

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

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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.

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 dinitrosalicylic acid (DNS) method [10] whereas colorimetry method was applied for analyzing the kojic acid quantitatively [11].

III. RESULTS AND DISCUSSION

Table I shows the effect of different levels of initial moisture content on the bioconversion of pineapple peel into kojic acid. Throughout the range tested, maximum kojic acid production of 0.415g/L was observed when the initial moisture content was adjusted to 70% (v/w). This is linked to a yield of about 0.263g/g. It was also observed that fermentation time corresponded slightly with the initial moisture content. At 70 to 75% (v/w), the fermentation time was the shortest as compared to other levels of initial moisture content. From these results, it can be suggested that, further enhancement of kojic acid production by *A. flavus* Link 44-1 from pineapple waste can be attained by optimizing the initial moisture content from 70% to 75% (v/w) with smaller interval in future studies.

TABLE I
KINETIC PARAMETERS OF KOJIC ACID FERMENTATION AT VARIOUS LEVELS OF INITIAL MOISTURE CONTENT

Initial moisture content (v/w)	Maximum production of kojic acid (g/L)	Fermentation time (h)	$Y_{p/s}$ (g kojic acid / g reducing sugar)
60%	0.290	384	0.181
65%	0.334	288	0.213
70%	0.415	240	0.263
75%	0.404	240	0.250
80%	0.357	288	0.225

The effect of pH on kojic acid production from pineapple waste by *A. flavus* Link 44-1 has been investigated and the results are outlined in Table II. Throughout the range of pH tested from 2.5 to 4.5, the highest production of kojic acid was generally found at pH 2.5 with the maximum concentration of 0.164g/L and yield of about 0.103g/g. Most of the reports on kojic acid production as available in the literature are concerned with the effects of culture pH for SmF. Limited information is available regarding optimal pH for kojic acid production via SSF.

The results from this study were nonetheless comparable with findings by some researchers. For example, Rosfarizan et al. [12] reported that maximum production of kojic acid by *A. flavus* via SmF was obtained when the pH of the initial culture was adjusted to 3. Moreover it was also found by Kitada et al. [13] that the pH range that favours optimal production of kojic acid in submerged culture is between 2 and 3. In general, filamentous fungi such as *A. flavus* show optimal growth and activities at acidic condition [12]. Severe increase in pH may affect the growth of the fungi and also their associated enzyme activities.

TABLE II
KINETIC PARAMETERS OF KOJIC ACID FERMENTATION AT VARIOUS LEVELS OF INITIAL CULTURE PH

pH	Maximum production of kojic acid (g/L)	Fermentation time (h)	$Y_{p/s}$ (g kojic acid / g reducing sugar)
2.5	0.164	192	0.103
3.5	0.155	120	0.097
4.5	0.147	144	0.092

The effect of incubation time on SSF of pineapple waste by *A. flavus* Link 44-1 was studied by sampling the cultures at different time interval from day 0 to 18. Figs. 1 and 2 depict the time course of kojic acid production attained at 70% (v/w) initial moisture content and pH 2.5 respectively which were both found to give the highest production of kojic acid in this study.

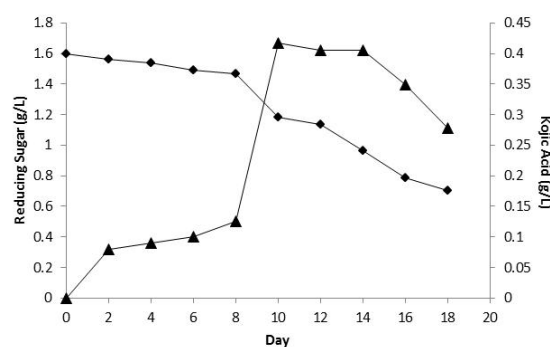


Fig. 1 Time course of kojic acid production by *A. flavus* Link 44-1 from pineapple residues at 70% (v/w) initial moisture content (◆) reducing sugar (▲) kojic acid

