

D-25: Preliminary investigation on pigment production by *Monascus purpureus* on rice and cassava (*Manihot esculentus*)

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The fungus *Monascus purpureus* responsible for the coloration of "Ank Kak" (Chinese red rice) was originally grown in China. This colour additive is a combination of 6 related pigments. The Japanese National Research Institute concluded in 1973-75 that the *Monascus* food colour was safe and this was also permitted by the Food and Drug Administration, USA as a food additive. At present the availability of natural food colouring agents in the market is limited. To date there have been no investigations that the mould of the genus *Monascus* could be successfully cultivated on a cheap starch substrate like cassava, though wheat, barley, oats, corn and glutinous rice, expensive substrates for Sri Lanka had been studied. The large varieties of yams grown in Sri Lanka provide a great opportunity for the production of this natural red (*Monascorubrin*, *Rubropuntamin*) food colour.

The objective of the current study on Solid Substrate fermentation with Sri Lankan rice and cassava is to explore their potential as renewable starch substrates of large scale production of *Monascus* red food colour for the market. Different conditions and different nitrogen sources have been studied to obtain the maximum red pigment production for the above mentioned substrate.

Monascus purpureus, the mold was maintained on PDA slants. YM and Sporulation Agar were used as working culture medium and sporulation medium respectively.

The composition of the pigment production medium is as follows. Rice 25 g; Soy meal 0.35 g; Zinc sulphate 0.01 g; Manganous sulphate 0.005g and Distilled water 14.5 ml. The pH of the medium was 3.5 - 4.0 Cooked cassava (*Manihot esculantus*) with no addition of water was used as a carbon substrate (25g) instead of rice, in the above solid medium.

Spores of $(200 \text{ to } 250) \times 10^8$ were transferred from Sporulation slant bottles with 2.5 ml of water into the sterilized pigment producing medium in a 250 ml flask. Test cultures were incubated at room temperature for 21 days.

After 14 days of incubation, a sample (10 g) from the pigment production medium was removed and ground with 20-25 ml of ethanol (65% v/v). The ethanolic mixture was then centrifuged at 3000 rpm for 15 min and vacuum filtered. Pigment concentration was estimated with the diluted extract at maximum absorption of OD 500 for the red colourant. The same procedure was followed after 21 days of incubation. The results are expressed as OD units per ml of ethanolic extract.

In Solid Substrate fermentation, after 21 days, the production of deep red-purple pigment concentration in white polished rice at OD 500 nm was 4.35 and for red rice was 1.19 using soy meal (1.4% w/w) as the nitrogen source.

The mycelium weight increased when soy meal concentration was lower than 1.2% w/w with rice. At these concentrations the pigment production was also reduced, but when the soy meal concentration was increased the pigment production was improved.

Cassava gave an OD₅₀₀ value of 1.55, 3.01 and 3.81 when soy meal concentrations were 1.4, 2 and 4% w/w respectively.

The rate of depletion of starch was higher in cassava than in rice. Analysis of starch content after 21 days indicated no traces of starch left in cassava.

It was observed that the presence of high water content had an effect on the pigment production.

The pH was an important factor for pigment production by *Monascus*. The soy meal gave best results with pH 3.5 - 4.0 and when pH was less than 2.0, it produced orange pigment for all varieties of cassava and rice tested.

A bleaching effect of colour by sunlight resulted if test cultures were exposed to sunlight for more than 21 days.

The cultured white rice grain was found to be rich red on the surface and with the same colour within. Dried cassava also yielded an excellent red coloured product.

Soy meal was a good N-source at pH 3.5-4.0 for red pigment production by the fungus. The concentration should not be less than 1.4% w/w for a good yield.

Therefore Sri Lankan rice and cassava can be utilized for the production of red food colourant by *Monascus purpureus* with a market value.