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RESEARCH PAPER

Stability Analysis of Food Bio-colour Extracts from Soybean Meal through Solid State Fermentation

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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 various conditions like 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 soybean meal fermented by *Monascus purpureus* (MTCC 410) in SSF was investigated under various conditions. The food bio-colour extracts were characterized for considerable stability and it was found that these extracts are stable in dark, at low temperatures (20°C, 40°C and 60°C) while the red, orange and yellow bio-colour extracts showed a 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.

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

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-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). Microorganisms have high growth rate and productivity of pigment (Babhita, 2009), which reduced the production time of bio-colour using a process with continuous operation (Hendry and Houghton, 1997). In addition, microbial production of biocolour is flexible and can be easily controlled as compared to plant or animal sources. It is great advantageous to use microbes for food bio-colours production 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 a 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. The application of *Monascus* bio-colours in food industry has been carried out traditionally in the oriental foods for hundreds of years (Teng and

Feldheim, 2001; Babhita *et al.,* 2004). Bio-colours from this fungus widely used in food and pharmaceutical industries for therapeutic uses (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 bio-colours. *Monascus* a 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-colours productivity at a low cost when compared with liquid fermentation (Cavalcante *et al.,* 2008).

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 food bio-colours through solid state fermentation by using *Monascus purpureus* (MTCC 410).

The optimization of medium components and fermentation parameters is of primary importance in any fermentation process. Soybean meal as substrate in solid state fermentation was employed for optimization of fermentation parameters namely moisture content, particle size, incubation temperature, inoculum age, inoculum size, incubation time and initial pH of the medium as well as the extra supplementation of carbon and nitrogen sources were optimized to maximize the bio-colours yield.

Surprisingly, only a 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, among other factors. 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 exist 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 these 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 (PDA) slants for seven days at 30°C and maintained at 4°C in a 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 soybean meal was suspended in a 250 ml Erlenmeyer flask with 25 ml of distilled water and autoclaved at 121°C for 20 minutes and cooled

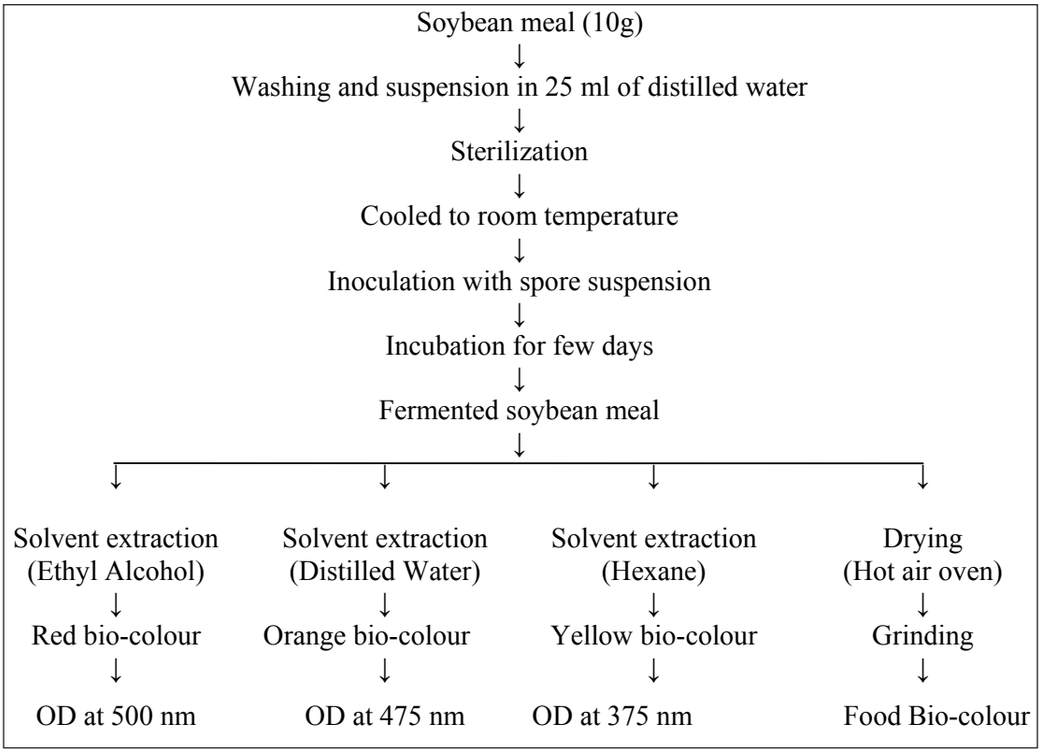


Fig. 1: Production of food bio-colours from soybean meal through solid state fermentation

to room temperature (Babitha *et al.*, 2006). The sterile soybean meal 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.

