

## Stability Analysis of Food Bio-Colour Extracts from Broken Rice through Solid State Fermentation

S. R. Mhalaskar<sup>1\*</sup>, S. S. Thorat<sup>2</sup> and A. A. Kulthe<sup>3</sup>

<sup>1,3</sup>Research Scholar, <sup>2</sup>Head

Dept. of Food Science and Technology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri Taluka- Rahuri District. -Ahmednagar (Maharashtra), Pin Code- 413 722

\*Corresponding Author E-mail: [mhalaskarsachin10@gmail.com](mailto:mhalaskarsachin10@gmail.com)

Received: 5.02.2018 | Revised: 12.03.2018 | Accepted: 27.03.2018

### ABSTRACT

*An attractive and stable colour is important in the marketability of foods and beverages. However, replacing synthetic dyes with natural colorants poses a challenge due to the higher stability of synthetic colorants with respect to light, temperature, pH and is therefore a major concern in colouring foods. However, the lower stability of natural colourants against environmental factors could pose restriction to their utilization as food colorants in industry. Therefore, the stability of food bio-colour extracts from broken rice by *Monascus purpureus* (MTCC 410) in SSF was investigated by optimizing the light, temperature and pH conditions. The food bio-colour extracts were characterized for considerable stability and it was found that these extracts are stable in dark light, at low temperatures (20°C, 40°C and 60°C) while the red, orange and yellow bio-colour extracts showed good stability at pH of 6, 4 and 2 respectively. The stability of *Monascus* food bio-colours compares well with other natural colours, so that these food bio-colours will be a promising colour additive for food industry.*

**Key words:** *Monascus, Broken rice, stability, Bio-colours, Colorants, Solid state fermentation.*

### INTRODUCTION

With the advent of strict legislative regulations and growing awareness among the consumers about the food safety, food bio-colours have become the choice in the foods as these are considered as safer than their synthetic counterparts. Bio-colours could be a dye, pigment or substance that can impart colour when added or applied to a food, drug, cosmetics etc. Bio-colours are of biological origin derived from plants, insects or microbes<sup>19</sup>. Micro-organisms have high

growth rate and productivity for pigment<sup>3</sup>, which reduced the production time of bio-colours using a process with continuous operation<sup>12</sup>. In addition, microbial production is flexible and can be easily controlled as compared to plant or animal sources. It is great advantageous to use microbes for the production of food bio-colours due to their intrinsic properties of high growth rate, no seasonal variation, high production rate and ease of manipulation<sup>14</sup>.

**Cite this article:** Mhalaskar, S.R., Thorat, S. S. and Kulthe, A.A., Stability Analysis of Food Bio-Colour Extracts from Broken Rice through Solid State Fermentation, *Int. J. Pure App. Biosci.* 6(6): 304-313 (2018). doi: <http://dx.doi.org/10.18782/2320-7051.3089>

The bio-colours have been produced from large number of bacterial, yeast and mold species. The microorganisms for use as bio-colours source should have some necessary features. Among the different microorganisms *Rhodotorula* spp., *Achromobacter* spp., *Blakeslea* spp., *Micrococcus* spp., *Chromobacter* spp., *Sarcina* spp. and *Monascus* spp. are common bio-colours producing microbes<sup>14</sup>. The application of *Monascus* bio-colours in food industry has been carried out traditionally in the oriental foods for hundreds of years<sup>4,21</sup>. Bio-colours from this fungus widely used in food and pharmaceutical industries for therapeutic uses also<sup>15</sup>.

At present, bio-colours production at an industrial scale is not economical since the cost of production is still high. Therefore, the development of low cost comparatively viable process is needed for production of food bio-colours. *Monascus* is probably a xerophilic fungus, which grows in a wide variety of natural substrates<sup>4</sup>. Several materials such as jackfruit seed powder, sesame oil cake, coconut oil cake, palm kernel cake, apple pomace and grape waste have been studied as substrates in solid state fermentation process<sup>1,2,5,6,13,20</sup>. The solid state fermentation approach gives higher productivity of bio-colours at a low cost when compared with liquid fermentation process<sup>8</sup>.

The economics of rice milling industries is largely dependent on the commercial utilization of its by-products. Broken rice is one of the most important by-product of rice milling industry. Broken rice has low economic value as compared to whole rice. This primary product could serve as the sustainable raw material for secondary value-added products through fermentation of *Monascus* molds. Broken rice can be utilized for the production of useful microbial metabolites at an inexpensive manner and applied to varying food products<sup>24</sup>. Rice by-products may serve an important source of raw material that could be used as an ingredient of functional food and nutraceuticals. They have great potential to be converted into human food to improve food security in the country<sup>9</sup>.

