Appl Microbiol Biotechnol (2006) 73:297–304
DOI 10.1007/s00253-006-0457-8

Production of red mold rice using a modified Nagata type koji maker

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Received: 21 February 2006 / Revised: 3 April 2006 / Accepted: 5 April 2006 / Published online: 7 October 2006

Abstract In this research, a commercial 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 modified koji maker, in which temperature and humidity profiles were kept at varied levels at different stages, respectively. Air was circulated to remove fermentation heat while the perforated bed rotated slowly for providing mild agitation. Lag phase of the *Monascus* sp. in the modified koji maker was determined to be 16 h by the time the koji temperature raised rapidly. Water was added into the koji bed by a water curtain at the 36th hour to keep the moisture content of the rice koji at 50% or above. At the final stage, temperature was adjusted to 34°C to direct red pigment production. After 7 days, 1,200-kg high quality red mold rice was harvested per batch. Labor costs, space, and fermentation time were reduced tremendously compared with those made by traditional methods.

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Introduction

Red mold rice (also known as red fermented rice or red yeast rice), produced from solid-state fermentation of cooked rice with *Monascus* sp., contains many high value substances, such as monacolin K, γ-aminobutyric acid (GABA), natural red pigment, and other unidentified active components (Aniya et al. 2000; Blanc et al. 1995; Su et al. 2003). These components are the secondary metabolites of fermentation and are medically proven to possess anti-cholesterol, anticarcinogenic activities (Endo 1979, 1980; Hawksworth and Pitt 1983; Lee et al. 2005), and antifatigue activities (Wang et al. 2005). The Chinese ancient pharmacopoeia, *Ben Tsao Gum Mu*, indicates the use of red mold rice to promote the health of the cardiovascular systems (Kao 1997; Su 2001). Red mold rice is used in many Chinese processed foods and is used for red color enhancement and nutroceutical supplements at least for more than thousands of years. However, the formal written records were not unveiled until two pharmacopoeias were published in the Post-Han and Yuan Dynasties, respectively, which first described the medicinal functions of red mold rice (Bau 1996; Su 2001). In ca 1590, another pharmacopoeia was published and released describing the method for making red mold rice (Bau 1996). This method

