DOI: https://doi.org/10.21323/2618-9771-2024-7-1-71-76

Received 30.11.2023
Accepted in revised 07.03.2024
Accepted for publication 11.03.2024


Available online at https://www.fsjour.com/jour
Original scientific article
Open access

# OPTIMIZATION OF PRODUCTION AND EVALUATION OF MICROBIAL KOJIC ACID OBTAINED FROM SUGARCANE MOLASSES (SCM) BY *ASPERGILLUS* SP.

Dalia E. Mohamed, Ahmed M. Alian, Reda M. Mohamed

Food Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt

*KEY WORDS:*
*kojic acid, agro-industrial wastes, fermentation, molasses, Aspergillus sp., antioxidant, antibacterial*

ABSTRACT

Kojic acid (KA) is an organic acid that is generated by various fungi, particularly by *Aspergillus* species, as a secondary metabolite. The current study is aimed to determine the optimal conditions for the production of kojic acid from various fungal strains grown on agro-industrial wastes. After testing six fungal strains for their suitability for kojic acid production, *Aspergillus oryzae* (AUMC.64) and *Aspergillus tamari* (AUMC.43) were found to be the highest producers of KA. Three different agro-industrial wastes were screened as a fermentation media and sugar cane molasses showed the highest productivity for (KA). *Aspergillus oryzae* (AUMC.64), and *Aspergillus tamari* (AUMC.43) achieved the maximal production of kojic acid (25.91, 18.95±0.001 g. $L^{-1}$respectively) from sugarcane molasses (SCM) under optimum conditions of growth (10% solution of sugarcane molasses, pH 4.0 and fermentation period of 10 days). Also, the antimicrobial activities of KA produced by *A. oryzae* AUMC64 and *A. tamari* AUMC43 against the selected test strains of microorganisms *Staphylococcus aureus*, *Bacillus cereus, Escherichia coli, Salmonella typhimurium* were recorded. The maximum growth inhibition zone (20–13.2 mm) was observed on the cultures of *Escherichia coli*. Meanwhile the antioxidant activities of KA produced by *A. oryzae* AUMC64 and *A. tamari* AUMC43 was 79.1 and 62.42%, respectively.

**Поступила 30.11.2023**
**Поступила после рецензирования 07.03.2024**
**Принята в печать 11.03.2024**


**https://www.fsjour.com/jour**
**Научная статья**
**Open access**

# ОПТИМИЗАЦИЯ ПРОИЗВОДСТВА И ОЦЕНКА МИКРОБНОЙ КОЙЕВОЙ КИСЛОТЫ, ПОЛУЧАЕМОЙ ИЗ ПАТОКИ САХАРНОГО ТРОСТНИКА (ПСТ) ПРИ ПОМОЩИ ГРИБКОВОЙ КУЛЬТУРЫ *ASPERGILLUS* SP.

Мохамед Д. Е., Алиан А. М., Мохамед Р. М.

Кафедра науки о питании, Сельскохозяйственный факультет, Каирский университет, Гиза, Египет

*КЛЮЧЕВЫЕ СЛОВА:*
*койевая кислота, агропромышленные отходы, ферментация, патока, Aspergillus sp., антиоксидант, антибактериальный*

АННОТАЦИЯ

Койевая кислота (КК) представляет собой органическую кислоту, вырабатываемую различными грибками, в частности, видами *Aspergillus*, в качестве вторичного метаболита. Целью настоящего исследования является определение оптимальных условий получения койевой кислоты из различных штаммов грибков, выращенных на отходах агропромышленного комплекса. После тестирования шести штаммов грибков на предмет их пригодности для производства койевой кислоты было обнаружено, что *Aspergillus oryzae* (AUMC.64) и *Aspergillus tamari* (AUMC.43) являются самыми высокими продуцентами КК. В качестве среды для ферментации были испытаны три вида различных агропромышленных отходов, из них патока сахарного тростника показала самую высокую продуктивность по производству КК. Были получены культуры *Aspergillus oryzae* (AUMC.64) и *Aspergillus tamari* (AUMC.43), обеспечившие максимальный объем производства койевой кислоты (25,91, 18,95±0,001 г/л соответственно) из патоки сахарного тростника (ПСТ) в оптимальных условиях культивирования (10% раствор патоки сахарного тростника), pH патоки 4,0, период ферментации — 10 дней). Также была зафиксирована антимикробная активность КК, продуцируемых *A. oryzae AUMC64* и *A. tamari AUMC43*, в отношении выбранных тест-штаммов микроорганизмов *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella typhimurium*. Максимальная зона подавления роста (20–13,2 мм) наблюдалась на культурах *Escherichia coli*. При этом антиоксидантная активность КК, продуцируемого грибками *A. oryzae AUMC64* и *A. tamari AUMC43*, составила 79,1 и 62,42% соответственно.

## 1. Introduction

Sugars, fibers, proteins, and minerals are the main ingredients of the goods of agricultural or animal origin that are produced by industrial processing. These agro-industrial wastes are mostly composed of lignocellulosic components, which include cellulose, hemicelluloses, and lignin [1]. Low-cost agro-industrial sources such as whey, sugarcane bagasse, maize steep liquor, wheat bran, soy bean meal, and others have been used in place of synthetic nitrogen and carbohydrate sources [2]. To make such wastes more palatable to microorganisms, they can undergo a range of physical, chemical, and enzymatic processing [3].

Organic acids can be manufactured industrially with the use of fermentative processes and variety of microorganisms. It is known that some *Aspergillus* fungi produce high concentrations of several organic acids, including citric acid, gluconic acid, and so on. However, some organic acids such as lactic acid, gibberellic acid, malic acid, and kojic acid are formed in trace amounts [4]. These acids are very advantageous for scientific, medical, and economic applications. In 1907, Saito used steamed rice to produce *Aspergillus oryzae* and isolated the kojic acid from the mycelial mat. Eventually, the rice was referred to as Koji, and the phrase Kojic acid was first used in 1913 by a scientist by the name of Yabuta [5].

Numerous research had proposed using agro-industrial wastes or by-products for the fungal production of beneficial products, the waste or byproducts included cheese whey, sugar cane molasses, fruits, vegetables, and maize steep liquor [3,6].

Kojic acid (5-hydroxy-2-hydroxymethylgamma-pyrone, or KA) is a significant secondary metabolite that can be generated from carbohydrates using a variety of carbon and nitrogen sources [7]. Using agricultural wastes and aerobic fermentation techniques, this task can be accomplished. Kojic acid is produced by *Aspergillus* species that are predominantly found in the section of *flavi*, namely *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus*

FOR CITATION: **Mohamed, D. E., Alian, A. M., Mohamed, R. M.** (2024). Optimization Production and Evaluation of Microbial kojic Acid from Sugarcane Molasses (SCM) by *Aspergillus* sp. *Food Systems,* 7(1), 71-76. https://doi.org/10.21323/2618-9771-2024-7-1-71-76

ДЛЯ ЦИТИРОВАНИЯ: **Мохамед, Д. Е., Алиан, А. М., Мохамед, Р. М.** (2024). Оптимизация производства и оценка микробной койевой кислоты, получаемой из патоки сахарного тростника (ПСТ) при помощи грибковой культуры *Aspergillus* sp. *Пищевые системы*, 7(1), 71-76. https://doi.org/10.21323/2618-9771-2024-7-1-71-76

*oryzae var effusus*, *Aspergillus tamari*, and *Aspergillus parasiticus*, in addition to *Penicillium* sp. and certain bacteria [3]. Kojic acid, which has a melting point within the range of 151–154 °C, crystallizes as colorless prismatic needles in water when cooled by centrifugation [8].

