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RESEARCH PAPER

Bio-utilization of Soybean Meal for the Production of Food Bio-Colours through Solid State Fermentation

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Abstract

Natural pigments are an important alternative to potentially harmful synthetic dyes used as colourant in food. The feasibility of soybean meal as a substrate for production of food bio-colour by *Monascus purpureus* (MTCC 410) in solid-state fermentation was investigated to optimize the fermentation conditions. The results showed that the highest yield of red, orange, yellow and total bio-colour were 29.36 Units/g dms at 500 nm, 23.06 Units/g dms at 475 nm, 9.80 OD Units/g dms at 375 nm and 62.22 OD Units/ g dms respectively achieved with soybean meal at optimized process parameters that include 65% (w/v) initial moisture content, 0.3-0.4 mm particle size, temperature 30°C, pH 6, inoculation with 3% spore suspension of 6 days old culture and an incubation period of 9 days. The yield of bio-colours indicated that soybean meal has good potentiality to be used as a substrate for the production of food bio-colours through solid state fermentation.

Keywords*: Monascus,* soybean meal, bio-colours, solid state fermentation, colourants

"Bio-colour" word consists of two words 'Bio' meaning natural and 'Colour' meaning anything which is used for colouring purpose. With the advent of strict legislative regulations and growing awareness among the consumers about 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, human body 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 can cut down the production time of bio-colour 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. Therefore, it is great advantageous to use microbes for food bio-colour production due to their intrinsic properties of high growth rate, no seasonal variation, high production rate and ease of manipulation (Joshi *et al.* 2003).

But 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 (Joshi *et al.* 2003). 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 (Joshi *et al.* 2012). 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 can be widely used in food and pharmaceutical industries for therapeutic use besides bio-colour (Kumar *et al.* 2012).

At present, bio-colour production at an industrial scale is not economical since the cost of production is still high. Therefore, the development of lowcost 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 (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 (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 high bio-colour productivity at a low cost when compared with liquid fermentation (Cavalcante *et al.* 2008). These reports indicated that utilization of cheaply available substrates in solid state fermentation could be a good strategy for attaining significant bio-colour production.

Today soybeans (*Glycine max*) are grown primarily for the production of vegetable oil for human consumption and soybean meal (SBM) is a by-product. The latter is considered to be the most nutritive plant protein and used as the major protein source in diets (El-Sayed, 1999) besides being an important source of other nutrients such as sucrose, oligosaccharide and minerals. Therefore, an attempt was made to address the nutritive potential of soybean meal for production of bio-colour through solid state fermentation by using *Monascus purpureus* (MTCC 410). The results are reported here.

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 the refrigerator and by periodically sub-culturing after every two months whenever needed

Preparation of inoculum

The *Monascus purpureus* (MTCC 410) strain was grown on potato dextrose agar 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 and was inoculated in 100 ml of potato dextrose broth and incubated in shaker at 30˚C at 120 rpm for 5 days.

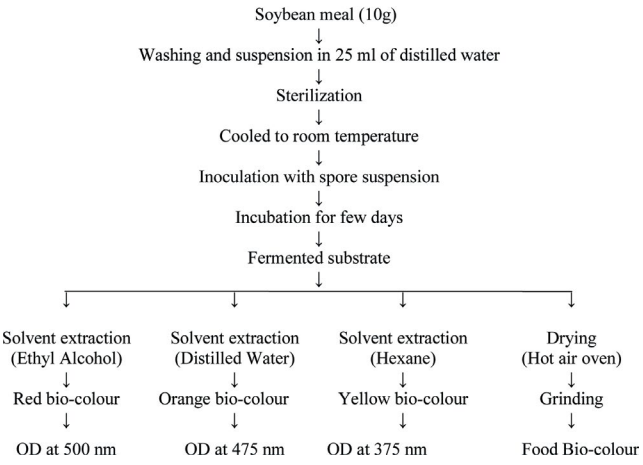

Fig. 1: Production of bio-colour using soybean meal as a substrate through solid state fermentation

Solid state fermentation

Ten gram of soybean meal was washed with tap water and was suspended in a 500 ml Erlenmeyer flask with 25 ml of distilled water and a salt solution (2 ml) containing (g/l): KH_2PO_4, 2; NH_4NO_3,5; NaCl, 1 and $MgSO_4$. $7H_2O$, 1, autoclaved at 121˚C for 20 minutes and cooled to room temperature (Babitha *et al.* 2006). With spore suspension, the sterile soybean meal medium was inoculated

under aseptic conditions, mixed with sterile rod to ensure uniform distribution of the spores. After inoculation, 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 (Vanajakshi, 2006). The solid state fermentation was performed as per the procedure depicted in Fig. 1.

