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Microbial Bioconversion of Rice Broken to Food Grade Pigments

[1]R. Vidyalakshmi, [1]R. Paranthaman, [2]S. Murugesh and [1]K. Singaravadivel

[1]Indian Institute of Crop Processing Technology, Thanjavur-613 005, Tamil Nadu, India
[2]SASTRA University, Thanjavur, Tamil Nadu, India

Abstract: Solid-state fermentation was carried out using rice broken as a substrate for the production of pigments using a fungal culture of *Monascus ruber* MTCC2326. Red, orange and yellow pigments were produced. The pigments were further purified, analysed and applied in the preparation of flavoured milk. On the sensory evaluation of the flavoured milk, the application of pigments was found to be acceptable. In this microbial bioconversion, in addition to food grade pigments, other compounds of significant importance were also identified.

Key words: Rice · Pigment · *Monascus*

INTRODUCTION

In the recent years, colouring of food with pigments produced from natural sources is of worldwide interest and is gaining importance. These pigments are looked upon for their safe use as a natural food dye in replacement of synthetic ones because of undesirable toxic effects including mutagenicity and potential carcinogenicity. Though many natural colors are available, microbial colorants play a significant role as food coloring agent, because of its flexibility in production and easy down streaming process. Among the various pigment-producing microorganisms, *Monascus* is reported to produce non-toxic pigments, which can be used as food colorant. Besides a colouring agent it enhances the flavour of the food and acts a food preservative.

Natural raw materials and by-products of industry have wide use as culture media infermentation processes because of their low cost since the medium components can represent from 38 to 73% of the total production cost [1]. Rice is one of the major agricultural product of economic importance. This primary product could serve as the sustainable raw material for secondary value-added products through fermentation of *Monascus* molds. Rice broken can be utilized for the production of these useful microbial metabolites at an inexpensive manner and can be applied to varying food products. In case of red pigment production by *Monascus* sp., the cost of the culture medium cost is certainly a significant fraction of the total cost, because of the low cost of the product and the lack of purification. The raw fermentation medium of semisolid cultures of *Monascus* sp. in rice, for example, is used directly as the red pigment in foods.

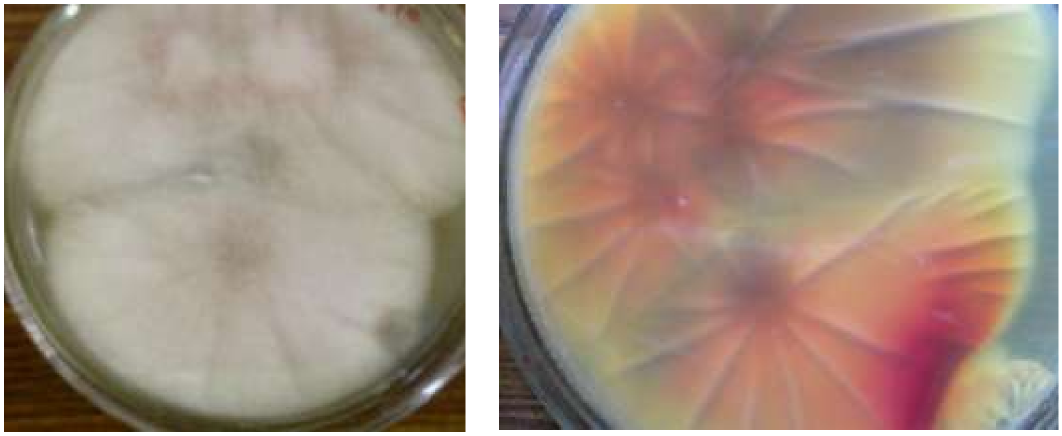
MATERIALS AND METHODS

Substrate: Rice brokens purchased from the local market of Thanjavur, Tamil Nadu, India was used as the substrate.

Inoculum: *Monascus ruber* MTCC 2326 (Fig. 1) obtained from Microbial Type Culture Collection Centre, Chandigarh, India and mass multiplied in the laboratory of Paddy Processing Research Centre, Thanjavur, Tamil Nadu, India was used for the production of pigments. *Monascus ruber* MTCC 2326 maintained in Potato Dextrose Agar (PDA) slants was sub-cultured in yeast phosphate soluble starch medium which composed of yeast extract - 4.0 g, soluble starch - 15.0 g, K_2HPO_4 - 1.0 g, KH_2PO_4 - 1.0 g, agar - 20.0 g, distilled water - 1000 ml, pH 6.5, was used as inoculum medium (Fig. 2).