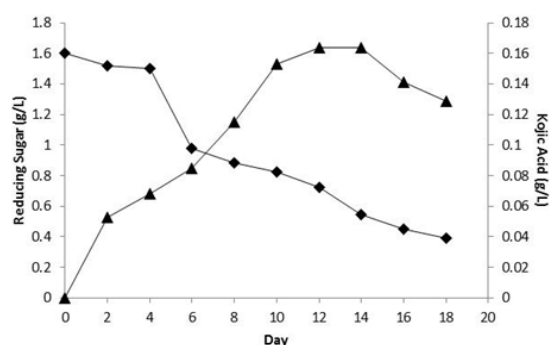


Fig. 2 Time course of kojic acid production by *A. flavus* Link 44-1 from pineapple residues at pH 2.5 (◆) reducing sugar (▲) kojic acid

IV. CONCLUSIONS

In view of the results obtained, pineapple residues have shown promising potentials to be exploited as alternative substrate for kojic acid production by *A. flavus* Link 44-1 via SSF. Enhancement of kojic acid production from pineapple waste may be achieved by further optimization of other associated parameters in future works.

ACKNOWLEDGMENT

The authors wish to thank the Department of Molecular Biology, Faculty of Resource Science and Technology, UNIMAS for providing facilities and support upon the successfulness of this research work. Deepest gratitude is also extended to Prof Dr Arbakariya Ariff from Universiti Putra Malaysia for providing the strain used in this study.

REFERENCES

- [1] S. Ketnawa, P. Chaiwut, and S. Rawdkuen, "Pineapple wastes: A potential source for bromelain extraction," *Food and Bioprocess Processing*, 90, pp. 385–391, 2012.
- [2] K. A. Buckle, "Biotechnology Opportunities in Waste Treatment and Utilisation for the Food Industry," *In Biotechnology and The Food Industry*, Ed. Rogers P.L. Gordon and Breach Science Publishers, New York, pp. 261-277, 1989.
- [3] Abdullah, "Solid and liquid pineapple waste utilization for lactic acid fermentation using *Lactobacillus delbrueckii*," *Reaktor*, vol. 11, no 1, pp. 50-52, 2007.
- [4] K. Tanaka, Z. D. Hilary, and A. Ishizaki, "Investigation of the utility of pineapple juice and pineapple waste material as low-cost substrate for ethanol fermentation by *Zymomonas mobile*," *Journal of Bioscience and Bioengineering*, vol. 87, pp. 642–646, 1999.
- [5] D. Kumar, V. K. Jain, G. Shanker, and A. Srivastava, "Utilisation of fruits waste for citric acid production by solid state fermentation," *Process Biochemistry*, vol. 38, pp. 1725–1729, 2003.
- [6] T. Chang, "An updated review of tyrosinase inhibitors," *International Journal of Molecular Sciences*, vol. 10, pp. 2440-2475, 2009.
- [7] A. J. Gomes, C.N. Lunardi, S. Gonzalez, and A. C. Tedesco, "The antioxidant action of Polypodium leucotomos extract and kojic acid: reactions with reactive oxygen species," *Brazilian Journal of Medical and Biological Research*, vol. 34, pp. 1487-1494, 2001.
- [8] G.A. Burdock, M. G. Soni, and I. G. Carabin, "Evaluation of health aspects of kojic acid in food," *Regulatory Toxicology and Pharmacology*, vol. 33, pp. 214-228, 2001.
- [9] M. Rosfarizan, "Kinetics, modelling and scaling-up of kojic acid fermentation by *Aspergillus flavus* 44-1 using different carbon sources," *PhD thesis. Universiti Pertanian Malaysia. 2000.*
- [10] G. L. Miller, "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemistry*, vol. 31, no. 3, pp. 426-428, 1959.
- [11] R. Bently, "Preparation and analysis of kojic acid," *Enzymo*, vol. 3, pp. 238-241, 1957.
- [12] M. Rosfarizan, A. B. Ariff, M.A. Hassan, and M. I. A. Karim, "Influence of pH on kojic acid fermentation by *Aspergillus flavus*," *Pakistan Journal of Biological Sciences*, vol. 6, pp. 977-982, 2000.
- [13] M. Kitada, H. Ueyama, and T. Fukimbara, "Studies on kojic acid fermentation (I) Cultural condition in submerged culture," *Journal of Fermentation Technology*, vol. 4, pp. 1101-1107, 1967.