Surprisingly, relatively few articles deal with stability of *Monascus* bio-colours considering that several industries produce these bio-colours. An attractive and stable colour is important in the marketability of foods and beverages. However, replacing synthetic dyes with natural colorants poses a challenge due to the higher stability of synthetic colorants with respect to light, oxygen, temperature and pH. Colour degradation is common for natural pigments and is therefore a major concern in colouring foods. Despite its poor stability, *Monascus* compares well with other natural colours, so that these bio-colours are still a promising colour additive. For the use of natural colours, it is important to have a complete understanding of the chemical and physical environment that exists in the product to be coloured during and after processing. Also the capabilities and limitations of natural colours apply to the product need to be taken into consideration. Instability of natural colours is one of the major limitations in the application of natural bio-colours.

## MATERIAL AND METHODS

### Microorganism

The freeze dried culture of *Monascus purpureus* (MTCC 410) was obtained from Institute of Microbial Technology (IMTECH) Chandigarh, India. The stock culture was grown on potato dextrose agar slants for seven days at 30°C and maintained at 4°C in refrigerator by periodically sub-culturing after every two months.

### Preparation of inoculum

The *Monascus purpureus* (MTCC 410) strain was grown on PDA slants for 7 days at 30°C. Spores were harvested from slants by adding 8 ml of 0.85% sterile saline to each of the tube and scrapping of spores gently into saline solution under strict aseptic conditions.

### Solid state fermentation

10g of cleaned broken rice was suspended in a 250 ml Erlenmeyer flask with 25 ml of distilled water and autoclaved at 121°C for 20 minutes and cooled to room temperature<sup>5</sup>. The sterile broken rice medium was inoculated with spore suspension under aseptic

conditions, mixed with sterile rod to ensure uniform distribution of the spores and the flask was incubated for 7 days. Each day, the inoculated substrate was manually shaken until all the substrate contents were separated from each other<sup>22</sup>. The solid state fermentation process was performed as per the procedure depicted in Fig.1.

#### **Stability analysis of food bio-colour extracts**

In an attempt to estimate how stable *Monascus* food bio-colours in several applications, extracts of food bio-colours were incubated at different light, temperature and pH conditions.

#### **Light stability**

To investigate the effect of light on stability, tubes containing 10 mL of bio-colour extracts were incubated under conditions including 8 hr in dark, 8 hr in sunlight and 8 hr in UV light.

#### **Thermal stability**

The thermal stability of food bio-colours was tested by subjecting sterile filtered extracts (10 mL) separately to different temperatures at 20°C, 40°C, 60°C, 80°C and 100°C for 8 hr.

#### **pH stability**

The stability of food bio-colours to pH was tested by subjecting sterile filtered extracts (10 mL) separately to different pH values i.e. pH 2, 4, 6, 8 and 10 for 8 hr in sterile tubes covered with aluminium foil. All tubes were held at room temperature (approximately 25°C). The various pH values of substrate medium were attained by adding a few drops of either 0.1 N HCL or 0.1 N NaOH.

The absorbance (OD) was recorded for all tubes against a blank original sample. Absorbance (OD) was measured using a spectrophotometer after time interval of 2, 4, 6 and 8 hr and result of stability was expressed as percentage of the initial absorbance remaining after exposure to particular light, temperature and pH conditions for 8 hr. Absorbance (OD) of food bio-colour extracts were measured spectrophotometrically at 500, 475 and 375 nm. Stability was measured as percentage of the initial absorbance remaining at any time.

## **RESULTS AND DISCUSSION**

### **Stability of food bio-colour extracts**

In an attempt to estimate the stability, the extracts of food bio-colours were incubated at

different light, temperatures and pH conditions. The extracts of bio-colours from solid state fermentation of broken rice were filtered and placed in sterile test tubes. The tubes were subjected to the various treatments as described earlier. Stability was measured as percentage of the initial absorbance remaining at any time.

#### **Effect of light**

Residual colouration of the red bio-colours after exposure to dark, UV and sunlight for 8 hr indicated greater sensitivity for red bio-colours, which had colour retention of 99.16%, 84.52% and 90.05% respectively (Fig.2). The orange bio-colour was retained to an extent of 98.98%, 89.83% and 90.67% when exposure to dark, UV and sunlight respectively for 8 hr (Fig.3). After 8 hr of exposure to dark, ultraviolet and sun light the yellow bio-colour extract retained upto 98.76%, 86.29% and 91.12% respectively (Fig.4). Natural colorants were affected by dark, UV and sunlight. It was inferred from the results that food bio-colour extracts were more stable in dark followed by sunlight and UV.

Similar results were reported by Nimnoi and Lumyong<sup>18</sup> who measured stability using a relative level of residual absorbance after incubation for 1 to 6 hr. They reported that the pigment decayed over time, as shown by an intolerance with long exposure to UV (>3 hr). The above findings were in contradiction with reports of Gunasekaran and poorniammal<sup>11</sup> who subjected the pigments of fungus to various physical and chemical conditions and concluded that the pigments were more stable in UV light (99.2%) compared to fluorescent light and sunlight.

