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Research Article

KOJIC ACID PRODUCTION USING MIXED CULTURES OF *ASPERGILLUS ORYZAE* AND *ASPERGILLUS TAMARII*

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ABSTRACT

Objective: This study aimed to find the optimum kojic acid fermentation conditions using combination cultures of *Aspergillus oryzae* and *Aspergillus tamarii*.

Methods: Screening of the best mixed cultures was performed using yeast extract medium with 5% (w/v) glucose. Fermentation conditions were optimized by varying carbon and nitrogen sources, pH of medium, inoculum ratio, and aeration. Aeration was varied using 50 and 100 mL of medium in 100 and 250 mL Erlenmeyer flasks, respectively. Kojic acid was analyzed using thin-layer chromatography-densitometry and UV-Vis spectrophotometry.

Results: Kojic acid produced from mixed cultures yielded 0.1396 gg^{-1}, while sole cultures of *A. oryzae* and *A. tamarii* yielded 0.0329 gg^{-1} and 0.1001 gg^{-1}, respectively. Of the nine fermentation mediums, the best carbon and nitrogen sources were sucrose and yeast extract. From the three variations of pH, pH 3.5 was the optimum pH value. From the three ratios of inoculum concentration, a ratio of 2:3 (*A. oryzae*:*A. tamarii*) was the best ratio. Aeration was varied using 50 and 100 mL of medium in 100 and 250 mL Erlenmeyer flasks, respectively. Aeration of 100 mL medium in 250 mL Erlenmeyer flask was selected as the best aeration that produced 6.559 g/L kojic acid.

Conclusion: The highest concentration of kojic acid was obtained by mixing cultures of *A. oryzae* and *A. tamarii* in a ratio of 2:3, using sucrose and yeast extract as the substrates at pH 3.5 and semiaerobic condition.

Keywords: *Aspergillus oryzae*, *Aspergillus tamarii*, Kojic acid, Optimization of fermentation.

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INTRODUCTION

Kojic acid is a compound that is widely used in various fields, including the food, chemical, and cosmetic industries, and it is commonly used as whitening agent in cosmetic products [1]. This compound can be produced from the fermentation of several microorganisms from the *Aspergillus* and *Penicillium* genera [2].

Numerous studies associated with the production of kojic acid have generally used *Aspergillus* as a sole culture. However, there was a study used a combination culture of *Aspergillus flavus* NSH9 and *A. flavus* Link 44-1 for kojic acid fermentation. The results showed that kojic acid produced by mixed cultures of *Aspergillus* molds showed higher yield value than was produced by the sole use of *Aspergillus* [3]. Other studies associated with the production of kojic acid using *A. flavus* showed that aflatoxin, which is carcinogenic, was also secreted while kojic acid was produced [4]. Aflatoxins are a kind of mycotoxin and are mostly produced by *A. flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* [5]. Therefore, *Aspergillus oryzae* and *Aspergillus tamarii* cultures, which are generally recognized as safe sources of mold, were used in this study due to their safety approval and the absence of aflatoxin production with their use [6].

Several conditions may affect the fermentation of kojic acid, including: Carbon and nitrogen sources as substrates, pH values, temperature, aeration conditions, and the speed of agitation [7]. In this study, several conditions were gradually changed to determine the optimum fermentation conditions. The inoculum concentration of each mold also had to be optimized for obtaining the ideal concentration ratio. The sugar concentration was also analyzed for determining the yield value of kojic acid from the use of sole and mixed cultures.

MATERIALS AND METHODS

The two *A. oryzae* and *A. tamarii* species were obtained from IPB Culture Collection (IPBCC) in Bogor, ITB Culture Collection in Bandung, and the University Laboratory of Microbiology, Depok, Indonesia. Stock cultures were maintained on potato dextrose agar (PDA) (Difco) at 28°C.

Instruments

The instruments used in this study were autoclave (Hirayama), oven (WTB Binder), analytical balance (Acculab), vortex mixer (Barnsted), hotplate stirrer (Corning), pH meter (Hanna), sentrifugator (Kubota 6800), incubator (Memmert), shaker (Orbit), pipettes, and other glasswares commonly used in laboratories. The instruments used for analysis were the thin-layer chromatography (TLC)-densitometer (Camag TLC Scanner 3) and UV-Vis spectrophotometer (Shimadzu).

