Appl Microbiol Biotechnol (2013) 97:8895–8902
DOI 10.1007/s00253-013-5106-4

Fermentation and quality of yellow pigments from golden brown rice solid culture by a selected *Monascus* mutant

Busaba Yongsmith · Panida Thongpradis · Worawan Klinsupa · Withida Chantrapornchai · Vichai Haruthaithanasan

Received: 11 April 2013 / Revised: 3 July 2013 / Accepted: 5 July 2013 / Published online: 4 August 2013

Abstract A single peak (λmax 370) yellow pigment-producing mutant derived from *Monascus* sp. TISTR 3179 was used for the pigment production in solid rice culture. Various factors affecting yellow tones were investigated. Hom-mali rice variety was the best amongst five Thai local varieties used for fungus culture. It was also better than corn, mungbean, soybean, potato, sweet potato, or cassava tubers. The moisture content and temperature were the key environmental factors affecting the color tones of creamy, tangerine, and golden brown rice solid cultures. The golden brown rice culture gave the highest yellow pigment concentration. Under an optimum room temperature of 28–32 °C, an initial moisture content of 42 %, and 7-day-old inoculum size of 2 % (*v*/*w*) the maximum yield at 2,224.63 A_{370}U/gdw of yellow pigment was produced. A mellow yellow powder at 550 A_{370}U/gdw could be obtained using spray-drying techniques. The powder had a moisture content of 5.15 %, a water activity value of 0.398, a hue angle of 73.70 ° (yellowish orange), high lightness (L*) of 74.63, color saturation (C*) of 28.97, a neutral pH of 7.42, 0.12 % acidity and solubility of 0.211 g/10 ml. It was noteworthy that the Chinese fresh noodle with spray-dried yellow powder showed no discoloration during 8-day storage.

Keywords *Monascus* mutant · Yellow pigment · Golden brown yeast rice · Solid culture

B. Yongsmith (✉) · P. Thongpradis · W. Klinsupa
Department of Microbiology, Faculty of Science, Kasetsart University, Chatuchak Bangkok 10900, Thailand
e-mail: fscibus@ku.ac.th

W. Chantrapornchai · V. Haruthaithanasan
Department of Product Development, Faculty of Agro Industry, Kasetsart University, Chatuchak Bangkok 10900, Thailand

Introduction

Yellow is one of the three primary colors along with red and blue. Synthetic yellow pigments permitted for use in food processing are limited to just tartrazine and sunset yellow FCF. The toxicity to health of both pigments is presently still in question, while natural yellow pigments from animals, plants, or microorganisms have gained more favorable consideration in recent years. *Monascus*, a traditional red fungus is used to produce *Monascus* red rice (angkak; ang = red, kak = seed) whose pigments are characterized by multiabsorption peaks (λmax 420, 500 nm) of red, orange, and yellow. However, *Monascus* yellow pigments need further chemical extraction of angkak (Sato et al. 1997; Sweeny et al. 1981) because both monascin (Fielding et al. 1961) and ankaflavin (Manchand et al. 1973) are minor components in the mixture. In our cassava starch-based *Monascus* improvement research program, a secondary mutant of *Monascus* sp. TISTR 3642 which is capable of producing yellow pigments at single peak (λmax 370 nm), was first reported (Yongsmith et al. 1990). It produced the yellow pigment in submerged cultivation using cassava starch and soybean flour as the main substrates (Yongsmith et al. 1994). Its glucoamylase activity on rice solid culture was compared among the color mutants (Yongsmith et al. 2000). Purification of ungkak (ung = yellow, kak = seed) was carried out and three new *Monascus* yellow azaphilones were reported in 2004 (Jongrungruangchok et al. 2004). Strong antimutagenic (Kruawan et al. 2005) as well as antioxidant properties of this ungkak were also investigated. Recently, research worldwide has focused on *Monascus* yellow pigment areas through mutagenesis techniques as well as medium formulation (Zhou et al. 2008, 2009; Yang 2009) or statistical optimization of nutritional and physical parameters (Jirasatid et al. 2013). It is our intention to investigate factors affecting ungkak fermentation regarding

its quality in order to enable development of novel applications in the future.

Materials and methods

Microorganism and cultivation

A yellow pigment-producing mutant, strain TISTR 3642 (formerly known as strain KB20M10.2), selected through UV secondary mutation of *Monascus purpureus* TISTR 3179 (formerly known as strain KB9) was used. The stock culture was maintained on MYS agar.

Solid-state fermentation of *Monascus* sp.