Microbial Bioconversion of Rice Brokens for the Production of Pigments: Rice brokens 100 g was soaked overnight in 250 ml tap water. Water drained rice was sterilized at 121°C for 30 min by autoclaving. It was allowed to cool and inoculated with 10 % of the inoculum and incubated for 15 days at room temperature. After incubation the flasks were sterilized at 121^0C for 30 minutes by autoclaving. The sterilized Monascus fermented rice (MFR) was dried at 50°C for 24 hours. Dried MFR was pulverized in an Udy cyclone mill and stored for further analysis.

Corresponding Author: R. Vidyalakshmi, Indian Institute of Crop Processing Technology, Thanjavur-613 005, Tamil Nadu, India



Dorsal view

Ventral view

Fig. 1: *Monascus ruber* MTCC 2326

Fig. 2: Growth of M. ruber MTCC 2326 on Yeast Phosphate Soluble Starch Medium

Determination of Pigments: MFR powder 0.5 g was extracted with 80 % ethanol and the final volume was made up to 50 ml. The mixture was kept on a rotary shaker at 200 rpm for one hour. Then it was centrifuged for 15 min at 10000 rpm and the supernatant was collected for pigment analysis.

Pigment estimation was done as described by Tseng [2] in which the optical density at its absorbance maxima were expressed as the concentration of pigment produced. The absorbance maxima of pigment extract was measured by spectral analysis at 510 and 410 nm to analyze the red and yellow pigments respectively using a double beam spectrophotometer (Shimadzu UV 1601), taking into consideration the dilution factor of the sample OD at its maxima, per gram dry fermented matter [3].

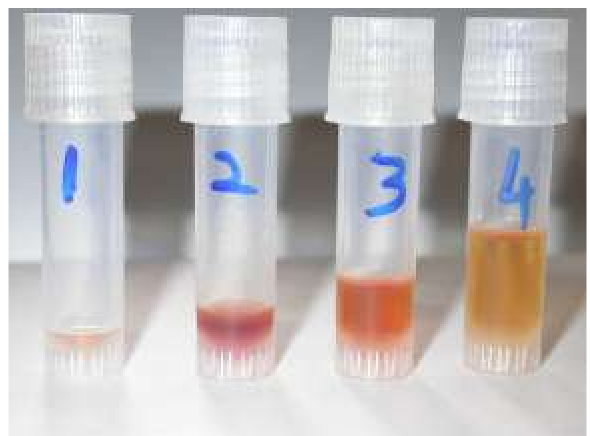


Fig. 3: Purification of Monascus pigments

Purification of Pigments Using Column Chromatography: The pigments were purified by silica gel (60-120 mesh) column chromatography. The column (10 x 210 mm) was packed with silica gel by slurry method. The column was pre eluted using the solvent chloroform: ethanol (9:1 v/v). Then 0.1 g powdered MFR sample was added and it was eluted using the same. The different fractions of the pigment were collected (Fig. 3). The column purified red pigments were further purified by prep-thin layer chromatography (TLC), by elution with a solution of chloroform: ethanol: water (65: 25: 4), red spots were recovered and dissolved in ethanol and then used for further studies.

HPTLC Separation of Pigments: Ethyl acetate 2 ml was added to 0.5 g of sample, vortexed and filtered through Whatman No.40 filter paper. Then it was again filtered through membrane filter and the extract collected was analysed in HPTLC. 5µl of concentrated crude extracts were applied on a pre-coated silica gel plate of size 20 x 10 cm (MERCK 60F 254), using CAMAG Automatic TLC applicator. The chromatograms were developed with mobile phase benzene:methanol:chloroform::30:10:9 and the developed plates were air dried. The plates were scanned at 256nm UV lamp using CAMAG TLC (model) Autoscanner with WINCATS software.

Nutrient Analysis of MFR: The moisture content, protein, crude fiber, ash, fat and starch content of the red yeast rice powder was determined by the procedures of AOAC [4]. MFR was also analysed for its various components in GC-MS.

Application of MFR in Food: MFR 1.2 % was used in the preparation of flavoured milk. The flavoured milk was subjected to sensory evaluation by a panel of ten members. Appearance, colour, taste, flavour and overall acceptability of flavoured milk were evaluated using score card.

RESULTS AND DISCUSSION

The MFR (Fig. 4) yield obtained was 44.51%. On analysis of the ethanol extract of MFR using UV Spectrophotometer 0.202 and 0.330 U/g MFR, for red and yellow pigments were obtained. The MFR produced when analysed for its pigment composition, using HPTLC, colour bands were distinguished based on their Rf values. Red (Rf 0.2), orange (Rf 0.7) and yellow (Rf 0.8) bands were visible. In this study, orange and yellow pigments were found to be maximum than red pigments.

