J. Appl. Biol. Sci., 19 (1): 13-, January 2025
DOI: https://doi.org/10.71336/jabs.1370
RESEARCH ARTICLE

Journal of Applied Biological Sciences
E-ISSN: 2146-0108; License: CC BY 4.0
Anatolia Academy of Sciences
Open Access

Production of kojic acid by *Aspergillus niger* (PP330720) and *Penicilium digitatum* (PP892864) grown on guinea corn (*Sorghum bicolor)* sheaf

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Received: August 2, 2024
Revised: January 15, 2025
Accepted: January 16, 2025

ABSTRACT: Kojic acid (KA) is globally relevant in the cosmetic, medical, and food industries, production optimization to mitigate cost, maximize profit and manage waste through eco-friendly and sustainable alternatives is imperative. This research sought to investigate the suitability of resident moulds grown on guinea corn sheaf (GCS) as sole carbon source for KA production. Resident moulds were isolated, identified and screened for KA production through standard methods. Promising moulds were adopted for kojic acid production in SSF for 9 days and assay was done daily using commercial kojic acid as standard. Response surface methodology was utilized to optimize some fermentation process variables. The functional groups in extracted KA were determined with FTIR. *Aspergillus niger, Rhizopus stolonifer,* and *Penicillium digitatum* were identified and confirmed with the accession number PP330720 and PP461997 for *A. niger* and *P. digitatum,* respectively. Fermentation for 9 days, substrate concentration of 30 g/l, inoculum size of approximately 2.1×10^9 spores/ml, moisture content of 30 ml, mineral supplement of 6 g/l, pH of 6.82 and temperature of 27.5 ^oC were identified as the best conditions for maximum kojic acid yield. *A. niger* and *P. digitatum* were used for fermentation. Kojic acid concentration was similar by the two isolates (1.85±0.07 – 6.80) until day 7 when *Penicillium digitatum* demonstrated higher kojic acid concentrations (7.28 mg/ml). Alkyl, hydroxyl and carboxylic acid groups were found in the KA produced. The research suggests the suitability of GCS as substrate for KA production and the isolated moulds as potential organisms.

KEYWORDS: Kojic acid, Guinea corn sheaf, Solid-state fermentation, Moulds, Optimization

INTRODUCTION

During centuries ago, it was found that divergent types of natural sources like animals, microorganisms, insects and some plants were capable of producing numerous active metabolites. Out of these sources, the microorganisms producing metabolites, occupy promptly the utmost industrial applications because they obtain energy from organic compounds through chemical reactions (chemoorganotrophic), possess a maximum growth rate within a short period of life cycle and produces high amounts of biomass in short time. Hence, generation of fungal metabolites industrially claims minor complex operational control process [1].

Kojic acid, a significant secondary metabolite, can be derived from carbohydrates utilizing various carbon and nitrogen sources, as well as agriculture wastes through aerobic fermentation techniques. The initial isolation of Kojic acid was accomplished by Satio [2] from the mycelium of *Aspergillus oryzae* on steamed rice. These isolated natural products; one of such compound, kojic acid covers a wide range of applications in various fields like food, pharmaceutical, cosmetics, medical, chemical industries etc.

Glucose, sucrose, acetate, ethanol, arabinose, and xylose have served as carbon sources in the production of kojic acid. Among these, glucose stands out as the most effective carbon source for kojic acid synthesis, attributed to its structural resemblance to kojic acid. It has been proposed that, in the fermentation process, kojic acid is synthesized directly from glucose without any breakdown of the carbon chain into smaller components. Numerous studies have suggested the use of agricultural and industrial residues for fungal production of valuable substances. Examples include cheese whey, sugar cane molasses, fruits, vegetables, corn steep liquor, rice husk, and guinea corn sheaf (GCS), etc. [3].

Guinea corn (*Sorghum bicolor*) belongs to the genus *Sorghum*, tribe *Andropogoneae*, of the *Poaceae* family. The species *S. bicolor* includes all cultivated sorghums as well as a group of semi wild and wild plants regarded as weeds. *Sorghum bicolor* (sweet sorghum) is a high yielding species and it is considered as an energy crop that is able to survive in water limited environments.

The harvesting of guinea corn generates a considerable amount of waste, presenting a significant challenge in terms of effective waste management strategies. GCS is an agricultural waste from milling of Guinea corn (*Sorghum bicolor*). FAO [4] reported that about 3.0 million tons of GCS is generated annually which has potential to increase as the economy is diversified to agriculture. Conventionally, GCS has been disposed of through incineration, landfills, or opens burning. However, these methods have been associated with several environmental concerns. Incineration releases greenhouse gases and air pollutants, contributing to climate change and air pollution. Open burning not only produces harmful emissions but also leads to the loss of organic matter and valuable nutrients that could otherwise be utilized beneficially [5].