Various local agricultural products such as rice, corn, mungbean, soybean, potato, sweet potato, and cassava tubers were used for solid-state culture of *Monascus* sp. strain TISTR 3642. Experiments were conducted in 500-ml Erlenmeyer flasks containing 100-g substrate. Pretreatments of potato, sweet potato, and cassava tubers involved peeling and cutting into diced pieces of 0.5×0.5×0.5 cm before drying at 60 °C overnight in a hot-air oven. Rice, corn, mungbean, soybean, and all pretreated tubers were soaked overnight. The water-drained substrates (100 g in each 500-ml Erlenmeyer flask) were sterilized at 121 °C for 15 min by autoclaving. Each of the substrates was then allowed to cool and inoculated with 2 % (*v*/*w*) of the inoculums and incubated for 15 days at room temperature (28–32 °C). After incubation, the flasks were steamed for 30 min. The steamed, fermented substrates were dried at 50 °C for 24 h before pulverization and stored before analysis.

A local, non-sticky rice, cultivar (Hom-mali) was selected and used mainly throughout the experiments. The preparation of the rice medium for solid culture was as follows: dehulled broken rice grains were soaked in tap water for 3 h. After the water was removed, the soaked rice grains were drained for 5–10 min and then 100 g were placed in a 500-ml flask, and autoclaved for 15 min at 121.5 °C and cooled to room temperature. Two milliliters of 10^6 spores/ml of *Monascus* molds were inoculated and incubated at room temperature. Unless otherwise indicated, these conditions were maintained throughout the experiment.

Rice preparation variables

Studies on factors effecting yellow pigment production from rice substrate were carried out in 500-ml flask. The following parameters were evaluated: four Thai rice varieties; incubation temperature (20 °C, room temperature of 28–32, 37, and 45 °C); initial moisture contents of the substrate (38, 44, 49, and 58 % *w*/*w*) with distilled water; and with varying initial pH levels in aqueous citric acid solutions (pH2, pH3, pH4).

Estimation of pigment concentration

The pigment concentration was measured by a spectrophotometer (UV-240, Shimadzu, Kyoto, Japan) at 370 nm for yellow pigment. Pigment in fermented rice samples corresponding to 1 g of initial rice substrate was extracted with 39 ml of 50 % ethanol for 3 h on a rotary shaker (300 rpm). The extract was then centrifuged at 7,000×*g* for 15 min to remove suspended solids and the supernatant was analyzed by the spectrophotometer against a 50 % ethanol blank. The moisture content of the rice samples was determined by heating the fermented rice in a hot-air oven overnight at 105 °C and the weight loss was measured. The extracted, yellow pigment yield was expressed as its λmax unit per gram dry weight of fermented matter (A_{370}U/gdw).

Biomass estimation

The growth of *Monascus* culture was estimated by determining the *N*-acetyl glucosamine released by the acid hydrolysis of the chitin present in the fungal cell wall (Van de Loo 1976). In brief, 0.5 g of dry fermented sample was mixed with 1 ml of concentrated H_2SO_4. Acetyl acetone reagent (1 ml) was added to the mixture, which was then placed in a boiling water bath for 20 min. After cooling the mixture down to room temperature, 6 ml of ethanol was added, followed by the addition of 1 ml of Ehrlich reagent (Sigma-Aldrich, Milwaukee, WI, USA) and incubation at 65 °C for 10 min. After cooling the mixture down, the optical density was measured at 530 nm against the reagent blank using *N*-acetyl glucosamine (Sigma) as the standard.

Crude recovery of pigment

Extraction

Forty-five percent (*v*/*v*) ethanol was used for crude pigment extraction (ground brown fermented rice: solvent ratio of 1:100 *w*/*v*) at 50 °C for 75 min. The extracted solution was concentrated; alcohol was evaporated in a water bath at 85 °C (4 h for 500-ml extracted solution) and then yellow water was obtained. The extraction was done in a hot water bath: (Schutzart DIN40050-IP20; Memmert, Germany).

Spray drying

First, 10 % maltodextrin DE10 was added to the yellow water to give a body to the mixture before spray drying. The mixture was then spray-dried at an inlet air temperature of 135–145 °C and an outlet air temperature of 90–100 °C

with a nozzle speed of 25,000 rpm and a nozzle air pressure of 2 kg/cm^2.

Spray-dried yellow powder and quality

The quality of the spray-dried yellow powder was investigated, using the following parameters:

- Moisture content (AOAC, 2000)
- Pigment; measured absorbance at 370 nm (diluted until the absorbance value was within 0.2–0.5). Report in absorbance units × dilution factor;
- Color value in the Commission International de L' Eclairage (CIE) L*C*h system; which is composed of L* or lightness (value ranges from 0 for white to 100 for black), C* or chroma or color saturation, and h or hue angle which ranges from 0 (red), 90 (yellow), 180 (green) to 270 ° (blue) was determined using a colorimeter: model CM-3500d, (Minolta, Japan) after Francis 1998;
- Water activity (a_w) using Novasina model TH200g (Switzerland);
- Total soluble solids;
- pH measurement by a pH meter (TOA model HM-16S Electronics Co. Ltd., Japan);
- Acidity: 1 g of yellow powder was dissolved in 100-ml distilled water, a 10-ml portion of the solution was poured into a 250-ml flask, diluted with distilled water till the color faded and then titrated with a standard solution of 0.1 N NaOH, using phenolphthalein as an indicator which gave a pale pink color at the end point and then the acidity (in percentage) was calculated,

$$\text{Acidity}(\%) = \frac{\text{volume of NaOH(ml)} \times \text{Normality} \times 70 \times 100}{1000 \times \text{weight of yellow powder (g)}}$$