Chemicals

PDA (Difco), glucose (Sigma-Aldrich), technical sucrose, fructose (Sigma-Aldrich), yeast extract (Merck), urea ($CO(NH_2)_2$) (Merck), ammonium sulfate ($(NH_4)_2SO_4$) (Merck), magnesium sulfate ($MgSO_4.7H_2O$) (Merck), dicalcium phosphate (KH_2PO_4) (Merck), ethanol (Brataco), chloride acid (HCl) (Merck), sodium hydroxide (NaOH) (Merck), ferric chloride ($FeCl_3$) (Merck), phenol (Merck), chloroform (Merck), methanol (Merck), toluene (Merck), formic acid (Merck), ethyl acetate (Merck), sulfuric acid (H_2SO_4) (Merck), distilled water, and double-distilled water were used.

Method of preparation

Culture maintenance and initial fermentation medium

A. oryzae and *A. tamarii* colonies were streaked on an aseptic agar slope of PDA medium then incubated at 28°C for 7 days. Most cultures were stored

as stock cultures at 4°C and the rest, used as working cultures, were stored at 30°C. Stock culture maintenance was performed every 2 months while working culture was performed every 2 weeks [8,9]. For screening, initial fermentation medium was prepared which contained (w/v): 5% glucose, 0.25% yeast extract, 0.10% KH_2PO_4, and 0.05% $MgSO_4.7H_2O$.

Preculture/inoculum development

After incubation for 7 days, a volume of 1.0 mL double-distilled water was added into the mold cultures on the agar medium. The spores were scraped and aseptically transferred into a reaction tube containing 9.0 mL double-distilled water and then homogenized using a vortex for obtaining spore suspension with 10-times dilution. 50 mL of pre-culture medium containing sucrose and yeast extract (YES) was poured into a 100 mL flask and 5.0 mL of spore suspension was added. They were then incubated for 2 days at room temperature with 180 rpm agitation [8].

Screening of best mixed cultures

A total of 5% (v/v) of each inoculum was mixed and suspended with 2 mL of an initial fermentation medium containing glucose, yeast extract, KH_2PO_4, and $MgSO_4.7H_2O$ in the reaction tube. They were incubated at room temperature for 7 days with 180 rpm agitation. The identification of kojic acid was performed using 1% $FeCl_3$ reagent. A total of 200 µL of fermented cultures were added to a drop plate and then administered with one drop of 1% $FeCl_3$. The cultures with a reddish-brown color, those that were more concentrated, and those which produced most cell biomass were selected as the best mixed cultures [4].

Preparation of the Kojic Acid Standard Calibration Curve

The standard solution of kojic acid was prepared with a concentration ranging from 20 to 80 ppm using chloroform: methanol (2:1). 2 µL of each concentration was spotted onto the silica gel plate. The plate was eluted with toluene: ethyl acetate: formic acid (3:6:1). The eluted plate was dried and the absorbance was measured by densitometer using a D2 lamp, UV-Vis detector, and the maximum wavelength was determined. On the basis of the maximum wavelength obtained, the calibration curve was made between the area and the concentration of the solution. A linear regression equation and correlation coefficient (r) were obtained for kojic acid determination [4].

Optimization of fermentation conditions

Variation of carbon and nitrogen sources

Kojic acid fermentation was carried out using a 50 mL mixture of three different carbon sources (5% of glucose, sucrose, and fructose, respectively) and three different nitrogen sources (0.25% of yeast extract, urea, and ammonium sulfate, respectively) in a 100 mL flask. A total of 10% (v/v) inoculum was suspended into each fermentation medium. They were incubated at room temperature for 9 days with 180 rpm agitation. The cell biomass and kojic acid concentration were determined.

Variation of pH medium

The pH variations in the fermentation processes with the selected medium were as follows: 3.5, 4.5, and 5.5. pH values were adjusted by adding a few drops of HCl 1 N on the fermentation medium [10]. They were incubated at room temperature for 9 days with 180 rpm agitation. The cell biomass and kojic acid concentration were determined.

