynasty (A.D. 1368-1644), described medicinal function of angkak for the treatment of indigestion and diarrhea, anthrax, bruised muscles, hangovers, colic dyspepsia in children and post-partum problems. Besides, it has been used for improving blood circulation and for promoting the function of the spleen and stomach. Moreover, several books including *Materia Medica for Daily Use*, *Supplements on Developments of Herb Medicine*, and *Compendium of Material Medica* also described the utilization of this pigment as a coloring agent and medicine in the treatment of various diseases [5].

Today, angkak is still used as traditional medicine and food colorant in Asia and in Chinese communities in North America. Considerable interest has been devoted to the application of angkak as a nitrite/nitrate substitute for the preservation of meats [9]. The synergistic effect of *Monascus* spp. pigment in lowering blood cholesterol could be observed in the consumption of high quality sausages which, in turn, enhancing human health [3].

3. THE MORPHOLOGICAL DATA

Monascus spp. belongs to the group of Ascomycetes and particularly to the family of Monascaceae. The genus *Monascus* can be divided into four species: *M. pilosus, M. purpureus, M. ruber* and *M. froridanus*, which account for the majority of strains isolated from traditional oriental food [10]. The common names of this fungal product are red yeast rice, red rice, angkak, red leaven, beni-koji (Japanese), hung-chu, hong qu, zhitai (Chinese), rotschimmelreis (Europe), red mould (USA). *M. purpureus* can be easily distinguished by its ascospores which appeared to be spherical in shape of 5 microns in diameter or slightly ovoid (6 x 5 microns) (Figure 1). The mycelium is white in the early stage. However, it rapidly changes to a rich pink and subsequently to a distinctive yellow-orange color. The production of yellow-orange hyphae reflects the increased acidity of the medium. A deep crimson color is formed as the culture ages [5]. Most of products could be used in powder form or pigment extracts for developing the color of products.

It is difficult to determine the growth of *Monascus* by counting mycelia using electron microscope. An easier method could be performed using glucosamine analysis. This compound is a monomer of chitin, which is the main component in fungal cell wall [11]. The growth of *Monascus* fungi is a key indicator in the synthesis of pigments and other metabolites. Yongsmith [8] explained that during the first stage of fermentation period, the fungi utilizes carbon and nitrogen source from substrate for its primary metabolites, bioconversion, energy, carbondioxide and water. On the last stage, fungi use the product produced on the first stage for producing secondary metabolites. Therefore, secondary metabolites such as pigment, citrinin and mevinolin can be detected after the first stage of fungal growth ended.

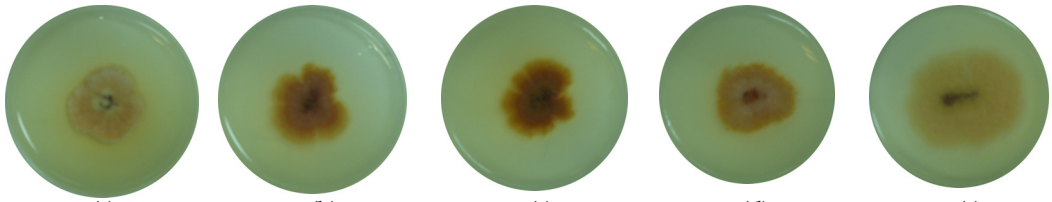


(a) (b)

Figure 1. Pedicellate ascomata with ascospores of *M. purpureus* Went, (a) Ascomata wall break down, (b) Ascomata with ascospores [12].

4. FERMENTATION CONDITIONS OF *MONASCUS* SPP.

Monascus spp. can be cultivated on potato-dextrose agar, Sabouraud's agar or Czapek's solution agar for 10 days at 29 to 32°C. The cultures grow rapidly and spread on the media. The diameter, color shade and area-texture of the mycelia are all depended on cultural media, strains of fungi and cultivation conditions (Figure 2) [7].



(a) (b) (c) (d) (e)

Figure 2. *Monascus* spp. cultivated on potato-dextrose agar for 10 days at 30°C, (a) *M. purpureus* ATCC 16365, (b) *M. purpureus* BCC 6131, (c) *M. purpureus* DMKU, (d) *M. purpureus* FTCMU, (e) *M. ruber* TISTR 3006 [13].