- Solubility: 10 g of yellow powder was dissolved in 100-ml distilled water in a 250-ml beaker, stirred for 90 s; a 10-ml portion of the solution was filtrated onto a filtrate paper (known weight). The filtrate paper was dried at 105 °C, until the weight was constant. Solubility (g/10 ml solution) was then calculated and compared to the weight of the filtrate (in grams) minus the known weight of the filtrate paper (Al-kahtani and Hassan 1990).

Stability study of spray-dried yellow powder and yellow water

A stability study of the spray-dried yellow powder and yellow water was carried out with the same ratio (1:100). A full factorial experimental design was used in this study. The factors studied were pH (3, 5, 7, and 9), processed heat level (control, pasteurized at 75 °C for 30 min, and sterilized at 121 °C for 15 min). Color value and absorbance values were determined.

Chinese noodle processing

Fresh Chinese noodles have a simplified formula, containing only flour, water, and salt at 100, 28, and 3 %, respectively. Ten percent of egg or soybean flour could be added for making yellow colors of egg noodles or soybean noodles (vegetable noodles). Spray-dried yellow powder at 0.5 g of *Monascus* sp. TISTR 3462 can be added in such a Chinese fresh noodles formula to give the noodles a yellow shade. The preparation for each type of noodles was started with mixing the raw materials for 20–40 min, resting the crumbly dough, sheeting the dough into two dough sheets, compounding the two sheets into one, gradually sheeting the dough and slitting each sheet into raw noodle strands of 1.5 mm thickness and 1.5 mm width. All types of noodle (egg and soybean fresh noodle with and without spray-dried powder) were kept at room temperature and color appearance was observed for 8 days.

Results

Factors affecting yellow pigment production on rice solid culture

Local agricultural products comprising rice, corn, mungbean, soybean, potato, sweet potato, and cassava tubers were evaluated for the best substrate for yellow pigment production. The highest yellow pigment production was achieved with rice (1,706.18 A_{370}U/gdw). The other products showed poorer yields, with potato 582.74, sweet potato 541.10, cassava 538.10, mungbean 410.27, corn 327.54, and soybean 24.14 A_{370}U/gdw, respectively (Fig. 1). Hence, rice varieties, either glutinous or non-glutinous types were selected and used for subsequent studies. Hom-mali (jasmine) rice (a non-

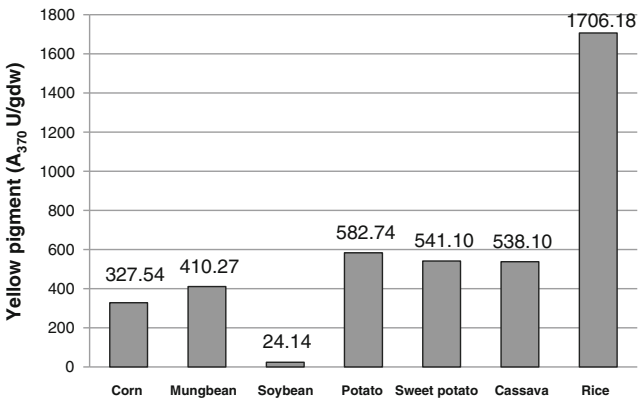

Fig. 1 Yellow pigment yield from different solid substrates incubated for 15 days at room temperature (28–32 °C). Rice, corn, mungbean, and soybean were soaked overnight and drained while potato, sweet potato, and cassava were peeled and cut into dices of 0.5×0.5 cm, each of 100-g substrates was filled in 500-ml flasks then sterilized before fermentation

glutinous type) gave the best yield (1,672.42 A_{370}U/gdw) compared with the others (Keo-ngu, 1,109.70, Sao-hai 380.82, Ta-haeng 303.72 A_{370}U/gdw, respectively) (Table 1). Hom-mali rice was then used for the subsequent experiments. This result agreed with that reported by Steinkraus (1995).

Table 1 shows that at least three key factors affected yellow pigment production by the strain TISTR 3642 in rice solid culture. Firstly, the non-glutinous Hom-mali rice variety was the best substrate to produce maximum yellow pigmentation at room temperature (28–32 °C) incubation compared with the other varieties of Sao-hai, Ta-haeng, or glutinous Keo-ngu. Secondly, an initial moisture content of Hom-mali rice samples of 38, 44, 49, and 58 % gave remarkably different results. An initial moisture content between 38–44 % was found to be suitable for yellow pigmentation with a brown rice color tone while a higher moisture content resulted in lower pigmentation with tangerine tones. Thirdly, when the initial pH ranged from acidic to neutral (pH 2.80, 5.05, 5.31, and 6.18) a similar brown rice tone was produced after 15 days of incubation at room temperature. However, after extraction, the concentration of yellow pigments was found maximum in a weak acidic pH (pH 5.31–6.18).