Variation of inoculum concentration ratio

For obtaining the ideal inoculum concentration ratio of *A. oryzae* and *A. tamarii*, ratios of 1:1, 3:2, and 2:3 were prepared. The cell biomass and kojic acid concentration were determined.

Variation of aeration conditions

A total of 10% (v/v) inoculum with selected inoculum concentration ratio was suspended with selected fermentation medium in various aeration volumes. For aeration optimization, the volume of medium used was varied: 50 mL in 100 mL flask and 100 mL in 250 mL Erlenmeyer flask. They were incubated for 9 days with 180 rpm agitation [11]. The cell biomass and kojic acid concentration were determined.

Quantitative analysis of kojic acid

Kojic acid determination

Each fermentation culture was centrifuged for 15 min at 6500 rpm at room temperature; 200 µL of each supernatant was drawn out and dissolved in 2 mL of chloroform: methanol (2:1). A total of 1 µL of each sample was spotted on a silica plate. The plate was eluted, dried, and analyzed for obtaining the area. The concentration of kojic acid was obtained by plotting the area into the regression equation of the kojic acid standard solution [8].

Cell biomass separation and determination of dry cell weight

The mycelial pellet was washed with distilled water [10], and then, the filter paper with the mycelial pellet was dried in the oven at 105°C and weighed to a constant weight. Dry cell weight was determined by the difference between the pre-weighed filter paper and its weight after (with cell biomass deposits) [4].

Qualitative analysis of kojic acid

The UV-Vis spectrophotometry and spectrocolorimeter method were used for analyzing kojic acid qualitatively. Using the UV-Vis Spectrophotometry, a standard solution of kojic acid was made with a concentration of 25.2 ppm. The solution absorbance was measured at a wavelength of 200–400 nm and the maximum wavelength was determined; 1 mL of the fermented filtrate was transferred into a 10 mL measuring flask and dissolved in distilled water for analyzing kojic acid in fermentation culture; 1 mL of this solution was transferred into a 50 mL measuring flask and distilled water was adjusted until 50 mL. The maximum wavelength was determined based on the absorbance obtained and compared with the standard maximum wavelength [8].

Using the spectrocolorimeter method, a standard solution of kojic acid was prepared with 100.8 ppm concentration. Up to 8 mL of standard solution was added, with 1 mL of 1% $FeCl_3$ solution. The solution absorbance was measured at a wavelength of 400–700 nm and the maximum wavelength determined. For analyzing kojic acid in fermentation culture, 1 mL filtrate was transferred into a 50 mL measuring flask and diluted with distilled water. Next, 8 mL of the solution was transferred and mixed with 1 mL of 1% $FeCl_3$ solution. The absorbance of the solution was measured, and its maximum wavelength was determined and then compared with the standard maximum wavelength [8].

Analysis of sugar concentration in fermentation medium

The optimum concentration of sucrose as the carbon source was determined using a standard solution with the selected concentration (4–10 ppm); 1 mL of sucrose standard solution from each concentration was transferred into a test tube. Next, 1 mL of 5% phenol solution and 5 mL of concentrated H_2SO_4 solution were added. After 10 min, the solution was homogenized by vortexing for 1 min at 6500 rpm and then left to stand for 20 min. The absorbance was then measured at a wavelength of 490 nm and the calibration curve was made using the absorbance obtained from each concentration [12].

For determining the concentration of sucrose in fermentation culture, the centrifuged fermentation culture was diluted up to 1000 times with distilled water; 1 mL of the solution was transferred into the reaction tube, and then, 1 mL of 5% phenol solution and 5 mL of H_2SO_4 solution were added. The solution was left to stand for 10 min, homogenized, and then left to stand for an additional 20 min. The absorbance was measured using UV-Vis spectrophotometer at 490 nm. The obtained absorbance was plotted into the regression equation of the sucrose standard solution [13].