Stability analysis of food bio-colour extracts

In an attempt to estimate how stable *Monascus* food bio-colours in several applications was extracts of 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 stress conditions including 8 h in dark, 8 h in sunlight and 8 h in UV light.

Thermal stability

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

pH stability

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

The absorbance was recorded for all the tubes against a blank original sample. Absorbance was measured using a spectrophotometer after time interval of 2, 4, 6 and 8 h and the result of stability was expressed as percentage of the initial absorbance remaining after exposure to particular light, temperature and pH conditions for 8 h. Absorbance (OD) values 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

Yield profile of food bio-colours after optimization

The result of optimization study proved that soybean meal 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 soybean meal and associated with growth of the *Monascus purpureus* (MTCC 410) strain. Finally, the topmost yield value for red, orange yellow and total bio-colours were 45.76 OD Units/g dms at 500 nm, 37.95 OD Units/g dms at 475 nm, 26.23 OD Units/g dms at 375 nm and 109.93 OD units/g dms respectively, recorded through SSF of soybean meal substrate medium at optimized process parameters including 65% (w/v) of initial moisture content, 0.3-0.4 mm of particle size, incubation temperature of 30°C, inoculation with 3% inoculum of 6 days old culture and an incubation period of 9 days at pH 6, comprised of sucrose (3% w/w) and yeast extract (1% w/w) as a carbon and nitrogen source respectively.

Stability of food bio-colour extracts

Effect of light

The effect of dark, UV and sun light conditions on absorbance level of bio-colours extracted from soybean meal are shown in Fig. 2, 3 and 4. The results showed that the residual colouration of the red bio-colours after exposure to dark, UV and sunlight for 8 h indicated retention of 99.02%, 84.51% and 88.41%, respectively (Fig. 2).

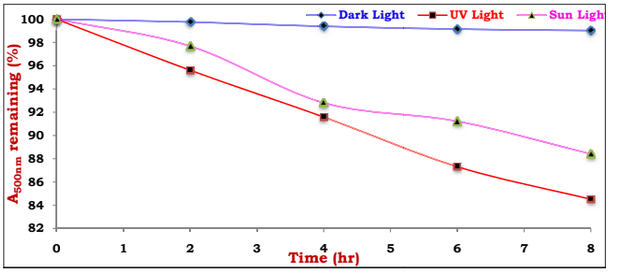


Fig. 2: Light stability of red bio-colour extract from soybean meal

Furthermore, the orange bio-colour was retained to an extent of 98.97%, 80.88% and 83.52% when exposure to dark, UV and sunlight respectively for 8 h (Fig. 3) was made.

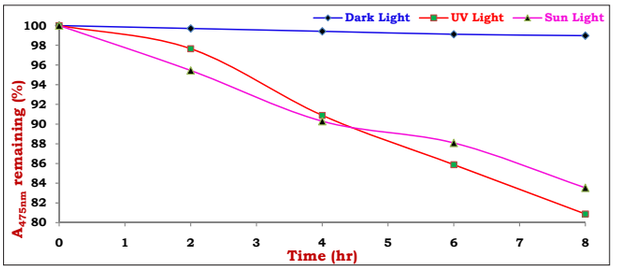


Fig. 3: Light stability of orange bio-colour extract from soybean meal

After 8 h of exposure to dark light, sun light and ultraviolet light, the yellow bio-colour extracts showed retention of 98.72%, 81.06% and 87.23%, respectively. It was revealed from the results that the food bio-colours were more stable in dark light compared to UV and sunlight.

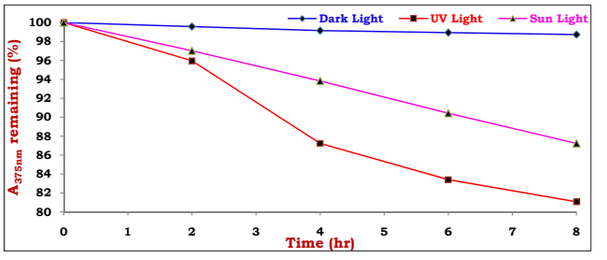
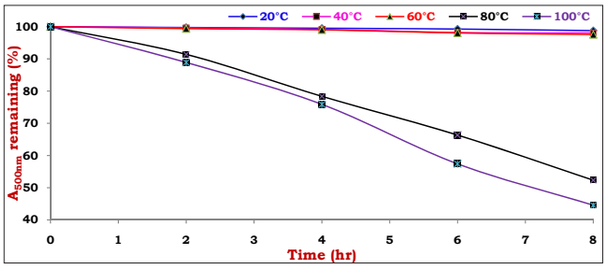


