An investigation on fermentative changes during the production of food bio-colours through solid state fermentation of broken rice by Monascus purpureus (MTCC 410)

S.R. Mhalaskar*, S.S. Thorat and Y.R. Deshmukh

Department of Food Science and Technology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri -413 722, Maharashtra, India.

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ABSTRACT

An attractive and stable colour is important in the marketability of foods and beverages. The utilization of broken rice as a by-product from agricultural farm can be of immense importance to convert relatively high-energy by-products into more useful and highly nutritious end product by use of microorganisms. In this respect the present investigation was aimed to address the fermentative changes in broken rice as a substrate during the production of food bio-colours through solid state fermentation by using Monascus purpureus (MTCC 410). The 7 days of SSF process was accompanied with the increase in the levels of protein, crude fat, crude fiber and ash content by values of 8.98% to 14.14%, 1.50% to 2.07%, 0.70% to 6.61% and 2.70% to 3.69% respectively while decrease in the level of carbohydrate by values of 74.32% to 27.71%, with lowering pH from 6.6 to 5.5. Present findings concluded that broken rice possesses good potentials for the bioconversion of the high energy organic materials into more useful and highly nutritious food bio-colours by Monascus purpureus (MTCC 410).

Key words: Bio-Colours, Broken Rice, Colorants, Monascus, Solid State Fermentation.

Abbreviations: SSF: Solid state fermentation, Spp.: Species, MTCC: Microbial Type Culture Collection, IMTECH: Indian Institute of Microbial Technology

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 (Sharma, 2014). Micro-organisms have high growth rate and productivity for pigment (Babhita, 2009), which reduced the production time of bio-colours using a process with continuous operation (Hendry and Houghton, 1997). 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 (Joshi et al., 2003).

The bio-colours have been produced from large number of bacterial, yeast and mold species. The microorganisms for use as a 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 (Joshi et al., 2003). The application of Monascus bio-colours in food industry has been carried out traditionally in the oriental foods for hundreds of years (Babhita et al., 2004; Teng and Feldheim, 2001). Bio-colours from this fungus widely used in food and pharmaceutical industries for therapeutic uses also (Kumar et al., 2012).

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 (Babhita et al., 2004). 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 (Attri and Joshi, 2005a,b; Babhita et al., 2006; Babhita et al., 2007; Joshi and Attri, 2006; Sandhu and Joshi, 1996; Silverira et al., 2008). The solid state fermentation approach gives higher productivity of bio-colours at a low cost when compared with liquid fermentation process (Cavalcante et al., 2008).

*Corresponding author’s e-mail: email Id -mhalaskarsachin10@gmail.com
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 (Vidyalakshmi et al., 2009). 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 (Esa et al., 2013). The utilization of the by-products from agricultural farm products can be of immense importance. Therefore, most attention today must be given to possible use of microorganisms to convert relatively high-energy by-products into more useful and highly nutritious end product. However, there are some important considerations necessary for microbial conversion i.e. which microorganism or microorganisms possess potentials for the bioconversion of the organic materials under consideration.

Therefore, the present investigation was aimed to address the fermentative changes in broken rice as a substrate during the production of food bio-colours through solid state fermentation process by using Monascus purpureus (MTCC 410).

**MATERIALS 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 (Babitha et al., 2006). 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 12 days. Each day, the inoculated substrate was manually shaken until all the substrate contents were separated from each other (Vanajakshi, 2006). The solid state fermentation process was performed as per the procedure depicted in Fig.1.

**Extraction of food bio-colours:** The orange bio-colour was extracted by suspending 10 gm of fermented broken rice in 25 ml of distilled water and autoclaved at 121°C for 20 minutes and cooled to room temperature (Babitha et al., 2006). 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 12 days. Each day, the inoculated substrate was manually shaken until all the substrate contents were separated from each other (Vanajakshi, 2006). The solid state fermentation process was performed as per the procedure depicted in Fig.1.

**Flow chart for the production of food bio-colours from broken rice through solid state fermentation**
extracts were pooled together individually and taken for spectrophotometric analysis (Vanajakshi, 2006).