RESULTS

Preparation

Culture maintenance and preculture

After 7 days, green colonies of *A. oryzae* and *A. tamarii* covered the entire agar surface (Fig. 1). The PDA medium expressed a yellow cloudy color,

which was originally a yellowish white. Each colony was inoculated in YES medium, and after preculturing for 2 days, white biomass pellet cells were obtained (Fig. 2).

Screening of best mixed cultures

The screening of fermentation cultures with 1% $FeCl_3$ solution is shown in Fig. 3. They expressed a similar brown color as in the sole culture, except the combination of *A. oryzae* A and *A. tamarii* A from IPBCC collection, which showed the most intensive brown color. The dry cell weight of each culture is shown in Table 1. The results show that a combination of both molds is also the heaviest compared with other batch combinations (42.20 g/L).

Preparation of kojic acid standard calibration curve

The standard absorbance spectrum of kojic acid was obtained by TLC-densitometer at a maximum wavelength of 318 nm. The regression equation was obtained as y=−435.55+89.352x with r=0.9993 (standard curve not shown).

Optimization of fermentation conditions

Variation of carbon and nitrogen sources

After the fermentation was completed (9 days), each fermentation culture was screened by the addition of 1% $FeCl_3$ solution, followed with the determination of kojic acid and dry cell weight (Table 2). The results of screening are shown in Figs. 4 and 5. The result shows that both sole cultures and mixed cultures produced the most intense brown color with sucrose and yeast extract as substrate. Kojic acid determination was performed and is shown in Table 3. It shows that 2.6133 g/L of kojic acid was produced in that substrate.

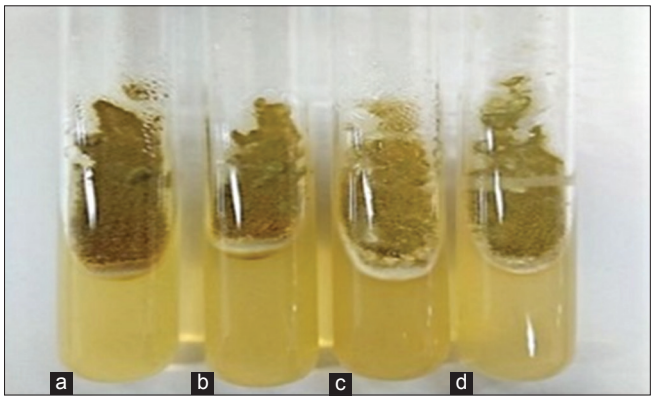

Fig. 1: Colonies of stock cultures incubated for 7 days: (a) *Aspergillus oryzae* A; (b) *A. oryzae* B; (c) *Aspergillus tamarii* A; (d) *A. tamarii* B

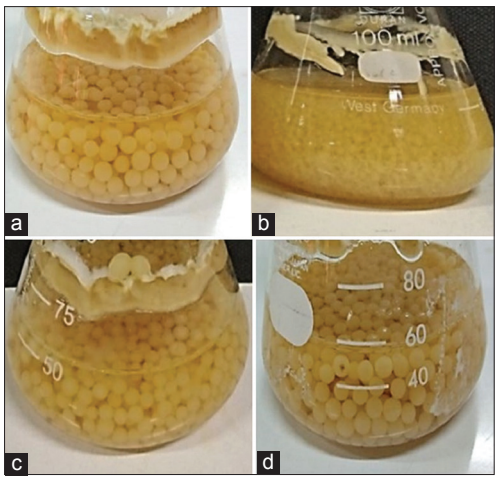

Fig. 2: Cell biomass after preculture for 2 days: (a) *Aspergillus oryzae* A; (b) *A. oryzae* B; (c) *Aspergillus tamarii tamarii* A; (d) *A. tamarii* B

Variation of pH medium

From the data presented in Table 4, it can be seen that the medium with a pH of 3.5 produced kojic acid at a higher concentration (2.6163 g/L) than the other media with different pH values. Based on the dry cell weight data in Table 4, a greater dry cell weight was obtained on the medium with a pH of 3.5, indicating that the more acidic fermentation medium was a more ideal environment for microbial cell growth.