In recent years, there has been a growing recognition of the need to develop sustainable waste management practices within the agricultural industry. Researchers and industry stakeholders have explored alternative approaches to agricultural waste management, aiming to minimize environmental impacts and promote resource efficiency. One such approach is the utilization of GCS as a substrate for the production of value-added products through various biotechnological processes [6].

MATERIALS AND METHODS

Sample collection and preparation

GCS was collected from local farmers and it was transported to the laboratory in polythene bags. The GCS was pulverized to 0.4mm in sizes using blendtec blender to increase surface area for microorganisms.

Sterilization techniques

All glass wares (conical flask, beakers, etc.) used for the experiment were washed with detergent, rinsed and allowed to dry, they were then wrapped with aluminum foil and sterilized in the hot air oven at 160 ^{o}C for one hour in order to prevent contamination. The inoculating needle was sterilized by heating to redness before and after each round of experiment. Cotton wool soaked in 70 % alcohol was used to disinfect the laboratory bench and this was done before and after each round of experiment [7].

Culture media

The type of agar that was used for the microbiological analyses was Potato Dextrose Agar (PDA), a selective medium which is used for the isolation and maintenance of Fungi. The medium was prepared according to the manufacturer's instructions and was sterilized by autoclaving at 121 ^{o}C temperature at 15 lbs. pressure for 15 minutes [8].

Isolation of fungi through spontaneous fermentation of corn sheaf

Five grams of the GCS was weighed and mixed with 5 ml of distilled water into a flask. It was left for spontaneous fermentation at 27 ^{o}C for 72 hours. Visible mycelia growth from the fermented substrate were inoculated on PDA. The plates were incubated at 28 ± 2 ^{o}C for 48 hours until fungal growth was observed. The cultures were sub-cultured repeatedly on PDA to obtain pure colonies. The pure cultures were kept on a PDA slant for further analysis at 4 ^{o}C [9].

Characterization and identification of isolates

The fungal morphology was studied macroscopically by observing the colony features (color, shape, size, hyphae), and microscopically using a compound microscope with a digital camera using a lactophenol cotton blue stained slide mounted with a small portion of the mycelium [10].

Determination of proximate composition of GCS

Proximate analysis was conducted to ascertain the mineral composition present in the sample which include; lipids, protein, carbohydrates, ash and moisture content.

Screening of the isolates for kojic acid production

Screening of kojic acid producers were determined according to colorimetric method using 1 % Ferric chloride solution. KA forms a chelated compound with ferric ions and subsequently generates a red color: The fungal isolates were subcultured onto PDA supplemented with 1% Ferric chloride solution and incubated for 72 hours at 28 ^{o}C. The appearance of a deep red colour after incubation was the indication of the presence of kojic acid production by the strains [11].

Molecular identification of fungi

Two of the fungal strain showing promise for kojic acid production was subsequently identified using molecular biology methods following screening for kojic acid production.

Kojic acid production by solid-state fermentation

Medium preparation

A minimal salts medium broth containing the substrate as the carbon source was prepared and dispersed, into clean conical flask and sterilized by autoclaving at 121 ^{o}C temperature at 15 lbs. pressure for 15 minutes [12]. The ingredients of the salt medium includes: 5 g/L of Yeast Extract, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4.7H_2O$, 50 g of GCS as the only carbon source.

Preparation of the kojic acid standard calibration curve

The standard solution was prepared by mixing 0.01 g of commercial kojic acid in 100 ml of distilled water. Backward dilution was carried out with 10 test tubes each having varying concentration of the standard solution mixed with varying concentration of distilled water. Each tube was reduced to 2 ml and 2 ml of 1 % Ferric chloride solution was added. The absorbance was measured at 540 nm [13].

Solid-state fermentation for kojic acid production

The medium suggested by Shifali *et al.* [14] was employed for the production of kojic acid. The medium consist of 5 g/Lof Yeast Extract, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4.7H_2O$, 50 g of GCS as carbon source in a 250 ml Erlenmeyer flask. From all isolated fungal cultures, spore suspension was prepared and adjusted to 0.5 % Macfarland standard solution (One ml of inoculum contains approximately 1.5×10^8 spores/ml) [15], 20 ml of spore suspension was used as inoculum in all experiment. The mixture was incubated at static conditions for 9 days.

Assay for kojic acid

Every 24 hours, 1 g of the fermented substrate was weighed into sterile conical flask with the addition of 100 ml of distilled water. It was placed on orbital shaker at 180 rpm for 1 hour to extract the kojic acid into the water. After that, it was filtered using number 1 Whatman filter paper. The filterate was taken as the crude kojic acid. Then, to 2 ml of the crude kojic acid was added 2 ml of 1 % Ferric chloride solution, this was mixed thoroughly by shaking. kojic acid was quantified by measuring the absorbance at 540 nm and calculating the concentration using the standard curve [16].

