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MiniReview

Recent research and development of *Monascus* fermentation products

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Abstract

Monascus fermentation products (MFPs) have been widely used by people mainly in Asian countries for many centuries as food colorant, preservative, food supplement and traditional medicine. With the cultivation of different strains of *Monascus* and fermentation in various substrates, MFPs are now widely available and are becoming popular worldwide. This review mainly introduces recent advances in research, technological development, production and application of *Monascus*. In recent years, the *Monascus* industry have progressed from the traditional, labored intensive and time consuming approach to more efficient production of *Monascus* products that allows the industry to overcome problems of space, scale-up and process control. Many studies on the molecular biology of *Monascus* are progressing gradually, providing us with more information and knowledge to enable us to explore the *Monascus* for designing novel functional foods as well as industrial applications. The production of natural *Monascus* dyes provides a platform for further improvement or related products, among which *Monascus* red pigments is the most promising product while *Monascus* yellow pigment has also gained a rapid development recently. To find a functional MFPs and an economic alternative to produce MFPs, research groups, mainly in Asia, develop non-rice MFPs i.e. *Monascus*-fermented soybean (MFS), *Monascus*-fermented adlay (MFA), *Monascus*-fermented dioscorea (MFD) and other new MFPs such as *Monascus*-fermented garlic, *Monascus*-fermented ginseng and *Monascus*-fermented durian seed. The physiological active components of functional *Monascus* products such as lovastatin and polysaccharides have become an important part of the *Monascus* industry. The "esterifying *Monascus* products" has been applied as a new biocatalyst which can be widely used in the Chinese spirits and soy sauce brewing. As with any industry, the safety and regulations on MFPs are also important to ensure its applications in foods and medicine are well accepted by the public.

Introduction

Monascus-fermented products (MFPs) are products of fermentation process by *Monascus* sp. through solid state fermentation or submerged fermentation methods. The *Monascus* species which are usually used for the fermentation are *Monascus purpureus*, *Monascus pilosus*, *Monascus anka* and *Monascus ruber*. The first MFPs which have been consumed over the centuries in Asian countries are *Monascus*-fermented rice (MFR), so called angkak, anka, beni koji, and red yeast rice. It has been used traditionally as food colorant and preservative, food supplement and in traditional medicine. During fermentation, *Monascus* sp. produces at least 6 pigments which were categorized into 3 colors i.e. yellow, orange and red (Blanc *et al*., 1994). On food colorant and preservative, MFR has been applied on food products such as Chinese cheese, bagoong, wine, tofu, sake, miso, pork, sausage and fish. On health care and medication, people consume MFR to improve blood circulation system and to reduce blood cholesterol level. Recently, *Monascus* products have been used for the treatment of dengue virus infection (Suharto and Triyono, 2012). Endo (1980) found a *Monascus* metabolite, monacolin K, which has a positive health promotion through inhibition of *3-hydroxy-3-methylglutaryl-coenzyme A* (HMG CoA) reductase, a key enzyme of cholesterol synthesis in human body. Kono and Himeno (2000) reported that γ-amino butyric acid (GABA) on MFR play an important role in reducing the blood pressure. Elsewhere Blanc *et a*l. (1995) showed that *Monascus* sp. produces metabolites which not only have

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health positive promotion, but also toxic for human body. Monascidin A was characterized as citrinin, a mycotoxin. In the last few decades, research worldwide have turn their focus on the health positive promotion (anticancer, antiobesity, antidiabetes, stimulating bone formation, etc) and the safety of MFR. MFPs has also been applied for functional food ingredient to produce beverage and bread (Kim *et al*., 2008; Tseng *et al*., 2011). Currently, more than 50 patents concerning the use of *Monascus* pigments for food have been issued in Japan, the United States, France and Germany (Hajjaj *et al.*, 2012).

This review mainly introduces recent advances in the research, technological development, production and application of *Monascus.* In recent years, there are great developments in *Monascus* industry from many aspects. The first aspect is *Monascus* strain research and development. The studies of taxonomy and molecular biology of *Monascus* are progressing gradually, which can give a basic understanding on the *Monascus* research. The second aspect is *Monascus*-fermented products research and development including *Monascus* pigments, functional MFPs and new other MFPs. The third aspect is safety of MFPs, which review in last section i.e. regulations on MFPs in Asia, United States and European Union.

Monascus strain research and development

Taxonomic study of Monascus spp.

As a result of historical reason and the disordered use of *Monascus* strains during research and production, the classification names of *Monascus* are still confused in current research and literatures. Although *Monascus* was first discovered and used in China, the first classification of *Monascus* strains was performed in other countries. There are multiple systems in the classification of *Monascus* around the world. In China, Zhong-Qing Li from The Chinese Academy of Sciences has long been committed to the basic research of *Monascus* (mainly focus on the collection, identification and classification of *Monascus*). In recent years, Li (2009) listed a distinct retrieval table on 12 *Monascus* species in China (Table 1), after a rearrangement of the *Monascus* classification systems and combination with the latest research results. Among these 12 *Monascus* species, 4 species (*M. albidus*, *M. rutilus*, *M. aurantiacus* and *M. fumeus*) were identified as new based on their rDNA sequences in the inherent ribosomal internal transcribed spacer (ITS).

