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Stimulation of *Monascus* Pigments by Intervention of Different Nitrogen Sources

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Abstract: Traditionally *Monascus* sp. are cultivated on steamed non-glutinous rice and the fermented product is used as a food colourant. The study was conducted with the aim to determine the pigment yield of *Monascus ruber* by the solid-state fermentation of rice supplemented with different nitrogen sources. Rice was added with 0.5 % of organic and inorganic nitrogen sources, sterilized, inoculated with *Monascus ruber* and allowed for solid state fermentation. In this study, mono sodium glutamate was found to yield higher pigment of 0.464 (OD_{510}) and 1.314 (OD_{410}) U/g Monascus fermented rice for both red and yellow pigments respectively. The pigments were identified as bands in HPTLC and were distinguished based on their Rf values. Monascin and monascorubin were found to be maximum than monoscorubramine. Monascus fermented rice was used as colorant in the preparation of food product *kesari* and its sensory qualities evaluated. Incorporation of Monascus fermented rice for coloring the *kesari*, showed appealing color, appearance and was overall acceptable.

Key words: Monascus · Rice · Pigment · Nitrogen

INTRODUCTION

Most of the natural dyes are extracts from plants, plant products or produced by microorganisms, which provides production advantages. Since the number of permitted synthetic colorants has decreased because of undesirable toxic effects including mutagenicity and potential carcinogenicity, interest focuses on the development of food pigments from natural sources. 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. The pigment of *Monascus* improves the coloring appearance of foods and their organoleptic characters. Rice is one of the major agricultural product of economic importance. This primary product could serve as sustainable raw material for secondary value-added products through fermentation of *Monascus* molds [1]. The filamentous fungus *Monascus* produces six major related pigments which could be categorized as (1) orange pigments: rubropunctatin ($C_{21}H_{22}O_5$) and monascorubin ($C_{23}H_{26}O_5$); (2) yellow pigments: monascin ($C_{21}H_{26}O_5$) and ankaflavin ($C_{23}H_{30}O_5$); and (3) red pigments: rubropunctamine ($C_{21}H_{23}NO_4$) and monoscorubramine ($C_{23}H_{27}NO_4$) [2].

The biomass growth, types and production of metabolites would be directly or indirectly affected by the cultivation environment and cultivation methods. As far as the cultivation methods were concerned, Lin [4] states that solid state cultivation results in higher pigment yield than cultivation in shake culture and this phenomenon is due to the fact that pigments are released into grains under solid state culture and the pigments are accumulated in the mycelium under submerged cultivation. Various derivatives of *Monascus* pigments are produced when nitrogen sources are added to the culture broth. This study was taken up with the objective to, produce natural food colorant by the solid-state fermentation of rice with *Monascus* sp. with the addition of different nitrogen sources.

MATERIALS AND METHODS

Materials: Raw rice purchased from the local market was used as the substrate. Different organic & inorganic

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nitrogen sources *viz*., peptone, yeast extract, ammonium nitrate and monosodium glutamate were directly added to the substrate at concentration of 0.5%.

Monascus ruber cultured at Indian Institute of Crop Processing Technology, Thanjavur, was used for the production of pigments. *Monascus ruber* maintained in Potato Dextrose Agar (PDA) slants and 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.

Production of Pigments by Solid State Fermentation: Rice 100 g was soaked overnight in 250 ml tap water. Water drained rice were added with 0.5 % of different nitrogen sources separately and sterilized at 121°C for 30 min by autoclaving. It was allowed to cool and inoculated with 10 % of the inoculum (*Monascus ruber)* and incubated for 15 days at room temperature. After incubation the flasks were sterilized at 121°C for 30 minutes by autoclaving. The sterilized fermented Monascus fermented rice (MFR) was dried at 50°C for 24hours. Dried MFR was pulverized in an Udy cyclone mill and stored for further analysis.

Determination of Pigments

Extraction of Pigments: MFR powder 0.5 g was extracted with 50 ml of 80 % ethanol and subject to rotary shaking at 200 rpm for one hour. It was then centrifuged for 15 min at 10000 rpm and the supernatant was collected for pigment analysis.

Estimation of Pigments: 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 [4].

Determination of Pigments Using HPTLC: 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 256 nm UV lamp using CAMAG TLC (model) Autoscanner with WINCATS software.

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

RESULTS AND DISCUSSION

Growth of *M. ruber* and Yield of Pigments: The growth was periodically monitored at intervals of 2, 7 and 15 days of incubation. Slight growth of *M. ruber* was observed in N-supplemented treatments on the second day. After 15 days of incubation, improved growth and pigment production was observed in N-supplemented samples. The treatment containing monosodium glutamate favored more growth of *M. ruber* when compared to other treatments. The results are presented in Table 1. Shepherd and Carels [6] reported that nitrogen source affect the growth and pigment production. The MFR yield analyzed are presented in the Fig. 1. The yield content varied from 44.67 to 50.33%, the control being 44.51%.