**Estimation of food bio-colours**: Each bio-colour extract was appropriately diluted with respective organic solvent and O.D. was measured using spectrophotometer against same solvent as blank. Optical density (absorbance) was measured at λ_{525}, λ_{475} and λ_{375} corresponding to red, orange and yellow bio-colours respectively. The bio-colours yield (OD Units/ g dry mouldy substrate) of individual fraction was calculated using the following formula.

\[
\text{Bio-colour yield} = \frac{\text{OD}_{\text{abs}} \times \text{Dilution factor} \times \text{Total volume of bio-colour}}{\text{Dry weight of mouldy substrate}}
\]

Finally, the total bio-colours yield from the fermented substrate was expressed as the sum of total red, orange and yellow bio-colour in OD Units/g dry mouldy substrate (Johns and Stuart, 1991)

**Estimation of dry weight of mouldy substrate**: The wet fermented mouldy broken rice (2 gm) was taken in a pre-weighed aluminium dish to which about 5 ml of ethanol was added. The drying of sample was performed in hot air oven maintained at 105°C. After 4 h the dish was transferred to a desiccator with the help of forceps for cooling upto ambient temperature, the dish with the dry mouldy broken rice was then weighed. Drying was continued till constant weight was obtained. The difference in the weight was recorded as the moisture content of mouldy broken rice and weight of residue was recorded as weight of mouldy substrate (Vanajakshi, 2006).

**Statistical analysis**: The data obtained in the present investigation was statistically analysed by using completely randomized design as per method of Panse and Sukhatme (1989).

### RESULTS AND DISCUSSION

SSF is a technology lesser explored than submerged fermentation systems, but that has been proved to be able to give higher product yields and productivities, which is of great interest for industrial activities. In addition, costs are much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower due to the efficient utilization and value addition of wastes or by-products. The use of agro-industrial wastes or by-products such as broken rice in SSF processes is of much lower du
Temperature of 30°C appeared to favour superior yield of 72.92 OD Units/g dms at 500 nm for red bio-colour relative to the yellow bio-colour, but at elevated temperature (i.e. >35°C), the red bio-colour was degraded and yellow bio-colour was produced preferentially with yield value of 47.32 OD Units/g dms at 10°C. The data showed that highest total bio-colour yield of 158.03 OD Units/g dms was obtained at 30°C, while production decreased drastically at higher temperatures due to the mesophilic nature of Monascus purpureus (MTCC 410).

The 6 days old culture gave peak yield of red, orange, yellow and total bio-colours viz., 65.13, 46.22, 30.46 and 141.81 OD Units/g dms respectively. The optimum inoculum size for production of bio-colours by Monascus purpureus (MTCC 410) was 2%. The higher yields of red, orange, yellow and total bio-colours were 71.28, 39.26, 28.41 and 138.95 OD Units/g dms respectively observed with 2% of inoculum. The higher yields of red, orange, yellow and total bio-colours were 71.35, 44.79, 17.60 and 143.75 OD Units/g dms respectively obtained on 7th day of solid state fermentation. At pH 3.0, 4.0 and 5.0, the maximum absorbance shifted to 375 nm and 475 nm respectively. The results showed that yield differed between pH 4 and 6; red bio-colour yield (58.26 OD Units/g dms) was maximal at pH 6 and yellow bio-colour yield (48.37 OD Units/g dms) was maximal at pH 4.

Among the carbon sources tested the maltose showed a better yield. Maltose (3% w/w) supplementation leads to higher yields of red, orange, yellow and total bio-colours having yield values of 161.46, 94.79, 70.83 and 317.08 OD Units/g dms respectively. Nitrogen sources greatly increased the yield of bio-colours in broken rice substrate medium. The greater yields of red, orange, yellow and total bio-colours were contributed to value of 186.46, 122.92, 92.71 and 402.08 OD Units/g dms respectively by supplementation of medium with MSG (1% w/w).

The result of optimization study proved that broken rice has potential to be a substrate for the production of food bio-colours through solid state fermentation. Food bio-colours production by Monascus purpureus (MTCC 410) strain under solid state fermentation was influenced by physiological and chemical nature of the broken rice and associated with growth of the Monascus purpureus (MTCC 410) strain.

Bio-colours yield after optimized conditions: The peak yield value of red, orange, yellow and total bio-colour were 186.46, 122.92, 92.71 and 402.08 OD Units/g dms respectively achieved through SSF of broken rice at optimized process parameters including 70% (v/w) initial moisture content, 0.2-0.3 mm particle size, temperature 30°C, inoculation with 2% spore suspension of 6 days old culture for incubation period of 7 days at pH 6 by supplementation of maltose (3% w/w) and MSG (1% w/w) as a carbon and nitrogen source respectively.