Research progress in molecular biology of Monascus

There are increasing researches on the molecular biology of *Monascus* in recent years around the world. For example, Shimizu (Osaka University, Japan) has isolated citrinin synthase gene (pksCT, AB167465) from *M. purpureus.* Taiwan has also launched whole genome sequence on *Monascus.* There are some related publications in recent years, mainly focused on certain aspects of molecular biology of *Monascus* metabolites such as pigments (Shao *et al.*, 2006; Chatterjee *et al*., 2009), citrinin and Monacolin K (Wang, 2011).

Table 1. Classification of *Monascus* species in China

Name	CAS* no.
M. eremophilus	-
M. albidus	AS3.568
M. pallens	AS3.5844
M. ruber	AS3.549
M.rutilus	AS3.2636
M. aurantiacus	AS3.4384
M. pilosus	AS3.4646
M. purpureus	AS3.4629
M. sanguineus	AS3.5845
M. lunisporas	-
M. floridanus	AS3.5843
M. fumeus	AS3.2093

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Shao *et al.* (2006) reported the construction of T-DNA insertional library of *Monascus ruber* mediated by *Agrobacterium tumefaciens*. 50 of 5132 transformants screened were found to have inserts for the genes for color-production by PCR amplification and the result showed that the T-DNA were integrated into the genome. 94% of the mutants screened were fully stable after five successive culture and showed resistance to hygromycin B. The abilities to produce pigment and citrinin were changed greatly which monitored by UV-VIS spectrophotometry and HPLC analysis. The method of T-DNA transformation mediated by *A. tumefaciens* should be very useful for further studies on the metabolites of *Monascus* spp. and the function of gene of interest. In a similar study by Ding *et al.* (2006), 200 citrinin-producing mutants were screened with the inhibition zone method from the 5,000 transformants library of *Monascus ruber* M-7 by *Agrobacterium*-mediated DNA transfer. When analysed using HPLC, 53 of the mutants have the capabilities of producing citrinin ranging from 0.04 μg/g to 154.57 μg/g in the *Monascus* fermented rice. Color values (color value is a concentration unit for the pigment content) of red fermented rice prepared by these mutants were also detected. The results showed that there was a positive correlation between the citrinin content and the color value among the mutants. These results provide materials and research bases for further study on the relationship between the production of citrinin and pigment of *Monascus ruber* at molecular level. In the study of Lai *et al.* (2005), two cDNA subtractive libraries of *Monascus* were constructed by suppression subtractive hybridization (SSH) and screened for correlative genes from the citrinin biosynthetic pathway in *Monascus* species. All clones in the two libraries were sequenced and the results were analyzed by bioinformatic softwares. There were 8 homologous ESTs in both libraries. Comparing with other ESTs by BLAST, these ESTs were considered as novel, and are now deposited into the Genbank. A further study by Fu *et al.* (2008) using fourteen citrinin-producing *Monascus* strains coming from seven *Monascus* species, they reported that the pksCT gene was a related gene responsible for citrinin biosynthesis in *Monascus purpureus.* Two DNA fragments from the transcriptional start region and the stop codon region of pksCT gene, which were 669 bp and 591 bp respectively, were obtained from the fourteen *Monascus* spp. by genomic PCR. Results of NCBI BLAST showed that the PCR products exhibited high identity with each other, and were in accordance with the partial sequences of pksCT gene in *M. purpureus* available in the Genbank. It was suggested that the pksCT gene was a general gene responsible for citrinin biosynthesis in *Monascus* species. It was also indicated that it was the effective way to distinguish the citrinin-producing *Monascus* strain by analyzing the pksCT gene with genomic PCR.

In the study of Shao *et al*. (2009), the feasibility of gene deletion mediated via *Agrobacterium tumefaciens* on the basis of homologous recombination was analyzed by studying on the deletion of the RGS domain of putative G-protein signaling regulator gene mrfA in *Monascus ruber.* The lengths of homologous arms of deletion vector pC805S were 958 bp and 824 bp, respectively. Homologous recombination of about 18% were observed for 26 of the 138 transformants analysed. The result showed it was feasible to identify the function of unknown gene in *M. ruber* with the targeted-deletion technology mediated via *A. tumefaciens.* Wang (2011) constructed the binary vector pCAMBIA 3300-gpdA-hph-trpC and transformed it into *Agrobacterium tumefaciens* GV3101. The exogenous gene gpdA-hph-trpC was transformed into *M. albidus* by ATMT. Some of the transformants were selected for PCR analysis, and the results further confirmed that the transformants tested were arbitrarily integrated with exogenous T-DNA in genome. Among many transformants, one transformant H1 with high Monacolin K production was selected, from which the flanking sequence mkWL (0.88 kb) of T-DNA by inverse PCR was obtained. The result of homology between the gene mkWL and Monacolin K biosynthetic gene cluster from *M. albidus* in the Genbank was analyzed, which revealed that there was identity of 97% at DNA sequence homology. The gene mkWL was deduced to be the related gene in Monacolin K biosynthetic pathway of *M. albidus.*