According to Rasmey and Abdel-Kareem [9], it is more soluble in water, ethanol, and ethyl acetate and less soluble in ether and chloroform. Kojic acid has a wide range of applications in chemistry, medicine, food and cosmetics, even though it has been on the market for 40 years, research on the microbial production and application of kojic acid is still ongoing. It also has antibacterial, anticoagulant and chelating properties [10]. The main objectives of this work were to identify the *Aspergillus* species that can manufacture kojic acid (KA) using glucose and agro-industrial waste media, as well as to optimize the conditions of kojic acid synthesis.

## 2. Materials and methods

### *2.1. Materials*

*2.1.1. Microorganisms and chemicals*

*Aspergillus oryzae* (AUMC.64), *Aspergillus tamari* (AUMC.43), *Aspergillus niger* (AUMC.42), *Aspergillus fumigatus* (AUMC.13602), *Aspergillus nidulans* (AUMC .13902), and *Aspergillus japonicas* (AUMC.14380) used in this work were obtained from Assiut University Moubasher Mycological Centre (AUMMC, Assiut, Egypt). Organisms were cultivated on potato dextrose agar slants (PDA) and kept at 4 °C.

The mold was inoculated on malt extract agar slants and incubated for five days at 30 °C in order to create spores. After incubation, the spores were taken out and re-injected into 50 ml of saline solution containing 0.1% of tween 80. The spores ($5 \times 10^8$ spores per $ml^{-1}$) were counted under a microscope, stored at 4 °C, and utilized as a stock inoculum. The chemicals were obtained from Sigma Chemical Company (St. Louis, Mo. USA).

*2.1.2. Screening medium and culture conditions*

The medium for kojic acid production consists of: ($gl^{-1}$) glucose, 100; yeast extract, 5; $KH_2PO_4$, 1.5; and $MgSO_4$, 0.5 combined at pH 4. 50 ml of the synthetic medium was added to 250 ml Erlenmeyer flasks, which were used to grow the experimental cultures. After being sterilized for 15 minutes at 121 °C, one milliliter of inoculum spore suspension ($5 \times 10^8$ spores per $ml^{-1}$) was added to the flasks after cooling. Then the cultures were incubated for ten days at 28 ± 2 °C.

*2.1.3. Agro-industrial wastes*

Various samples of agro-industrial wastes sugarcane molasses (SCM) were purchased from Sugars and Integrated Industries Egyptian Distillation Plants in Hawamdeia city, Giza, Egypt. Unsalted cheese whey as a by-product precipitated from milk during the production of cheese samples was kindly provided by the Dairy Department, Faculty of Agricultural-Cairo University. Also, the potato washing water resulting from the manufacture of chips was used in the fermentation process.

### *2.2. Methods*

*2.2.1. Screening of Aspergillus sp. for kojic acid production*

The ability of the following fungal strains to produce kojic acid from glucose as the only carbon source in a liquid synthetic medium under aerobic conditions was tested: *Aspergillus oryzae* (AUMC.64), *Aspergillus tamari* (AUMC.43), *Aspergillus niger* (AUMC.42), *Aspergillus fumigatus* (AUMC.13602), *Aspergillus nidulans* (AUMC.13902), and *Aspergillus japonicas* (AUMC.14380) were tested. Since glucose is the most basic sugar, it was utilized to screen for *Aspergillus* species that produce kojic acid [8].

*2.2.2. Treatment of sugarcane molasses*

Molasses from sugarcane was kept at 4 °C. To create treated molasses, the molasses was diluted 1:1 with distilled water, and concentrated sulfuric acid was added to bring the diluted molasses' pH to 4. After being heated to approximately 80 °C in a water bath for 1.5 hours, the molasses was centrifuged for 10 minutes at 4,000 rpm to precipitate the sludge while cooling. After filtering the treated molasses, the supernatant was moved to the fermentation flasks [11].

*2.2.3. Screening of agro-industrial wastes for kojic acid production*

Processed sugar cane molasses, whey, and potato water were used as fermentation media to produce kojic acid without adding any growth supplements. The previous media were sterilized at 121 °C for 15 minutes, followed by inoculation with tested *Aspergillus* strains and incubation at 28 ± 2 °C for 10 days. The ability of each experiment to yield kojic acid was evaluated [12].

*2.2.4. Chemical composition of agro-industrial wastes*

The chemical composition of agro-industrial wastes (sugarcane molasses, unsalted cheese whey, and potato water**)** were evaluated. Total carbohydrates were determined by the phenol sulfuric acid method [13]. Reducing the content of sugar was determined by DNSA method Utekar et al. [14]. Protein was also estimated using the Bradford method [15].

*2.2.5. Determination of kojic acid content*

According to Bentley, the amount of formed kojic acid in the supernatant culture was measured using a colorimetric technique with ferric chloride ($FeCl_3$) reagent. To prepare the reagent, one gram of $FeCl_3.6H_2O$ was dissolved in 100 milliliters of 0.1 N HCl. The functional groups of the hydroxyl and pyrone rings in the samples react with the reagent to produce a deep red color. A spectrophotometer was used to measure the mixture's absorbance at 500 nm. The kojic acid standard curve was used to calculate the kojic acid equivalent [9].

*2.2.6. Fast detection of kojic acid*

Screening the kojic acid producer was done fast (qualitatively), and the fungal-grown PDA plates were covered with a ferric chloride solution. The appearance of a deep red color during this procedure indicates the presence of kojic acid produced by the strain [10].

*2.2.7. Fourier-transform infrared spectroscopy (FTIR)*

For the further detection of the presence of kojic acid in extracted crystals, a Fourier-transform infrared spectroscope (FTIR) was used. The samples were analyzed on a Nicolet 380 FTIR using the KBr disk technique. Resolution was 4, and 16 scans were accumulated for each spectrum in a range of 500~4000 $cm^{-1}$, IR was used as a source and the detector is DTGS KBr. NICOLET 380 FT-IR, Thermo Scientific, made in China [16].

*2.2.8. High performance liquid chromatography (HPLC)*

To determine the KA content HPLC, Smart line, Knauer, Germany, was used, as well as a Kinetex XB-C18 Column (150 mm × 4.6 mm (Phenomenex®, USA)), operated at 40 °C. The injected volume was 20 µl in mobile phase of 0.1% acetic acid aqueous solution. Methanol 90:10 (v/v) Detection: VWD detector set at 270 nm was used. VWD: Variable Wavelength Detector [17].

