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Research Article

Gibberellic Acid Production from Corn Cob Residues via Fermentation with *Aspergillus niger*

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Following numerous biotechnological innovations, a variety of agricultural by-products can now be employed as low-cost substrates for the production of secondary metabolites, such as antibiotics, phytohormones, biofuels, pesticides, and organic acids. As an example, gibberellin (GA) growth phytohormones can be obtained by such means, wherein gibberellic acid (GA_3) is of great interest worldwide in the agricultural sector. The central aspect of this research therefore focused on the bioconversion of agricultural by-products, such as corn cob, to obtain GA_3 phytohormone via solid-state fermentation (SSF) with *Aspergillus niger*. The chemical characterization of the obtained material showed that the corn cob possessed glucose, mannose, arabinose, and lignin contents of 34, 26, 8, and 16%, respectively. Our results also indicated an appreciable carbon content (47%), in addition to the mineral elements of nitrogen (4%), potassium (1.2%), iron (0.03%), sodium (0.01%), calcium (0.06%), and Al (0.02%). Following SSF for 11 d in the presence of *A. niger* at pH 5, 30°C, and 24% sample consistency, a GA_3 production of >6.1 $g{\cdot}kg^{-1}$ was obtained. This value is higher than those previously reported for different by-products of the food industry, such as coffee husk, wheat bran, cassava, pea pods, and sorghum straw (i.e., 0.25–5.5 $g{\cdot}kg^{-1}$) following SSF. The production of GA_3 from corn cob residues not only contributes to reducing the negative impact of agricultural by-products but also represents a new source of a key raw material for phytohormone production, thereby contributing to the development of processes to convert agricultural residues into biologically active compounds of commercial interest.

1. Introduction

The gibberellin (GA) growth phytohormones are a large family of isoprenoid phytohormones that are of great interest in the agricultural sector [1, 2]. Among the gibberellins of greater industrial and economic importance, gibberellic acid (GA_3), a tetracyclic dihydroxy lactonic acid, is widely employed in worldwide agriculture due to its ability to increase the germination rate of seeds, increase the size of seedless plants, increase vegetative growth, stimulate cell division, and promote flowering, sex expression, enzyme induction, and senescence of fruits [3–8].

Industrially, GA_3 is produced via fermentation with the fungus *Gibberella fujikuroi*, which is also known as *Fusarium fujikuroi* [4], although other microorganisms such as *Fusarium moniliforme*, *Bacillus siamensis*, and *Aspergillus niger* are also capable of producing this desired product [9–11]. Due to its widespread usage, the annual production of GA_3 is 100 tons, with prices ranging from 150 to 500 U.S. dollars per kilogram [12]. The main fermentation process used to obtain GA_3 is submerged fermentation (SmF); however, the production costs of this process are high owing to low yields and extensive separation steps. In addition, although GA_3 can be isolated from plant tissues and by chemical synthesis, these processes are complicated, give low yields, and are not profitable in an industrial context [13]. Thus, in recent years, the possibility of using solid-state fermentation (SSF) has attracted significant attention,

particularly due to the series of economic advantages boasted by this in terms of the production of microbial biomass and metabolites and the valorization of agro-industrial by-products [1, 9]. Moreover, the SSF technology can produce a high concentration of easily recovered products with low bacterial contamination and with reduced wastewater generation. The low energy requirement of this process is also important in the current climate, as is the applicability of a variety of low-cost agroindustrial sub-products to this transformation [8, 14, 15].

Agricultural by-products constitute some of the most abundant and potentially valuable materials on the planet due to their renewable nature and the fact that they can be used to obtain numerous useful biological and chemical products. Currently, much of the agroindustrial waste produced globally remains unused and is discarded as waste, for example, 144 million tons of corn cob is generated annually as waste during corn processing and is either discarded and/or burned without any benefit [16]. To indicate the potential of such by-products, wheat straw, rice straw, coconut waste, corn cob, bagasse, soybean waste, and rice husk/bran waste can be used as raw materials for the production of products with high-added values, including antibiotics, steroids, hormones for plant growth, biofuels, herbicides, organic acids, mycotoxins, enzymes, biosorbents, immunosuppressants, pesticides, and alkaloids [1, 17–20].

This study therefore aims to evaluate the growth of GA_3 phytohormones from corn cobs by means of SSF, taking into account diverse operational parameters and using the fungus *A. niger*.

2. Materials and Methods

2.1. The Raw Material. The samples of corn cob provided by a local producer were air-dried until reaching 10% (w/w) moisture, after which they were milled and stored in plastic bags under dry conditions until required for use. All chemicals were of analytical grade.

