*Intl. J. Food. Ferment. Technol. 6(1): 185-187, June, 2016*


**DOI:** 10.5958/2277-9396.2016.00041.6

**RESEARCH NOTE**

# Chemical Conversion of Pigment of *Monascus purpureus* to Water Soluble Pigment

Sangeeta Sharma, V.K. Joshi* and Naveen Kumar

*Corresponding author: editorijf @gmail.com

Paper No. 127 Received: 17-9-2015 Accepted: 23-4-2016

**Abstract**

The microbial pigments are useful as biocolour as they are safe. But the biocolour produced, most of the times, are insoluble water so cannot be used in food products and the pigment was produced using solid state fermentation on a medium with rice grits, apple pomace or combination, glucose monosodium glutamate and magnesium sulphate. The pH of the medium used was 6.0 and a temperature of 30 °C was used for incubators. Results of investigation carried out on the conversion of biocolour produced by *Monascus purpureus* MTCC 410 under incubation solid state conditions, into water soluble pigment are reported here. The dried culture medium was used and the pigment was extracted using hexane, ethanol, methanol, petroleum ether, acetone and water. It was found that maximum conversion took place using amino glutamic acid (72%) in comparison to amino acetic (58.0%) acid and gelatin (64.0%). The optimum pH of conversion was found to be 9.2.

**Keywords:** Pigments, water solubility, *Monascus purpureus,* solid state fermentation, chemical conversion

Microorganisms are found to be a good source of pigments and several microorganisms like *Rhodotorula, Monascus, Sarcina, Achromobacter* have been investigated to produce biocolour including the use of the waste bioproducts as a substrate for solid state fermentation (Joshi and Sandhu, 1996; Joshi *et al.* 2003; Joshi and Attri, 2006; Joshi *et al*. 2011 and Joshi *et al*. 2012). *Monascus* as a colour producing microorganism mainly produces 3 types of colours, yellow, orange and red. *Monascus* is traditionally used in oriental countries, originally in China and Thailand, to prepare fermented rice with strong red colour. The biocolour finds several applications ranging from conferring colour to a wide range of products such as wine, cheese and meat, to medicinal uses and as a meat preservative (Wong *et al.*, 1981). *Monascus purpureus* is normally cultivated on cooked rice to produce a range of metabolite, statin viz. Lovastatin, monacolins J, pravastatin and mevastatin. Lovastatin (Monacolin K) has cholesterol lowering properties (Panda *et al*., 2008). But the biocolours produced by the microorganisms are not soluble in water so these cannot be used in food items as food mainly contains water. It is therefore, very essential that these colours be made water soluble. In our earlier studies, pigments produced by *Rhodotorula, Micrococcus, Sarcina* and *Achromobacter* were successfully converted into water soluble pigments, through the conversion extent was variable and varied with the chemicals employed (Joshi and Attri, 2013). These colour were successfully used in model food systems also. Since in our case, the substrate was different, it could have possibly altered the composition, hence solubility. So in the present experiment, efforts were made to make these colours water soluble by chemical conversion.

## Materials and Methods

### *Microbial culture*

*Monascus purpureus* MTCC 410 used as a source of biocolour. The strain was obtained from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

### *Biocolour production*

**Inoculum preparation:** Culture of *Monascus* was maintained on malt extract agar slants at 4°C and then, sub-cultured as and where needed. Seed culture was prepared by adding 5 ml of Tween 80 in petri plates and used for further colour production in solid state fermentation.

**Substrates and carbon, nitrogen and mineral sources:** Mainly two carbon substrates were used and i.e. rice (4.8%) and apple pomace (4.8%), third substrate was the combination of these two substrates (2.4% each) for production of pigment by *Monascus*. Glucose at a concentration of 4 % was used as a carbon source. Monosodium glutamate (0.2g/l) was used as a nitrogen source while magnesium sulphate (0.02 mg/l) was used as mineral source, as optimized earlier (Sharma, 2014).

**pH, temperature and incubation time:** Pigment, biomass (glucosamine) and monacolin K were estimated for 14 days after inoculation of *Monascus* to monitor the growth and production of pigment. pH of the medium was set to 6 and optimum temperature of 30°C was used for pigment production.

### *Pigment extraction and estimation*

The culture medium was harvested and dried for 48 hours at 50°C. One gram of solid substrate (fermented) was taken for extraction of the pigment using different solvents viz., ethanol, methanol, hexane, petroleum ether, acetone and water with a vortex mixture for 20 min. Pigment absorbance was measured with a spectrophotometer over a wide range of wavelength i.e. 200-1100 nm.

### *Chemical modification of microbial pigments*

To 1 g of pigment, 50 per cent of 50 ml ethanol was added in a flask connected with a long refluxing glass tube for conversion of pigment as described earlier for extraction of pigment from *Rhodotorula* (Joshi and Attri, 2013). 2 mg of amino acetic acid or 2 mg of amino benzoic acid or gelatin was added along with 2-3 drops of 1N NaOH and refluxed at 100°C for 20 minutes. After cooling, the extract was filtered. The filtrate were dried and redissolved in 50 ml of 0.01 m sodium citrate, phosphate or carbonate buffer at pH 3, 7 and 9.2 and measured optical density at different wavelengths. Observations on optical density (λ-max) and % water solubility of the pigment was recorded and the data were accordingly analyzed and interpreted.