#### **Effect of temperature**

The colour intensity of the red bio-colour retained to 98.88%, 95.75%, 98.15%, 51.31% and 41.66% after 8 hr of exposure to 20°C, 40°C, 60°C, 80°C and 100°C respectively (Fig.5). After 8 hr of exposure to 20°C, 40°C, 60°C, 80°C and 100°C temperature indicated that amount of orange bio-colours remained stable about 98.89%, 97.54%, 97.45%, 49.44% and 37.52% respectively (Fig.6). A 98.65%, 97.52%, 97.64%, 46.12% and 37.21%

retention of yellow bio-colours occurred when subjected to 20°C, 40°C, 60°C, 80°C and 100°C temperature respectively over 8 hr (Fig.7). Results of stability analysis indicated that bio-colour extracts obtained from *Monascus purpureus* (MTCC 410) under solid state fermentation of broken rice were more stable at 20°C, 40°C and 60°C than 80°C and 100°C temperature.

Nimnoi and Lumyong<sup>18</sup> observed that the pigment decayed over time, as shown by an intolerance to high temperature (>40°C) and the colour intensity of the red pigment after autoclaving and pasteurization decayed to 30.57% and 5.41% respectively. Moreover, the stability of bio-colours from *Monascus* has been widely studied by others such as Carvalho *et al.*<sup>7</sup>, who reported that the pigments are unstable at high temperature and possibly due to the fact that the extract was a mixture of pigments, whose degradation may present different decaying behaviour. Contrary to the above findings Lin and Demain<sup>17</sup> reported that the pigments were stable over a wide range of pH and autoclaving. Velmurugan *et al.*<sup>23</sup>, reported that the bio-colours were stable at high temperature which was precisely similar to the results of current investigation. They postulated that constancy of absorbance at 500 nm indicated thermo stability when the pigments were subjected to steaming and sunlight. However, pigment colour changed to brown during 12 hr of exposure to hot air.

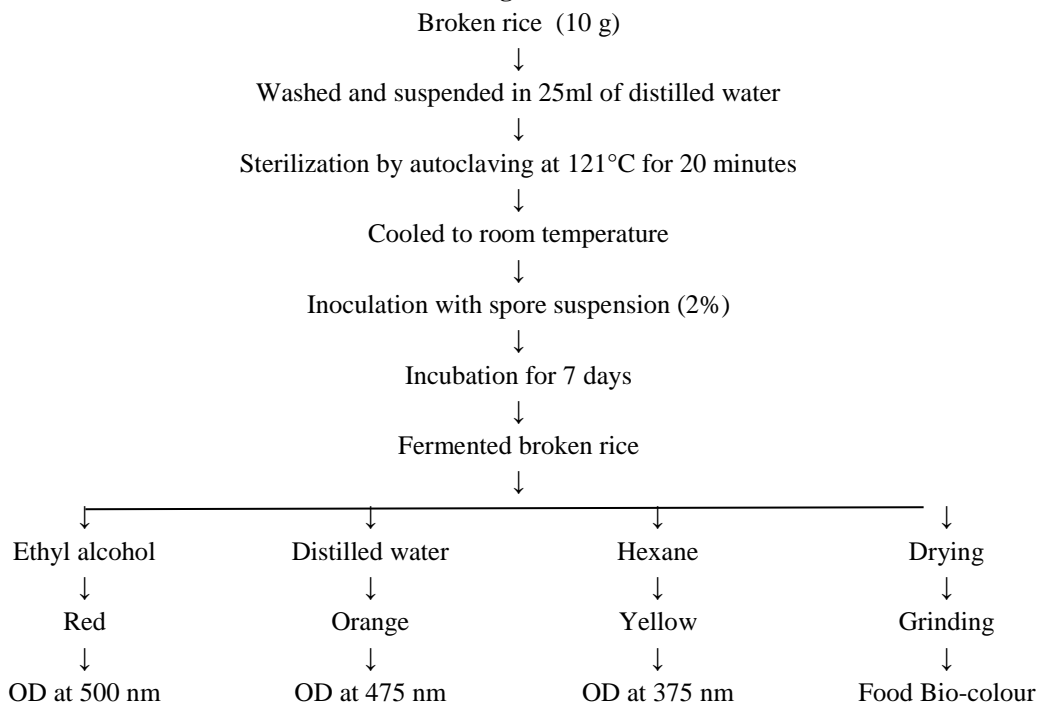
#### Effect of pH

The pH of the medium had a major influence on stability of the pigments in solution sometimes inducing a modification in their structure. It was observed that in lower pH the colour degradation was more significant. A 76.42%, 81.95%, 98.88%, 90.67% and 87.37% retention of red bio-colour occurred when subjected to pH of 2, 4, 6, 8 and 10 respectively over 8 hr (Fig.8). After 8 hr incubation of orange bio-colour at pH of 2, 4, 6, 8 and 10 their colour remained stable upto 91.61%, 98.89%, 95.76%, 93.30% and 88.98% respectively (Fig.9). A 98.97%, 94.49%, 85.16%, 90.67% and 89.55% retention was