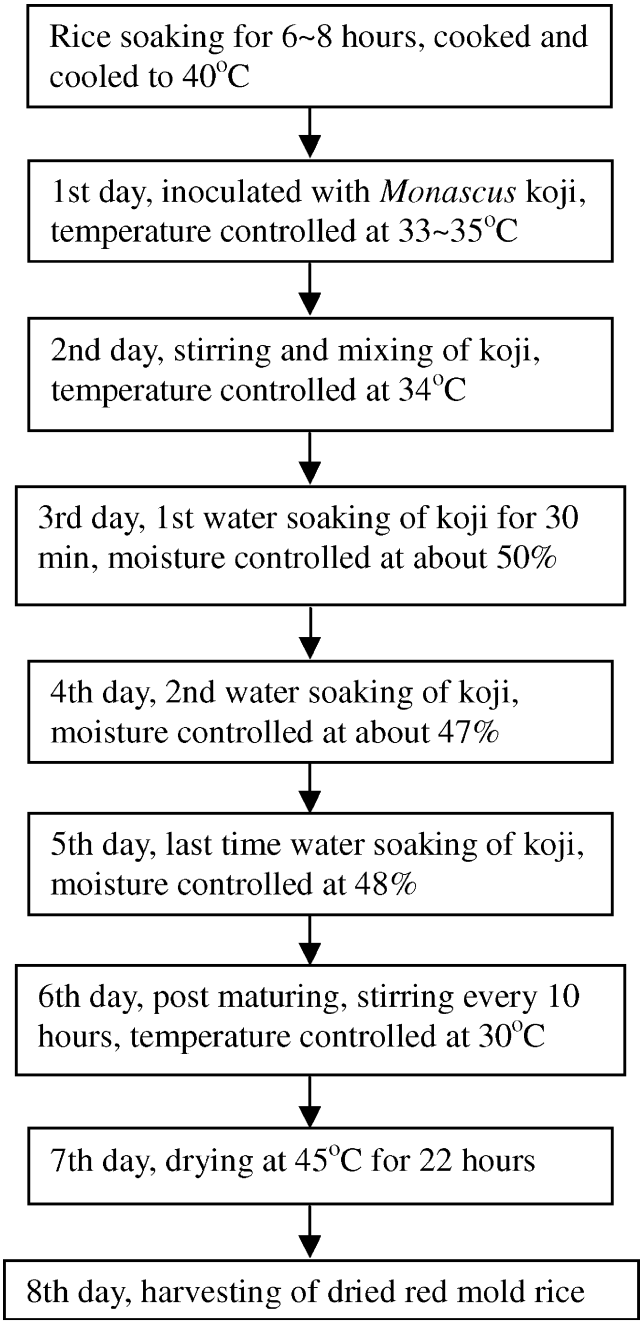

Fig. 1 Red mold rice production procedure by traditional process

has ever since been adopted as the standard fermentation process until recent years, as shown in Fig. 1.

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; Su 2001).

Microbiological studies of red molds was first conducted in 1884 by van Tieghem, a French microbiologist, and were categorized as the genus *Monascaceae* (Huang 1985; Su et al. 1970). 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 et al. 1970). This finding led to the use of pure culture in commercial production of red mold rice. However, due to the strict limit of the "Monopoly Law for Production and Selling of Tobacco and Liquor," commercial production of red mold rice was only authorized to Taiwan Tobacco and Liquor Monopoly Corporation. Most researches for red mold rice production were only conducted in its affiliated Taiwan Wine Research Institute (TWRI), including species selection and process modification.

Literature survey on red mold rice production in a closed environment using a koji maker shows no successful cases. Lin and his associates in TWRI tried it with a pilot scale (50 kg in capacity) Nagata type rotary bed solid-state fermentor and ended up with unsatisfactory results (Lin 1982a,b, 1987). The result was attributed to the difficulties of water addition and fermentation heat removal in the site. Since then, no further studies had been conducted. On the other hands, reports on microbial strains used in red mold rice production and functional properties of metabolites were easily found (Endo 1980; Hawksworth and Pitt 1983; Lizuka and Lin 1980). Most of these works focused on the production capacities of monacolin, pigment, GABA, etc., and their applications as dietary supplements in healthy foods.

Twenty nine *Monascus* strains have been named and found possessing various production capacities of monacolin K, GABA, flavonoids, citrinin and red pigment, etc., as their primary and secondary metabolites, although citrinin is usually regarded as a hazardous factor to health. Among these strains, *Monascus purpureus*, *M. anka*, and *Monascus ruber* are the most often used for research and industrial production of red mold rice. However, their capacities for producing monacolin K, GABA, and red pigments are the major concerns (Su 2001).

In the mid-1950s, mechanization on koji production was just started in Japan. A Nagata type rotary bed koji maker (as shown in Fig. 2) was developed for making rice, soybean, and wheat koji for sake (Japanese rice wine) or soy sauce production using *Aspergillus* sp. as cultures. Major components of the rotary bed koji maker are a round