Fig. 4: Light stability of yellow bio-colour extract from soybean meal

The above findings are in good conformity with Nimnoi and Lumyong (2011) who measured stability using a relative level of residual absorbance after incubation for 1 to 6 h. The results showed that the pigment decayed over time as shown by long exposure to UV (>3 h). The dissimilar results to above findings were reported by Gunasekaran and Poorniammal (2008) who subjected the pigments of fungus to various physical and chemical conditions. It was inferred from the results that the pigments were more stable in UV light (99.2%) compared to fluorescent light and sunlight. However, the bio-colours were unstable to light (only 20% residual colour after 50 days).

Effect of temperature

The colour intensity of the red bio-colour retained upto 98.78% 98.04%, 97.56%, 52.34% and 44.56% after 8 h exposure to 20, 40, 60, 80 and 100°C, respectively (Fig. 5).



After 8 h of exposure to 20, 40, 60, 80 and 100°C temperature indicated that amount of orange bio-colour remained stable was about 98.97%, 98.52%, 97.94%, 47.34% and 41.37%, respectively (Fig. 6). A 98.93%, 94.04%, 97.87%, 45.61% and 38.60% retention of yellow bio-colour occurred when kept at 20, 40, 60, 80 and 100°C temperature respectively over 8 h (Fig. 7). It was found that destruction of bio-colours at 20, 40 and 60°C temperature were comparatively less when compared to the exposure of bio-colours under temperature of 80 and 100°C. The effect of temperature was similar to that observed in other thermal degradations, in which higher temperature greatly increases the effect. However, an Arrhenius kinetic model of exponential decay does not represent the system well. This is possibly due to the fact that the extract is a mixture of colours, whose degradation may present different decaying behaviour.

Fig. 5: Thermal stability of red bio-colour extract from soybean meal

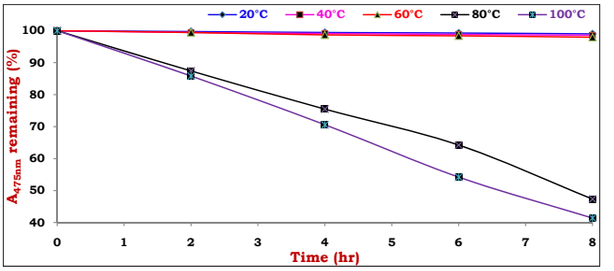


Fig. 6: Thermal stability of orange bio-colour extract from soybean meal

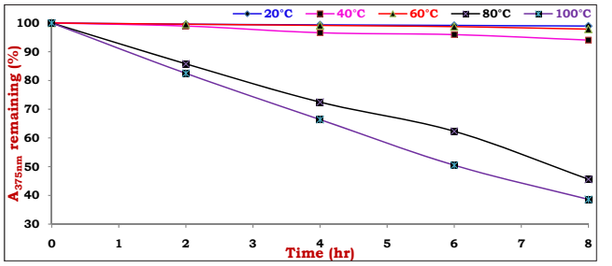


Fig. 7: Thermal stability of yellow bio-colour extract from soybean meal

The stability of bio-colours from *Monascus* has been widely studied by several authors such as Carvalho *et al*. (2005) who found that the pigments are unstable at high temperature possibly due to the fact that the extract is a mixture of pigments, whose degradation may present different decaying behaviour. Similar results to the these findings were reported by Nimnoi and Lumyong (2011) who measured stability using a relative level of residual absorbance after incubation for 1 to 6 h. The results indicated that the pigment decayed over time, as shown by an intolerance to high temperature (>40°C). The colour intensity of the red pigment after autoclaving and pasteurization decayed to 30.57% and 5.41%, respectively.

Effect of pH

The pH of the substrate medium had a major influence on colouration of the pigments in solution inducing

sometimes a modification in their structure (Berset, 1990). The results are shown in Fig. 8, 9 and 10.

