Solid-State Bioconversion of Pineapple Residues into Kojic Acid by *Aspergillus flavus*: A Prospective Study

S. Nurashikin, E. Z. Rusley, and A. Husaini

***Abstract*—**Kojic acid is an organic acid that is widely used as an ingredient for dermatological products, precursor for flavor enhancer and also as anti-inflammatory drug. The present study was undertaken to test the feasibility of pineapple residues as substrate for kojic acid production by *Aspergillus flavus* Link 44-1 via solid-state fermentation. The effect of initial moisture content, pH and incubation time on kojic acid fermentation was investigated. The best initial moisture content for kojic acid production from pineapple residues was observed at 70% (v/w) whereas initial culture pH 2.5 was identified to give high production of kojic acid. The optimal range of incubation time was identified between 8 and 14 days of incubation which corresponded to highest range of kojic acid produced. The results from this study pronounce the promising usability of pineapple residues as alternative substrate for kojic acid production by *A. flavus* Link 44-1.

Keywords*—Aspergillus flavus*, kojic acid, pineapple residues, solid state fermentation.

I. Introduction

EACH year, pineapple-canning industries throughout the region generate bulk amount of residues. Conventionally, the residues are frequently used as fertilizer and animal feed [1]. However, large amount of the residues are still remained under utilization. Current disposal of pineapple residues poses tremendous environmental hazards due to the high content of organic material and suspended solid [2]. The high amount of residual sugars such as sucrose, glucose and fructose present in the pineapple residues [3] may possibly create potential on its usage for the production of various value-added products. Throughout the years, various metabolites have been produced from pineapple residues such as ethanol [4] and citric acid [5]. One of the bioproducts that can also be potentially initiated from pineapple waste is kojic acid. This organic acid has high commercial values due to its depigmenting [6], antioxidant [7] and anti-tumor [8] properties. Despite the initial discovery of kojic acid in some oriental foods via solid-state fermentation (SSF) process during ancient times, submerged fermentation (SmF) has been continuously opted as a favorable method for commercial production for decades. As at to date, little interest has been given to SSF as the renewed mean for kojic acid production. In addition, no work has yet been reported on the use of pineapple residues as carbon source for kojic acid production. The current study was the first effort in employing SSF as a renewed mean for kojic acid production. The objectives of this work were to study the usability of pineapple waste as substrate for kojic acid production by *A. flavus* Link 44-1 via SSF and to discover the appropriate conditions for the fermentation process.

S. Nurashikin is with the Universiti Malaysia Sarawak (UNIMAS), 94300 Kota Samarahan, Sarawak, Malaysia (phone: 6082-583023; fax: 6082-583160; e-mail: snurashikin@frst.unimas.my).

E. Z. Rusley was with Universiti Malaysia Sarawak (UNIMAS), 94300 Kota Samarahan, Sarawak, Malaysia (e-mail: pinky_roselluv@yahoo.com).

A. Husaini is with the Universiti Malaysia Sarawak (UNIMAS), 94300 Kota Samarahan, Sarawak, Malaysia (e-mail: haahmad@frst.unimas.my).

II. Materials and Methods

A. Substrate

Pineapple waste was collected from Kota Samarahan, Sarawak. The peels were oven-dried at 60°C. The dried pineapple peels was milled and sieved prior to use.

B. Microorganism

Aspergillus flavus Link 44-1 was obtained from Department of Bioprocess Technology, UPM. The strain was grown on Potato Dextrose Agar (PDA) for 7 days at 30±2°C. Inoculum for SSF was prepared in the form of spore suspension which was harvested using 0.001% (v/v) Tween-80. The spore concentration was standardized at 1×10^5 spore/ml [9] for all SSF runs.

C. SSF

The medium for SSF was prepared by using 5g of sieved pineapple peels placed in petri dish. Once inoculated, all plates were incubated at 30±2°C for 18 days in a static condition. The effect of several parameters namely initial moisture content, pH and incubation time on kojic acid fermentation was investigated. The cultures were sampled at regular time interval for reducing sugar and kojic acid determination.

D. Extraction

Extraction was performed by introducing 50ml of distilled water to the sampled cultures. The slurry suspension was centrifuged at 6000rpm for 20 minutes at 4°C. Following that, the suspension was filtered through 0.45µm filter for subsequent reducing sugar and kojic acid assays.

E. Analyses

The reducing sugar and kojic acid was analyzed based on dinitrosalicyclic acid (DNS) method [10] whereas colorimetry method was applied for analyzing the kojic acid quantitatively [11].