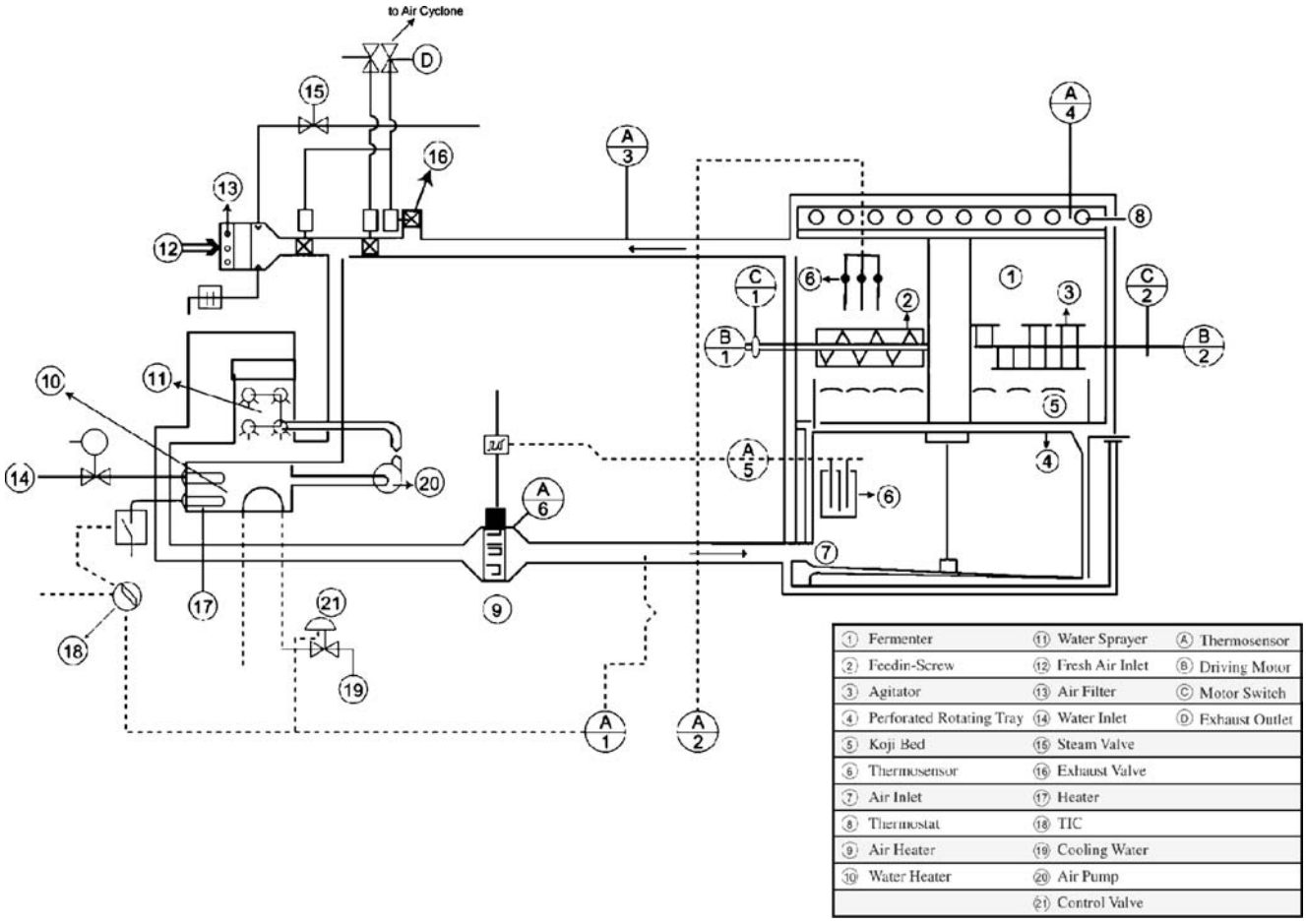

Fig. 2 The Nagata type large scale koji maker (Source: Lin 1987)

bed with at least 30 cm in depth and a perforated bottom plate for up-flow aeration; a set of adjustable speed mixer for plowing up rice koji during fermentation for assisting aeration, heat removal, and preventing koji from agglomeration; and a set of screw for cooked rice feed-in and koji discharge. Aeration system includes an air sterilizer and humidifier before charging into the bed, and a cyclone separator for the exhausted air. Temperature and humidity sensors are inserted for monitoring and control.

However, this Nagata type koji maker was originally designed for making koji with *Aspergillus* sp., which is physiologically and morphologically different from *Monascus* sp., especially on the aspects of moisture need and temperature rising speed of rice koji during solid-state fermentation. Therefore, modification for mass production of red mold rice in a more hygienic and controllable condition with mechanized koji making facilities became the motivation of this research. Controls of rice koji moisture content and fermentation temperature, and hence, modification of the koji maker, are definitely the cores of this research. The ultimate goals are to develop an optimal process for red mold rice mass production with high and consistent quality and low cost to meet the healthy food market demands.