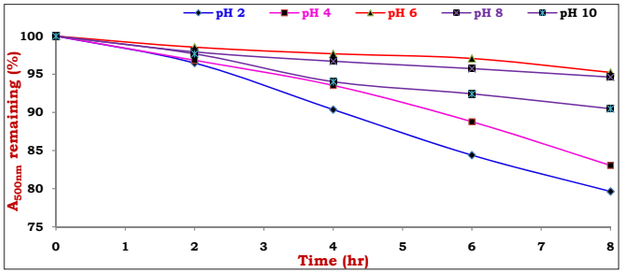


Fig. 8: pH stability of red bio-colour extract from soybean meal

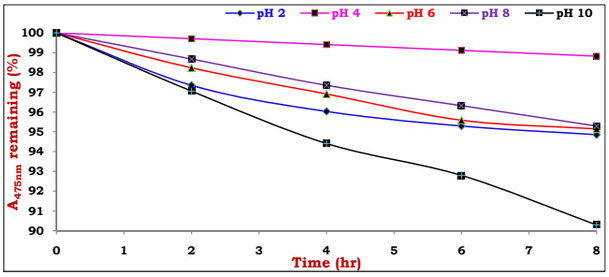


Fig. 9: pH stability of orange bio-colour extract from soybean meal

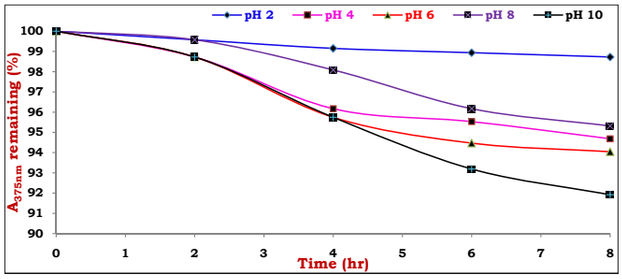


Fig. 10: pH stability of yellow bio-colour extract from soybean meal

It is revealed that, 79.63%, 83.04%, 99.02%, 94.63% and 90.48% retention of red bio-colour occurred when kept at pH of 2, 4, 6, 8 and 10, respectively over 8 h (Fig. 8). After 8 h of incubation of orange bio-colour extract at pH of 2, 4, 6, 8 and 10 showed that their colour remained stable upto 94.85%, 98.82%, 95.14%, 95.29% and 90.29% respectively (Fig. 9). A 98.72%, 94.68%, 94.04%, 95.31% and 91.91% retention was observed for yellow bio-colour at pH 2, 4, 6, 8 and 10 respectively (Fig. 10) and the highest degradation of yellow bio-colour was observed at pH of 6.

Lower pH in soybean meal substrate medium can cause fading of red bio-colour and decreases in stability of the colour while the red bio-colours were more stable at pH nearer to neutrality. Our results showed that decreasing pH causes greater destruction of red bio-colours in samples. Red bio-colour extract was stable under neutral and weekly acidic condition, while yellow bio-colour was stable under strongly acidic conditions. The stability of orange bio-colour extract was most predominant at pH 4 while further decrease or increase in absorbance leads to degradation of extract. It is thus, apparent that the orange bio-colour extract was most stable at highly acidic conditions. When the pH of red bio-colour extract is lower than 6.0, the absorbance decreased slowly and the intensity of bio-colour extract became very fade, which is the typical acidic reaction feature of bio-colours. The colour decreases more rapidly in low pH values. This effect may be due to the acid acceleration of water interaction with pigments, such as breaking of an ester linkage in rubropunctamine or monascorubramine. 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.

Faber *et al*. (1993) who studied the pigment of *Monascus ruber* and found that it was seriously affected by pH. The red pigment was more stable at basic or neutral pH. Moreover, the stability of pigment from *Monascus* has been widely studied by others such as Carvalho *et al*. (2005) who reported that the red pigments are unstable at low pH possibly due to the fact that the extract is a mixture of pigments, whose degradation may present different decaying behaviour. The above findings are in good conformity with Velmurugan *et*

al. (2011) who found that the yellow bio-colour was stable at acidic pH. The original colour of red bio-colour was retained at pH 5, 6 and 7.

CONCLUSION

It is concluded that, food bio-colour 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 show good stability at pH of 6, 4 and 2 respectively. The red, orange and yellow bio-colour extracts maintained colour retention equivalent to 99.02%, 98.97% and 98.72%, respectively after exposure to dark light for 8 h, reported good stability compared to UV and sunlight. A better stability was obtained after exposure to a temperature of 20°C, 40°C and 60°C for 8 h with (98.78% 98.04% and 97.56%), (98.97%, 98.52% and 97.94%) and (98.93%, 94.04% and 97.87%) retention for red, orange and yellow bio-colours, respectively whereas colour retention equivalent to 99.02%, 98.82% and 98.72% were recorded after 8 h exposure to pH 6, 4 and 2 for red, orange and yellow bio-colours respectively.

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