Time course of growth and yellow pigment production of *M. purpureus* TISTR 3642

Figure 2 shows the results of the analysis of the 15-day course of growth and yellow pigment production of *M. purpureus* TISTR 3642 on Hom-mali rice solid cultivation at room temperature (28–32 °C). Nearly 40 % (*w*/*w*) initial moisture content of rice substrate promoted growth of this yellow mutant. The mold grew quickly up to the eighth day of cultivation and slowed down after that. Yellow pigments were not seen during the first 2 days of cultivation, whereas its production appeared after 3 days of cultivation and increased rapidly after 5 days of cultivation along with increased humidity, both of which reached their respective maxima of 2,224.63 A_{370}U/gdw and 48.98 % (*w*/*w*) respectively, while the initial pH at 6.42 slowly decreased to its lowest value of 4.12 at 15 days of incubation. A light reddish-brown rice culture appeared at 7 days of incubation

Table 1 Factors affecting on yellow pigment production of *M.kaoliang* KB20M10.2 on rice solid cultures

Factor	Moisture content		pH		Yellow pigments	
	Initial	Final	Initial	Final	A_{370} U/gdw	Tone
Rice variety[a,c]						
Non-glutinous rice						
Hom-mali	38.87	47.86	6.20	3.84	1,672.42	Brown
Sao-hai	33.50	35.72	6.19	5.38	380.82	Tangerine
Ta-haeng	30.29	31.00	6.21	5.78	303.72	Tangerine
Glutinous rice						
Keo-ngu	37.55	45.57	6.13	3.92	1,109.70	Brown
Incubation temperature (°C)[a,b]						
20	35.02	36.51	6.00	4.62	110.26	Light brown (creamy)
Room temperature (28–32)	35.02	49.30	6.00	4.03	1,451.73	Brown
37	35.02	47.68	6.00	5.04	49.85	Tangerine
45	35.02	24.66	6.00	4.21	ND	Tangerine
Water addition (ml)[b,c]						
None	38.02	49.30	6.30	3.93	1,451.73	Brown
10	43.73	59.26	6.31	3.99	2,106.19	Brown
20	48.75	68.30	6.31	4.48	441.65	Tangerine
30	58.05	64.62	6.31	5.10	141.90	Tangerine
Soaking solution (citric acid)[a,b,c]						
pH2	37.31	43.94	2.80	2.71	809.79	Brown
pH3	39.22	46.55	5.05	4.18	1,526.72	Brown
pH4	36.61	47.89	5.31	4.29	2,118.68	Brown
Control (pH 6 with water)	34.53	44.72	6.18	4.36	1,584.53	Brown

[a] Rice variety substrates were soaked, drained and fermented without water addition at room temperature.

[b] Only Hom-mali rice was used.

[c] Incubation was at room temperature (28–32 °C)

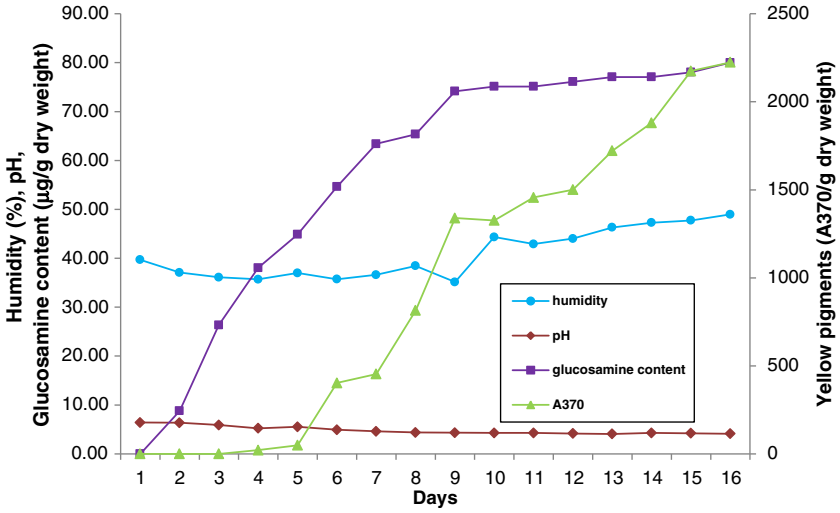

Fig. 2 Fermentation time course of *Monascus* yellow mutant TISTR 3642 on rice solid cultivation

and turned to a darker golden brown rice culture at 14 days of incubation at room temperature.