Materials and methods

Microbial strain

M. purpureus BCRC 31499 was bought from Bioresources Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan.

Media and substrates

Indica rice medium was a mixture of Indica rice powder, 100 g, KH_2PO_4, 0.5 g, monosodium glutamate (MSG), 1.5 g, and lactic acid, 2 ml, in 850-ml deionized water. Bread koji medium applied steamed white bread as raw material. Indica rice powder, 15 kg, MSG, 110 g, KH_2PO_4, 30 g, and $CaHPO_4$, 15 g, were liquefied and dissolved in 65-l water as liquid culture medium for the 120-l submerged

fermentor. Cooked Indica rice was used for red mold rice mass production.

Reagents and materials

All chemical reagents were of reagent grade. Indica rice was of local variety, 90% polished with 74% starch value. Novo Termamyl (Novo, South Carolina, USA) was used as a liquefaction enzyme.

Equipment

Rice cooker with a capacity of 1,000 kg was capable of cooking rice with steam in 30 min and cooling rice from 100 to 30°C in 5 min (Miaoli Machine Shop, Miaoli, Taiwan). Submerged type fermentor for the production of inocula was 120 l with agitation, air aseptic, and temperature control systems. Nagata type koji maker containing a rotary bed with a capacity of 1,500 kg as shown in Fig. 2 was applied as a solid state fermentor for mass production (Agro-Industrial Machine, Chiayi, Taiwan). This fermentor was modified with different water-addition and temperature-control systems for application on red mold rice mass production.

Liquefaction methods and inocula used

In the 120-l submerged type fermentor, 15-kg rice powder was added to 65-l water and heated to 121°C for 50 min. Lactic acid or Novo Termamyl was used as liquefaction agent and added into the medium (described below). Bread koji of 120 g or 2% (v/w) liquefied rice powder culture was inoculated when medium temperature was lowered to 39°C. Agitation at 60 rpm and aeration of aseptic air at 2 vvm were maintained during the cultivation period.

Six kinds of liquefaction methods were used in this study: (A) 2% (v/w) lactic acid was used as liquefaction agent and 2% (v/w) liquefied rice powder culture was used as inocula; (B) 0.2% (v/w) Termamyl was added at 85°C and heated to 121°C for 50 min during liquefaction and 2% (v/w) liquefied rice powder culture was used as inocula; (C) 0.1% (v/w) Termamyl was added at 85°C and heated to 121°C for 50 min, then, another 0.1% (v/w) Termamyl was boosted when temperature was cooled down to 90°C during liquefaction and 2% (v/w) liquefied rice powder culture was used as inocula; (D) 2% (v/w) lactic acid was used as liquefaction agent and bread koji, 120 g, was used as inocula; (E) 0.2% (v/w) Termamyl was added at 85°C and heated to 121°C for 50 min and bread koji, 120 g, was used as inocula; (F) 0.1% (v/w) Termamyl was added at 85°C and heated to 121°C for 50 min, then another 0.1% (v/w) Termamyl was boosted when temperature was cooled down to 90°C during liquefaction and bread koji, 120 g, was used as inocula.

Analysis

Red pigment, pH value, activity of glucoamylase, specific viscosity, and total acidity of red mold rice were analyzed based on the standard methods of TWRI (Anonymous 1994). Monacolin K and citrinin were analyzed following the methods developed by Hsieh and Pan (Hsieh and Pan 2002).

Results

Preparations of high concentration liquid culture

Viscosity of the porridge-like medium in liquid culture preparation will be lifted as increasing the concentration of rice porridge, which, in turn, will retard the growth of

Table 1 High concentration liquid koji inocula cultivated for 5 days

Methods	Cell conc% (w/v)	pH value	Total acids (ml)	End point volume (l)	Residual sugars (g/100 ml)	Spec visc[a]
A[b]	3.59±0.70(b)	4.59±0.44(b)	40.5±1.5(ab)	49.5±3.9(cd)	3.17±0.44(a)	3.90±0.38(a)
B[b]	3.78±0.85(ab)	5.30±0.32(a)	34.4±2.4(b)	59.0±2.3(b)	2.51±0.45(ab)	2.90±0.12(abc)
C[b]	4.44±0.43(ab)	5.37±0.44(a)	34.2±1.3(b)	70.0±4.5(a)	1.50±0.69(ab)	1.50±0.06(c)
D[c]	4.32±0.20(ab)	4.51±0.06(b)	42.4±1.4(a)	43.0±4.5(d)	3.00±0.34(a)	3.60±0.25(ab)
E[c]	4.63±0.12(ab)	5.29±0.13(a)	34.7±1.1(b)	56.0±4.4(bc)	1.72±0.33(ab)	2.20±0.69(bc)
F[c]	4.86±0.27(a)	5.31±0.57(a)	33.8±0.8(b)	70.5±1.0(a)	1.11±0.77(b)	1.70±0.25(c)