The utilization of rice carbohydrate by *Monascus* for its metabolism and production of the secondary metabolite namely the pigment has also resulted in an increased protein and crude fibre content of MFR (Table 1). A protein content of 17.16 %, 6.71 % crude fibre,

Fig. 4: Monascus fermented rice (MFR)

Fig. 5: MFR incorporated flavoured milk

Table 1: Nutrient analysis of MFR

S.No.	Nutrient	Raw substrate (%)	MFR (%)
1	Carbohydrate	72.00	25.39
2	Protein	12.00	17.16
3	Crude fibre	0.80	6.71
4	Fat	1.41	1.98
5	Ash	0.66	1.65

Table 2: GC-MS analysis of MFR

S.No.	Compounds present in MFR	Molecular formula	Molecular weight	Retention Time	Property
1	Dehydromevalonic lactone	$C_6H_8O_2$	112	7.68	Fragrance
2	2-furancarboxaldehyde, 5(hydroxymethyl)	$C_6H_6O_3$	126	9.09	Antimicrobial
3	Palmitic acid	$C_{16}H_3O_2$	256	26.14	Antioxidant
4	Oleic acid	$C_{18}H_{34}O_2$	282	26.59	Medicinal

Table 3: Sensory evaluation of flavored milk incorporated with MFR

S.No.	Parameters	Average Grade
1	Appearance	8.5
2	Color	8.2
3	Taste	8.7
4	Flavor	7.9
5	Overall Acceptability	8.3

1.98 % fat and 1.65 % ash was found to be present in MFR. Xu [5] reported that 8 to 10% of protein was obtained when corn was used as substrate. Rasheva [6] reported that 0.5 to 12% of protein was obtained when milk permeate was used as substrate for fermentation. Heber [7] reported that *Monascus* fermented rice is a low fat product and it reduces the cholesterol levels. Fermentation resulted in the formation of glutamic acid as the major amino acid in MFR. On GC-MS analysis of MFR, four major components *viz*., dehydromevalonic lactone, 2-furancarboxaldehyde, 5(hydroxymethyl), Palmitic acid and oleic acid were detected, the properties of which are given in Table 2.

Food products gain more intense and stable red color and improved organoleptic characteristics when *Monascus* pigment is used. Incorporation of MFR for coloring flavored milk (Fig. 5), showed appealing color, appearance and was overall acceptable (Table 3). The pigment distributed evenly in the food product giving a pleasing appearance. Moreover application of the natural pigment promotes consumers health protection and allows manufacturing fully natural food without any synthetic additives [8]. Red yeast rice is generally regarded as a safe product.

CONCLUSION

The interest in red pigments produced by *Monascus* sp. in the food industry has been growing because of their wide application in meat, fish, ketchup, wine, etc. and also due to the carcinogenic and teratogenic effects of some synthetic colorants, like nitrosamines formed from nitrites and nitrates in cured meats. Food products treated with red yeast rice extract are thought to have nutritional and pharmacological benefits. According to the American Heart Association, due to elevated level of cholesterol, more than one million people die each year of cardiovascular diseases. The available medications are very expensive posing side effects. Red yeast rice is a natural colourant, containing naturally produced monacolins which has the ability to reduce LDL-cholesterol and increase HDL-cholesterol thus improving heart health, besides a food colourant.

REFERENCES

1. Schmidell, W., 2001. Microrganismos e meios de cultura para utilização industrial. In: Schmidell, W., Lima, U.A., Aquarone, E. and Borzani, W, Biotecnologia Industrial. Editora Edgard Blucher Ltda, Sao Paulo.
2. Teng, S.S. and W. Feldheim, 2001. Anka and anka pigment production. Journal of industrial microbiology and biotechnology, 26: 280-282.
3. Johns, M.R. and D.M. Stuart, 1991. Production of pigments by *Monascus purpureus* in solid culture. J. Ind. Microbiol., 8: 23.
4. AOAC. 2000. Official methods of analysis. 17^{th} edition. AOAC International, USA
5. Xu Gangrong, Chen Yun and Y.U. Huiling, 2002. Prevention of pigment interference in HPLC analysis of *Monascus* citrinin and monacolins. Food and Fermentation Industry, 28: 59-64.
6. Rasheva, T., J.N. Hallet and A. Kujumdizeiva, 1998. Isolation and characterization of mutants from *Monascus purpureus,* 9^{th} Congress of Bulgarian Microbiologists Sofia, pp: 15-17.
7. Heber, D., Ian Yip, Judith Ashley, David Elashoff, Robert Elashoff and W.G. Vay Liang, 1999. Cholesterol lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. American Journal of Clinical Nutrition, 69: 231-236.
8. Maria Barnova, Pavel Mala, Olga Burdova, Michal Hadbavny and Gabriela Sabolova, 2004. Effect of natural pigment of Monasus purpureus on the organoleptic characters of processed chesses. Bull. Vet. Pulawy, 48: 59-62.