Optimization of kojic acid production using the response surface methodology

The Response Surface Methodology (RSM) was used to establish the best parameters for both fungal growth and the production of kojic acid. This involved analyzing data (Table 1) from experiments that optimized factors such as substrate concentration, inoculum size, mineral supplements and moisture content, all of which were previously studied to pinpoint the conditions that would maximize fungal growth and improve kojic acid production. Using RSM, a statistical technique for modeling and optimization, the association between the chosen factors and the response variables (kojic acid production) was examined to pinpoint the best levels of each factor [17]. This enabled the identification of the advantageous mix of substrate concentration, inoculum size, mineral supplements and moisture content that would result in the greatest production of kojic acid by fungi. The best optimization parameters were Inoculum size of 2.1 x 10^9 spores/mL, Substrate concentration of 30 g/L, 30ml of Moisture content, 6 g/L of Mineral supplement, 27.5 ^oC of Temperature, and a pH of 6.82.

Table 1. Four Variables Screened for RSM Design

S/N	Factors	Range (minimum)	Range (Maximum)	Unit
1	Substrate Concentration	10	50	Gram per litre (g/L)
2	Inoculum size	1.5 x 10^8	2.1 x 10^9	Spores/mL
3	Mineral supplements	5	7	g/L
4	Moisture Content	20	40	ml

Extraction of kojic acid from fermentation medium

In order to determine the effect of different amounts of ethyl acetate on the yield of kojic acid extraction, 300 mL of fermentation broth (containing 12.45 g of kojic acid) was added to ethyl acetate for extraction according to the fermentation broth: ethyl acetate (volume ratio) = 1:0.8, 1:1.0, 1:1.2, 1:1.4. After extracting with ethyl acetate, the mixture was shaken for 1 minute every 2 minutes and repeated for 10 times, and then allowed to stand for 1 hour. The ethyl acetate layer was separated, and subjected to a rotary evaporator to recover ethyl acetate. The obtained product was vacuum dried, weighed, and analyzed to determine the purity of the product, and the extraction rate was calculated [18].

Characterization of the extracted kojic acid

Analysis was done to acquire the elemental composition of kojic acid. Purified kojic acid samples underwent Fourier Transmission Infra-red spectroscopy (FTIR) to identify the constituents and functional groups present in the samples [19].

RESULTS

Fungi isolation

A total of three unique fungal isolates were acquired during the isolation process. They were found as members of three genera, which were identified as *Aspergillus*, *Penicillium* and *Rhizopus*

Microscopic and macroscopic characterization and identification

The isolates exhibited diverse colonial morphological properties and microscopic characteristics. *Aspergillus niger* has dark brown spore colors, presence of conidia, spreading mycelia, the stipe of its conidiophore was double walled while *Penicillium digitatum* has green color of spore, finger-like conidia head, septate hyphae, spreading mycelium, while *Rhizopus stolonifer* has grayish white spore color, roughened sporangiophore, rising mycelia, etc. as shown in Table 2.

Molecular identification of fungi

The molecular identification process, confirms the isolates to be *Aspergillus niger* (PP330720) and *Penicillium digitatum* (PP4892864). The names with the accession numbers have been deposited at the Genbank as shown in Figure 2.

GCS proximate composition

The proximate results of the GCS indicated that Crude Protein (%) was the lowest (1.660227 ± 0), while CHO (%) had the highest value (45.42475 ± 2.141756). The Caloric Value was found to be 929.66205 ± 40.72079 kcal/100 g as shown in Table 3.

Plate screening of fungal isolates for kojic acid production

Two of the fungal isolates which are: *Aspergillus niger* and *Penicillium digitatum* were positive exhibiting a deep red zone of clearance while *Rhizopus stolonifer* was negative. The growth of *A. niger* and *P. digitatum* was numerically represented in relation to the zone of clearance as displayed in Table 4.

Kojic acid production by fungal isolates

The concentration of kojic acid obtained for each isolate. The results, presented in Table 5, show the mean kojic acid concentration (mg/ml) for both *A. niger* and *P. digitatum* at each time point. Over the nine-day period, both organism exhibited similar concentrations on the first 7 days while *P. digitatum* exhibited higher kojic acid concentrations compared to *A. niger* on the last two days of the fermentation period. The highest concentration for both *A. niger* and *P. digitatum* were 6.81 (day 8) and 7.28 (day 8) respectively and the lowest concentration for both *A. niger* and *P. digitatum* were 1.93±0.11 and 1.85±0.07 respectively on day 1.