Variation of inoculum concentration ratio

The results in Table 5 show that a 2:3 ratio of inoculum *A. oryzae*:*A. tamarii* was more optimum than the other ratio which produced 2.8889 g/L of kojic acid. Based on the dry cell weight data in Table 5, more cell biomass was obtained at that inoculum concentration ratio.

Variation of aeration

The results in Table 6 show that the amount of kojic acid produced from the medium with a 100 mL volume in a 250 mL Erlenmeyer flask was higher than that of the 50 mL volume of medium in 100 mL in a Erlenmeyer flask. However, based on the data of dry cell weight in Table 6, cell biomass in the 100 mL volume medium was less than cell biomass in the 50 mL volume medium.

Qualitative analysis of kojic acid

The standard solution of kojic acid with a concentration of 25.2 ppm gave a maximum wavelength at 268.8 nm with an absorbance of 1.316 A (Fig. 6). Meanwhile, the kojic acid spectrum from the mixed cultures supernatant had the same maximum wavelength at 268.8 nm with absorbance at 1.163 A. It shows that kojic acid was positively identified in the fermentation culture by spectrophotometry UV.

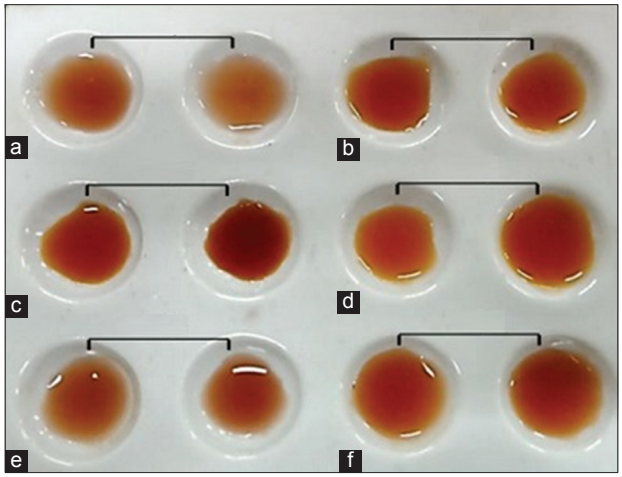

Fig. 3: Best mixed cultures screening: (a) *Aspergillus oryzae* B and *Aspergillus tamarii* A; (b) *A. oryzae* B and *A. tamarii* B; (c) *A. oryzae* A and *A. tamarii* A; (d) *A. oryzae* A and *A. tamarii* B; (e) *A. oryzae* A (comparison); (f) *A. tamarii* B (comparison)

Table 1: Dry cell weight determination of screening of best mixed cultures

No	Inoculum	Dry cell weight (g/L)
1	*A. oryzae* A	22.50
2	*A. tamarii* B	30.25
3	*A. oryzae* A and *A. tamarii* A	42.20
4	*A. oryzae* A and *A. tamarii* B	27.50
5	*A. oryzae* B and *A. tamarii* A	35.45
6	*A. oryzae* B and *A. tamarii* B	12.85

A. oryzae: Aspergillus oryzae, A. tamarii: Aspergillus tamarii

Table 2: Effect of fermentation medium on dry cell weight concentration

Dry cell weight (g/L)					
No	**Carbon source (50 g/L)**	**Nitrogen source (2,5 g/L)**	***A. oryzae* (comparison)**	***A. tamarii* (comparison)**	***A. oryzae* and *A. tamarii***
1	Glucose	Yeast extract	13.25	13.668	10.342
2	Glucose	$CO(NH_2)_2$	8.638	8.942	8.606
3	Glucose	$(NH_4)_2SO_4$	10.53	10.732	10.09
4	Sucrose	Yeast extract	13.508	13.726	14.908
5	Sucrose	$CO(NH_2)_2$	10.806	13.006	10.796
6	Sucrose	$(NH_4)_2SO_4$	13.692	9.602	11.128
7	Fructose	Yeast extract	11.998	10.474	10.868
8	Fructose	$CO(NH_2)_2$	10.97	10.462	9.194
9	Fructose	$(NH_4)_2SO_4$	8.078	8.048	9.382