From the spectral analysis, slight shift in absorbance maxima was observed for different nitrogen sources. In this study, mono sodium glutamate was found to yield maximum pigment 0.464 (510nm) and 1.314 (410nm) U/g of MFR for both red and yellow pigments respectively (Fig. 2). Ammonium nitrate yielded 0.402 and 0.890 U/g of MFR production. The pigment yield was higher in

Table 1: Growth of M. ruber in rice supplemented with different nitrogen sources

Treatments	After 2 days	After 7 days	After 15 days
Rice + peptone	+	++	++
Rice + yeast extract	+	++	+++
Rice + ammonium nitrate	+	++	++
Rice + mono sodium glutamate	+	++	+++
Rice alone (control)	-	+	++

- No growth + Slight growth ++ Dense growth +++ Dense growth with dark pigmentation

Table 2: Identification of Monascus Pigments using HPTLC

		Rf values	
Sample Details	Monascorubrin (red)	Monascin (yellow)	Monascorubin (orange)
Rice + peptone	0.23	0.78	-
Rice + yeast extract	0.23	0.78	0.69
Rice + ammonium nitrate	0.21	0.79	0.70
Rice + monosodium glutamate	0.21	0.79	0.70
Rice alone (control)	0.20	0.79	0.70

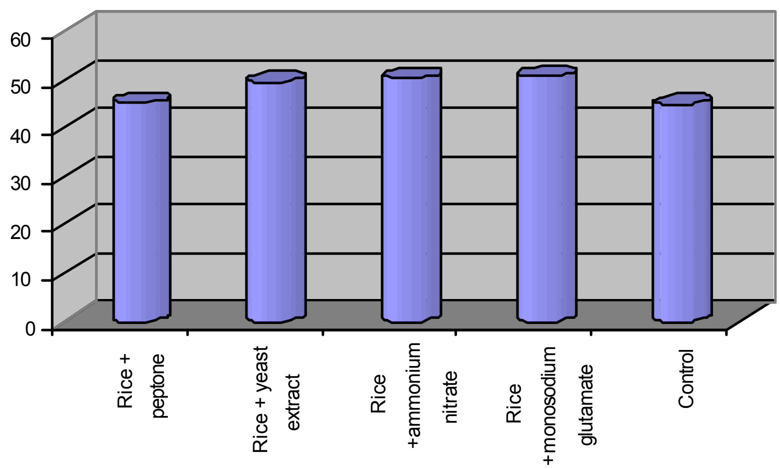

Fig. 1: Influence of different nitrogen sources on MFR Yield

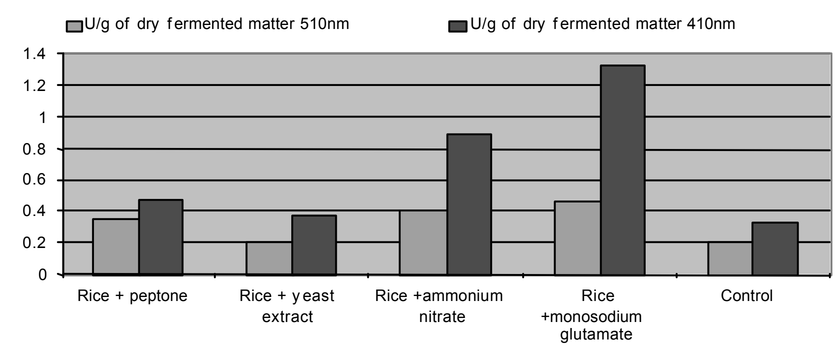

Fig. 2: Influence of different nitrogen sources on Pigment Yield by *M. ruber*

inorganic nitrogen sources than organic nitrogen sources. Control recorded the least pigment yield of 0.202 and 0.330 U/g of MFR. Compared to the control addition of nitrogen sources increased the yield of pigment. The nitrogen source, mono sodium glutamate (MSG) increased 56% pigment production by *M. ruber*.

The nitrogen sources monosodium glutamate and yeast extract favoured the growth of *M. ruber* strains [1]. Addition of ammonium chloride, sodium nitrate, peptone and monosodium glutamate has different effects on metabolite formation [7]. It is suggested that only the orange pigments, monascorubrin and rubropunctatin, are produced biosynthetically and that the other pigments are formed from these by chemical transformations depending on the cultural conditions.

Identification of Monascus Pigments Using HPTLC: The pigments were identified in HPTLC as visible bands of red, orange and yellow. They were distinguished based on their Rf values (Table 2). With the Rf value of 0.70 orange pigment Monascorubin, was recorded in most treatments except peptone. In this study, orange and yellow pigments were found to be maximum than red pigments. Monascorubrin is usually mixed with rubropunctatin, but the latter is orange colored. Rubropunctatin can be oxidized to form monascin [8].

Sensorily Evaluation of Kesari: The *kesari* prepared using MFR as colorant was sensory evaluated for color, appearance, flavor, texture, taste and overall acceptability. A panel of ten members scored the product by comparing with the control. The average score for color was 7.4, flavor 8, appearance 7.5 and overall acceptability 8 scored to be as 'liked very much'. The result shows that the incorporation of MFR for coloring kesari has proved to be good. The pigment distributed evenly in the food product giving a pleasing appearance. Moreover application of the natural pigment promotes consumers health protection [9] and allows manufacturing fully natural food without any synthetic additives.

CONCLUSION

Food products gain more intense and stable red color and improved organoleptic characteristics when *Monascus* pigment is used. Furthermore the production of pigments could be augumented with the addition of nitrogen source. The study reveals that the addition of nitrogen source improves the metabolic activity of the organism to produce increased quantity of the pigment. The production of natural food colour through bioprocessing would be of preference nowadays due to the phasing out of synthetic colourants.

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