Means±SD followed by different letters in parentheses are significantly different at 5% level by Duncan's Multiple Range Test

Liquefaction methods: *A* 2% (v/w) lactic acid was used as liquefaction agent, *B* 0.2% (v/w) Termamyl was added at 85°C and heated to 121°C for 50 min during liquefaction, *C* 0.1% (v/w) Termamyl was added at 85°C and heated to 12°C for 50 min, then another 0.1% (v/w) Termamyl was boosted when temperature was cooled down to 90°C during liquefaction, *D* 2% (v/w) lactic acid was used as liquefaction agent, *E* 0.2% (v/w) Termamyl was added at 85°C and heated to 121°C for 50 min, *F* 0.1% (v/w) Termamyl was added at 85°C and heated to 12°C for 50 min, then another 0.1% (v/w) Termamyl was boosted when temperature was cooled down to 90°C during liquefaction

[a]Specific viscosity

[b]2% (v/w) liquefied rice powder culture was used as inocula

[c]120-g bread koji was used as inocula

Table 2 Effect of inoculum amounts on lag phase during solid-state fermentation of *Monascus purpureus*

Inoculum ratio (%, v/w)	Lag phase (h)
0.7	25.70±0.26(a)
1.0	20.80±0.53(b)
2.0	16.50±0.38(c)
4.0	16.00±0.30(c)

Cell concentration of the liquid inocula was 4.78% (w/v); means±SD followed by different letters in parentheses are significantly different at 5% level by Duncan's Multiple Range Test

filamentous red mold culture. In eliminating this effect, different liquefaction methods were compared based on the final cell concentration, pH value, total acidity, residual sugars, end point volume, and specific viscosity.

Results were shown in Table 1, which concluded that preparation based on method F would provide the best harvest of *M. purpureus* (4.86±0.27%, w/v) after a 5-day period and was adopted, hereafter, in this research as inocula.

Amounts of liquid inocula on lag phase

Solid-state fermentation is difficult to control, especially on the aspect of prevention of microbial contamination from the surroundings. The growth rate of *Monascus* sp. is much slower than that of other two fungi, *Aspergillus* sp. and *Rhizopus* sp., which are commonly found as contaminant strains in red mold rice fermentation. For providing an environment for *M. purpureus* to prevail over the other competitors, the optimal quantities of liquid inocula were tested by comparing the lag phase of the solid-state fermentation. Increase of *Monascus* inocula may have the effect of predominating the growth under a semi-open circumstance for solid state fermentation. Lag phase was determined based on the time for the inoculated cooked rice to raise its central temperature up to 38°C. Table 2 showed that the inocula at 2.0% v/w would provide an acceptable lag phase of 16 h.

Table 3 The influence of soaking time on koji moisture content

Soaking time[a]	Moisture content (%)
0 s	30.00±0.00(a)
10 s	40.30±0.58(b)
30 s	44.60±0.46(c)
60 s	47.20±0.40(d)
2 min	48.70±0.21(e)
4 min	49.80±0.35(f)
6 min	50.30±0.31(gf)
10 min	51.00±0.15(gh)
15 min	51.40±0.30(h)
20 min	51.60±0.17(h)
25 min	51.70±0.12(h)

Means±SD followed by different letters in parentheses are significantly different at 5% level by Duncan's Multiple Range Test

[a]Soaking condition was: 1-kg koji (moisture content ca 30%) was soaked in a 20-l water bath to simulate koji soaking by traditional method

Water sorption capacity of red mold rice

Moisture content of the rice koji decreased dramatically during solid-state fermentation due to aeration. This would cause drying and hardening of the rice, which, in turn, impede penetration of fungal filaments into rice grain, and thus, hamper the growth of *M. purpureus*. As indicated in Fig. 1, the traditional method for making red mold rice involves at least three times of water soaking during fermentation to maintain the moisture content of the rice grains. Therefore, it is necessary to decide water sorption capacity of the cooked rice grain when developing a water-adding system in the solid-state fermentor. Table 3 revealed that a soaking time of 6 min would reach a moisture content of around 50%. Extending soaking time to longer than 6 min did not increase moisture content significantly. Understanding of this characteristic would help in developing a suitable water-adding system to keep the rice koji at the optimal moisture content for fermentation.