## Results and Discussion

The following complete sentence has not been added. So add it now. The extracted pigment was characterized by measuring optical densities a different wave lengths (200-1100 nm) in a spectrophotometer (UV-Vis spectrophotometer Shimadzu make). The results show that the pigment was carotenoid in nature. Similar results have been reported for Rhodotorula, Chromobacter, Sarcina and Micrococcus (Joshi and Attri, 2013). Since the pigment was carotenoid in nature, it needs to be made into water soluble for use in foods.

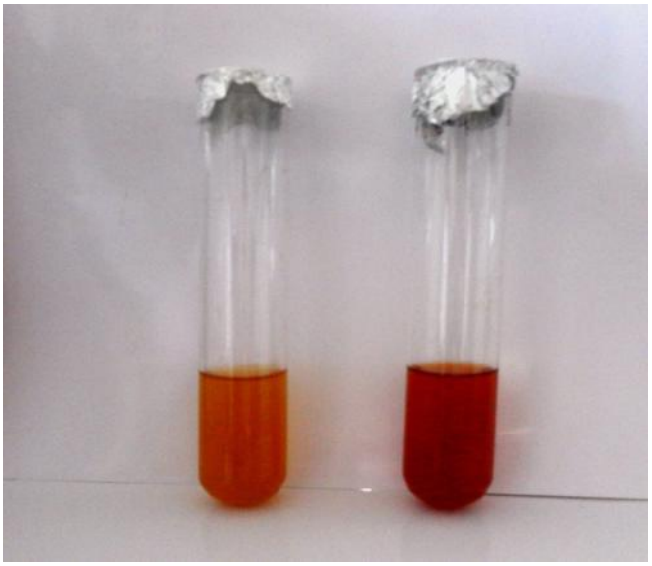

**Plate 1:** Pigment in water before and after conversion

So the pigment produced was modified by chemicals

like amino acetic acid, amino benzoic acid and gelatin to a variable extent. Maximum conversion of pigment was found in amino benzoic acid (72%) at 9.2 pH followed by 7. However, there was lesser solubility with amino acetic acid and gelatin (Table 1). The difference could be correlated with type, behaviour and reaction with solubilising pigment. With respect to water solubility of pigment from *Monascus*, these findings are similar to those reported by Wong and Koehler (1983).

So it can be inferred that substrate used for the growth of *Monascus* did not interfere with the composition or properties of pigment. However, the behaviour of pigment, though carotenoid in nature was different from that produced by *Rhodotorula*, *Micrococcus*, *Cronobacter* and *Rhodotorula* was different (Joshi and Attri, 2013). Still, the fact remains that the higher conversion would have facilitated better application, cannot be ignored, which call more indepth studies. The pigment produced by *Monascus* and dissolved in water before and after conversion is shown in Plate 1.

**Table 1: Chemical modification of pigment for water solubility**

| Chemical | pH | OD | % conversion |
|---|---|---|---|
| Amino acetic acid | 4 | 0.46 | 52% |
| | 7 | 0.48 | 56% |
| | 9.2 | 0.53 | 58% |
| Amino benzoic acid | 4 | 0.78 | 66% |
| | 7 | 0.83 | 68% |
| | 9.2 | 0.88 | 72% |
| Gelatin | 4 | 0.56 | 58% |
| | 7 | 0.63 | 62% |
| | 9.2 | 0.75 | 64% |

## Conclusion

The pigment produced could successfully be converted into water soluble pigment by the method employed though there were large variations in the efficiency of conversions of the chemical employed. The percent conversion of pigments into water soluble pigments ranged from 52-72%. Out of the chemical used, amino benzoic acid gave maximum conversion of 72%. At pH 9.2, maximum conversion occurred followed by 7 and 4.

## References

Joshi, V.K. and Attri, D. 2013. Characterization and conversion of microbial pigments into water soluble pigments for application in food products. *Proc. Nati. Acad. Sci.* **260**: 6.

Joshi, V.K., Atrri, D. and Rana, N.S. 2011. Optimization of apple pomace based medium and fermentation condition for pigment production by *Sarcina* species, *Indian Journal of Natural Products and Resources* **2**(4): 421-427.

Joshi, V.K., Attri, D., Anju Bala and Shashi Bhushan 2003. Microbial pigments. *Ind. J. Biotechnol*. **2**: 362-369.

Joshi, V.K., Devi, M.P., Attri, D. and Sharma R 2012. Biocolours: chemistry, production, safety and market potential. In: Food Biotechnology: Principles and Practices, Joshi, V.K. and Singh, R.S. (2011) eds. Ik International Publishing House, New Delhi, pp. 641-690.

Panda, B.P., Javed, S. and Ali, M. 2008. Fermentation process optimization, *Res. J. Microbiol.* **2**: 201-208.

Sandhu, D.K. and Joshi, V.K. 1996. Development of apple pomace based medium, optimizing pigment production by *Rhodotorula* and its characterization. *Adv. Food Sci* (CMTC), **19**: 31-34.

Sharma, S. 2014. Production and Evaluation of Biocolour from *Monascus*, using apple pomace. A thesis submitted to Dr Y.S. Parmar University of Horticulture and Forrestry, Nauni, Solan (H.P), India.

Wong, H.C and Koehler, P.E. 1983. Production of red water soluble *Monascus* pigments. *J. Food Sci.,* 1200-1203.

Wong, H.C., Lin, Y.C. and Koehler, P.E. 1981. Regulation of growth and pigmentation of *Monascus purpureus* by carbon and nitrogen concentration. *Mycologia.* **73**: 649-654.