The procedure for preparing angkak on a laboratory scale is carried out by placing 50 g of polished rice in 8 x 12 square inches autoclavable plastic bag with 50 ml water. The plastic bag opening was inserted through a two inches long and one inch diameter autoclavable plastic tube followed by being manipulated as if an Erlenmeyer flask's neck in which it was later plugged with cotton wool. The bags were then autoclaved at 121° C for 15 min prior to cooling down at room temperature. Each bag was inoculated with 1 cm diameter of the agar-cultivated culture. The culture was incubated for 14 days at room temperature. After incubation, the fermented rice was removed and dried at 55°C for 3 days, then ground into powder and used in coloring of various foods [3].

For centuries rice have been used as substrate for making angkak. All varieties of rice are suitable except the glutinous rice such as Malagkit Sungsung, which is considered unsatisfactory because of the glue texture and the agglomeration of rice grains [7]. Up to now, several cereal substrates such as oat, wheat, barley and corn had been studied on their possible uses as alternative substrates for *Monascus* spp. cultivation. Each cereal had different influence on angkak production due to the variation in its composition [8].

Corn was used as a substrate for angkak production because it is cheaper than rice and was not consumed as a main dish in China. Palo *et al.* [7] illustrated that corn, as well as rice, may be used to grow *Monascus* spp. to provide the red color. Ganrong *et al.* [14] reported the method of preparing corn for angkak production. Firstly, the corn was cracked and the outer skin was removed. This step was carried out because the mycelium of *M. anka* could not effectively penetrate the outer skin. After that, the corn kernels were steeped in water or diluted acetic acid solution for a period of 4 days at room temperature.

The soaked corn kernels of 15 g were filled in a 250 ml Erlenmeyer flask with the cotton-plugged before sterilizing at 121°C for 30 min in an autoclave. The sterilized corn kernels were allowed to cool down until its temperature dropped to 32°C prior to inoculation of 2 ml *M. anka* inoculum. The inoculated medium was incubated at the same temperture for 7-10 days, after which the fermented corn kernels were dried and ground.

Adlay is a new substrate for *M. purpureus*. According to the ancient Chinese medical book *Pen-Tsao-Kang-Mu* [15, 16], the seed of adlay was used in China for the treatment of warts, chapped skin, rheumatism and neuralgia and as an anti-inflammatory or antihelmintic agent. The dual benefits to human health may thus be attained because both *Monascus* spp. culture and adlay substrate are effective functional foods.

The preparation of *Monascus* adlay began by inoculation of the fungi on the malt extract agar and incubated at 25°C for 72 h. The pure culture was then re-inoculated into potato dextrose broth and incubated further at 25° C for one week. The collected culture was subsequently homogenized in a blender and added to the sterilized adlay and incubated again in the same conditions and duration as previously described. The mycelia was air-dried at 40°C before being ground in a mill and sieved at 20 mesh to obtain a coarse powder.

Several cereals may be used as substrates for angkak production but the high quality product can only be produced under suitable conditions. Moreover, the addition of carbon/nitrogen source can influence the production of *Monascus* metabolites such as pigment, mevinolin, citrinin and amylase enzyme [17 – 21]. Pattanagul [13] investigated the optimum level of carbon and nitrogen sources for supplemented in adlay angkak. It was found that the optimum level of lactose and yeast extract, which should be added in adlay as substrates for producing the highest level mevinolin, was 1.00% and 0.50%, respectively. This concentration could forecast the highest mevinolin content in adlay angkak of 47.40 ppm. Moreover, the addition of 1-5% glucose and 0.1-0.5% peptone in adlay affected on a value, orange and red pigments of adlay angkak cultivated with *Monascus ruber* TISTR3006 while 1-5% lactose and 0.1-0.5% yeast extract affected on mevinolin content, L and a values.

5. THE PIGMENT OF *M. PURPUREUS*

During growth, *Monascus* spp. breaks down starch substrate into several metabolites. The structures of pigments as secondary

metabolites depend on substrate types and other specific factors during cultivation such as pH, temperature, and moisture content.

