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Broken Rice – A Novel Substrate for the Production of Food Bio-Colours through Solid State Fermentation

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

Dept. of Food Science and Technology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri Tal.- Rahuri Dist.-Ahmednagar (M.S.) 413 722, India

*Corresponding Author E-mail: mhalaskarsachin10@gmail.com

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ABSTRACT

Natural pigments are an important alternative to potentially harmful synthetic dyes used as colourants in food. The toxicity problems caused by those of synthetic colours to the consumers have created a mounting interest towards natural colours. The food bio-colours are gaining importance and have become the focus of attention of many scientists all over the world. The feasibility of broken rice as a substrate for production of food bio-colours by Monascus purpureus (MTCC 410) in solid state fermentation was investigated by optimizing the fermentation conditions. The higher yield of red, orange and yellow bio-colours achieved were 186.46, 122.92 and 92.71 OD Units/g dms respectively with broken rice at optimized process parameters viz. 70% (w/v) initial moisture content, 0.2-0.3 mm particle size, pH 6, incubation at 30°C, inoculation with 2 ml of spores/gds of 6 days old and an incubation period of 7 days with supplementation as maltose (3% w/w) and MSG (1% w/w) as a carbon and nitrogen source respectively. The enhanced yield of bio-colours indicated that broken rice has good potentiality for the production of food bio-colours through solid state fermentation.

Key words: *Monascus, broken rice, solid state fermentation, bio-colours, colourants.*

INTRODUCTION

With the advent of strict legislative regulations and growing awareness among the consumers about the food safety, bio-colours have become the choice in the foods as these are considered as safer than their synthetic counterparts. Bio-colour 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[34]. Micro-organisms have high growth rate and productivity for pigment[5], which reduced the production time of bio-colours using a process with continuous operation[17]. 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[21].

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The bio-colours have been produced from large number of bacterial, yeast and mold species. The microorganisms for use as bio-colour source should have some necessary features[21]. Among the different microorganisms, *Rhodotorula* spp., *Achromobacter* spp., *Blakeslea* spp., *Micrococcus* spp., *Chromobacter* spp., *Sarcina* spp. and *Monascus* spp. are common bio-colour producing microbes. The application of *Monascus* bio-colours in food industry has been carried out traditionally in the oriental foods for hundreds of years[37]. Bio-colours from this fungus widely used in food and pharmaceutical industries for therapeutic uses also[22].

At present, bio-colour 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 bio-colours. *Monascus* is probably a xerophilic fungus, which grows in a wide variety of natural substrates. 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[3,4,20,33,35]. The solid state fermentation approach gives high bio-colours productivity at a low cost when compared with liquid fermentation[8].

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[40]. Rice by-products may serve an important source of raw material that could be used as ingredient of functional food and nutraceuticals. They have great potential to be converted into human food to improve food security in the country[13].

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[5]. 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 flasks were incubated for 12 days. Each day, the inoculated substrate was manually shaken until all the substrate contents were separated from each other[38]. The solid state fermentation process was performed as per the procedure depicted in Fig.1.

Optimization of fermentation parameters for the production of bio-colours

Effect of initial moisture content: The effect of initial moisture content in broken rice on yield was studied by varying moisture content at 60%, 65%, 70%,75% and 80% (w/v) level.

Effect of particle size: To study the effect of broken rice particle size on yield was observed by varying particle sizes such as 0.09-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4 and 0.4-0.6 mm.

Effect of temperature: The effect of incubation temperature on yield of bio-colours was studied by varying temperatures viz. 20°C, 25°C, 30°C, 35°C and 40°C using broken rice as a substrate.

Effect of inoculum age: The effect of inoculums age on the yield of bio-colours was studied by using 3, 4, 5, 6 and 7 days old cultures.

Effect of inoculum size: The effect of inoculum size on yield of bio-colours was studied using different volumes of inoculum (1, 2, 3, 4 and 5 ml of spores/gds).

Effect of incubation time: The effect of incubation time on yield of bio-colours was studied by using 3, 5, 7, 9 and 12 days of incubation.

Effect of pH: The effect of pH was studied by varying initial pH of medium ranging from 3.0, 4.0, 5.0, 6.0 and 7.0 using 0.1N HCl.

Effect of carbon sources: The effect of different carbon sources such as glucose, maltose, fructose, sucrose and lactose (3% w/w) as a supplement on yield of bio-colours was tested with optimized fermentation parameters.

Effect of nitrogen sources: The effect of different nitrogen sources such as peptone, urea, ammonium sulphate, yeast extract and MSG (1%) as a supplement on yield of bio-colours was evaluated with optimized fermentation parameters.