Crude recovery of yellow pigments and application

Since brown rice solid culture produced the highest yellow pigment yield, it was then used for crude extraction of the yellow pigment. Pigment powder of a mellow yellow shade with a yield of about 550 A_{370}U/gdw was obtained using spray-drying technique. The yellow powder had a color value in the CIE system for L*C* and h of 74.63, 28.97, and 73.70 °, respectively. The yellow powder was added at 0.5 % (*w*/*w*) to the Chinese noodles formula. The resulting noodles showed satisfactory yellowish shade appearance with no browning of fresh noodles for 1 week at room temperature, which was four times longer than the control of fresh noodles (Table 2).

Spray-dried yellow powder and its quality

Pigment powder of a mellow yellow shade with a yield of about 550 A_{370}U/gdw was obtained. The quality of spray-dried yellow powder compared to the quality of the yellow water is shown in Table 3.

Table 3 shows that the spray-dried yellow powder had a moisture content of 5.15 %, a water activity value of 0.398, a color h value of 73.70 ° (yellowish orange), a high lightness (L*) of 74.63, and color saturation (C*) of 28.97. Compared with the yellow water, which had a reddish orange color (h value of 40.50 °), higher color saturation (C* of 41.45), but much lower lightness (L* of 15.71), the total soluble solids of the spray-dried yellow powder were higher than those of yellow water by 10 % which probably resulted from the addition of maltodextrin in the extract prior to spray drying. Both the spray-dried yellow powder and the yellow water had a neutral pH (7.42 and 6.62, respectively), their acidity was 0.12 and their solubility was 0.200–0.211 g/10 ml. Table 4 shows the color value and absorbance of the color solution under different pH conditions (pH 3–9). This yellow powder

Table 2 Color appearance of Chinese fresh noodles with and without spray-dried yellow powder (SYP)

	Color appearance (days)							
	1	2	3	4	5	6	7	8
Non-SYP fresh noodles								
Egg noodles	Y	Y	B	B	DB	DB	DB	DB
Soybean noodles	Y	Y	B	B	DB	DB	DB	DB
SYP fresh noodles								
Egg noodles	Y	Y	Y	Y	Y	Y	Y	Y
Soybean noodles	Y	Y	Y	Y	Y	Y	Y	Y
Non-egg, non-soybean noodles	Y	Y	Y	Y	Y	Y	Y	Y

Y yellow, *B* brown, *DB* dark brown

Table 3 Comparative quality of spray-dried yellow powder and yellow water

Quality parameter	Spray-dried yellow powder	Yellow water
Moisture content (%)	5.15	ND
Pigments (A_{370}U/gdw or A_{370}U/ml)	550.0	2,082
Color value L*	74.63	15.71
a*	8.13	31.52
b*	27.81	26.92
C*	28.97	41.45
h°	73.70	40.50
a_w value	0.398	ND
Total soluble solids (%)	13.25	3.26
pH	7.42	6.62
Acidity (%)	0.12	0.12
Solubility (g/10 ml)	0.211	0.200

ND not detected

Table 4 Color value (L*, C*, h°) and absorbance (abs) value of color solution (ratio yellow powder or yellow water/water=1:100) under different pH conditions

Yellow powder/water=1:100					Yellow water/water=1:100				
pH	L*	C*	h°	abs	pH	L*	C*	h°	abs
3	72.54	34.96	90.01	730	3	23.91	52.67	51.12	2,246
5	71.78	35.57	88.34	848	5	19.43	46.34	46.00	2,274
7	66.66	40.34	79.23	532	6.62*	15.72	41.46	40.51	2,062
7.42*	66.34	40.31	78.98	556	7	16.93	45.47	39.65	2,082
9	65.44	45.91	77.81	564	9	6.04	30.40	19.70	2,232

*Actual pH of color solution

showed good resistance to pH but moderate resistance to temperature (Table 5).

The pH level did affect the color value and the absorbance of the color solution. The yellow powder, solution (yellow powder/water ratio of 1:100) had a neutral pH (7.42) and its lightness tended to decrease as the pH increased, in contrast to the color saturation. Hue angle values were in the range of 77–90 ° (which were in the first quadrant, 0 ° means redness and 90 ° means yellowness), so that the color solution from the yellow powder was orange yellowish. The absorbance result showed that the acidic solution had a higher absorbance value than that of a basic solution.

The yellow water solution diluted with water (1:100) had a pH value of 6.62 and an orange-reddish color (h value of 19–51 °). Its lightness and chroma tended to decrease as the pH increased. The absorbance was lower at a neutral pH and tended to increase as the pH moved away from neutral (to either more acidic or more basic). Moreover, its absorbance value was much higher compared to that of the yellow powder solution.

The color value (L*, C*, h) in Table 5 revealed that a heat-processed condition did not have much effect on the color value of the color solution, but had more effect on the absorbance. The absorbance of the color solution from the yellow powder decreased as the solution was processed under either pasteurization or sterilization. On the contrary, under heat-processed conditions, the color solution from the yellow water tended to have a higher absorbance value.