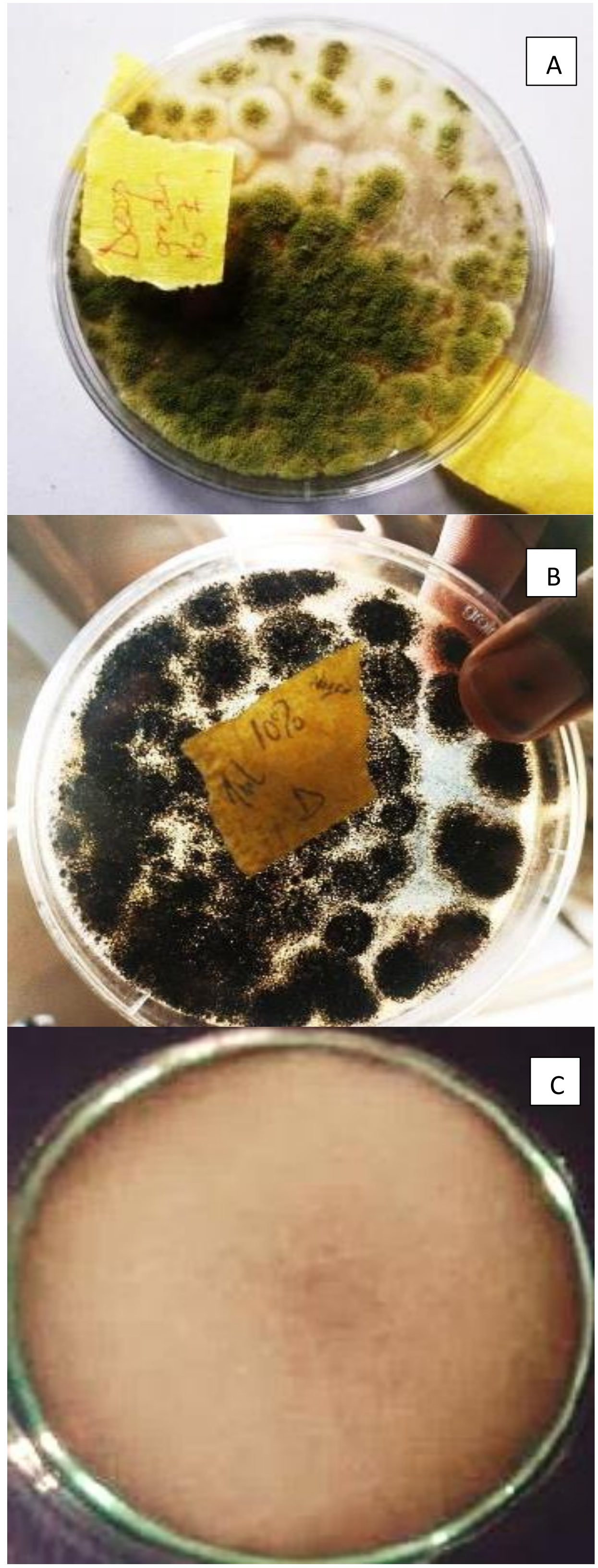

Figure 1. Fungi Isolates Acquired during Isolation Process. **A)** *Penicillium digitatum*; **B)** *Aspergillus niger*; **C)** *Rhizopus stolonifer*

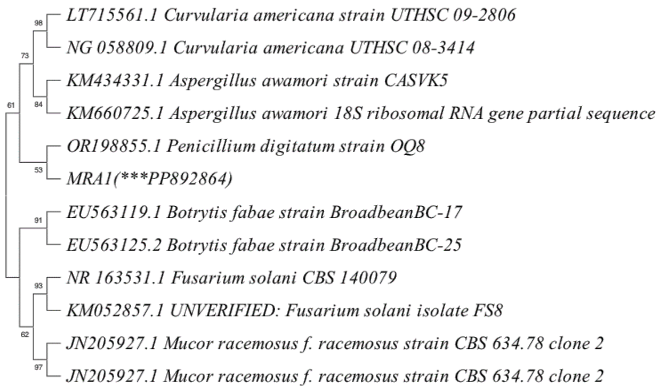

Figure 2. Phylogenetic tree of kojic-acid producing fungal isolate and related sequences obtained from NCBI Database