A. oryzae: Aspergillus oryzae, A. tamarii: Aspergillus tamarii

Table 3: Effect of fermentation medium on kojic acid concentration

Kojic acid concentration (g/L)					
No	**Carbon source (50 g/L)**	**Nitrogen source (2,5 g/L)**	***A. oryzae* (comparison)**	***A. tamarii* (comparison)**	***A. oryzae* and *A. tamarii***
1	Glucose	Yeast extract	0.4337	0.4261	0.1051
2	Glucose	$CO(NH_2)_2$	-	-	-
3	Glucose	$(NH_4)_2SO_4$	0.3114	0.1292	-
4	Sucrose	Yeast extract	1.4539	1.4844	2.6163
5	Sucrose	$CO(NH_2)_2$	0.1905	1.4349	0.2774
6	Sucrose	$(NH_4)_2SO_4$	0.4574	-	0.2195
7	Fructose	Yeast extract	0.2042	-	-
8	Fructose	$CO(NH_2)_2$	0.1722	0.3471	-
9	Fructose	$(NH_4)_2SO_4$	-	-	-

A. oryzae: Aspergillus oryzae, A. tamarii: Aspergillus tamarii

Table 4: Effect of pH of medium on kojic acid and dry cell weight concentration

No	**pH of fermentation medium**	***A. oryzae* (comparison)**	***A. tamarii* (comparison)**	***A. oryzae* and *A. tamarii***
Kojic acid concentration (g/L)				
1	3.5	1.4539	1.4844	2.6163
2	4.5	1.7057	1.249	1.0274
3	5.5	1.3195	2.7006	0.7970
Dry cell weight (g/L)				
1	3.5	13.508	13.726	14.908
2	4.5	14.566	13.024	13.544
3	5.5	13.872	15.544	12.558

A. oryzae: Aspergillus oryzae, A. tamarii: Aspergillus tamarii

Table 5: Effect of inoculum ratio on kojic acid and dry cell weight concentration

No	**Inoculum**	**Kojic acid concentration (g/L)**	**Dry cell weight (g/L)**
1	*A. oryzae* (comparison)	1.4637	13.508
2	*A. tamarii* (comparison)	1.4751	13.726
3	*A. oryzae* +*A. tamarii* (1:1)	2.6667	14.908
4	*A. oryzae* +*A. tamarii* (3:2)	2.3110	14.53
5	*A. oryzae* +*A. tamarii* (2:3)	2.8889	16.91

A. oryzae: Aspergillus oryzae, A. tamarii: Aspergillus tamarii

When compared with the spectrocolorimetry result, the mixed cultures supernatant of fermentation gave a spectrum with a maximum wavelength at 503.6 nm which was similar to the standard solution of kojic acid of 100.8 ppm (data not shown).

Analysis of sugar concentration in fermentation medium

The regression equation of the standard solution of sucrose was: y=–0.005+0.0682x with r=0.998. Based on the data obtained in Table 7, the yield value and productivity of kojic acid produced from mixed cultures of molds were higher than kojic acid produced from sole cultures. The yield value of kojic acid produced from mixed cultures of molds was 0.1396 gg^{-1}, with a productivity of 0.0304 gl/h. Meanwhile, the yield value of kojic acid derived from the sole cultures was 0.0329 gg^{-1}, with a productivity of 0.0070 gl/h^1 for *A. oryzae*, and the yield of kojic acid derived from *A. tamarii* was 0.1001 gg^{-1} with the productivity of 0.0219 gl/h. This suggests that the use of mixed cultures is more potent in the productivity of kojic acid than the use of sole cultures.

DISCUSSION

Green colonies of *A. oryzae* and *A. tamarii* that covered the entire agar surface were inoculated in YES medium. This stage aimed to obtain the optimum number and quality of the inoculum to quickly enter the growth phase during the fermentation process [8]. After preculture was performed with incubation for 2 days, white biomass pellet cells were obtained. A combination of *A. oryzae* and *A. tamarii* from IPBCC produced the heaviest cell biomass compared to other combinations and the sole cultures. It showed that microbial cells produced from the mixed cultures had the fastest growth which could increase the

productivity of the secondary metabolites. Meanwhile, the screened supernatants expressed a reddish-brown color. This color formation indicated an oxidation reaction between $FeCl_3$ and the OH group from kojic acid. The more intense the color formation, the more was the kojic acid production, which correlated with the weight of cell biomass. Therefore, *A. oryzae* and *A. tamarii* derived from the IPBCC collection were selected as the best mixed cultures for the fermentation process (Fig. 3c).