*2.2.9. Determination of dry weight*

After the fermentation period ended, the biomass was recovered by filtering it through Whatman filter paper (No. 113), washed three times with distilled water, and dried for an entire night at 70 °C to determine its dry mass (DM) [18].

*2.2.10. Optimization production conditions of kojic acid from sugarcane molasses*

The study examined the effects of varying cultivating conditions on the biosynthesis of kojic acid, such as molasses concentration, initial pH value, and incubation period. Kojic acid was assayed to determine the impact of varying of the initial pH values (3.0, 4.0, 5.0, 6.0, and 7), varying molasses concentrations (3 to 10% with a 1% interval) and varying fermentation times (7 to 21 days) at the conclusion of each parameter [9].

*2.2.11. Kojic acid extraction and crystallization*

The process of extracting Kojic acid crystal involved filtering off the culture media using Wattman filter paper No. 1. The remaining solution was treated with ethyl acetate and refrigerated for 24 hours at 5 °C, causing the KA crystals to precipitate. The crystal was processed and dried at 80 °C for an hour the following day [10].

*2.2.12. Determination of kojic acid antibacterial activity*

Kojic acid's antimicrobial activity was assessed using the well diffusion assay. Every 24 hour test strains of microorganisms with $1.0 \times 10^7$ CFU $ml^{-1}$ was spread plated in 0.1 ml volume on the plate count agar, PCA medium (Oxoid, England). Subsequently, 50 µl of Kojic acid was added to each well. The plates were then incubated for 24 hour at 37 °C, and the inhibition of test strains of microorganisms was measured as the inhibition zone in millimeters [19].

*2.2.13. Determination of kojic acid antioxidant activity*

The degree of KA antioxidant activity is its capacity to scavenge 1.1-diphenyl-2-picrylhydrazyl (DPPH). Radical scavenging (%) = [(A0 – A1/A0) × 100] is the formula used by [20,21,22] to determine the percentage of radical scavenging activity. The absorbance of the sample extracts is A1, and the absorbance in the control sample is A0 [23].

*2.2.14. Statistical analysis*

The XLSTAT software version 2014, 5.03 (Addinsoft, New York, NY, USA) was used to analyze the experimental results using analysis of variance (ANOVA) in three repeats. The results were expressed as the mean ± standard error of the mean. When calculating the significance of mean differences between samples, a p-value of less than 0.05 was deemed as significant.

## 3. Results and discussion

### *3.1. Screening of Aspergillus sp. for kojic acid production*

The ability of the *Aspergillus* species *A. oryzae, A. tamari, A. niger, A. fumigatus, A. nidulans,* and *A. japonicas*, to produce kojic acid was tested, and the results are recorded in the Table 1. The obtained results demonstrate that after ten days of cultivation, the various strains' abilities to produce kojic acid varied. The highest kojic acid production was recorded for *A. oryzae* AUMC64 (12.599 g. $L^{-1}$) followed by *A. tamari* AUMC43 (7.003 g. $L^{-1}$), *A. nidulans* AUMC13902 (1.527 g. $L^{-1}$), *A. fumigatus* AUMC13602 (0.694 g. $L^{-1}$), and A. *japonicas* AUMC14380 (0.276 g. $L^{-1}$), while the lowest value was for *niger* AUMC42 (0.115 g. $L^{-1}$). Therefore, *A. oryzae* AUMC64 and *A. tamari* AUMC43 were chosen for additional testing (Table 1). The results show a high correlation (r = 0.97) between the two approaches when it comes to the relation between the qualitative and quantitative methods. These results are consistent with those of [9,11,24]. These variations in the biomass and kojic acid production can be attributed to species differences or culture conditions. *Aspergillus* oryzae was a great starting strain for the safe synthesis of kojic acid because it has been proven in several studies to have low pathogenicity and to not produce aflatoxins or any other carcinogenic metabolites. *Aspergillus* species can produce the amylase enzyme, which hydrolyses starch into simple sugars that can then be converted into kojic acid, according to research by [25]. The type of fermentable sugars present in the culture medium determines the amount of kojic acid being produced (g. $L^{-1}$).

### *3.2. Chemical composition of agro-industrial waste*

The chemical composition of agro-industrial wastes (sugarcane molasses, unsalted cheese whey, and potato water**)** was evaluated. Total content of carbohydrates was determined, and the results were as follows: 56.01, 12, and 20% for molasses, whey, and potato water, respectively. Meanwhile, reduction sugar was found to be 24.90, 4, and 0.2% for molasses, whey, and potato water, respectively. Protein content was also estimated, and the results were as follows: 5, 2, and 0% for molasses, whey, and potato water, respectively.

### *3.3. Screening of the agro-industrial waste for kojic acid production*

Various samples of agro-industrial wastes (cheese whey, potato water, and sugarcane molasses) were tested as a carbon source for the growth of *A. oryzae*, and *A. tamari* and as a natural medium for kojic acid production. The results are shown in the Table 2. The results indicated that the highest productivity of kojic acid was obtained from SCM (14.28 g. $L^{-1}$), followed by cheese whey and potato water (5.34 g. $L^{-1}$ and 0.55 g. $L^{-1}$, respectively). These results were in harmony with those previously obtained by [3,11]. Egyptian SCM has detectable levels of several vitamins, including riboflavin and thiamin, and roughly 52% total sugar (glucose, sucrose, and fructose), as well as 0.46% total nitrogen. These substances may encourage the synthesis of kojic acid in each type of molasses [12].

Table 1. **Selection and screening of various *Aspergillus* sp. for kojic acid production**

Таблица 1. **Отбор и анализ различных видов *Aspergillus* sp., используемых для производства койевой кислоты**

| Strains | Kojic acid production (g. $L^{-1}$) | Red zone (mm) |
|---|---|---|
| *Aspergillus nidulans* AUMC13902 | $1.527^{c} \pm 0.03$ | $3.47^{c} \pm 0.06$ |
| *Aspergillus oryzae* AUMC64 | $12.599^{a} \pm 0.06$ | $4.97^{a} \pm 0.05$ |
| *Aspergillus fumigatus* AUMC13602 | $0.694^{d} \pm 0.02$ | $2.13^{d} \pm 0.05$ |
| *Aspergillus japonicas* AUMC14380 | $0.276^{e} \pm 0.04$ | $2.01^{d} \pm 0.09$ |
| *Aspergillus niger* AUMC42 | $0.115^{f} \pm 0.07$ | $1.03^{e} \pm 0.01$ |
| *Aspergillus tamari* AUMC43 | $7.003^{b} \pm 0.04$ | $4.07^{b} \pm 0.06$ |

The experimental values (means and SD for n = 3) with small letter are significantly different (P ⩽ 0.05).

