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Solid-state fermentation for the production of *Monascus* pigments from jackfruit seed

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Abstract

The aim of the present work was to investigate the feasibility of jackfruit seed powder as a substrate for the production of pigments by *Monascus purpureus* in solid-state fermentation (SSF). A pigment yield of 25 OD Units/g dry fermented substrate was achieved by employing jackfruit seed powder with optimized process parameters such as 50% initial moisture content, incubation temperature 30 °C, 9×10^4 spores/g dry substrate inoculum and an incubation period of seven days. The color of the pigments was stable over a wide range of pH, apparently due to the buffering nature of the substrate, which could be a significant point for its scope in food applications. To the best of our knowledge this is the first report on pigment production using jackfruit seed powder in solid-state fermentation (SSF).

Keywords: Solid-state fermentation; *Monascus purpureus*; Pigments; Jackfruit seed powder

1. Introduction

Color and flavor are the signals that are immediately perceived by the optical and chemical senses of humans and these attributes determine whether a certain food is appealing. Attractive food colors and flavors are usually translated into increased consumption, which is a fundamental behavioral response. However, color and flavors are often sensitive to heat, oxygen, light and acid and thus changed or lost during processing and storage. Natural colorants and flavors mainly derived from plants and chemosynthetic compounds are used by the food industry to replenish and sometimes raise the genuine stock (Pandey et al., 2001). Recent increasing concern on the use of edible coloring agents has banned various synthetic coloring agents, which have a potential of carcinogenicity and/or teratogenicity (Fabre et al., 1993). This circumstance has inevitably increased demands for highly safe, naturally occurring edible coloring agents, one of which is *Monascus*-pigment (Francis, 1987).

It has long been known that microorganisms of the genus *Monascus* produce red pigments, which can be used for coloring foods. In certain oriental countries, microorganisms of this type are grown on grains of rice and once the grains of rice are penetrated by the red mycelium, the whole matter is finely ground. Although for years it has been known that there are six pigments, in the last decade some new pigments have been discovered, which included xanthomonascin and yellow II, possibly derived from rubropunctatin (Sato, 1992; Juzlova et al., 1996; Watanabe et al., 1997). According to some authors, there are more than ten pigments, although only some of them have the structure elucidated (Shin et al., 1998). The orange pigments,

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monascorubrin and rubropunctatin, are synthesized on the cytosol from acetyl coenzyme A, through the multienzymatic complex polyketide synthase. These pigments have a structure responsible by their high affinity to compounds containing primary amino groups (thus called aminophiles). Reactions with amino acids lead to formations of hydro soluble red pigments, monascorubramine and rubropunctamine. The mechanism of yellow pigment formations is not yet clear; some authors consider that these are product of the alteration of orange pigments, as others believe it to be pigments with their own metabolic pathway (Carvalho et al., 2003).

In this regard, recent research has been devoted to the studies on general culture conditions and substrates evaluation for pigments production, and among these, most of the studies have been performed at laboratory-scale using liquid culture medium, which presents easily controllable conditions. Due to high cost of currently used technology of pigment production on an industrial-scale, there is a need for developing low cost process for the production of pigments, which could replace synthetic pigments. From the literature, it is evident that utilization of a cheaply available substrate through solid-state fermentation (SSF) could attain such an objective. In SSF process, the solid substrates not only supplies the nutrients to the microbial culture growing in it, but also serves as an anchorage for the cells (Pandey, 2003). In recent years, SSF has gained much interest for the production of primary and secondary metabolites. SSF presents a more adequate habitat for fungus, with high pigment productivity in a relatively low-cost process by using agro-industrial residues as substrates (Pandey et al., 2000, 2001).

Various agro-industrial residues such as rice bran, wheat bran, cassava, etc. have been used for pigment production. However, no effort has been made so far to utilize jackfruit seed as a substrate for pigment production. Largest of all tree-borne fruits, the jackfruit could be 20–90 cm long and 15–50 cm wide, and the weight could be from 4.5 to 20 kg, or even as much as 50 kg (Morton, 1987). Jackfruit is popular in several tropical countries and is an excellent example of a food prized in some areas of the world (mostly tropical) and is available in large part of the year at the places where produced. However, in some locations is the world, it is not used as food material and allowed to go to waste. There may be 100, or up to 300 seeds in a single fruit. Seeds make up around 10–15% of the total fruit weight and have high carbohydrate and protein contents (Bobbio et al., 1978; Kumar et al., 1988). Jackfruit seeds are discarded as waste from different agro-industries and higher percentage of fruits. Seeds also go waste from the fallen fruits. Since the seeds are rich in carbohydrate, protein and trace elements, it can be used as a potential substrate for the production of food grade red pigments.

Thus, the main objective of this study was to develop a potential fermentation process for the production of pigments employing SSF using non-conventional agro-residues and also studying the effect of different parameters in an attempt to maximize pigment production.

2. Methods

2.1. Culture

A culture of *Monascus purpureus* LPB 97, available in the Process Biotechnology Laboratory, Federal University of Parana, Brazil, was used in the present study. It was maintained on yeast extract–peptone–glucose medium (Hi-Media, Mumbai, India); preserved at 4 °C and sub-cultured once in every three weeks.

2.2. Inoculum preparation

To fully sporulated (6–8 days old) agar slope culture, 10 ml of sterile distilled water was added. Then the spores were scrapped under aseptic conditions. The spore suspension obtained was used as the inoculum (1.5×10^5 spores/ml).

2.3. Substrate and solid-state fermentation

Experiments were conducted in 250 ml Erlenmeyer flasks containing 5 g substrate (powdered jackfruit seed). The substrate was moistened with salt solution and distilled water in such a way as to obtain final moisture content of 60% After thorough mixing, the wet substrates were autoclaved at 121 °C for 20 min and cooled to room temperature. It was inoculated with 2 ml the spore suspension containing 1.5×10^5 spores/ml of *M. purpureus* LPB97 and incubated at 30 °C for 7 days. Unless and otherwise mentioned, these conditions were maintained throughout the experiment.

2.4. Pigment extraction

From the fermented solid substrate, a known amount was taken for pigment extraction using 90% methanol (5 mL of solvent per gram of wet fermented material). The mixture was kept on a rotary shaker at 200 rpm for one hour, allowed to stand for 15 min and filtered through Whatman #1 filter paper.

2.5. Pigment estimation

Pigment estimation was done as described by Tseng et al. (2000) in which the optical density at its absorbance maxima were expressed as the concentration of pigment produced. The analysis of pigment production was done by measuring absorbance maxima (λ max) of pigment extract by spectral analysis (Lin and Demain, 1992) using a double beam spectrophotometer (Shimadzu UV 1601) taking in to consideration the dilution factor of the sample (Chiu and Poon, 1993). Only extra-cellular pigments were considered in this study. Pigment yield was expressed as