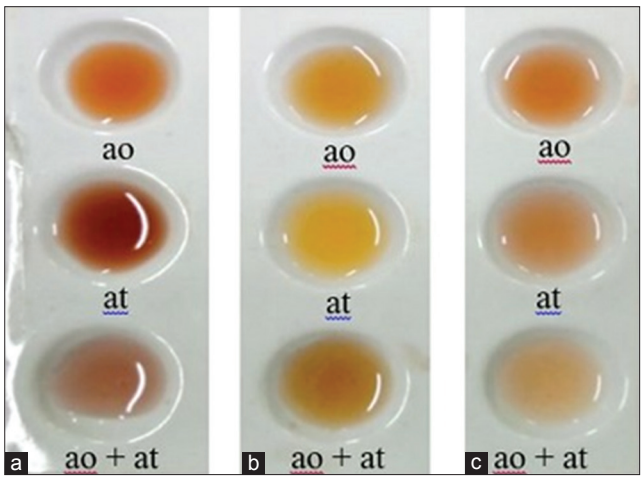

Fig. 4: Screening of fermentation medium with glucose as carbon source: (a) Yeast extract; (b) $CO(NH_2)_2$; (c) $(NH_4)_2SO_4$ as nitrogen source; ao = *Aspergillus oryzae*; at = *Aspergillus tamarii*; ao + at = *A. oryzae* + *A. tamarii*

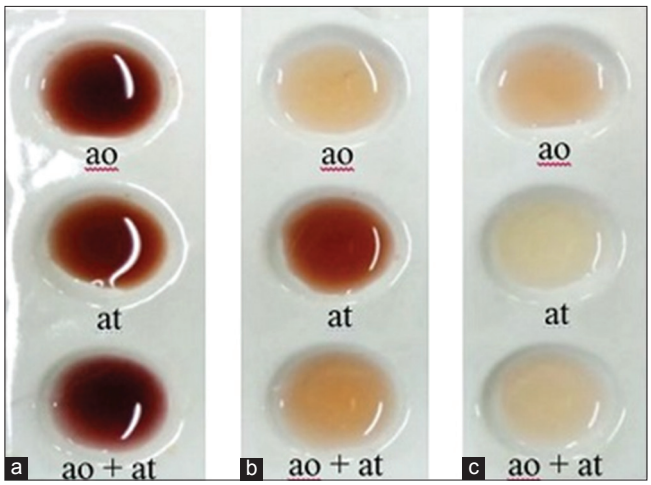

Fig. 5: Screening of fermentation medium with sucrose as carbon source: (a) Yeast extract; (b) $CO(NH_2)_2$; (c) $(NH_4)_2SO_4$ as nitrogen source; ao = *Aspergillus oryzae*; at = *Aspergillus tamarii;* ao + at = *A. oryzae* + *A. tamarii*

The composition of fermentation medium can influence the supply of nutrients and cell metabolism. Carbon and nitrogen sources have dominant roles in fermentation processes because these nutrients are directly associated with the cell biomass and metabolite formation. In addition, the fermentation productivity depends on the culture medium used. Therefore, the substrates were optimized using three various carbon and nitrogen sources [14]. The results showed that sucrose and yeast extract was the optimum substrate to produce kojic acid and was therefore selected. Sucrose is a disaccharide which can be hydrolyzed to glucose and fructose. In kojic acid biosynthesis, these two monosaccharides act simultaneously: Glucose acts as a precursor of kojic acid and fructose contributes to microbial cell growth [15-17]. These findings were also demonstrated in another study that stated that yeast extract is a complex nitrogen source containing vitamins that can act as a precursor of kojic acid formation [18]. As can be seen in the dry cell weight data in Tables 2 and 3, obtained cell biomass was proportional to the concentration of kojic acid produced by each medium where the greater the weight of the dry cell, the more kojic acid was produced.

