OPTIMUM CONDITIONS FOR THE PRODUCTION OF KOJIC ACID FROM THE FUNGUS ASPERGILLUS FLAVUS

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ABSTRACT

The current study aimed to determine the optimal conditions for the production of kojic acid from the fungal isolate Aspergillus flavus, as the fungus was grown on a plant medium consisting of a carbon source (glucose, sucrose, corn starch) and a nitrogen source (yeast extract, peptone, urea, ammonium sulfate) and PH (4, 5, 6, 7, 8), a temperature (20, 25, 30, 35, 40) °C, and an incubation period of (7, 12, 18, 24) days, dihydrogen potassium phosphate and magnesium sulfate seven-molecule water, as it was found that the optimal conditions for the production of Acid from the fungal isolate A.flavus (70 g/L sucrose, 5 g/L yeast extract, pH 6, temperature 30°C and incubation period of 12 days).

Keywords: kojic acid, A.flavus

I. INTRODUCTION

Organic acids production processes have tended to develop microorganisms by using cheap raw materials to produce the highest amount of acid required at the lowest costs. It helps to take advantage of these wastes and exploit them efficiently in the production of organic acids. On the other hand, some changes have been made in environmental legislation, forcing these industries to find alternative uses for their waste, especially agricultural and industrial (Alil and Zulkali., 2011).

Although it is possible to produce organic acids through chemical manufacturing or from animal and plant sources, the commercial production of these acids is done through microbial fermentation, because the latter method is characterized by the possibility of increasing production by improving environmental conditions or manipulating some genetic genes of the microorganism (Demain and Dana., 2007).

In general, the industrial production of organic acids was done by employing various microorganisms using different fermentation techniques and it was common to overproduce various organic acids such as citric acid, itaconic acid, gluconic acid etc. However, malic acid, gibberellic acid, kojic acid were only slightly produced. These acids are very useful for therapeutic, research and commercial purposes (Devi et al., 2014).

Kojic acid is a natural organic acid that was synthesized during the aerobic fermentation process of carbohydrates. It produces a secondary metabolic complex by the fermentation of various types of fungi, including Aspergillus oryzae, Aspergillus flavus, Aspergillus tamarii, Aspergillus niger, penicillum and some types of bacterial strains such as Acetobacter, Brevibacterium (Hazzaa et al .,2013; Rasmey and Abdel-Kareem ., 2021).

Kojic acid has many uses in the fields of chemistry, health, food and cosmetics, despite its presence in the market for 40 years. It is also used as an anti-bacterial, anti-coagulant and chelating agent (Badar et al., 2021) and also has a role in inhibiting phagocytic cells and their functions and microbial killing, Kojic acid is produced naturally by many microorganisms such as some species of the genus Aspergillus from the filamentous fungi that produce kojic acid (Al-Daami , 2014).
II. MATERIALS AND WORKING METHODS

Production media preparation

The medium described by Ariff et al (1996) was used for the purpose of producing kojic acid.

Pollination of production medium

Taken 2 ml of the inoculum (one ml contains $10^5$ cells/ml) was taken and transferred to a flask containing 50 ml of the production medium described by (Arrif et al., 1996) in two flasks and incubated in a Shaker Incubator at 25 °C for 10 days and after the incubation period ended Centrifugation was conducted at a speed of 5000 rpm to separate the biomass from the fungal filtrate and the amount of kojic acid was estimated in the studied samples.

Determination of the amount of kojic acid produced from isolates

The acid was estimated using $(1\%) \text{ FeCl}_3$ iron chloride solution as a chromatography reagent, mixing 1 ml of farm filtrate and transferred to a volumetric flask containing $(2.5) \text{ ml of (0.1) M HCl and 0.2 ml of (0.2) M FeCl}_3$. The mixture was shaken well and the absorbance was read at the wavelength 500 nm using a spectrometer Photometry (Spectrophotometer), (Sulistyaningrum., 2008 ; Suryadi et al., 2018)

III. DETERMINATION OF OPTIMAL CONDITIONS FOR THE PRODUCTION OF KOJIC ACID FROM A. FLAVUS

Effect of carbon source

The method described by Rasmey and Basha (2016) was followed with some modification. The 250 ml flasks were inoculated on the production medium and the carbon source of the medium was replaced with 3 other carbon sources if glucose, sucrose and corn starch were used in concentrations of $(50, 70, 100) \text{ g/L}$ with stabilization of the pH at pH 6. The media were inoculated with 5 ml of fungus inoculum so that each ml contains $10^5$ cells/ml, incubated with three replications for each beaker, and all of them were incubated at 30°C for 8 days at a rate of shaking 4 hours per day at a speed of 150 cycles/min.

Effect of nitrogen source

Prepare the culture medium for the production of kojic acid and replace the original nitrogen source with nitrogen sources to determine the efficiency of them in the production of acid and these sources (yeast extract, urea, peptone and ammonium sulfate) at three concentrations $(3, 5, 7) \text{ g/L}$ and three replicates for each beaker (El-Aasar., 2006).

Effect of PH

The flasks containing the production medium were inoculated with different pH numbers $(4, 5, 6, 7, 8)$.