Water adding timing on red pigment development

Rice koji has to be taken out from the fermentation room 3 days after inoculation and soaked in water to keep the moisture content at a certain level. This is the most labor-intensive step in the traditional method. However, when a large-scale koji maker is in use, aeration and the fermentation heat generated from growth speed up the loss of moisture content. Therefore, the timing for adding water to make up the moisture content for further fermentation should be earlier than that of the traditional method.

Based on the analysis of moisture contents of rice koji during fermentation at the time of water adding and the relationship between water adding time and final red pigment production, it was decided according to Fig. 3 that the 36th hour after inoculation was the optimal water adding time when using the modified large scale koji maker.

Effects of different methods of water adding

Three different prototypes for effective water sorption of rice koji were tested with the Nagata koji maker, namely, atomized water spray, direct water pouring, and water curtain, respectively. A spray nozzle was applied for atomized water spray, while a water hose was used, instead,

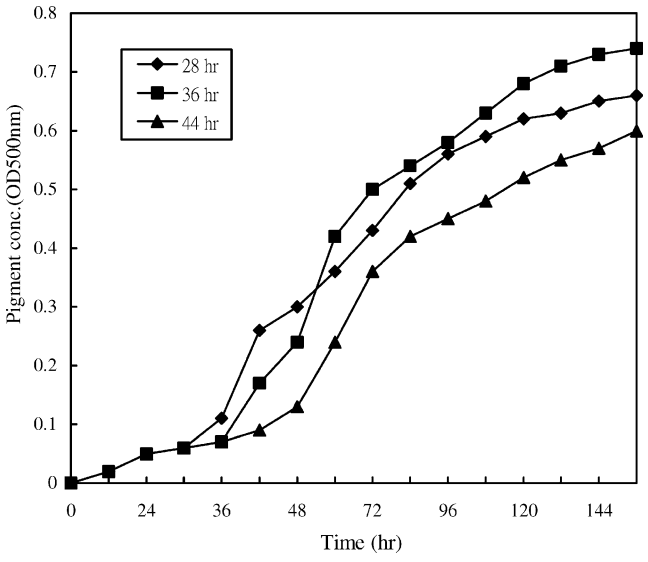

Fig. 3 Effect of water adding time on production of red mold rice pigment

for direct water pouring. However, a self-designed overflow device was employed to provide water curtain when needed. The apparatus consists of a rectangular water container. When water is overfilled, overflow becomes a thin curtain falling down directly to the koji bed. Results of these tests would be used later to modify the original Nagata koji maker as an invention. Water was added at the 36th hour after fermentation began; moisture content of red mold rice koji sampled from different spots of the rotary bed were analyzed. Data shown in Table 4 suggested that the method of water curtain was the most effective way for water adding. Atomized water by spray nozzle only provided atomized water on the surface of koji, not into the bed. Direct water pouring only provided some spots for water to flow through the bed. However, water curtain could provide an even water fall through the whole koji bed and resulted in better water sorption of the koji. However, water collecting and recycling devices under the perforated bed were also needed when using this method as water was added in great excess of the amount absorbed by the koji.

Table 5 Influence of agitation on white rice koji, red pigment production and broken rice percentages

Agitation[a]	White rice koji (%)	Red pigment ($OD_{500\ nm}$)	Broken rice koji (%)
A	3.70±0.28(a)	0.85±0.07(a)	29.10±0.42(a)
B	3.10±0.21(a)	0.90±0.02(a)	30.10±0.35(a)
C	0.90±0.14(b)	1.01±0.14(a)	31.20±0.77(a)

Means±SD followed by different letters in parentheses are significantly different at 5% level by Duncan's Multiple Range Test

[a] *A* Mixing of koji bed co-currently three rounds right after feed in, *B* mixing of koji bed co-currently three rounds 12 h after feed in, *C* mixing of koji bed counter-currently three rounds 12 h after feed in

In addition, this water recycling system functioned, recycling the wash-off fungal filaments back to the koji.