Optimization of fermentation parameters for production of bio-colour

Effect of initial moisture content: The effect of initial moisture content in soybean meal 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 particle size on yield, the soybean meal substrate of varying particle sizes, 0.09-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4 and 0.4-0.6 mm were used.

Effect of temperature: The effect of incubation temperature on yield of bio-colour was studied by varying temperature viz. 20, 25, 30, 35 and 40°C was used for incubation of soybean meal substrate.

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

Effect of inoculum size: The effect of inoculum size on yield of bio-colour was studied using different volumes of inoculum (1, 2, 3, 4 and 5%).

Effect of incubation time: The effect of incubation time on yield of bio-colour was studied by using of 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 and 0.1N NaoH

Extraction of bio-colours

Ten gram of the fermented soybean meal was suspended in 25 ml of distilled water, incubated on a rotary shaker at 28 ± 2°C for 15 minutes. The extracted orange bio-colour was decanted and this step was repeated till the orange bio-colour soluble in water was extracted completely from the fermented substrate. Similarly, the extraction of red and yellow bio-colours were performed using absolute alcohol and hexane as a solvent, respectively. The alcohol, water and hexane extracts were pooled together individually and taken for spectrophotometric analysis (Vanajakshi, 2006).

Estimation of bio-colours

Each bio-colours extract was appropriately diluted with respective organic solvent and O.D. values were measured using spectrophotometer against same solvent as a 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-colour 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-colour yield from the fermented substrate was expressed as the sum of total red, orange and yellow bio-colour OD Units/ g dry mouldy substrate (Johns and Stuart, 1991)

Estimation of dry weight of mouldy substrate

Two gram of wet fermented mouldy soybean meal 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 hr, the dish was transferred to a desiccator with the help of forceps for cooling upto ambient temperature, the dish with the dry mouldy soybean meal was then, weighed. Drying was continued till constant weight was obtained. The difference in the weight was recorded as the moisture content of mouldy soybean meal 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).

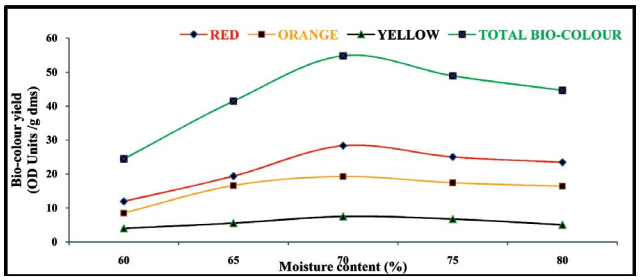

Fig. 2: Effect of moisture content on bio-colour yield at 30°C with 2% inoculums of 7 days old of pH 6 for 12 days of incubation

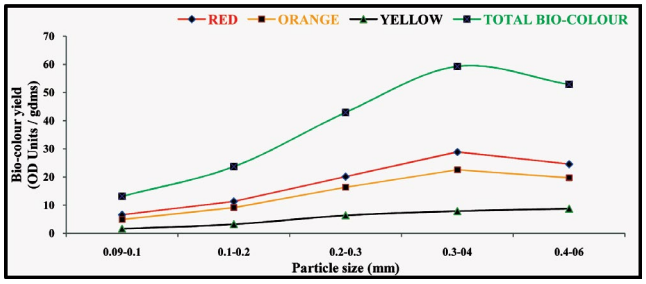

Fig. 3: Effect of particle size on bio-colour yield at 30°C with 2% inoculums (7 days old) pH 6 with 12 days of incubation and 70% (w/v) moisture content

Results and Discussion

Effect of different fermentation parameters

Initial Moisture Content: Optimization of fermentation parameters using soybean meal as a substrate with respect to initial moisture, particle size, temperature, inoculum age, inoculum size, incubation time and pH were carried out. Effect of moisture content of soybean meal substrate on bio-colours yield was presented in Fig. 2, showed that the higher yield of red, orange and yellow bio-colours obtained were 18.16 OD Units/g dms at 500 nm, 16.56 OD Units/g dms at 475 nm and 5.88 OD Units/g dms at 375 nm, respectively at 65% (w/v) moisture content compared to other levels of initial moisture contents. Similar results have been reported earlier by Johns and Stuart (1991) and Babitha *et al.* (2006), who reported reduction in bio-colour production at substrate moisture content below 60%. The moisture content of substrate are known to play a major key role in fungal growth, enzyme activity and metabolite production in solid state fermentation (Pandey, 2003; Wong *et al.* 1975; Yongsmith *et al.* 2000).

