Thesis Title Some Factors Affecting the Decolorization of Stevia (Stevia rebaudiana Bertoni) Extract by Microorganism

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Abstract

Stevioside a natural sweetener extracted from stevia (Stevia rebaudiana Bertoni) has been used in food industry and medicine. However, the crude extract of stevia is greenish-black. Therefore decolorization has to be done prior to extraction. This research was aimed to determine some factors affecting the decolorization of stevia extract. Preliminary study of stevia extract revealed that it is weakly acidic with pH 5.3-5.5, maximum absorption wavelength 420 nm, colour intensity 21.5 unit and the amount of reducing sugar 2.105 gram/litre. The microorganisms which were found from a previous study to be able to decolorize stevia extract, i.e 3 strains of bacteria : Micrococcus sp., M. luteus and Klebsiella sp. and 3 strains of fungi : Penicillium sp., Aspergillus niger and Fusarium sp. (wheat) were then

grown in liquid media containing the stevia extract. It was found that Micrococcus sp. and Fusarium sp. (wheat) was not able to decolorize stevia extract in any of the media used. A 27.3% decolorization by M. luteus was observed in nutrient stevia extract broth. A 35.4% decolorization by Klebsiella sp. occurred in M stevia extract broth. A 20.5% decolorization by Penicillium sp. and a 57.3% by A. niger were observed in S stevia extract broth. When two strains of microorganisms were used together, decolorization was better with certain strain combination. However, other combinations produced less percentage of decolorization.

A. niger, the best decolorizing microorganism among the organisms tested, was cultivated in liquid medium to study various factors affecting decolorization, i.e. glucose content, kind and amount of nitrogen sources, amount of KH_2PO_4, $MgSO_4.7H_2O$, pH of media, temperature and duration of decolorization including age and amount of inoculum. Optimum conditions for decolorization were found to be the following : 1.5% glucose, 0.1% $(NH_4)_2SO_4$ as nitrogen source, 0.1% KH_2PO_4, 0.05% (w/v) $MgSO_4.7H_2O$, pH of medium 6, temperature $28\pm2^{\circ}C$ for 5 days, age of inoculum 5 days and the amount of inoculum $4x10^7$ spores per 100 ml of the medium. When A. niger was grown in the modified medium at various optimal conditions, up to 85% decolorization was obtained.

The effect of supplement nutrients on decolorization indicated that decolorization was improved when these supplements were added. If any one of the supplements was omitted, eg. glucose, $(NH_4)_2SO_4$, KH_2PO_4 or $MgSO_4.7H_2O$, decolorization were 38.9%, 53.6%, 66.5% and 50.1% respectively. However if none of the supplements were added decolorization was only 35.2%

Decolorization by semi-continuous cultivation of A. niger in a 2 litre fermenter provided a reasonable result. A 62.3% decolorization was obtained in the initial batch of cultivation with a cell mass of 0.113 gram/100 ml in 5 days. When half of the medium was replaced by the freshly prepared lot, a 56.8% decolorization occurred with a cell mass of 0.173 gram/100 ml in one day. When half of the medium was then replaced for the second time, a 54.5% decolorization was observed with a cell mass of 0.210 gram/litre in one day.

Stevioside content was also analysed by HPLC. No significant change of stevioside content before and after decolorization was apparent.