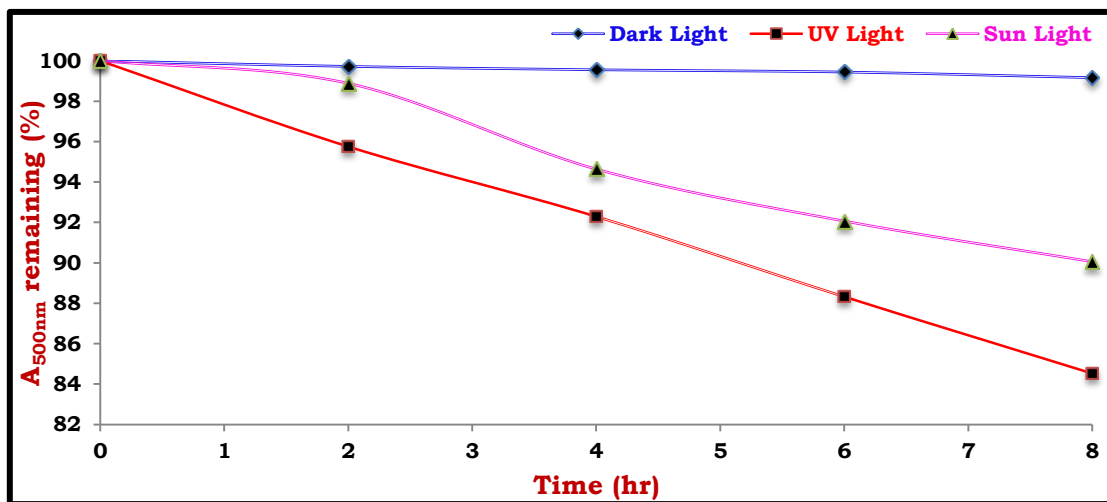
observed for yellow bio-colour at pH of 2, 4, 6, 8 and 10 respectively and highest degradation of yellow bio-colour was observed at pH of 6 (Fig.10). Lower pH in broken rice substrate medium can cause fading of bio-colour extracts and decrease in stability of the colour, while the bio-colours extract were more stable at pH nearer to neutrality (pH 6). The results showed that decrease in pH caused greater destruction of red bio-colours. Red bio-colour solutions were stable under neutral and weakly acidic conditions, while yellow bio-colour extract was stable under very strong acidic conditions. The orange bio-colour extract was more stable at pH 4 and the stability was reduced at pH 2 and 6 thus concluding that this extract was more stable in highly acidic conditions. When the pH of red bio-colour extract was lower than 6.0, the absorbance decreased slowly and the colour extract became very fade, which is the typical acidic reaction feature of bio-colours. The decrease in colour may be due to the acid acceleration of water interaction with pigments, such as breaking of an ester linkage in rubropunctamine or monascorubramine. The colours were more stable at neutral or basic pH<sup>10,16</sup>. The colour change can be attributed to protonation or dissociation, below or above the molecular dissociation constant of the pigment molecules. The presence or absence of colour for a specific pigment is a function of pH due to ionization of aromatic—OH groups and tautomerism of —O(—) with =O. Changes in the relative proportions of dissociated or undissociated molecules (with respective colours) would produce the resulting coloration, such as orange at pH 9, yellow at pH 10 and red at 14.

Moreover, the stability of pigment from *Monascus* has been widely studied by Carvalho *et al.*<sup>7</sup>, who reported that the red pigments were unstable at low pH possible due to the fact that the extract was a mixture of pigments, whose degradation may present decaying behaviour. Velmurugan *et al.*<sup>23</sup>, reported that the yellow bio-colour was stable at acidic pH which is in conformity with present findings.