Extraction of bio-colours: The orange bio-colour was extracted by suspending 10 gm of fermented broken rice in 25 ml of distilled water and mixing on a rotary shaker at 28 ± 2°C for 15 minutes. This step was repeated till the orange bio-colour soluble in water was extracted completely from the fermented substrate. Similarly, the red and yellow bio-colours were extracted using absolute alcohol and hexane as a solvent respectively. The water, alcohol and hexane extracts were pooled together individually and taken for spectrophotometric analysis[38].

Estimation of 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_{500}, 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.

$$\text{Bio-colour yield} = \frac{OD_{(abs)} \times \text{Dilution factor} \times \text{Total volume of bio-colour}}{\text{Dry weight of mouldy substrate}}$$

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[19].

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[38].

Statistical analysis: The data obtained in the present investigation was statistically analysed by using completely randomized design as per method of Panse and Sukhatme[30].

RESULTS AND DISCUSSION

Proximate composition of broken rice

The data pertaining to various chemical properties of broken rice are depicted in Table 1. The proximate composition of broken rice plays an important role for deciding its nutritional and functional qualities. It was observed that carbohydrates content of broken rice was 74.32%, protein 8.98%, fat 1.50%, fiber 0.70%, ash 2.70% and moisture 11.80% with pH of 6.6. The chemical composition reflects that broken rice has a potential as a conventional substrate for the production of

food bio-colours through solid state fermentation.

Effect of different fermentation parameters on yield of bio-colours

The results obtained during optimization of fermentation parameters using broken rice as a substrate with respect to its moisture, particle size, temperature, inoculum age, inoculum size, incubation time, pH also supplementation with carbon and nitrogen sources are discussed.

Effect of initial moisture content on bio-colours yield

The higher yield of red, orange and yellow bio-colours obtained were 72.45 OD Units/g dms at 500 nm, 56.40 OD Units/g dms at 475 nm and 20.20 OD Units/g dms at 375 nm respectively at 70% (w/v) initial moisture content of broken rice as compare to other levels of initial moisture (Fig. 2). The present findings are comparable with the findings reported by Mitchell *et al*[25]., Gautam *et al*[15]., and Perez-Guerra *et al*[31]., for initial moisture content of various agricultural wastes or by-products as a substrate in SSF. The moisture content of substrate plays a key role in fungal growth, enzyme activity and metabolite production in solid state fermentation[29,41,42]. The optimum initial moisture content favoured the mass transfer, intake of oxygen and release of carbon dioxide[4,14,24] and also facilitates effective absorption of the nutrients from the substrates for growth and metabolic activities[18].

Effect of particle size on bio-colours yield

The broken rice of particle size in between 0.2-0.3 mm was optimal for bio-colours yield. The peak yield of red, orange and yellow bio-colours were recorded as 70.80 OD Units/g dms at 500 nm, 47.25 OD Units/g dms at 475 nm and 18.88 OD Units/g dms at 375 nm respectively at 0.2-0.3 mm particle size. Generally, smaller substrate particles provide a larger surface area for microbial activity and thus it should be considered as a desirable factor for higher bio-colour production. However, too small particles may result in substrate agglomeration, which may interfere with aeration (due to less interparticle space) and thus may result in poor microbial growth and bio-colour yield. At the same time, larger particles provide better aeration efficiency (due to increased interparticle space), but provide limited surface for microbial activity[12]. Among the several factors in solid state fermentation which are important for microbial growth activity, the substrate particle size is one of the most critical parameters[28,43].

Effect of temperature on bio-colours yield

The yield obtained for red, orange and yellow bio-colours were 72.92 OD Units/g dms at 500 nm, 40.80 OD Units /g dms at 475 nm and 47.32 OD Units/g dms at 375 nm respectively at 30, 35 and 40^{o}C (Fig. 4). The production of total yield of bio-colours decreased drastically at higher temperatures due to the mesophilic nature of *Monascus* spp. The maintenance of an optimal process temperature is one of the major factors for getting higher yields of microbial metabolites. The temperature affects microbial cellular growth, spore formation, germination and microbial physiology, thus affecting bio-colours formation. Results are in agreement with Domsch *et al*[12]., and Babitha *et al*[5]., reported an optimum temperature of 30°C to 37°C for growth of *Monascus* spp.