Discussion

Normally, yellow, orange and red pigments are present as a mixture in *Monascus* culture having a reddish shade with absorption maxima at 400 or 420 and 500 nm in both the submerged culture and the solid-state culture (Table 6). Both red and yellow *Monascus* preparations are commercially available. However, multiple steps of solvent extraction and chemical modification are needed to obtain the yellow fraction (Fielding et al. 1961; Manchand et al. 1973). In contrast, our studies have led to the production of yellow pigments at a single absorption maximum by a specific strain of *Monascus* spp. Previously, our study in 1993 found that under stressed conditions of low initial pH (pH 4.0 or 2.5) and a certain medium formulation, the production of orange-red pigments having absorption maxima at 370, 420, and 500 nm by a wild type *Monascus barkari*, the wild type could be changed to that of yellow pigment with a single absorption maximum at 370 or 330 nm without orange and red pigments (Yongsmith et al. 1993). However, studies on this particular strain of *M. barkari* have been discontinued due to its pH-dependent property which made it impossible to scale up yellow pigment production.

Later in 1994, we reported another mutant strain TISTR 3642, which was formerly known as KB 20 M10.2 (a second-generation mutant), which produced yellow pigment with the predominating single absorption maximum at 370 nm in a similar optimal medium at various pH levels ranging from 2.5 to 7.0. The maximum concentration of pigments was obtained in the broth of TISTR 3642 (693 A_{370}U/ml). It seemed that the wild-type strain (*Monascus* sp. TISTR 3179) and its first generation mutant (TISTR 3643) produced high amount of such red pigments in the nitrogen-rich medium, whereas the second-generation mutant (TISTR 3642) could exclusively produce the yellow pigments in the same nitrogen-rich medium in wide pH ranges (2.5–7.0). Therefore, this phenomenon of yellow pigment production of strain

Table 5 Color value (L*, C*, h°) and absorbance (abs) value of color solution (ratio yellow powder or yellow water/water=1:100) under different heat-processed conditions

Heat-processed condition	Yellow powder/water=1:100				Yellow water/water=1:100			
	L*	C*	h°	abs	L*	C*	h°	abs
Pasteurization (75 °C 30 min)	66.01	36.07	81.49	382	18.45	45.67	43.86	2,336
Sterilization (121 °C 15 min)	65.22	37.22	80.44	436	16.28	41.69	42.04	2,264
Control	66.34	40.31	78.98	566	15.71	41.46	40.51	2,062

Table 6 Characterization of *Monascus* yellow pigments

Strain	λ_{max} (nm)	Purified yellow pigment	Formula	M.W.	λ_{max} (nm)	References
M. purpureus	400, 500	Monascin	$C_{21}H_{26}O_5$	358	370	Fielding et al. 1961
		Ankaflavin	$C_{23}H_{30}O_5$	386	370	Manchand et al. 1973
Monascus anka U-1	400, 500	Xanthomonascin	$C_{21}H_{24}O_7$	389	460	Sato et al. 1992
M. barkeri	330,370	Unknown	ND	ND	ND	Yongsmith et al. 1993
Monascus kaoliang KB 20 M10.2 (or strain TISTR 3642)	370	Monascusone A Monascusone B	$C_{13}H_{18}O_5$ $C_{17}H_{18}O_5$	277.1 303.1	243,346 243,375	Jongrungruangchok et al. 2004
		Monascin	$C_{21}H_{26}O_5$	358	370	
		FK 17-P2b2	$C_{21}H_{16}O_4$	236.3	376	
M. anka	410, 510	Unknown	ND	ND	ND	Zhou et al. 2009
M. purpureus TISTR 3514	400, 500	Unknown	ND	ND	ND	Jirasatid et al. 2013

ND Not detected

TISTR 3642 was proven to be independent of environmental factors such as the medium formulation or pH and is believed to be strain-specific (Yongsmith et al. 1994).