Agitation for reducing white rice koji percentage

White rice koji is the rice grain that is not fermented by *M. purpureus* and is regarded as a major defect of red mold rice production. The most possible reasons for this defect are block formation due to cooked rice agglomeration and loss of moisture. The latter results in hardening of the cooked rice, and it becomes too hard for red mold growth. Influences of factors causing white rice koji could be reduced by improved agitation timing and method. A perforated paddle-type blender capable of both co- and counter-current agitation relative to the direction of bed rotation was applied, which was also a major part of this research. Table 5 showed the effects of various agitations and timings on the reduction of the percentage of white rice koji. Results suggested that one round of bed rotation with counter-current agitation at the 12th hour after fermentation began could harvest red mold rice with higher red pigment content and less percentage of white rice koji as shown in Fig. 4.

Temperature control at the final stage of fermentation

Temperature has been found as the important factor influencing productions of red pigment and other secondary metab-

Table 4 Effect of different methods of water addition to the moisture content of red mold rice koji during solid state fermentation

Water adding methods	Original moisture content (%)	Moisture content (%)		
		1st round[a]	2nd round	3rd round
Atomized water	30.1±1.2(aA)	37.7±1.9(cB)	41.6±2.6(cC)	45.1±2.3(bD)
Direct water pouring	30.0±1.2(aD)	39.8±1.6(bC)	46.5±1.6(bB)	50.0±1.3(aA)
Water curtain	30.3±1.0(aC)	46.1±1.7(aB)	51.2±2.2(aA)	51.6±2.5(aA)

Means±SD followed by different uppercases in the same row and different lowercases in the same column, in parentheses, are significantly different at 5% level by Duncan's Multiple Range Test

[a] Rotating time per round is about 12 min

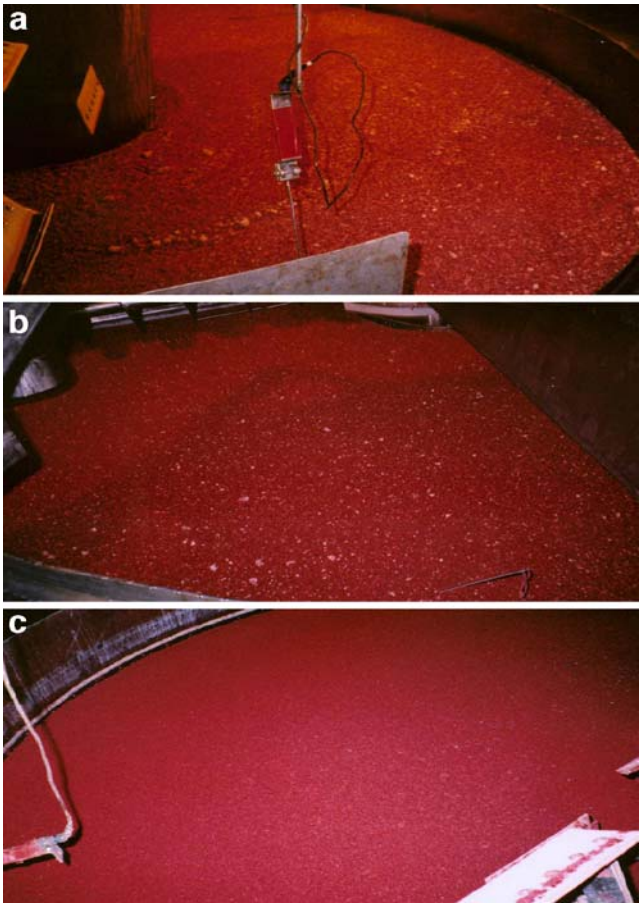

Fig. 4 Reducing white koji using different ways of agitation: **a** mixing of koji bed co-currently three rounds right after feed in, **b** mixing of koji bed co-currently three rounds 12 h after feed in, **c** mixing of koji bed counter-currently three rounds 12 h after feed in