Table 2. **Screening of the agro-industrial waste for kojic acid production (g. $L^{-1}$) by *A. oryzae* and *A. tamari***

Таблица 2. **Анализ агропромышленных отходов, используемых для производства койевой кислоты (g. $L^{-1}$) при помощи *A. oryzae* и *A. tamari***

| Agro-industrial waste | *Aspergillus oryzae* AUMC64 | | *Aspergillus tamari* AUMC43 | |
|---|---|---|---|---|
| | Kojic acid production (g. $L^{-1}$) | Dry weight (g. $L^{-1}$) | Kojic acid production (g. $L^{-1}$) | Dry weight (g. $L^{-1}$) |
| Synthetic media | $12.59^{b} \pm 0.01$ | $41.9^{b} \pm 0.07$ | $7.01^{b} \pm 0.06$ | $38.3^{b} \pm 0.31$ |
| Potato water | $0.55^{d} \pm 0.03$ | $7.9^{d} \pm 0.49$ | $0.66^{d} \pm 0.04$ | $5.7^{d} \pm 0.42$ |
| Cheese whey | $5.34^{c} \pm 0.02$ | $24.9^{c} \pm 0.34$ | $4.93^{c} \pm 0.03$ | $25.0^{c} \pm 0.40$ |
| Sugarcane molasses | $14.28^{a} \pm 0.04$ | $52.7^{a} \pm 0.36$ | $9.61^{a} \pm 0.06$ | $50.3^{a} \pm 0.86$ |

The experimental values (means and SD for n = 3) with small letter are significantly different (P ⩽ 0.05).

### *3.4. Optimization of kojic acid production in SCM media by A. oryzae, and A. tamari*

#### *3.4.1. Effect of duration of fermentation period on kojic acid production*

The effects of the duration of fermentation period on the production of kojic acid from SCM by *A. oryzae*, and *A. tamari* were investigated. The data presented in the Table 3 indicate that the production of kojic acid increased gradually along with the duration of the incubation period, reaching its maximum value (25.9 g. $L^{-1}$) at 10 days before declining. These outcomes corroborated those previously attained by [12,26]. According to Rasmey et al. [11], the mycelium's breakdown of kojic acid into oxalic and acetic acid when glucose levels are low may be the cause of the decrease in kojic acid that occurs with longer period of incubation.

#### *3.4.2. Effect of SCM concentrations on kojic acid production*

The Table 4 presents the results of a test conducted to determine the effect of SCM concentrations in the medium on the production of kojic acid by *A. oryzae* and *A. tamari*. The outcome demonstrated that the production of kojic acid increased as molasses sugar concentrations rose, peaking at 10% sugar content and being produced by *A. oryzae*, and *A. tamari* (25.9–18.9 g. $L^{-1}$). The production of the acid decreased as the concentration of sugar molasses (15–20–25%) was increased further. Fermentation rates decreased as a result of both decreased water activity and the beginning of plasmolysis. Elevating the initial concentration of sugar led to a noteworthy rise in residual sugar content, potentially resulting from the microorganisms' incapacity to metabolize elevated sugar levels. It was found that the osmotic pressure had a negative impact because the acid production abruptly stopped. These outcomes concurred with the preliminary findings of [11]. Once the fungal growth reached the stationary phase, the synthesis of kojic acid ceased when the certain concentration of medium's glucose was reached. According to studies by [25,26], the synthesis of kojic acid was impacted by an excessive concentration of carbon sources.

#### *3.4.3. Effect of pH on production of kojic acid*

The Table 5 presents the results of a study conducted to examine the effect of the initial pH value in the molasses fermentation medium on the production of kojic acid from SCM by *A. oryzae*, and *A. tamari* The

Table 3. **Effect of various fermentation periods on yield of kojic acid being produced from SCM by A. *oryzae,* and *A. tamari***

Таблица 3. **Влияние различных периодов ферментации на объем койевой кислоты, получаемой из ПСТ при помощи *A. oryzae* и *A. tamari***

| Days | *Aspergillus oryzae* AUMC64 | | *Aspergillus tamari* AUMC43 | |
|---|---|---|---|---|
| | Kojic acid production (g. $L^{-1}$) | Dry weight (g. $L^{-1}$) | Kojic acid production (g. $L^{-1}$) | Dry weight (g. $L^{-1}$) |
| 3 | $10.22^{d} \pm 0.06$ | $8.7^{g} \pm 0.06$ | $8.19^{d} \pm 0.07$ | $2.7^{g} \pm 0.06$ |
| 7 | $21.73^{b} \pm 0.09$ | $22.4^{f} \pm 0.01$ | $15.37^{b} \pm 0.09$ | $8.3^{f} \pm 0.12$ |
| 10 | $25.91^{a} \pm 0.06$ | $26.3^{e} \pm 0.06$ | $18.04^{a} \pm 0.08$ | $15.3^{e} \pm 0.12$ |
| 13 | $20.02^{c} \pm 0.09$ | $22.7^{d} \pm 0.12$ | $13.90^{c} \pm 0.09$ | $21.0^{d} \pm 0.10$ |
| 15 | $15.71^{e} \pm 0.07$ | $27.7^{c} \pm 0.06$ | $10.70^{e} \pm 0.06$ | $25.7^{c} \pm 0.06$ |
| 18 | $13.20^{f} \pm 0.08$ | $32.7^{b} \pm 0.06$ | $9.92^{f} \pm 0.05$ | $36.3^{b} \pm 0.06$ |
| 21 | $10.90^{g} \pm 0.01$ | $35.7^{a} \pm 0.06$ | $7.04^{g} \pm 0.08$ | $42.3^{a} \pm 0.06$ |

The experimental values (means and SD for n = 3) with small letter are significantly different (P ⩽ 0.05).

Table 4. **Effect of various concentrations of sugarcane molasses on the yield of kojic acid being produced by *A. oryzae* and *A. tamari***

Таблица 4. **Влияние различных концентраций патоки сахарного тростника на объем койевой кислоты, получаемой при помощи *A. oryzae* и *A. tamari***

| SCM concentration% (V/V) | *Aspergillus oryzae* AUMC64 | | *Aspergillus tamari* AUMC43 | |
|---|---|---|---|---|
| | Kojic acid production (g. $L^{-1}$) | Dry weight (g. $L^{-1}$) | Kojic acid production (g. $L^{-1}$) | Dry weight (g. $L^{-1}$) |
| 5% | $16.70^{d} \pm 0.01$ | $14.2^{e} \pm 0.1$ | $10.90^{d} \pm 0.01$ | $12.7^{e} \pm 0.38$ |
| 10% | $25.90^{a} \pm 0.05$ | $26.3^{d} \pm 0.12$ | $18.91^{a} \pm 0.07$ | $23.7^{d} \pm 0.21$ |
| 15% | $23.13^{b} \pm 0.05$ | $32.3^{c} \pm 0.16$ | $17.92^{b} \pm 0.04$ | $31.3^{c} \pm 0.35$ |
| 20% | $17.59^{c} \pm 0.06$ | $45.3^{b} \pm 0.25$ | $12.58^{c} \pm 0.05$ | $44.0^{b} \pm 0.26$ |
| 25% | $10.19^{e} \pm 0.05$ | $52.7^{a} \pm 0.12$ | $8.03^{e} \pm 0.07$ | $53.7^{a} \pm 0.31$ |