Carbon (glucose, maltose, ethanol) and nitrogen sources (peptone and ammonium nitrate) may be used to induce pigment production in *M. purpureus* [17, 18, 21, 22].

Monascus fungi produce at least six major related pigments which can be categorized into 3 groups based on color as follows (Figure 3) [23];

(1) yellow pigments: monascin ($C_{21}H_{26}O_5$) and ankaflavin ($C_{23}H_{30}O_5$)
(2) orange pigments: monascorubrin ($C_{23}H_{26}O_5$) and rubropunctatin ($C_{21}H_{22}O_5$)
(3) red pigments: monascorubramine ($C_{23}H_{27}NO_4$) and rubropuntamine ($C_{21}H_{23}NO_4$)

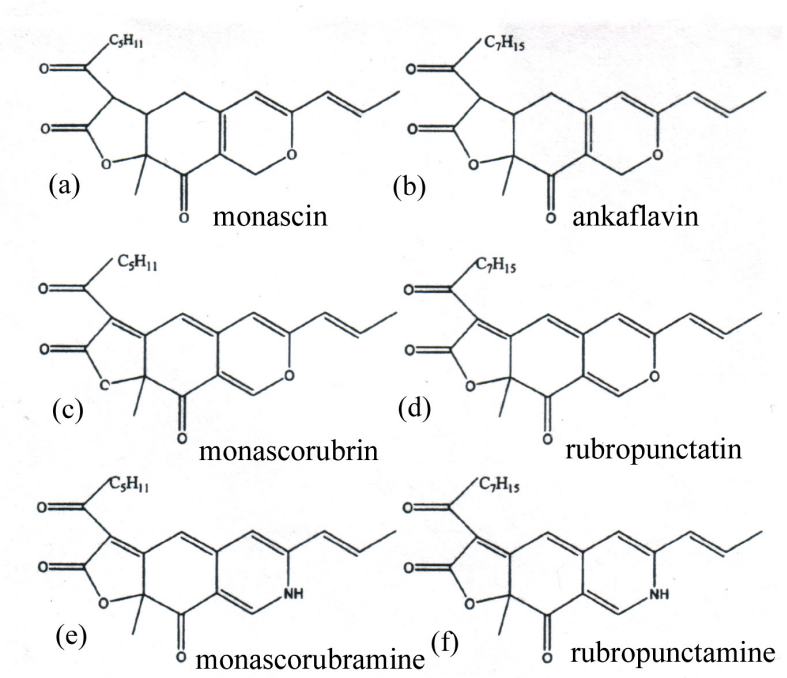


Figure 3. Chemical structure of *Monascus* pigments, (a) yellow pigments, (b) orange pigments, (c) red pigments [24].

The yellow, orange and red pigments of *Monascus* spp. can be detected by a spectrophotometer at 400, 470, 500 nm, respectively [22].

Nowadays, *Monascus* spp. pigments are used increasingly in meat product to replace nitrate or nitrite and improve quality of product. Pattanagul [3] applied angkak as a red pigment to enhance color of meat sausages. The optimum level of angkak was 1.60%(w/w). Shehata *et al.* [25] studied natural colorants used in Egyptian fresh beef sausage. The consumers preferred sausages with addition of both *Monascus* spp. pigment and nitrite which was added to improve color stability.

6. EFFECTS ON CHOLESTEROL AND LIPID METABOLISM

Hypercholesterolemia is a well-known risk factor for coronary artery disease, cerebrovascular disease and peripheral artery

disease. Moreover, reduced plasma cholesterol level coincides with a reduced incidence of cardiovascular complication (myocardial infarction, stroke, peripheral obstructive arterial disease). The treatment of hypercholesterolemia with a specific drug is costly, while in primary prevention life-style change and dietary habits such as reducing dietary saturated fatty acids, cholesterol, and excess body weight, appear to be more cost-effective than any pharmacological treatment. The inclusion criteria were the following: estimated 10-years cardiovascular disease risk <20%, moderate hypercholesterolemia (TC <300 mg/dL), normal triglyceridemia (TG <250 mg/dL), normal HDL cholesterol (>40 mg/dL) [26].