In the current study, we grew the strain TISTR 3642 on rice solid culture which resulted in the fermented rice showing a variety of pigment tones, with creamy, orange, and golden brown colors obtained (Fig. 3a). Maximum yellow pigments of more than 2,000 A_{370}U/gdw were found in golden brown rice using Thai Hom-mali rice fermented under optimal conditions: 43 % (*w*/*w*) initial moisture content, room temperatures of 28–30 °C and an initial neutral pH. While, the lowest yield of yellow pigments (110.26 A_{370}U/gdw) was obtained in creamy rice with a 20 °C incubation temperature, higher incubation temperatures at 37–45 °C, as well as higher initial moisture content levels of 49–58 % (*w*/*w*) turned the fermented rice to an orange tone with the yellow pigments concentration below 500 A_{370}U/gdw. The following fermentation time course of yellow pigment production in golden brown rice for 2 weeks incubation at room temperature (28–32 °C) under static flask culture revealed that yellow pigments appeared after 3 days of incubation, gradually increased and reached 2,000 A_{370}U/gdw around 14 days of incubation (Fig. 2). Its crude CH_2Cl_2 extract, sequentially subjected to Sephadex LH-20 and silica gel chromatography, yielded two new yellows, monascusones A and B, together with two known yellow compounds, monascin and FK 17-P2b2 (Jongrungruangchok et al. 2004). Characteristics of *Monascus* yellow pigments are presented in Table 6. It showed that our new yellow pigments, monascusones A and B having lower molecular weight as well as lower absorption maxima than those of monascin, ankaflavin, or xanthomonascin reported by other researchers (Fielding et al. 1961; Manchand et al. 1973; Sato et al. 1992; and Zhou et al. 2009). Our *Monascus* sp. TISTR 3642 which produced all four types of yellow pigments showed an absorption maximum at a single peak of 370 nm obtained from either submerged fermentation (Yongsmith et al. 1994) or solid-state fermentation of this study. The spray-dried powder of the crude ethanol extract from golden brown rice showed its mellow yellow color of 550 A_{370}U/gdw (Fig. 3b) with its water soluble property, and could be thus applied to fresh noodle processing at 0.5 % (*w*/*w*) with a good anti-browning property (Table 2). The results showed that the spray-dried yellow powder had antioxidative potential which also agreed with our group who found that the major purified yellow monascusone A showed strong antioxidative activity by the ferric reducing antioxidant power method (data not provided). Aniya et al. (1998) and Zochling et al. (2002) also found that red yeast rice powder extract contained antioxidatives by using the lipid radical 1,1 diphenyl 2-picrylhydrazyl. Furthermore, the yellow colors themselves showed no mutagenic activity but rather strong antimutagenic activity, inhibiting (>60 % inhibition) the mutagenicity of either

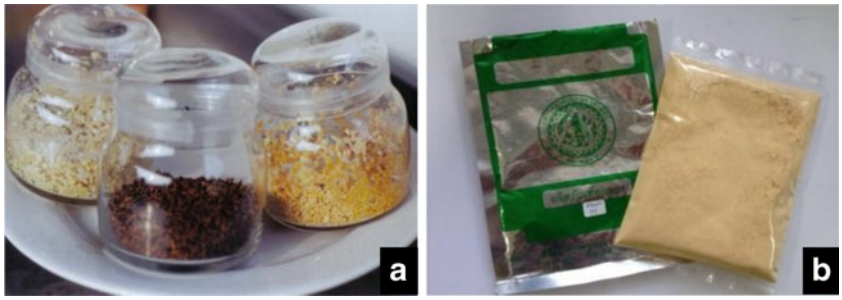

Fig. 3 *Monascus*-fermented rice for yellow pigments by *Monascus* sp. TISTR 3642. **a** Creamy, tangerine, and golden brown fermented rice by *Monascus* sp. TISTR 3642. **b**. Spray-dried yellow powder and crude ethanol extract from golden brown rice on aluminum package

nitrite-treated 1-amino pyrene or nitrite-treated fish extract on both *Salmonella typhimurium* strains TA98 and TA100 (Kruawan et al. 2005).

In conclusion, to our knowledge, this paper is the first report on the optimization of golden brown *Monascus* rice production as a source of natural yellow pigments showing its single absorption maximum at 370 nm (UVA absorbing ability) instead of the multi higher absorption maxima of the YOR mixture at 400 and 500 nm by other reports or a commercially purified oil-soluble yellow pigment, xanthomonascin, with a single absorption maximum at 460 nm in oil solution. The spray-dried yellow powder from our study had a moisture content of 5.15 %, a water activity value of 0.398, a hue angle of 73.70 ° (yellowish orange), high lightness (L*) of 74.63, color saturation (C*) of 28.97, a neutral pH of 7.42, 0.12 % acidity and solubility of 0.211 g/10 ml. The results from our investigation and those reported by Jongrungruangchok et al. (2004) and Kruawan et al. (2005) confirmed that yellow pigments of *Monascus* sp. strain KB20M10.2 or strain TISTR 3642 are not toxic. Another UV-absorbing compound, FK17-P2b2, extracted from *Aspergillus* sp. has been patented as an additive in cosmetics (Takayuki and Hiroshi 1994). It is believed that our yellow pigments with excellent in UV-absorbing ability are very useful for the protection of food products (fresh Chinese noodles) or non-food products, such as cosmetics, from deterioration. Future development on golden brown *Monascus* rice should focus on strain improvement for higher production of yellow pigments as well as process scaling up. At present, protoplast fusion of a yellow pigment-producing *Monascus* sp. TISTR 3642 and a fast-growing *Monascus* white mutant (TISTR 3644) has been undertaken successfully. Hopefully, the production of golden brown *Monascus* rice can be commercialized and result in a variety of applications.

Acknowledgments This research work was financially supported by the Kasetsart University Research and Development Institute and the Center for Advanced Studies in Tropical Natural Resources, National Research Universities.