The experimental values (means and SD for n = 3) with small letter are significantly different (P ⩽ 0.05).

tested strains produced kojic acid at the best level at a pH of 4.0. Beyond pH 4.0, kojic acid production started to decline. Based on the initial pH of culture, the majority of research on how culture pH affects kojic acid production and growth was done. *A. oryzae*, and *A. tamari* are two examples of fungi that can produce kojic acid within the pH range of 3 to 7. High pH had a major impact on the metabolism of fungi. The fungus's metabolism shifted to other pathways rather than producing kojic acid. Thus, a high pH further decreased the yield of kojic acid [27]. At pH 4.0, *A. oryzae* and *A. tamari* demonstrated the highest yield of crystals of kojic acid. The growth of the fungus and the yield of kojic acid decreased as the pH of the culture medium increased [10]. According to [9], the structure and function of enzymes were influenced by the pH of the culture medium because the enzymes are proteins with ionizable groups. Determining the perfect pH is crucial because it has a significant impact on the best way to produce the enzymes needed to obtain kojic acid.

### *3.5. Production of kojic acid in optimal conditions*

According to this study, 10% concentration of SCM provided the best conditions for the fermentation of *A. oryzae* AUMC64 and A. tamari AUMC43, which produce kojic acid. In perfect environmental conditions, the pH and incubation period were 4.0 and 10 days, respectively. These outcomes are exactly the same as those noted by multiple researchers [9,10,12,27].

### *3.6. Extraction and characterization of produced kojic acid*

The process of extracting kojic acid crystals involved filtering culture media using Wattman filter paper No. 1. As previously mentioned, the left mycelial mass was also weighted according to the procedures to determine wet and dry weight. After being treated with ethyl acetate, the remaining solution was kept in a refrigerator at 5 °C for a full day. The following day, crystals of KA were visible at the beaker's bottom. To o btain such crystals, filtration was done, and they were then oven dried for one hour at 80 °C [10].

Table 5. **Effect of various initial pH values on production of kojic acid from SCM by *A. Oryzae* and *A. tamari***

Таблица 5. **Влияние различных начальных значений pH на объем койевой кислоты, получаемой из ПСТ при помощи *A. oryzae* и *A. tamari***

| pH | *Aspergillus oryzae* AUMC64 | | *Aspergillus tamari* AUMC43 | |
|---|---|---|---|---|
| | Kojic acid production (g. $L^{-1}$) | Dry weight (g. $L^{-1}$) | Kojic acid production (g. $L^{-1}$) | Dry weight (g. $L^{-1}$) |
| 3 | $19.65^{c} \pm 0.05$ | $15.7^{b} \pm 0.04$ | $14.30^{c} \pm 0.04$ | $16.0^{b} \pm 0.30$ |
| 4 | $25.91^{a} \pm 0.05$ | $26.8^{a} \pm 0.25$ | $18.95^{a} \pm 0.09$ | $23.7^{a} \pm 0.50$ |
| 5 | $21.65^{b} \pm 0.06$ | $15.6^{b} \pm 0.05$ | $15.91^{b} \pm 0.08$ | $17.3^{b} \pm 0.15$ |
| 6 | $18.12^{d} \pm 0.07$ | $11.5^{c} \pm 0.31$ | $11.21^{d} \pm 0.01$ | $13.3^{c} \pm 0.15$ |
| 7 | $15.04^{e} \pm 0.05$ | $7.9^{d} \pm 0.26$ | $8.31^{e} \pm 0.06$ | $8.3^{d} \pm 0.06$ |

The experimental values (means and SD for n = 3) with small letter are significantly different ($P \leq 0.05$).

#### *3.6.1. Infrared spectroscopy via Fourier-transform infrared spectroscopy (FTIR)*

The sample of kojic acid's FTIR spectrum displays peak wave number values peculiar for the functional groups that are comparable to those of regular kojic acid (Figure 1). Functional groups 3270.8 $cm^{-1}$, 3179.43 $cm^{-1}$ (OH), 2925.17 $cm^{-1}$, 2854.05 $cm^{-1}$ (aliphatic-CH), 1660.59 $cm^{-1}$ (cyclic-C=O), 1611.11 $cm^{-1}$ (C=C), 1472.61 $cm^{-1}$ (deformation of-CH2), 1074.04 $cm^{-1}$ (cyclic C-O-C), 943.58 $cm^{-1}$, 863.66 $cm^{-1}$, and 775.65 $cm^{-1}$ (1, 4 α -disubstituted ring) are among the functional groups where the bands are present [17].

#### *3.6.2. High performance liquid chromatography (HPLC)*

The compound with the same retention time as the standard kojic acid was found among the best HPLC results for *A. oryzae* (Figure 2). All the experimental analysis (HPLC and FTIR) indicated that kojic acid was produced by both fungus [10].