2.2. Chemical Characterization. The milled samples (40/60 mesh) were extracted with acetone (90% v/v) according to the TAPPI method T280 pm-99. The extracted samples were analyzed for their carbohydrate, nitrogen, and mineral contents. All analyses were performed in triplicate. All samples were also analyzed by Fourier transform infrared (FTIR) spectroscopy. Further details regarding these characterization techniques can be found as follows.

The carbohydrate contents of the samples were determined using the methodology described by Ferraz et al. [21]. More specifically, in a test tube, the extractive-free milled (300 mg) was weighed and 72% (w/w) H_2SO_4 was added (3 mL). Hydrolysis was performed in a waterbath at 30°C for 1 h with stirring every 10 min. Subsequently, the hydrolyzed was diluted to 4% (w/w) with distilled water (79 mL), and the mixture was transferred to a 250 mL Erlenmeyer flask and autoclaved for 121°C for 1 h. After this time, the residual material was cooled at 25°C and filtered through a sintered glass filter (no. 4). The solid fraction, i.e., the insoluble lignin, was then dried at 105°C and weighed. The concentration of monomeric sugars in the soluble fraction was determined using high-performance liquid chromatography (HPLC) with a refractive index detector (Hitachi High-Tech D-7000-L-7490, Tokyo, Japan) and an Aminex HTX-87H column (Bio-Rad, Hercules, CA, USA) at 45°C and with elution at 0.6 mL/min using a 5 mM aqueous H_2SO_4 solution. Glucose and xylose + mannose were used as external calibration standards.

The carbon and nitrogen contents were measured using an elemental analyzer (Fisons Instrument EA 1108, Milano, Italy), wherein sulfanilamide was used as the standard. To determine the mineral content, the sample was digested in an acid solution and analyzed using inductively coupled plasma optical emission spectroscopy (Perkin Elmer Optima 5300 DV, USA).

The FTIR spectra of the samples incorporated into KBr pellets were measured using the direct transmittance mode. The spectra were recorded between 4000 and 500 cm^{-1} using a Shimadzu IRAffinity-1 FTIR spectrometer equipped with a deuterated L-alanine-doped triglycine sulfate detector. The background was recorded using a freshly prepared KBr pellet. All spectra were measured at a resolution of 2 nm, and 32 scans were taken per sample.

2.3. Growth and Inoculum Preparation of the A. niger Strain. The employed *A. niger* ATCC 6275 strain was conserved in Petri dishes containing 4 wt/vol% potato dextrose agar incubated at 30°C. To obtain the inoculum, spores were harvested from PDA cultures after 7 d of growth using a 0.1% Tween 80 solution. The resulting suspensions were used as the inocula. Spore counting was carried out in a Neubauer chamber until the required final concentration of 10^6 mL^{-1} was obtained. The inoculum mass was determined by gravimetry, and a determined volume of the spore suspension was placed on a filter paper. The remaining residue was dried at 105°C for 1 h, and the dry weight was calculated.

2.4. Solid-State Fermentation (SSF). The SSF process was carried out at a range of sample consistencies (10–30%) and pH values (4–6) in an Erlenmeyer flask (25 mL, the fermenter). For this purpose, the fermenter was charged with the sample (0.75 g, dry base), a nutrient solution previously autoclaved at 121°C for 15 min (50 mL; NH_4Cl, (1 g·L^{-1}), KH_2PO_4 (3 g·L^{-1}), $MgSO_4{\cdot}7H_2O$ (2.2 g·L^{-1})), and the inoculum solution (1 mL, containing 15 mg of the inoculum). The effects of the pH and sample consistency were evaluated using a central composite design to optimize the GA_3 yield. The reaction mixture was incubated at 30°C and 150 rpm for 11 d.

2.5. GA_3 Extraction and Determination. Following completion of the 11-day fermentation process, a small volume of water (5 mL) was added to the fermentation culture, stirred at 150 rpm for 30 min, and filtered through Whatman No. 41 filter paper. The supernatant volume was then adjusted to 25 mL using water and subjected to centrifugation

at 3500 rpm for 10 min. Subsequently, the samples were acidified to pH 2 using HCl and extracted with ethyl acetate [22]. The organic (ethyl acetate) extract was then evaporated, and the residue was resuspended in ethanol. Finally, the GA_3 content was determined by ultraviolet (UV) spectrophotometry at a wavelength of 254 nm and using a calibration curve prepared from the GA_3 standard.

2.6. Statistical Analysis. The influences of the various experimental design variables were determined using the response surface methodology approach [23, 24]. The models were based on a circumscribed composite central design consisting of factorial design and star points. The second-order function that best described the system behavior was determined using the multiple linear regression method. Statistical validation was carried out using an ANOVA test at a 95% confidence level. The generation and evaluation of the experimental design were performed using the MODDE® program, version 12 (Umetrics, USA).