Effect of inoculum age on bio-colours yield

The results indicated that the broken rice inoculated with 6 days old culture gave better yield of bio-colour i.e. red colour (65.13 OD Units/g dms at 500 nm), followed by orange colour (46.22 OD Units/ g dms at 475 nm) and yellow colour (30.46 OD Units/ g dms at 375 nm) as shown in Fig. 5. An increased in inoculum age resulted in decreased mycelial growth. Amongst several fungal physiological properties, the inoculum age usually plays an important role in fungal activity[6,16].

Effect of inoculum size on bio-colours yield

The results (Fig. 6) indicated that inoculating medium with 2 ml of spores/gds reported considerably higher yield of red colour (71.28 OD Units/g dms at 500 nm), followed by

orange colour (39.26 OD Units/ g dms at 475 nm) and yellow colour (28.41 OD Units/ g dms at 375 nm). The lower levels of inoculum resulted in insufficient biomass and lower yield of bio-colour, whereas too much inoculum produced excessive biomass and depleted the nutrients required for bio-colour formation[5]. The results are in agreement with previous studies[5,9,27]. A suitable inoculum size was needed for higher yield of bio-colours[32].

Effect of incubation time on bio-colours yield

The higher yield of red bio-colour on 7th day of fermentation was 71.35 OD Units/g dms at 500 nm, while yield of orange and yellow bio-colours were 44.79 OD Units/g dms at 475 nm and 27.60 OD Units/g dms at 375 nm respectively (Fig. 7). Velmurugan *et al*[39]., reported the maximum yield of red colour 33.42 OD Units/g dms, while yellow colours it was 15.28 OD Units/g dms on 7th day of fermentation by *Monascus purpureus* (KACC 42430). The production of bio-colour decreased after 9th day of incubation which might be due to the decline in growth of the fungus due to the depletion of medium ingredients. More or less similar results were reported by Carvalho *et al*[7].

Effect of pH on bio-colours yield

The results showed that red bio-colour yield was maximum (58.26 OD Units/g dms at 500 nm) at pH 6 and orange bio-colour (47.41 OD Units/g dms at 475 nm) was maximum at pH 5 (Fig. 8). These results are consistent with Babitha *et al*[5]. (2006), who reported maximum bio-colours production by *Monascus purpureus* (MTCC 410) at pH 4.5 to 7.5, with jack fruit seed powder as substrate in solid state fermentation. Yongsmith *et al*[42]., reported that a lower pH promotes synthesis of yellow colour, whereas a higher pH favours red colour.

Effect of carbon sources on bio-colours yield

Among the various carbon sources tested maltose (3% w/w) promoted maximal yield of bio-colours for *Monascus purpureus* (MTCC 410) strain. Glucose and fructose were found to be good supporters for bio-colours production next to maltose. The lactose showed little effect on yield of bio-colours. It was observed that maltose when used to 3% (w/w) concentration, the highest total yield of bio-colour was observed to be 327.08 OD Units/g dms. The higher yield of red, orange and yellow bio-colours were (161.46 OD Units/g dms), (94.79 OD Units/g dms) and (70.83 OD Units/g dms) respectively, when maltose (3% w/w) was supplemented in broken rice medium. The findings in this study were also confirmed with an earlier study that hexoses and disaccharides, except lactose, supported good growth for virtually all cultivated fungi[10].

Effect of nitrogen sources on bio-colours yield

Nitrogen sources greatly increased the yield of bio-colours produced by broken rice substrate medium (Fig. 10). There was remarkable increase in the total yield of bio-colours by broken rice medium supplemented with MSG. MSG as the nitrogen source was found to gave the outstanding yield of 186.46, 122.92 and 92.71 OD Units/g dms for red, orange and yellow bio-colours respectively. The results obtained in the present study are confirmed with Vidyalakshmi *et al*[40]., who found that mono sodium glutamate resulted in maximum pigment 0.464 U/g (510 nm) and 1.314 U/g (410 nm) of *Monascus* fermented rice for both red and yellow pigments respectively. Based on the results it was found that MSG was the best organic nitrogen source and its supplementation increased total yield of bio-colours to 402.08 OD Units/g dms.

Bio-colours yield after optimized conditions

The peak yield value of red, orange, yellow and total bio-colours 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% (w/v) initial moisture content, 0.2-0.3 mm particle size, temperature 30°C, inoculation with 2 ml of spores/gds of 6 days old 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 data pertaining to various chemical parameters of fermented broken rice is depicted in Table 1. 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 1).

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 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 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 fungal biomass. Moraes[26]; Anupama and Ravindra[1] and Laufenberg *et al*[23]., demonstrated that some fungal species were able to increase the protein level in agro-industries wastes. Cristina and Eliana[11] observed an increase in the protein content during the fermentation.