The conditions for efficient protoplast formation and regeneration from transformant H1 and *M. fumeus* 9908 were further studied by Wang (2011). *Monascus fusants* HH1 with high Monacolin K production were selected via protoplast fusion between tansformant H1 and *M. fumeus* 9908, and the concentration of Monacolin K in fermented millet by fusant HH1 increased by 404% and 27%, respectively, compared with that produced by parental *Monascus* spp. and transformant H1. In the study of Xu *et al.* (2009), about 8 000 transformants were obtained by T-DNA insertional library of *Monascus ruber* mediated by *Agrobacterium tumefaciens.* 230 of them with significant changes were classified into seven groups according to characteristics of the colony and micro-morphological observation. On this basis, pigment, Monacolin K, citrinin and amylase of the representatives of the 14 mutants were analyzed. The capacity to produce pigment of all the mutants were poorer than the original strain M-7; 806# and 2553# were the poorest, and the pigment-value of them were less than tenth of M-7. Monacolin K output of 10 mutants was higher than M-7; 3245# was almost 10 times that of M-7, which amounts to 679.25 μg/g. The citrinin production (dry weight of Hongqu) of 1822# was the highest, reaching 60.01 ng/g, nearly 30 times that of M-7; the citrinin production of 178# and 806# was not detected under this experimental condition. The amylase activity among the 13 mutants were higher than that of M-7; while the activity of amylase of 1067# was the highest, up to 3227.10 U/g and was 20 times that of M-7. These results showed that some variant *Monascus* strains could be obtained by the use of insertion method.

As mentioned above, Chinese scholars have carried out some researches on the molecular biology of *Monascus* and preliminary built up several genetic transformation methods for further study of Monascus, mainly focusing on functional genes for three important metabolites (pigment, citrinin and Monacolin K). Although the breeding of mutant transformant has been achieved by insertional inactivation in *Monascus* chromosomal genes and even the site-directed knockout technology has been built up for harmful citrinin synthetase gene, many works on the molecular biology of *Monascus* are still needed in the future. For example, although the insertional library and subtractive library has been set up by using molecular biological methods for insertion of foreign gene into the chromosome of *Monascus*, the functional gene identification on the transformants were still conducted by traditional morphological observation or metabolite analysis. Hence, it was not significantly different with the traditional breeding methods.

Until now, the studies only investigated some functional genes responsible for key metabolites (citrinin or Monacolin K synthase gene), in which only some DNA fragments consist in these functional genes were obtained or preliminarily studied. The research on *Monascus* molecular biology also lacks a unified proposal in China. For example, correlation between citrinin synthase genes and citrinin synthesis has not been elucidated. Research on the relationship between three important metabolites (pigment, Monacolin K and citrinin) and the polyketide compounds, polyketide synthase gene and their regulation genes has not been conducted. There is also a lack of in-depth understanding on the structure and regulation of some key *Monascus* metabolites synthetase gene. All these defects are mainly due to the lack of information on the *Monascus* genes and immature application of the genetic transformation system. Moreover, the gene cluster responsible for the synthetase system is relatively complex, which is made up of many protein structural domains. Hence, it is difficult to carry out more in-depth investigation by the existing technology and it is a long way to understand and control the synthesis of key *Monascus* metabolites from the gene level.

Monascus-fermented products research and development

Monascus pigments

There are two kinds of methods for the production of *Monascus* pigment in China, including solid-state fermentation and submerged fermentation. Most of the *Monascus* pigment products are rich in red pigment. They are called "*Monascus* red rice" and "*Monascus* red pigment". "*Monascus* red rice" is the product from solid-state fermentation in which rice is used as raw material and then inoculated with *Monascus* seed culture. "*Monascus* red rice" contains many kinds of pigments such as red, yellow and orange and a mixture of residue starch, protein and other substances. The color purity of "*Monascus* red rice" is relatively low but the color tune is red in general. On the other hand, "*Monascus* red pigment" is the product from submerged fermentation in which grain (mainly rice and soybean) is used as raw material for the preparation of fermentation medium and then inoculated with *Monascus* seed culture. And then the "*Monascus* red pigment" is obtained by submerged fermentation, followed with separation, purification, concentration and spray drying. Some pigment in concentrated liquids are not spray dried but directly put into paste for food industry. In addition, some Chinese companies begin producing "*Monascus* yellow pigment" mainly by submerged fermentation and special "*Monascus* red rice" with a yellow tone by solid-state fermentation.

Monascus red pigment

The development of *Monascus* pigment production by submerged fermentation process began in about 1990s, and the production technology achieved success in about 2000 (Chen, 2000). The pigment produced through the below process: preparation of raw material → sterilization → fermentation (inoculated by Monascus seed)→ 1st filtration → enzymolysis and immersion of filtrated residues → 2nd filtration → vacuum concentration → spray drying → sieve powder → product packaging → storage. The pigment yield can reached a very high level by submerged fermentation of *Monascus.* The color value of fermentation broth can reach above 700 U/mL while the color value of *Monascus* red pigment products can reach 10000 U/g ~ 15000 U/g in current industry scale. The citrinin content in most of the *Monascus* pigment products by Chinese companies can meet specification requirement in the Japanese standard. Therefore, it can be said that in the production of natural pigment, *Monascus* red pigment produced by industrial scale fermentation is one of the most successful applications. Because of the good solubility of *Monascus* red pigment products in water or ethanol, it can be added in most foods, especially in meat, cakes and drinks. The production of *Monascus* red pigment by submerged fermentation is easy to realize by large-scale industrialization. Meanwhile, manpower cost in the production process is low and the process is not easy to be contaminated. At present, the production of *Monascus* red pigment by submerged fermentation is rapidly developing. In China, there are mainly three companies for the production of *Monascus* red pigment. However, the *Monascus* pigment production scale and the actual output are still less than 1000 tons (color value 10000 U/g ~ 15000 U/g). It is expected that the production scale will reach more than 1500 tons after 2013.