Several *Monascus* metabolites have been subjected to investigation in order to confirm its pharmacological effects. Monacolin K, called mevinolin, is the only metabolite which is able to decrease blood cholesterol. It acts by competitively inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG Co-A) which catalyzes the rate limiting step of cholesterol biosynthesis [27]. Monacolin K has two different structures: beta-hydroxy acid and lactone forms. The ratio of the acid form to the lactone form varies depending on the *Monascus* strains being used, pH, culture media, temperature and initial moisture content. The higher ratio of the acid form was regarded as a higher quality product [14]. Furthermore, Monacolin J, L, X, M and their derivative forms were found from *M. purpureus*-fermented rice. Each of these monacolins was found to be a potent hypocholesterolemic agent [28].

Heber *et al.* [29] reported that angkak significantly decreased total cholesterol (TC), low-density lipoprotein cholesterol (LDL) and total triacylglycerol (TG) concentrations in human blood in comparison with the placebo. They evaluated the lipid-lowering effects of angkak dietary supplement in US adults. Eighty-three healthy subjects with hyperlipidemia who were not being treated with lipid-lowering drugs participated in the experiments. Subjects were treated with angkak (2.4 g/day) or placebo and instructed to consume a diet providing 30% of energy from fat with saturated fat of less than 10% and cholesterol (<300 mg). The TC, TG, high-density lipoprotein cholesterol (HDL) and LDL cholesterol were measured at weeks 8, 9, 11 and 12. TC concentration decreased significantly between the baseline and 8 weeks in the angkak treated group compared with the placebo-treated group. LDL cholesterol and total triacylglycerol were also dropped with the supplement while the level of HDL cholesterol was not changed significantly. Therefore, angkak provided a novel approach of lowering cholesterol in the general population by applying it in food.

Angkak also affected blood lipids and lipoprotein concentrations in rabbits whose diet was 25% casein to induce endogenous hypercholesterolemia. Within 60 days, its serum cholesterol concentration increased from approximately 1.81 to 7.51 mmol/L. Treatment with angkak for 30 days at dosages of 0.4 and 0.8 g/kg/day had significantly lowered serum TC concentration and TC:HDL-c ratio. Moreover, hypercholesterolemia in rabbits could also be induced exogenously with a diet consisting of 0.5% cholesterol, 15% yolk powder and 5% lard. Rabbits were fed angkak at doses of 0.8 g/kg/day for 40 days which prevented the increase of serum TC, TG concentration and TC:HDL-c ratio. In the hyperlipidemia induced quail with 1% cholesterol, 14% lard, 6% soya-bean oil, angkak was fed orally at doses of 0.1, 0.2 and 0.4 g/kg/day for 2 weeks. It was found that angkak largely prevented the increase of serum TC and TG.

These studies demonstrated that angkak could be used in the reduction of serum TC and TG in rabbits and quail [30].

Xuezhikang Jiaonang, is angkak in the capsule form for the treatment of serum TC, TG and LDL cholesterol with the exception for HDL. This drug can be used in the treatment of hyperlipidemia and cardio-cerebro-vascular diseases caused by high blood cholesterol. Furthermore, there are several commercialised names dedicated to blood cholesterol medicines containing mevinolin such as Mevacor, Cholestin, Lovastatin, Zocor, Lipiton, Mevalotin. These are commonly sold in drugstores in China, Japan, United States, Indonesia, Taiwan and the Philippines [31]. It has relatively mild side effects such as heartburn, wind and dizziness, which may occur, in some groups of patient taking drug. Therefore, patients should consult a physician before taking angkak to lower their blood cholesterol. It is recommended to take 5-10 mg monacolins per day in a divided dosage for 12 weeks [29, 31].

7. TOXICOLOGY

Angkak production may be contaminated by citrinin, a potent mycotoxin formerly known as monascidin A which could damage kidney and liver. Blanc *et al.* [1] isolated and identified monascidin A from various species of *Monascus* spp. Mass spectroscopic analysis indicated that its structure was identical to citrinin. The antibacterial effects of *Monascus* had been confirmed by Wong and Koehler [32]. Monascidin A, isolated from *M. purpureus* cultures, was able to inhibit *Bacillus* spp., *Streptococcus* spp. and *Pseudomonas* spp. [33].