The crude fat content showed a 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.

For fermented broken rice, the increase in fiber content was from 0.70% to 6.61% after 7th 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 high as reported by Singh *et al*[36].

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.

Table 1. Proximate composition of unfermented and fermented broken rice

Sr. No.	Chemical parameter	Measurement/Value	
		Unfermented	Fermented
1	Carbohydrates (%)	74.32	27.71
2	Protein (%)	8.98	14.14
3	Fat (%)	1.50	2.07
4	Crude fibre (%)	0.70	6.61
5	Ash (%)	2.70	3.69
6	Moisture (%)	11.80	45.78
7	pH	6.6	5.5

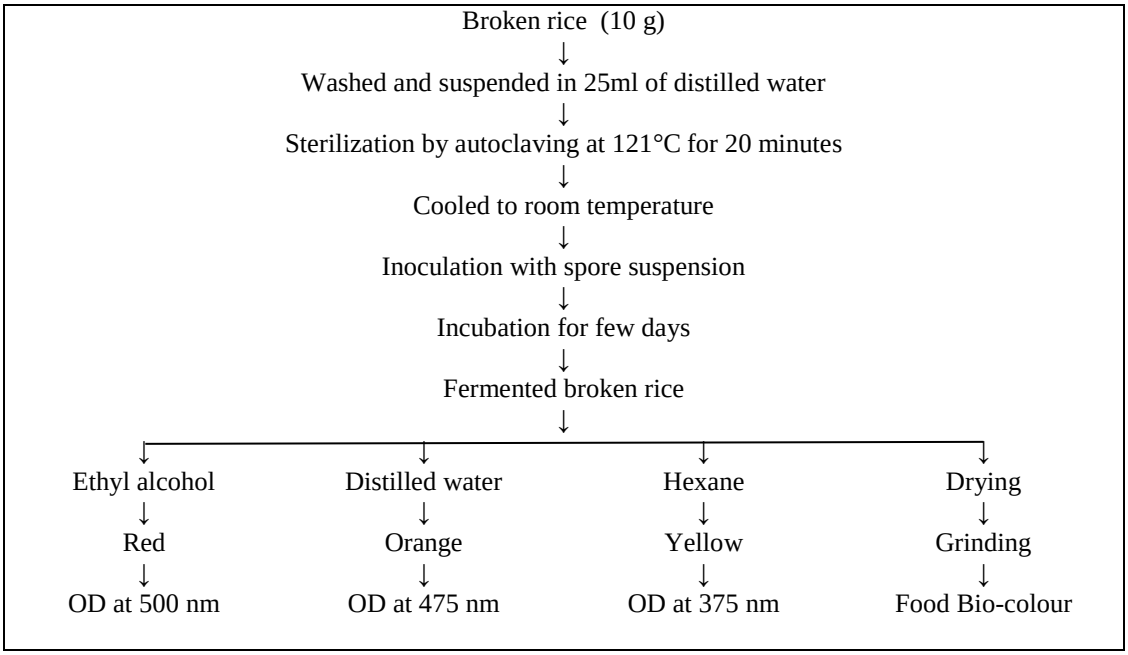

Fig.1: Flow chart for the production of food bio-colours from broken rice through solid state fermentation