Technical information about the production of *Monascus* red pigment by submerged fermentation is rarely published due to commercial secrecy. In general, the development of production technology are mainly carried out by constant breeding of

Monascus strains, optimization of fermentation medium and fed-batch fermentation process, and the improvement of ventilation and mixing technology in fermentation. Breeding of *Monascus* strains of non-citrinin and production of *Monascus* pigment without citrinin have become the goal in both academic and industrial field in recent years in China. Chan *et al.* (2006) carried out a related study in which *Monascus purpureus* 9908 was mutated by ^{60}Co irradiation. One *Monascus purpureus* strain that does not produce citrinin was obtained after a series of screening. The color value was above 1300 U/g by fermentation at 32°C or 6 days. In addition, the results showed that this strain did not produce citrinin in both submerged fermentation and solid-state fermentation in YES and MSG. The color value of the red rice in solid-state fermentation reached 2000 U/g without citrinin. Histidine was found to be the most valuable amino acid as it resulted in the highest production of red pigments and almost completely eliminated the formation of citrinin (Hajjaj *et al.*, 2012).

Jiangnan University and Tianyi Biological Engineering Company Limited (Dongguan, Guangdong province, China) cooperated to carry out the production of *Monascus* pigment with high color value and without citrinin in submerged fermentation. Experts believed that this project has reached its goal and the achievements lead in international advanced level at the industry scale. Tianyi Biological Engineering Company Limited (Dongguan, Guangdong province, China) has applied for national invention patent. In addition, oil soluble *Monascus* pigment and acid soluble *Monascus* pigment have already been available by Tianyi Biological Engineering Company Limited (Dongguan, Guangdong province, China) and also access to China's invention patent.

Monascus yellow pigment

At present, most of the *Monascus* pigments are rich in red pigments, which in fact are mixture of red, yellow and orange pigment with different proportions and the red one is the major composition in the existing *Monascus* pigments. The so called *Monascus* yellow pigment is actually the mixture product that is rich in yellow pigment. The yellow pigment has been widely used in the food industry (e.g. edible oil, biscuits, bread, cakes and beverages) because it is one of the basic colors. It is anticipated that yellow pigment may has a wider application range than red pigment. Scientists in Thailand carried out the breeding of *Monascus* for production of yellow pigment as early as 2000 and have gained the *Monascus* strain which produced mainly yellow pigment. Recently, China has also made progress in this area. For example, Zhou *et al.* (2008) has obtained a *Monascus* mutant with high yield of yellow pigment by using conventional mutation techniques, e.g. treating with physical mutagens (such as UV light) and chemical substances (such as N-methyl-N'-nitro-N-nitrosoguanidine). The growth characteristic of *Monascus* mutant was stable and the yellow pigment value and color tone in submerged fermentation reached 100 U/mL and 3.5, respectively. The maximal absorption of yellow pigment was determined at 410 nm and it was stable from pH 3 to pH 8. In order to improve the ratio of yellow pigment in *Monascus* pigment mixture, researchers mainly achieve the objective by optimization of the composition of culture media.

In order to improve the ratio of yellow pigment in *Monascus* pigment mixture, researchers carried out the optimization of culture medium and tried to improve the yield of yellow pigment. Zhou *et al.* (2008) investigated the effect of nitrogen source on the production of yellow pigment in submerged fermentation and found that the organic nitrogen source corn syrup and inorganic nitrogen ammonium chloride were beneficial for the production of yellow pigment. Tang *et al*. (2006) studied a *Monascus* strain which produced yellow pigment and found that its color tone was yellow with the maximum absorption peak at 370 nm. The suitable carbon source was corn powder and the nitrogen source was NH_4NO_3. In the study of Zhou *et al.* (2009), six ammonium salts including ammonium chloride, ammonium acetic, ammonium sulfate, ammonium dihydrogen phosphate, ammonium citrate tribasic and ammonium nitrate were investigated as inorganic nitrogen sources for production of *Monascus* yellow and red pigments. The results demonstrated that the yellow pigments were not affected significantly by the ammonium salts; however, the production of red pigments and citrinin were influenced noticeably, which resulted in the remarkable difference of colour tune. High pH caused by consumption of ammonium salts, inhibited citrinin production but promoted production of extracellular yellow and red pigments. pH environment ranging from 3 to 5, resulted from the consumption of ammonium salts, were beneficial for citrinin production, whereas citrinin production was prevented by low pH below 3. Actually, citrinin is also a yellow pigment. Hence, the specific components of yellow pigment should be carefully analyzed in the production of *Monascus* yellow pigment. In the study of Yang (2009), the effects of several important factors such as *Monascus* strains, initial pH and the concentration of inorganic salt were investigated for the production of *Monascus* yellow and red pigments. It was found that the addition of acetic acid into the soaking rice water increased the yellow tune although the color value was slightly decreased. The color value and tune were greatly increased by change of concentration of inorganic salt. The color value was gradually increased in the fermentation period and the yellow pigment was increased in day 7-10 and decreased slightly in day 10-13. By using the orthogonal test and variance analysis, the optimal conditions for production of the yellow pigment were as follows: strains116-R, 0.2% inorganic salt and initial pH 5.8.