**Figures**



**Fig. 1: Flow chart for the production of food bio-colours from broken rice through solid state fermentation**



**Fig. 2: Light stability of red bio-colour extract from broken rice**

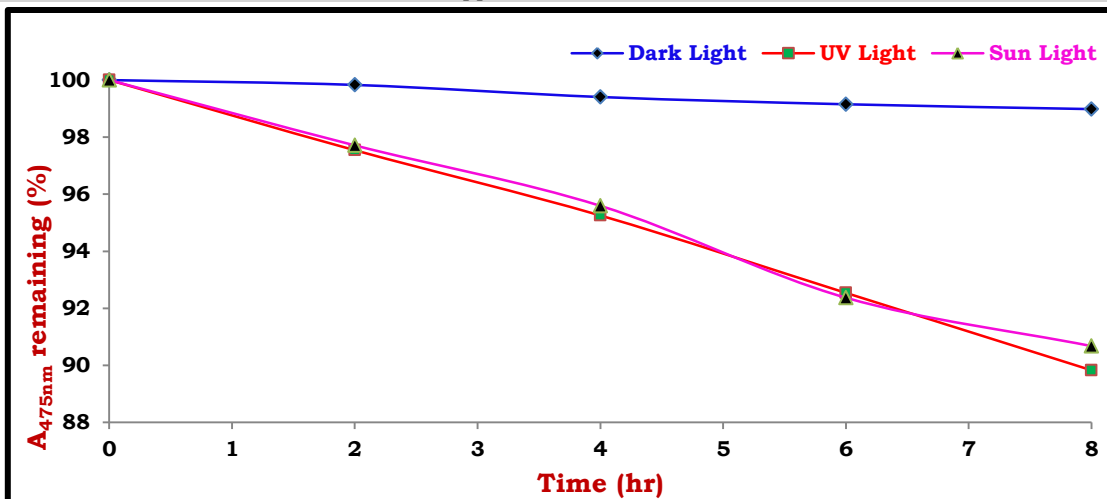


Fig. 3: Light stability of orange bio-colour extract from broken rice

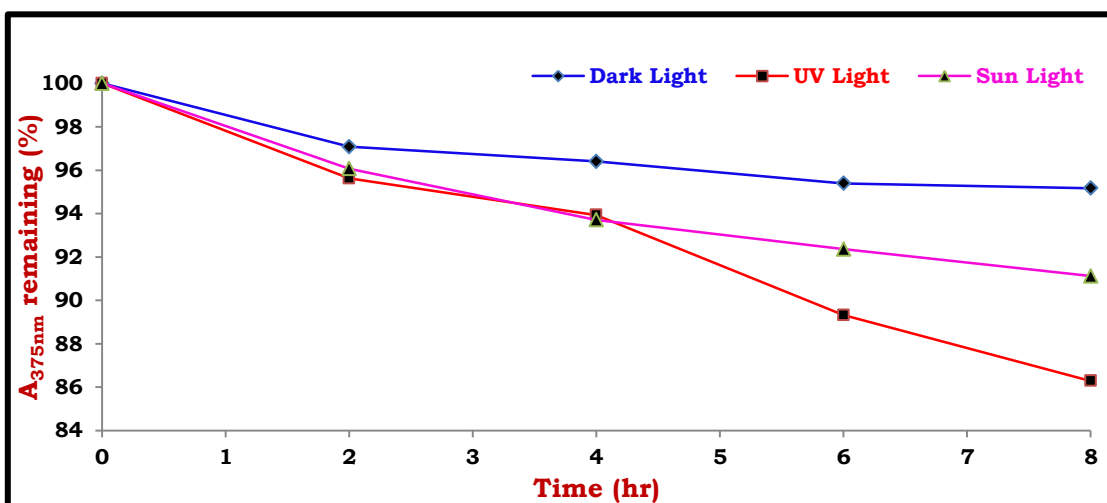


Fig. 4: Light stability of yellow bio-colour extract from broken rice

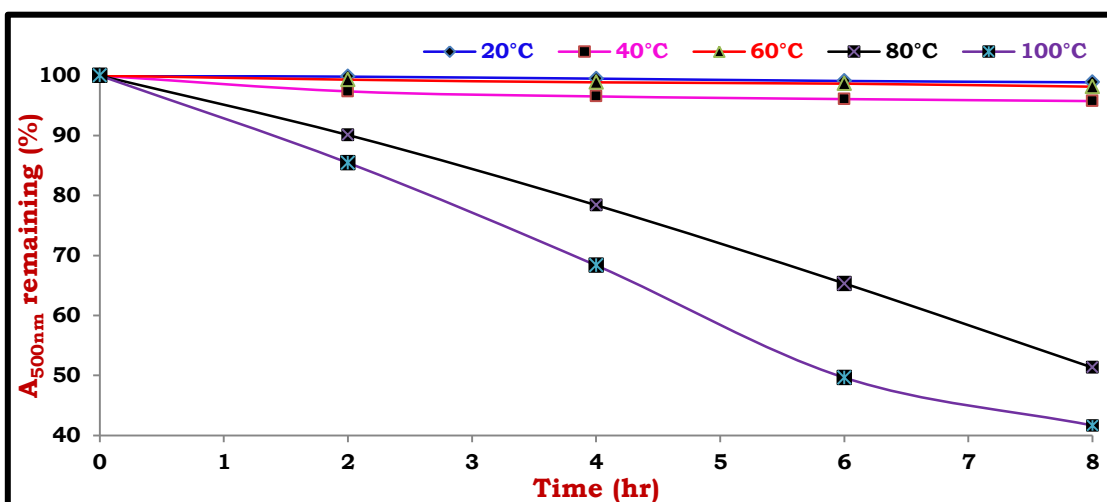


Fig. 5: Thermal stability of red bio-colour extract from broken rice

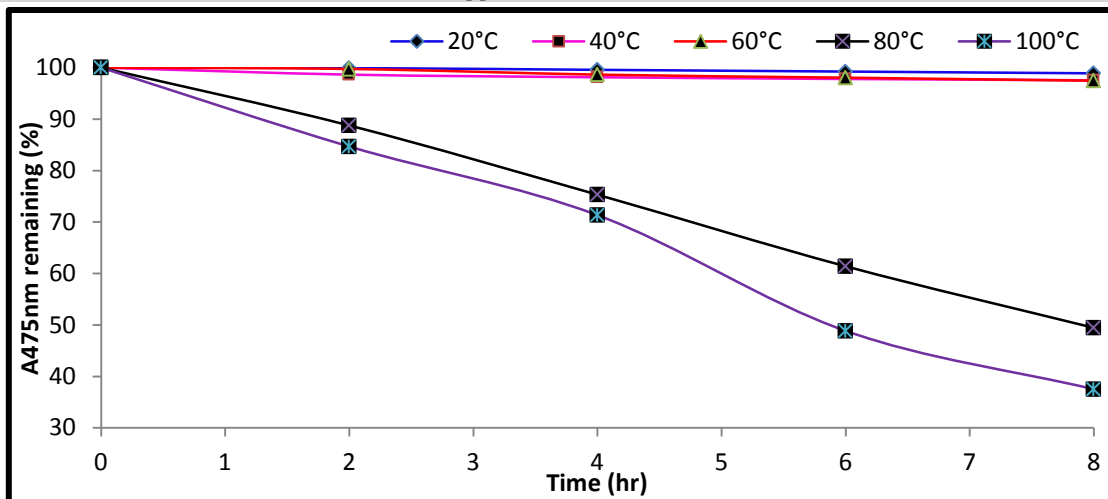


Fig. 6: Thermal stability of orange bio-colour extract from broken rice

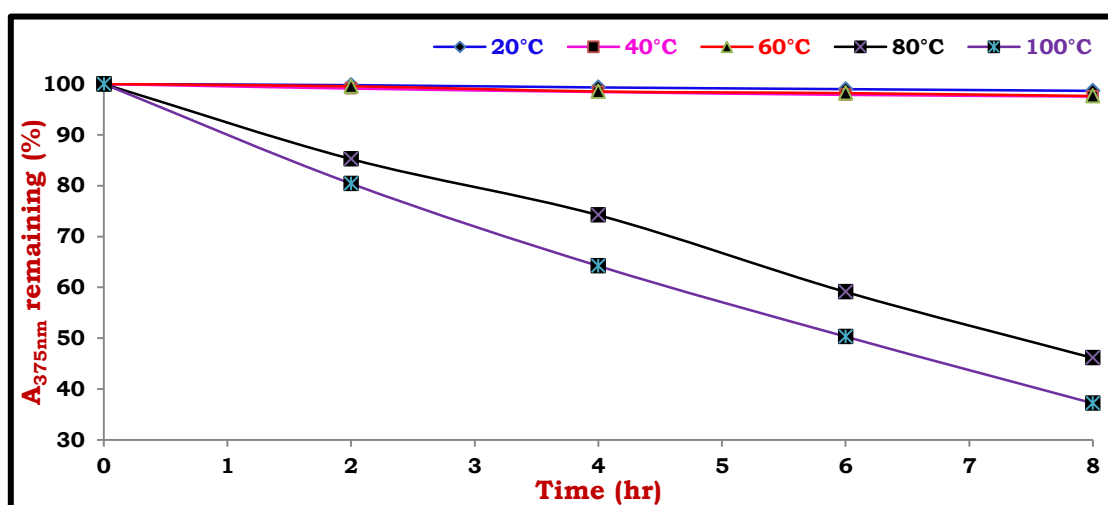


Fig. 7: Thermal stability of yellow bio-colour extract from broken rice