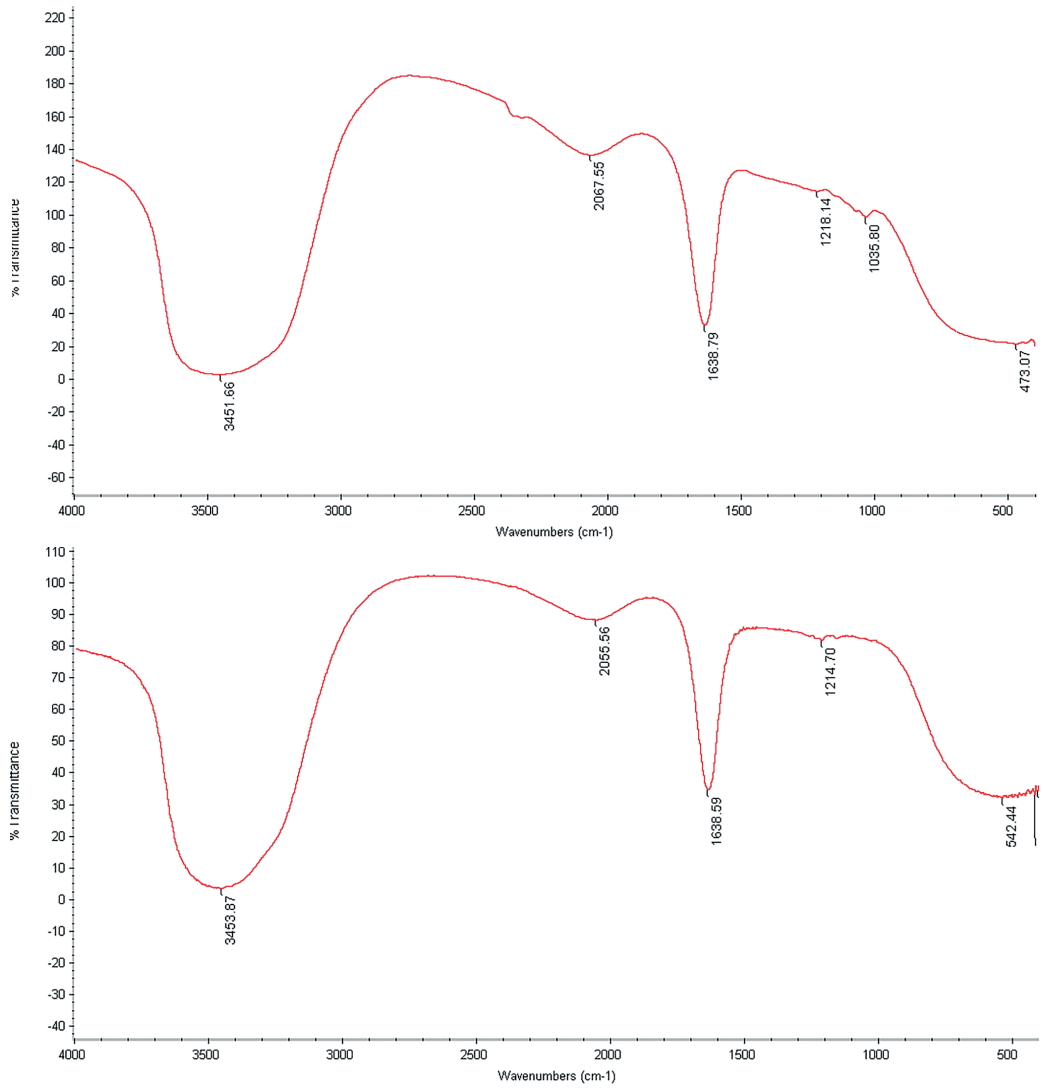



Figure 1. **Peak wave number values of FTIR for standard kojic acid**

Рисунок 1. **Значения пиковых волновых чисел по ИК-Фурье спектроскопии у стандартной койевой кислоты**

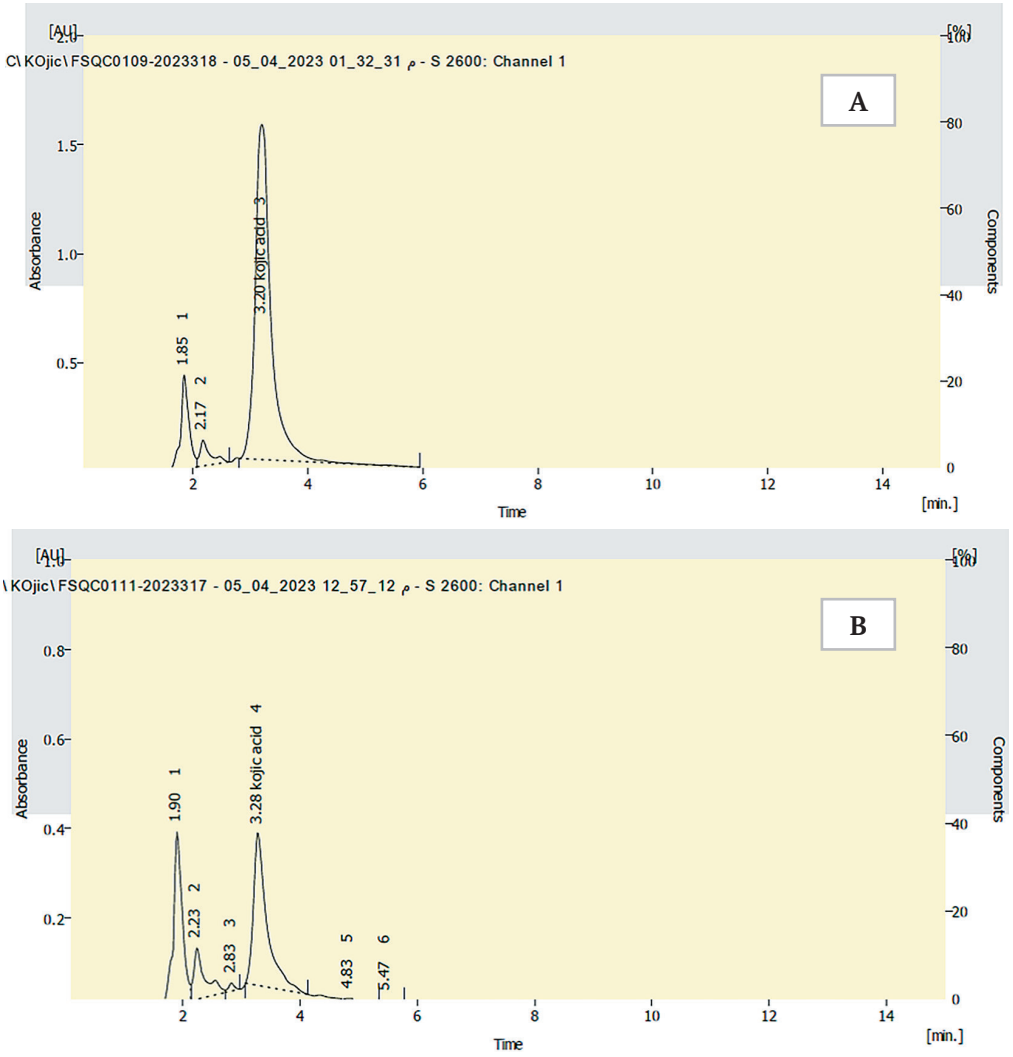



**Figure 2. HPLC of kojic acid obtained from *A. oryzae* (A) and *A. tamari* (B)**

Рисунок 2. **ВЭЖХ койевой кислоты, получаемой при помощи *A. oryzae* (A) и *A. tamari* (B)**

*3.6.3. Antibacterial activity of kojic acid*

The antimicrobial activities of KA produced by *A. oryzae* AUMC64 and *A. tamari* AUMC43 against the selected test strains of microorganisms (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Salmonella typhimurium)* was investigated. Our results are presented in the Table 6. The maximum zone of inhibition (20–13.2 mm) was observed with the cultures, *Escherichia coli* and *Salmonella typhimurium,* followed by *Staphylococcus aureus* and *Bacillus cereus* (9.6–8.3 mm) for KA produced by *A. oryzae* AUMC64. Such a result indicates that these organisms were highly sensitive to antimicrobial compounds and kojic acid. These results agreed with those previously obtained by [5,19].