The production of *Monascus* yellow pigment is just beginning in mainland China by submerged fermentation, solid-state fermentation and conversion method. Tianyi Biological Engineering Company Limited (Dongguan, Guangdong Province, China) has applied for the Chinese invention patent of *Monascus* pigment which is rich yellow pigment (Tianyi Biological Engineering Company Limited 2010) and acid soluble *Monascus* pigment (Tianyi Biological Engineering Company Limited 2008).

Functional Monascus fermentation products

Monascus metabolites such as lovastatin, polysaccharide, γ-aminobutyric acid (GABA), ergosterol have been considered to have certain physiological function. In China, the development of these functional *Monascus* components has attracted much attention in recent years.

Monascus product containing lovastatin

Lovastatin (also known as Monacolin K), which is the main bioactive component of functional *Monascus* products, has got a considerable development in the recent ten years. There are about ten companies producing *Monascus* products containing lovastatin. These products are used as raw materials for the health-promotion products in China. In addition, there are two companies using *Monascus* lovastatin as the main raw materials for the production of blood cholesterol reducing drug. At present, about 1000 tons functional *Monascus* raw materials can be achieved each year in China, which are almost produced by solid-state fermentation. Generally, cereals such as rice, millet and wheat are used as raw materials. Tian *et al.* (2006) reported the use of wheat bran, which has many advantages such as rich in nutrition, porous and good air permeability for solid-state fermentation process, for efficient production of *Monascus* lovastatin.

It needs about 20 days for producing lovastatin by solid-state fermentation of *Monascus.* Ristiarini *et al*. (2011) found that lovastatin level in 3 weeks fermentation was significantly higher (almost two fold) than that of 2 weeks fermentation. Therefore, it is difficult to realize the large scale production due to the long fermentation time and difficulty in preventing contamination. At present, almost all manufacturers are using bottles for solid-state fermentation, in which the manpower cost is very high, and also difficult to control microbial contamination.

There is only limited publication on the production of lovastatin in submerged fermentation of *Monascus.* Chen *et al*. (2004) reported the concentration of lovastatin could reach 1600 mg/L in shake flask fermentation. However, the corresponding level was only 1000 mg/L in 15 L fermentor. Submerged fermentation should be one expected method for large-scale industrialized production of *Monascus* lovastatin, although the concentration is still low and restricts its application when uses grain as raw material. Additionally, the *Monascus* mycelia are easily interrupted or adhered to the fermentation tank wall and on the stirring paddle, which may also cause a low level of fermentation production. Besides fermentation broth, the *Monascus* mycelia were also reported to have bioactive function and there are Chinese companies exploring the use of *Monascus* mycelial powder as raw material in functional food.

Monascus polysaccharide

There are a large amount of polysaccharides present in *Monascus* fermentation broth. Tian *et al*. (1998) analyzed the chemical structure of *Monascus* polysaccharide and found that *Monascus* polysaccharide was mainly consists of mannose, glucose and galactose with a molar ratio 1:2:4. The polysaccharide was mainly composed of 1→3 glucosidic bond, as well as 1→6 and 1→2 glucosidic bonds. The production of polysaccharide by submerged fermentation of *Monascus* spp. ZKOA was investigated by Li *et al.* (2009). The fermentation conditions were optimized as follows: sucrose 40 g/L, yeast extract 4.5 g/L, $KH_2PO_4{\bullet}3H_2O$ 3.5 g/L, $MgSO_4$ 0.4 g/L, vegetable oil 2 mL/L, seed culture age 30 h, inoculation volume 8%, initial pH of fermentation medium 5.0, and fermentation lasted for 90 h. Under these conditions, the yield of *Monascus* polysaccharides reached 7.6 g/L and 7.34 g/L in shaking flask and 20 L fermenter, respectively. However, the extracted polysaccharide was crude and its specific structure was not clear. In the study of Ding (2007), the purity of *Monascus* polysaccharide reached 78% after a series of purification. Kunming mice were fed with polysaccharide for two weeks after replanted by S180 tumor and then the tumors and spleens of experimental mice were removed and weighted. Then tumor suppressing rates and spleen index were calculated and analyzed with SPSS software. Results showed that *Monascus* polysaccharide inhibited the growth of tumors in mice obviously. The highest tumor suppressing rate was 39.82%. The effect of the polysaccharide showed a dose dependent manner. Because *Monascus* polysaccharide can enhance the immunity, the average weight of mice spleens increased markedly with the high dose of polysaccharide. Consequently, the antitumor effect of *Monascus* polysaccharide was affirmed, and it is worth exploiting this activity. Wang *et al.* (2011) investigated the conditions for producing exopolysaccharide by submerged fermentation with *Monascus* Mr-70 and its antioxidant activity. Under the optimal fermentation conditions for 96 h, the yield of crude exopolysaccharide reached as much as 10.15 g/L in shaker flask. The scavenging rate for DPPH• was 82.24% with 5 mg/mL of crude exopolysaccharide. It was manifested that the crude exopolysaccharide of *Monascus* Mr-70 had strong antioxidant activity.