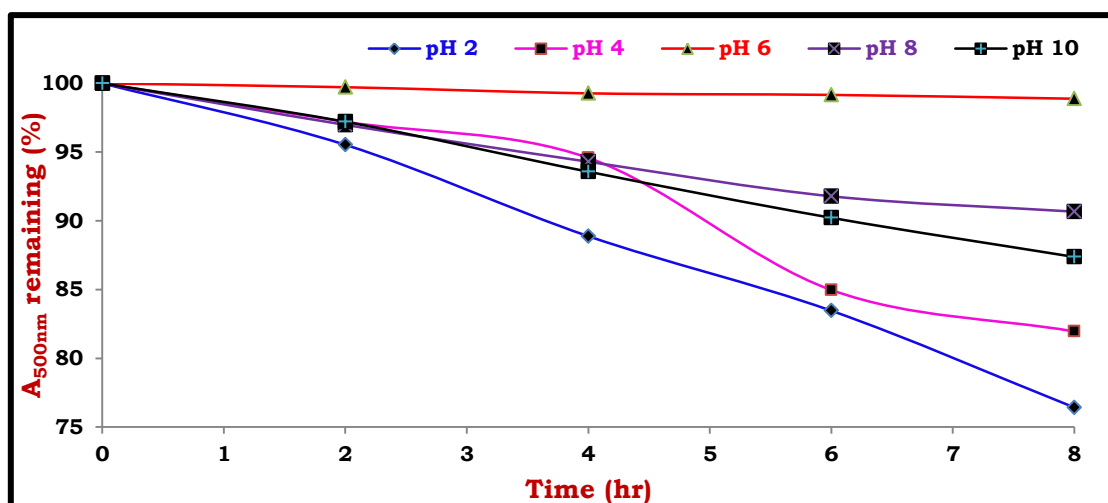


Fig. 8: pH stability of red bio-colour extract from broken rice

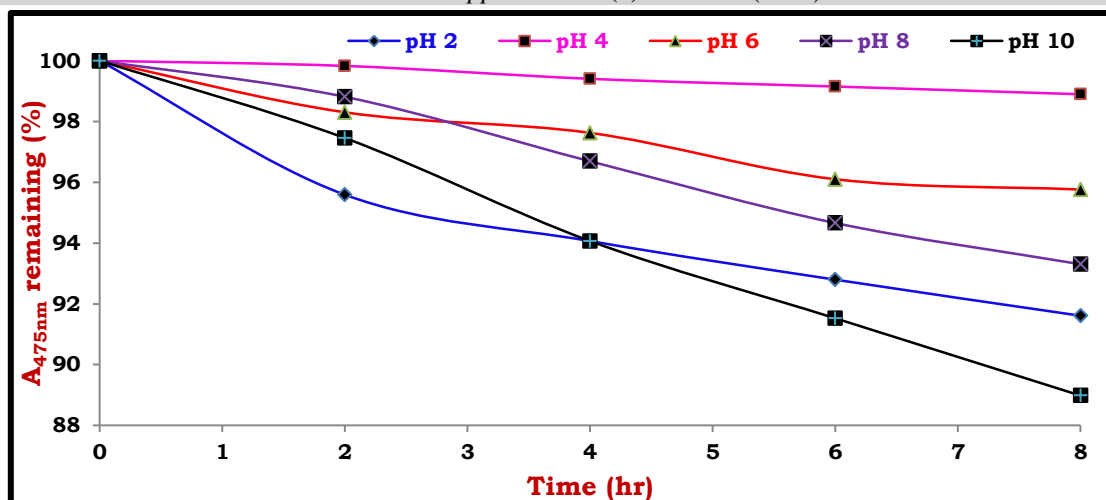


Fig. 9: pH stability of orange bio-colour extract from broken rice

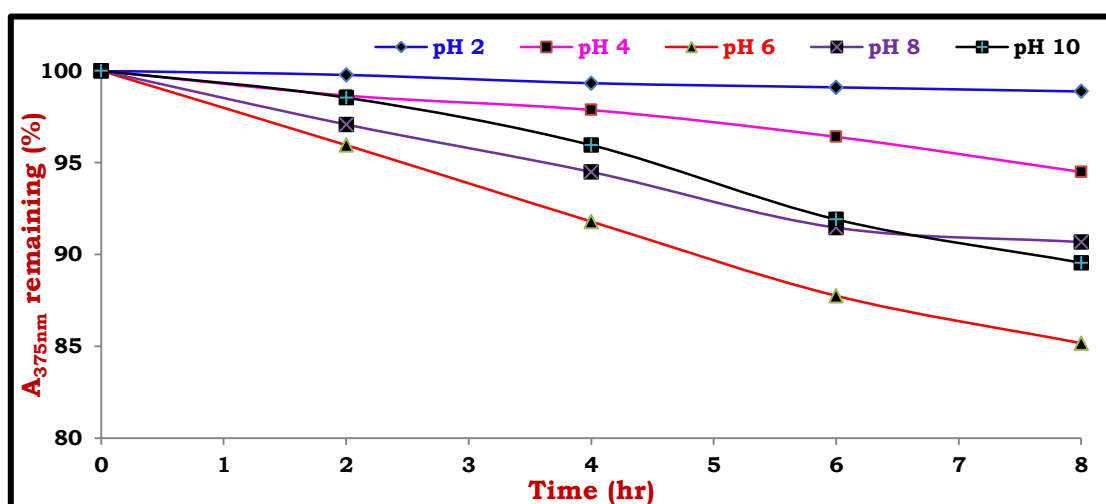


Fig. 10: pH stability of yellow bio-colour extract from broken rice

### CONCLUSION

The food bio-colour extracts from broken rice were highly or moderately sensitive to the light, temperature and pH. The colour intensity of the red, orange and yellow bio-colours retained to 99.16%, 98.98% and 98.76% after 8 hr exposure under dark light which indicates that the bio-colour extracts were more stable in dark light compared to UV and sunlight. The retention of the red bio-colour (51.31%, 41.66%), orange bio-colour (49.44%, 37.52%) and yellow bio-colour (46.12%, 37.21%) after exposure to 80°C and 100°C for 8 hr indicated greater sensitivity and degradation for bio-colours extracts compared to 20°C, 40°C and 60°C. After 8 hr incubation of red, orange and yellow bio-colours at pH of 6, 4 and 2, it was observed that colour remained stable upto 98.88%, 98.89% and 98.97% respectively.

This showed that the red, orange and yellow bio-colour extracts were stable to neutral, acidic and strongly acidic conditions. Bio-colour extracts from broken rice were more stable in dark light and temperature at 20°C, 40°C and 60°C while the red, orange and yellow bio-colour extracts showed good stability at pH of 6, 4 and 2 respectively.