Citrinin could induce a mutagenic response in the *Salmonella*-hepatocyte-assay applying strain TA-98. However, no mutagenicity could be detected in the *Salmonella*-microsome assay. These findings provide further evidence that citrinin requires complex cellular biotransformation to exert mutagenicity [10]. However, *Monascus* extracts and angkak were used as food colorants for centuries without the known case of adverse effects due to the low concentrations applied in food. In all commercial *Monascus* samples, the citrinin level of 0.2 to 1.71 ug/g were detected [10].

Although recent research confirmed that angkak did not pose any adverse health effect, most researchers supported the idea that some actions should be taken to control citrinin concentration in angkak. In Japan, the maximum citrinin in angkak must not exceed 200 ng/g. In China and the European Economic community, the maximum allowable citrinin in angkak is still under debate [34]. Therefore, the investigation should focus on the conditions of angkak production that yields no citrinin or possible lowest concentration before using angkak as food additives or dietary supplement.

8. THE PRODUCTION OF ANGKAK FOR SAFE CONSUMPTION

Due to popularity of angkak, it is crucial to develop a safe process for angkak production. The level of citrinin being produced depended on several factors during fermentation process such as carbon/nitrogen source, *Monascus* spp. strains, amino acids, trace elements, water activity and temperature [1, 32, 35-37]. In addition, these factors also influenced on mevinolin and enzyme production [17, 19, 22, 38, 39]. Therefore, production of angkak with low level of citrinin, high level of mevinolin and pigments by using the optimum fermentation condition is the main aim to up-grade the quality of angkak into commercial market.

Moreover, mutation of *Monascus* strain was the way to reduce the quantity of its mycotoxin and encourage the production of mevinolin and pigments. Chen and Hu [34]

developed a mutant strain, *Monascus* spp. M 12-69, fungal spores of wild strain were treated with mutagenic agent (dimethyl sulphate), UV irradiation and ^{60}Co gamma irradiation, respectively. This mutant strain could be used to produce angkak with high concentration of mevinolin (2.52 mg/g) and low concentration of citrinin (0.13 ng/g). Yongsmith *et al.* [40] developed the mutagenesis of *Monascus* spp. KB9 strain by using UV light. Three mutant strains were obtained from wild type of *Monascus* spp. KB9 were red, yellow and white mutants. Red and yellow mutants favoured the highest initial rice content at 43% for glucoamylase activity and accumulated high glucose with some ethanol that could inhibit their pigmentation to some extent, while wild type strain favoured initial rice moisture content at 38% for its glucoamylase as well as pigmentation. Unlike these three strains, white mutant could not synthesize any pigment, however, it synthesized glucoamylase.

Natural factors may also contribute to the safety of angkak. Adlay could induce a low citrinin concentration in angkak. Yang *et al.* [16] reported that citrinin concentration of less than 1 ppm was achieved in monascal adlay substrate. The presence of 2%(w/v) lactose with 0.1-0.4%(w/v) soybean meal or yeast extract could improve high mevinolin production in angkak [19]. Pattanagul [13] screened the strains of *Monascus* which produced low citrinin and high mevinolin contents on adlay angkak. It was found that *Monascus purpureus* DMKU was a strain, which produced the highest level of mevinolin (25.03 ppm). However, this strain produced the level of citrinin as low as the other strains (ATCC16365, BCC6131 and FTCMU) while *Monascus ruber* produced the highest level of citrinin (14.64 ppm).

When a new angkak is safe for consumption with the lowering blood cholesterol property, the next step is to improve the quantity of *Monascus* pigments by using ethanol, maltose, glucose about 1-10%(w/v) as a supplement carbon sources and adding either peptone or ammonium nitrate about 0.1-0.5% as an additional nitrogen source [17, 18, 21, 22].

9. CONCLUSION

Fungi from the genus *Monascus* are a promising source for natural color additive and reducing blood cholesterol. However, before effectively applying *Monascus* to foods or dietary supplement, it is important to select and control the fermentation condition to obtain large amounts of required substances such as pigment or mevinolin but with little or no citrinin. Strains of *Monascus*, types of substrate, fermentation temperature, pH and moisture content are important factors to indicate different production of their metabolites in angkak. A suitable condition for angkak fermentation is critical for manufacture success of this product.

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