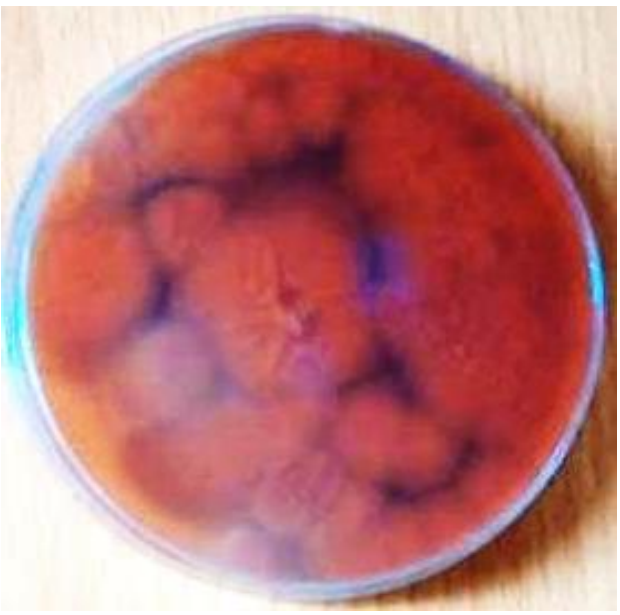

Figure 3. Positive for Kojic acid production

Figure 4. Fermented Medium

Optimization of kojic acid production using response surface methodology (RSM)

Results in Tables 6 and 7 reveal that kojic acid yield ranges from 6.20 – 8.74 mg/mL. The highest yield (8.74 mg/mL) was obtained at 9 days fermentation time, using 30.0 g/L of substrate concentration, 2.1 × 10^9 spores/mL of Inoculum size at a pH of 6.82, moisture content of 30 mL, mineral supplement of 6.0 g/L and temperature of 27.5 ^{o}C (Run 7 using *P. digitatum*), while the least value (6.23 mg/mL) was observed in experimental run 15 using *A. niger*: 9 days of fermentation period, 50.0 g/L of substrate concentration, 3.0 x 10^8 spores/mL of Inoculum size at pH of 5.77, moisture content of 40 mL, mineral supplement of 5.0 g/L and temperature of 30.0 ^{o}C.

Table 2. Microscopic and macroscopic characteristics of the isolates

Characteristics	**Isolate A**	**Isolate B**	**Isolate C**
Colony color before Sporulation	White	Olive-Green	White
Color of Spore	Dark Brown	Green	Grayish White
Reverse View	Black	Creamy	Dark Gray
Days before Sporulation	2 Days	3 Days	2 Days
Type of Spore	Conidia	Conidia	Sporangiospores
Conidia Head/ Sporangium	Radiate	Finger-like	Rounded
Conidiophore/ Sporangiophore	Roughened	Hyaline and Smooth	Roughened
Stipe	Double Walled	Single Walled	Single Walled
Conidia / Sporangiospores	Double Walled	Double Walled	Single Walled
Hyphae	Aseptate	Septate	Aseptate
Mycelia	Spreading	Spreading	Rising
Probable Organism	*Aspergillus niger*	*Penicillium digitatum*	*Rhizopus stolonifer*

Table 3. Proximate content of GCS samples

Proximate composition	GCS samples (Mean ± SD)
Moisture (%)	8.936339 ± 0.05
Ash (%)	5.12195± 0.05
CHO (%)	45.42475 ± 2.14
Caloric Value (Kj/100 g)	929.66205 ± 40.72
Crude Lipid (%)	3.8020985 ± 0.13
Crude Fibre (%)	35.054405 ± 2.27
Crude Protein (%)	1.660227 ± 0.00

CHO: Carbohydrate; Data are means of two replicates±SEM

Table 4. Kojic Acid Production Ability of the Fungal Isolates as Depicted by Zones of Red Clearance after Screening

Fungal isolates	Zones of red clearance (mm)
A. niger	8.00
P. digitatum	8.50

Table 5. Kojic acid production by *A. niger* and *P. digitatum*

Incubation period (Days)	Kojic acid concentration (mg/ml) Mean±SD	
	A. niger	*P. digitatum*
0	0	0
1	1.93±0.11[a]	1.85±0.07[a]
2	1.85±0.00[a]	2.15±0.35[b]
3	5.10±0.14[b]	5.6±0.28[b]
4	6.44±0.05[c]	6.44±0.06[c]
5	6.48±0.00[c]	6.45±0.07[c]
6	6.75±0.07[c]	6.58±0.04[c]
7	6.80±0.00[c]	6.80±0.00[c]
8	6.81± 0.00[c]	7.28±0.00[c]
9	6.44±0.06[c]	7.04±0.34[c]

Data are means of two replicates ±SEM. Groups with the same letter (a, b, c) within each column are not significantly different from each other.