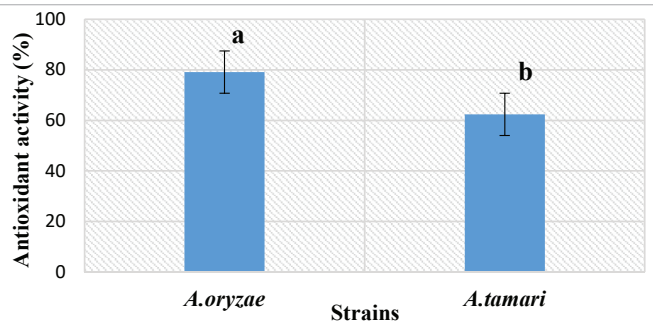
Table 6. **Antibacterial activities of kojic acid produced by *A. oryzae* and *A. tamari***

Таблица 6. **Антибактериальная активность койевой кислоты, получаемой при помощи *A.oryzae* и *A. tamari***

| Test strains of microorganisms | Kojic acid concentration (mg/ml) | | | Inhibition zone (mm) | | |
|---|---|---|---|---|---|---|
| | *A. oryzae* | | | *A. tamari* | | |
| | 0.5 | 1 | 2 | 0.5 | 1 | 2 |
| *S. aureus* | 2.5 | 6.6 | 9.6 | 1.6 | 4.5 | 7.1 |
| *B. cereus* | 1.8 | 6.1 | 8.3 | 1.1 | 3.8 | 6.4 |
| *E. coli* | 4.2 | 8.4 | 20 | 2.5 | 5.7 | 13.2 |
| *Sal. typhimurium* | 3.2 | 7.3 | 11.1 | 1.9 | 5.1 | 7.6 |

*3.6.4. Antioxidant activity of kojic acid*

The antioxidant activity of KA produced by two fungal strains was tested by their scavenging effect on DPPH radicals under cultural conditions, and the results are recorded in the Figure 3. The results indicated that the antioxidant activity of KA extract produced by *A. oryzae* AUMC64 and *A. tamari* AUMC43 was 79.1 and 62.42%, respectively. These results indicate that the antioxidant percentage is correlated with amounts of KA in the culture medium. KA is a good chelator of transitional metal ions and a good scavenger of the free radical DPPH. Moreover, KA has potential activity in depigmentation processes through chelating the copper ion present in the active site of tyrosinase, which mediates the formation of melanin from the amino acid tyrosine [28]. The potential antioxidant activity of KA approves the benefits of its application in the food industry, where it has been used in the post-harvest process as an anti-speck and an anti-browning agent for the agricultural products [29].




The experimental values (means for n = 3) with small letter are significantly different (P ⩽ 0.05).

Figure 3. **Antioxidant activity of kojic acid produced by *A. oryzae* and *A. tamari***

Рисунок 3. **Антиоксидантная активность койевой кислоты, получаемой при помощи *A.oryzae* и *A.tamari***

## 4. Conclusion

During the study of the possibility of utilizing some agro-industrial wastes to produce microbial kojic acid using both *A. oryzae* and *A. tamari*, the results demonstrated the possibility of using SCM as a fermentation

medium and obtaining high level of kojic acid production compared to the synthetic medium. Fermentation conditions were also controlled to maximize the productivity. Some properties of kojic acid were studied, such as its effect as an antioxidant and antibacterial substance, and the results positively proved its ability to do this. In the future, we can continue to study the possibility of using waste for fermentation producing biological compounds and running works to reduce the pollution resulting from these wastes on the path to sustainable development.