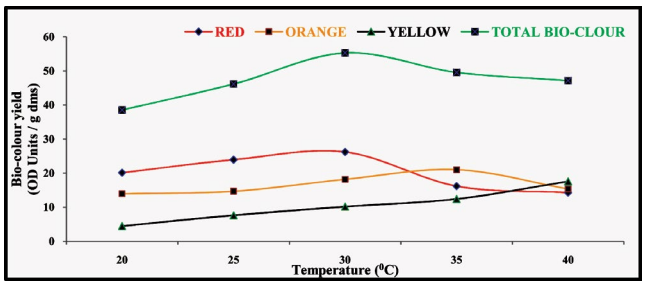

Fig. 4: Effect of temperature on bio-colour yield at 70% (w/v) moisture content, 0.3-0.4 mm particle size with 2% inoculums of 7 days old of pH 6 for 12 days of incubation

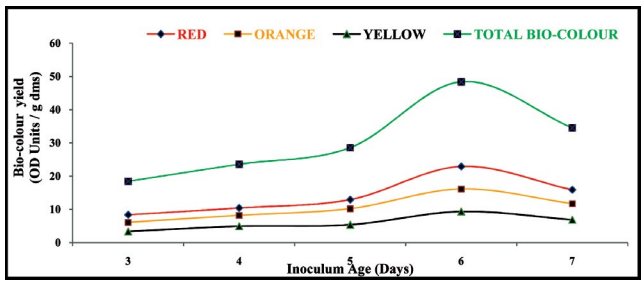

Fig. 5: Effect of inoculums age on bio-colour yield at 70% (w/v) moisture content, 0.3-0.4 mm particle size, 30°C with 2% inoculum of pH 6 for 12 days of incubation

Particle Size: The results (Fig. 3) indicated that soybean meal substrate of particle size in between 0.3 and 0.4 mm were optimal for bio-colour yield. The higher yield of red, orange and yellow bio-colours were recorded 28.89 OD Units/g dms at 500 nm, 22.58 OD Units/g dms at 475 nm and 7.88 OD Units/g dms at 375 nm, respectively at 0.3-0.4 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 (Domsch *et al.* 1980).

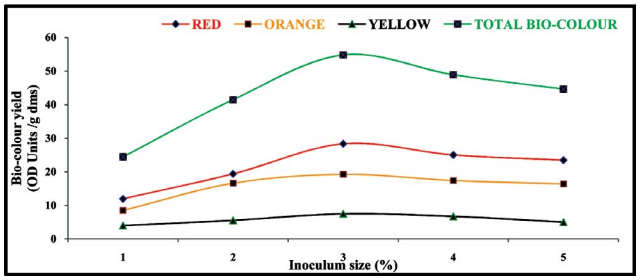

Fig. 6: Effect of inoculums size on bio-colour yield at 70% (w/v) moisture content, 0.3-0.4 mm particle size, 30°C with 6 days old inoculums of pH 6 for 12 days of incubation

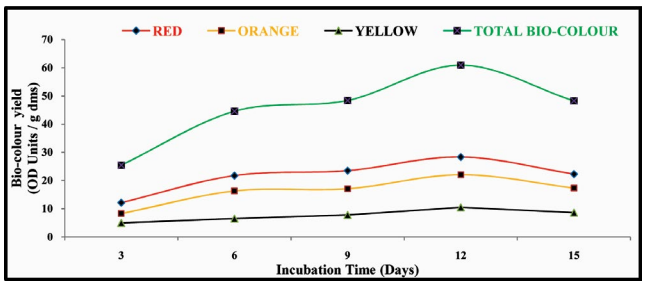

Fig. 7: Effect of incubation time on bio-colour yield at 70% (w/v) moisture content, 0.3-0.4 mm particle size, 30°C with 3% spore suspension of 6 days old inoculums of pH 6

Temperature of Incubation: The higher yield obtained for red, orange and yellow bio-colours were 26.18 OD Units/g dms at 500 nm, 21.01 OD Units /g dms at 475 nm and 17.54 OD Units/g dms at 375 nm, respectively at 30, 35 and 40°C (Fig. 4). The production of total yield of bio-colours decreased drastically at higher temperatures might be due to the mesophilic nature of *Monascus* spp. The maintenance of an optimal process temperature is one of the major factors in the economics of a process. The temperature affects microbial cellular growth, spore formation, germination and microbial physiology, thus affecting bio-colour formation. The results are in agreement with Domsch *et al.* (1980) and Babitha *et al.* (2006), who found an optimum temperature of 30°C to 37°C for the growth of *Monascus* spp.