Table 6. The yield of kojic acid as affected by process variables with *A. niger*

Run	Inoculum size (spores/mL)	Substrate concentration (g/L)	Moisture content (ml)	Mineral supplement (g/L)	Temperature (^oC)	pH	Yield (mg/mL)
1	2.1×10^9	30.00	50.00	6.00	25.00	6.14	6.73
2	2.1×10^9	00.00	30.00	6.00	27.50	7.23	0.00
3	1.5×10^8	10.00	40.00	5.00	30.00	4.34	6.83
4	2.1×10^9	30.00	30.00	6.00	30.00	6.10	7.08
5	1.5×10^8	10.00	20.00	5.00	25.00	6.36	6.80
6	2.1×10^9	30.00	30.00	6.00	27.50	6.05	6.83
7	1.5×10^8	10.00	20.00	7.00	23.60	6.27	6.80
8	1.5×10^8	10.00	40.00	7.00	25.00	5.94	6.80
9	3.0×10^8	50.00	20.00	5.00	30.00	6.18	6.80
10	2.1×10^9	30.00	30.00	4.00	31.40	6.92	6.80
11	0	30.00	40.00	6.00	27.50	6.58	0.00
12	1.5×10^8	50.00	40.00	5.00	25.00	5.68	6.38
13	3.0×10^8	10.00	20.00	5.00	30.00	5.83	6.79
14	1.5×10^8	50.00	20.00	5.00	30.00	6.01	6.58
15	3.0×10^8	50.00	40.00	5.00	30.00	5.77	6.23
16	2.1×10^9	30.00	30.00	8.00	27.50	5.76	6.45

Table 7. The yield of Kojic acid as affected by process variables with *P. digitatum*

Run	Inoculum size (spores/mL)	Substrate concentration (g/L)	Moisture content (ml)	Mineral supplement (g/L)	Temperature (^oC)	pH	Yield (mg/mL)
1	2.1×10^9	30.00	30.00	6.00	25.00	5.70	6.41
2	2.1×10^9	30.00	30.00	6.00	25.00	6.27	6.73
3	2.1×10^9	70.00	30.00	6.00	25.00	6.64	6.44
4	3.0×10^8	50.00	40.00	7.00	27.50	6.10	6.78
5	3.0×10^8	50.00	20.00	7.00	27.50	6.62	6.77
6	3.0×10^8	10.00	20.00	7.00	30.00	6.21	6.44
7	2.1×10^9	30.00	30.00	6.00	27.50	6.82	8.74
8	2.1×10^9	30.00	30.00	8.00	27.50	6.71	6.32
9	3.0×10^8	10.00	40.00	5.00	27.50	4.27	7.80
10	1.5×10^8	50.00	20.00	7.00	25.00	6.53	6.70
11	2.25×10^8	30.00	30.00	6.00	30.00	6.34	8.04
12	2.1×10^9	30.00	10.00	6.00	27.50	6.99	6.50
13	2.1×10^9	30.00	30.00	6.00	27.50	6.45	6.63
14	3.0×10^8	10.00	40.00	7.00	27.50	5.17	6.75
15	1.5×10^8	50.00	40.00	7.00	27.50	6.25	7.28
16	2.1×10^9	00.00	30.00	6.00	27.50	6.25	0.00
17	0.0×10^9	30.00	30.00	6.00	27.50	6.07	0.00

Fourier transform infrared spectroscopy characterization

Graphs were used to represent the results of the FTIR characterization, displaying the infrared spectra of kojic acid produced by fungi organisms alongside the infrared spectrum of commercially obtained kojic acid, which has a complex chemical composition. The two samples share common functional groups, such as hydroxyl, alkane groups, alkene groups, ethers, etc. indicating their similarity (Table 9).

Table 8. Ethyl acetate for Koji acid extraction

Fermented broth: ethyl acetate (v/v)	**Ratio of fermented broth to ethyl acetate**		
	1:1.0	**1:1.2**	**1:1.4**
Final product mass (g)	2.63	3.25	3.72
Purity (%)	94.72	97.31	98.20
Extraction rate (%)	15.93	19.55	23.04