Effect of incubation temperature

The production medium was inoculated as in the previous methods and incubated at temperatures $(20, 25, 30, 35, 40) \text{ °C}$ for 8 days at a shaking rate of 4 hours per day at a speed of 150 rpm.

Effect Time of incubation

The flasks containing the production medium were incubated in a Shaker incubator for different times $(7, 12, 18, 24)$ days while fixing the optimal conditions obtained from previous experiments.

Results and discussion

When estimating the amount of kojic acid, the isolate $A. flavus$ gave $(19.99) \text{ g/L}$ of acid. Determining the optimal conditions for the production of kojic acid from $A. flavus$.

IV. EFFECT OF CARBON SOURCE

The current study showed the ability of the fungal isolate $A. flavus$ to produce kojic acid when using multiple carbon sources such as glucose, sucrose and starch at concentrations of $(50, 70, 100) \text{ g/L}$. Whereas the fungal isolate $A. flavus$ when using sucrose at a concentration of 70 g / L gave the highest amount of Kojic acid, which amounted to $(22.5) \text{ g/L}$ Figure (1-4). While indicated Rasmey and Basha (2016) that glucose as a carbon source was effective
for the fungi used in his study, as *A. oryzae* gave an amount of (40.73) g / L and *A. flavus* (19.92) g / L, followed by sucrose and starch, while lactose and cellulose did not give any acid production.

![Image of bar chart showing carbon source for kojic acid production]

Figure (1-4) shows the best carbon source for producing kojic acid from *A. flavus*.

**Effect of nitrogen source**

The results shown in Figure (2-4) indicated that the best nitrogen source for acid production from *A. flavus* is yeast extract of (26.8) g/L at a concentration of 5 g/L. As for peptone, the concentration of 5 g/L gave an amount of acid of (19.3) g / L, while ammonium sulfate gave lower amounts by (7.8, 9.3, 8.7) g / L for concentrations (3,5,7) g / L respectively, while when using urea as a nitrogen source was the amount of acid produced from fungus (14.2, 9.5, 6) g / l for concentrations (3,5,7) g / L, respectively.

While Promsang *et al* (2019) indicated that the use of a nitrogen source of 0.05% of yeast extract and ammonium sulfate to extract kojic acid from the fungus ATCC 10124 *A. oryzae* gave an amount of (1.58) g/L for the incubation period of 4 days, while the ammonium sulfate gave the acid amounts of Less when ammonium sulfate is used as another nitrogen source.

![Image of bar chart showing nitrogen source for kojic acid production]

Figure (2-4) shows the best nitrogen source for producing kojic acid from *A.flavus*

**Effect of pH**

The results in Figure (3-4) indicate that the highest amount of kojic acid production for *A.flavus* fermentation medium was (27.7) g / L at PH 6, while the fermenting medium gave the lowest amount of kojic acid at PH 8, reaching 10.8 g / L. The difference in the production of the amount of kojic acid according to the carbon and nitrogen source, in general, the fungi are characterized by their tolerance to acidic pH, as some of them have a pH (6-5) for cellular growth And other metabolic activities, in addition to the action of the enzymes responsible for the production of organic acids (Rosfarizan *et al.*, 2000) and Hassan *et al.* (2014) indicated that the optimum pH for
the production of kojic acid from Var effusus NR c14 A. oryzae was at (4) pH When using glucose as a carbon source and ammonium nitrate as a nitrogen source.

![Figure 3-4](image1.png)

**Figure (3-4) shows the best pH for the production of kojic acid**

**Effect of incubation temperature**

The results of the current study, which are shown in Figure (4-4), showed that the best temperature was 30°C, as the amount of acid produced was (28.9) g/L. While the lowest amount of production was at a temperature of 20 °C, reaching (13.8) g / L, and the ideal temperature for most fungi (28 - 30) °C, which is also optimal for the production of kojic acid in the fermentation medium (El-kady et al., 2014).

![Figure 4-4](image2.png)

**Figure[ 4-4] shows the best temperature for the production of kojic acid**

**Effect Time of incubation**

The results shown in Figure (5-4) showed that the incubation period had an effect on the production of kojic acid, as it differed from one isolate to another, as the best incubation period was for isolate A. flavus 12 day for the production of kojic acid, as the amount of acid produced was (29.6) g/L. As for the incubation periods (7, 18, and 24) days, the amount of acid was (27.1, 25.5, and 20.4) g/L, respectively. While Lin (2001) indicated that the best incubation period was 11 days for A. oryzae A. flavus. Kamaroddin (2007) explained that the incubation periods vary to obtain kojic acid, which may be (18-24) day, which is an appropriate and ideal incubation period for most fungi such as A. Candidus, while it is 4 days for A. flavus.
REFERENCES


23. Promsang, A.V.,Rungsardthong ; B.Thumthanaruk ; C.Puttanlek ; D.Uttapat ; T.Foophow ; V.Phalathanaporn and Vatanyoopaisarn S. (2019) Effect of culture conditions and medium compositions on kojic acid production by Aspergillus oryzae ATCC 10124