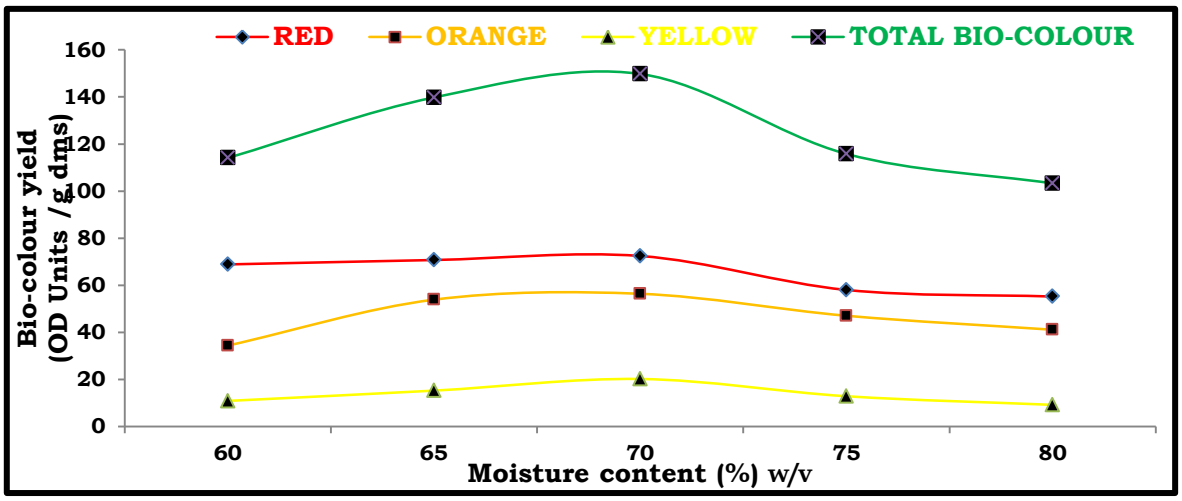

Fig. 2: Effect of initial moisture content on bio-colours yield

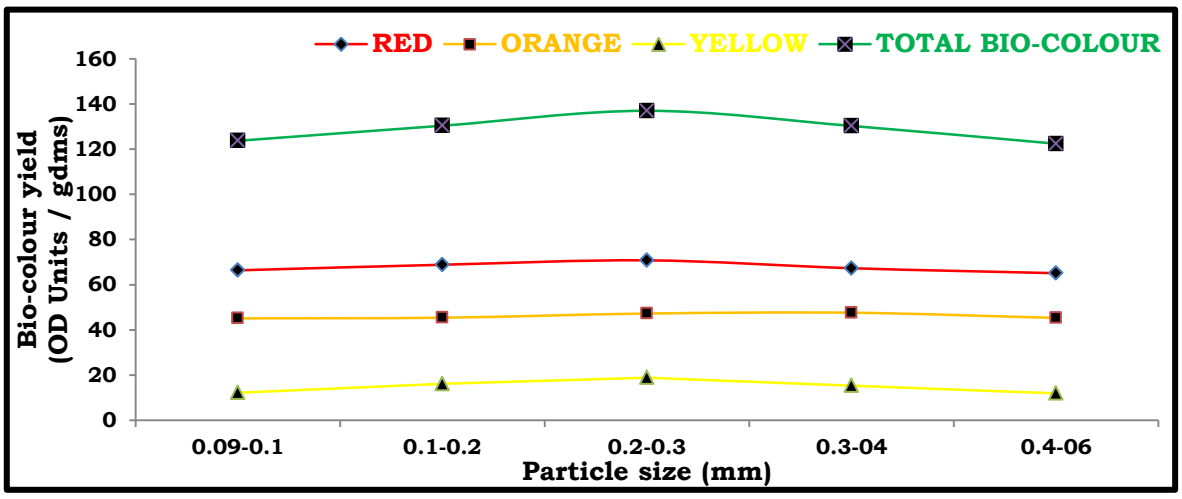

Fig. 3: Effect of particle size on bio-colours yield

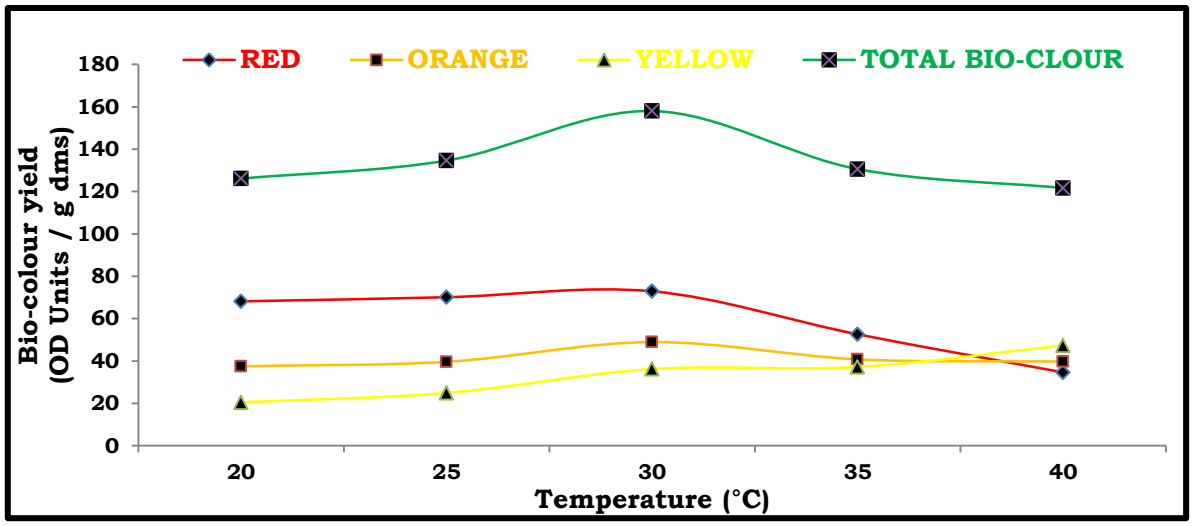

Fig. 4: Effect of temperature on bio-colours yield

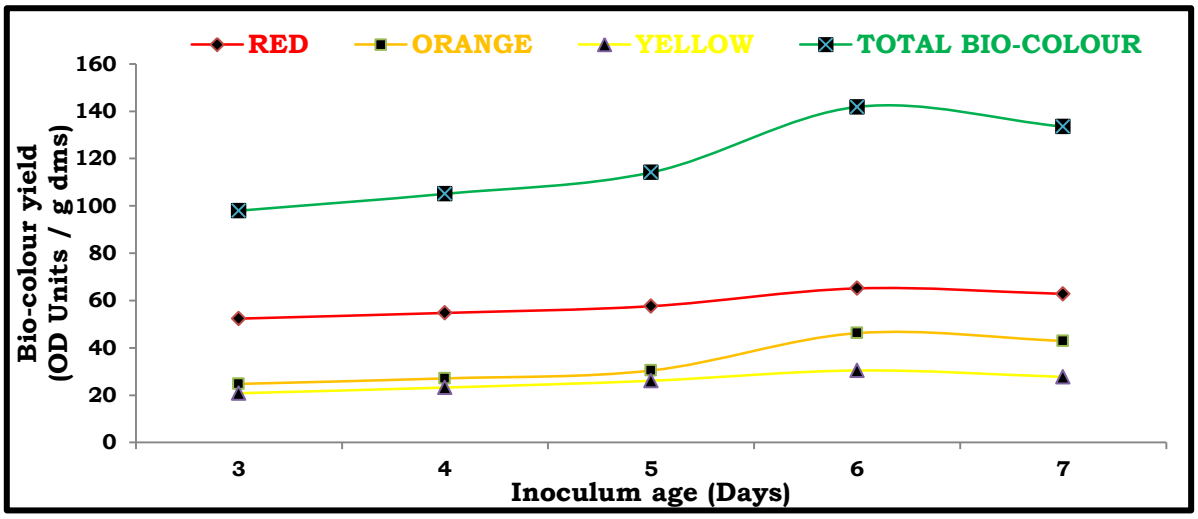

Fig. 5: Effect of inoculums age on bio-colours yield