In order to increase the yield of chitosan, *Monascus purpureus* was induced by ^{60}Co and UV (Chen *et al*., 2009). The death rate and the mutation rate of bacterial cell under the different doses of mutagen were investigated to determine the optimal conditions for mutation, and ultimately to achieve a stable mutant *Monascus purpureus* C16. Through the extraction of chitosan after submerged fermentation, the chitosan yield increased by 5.46%. The chitosan yield enhanced when characteristic resources in Guizhou, such as Chinese yam, bitter buckwheat and the gastrodia elata, were used as substrates for the fermentation of *Monascus purpureus* C16.

Monascus vinegar

The so called *Monascus* vinegar product is characterized by adding *Monascus* red rice as saccharifiying and fermenting agent in the process of making vinegar. Wen *et al.* (2007) improved the process for preparing functional *Monascus* vinegar based on traditional brewing techniques. The effect of reducing blood lipids level of the new product was validated through animal experiments. The process for preparing the functional *Monascus* vinegar was as follows: rice → washed out → comminuted → added water → cooked → added *Monascus* and functional *Monascus* rice → liquefied → saccharified → *Acetobacillus* fermented → filtered → sterilized. The high blood lipids models of rats and mice were administered with traditional *Monascus* vinegar and functional *Monascus* vinegar separately for 60 days. Then the total contents of cholesterol tri-acyl glycerol and high density lipoprotein-cholesterol in serum of rats and mice were determined. There was no significant difference on the levels of the three indicators when administered with the traditional *Monascus* vinegar. In contrast, the levels of total cholesterol and tri-acyl glycerol decreased significantly ($p < 0.05$) when administred with the functional *Monascus* vinegar. Therefore, it was concluded that the improved techniques for preparing functional *Monascus* vinegar showed definite function of reducing blood lipids level.

“Esterifying Monascus qu”

Qu is the name of the starter and it contains various hydrolysis enzymes. It is often used as saccharifying and fermenting agent in fermentation of Chinese traditional spirit. *Monascus* qu was one of the common used saccharifing and fermenting agents. Studies have shown that *Monascus* qu contain a variety of enzymes including esterifying enzymes. Besides the normal effect of liquefaction and saccharification, the crude enzyme preparations in Chinese spirit production by fermentation of *Monascus* also have the obvious enhancing flavor. In recent years, esterifying starter has been industrialized and used in Chinese spirit production. Some institutions such as Jiangnan University and Hubei University of Technology have carried out investigation on the breeding of *Monascus* strains for producing esterifying enzymes. Xiao (2012) has obtained a mutant *Monascus* strain after UV mutagenesis processing of *Monascus* AG-4as, and the mutant possessed a better esterifying enzyme activity than *Monascus* AG-4as 29% strains {29% increase, 90.88 mg/(g 100 h)}. After a series of domestication experiments and the optimization of fermentation conditions, the final esterifying enzyme activity reached 117.5 mg/(g 100 h).

In addition to the action of the esterifying enzyme, there may be other substances from *Monascus* qu enhancing the properties of Chinese spirit. For example, a certain amount of octanoic acid, capric acid, lauric acid, myristic acid, oleic acid, linoleic acid and acetic acid can be produced during the Chinese spirit production by adding small part of esterifying *Monascus* qu. Moreover, the *Monascus* pigment may also exert certain effects on the flavor of Chinese spirit. Therefore, it still needs further study on the mechanism of esterifying *Monascus* qu during the fermentation process of Chinese spirit. Cheng *et al.* (2006) reported the production of esterifying *Monascus* qu using wheat bran as main raw material. The solid-state fermentation process was carried out in shallow tray fermenter or heavy layer ventilation pool, in which the fermentation usually lasted for about 3-4 days. The esterifying enzyme activity reached 220 mg/(g 100 h) by GC analysis. In addition to the solid-state fermentation, there were also some reports on the esterifying enzyme production by submerged fermentation of *Monascus* (Ma *et al*., 2007). *M. fumeus* (Fang *et al*., 2011) and *M. pilosus* (Tang *et al.*, 2005) were the two common used *Monascus* strains for production of esterifying *Monascus* qu. Esterifying activity of esterifying *Monascus* qu is usually measured by the classical titration method which takes five days to get results. In brewing industry, it is common to use the esterifying ability to express the esterifying activity. There have been reports of using GC or HPLC for the determination of esterifying activity which are distinct to the classical acid-base titration method. Currently, esterifying *Monascus* qu has been mainly used for spirit and soy sauce production. There are several different ways in the application of esterifying *Monascus* in Chinese spirit: add the esterifying *Monascus* qu in the preparation process of starter; add into the esterifying solution; add during the fermentation.