olites, such as monacolin K and citrinin. Literature shows that temperatures at the range of 25–35°C are good for productions of red pigment and monacolin K by *M. purpureus* (Endo 1979). However, in the solid-state fermentation of *M. purpureus* using a large scale koji maker, temperature was kept at 37–38°C for effectively facilitating reproduction and growth. In increasing red pigment and monacolin K in the product, temperature at the final stage of fermentation has to be kept at an optimum. Results were listed in Table 6. When temperatures at the 86th hour till the end of fermentation were maintained at 26, 30, and 34°C, respectively, there was not much significant difference in the productions of red pigment, monacolin K, and citrinin. From the energy point of view, 34°C was accepted as the temperature wherein there is no need in lowering the temperature too much at the final stage.

Discussion

In the traditional process, agitation with hands is needed in flipping over the bottom part of rice koji and removing fermentation heat. Each tray is taken out many times from the fermentation room and soaked in water to maintain the proper moisture content of rice koji. So it is easy to control the temperature and moisture content during fermentation.

However, the traditional method needs large space, high labor costs for koji agitation by hands and water soaking, and a long process time. At the same time, red mold rice is easily contaminated by the open environmental factors, which always results in inconsistent and unsatisfactory quality.

Literature survey on red mold rice production in a closed environment using a koji maker shows no successful cases. The result of this study was attributed to the difficulties of water addition and fermentation heat removal.

Reports on microbial strains used in red mold rice production and functional properties of metabolites were easily found. Most of these works focused on the production capacities of monacolin, pigment, GABA, etc., and their applications as dietary supplements in healthy foods (Endo 1979, 1980; Hawksworth and Pitt 1983, Lee et al. 2005, Wang et al. 2005). So it is a very important issue to develop the process for mass production of red mold rice.

Nagata type koji maker was originally designed to making koji with *Aspergillus* sp. The physiological and morphological characteristics of *Aspergillus* sp. were different from *Monascus* sp., especially on the aspects of moisture need and temperature rising speed of rice koji during solid-state fermentation.

We studied the modification for mass production of red mold rice in a more hygienic and controllable condition with mechanized koji-making facilities in this research. Controls of rice koji moisture content and fermentation temperature, and hence, modification of the koji maker, are

Table 6 Effect of temperature control at the final stage of fermentations on metabolites productions

Temperature[a] (°C)	Glucoamylase (units)	Pigment ($OD_{500\ nm}$)	Monacolin K (ppm)	Citrinin (ppb)
34	135.0±18.2(a)	0.91±0.27(a)	46.5±9.5(a)	616.5±10.8(a)
30	78.0±11.4(ab)	0.87±0.18(a)	53.0±20.3(a)	467.0±85.1(a)
26	48.5±7.0(b)	0.75±0.50(a)	53.5±5.7(a)	331.5±263.6(a)

Means±SD (n=3) followed by different letters in parentheses are significantly different at 5% level by Duncan's Multiple Range Test
[a]Temperature was lowered at the 86th hour to 26, 30, 34°C, respectively, in 4 h and maintained until the end of fermentation

definitely the cores of this research. The ultimate goals are to develop an optimal process for red mold rice mass production with high and consistent quality and low cost to meet the increasing market demand of healthy foods.

In this work, we focused mainly on the mass production of red mold rice using a large scale koji maker which we modified from a commercial one. Compared to the traditional method which needs longer time, more workforce and production space, we had successfully reduced process time, workforce, and space in a compact solid state fermentor. The results indicated that the modifications were promising and potentially useful for industrial application.

Red mold rice production using the modified Nagata type koji maker was concluded feasible with appropriate hardware modifications on water-adding and agitation systems and software preprogramming for optimal temperature and humidity conditions from this research. Further investigation was suggested to integrate with a programmable logic controller/personal computer (PLC/PC)-based on-line man–machine interface (MMI) control system for more automatic and accurate temperature, moisture content, and timing control.

Acknowledgements This research was granted by the National Science Council, Taiwan (NSC 92-2214-E-020-002). Special thanks are due to the Taiwan Tobacco and Liquor Corporation (Pingtung, Taiwan) for providing the equipment and support.

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