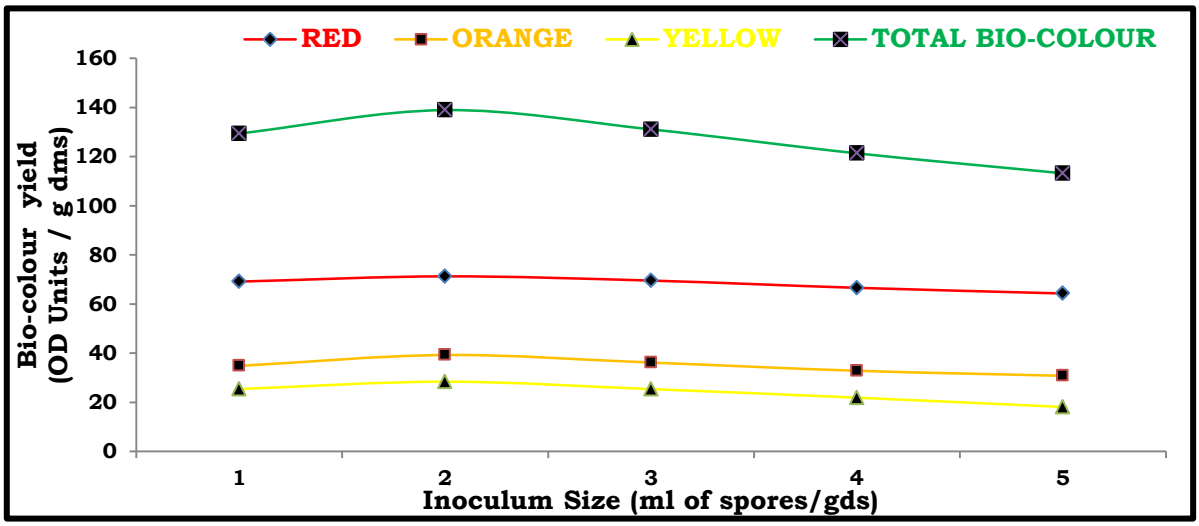

Fig. 6: Effect of inoculum size on bio-colours yield

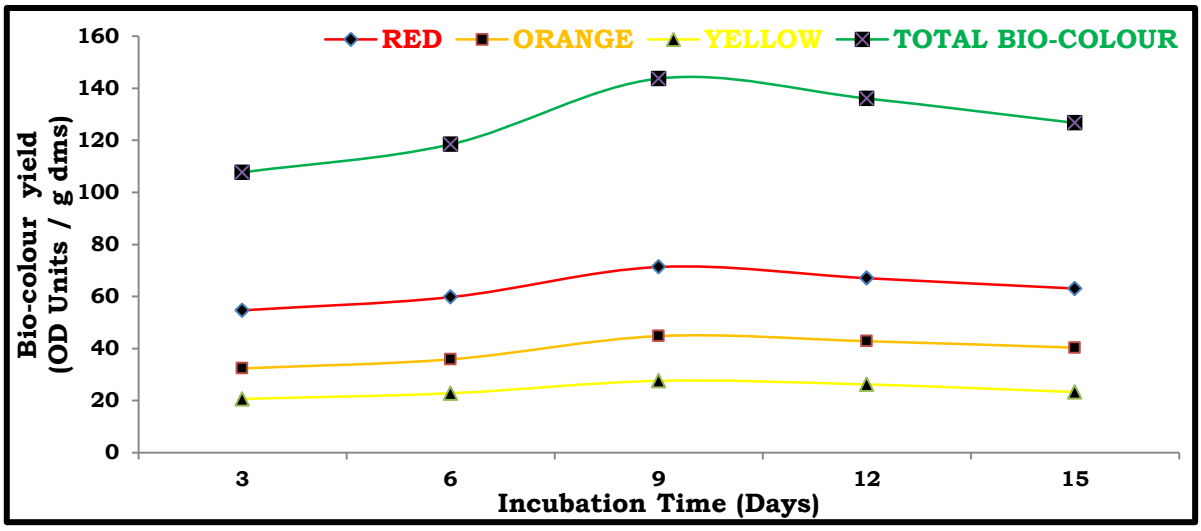

Fig. 7: Effect of incubation time on bio-colours yield

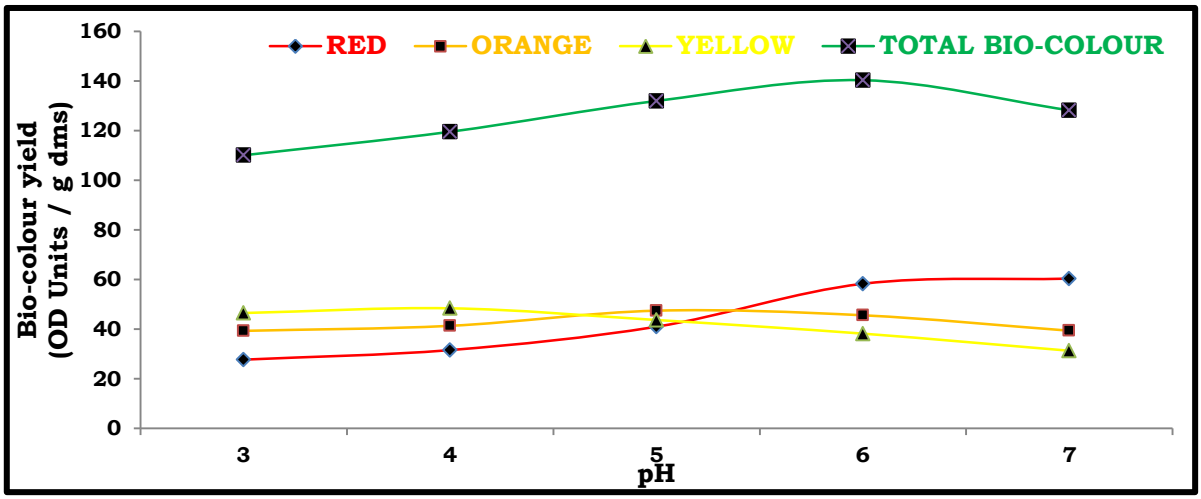

Fig. 8: Effect of pH on bio-colours yield

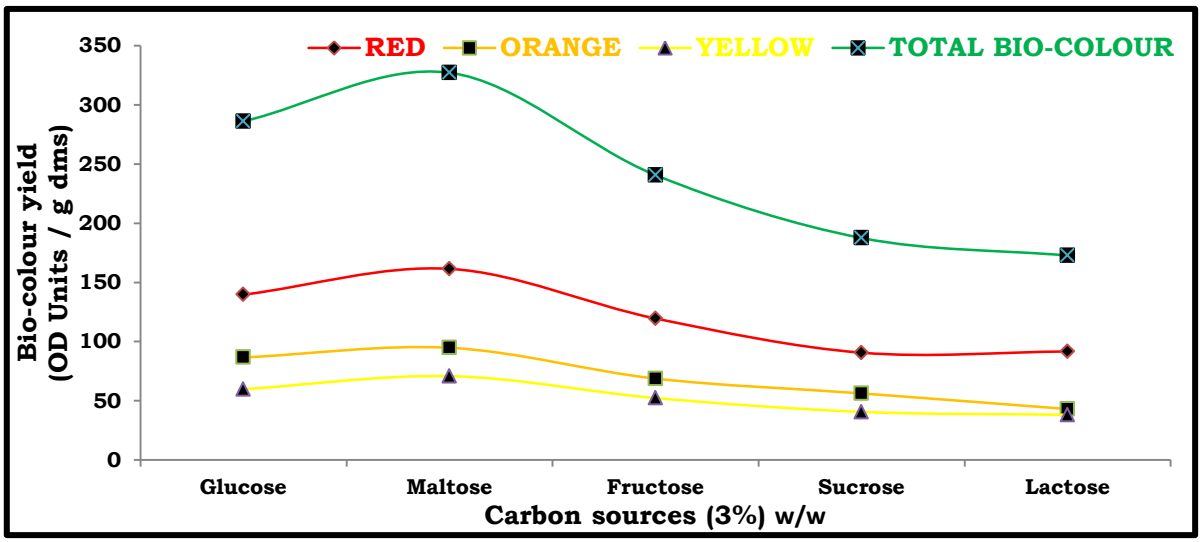

Fig. 9: Effect of carbon sources on bio-colours yield

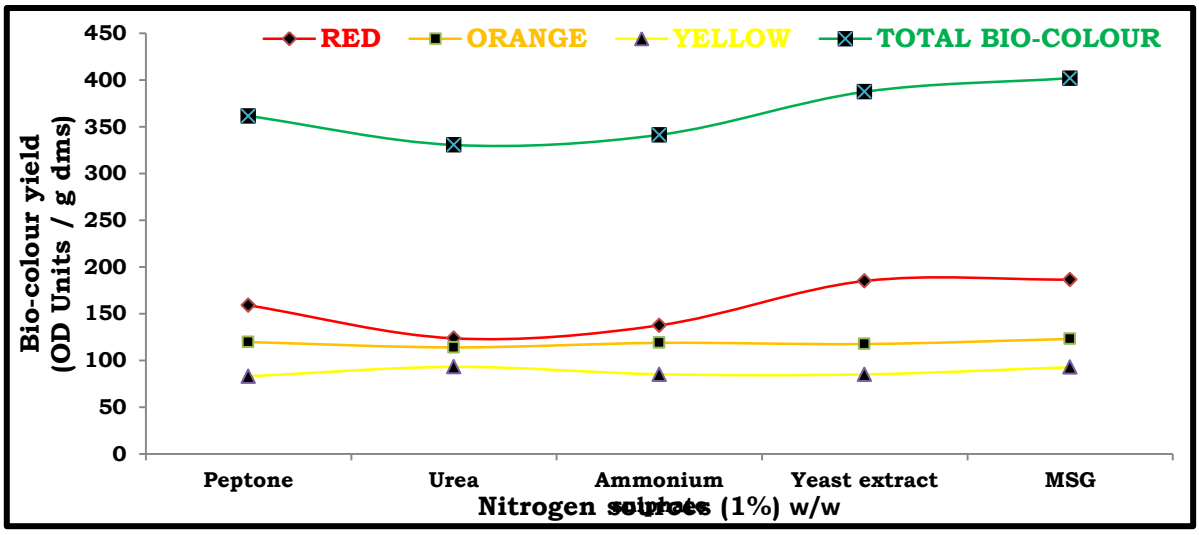

Fig. 10: Effect of nitrogen sources on bio-colours yield

CONCLUSION

From the results it can be concluded that broken rice could be an effective substrate for the production of food bio-colours by fungal culture of *Monascus purpureus* (MTCC 410) through solid state fermentation. The peak yield value of red, orange, yellow and total bio-colours 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% (w/v) initial moisture content, 0.2-0.3 mm particle size, 30°C temperature with 2 ml of spores/gds of 6 days old 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. It could also be established that by varying the fermentation conditions, the fungal metabolism changed to produce red, orange and yellow bio-colours in varying concentrations.

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