Proximate composition of fermented broken rice: The knowledge of chemical parameters is very interesting for the industrial and nutritional evaluation of fermented products, because a number of different chemical or physical alterations can occur during the process. These alterations can cause positive or negative effects on the final product quality. The data pertaining to various chemical parameters of fermented broken rice is depicted in Table 2. It was revealed that carbohydrates content of fermented broken rice was 27.71%, protein 14.14%, fat 2.07%, fiber 6.61%, ash 3.69% and moisture 45.78% with pH of 5.5 (Table 2).

<table>
<thead>
<tr>
<th>Chemical parameter</th>
<th>Measurement/Value</th>
<th>Unfermented</th>
<th>Fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates (%)</td>
<td>74.32</td>
<td>27.71</td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>8.98</td>
<td>14.14</td>
<td></td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.50</td>
<td>2.07</td>
<td></td>
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<tr>
<td>Crude fibre (%)</td>
<td>0.70</td>
<td>6.61</td>
<td></td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.70</td>
<td>3.69</td>
<td></td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>11.80</td>
<td>45.78</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.6</td>
<td>5.5</td>
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</tr>
</tbody>
</table>

Each value represents average of three determinations

Over the 7 days of fermentation of broken rice, carbohydrate content and pH value decreased considerably. The reduction in these demonstrated the intensive metabolism and growth of fungus. During the 7 days of fermentation, the carbohydrate content of broken rice was reduced from 74.32% to 27.71% i.e. 62.72% of the carbohydrate in broken rice substrate was metabolized by fungus. The utilization of rice carbohydrate by Monascus for its metabolism and production of the secondary metabolite namely the bio-colour has also resulted in an increased protein and crude fibre content of fermented broken rice. Since carbohydrate is the main component of broken rice, the changes in carbohydrate content confirmed the metabolism of the fungus. The total protein content of fermented broken rice was increased from 8.98% to 14.14% i.e. 57.46% increase during the 7 days of fermentation. The increase in total protein content of fermented broken rice during period of cultivation resulted from the increase of fungus biomass. Moraes (1999); Anupama and Ravindra (2000) and Laufenberg et al. (2003) demonstrated that some fungal species were able to increase the protein level in agro-industries wastes. Cristina and Eliana (2009) observed an increase in the protein content during the fermentation.

Fermentation resulted in the formation of glutamic acid as the major amino acid in fermented broken rice. The crude fat content showed an slight increase of about 1.50% to 2.07% on day 7 compared to that of the initial. The increase in crude fat content was contributed to about 38%. The
increase in crude fat content in the fermented broken rice during SSF till day 7 may be attributed to the production of fungal fatty acids during fermentation. Fungi are reported to produce fatty acids at varying levels during SSF (Higashiyama et al., 2002). Heber et al. (1999) reported that Monascus fermented rice is a low fat product and it reduces the cholesterol levels. Considerable increase in the crude fiber content was observed in fermented broken rice. For fermented broken rice, the increase in fiber content was from 0.70% to 6.61% after 7 days of fermentation. In fermented broken rice the increase in fiber content contributed to about 65.88%. It may be attributed to the utilization of easily digestible soluble carbohydrates by the growing fungus, leaving the indigestible fiber content as high as reported by Singh et al. (1990).

Crude ash level in fermented broken rice increased from the initial by about 36.66%. The increase in ash content was from 2.70% to 3.69% after completion of 7 days of fermentation. The increase observed in crude ash may be due to the dry matter loss during fermentation causing a relative increase in the unaltered components of the fermented product, especially the fiber and ash contents. The initial pH of the broken rice substrate medium was 6.6. It decreased slowly throughout the fermentation period of 7 days. The lowest pH (5.5) value was observed in fermented broken rice after 7 days of fermentation. The changes in pH of broken rice content confirmed the metabolism of the fungus. Vidyalakshmi et al. (2009) reported that fermented broken rice has been found to contain an average protein content of 17.16%, fat 1.98%, crude fibre 6.71% and ash 1.65%.

CONCLUSION

Present findings concluded that broken rice possesses good potentials for the bioconversion of the high energy organic materials into more useful and highly nutritious food bio-colours by Monascus purpureus (MTCC 410). The 7 days of SSF process was accompanied with the increase in the levels of protein, crude fat, crude fiber and ash content by values of 8.98% to 14.14%, 1.50% to 2.07%, 0.70% to 6.61% and 2.70% to 3.69% respectively while decrease in the levels of carbohydrate by values of 74.32% to 27.71%, with lowering pH from 6.6 to 5.5. Broken rice has potential to be utilized as raw material for SSF, as carbohydrate content is quite high with proportionate presence of protein, crude fiber, mineral etc. can be readily converted towards the synthesis of food bio-colours.

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REFERENCES