## REFERENCES


1. Mussatto, S.I, Ballesteros, L.F, Martins, S.F, Teixeira, J.A. (2012). Use of agro-industrial wastes in solid state fermentation processes. Chapter in a book: Industrial waste. InTech Open Access Publisher, 2012. http://doi.org/10.5772/36310
2. Fatima, B, Hussain, Z, Khan, M.A. (2014). Utilization of agroindustrial waste residues for the production of amylase from *Aspergillus* oryzae IIB-62014. *British Biotechnology Journal*, 4(4), 350–365. http://doi.org/10.9734/BBJ/2014/7915
3. Zohri, A. A.-N., Mahmoud, G. A.-E., Saddek, N. H., Hanafy, R. A. (2018**).** Optimization of kojic acid production conditions from cane molasses using plackett — burman design. *European Journal of Biological Research*, 8, 56–69. http://doi.org/10.5281/zenodo.1211517
4. de Caldas Felipe, M. T., do Nascimento Barbosa, R., Bezerra, J. D. P., de Souza-Motta, C. M. (2023). Production of kojic acid by *Aspergillus* species: Trends and applications. *Fungal Biology Reviews*, 45, Article 100313. https://doi.org/10.1016/j.fbr.2023.100313
5. Devi, K. B. D., Vijayalakshmi, P., Shilpa, V., Kumar, B. V. (2015). Optimization of cultural parameters for cost effective production of kojic acid by fungal species isolated from soil. *Microbiology Research Journal International*, 7(5), 255–268. https://doi.org/10.9734/BMRJ/2015/15325
6. Khamaruddin, N.R., Basri, M., Lian, G.E.C. (2008). Enzymatic synthesis and characterization of palm-based kojic acid ester. *Journal of Oil Palm Research,* 20, 461–469.
7. Li, C., Ji, X., Li, J., Wu, D., Qi, L., Wang, A. et al. (2022). Measurement and correlation of the solubility of kojic acid in pure and binary solvents. *The Journal of Chemical Thermodynamics*, 167, Article 106712. https://doi.org/10.1016/j.jct.2021.106712
8. Chib, S., Jamwal, V. L., Kumar, V., Gandhi, S. G., Saran, S. (2023). Fungal production of kojic acid and its industrial applications. *Applied Microbiology and Biotechnology*, 107(7–8), 2111–2130. https://doi.org/10.1007/s00253-023-12451-1
9. Rasmey, A. -H. M., Abdel-Kareem, M. (2021). Optimization of culture conditions for Kojic acid production in surface fermentation by *Aspergillus oryzae* isolated from wheat grains. *Bulletin of Pharmaceutical Sciences Assiut*, 44(1), 201–211.
10. Chib, S., Dogra, A., Nandi, U., Saran, S. (2019). Consistent production of kojic acid from *Aspergillus sojae* SSC-3 isolated from rice husk. *Molecular Biology Reports*, 46, 5995–6002. https://doi.org/10.1007/s11033-019-05035-8
11. Rasmey, A.-H. M., Basha, A.H. (2016). Isolation and screening of kojic acid producing isolate of *Aspergillus oryzae* potentially applicable for production from sugarcane molasses. *International Journal of Biological Research*, 4(2), Article 119. http://doi.org/10.14419/ijbr.v4i2.6434
12. El-Kady, I. A., Zohri, A. N. A., Hamed, S. R. (2014). Kojic acid production from agro-industrial by-products using fungi. *Biotechnology Research International*, 2014, Article 642385. https://doi.org/10.1155%2F2014%2F642385
13. Mansfield, S. D. (2020). Determination of Total Carbohydrates. Chapter in a book: Methods to Study Litter Decomposition: A Practical Guide. Springer Cham, 2020. https://doi.org/10.1007/978-3-030-30515-4_14
14. Utekar, P. G., Kininge, M. M., Gogate, P. R. (2021). Intensification of delignification and enzymatic hydrolysis of orange peel waste using ultrasound for enhanced fermentable sugar production. *Chemical Engineering and Processing-Process Intensification*, 168, Article 108556. http://doi.org/10.1016/j.cep.2021.108556
15. Rekowski, A., Langenkämper, G., Dier, M., Wimmer, M. A., Scherf, K. A., Zörb, C. (2021). Determination of soluble wheat protein fractions using the Bradford assay. *Cereal Chemistry*, 98(5), 1059–1065. http://doi.org/10.1002/cche.10447
16. Käppler, A., Fischer, D., Oberbeckmann, S., Schernewski, G., Labrenz, M., Eichhorn, K.-J. et al. (2016). Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Analytical and Bioanalytical Chemistry*, 408, 8377–8391. https://doi.org/10.1007/s00216-016-9956-3
17. Chib, S., Jamwal, V. L., Kumar, V., Gandhi, S. G., Saran, S. (2023). Fungal production of kojic acid and its industrial applications. *Applied Microbiology and Biotechnology*, 107(7–8), 2111–2130. https://doi.org/10.1007/s00253-023-12451-1
18. Quiterio-Gutiérrez, T., González-Morales, S., González-Fuentes, J. A., Benavides-Mendoza, A., Fernández-Luqueño, F., Medrano-Macías, J. et al. (2023). Production of kojic acid by *aspergillus* niger m4 with different concentrations of yeast extract as a nitrogen source. *Processes*, 11(6), Article 1724. http://doi.org/10.3390/pr11061724
19. Khodabandlou, S., Saraei, M. (2021). Synthesis of novel isoxazole derivatives bearing kojic acid moiety and evaluation of their antimicrobial activity. *Chemistry of Heterocyclic Compounds*, 57, 823–827. https://doi.org/10.1007/s10593-021-02986-4
20. Elmastas, M., Isildak, O., Türkekul, I., Temur, N. (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis*, *20*(3–4), 337–345. http://doi.org/10.1016/j.jfca.2006.07.003
21. Ali, M.R., Mohamed, R. M., Abedelmaksoud, T. G. (2021). Functional strawberry and red beetroot jelly candies rich in fibers and phenolic compounds. *Food Systems*, 4(2), 82–88. https://doi.org/10.21323/2618-9771-2021-4-1-82-88
22. Wang, Y.-X., Wang, S.-Y., Beta, T., Shahriar, M., Laborda, P., Herrera-Balandrano, D. D. (2023). Kojic acid induces resistance against Colletotrichum brevisporum and enhances antioxidant properties of postharvest papaya. *Food Control*, 144, Article 109405. https://doi.org/10.1016/j.foodcont.2022.109405
23. Wang, R., Hu, X., Agyekumwaa, A. K., Li, X., Xiao, X., Yu, Y. (2021). Synergistic effect of kojic acid and tea polyphenols on bacterial inhibition and quality maintenance of refrigerated sea bass (*Lateolabrax japonicus*) fillets. *LWT*, 137, Article 110452. https://doi.org/10.1016/j.lwt.2020.110452
24. Abd El-Aziz, A. B. (2013). Improvement of kojic acid production by a mutant strain of *Aspergillus flavus*. *Journal of Natural Sciences Research*, 3(4), 31–41.
25. Rosfarizan, M., Ariff, A.B. (2000). Kinetics of kojic acid fermentation by *Aspergillus* flavus using different types and concentrations of carbon and nitrogen sources. *Journal of Industrial Microbiology and Biotechnology*, 25(1), 20–24. http://doi.org/10.1038/sj.jim.7000017
26. El-Aasar, S.A. (2006). Cultural conditions studies on kojic acid production by *Aspergillus* parasiticus. *International Journal of Agriculture and Biology*, 8(4), 468–473.
27. Mohamad, R., Mohamed, M. S., Suhaili, N., Salleh, M. M., Ariff, A.B. (2010). Kojic acid: Applications and development of fermentation process for production. *Biotechnology and Molecular Biology Reviews*, 5(2), 24–37.
28. Gonçalez, M. L., Correa, M. A., Chorilli, M. (2013). Skin delivery of kojic acid-loaded anotechnology-based drug delivery systems for the treatment of skin aging. *BioMed Research International*, 2013, Article 271276. https://doi.org/10.1155/2013/271276
29. Chaudhary, J., Pathak, A.N., Lakhawat, S. (2014). Production technology and applications of Kojic acid. *Annual Research and Review in Biology*, 4(21), 3165–3196. https://doi.org/10.9734/ARRB/2014/10643



| AUTHOR INFORMATION | СВЕДЕНИЯ ОБ АВТОРАХ |
|---|---|
| **Affiliation** | **Принадлежность к организации** |
| **Dalia E. Mohamed,** Teaching Assistant, Department of Food Science, Faculty of Agriculture, Cairo University<br>1 Gamaa Street, 12613, Giza, Egypt<br>Tel.: +2–0102–273–63–22<br>E mail: Dalia.header@agr.cu.edu.eg<br>ORCID: https://orcid.org/0009-0004-3487-8759 | **Мохамед Д. Е.** — ассистент преподавателя, кафедра науки о питании, сельскохозяйственный факультет, Каирский Университет<br>12613, Египет, Гиза, ул. Гамаа, 1<br>Тел.: +2–0102–273–63–22<br>E mail: Dalia.header@agr.cu.edu.eg<br>ORCID: https://orcid.org/0009-0004-3487-8759 |
| **Ahmed M. Alian,** Professor, Department of Food Science, Faculty of Agriculture, Cairo University<br>1 Gamaa Street, 12613, Giza, Egypt<br>Tel.: +2–0122–362–31–40<br>E mail: dr.alian@hotmail.com<br>ORCID: https://orcid.org/0009-0003-1846-5375 | **Алиан А. М.** — профессор, кафедра науки о питании, сельскохозяйственный факультет, Каирский Университет<br>12613, Египет, Гиза, ул. Гамаа, 1<br>Тел.: +2–0122–362–31–40<br>E mail: dr.alian@hotmail.com<br>ORCID: https://orcid.org/0009-0003-1846-5375 |
| **Reda M. Mohamed,** Associate Professor, Department of Food Science, Faculty of Agriculture, Cairo University<br>1 Gamaa Street, 12613, Giza, Egypt<br>Tel.: +2–0115–659–47–47<br>E mail: reda_karrim@agr.cu.edu.eg<br>ORCID: https://orcid.org/0000-0001-5817-9187 | **Мохамед Р. М.** — адъюнкт-профессор, кафедра науки о питании, сельскохозяйственный факультет, Каирский Университет<br>12613, Египет, Гиза, ул. Гамаа, 1<br>Тел.: +2–0115–659–47–47<br>E mail: reda_karrim@agr.cu.edu.eg<br>ORCID: https://orcid.org/0000-0001-5817-9187 |
| **Contribution** | **Критерии авторства** |
| Authors equally relevant to the writing of the manuscript, and equally responsible for plagiarism. | Авторы в равных долях имеют отношение к написанию рукописи и одинаково несут ответственность за плагиат. |
| **Conflict of interest** | **Конфликт интересов** |
| The authors declare no conflict of interest. | Авторы заявляют об отсутствии конфликта интересов. |