References

Al-kahtani A, Hassan H (1990) Spray drying of roselle (*Hibicus Sabdariffa* L.) extract. J Food Sci 55(4):1073–1076

Aniya Y, Yokomakura T, Yonamine M, Nagamine T, Nakanishi H (1998) Protective effect of the mold *Monascus anka* against acetaminophen-induced liver toxicity in rats. Jpn J Pharmacol 78(1):79–82

Fielding BC, Holker JSE, Jone DF, Powell ADG, Richman KW, Robertson A, Whalley WB (1961) The chemistry of fungi. Part XXXIX. The structure of monascin. J Chem Soc 1961:4579–4589

Francis FJ (1998) Color analysis. In: Nielsen SS (ed) Food Analysis, 2nd edn. Aspen Publishers Inc, Maryland, USA, pp 599–612

Jirasatid S, Nopharatana M, Kitsubun P, Vichitsoonthonkul T, Tongta A (2013) Statistical optimization for monacolin K and yellow pigment production and citrinin reduction by *Monascus purpureus* in solid-state fermentation. J Microbiol Biotechnol 23(3):364–374

Jongrungruangchok S, Kittikoop P, Yongsmith B, Bavavoda R, Tanasupawat S, Lartpornmatulee N, Thebtaranonth Y (2004) Azaphilone pigments from a yellow mutant of the fungus *Monascus kaoliang*. Phytochem 65:2569–2575

Kruawan K, Kangsadalampai K, Yongsmith B, Sriyapai T (2005) Effects of *Monascus* colorants on the mutagenicity of nitrite treated 1-aminopyrene using Ames test. Thai J Pharmal Sci 29(1–2):29–41

Manchand PS, Whalley WB, Chen FH (1973) Isolation and structure of ankaflavin: a new pigment from *Monascus anka*. Phytochem 12:2531–2532

Sato K, Iwakami S, Goda Y, Okuyama E, Yoshihira K, Ichi T, Odake Y, Noguchi H, Sankawa U (1992) Novel natural colorants from *Monascus anka* U-1. Heterocycles 34(11):2057–2060

Sato K, Goda Y, Sakamoto SS, Shibata L, Maitami T, Yamada T (1997) Identification of major pigments containing D-amino acid units in commercial *Monascus* pigments. Chem Pharm Bull 45:227–229

Steinkraus KH (1995) Miscellaneous oriental fermentations, Chinese soy sauce, pastes and related fermented foods. In: Steinkraus KH (ed) Handbook of indigenous fermented foods, 2nd edn. Marcel Dekker, Inc., New York, pp 626–627

Sweeny JG, Estrada-Valdes MC, Lacobucci GA, Sato H, Sakamura S (1981) Photoprotection of the red pigments of *Monascus anka* in aqueous media by 1,4,6-trihydroxy naphthalene. J Agric Food Chem 29(6):1189–1193

Takayuki A, Hiroshi S (1994) Novel UV-absorbing compounds TK17-P2a, FK17-P2b1, FK17-P2b2, and FK17-P3 and manufacture of the compounds with *Aspergillus* sp., Patent No. JP6329576.

Van de Loo HM (1976) An improved method for the quantitative determination of hexosamines according to Elson and Morgan. Anal Biochem 76:556–560

Yang CL (2009) Fermentation conditions for partial yellow, high color value red rice. Fujian J Agric Sci 4:361–364

Yongsmith B, Tabloka W, Krairak S, Bavavoda R (1990) Cassava fermentation of yellow pigments and amylolytic enzymes by a mutant of *Monascus* spp. in submerged cultivation. Microb Uti Renewable Resour 7:354–363

Yongsmith B, Tabloka W, Yongmanitchai W, Bavavoda R (1993) Culture conditions for yellow pigment formation by *Monascus* sp. KB10 grown on cassava medium. World J Microbiol Biotechnol 9:85–90

Yongsmith B, Krairak S, Bavavoda R (1994) Production of yellow pigment in submerged culture of a mutant of *Monascus* sp. J Ferment Bioeng 78:223–228

Yongsmith B, Kitpreechavanich V, Chitradon L, Chaisrisook C, Budda N (2000) Color mutants of *Monascus* sp. KB9 and their comparative glucoamylases on rice solid cultures. J of Mol Cat B Enzym 10:263–272

Zhou B, Pu YW, Zhu MJ, Liang SZ (2008) Effect of nitrogen sources on *Monascus* yellow pigment production by *Monascus* mutant. Mod Food Sci and Technol 2:123–127

Zhou B, Wang J, Pu Y, Zhu M, Liu S, Liang S (2009) Optimization of culture medium for yellow pigments production with *Monascus anka* using response surface methodology. Eur Food Res Technol 228:895–901

Zöchling S, Murkovic M, Pfannhauser W (2002) Effects of industrially produced flavours with pro- and antioxidative properties on the formation of the heterocyclic amine PhIP in a model system. J Biochem Biophys Method 53:37–44