Age of Inoculum: The effect of inoculum age on bio-colour yield was studied by using inoculums culture with varying ages. The results revealed that the soybean meal substrate medium inoculated with 6 days old culture gave maximum yield of bio-colour i.e. red colour (22.92 OD Units/g dms at 500 nm), followed by orange colour (16.14 OD Units/ g dms at 475 nm), with yellow colour (9.36 OD Units/ g dms at 375 nm) as shown in Fig. 5. An increase in the age of inoculums decreased the mycelial growth. Amongst several fungal physiological properties, the age of inoculum usually plays an important role in fungal activity (Glazebrook *et al.* 1992; Bae *et al.* 2000).

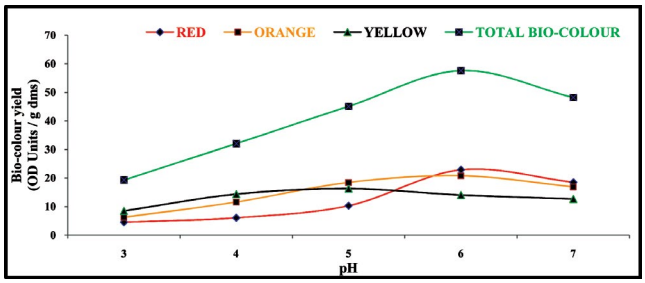

Fig. 8: Effect of pH on bio-colour yield at 70% (w/v) moisture content, 0.3-0.4 mm particle size, 30°C with 3% spore suspension of 6 days old inoculums for 9 days of incubation

Size of Inoculum: The results (Fig. 6) indicated that inoculating medium with 3% of spores suspension reported higher yield of red bio-colour (28.31 OD Units/g dms at 500 nm), followed by orange bio-colour (19.23 OD Units/ g dms at 475 nm), with yellow bio-colour (7.48 OD Units/ g dms at 375 nm). The lower levels of inoculum might have resulted in production of insufficient biomass and have lower yield of bio-colour, whereas too much inoculum might have produced excessive biomass and also depleted the nutrients required for bio-colour formation (Babitha *et al.* 2006). The results are in agreement with previous studies (Babitha *et al.* 2006; Chakradhar *et al.* 2009; Pandey *et al.* 2005).

Incubation Time: As shown in Fig. 7. the higher yield of red bio-colour on 9th day of fermentation was 28.36 OD Units/g dms at 500 nm, while yield of orange and yellow bio-colours were 22.06 OD

Units/g dms at 475 nm and 10.50 OD Units/g dms at 375 nm, respectively. Velmurugan *et. al*., (2011) have reported that the maximum yield of red colour was 33.42 OD Units/g dms, while yield of yellow colours was 15.28 OD Units/g dms respectively, 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 growth phase of the fungus as well as due to the depletion of medium ingredients.

Initial pH: *Monascus purpureus* (MTCC 410) biomass and bio-colour yields were reported at different initial substrate pH levels (Fig. 8). At pH 3.0 and 4.0, the maximum absorbance shifted to 375 nm. The results (Fig. 8) showed that red bio-colour yield was maximal (22.92 OD Units/g dms at 500 nm) at pH 6 and orange bio-colour (18.48 OD Units/g dms at 475 nm) was maximal at pH 5. These results are consistent with Babitha *et al.* (2006), who found maximum bio-colour production by *Monascus purpureus* (MTCC 410) at pH 4.5 to 7.5, while using jack fruit seed as substrate in solid state fermentation. Yongsmith *et al.* (2000) reported that a lower substrate pH promotes synthesis of yellow colour, whereas a higher pH promotes red colour.

The outstanding yield of red, orange, yellow and total bio-colour 29.36 OD Units/g dms at 500 nm, 23.06 OD Units/g dms at 375 nm, 9.80 OD Units/g dms and 62.22 OD Units/ g dms respectively achieved with soybean meal substrate medium at optimized process parameters including 65% (w/v) initial moisture content, 0.3-0.4 mm particle size, pH 5.0 to 6.0, temperature 30°C, inoculation with 3% spore suspension of 6 days old culture and an incubation period of 9 days.

Conclusion

It can be concluded from the results that soybean meal could be an effective substrate for the production of bio-colour by fungal culture of *Monascus purpureus* (MTCC 410). Soybean meal with 65% (w/v) initial moisture content, 0.3 and 0.4 mm particle size, pH 5.0 to 6.0 and 3% inoculum of 6 days old culture recorded superior yield of red, orange, yellow and total bio-colours having yield values of 29.36 OD Units/g dms at 500 nm, 23.06 OD Units/g dms at 375 nm, 9.80 OD Units/g dms and 62.22 OD Units/ g dms respectively after 9 days of fermentation. It could also be concluded 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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