Medium pH was optimized with three various pH values, in which we found that a high amount of kojic acid was produced in a medium of pH 3.5. This correlates with an experiment which found that the optimum pH for the production of kojic acid depends on the type of carbon and nitrogen source used, where a pH in the range of 2–3 was the optimum when sucrose and yeast extract were used as a source of carbon and nitrogen [18].

Along with medium conditions, it was important to optimize inoculum concentrations of *A. oryzae* and *A. tamarii* to find which concentration has more potential in producing kojic acid. The results indicated that *A. tamarii* had a greater role than *A. oryzae* when both were combined. However, in this study, the growth curve of each mold could not be observed due to technical limitations, and optimum time for the growth of these two molds cells could not be determined.

Oxygen is necessarily required in aerobic fermentation processes. The mold cultures must be supplied with oxygen to satisfy the demand for growth. Oxygen and nutrients must be distributed uniformly by aeration and agitation. Aeration provides the required oxygen for microorganism growth, while agitation helps in mixing of the nutrients and also has a major role in microorganism growth [19]. This experiment showed that kojic acid from 100 mL medium was produced at a higher rate than the 50 mL medium; however, the cell biomass showed the opposite. Based on the concentration of kojic acid produced, the optimum aeration condition for kojic acid fermentation achieved was the medium with 100 mL volume in 250 mL flask, which was close to semi-aerobic conditions.

CONCLUSION

The best combination of fungi isolates for kojic acid fermentation was *A. oryzae* and *A. tamarii* from IPBCC. The best carbon and nitrogen source in this experiment was sucrose and yeast extract with a medium pH of 3.5. In addition, the best ratio of mixed cultures inoculum was 2:3 of *A. oryzae* and *A. tamarii* using 100 mL volume medium in 250 mL

Table 6: Effect of aeration on kojic acid production and dry cell weight concentration

No	Volume of fermentation medium (mL)	*A. oryzae* (comparison)	*A. tamarii* (comparison)	*A. oryzae* and *A. tamarii*
Kojic acid concentration (g/L)				
1	50	1.4634	1.4921	2.8932
2	100	1.5176	4.7202	6.5594
Dry cell weight (g/L)				
1	50	13.537	13.759	16.954
2	100	12.056	12.301	12.424

A. oryzae: Aspergillus oryzae, A. tamarii: Aspergillus tamarii

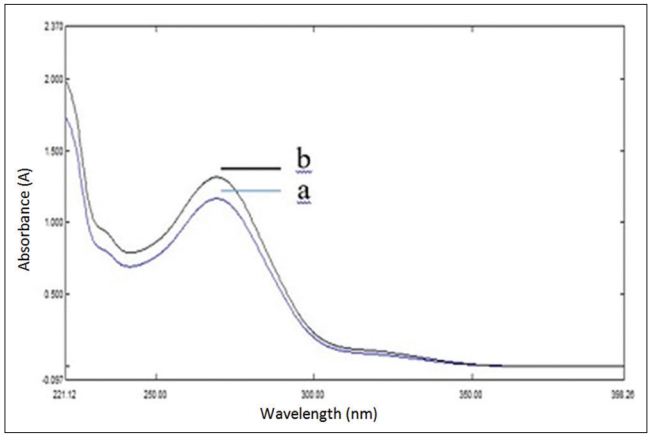

Fig. 6: Kojic acid spectrum on UV-Vis spectrophotometer: (a) Kojic acid from fermentation culture; (b) standard of kojic acid

Table 7: Yield and productivity of kojic acid from fermentation cultures

Inoculum	Pmax (gL−1)	Yp/s (gg−1)	t (h)	P (gL/h−1)
A. oryzae	1.5176	0.0329	216	0.0070
A. tamarii	4.7202	0.1001	216	0.0219
A. oryzae + A. tamarii	6.5594	0.1396	216	0.0304

A. oryzae: Aspergillus oryzae, A. tamarii: Aspergillus tamarii

Erlenmeyer flask. The yield value of kojic acid using mixed cultures was higher than that when using a sole culture.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest in this research.

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