Monascus-fermented soybean

Soybean (*Glycine max*) has been consumed worldwide as fermented (tempe, soysauce, tofuyo, tauco, soy yoghurt) and non-fermented foods (soymilk, tofu). Soyfoods were consumed as protein and antioxidant rich foods. *Monascus*-fermented soybean (MFS) were recently developed to combine functional of soyfood and *Monascus* metabolites. MFS was more effective in reducing power and scavenging ability on hydroxyl radicals than uninoculated soybean. Based on the results obtained, MFS was relatively effective in the antioxidant properties assayed and might be potential antioxidants for application in food products (Lee *et al*., 2008; 2009). Pyo and Seong (2009) found that MFS extract perform an additive hypolipidemic effect in hyperlipidemic rats than unfermented soybean extract. The oral administration of MFSE (200 and 400 mg/Kg body weight) significantly lowered the serum total cholesterol (LDL-C) level and raised high-density lipoprotein cholesterol (HDL-C) levels in hyperlipidemic rats. Pyo and Seong (2009) develop a medicinal soy yogurt (sogurt) containing high levels of γ-aminobutyric acid (GABA), free amino acids (FAAs), statins, and isoflavone aglycones using *Monascus*-fermented soybean extract (MFSE, 1.5%, w/v). Kim *et al.* (2010) produced whole soymilk from MFS and studied its physicochemical.

Monascus-fermented Adlay

Adlay (Chinese pearl barley, soft-shelled Job's tears, *Coix lachrymal-jobi* L. var. ma-yuen Stapf) is a grass crop that has long been used in traditional Chinese medicine and a nourishing food due to its high nutritional value and special biological and functional effects on the human body. The seed of adlay has been used in Asian countries for the treatment of warts, rheumatism, female endocrine system and neuralgia from ancient times and reported to exhibit anti-inflammatory, stomachic, diuretic, antispastic and hypolipidemic effects (Shih *et al.*, 2004). Both the fungus *Monascus* species and adlay possess functional components effective in maintaining human health. *Monascus*-fermented adlay (MFA) has been produced by inoculation of *Monascus* culture into cooked adlay. The taste quality, storage stability, and the antioxidant of MFA have been evaluated (Tseng *et al.*, 2004; 2005; Yang *et al.*, 2005). Pattanagul *et al.* (2008) studied the cultivation of *Monascus purpureus* (ATCC 16365, BCC 6131, DMKU and FTCMU) and *Monascus ruber* TISTR 3006 on the adlay substrate for mevinolin, citrinin, pigments and glucosamine synthesis at room temperature (32 – 35°C) for 28 days. *M. purpureus* DMKU produced the lowest citrinin content of 0.26 ppm (within an authorized citrinin level) and the highest mevinolin content of 25.03 ppm. Shi and Pan (2010) showed that MFA was useful as chemo-preventive agent and has activity to reduce plasma glucose, amylase, triglyceride and cholesterol levels.

Monascus-fermented dioscorea

Dioscorea (*Dioscorea batatas*) root is regarded as a functional food or a worthful herb because of the inclusion of many functional ingredients for the prevention of various diseases. Dioscorea exhibit great antioxidative ability, anti-inflammatory and hypolipidemic abilities. RMD comprises a dioscorea root substance besides *Monascus* metabolite. Therefore, the function of dioscorea should strengthen the hypolipidemic and antiatherosclerotic effect of RMD. Lee *et al.* (2006) used sweet potato (*Ipomoea batatas*), potato (*Solanum tuberosum*), casava (*Manihot esculenta*), and dioscorea (*Dioscorea batatas*) as the *Monascus* substrates to produce monacolin K. The results show that *Monascus purpureus* NTU 301, with dioscorea as the substrate, can produce monacolin K at 2,584 mg kg^{-1}, which is 5.37 times to that resulted when rice is used as the substrate. *Dioscorea* is concluded to be the best substrate for *Monascus* species to produce the cholesterol-lowering agent - monacolin

K and antiinflammation agent - monascin. Recently, various research group conducted researches on MFD intensively especially on potential of MFD for antihypertriglycemia, antiatherosclerosis, antihypertension, antihyperlipidemia dan anticancer (Lee *et al.*, 2007; Wu *et al.*, 2009; Hsu *et al.*, 2010; 2011; Shi *et al.*, 2011; Chen and Pan, 2012).

New MFPs

Research groups, mainly in Asia, develop new MFPs such as *Monascus*-fermented red ginseng, *Monascus*-fermented garlic and *Monascus*-fermented durian seed (Table 2).

Hong *et al.* (2011) developed *Monascus*-fermented red ginseng as a novel functional food that contains better profile of ginsenosides. Most of ginsenosides were converted to deglycosylated ginsenocides. The product also contained monacolin K which contribute an additional functionality. The product can be applied in the development of several functional foods. Park *et al.* (2012) and Higashikawa *et al.* (2012) reported that combination of functionality of *Monascus* metabolites and garlic components has good potentiality as a food supplements for prevention and treatment of obesity and metabolic syndrome. Currently, Srianta *et al.* (2012a; 2012b) reported that durian seed has good potentiality as a substrate for angkak production. Besides pigments, the product contained monacolin K and phenolic compounds.