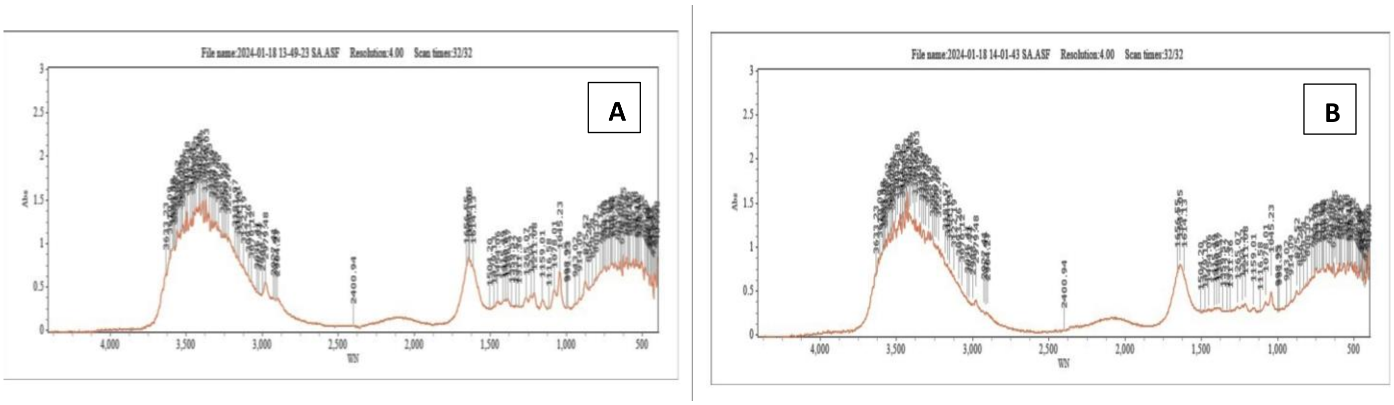

Figure 5. FTIR Characterization of different sources of Kojic acid**. A)** FTIR characterization of commercial Kojic acid; **B)** FTIR Characterization of extracted Kojic acid with ethyl acetate

Table 9. Functional groups in kojic acid as revealed by FTIR using penicilium digitatum

Wavelength	**Functional groups**	**Names**	**Commercial Kojic Acid**	**Extracted Kojic Acid**
3500-3400cm^-1	O-H	Hydroxyl Groups	Present	Present
2979.48 cm^-1	C-H	Alkane and Alkyl Groups	Present	Present
1643.05 cm^-1	C=C	Alkene and Aromatic Compounds	Present	Present
1045.23 cm^-1	C-O	Ethers, Esters, and Carboxylic Acids	Present	Present

DISCUSSION

This research sought to investigate the possibility of generating kojic acid by employing indigenous fungal strains in the solid-state fermentation of GCS. The emphasis was placed on assessing the effectiveness of production and the suitability of indigenous fungi for synthesizing kojic acid in a regulated environment.

The outcomes of the isolation, characterization and successful identification of fungi from the GCS sample indicate the three genera. The isolates which were *Aspergillus niger*, *Rhizopus stolonifer*, and *Penicillium* sp. The successful isolation of *Aspergillus niger*, *Rhizopus stolonifer*, and *Penicillium digitatum* from GCS can be attributed to the natural presence and growth of these fungi in the environment, particularly in materials such as plant matter and grains [20]. These fungi are commonly found in the soil, air, and decaying organic matter [21]. GCS provides a suitable environment for the growth of these fungi due to its composition, which includes carbohydrates, proteins, and other nutrients that serve as a food source for these organisms. Additionally, the physical structure of the GCS may provide a condusive habitat for the colonization and growth of these fungi. Fungi like *Aspergillus niger*, *Rhizopus stolonifer*, and *Penicillium digitatum* are known for their ability to break down complex organic compounds and utilize them for growth and reproduction [22]. Therefore, it is not uncommon to find these fungi present in agricultural residues such as GCS where they play a role in the natural decomposition process. These fungi genera isolated were similar to the report of Ajiboye and Said [23]; Kawata *et al*. [24] who reported the presence of similar fungi in fermented agricultural wastes.

The proximate composition of the GCS samples as shown in Table 2 indicate that GCS is predominantly rich in carbohydrate and has relatively low protein content. The high carbohydrate content and relatively low protein makes it suitable for the production of kojic acid using fungi because high amount of sugar is needed to synthesize kojic acid [14]. Carbohydrates serve as the primary energy source for fungi during fermentation processes, and they can be metabolized by fungi to produce various metabolites, including kojic acid [25]. Although proteins are important for the growth and metabolism of fungi, the low protein content in GCS may not be a limiting factor for kojic acid production. Fungi are versatile microorganisms that can adapt to different nutrient sources and can efficiently utilize carbohydrates present in substrates like GCS for their metabolic processes. This report corroborates those of Gbadebo *et al*. [26].

The ability of two of the isolates to produce kojic acid as presented in Table 3 is attributable to the fact that filamentous fungi have been incriminated to carry out enzymatic degradations resulting in the release of different types of organic acid [27]. *A. niger* and *P. digitatum* produced kojic acid reacts with ferric ions to create a chelated complex, resulting in the production of a red hue [28, 29]. Conversely, *R. stolonifer* was negative in this study, indicating a lack of suitability to use this substrate in terms of kojic acid production. *A. niger* and *P. digitatum* were reported to have the genetic capability to produce kojic acid due to the presence of specific enzymes and metabolic pathways that enable them to synthesize this compound [30]. On the other hand, *Rhizopus stolonifer* might not naturally produce kojic acid because it lacks the necessary enzymes and metabolic pathways for kojic acid biosynthesis [31]. The ability of a fungus to synthesize a specific compound like kojic acid is determined by its genetic makeup and metabolic capabilities. This was similar to report of Xiao *et al*. [32].