### Acknowledgements

Dr. Sachin R. Mhalaskar acknowledges the financial support given by **University Grants Commission (UGC), New Delhi, India.**

### REFERENCES

- Attri, D. and Joshi, V. K., Optimisation of apple pomace based medium and fermentation conditions for pigment production by *Chromobacter* spp. *J. Food*



- Sci. Technol.*, **4**: 515-520 (2005a).
2. Attri, D. and Joshi, V. K., Optimisation of apple pomace based medium and fermentation conditions for pigment production by *Micrococcus* spp. *J. Sci. Ind. Res.* **64**: 598-601 (2005b).
  3. Babitha, S., Microbial Pigments. In: Singh nee Nigam, P. and A. Pandey, (Eds), *Biotechnology for Agro-industrial Residues Utilization*. Springer Science & Business Media B. V., Netherlands. 147-162 (2009).
  4. Babitha, S., Sandhya, C. and Pandey, A., Natural Food Colorants. *Applied Botany Abstracts*, **23**: 258-266 (2004).
  5. Babitha, S., Soccol, C. R. and Pandey, A., Jackfruit Seed – A Novel Substrate for the Production of *Monascus* Pigments through Solid-State Fermentation. *Food Technology and Biotechnology*, **44**: 465–471 (2006).
  6. Babitha, S., Soccol, C. R. and Pandey, A., Solid-state fermentation for the production of *Monascus* pigments from jackfruit seed. *Bioresource Technology*, **98**: 1554–1560 (2007).
  7. Carvalho, J. C., Oishi, B. O., Pandey, A. and Soccol, C. R., Biopigments from *Monascus*: Strain selection, citrinin production and color stability. *Braz. Arch. Biol. Technol.*, **48**: 885–894 (2005).
  8. Cavalcante, R. S., Lima, H. L. S., Pinto, G. A. S., Gava, C. A. T. and Rodrigues, S., Effect of moisture on *Trichoderma conidia* production on corn and wheat bran by solid state fermentation. *Food and Bioprocess Technology*, **1**: 100–104 (2008).
  9. Mohd, E. N., Ling, T. B. and Peng, L. S., By-products of rice processing: An overview of health benefits and applications. *J. Rice Res.*, **1**: 1 (2013).
  10. Fabre, C. E., Goma, G. and Blanc, P. J., Production and food applications of the red pigments of *Monascus ruber*. *J. Food Sci.*, **58**(5): 1099-1110 (1993).
  11. Gunasekaran, S. and Poorniammal, R., Optimization of fermentation conditions for red pigment production from *Penicillium* spp. under submerged cultivation. *African J. Biotech*, **7**(12): 1894-1898 (2008).
  12. Hendry, G. A. F. and Houghton, D., National Food Colorants. Blackie & Sons, Glasgow (1997).
  13. Joshi, V. K. and Attri, D., Optimisation of apple pomace based medium and fermentation conditions for pigment production by *Rhodotorula* spp. *Proceedings of National Academy of Sciences.*, **76B**: 171-176 (2006).
  14. Joshi, V. K., Attri, D., Bala, A. and Bhushan, S., Microbial pigments. *Indian J. of Biotechnol.*, **2**: 362-369 (2003).
  15. Kumar, S., Verma, U. and Sharma, H., Antibacterial Activity *Monascus purpureus* (red pigment) Isolated from Rice malt. *Asian Journal of Biology and Life Sciences*, **1**: 252-255 (2012).
  16. Lee, Y. K. and Chen, D. C., Application of *Monascus* pigments as food colourants. [www.allok.com/literature](http://www.allok.com/literature) (2000).
  17. Lin, T. F. and Demain, A. I., Effect of nutrition of *Monascus* spp. on formation of red pigments. *App. Microbiol. Biotech*, **36**(1): 70-75 (1992).
  18. Nimnoi, P. and Lumyong, S., Improving solid state fermentation of *Monascus purpureus* on agricultural products for pigment production. *Food Bioprocess Technol.*, **4**: 1384-1390 (2011).
  19. Dipti, S., Understanding Bio-colour- A Review. *International Journal of Scientific & Technology Research*, **3**(1): 2277-8616 (2014).
  20. Silverira, S. T., Daroit, D. J. and Brandelli, A., Pigment production by *Monascus purpureus* in grape waste using factorial design. *Food Science and Technology*, **41**: 170–174 (2008).
  21. Teng, S. S. and Feldheim, W., Anka and Anka Pigment Production. *Journal of Industrial Microbiology and Biotechnology*, **26**: 280-282 (2001).
  22. Vanajakshi V., A Thesis on polyketide production by *Monascus pupureus*. Submitted to university of Mysore for the award of the degree of Master of Science

- in Food Science (By Research) (2006).
23. Palanivel, V., Hur, H., Balachandar, V., *Monascus* pigment production by solid-state fermentation with corn cob substrate. *Journal of Bioscience and Bioengineering*, **112(6)**: 590–594 (2011).
24. Vidyalakshmi, R., Paranthaman, R., Muruges, S. and Singaravadivel, K., Stimulation of *Monascus* pigments by intervention of different nitrogen sources. *Global J. Biotech. Biochem*, **4(1)**: 25-28 (2009).