Table 2. New *Monascus* fermentation products

MFPs	Research	Source
Monascus fermentation Ginseng	Enrichment of deglycosylated ginenosides and monacolin K in red ginseng by fermentation with *Monascus pilosus*	Hong *et al.* (2011)
Monascus fermentation Garlic	The ability of red yeast rice-garlic to inhibit the markers of differentiation in 3T3-L1 preadipocytes Clinical trial: reduction of serum lipids by the intake of extract of garlic fermented with *Monascus pilosus*	Park *et al.* (2012) Higashikawa *et al.* (2012)
Monascus fermentation Durian seed	Durian seed as a new substrate to produce monascus pigments and monacolin K	Srianta *et al.* (2012a; 2012b)

Regulations on MFPs

Asia

MFPs have been consumed for many centuries in Asian countries. MFPs are widely distributed commercially in various markets such as in food ingredients shops, Chinese traditional medicines and internet marketing. At present, there are three national standards in China related to *Monascus* products including national standard for *Monascus* pigment, red kojic rice (powder) and citrinin determination. In addition, there is also one industry standard for functional *Monascus* product. The existing China national standard (GB15961-2005) for *Monascus* red pigment was enacted in 2005. This standard requested specific quality for *Monascus* red pigment from two aspects (sensory requirements and physicochemical indexes). However, this standard did not mention the limit index for citrinin. Currently, a new national standard for *Monascus* red pigment is underway. The existing national standard (GB 4926-2008) for red kojic rice (powder) was enacted in 2008. This standard requested specific quality for red kojic rice (powder) from three aspects (sensory requirements, physicochemical indexes and health requirements). However, this standard also did not mention the limit index for citrinin although made a strict requirement on aflatoxin B1 (5 µg/kg). Since Blanc *et al.* (1995) found citrinin in *Monascus* pigment, it has attracted much attention in China. In 2000, research on the determination method of citrinin was listed in national fifteen projects. After that, a number of studies were carried out on the determination method of citrinin and the current situation of citrinin in the *Monascus* products was introduced by Xu (2003; 2004) and Li (2003; 2005). Eventually the national standard for *Monascus* citrinin determination was developed. From value perspective, the production of functional *Monascus* products will become one of the largest industries in the Chinese *Monascus* industry. At present, there are more than ten companies engaged in functional *Monascus* products. Therefore, China Light Industry has developed an industry standard for functional *Monascus* products. The standard requires a minimum of physiologically active ingredient content (4 mg/kg) in functional *Monascus* products and meanwhile a limit index of citrinin (50 µg/kg). However, in fact this content of citrinin cannot be accurate quantitative in the existing HPLC detection method and hence citrinin content cannot be accurate controlled. As a result of the later investigation on *Monascus* yellow pigment, the products are not appeared in the market at present. The company is still preparing the national standard for *Monascus* yellow pigment.

In Japan, *Monascus* color has been included among other food colors i.e. Beet Red, Black Currant Color, Caramel I, Caramel II, Caramel III, CaramelIV, Carrot Carotene, Carthamus Red, Carthamus Yellow, Chlorophyll, Cochineal Extract, Dunaliella Carotene, Grape Skin Extract, Marigold Color, Palm Oil Carotene, Paprika Color, and Turmeric Oleoresin which are cathegorized in existing food additives. Its specification has been established in the Japan's Specifications and Standards for Food Additives, 7th Edition with citrinin level limit of 0.2 µg/g.

United States

FDA has not extensive testing dietary supplements, including red yeast rice. Without adequate testing, red yeast rice products are not guaranteed to contain ingredients stated on the product label. In 2007, the FDA warned consumers to avoid red yeast rice products promoted on the internet to lower cholesterol because of the possibility of myopathy, leading to kidney impairment. Inconsistencies on monacolins levels led the FDA to require current good manufacturing practices (CGMPs) for dietary supplements. Final CGMPs were effective in June 2008 for large companies and are to be implemented in June 2009 for companies with fewer than 500 employees. Under this ruling, all domestic and foreign supplements must be processed in a consistent manner and to meet quality standards. To demonstrate quality and consistency, tests will be performed on all supplements to ensure their identity, purity, strength, and composition (Klimek *et al*., 2009).

European Union

Final evaluation by DFG-Senate Commission on Food Safety concluded that red mould rice is not suitable for use as a foodstuff/food supplement because basic toxicological data for a safety evaluation of red mould rice or its constituents are missing. Standards and specifications to ensure purity and identity as well as the absence of toxic constituents are completely missing (Eisenbrand 2006). In 2011 the European Food Safety Authority (EFSA) has provided a scientific opinion on the health claims related to monacolin K from red yeast rice based on human intervention studies. The EFSA has not classified such products as food or medicine. The EFSA's opinion should not be interpreted as an approval of red yeast rice for food or medicinal use (Lachenmeier *et al.*, 2012). In summary, recent research and development of MFPs showed great progress and impact on wide aspects: science, technology, health, economic and regulation. We estimate that future research and development of MFPs will increase progressively.

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