The results, presented in Table 4, show the mean kojic acid concentration (mg/mL) for both *A. niger* and *P. digitatum* at each time point. The two isolates were able to synthesize kojic acid at a similar rate until the he last two days of the fermentation period. This observation was similar to the report of previous authors on kojic acid production [33]. The steady increase in kojic acid concentration over the nine-day period for both isolates can be attributed to several factors such as enzyme production needed that help catalyze the conversion of precursor molecules in the GCS into kojic acid [30], metabolic activity of the fungi whereby they utilize nutrients present in the GCS to fuel their growth and biosynthetic pathways leading to the production of kojic acid as a byproduct. This was also reported by Ibrahim [34].

Table 5 and 6 shows the yield of the kojic acid generated in a detailed manner as affected by process variables of substrate concentration, Inoculum size, pH, temperature, mineral supplements and moisture content. The variations in kojic acid yield may be attributed to several factors such as variations in substrate concentration which could affect the availability of essential nutrients for the fungi, impacting their ability to produce kojic acid efficiently, changes in parameters like moisture content, pH, temperature, and aeration can influence the growth and metabolic activity of the fungi, the metabolic pathways responsible for kojic acid biosynthesis in *A. niger* and *P. digitatum* may be influenced by the fermentation medium composition [35]. Altering parameters like substrate concentration can affect the flux through these pathways, potentially impacting the yield of kojic acid. This was similar to report of Abdel-Hamied *et al.* [17] who reported the optimum temperature for formation of kojic acid by different fungal species to be in the range 25-30 °C. Zohri et al. [36] reported the best incubation days to be 9 days as earlier reported in this study.

The results of Fourier Transmission Infra-Red spectroscopy (FTIR) study for both the commercial and produced kojic acid study (Table 8) revealed O-H (hydroxyl) functional group, which are commonly found in alcohols and carboxylic acids. The two samples also contain a C-H (alkyl group) stretching vibration which indicates the presence of hydrocarbons in both samples. Presence of C=C (alkene) stretching vibrations was also detected in both samples. Previous authors have documented the presence of these functional groups and others (C-O, ethers, esters, hydroxyl and carboxylic acids) stretching vibrations in KA [10]. Functional groups in molecules are responsible for the chemical properties of compounds. Therefore, the presence of similar functional groups in the FTIR spectra of extracted kojic acid in this study and commercially available kojic acid confirms that both samples are likely composed of the same compound. This similarity was also reported by Kayitha *et al*. [37] and Prantika *et al*. [19].

CONCLUSION

Based on the findings of this study, the following recommendations are suggested:

The adoption of carbohydrate rich agricultural waste in the production of KA and other important industrial raw materials to reduce production and importation cost. This will enhance effective agricultural waste management in no small measure and mitigate waste burning which could lead to generation of greenhouse gas. It is also a way of value addition with strings of benefits in value addition to waste. There is an urgent need to continuously optimize fermentation process to maximize kojic acid production. This may involve monitoring and adjusting parameters such as aeration, pH etc. Locally developed methods for recovery and purification of the produced kojic acids are very essential and must be prioritized. Finally, there should be consideration on the utilization of the fungal biomass and fermentation residues as biofertilizers, animal feed additives, or for biogas production to ensure minimal waste generation.

This study highlights the potential of using indigenous fungi and GCS as a substrate for kojic acid production, contributing to the interest in converting waste materials into valuable products for various applications. The findings indicated that kojic acid biosynthesis is highly affected by substrate concentration, inoculum size, mineral supplement and moisture content in the fermentation medium. The optimization process in this study identified the best conditions for maximum kojic acid yield, including a fermentation time of 9 days, substrate concentration of 30 g/L, inoculum size of approximately 2.1 x 10^9spores/mL, moisture content of 30 ml, mineral supplement of 6 g/L, pH of 6.82 and Temperature of 27.5 ^oC. The FTIR analysis revealed the presence of similar functional groups in the produced kojic acid and the commercial kojic acid.

DECLARATIONS

Acknowledgement

The authors acknowledge laboratory of Microbiology of the Faculty of Pure an Applied Sciences, Kwara State University, Nigeria for research facilities.

Authorship Contribution

Concept: M.A., Work Supervision: M.A., Manuscript Editing: M.A., Research: T.A., Data Collection and Analysis: T.A., Manuscript Writing: T.A.

Funding

This research received no grant from any funding agency/sector.

Competing interests

The authors declared that there is no conflict of interest.

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