3. Results and Discussion

3.1. Chemical Composition of the Corn Cob. The chemical composition of the corn cob raw material employed in this study is given in Table 1. As indicated, corn cob is a lignocellulosic material composed mainly of glucose (from cellulose), mannose, arabinose (from hemicellulose), and lignin. However, we note that the obtained glucose and xylose contents were slightly lower than those reported by other authors. More specifically, Van Dongen et al. [25] and Lili et al. [26] reported values of 34–34.6 and 27–31.1 for glucose and xylose, respectively, in corn cobs. However, the arabinose contents (2.4–3.6) reported by these authors were lower than the value determined in our study. In addition, the determined lignin content given in Table 1 is higher than that reported by Lili et al. (9.4) but lower than that reported by Van Dongen et al. (18.3).

In addition, elemental analysis indicated that corn cobs have an appreciable carbon content but a low nitrogen content, thereby giving a high C/N ratio, which is favorable for the production of GA_3. In addition, the mineral content of the corn cob was low, as previously reported by Berber-Villamar et al. [16]. The above results are also consistent with those of other studies, which indicate that the main components of corn cob are cellulose, hemicellulose, and lignin.

The FTIR spectra of the corn cob are shown in Figure 1, wherein the characteristic bands of lignocellulosic materials can be seen. More specifically, the peak at 3424 cm^{-1} was attributed to the O–H stretching vibrations of the phenol, alcohol, and carboxylic acid functional groups [27], while those at 2927 and 1645 cm^{-1} represent the C–H and OH^- stretching vibrations, respectively [28]. In addition, the peak at 1549 cm^{-1} was assigned to N–H bending mixed with C–N stretching, which is characteristic of the protein amide I band [28]. Furthermore, the peaks observed at 1456 and 1397 cm^{-1} corresponded to C–C stretching in the lignin aromatic ring and C–H asymmetric deformation, respectively, while those at 1456–1251 cm^{-1} were assigned to C–O stretching and O–H deformation vibrations; according to Lu et al. [29], the peak at 1251 cm^{-1} represents the C–O stretching band of the hemicellulose structure. Moreover, the band at 1038 cm^{-1} corresponds to the cellulose (polysaccharide) C–O stretching [27], while that at 610 cm^{-1} was attributed to the O–H out-of-plane vibrations.

3.2. GA_3 Production via SSF with A. niger. As given in Table 2, the production of GA_3 in the presence of *A. niger* varied from 2.1 to 6.2 $g{\cdot}kg^{-1}$ depending on the fermentation conditions employed during the SSF process (Table 2). As mentioned above, the effects of the pH and sample consistency on GA_3 production were evaluated because pH is one of the most important factors affecting the biomass and yield obtained using *A. niger*, and the sample consistency is related to the amount of substrate available for GA_3 production [13]. According to Omajasola and Adejoro [13], a balanced amount of substrate is necessary for good GA_3 production. These authors indicate that GA_3 production and maintenance of biomass require low glucose concentration (<4%) in the culture medium.

Based on the experimental design data and GA_3 content obtained under each condition, a quadratic polynomial was determined (equation (1)), which was subsequently validated using ANOVA.

$$GA_3\left(gkg^{-1}\right) = 6.1 \pm 0.2 + 0.3 \pm 0.1X_1 + 0.44 \pm 0.1X_2 - 1.4 \pm 0.1X_1^2 - 0.5 \pm 0.1X_2^2, \quad (1)$$

where X_1 is the pH and X_2 is the consistency (%). The error values correspond to a 95% confidence level.

The linear terms showed a positive coefficient for pH and consistency, indicating that GA_3 production increased with an increase in these variables until a maximum value was reached, as shown by the negative quadratic terms for the two variables. According to the polynomial, pH was the main determining factor for maximizing GA_3 production. In addition, considering the confidence intervals, the interaction between the variables did not have a significant effect on GA_3 production. Thus, the effects of these fermentation conditions on GA_3 production are shown in the response surfaces for the polynomials shown in Figure 2.

Based on the experimental conditions employed during experimental design, the polynomial was used to predict GA_3 production, and the responses were close to the experimental values given in Table 2, with a correlation coefficient (r^2) of 0.99 being obtained. These values, together with the ANOVA test (Table 3), statistically validated this model. The response surface methodology (RSM) model' p value for the production of GA_3 was 0.000, which demonstrated the high significance of the model. In addition, the p value for the lack-of-fit was 0.141, which indicates no significant difference compared to the pure error.

Subsequently, the optimum values of the various experimental variables to maximize the amount of GA_3 production were determined by the Simplex method using the maximum values of the response surface, wherein the

Table 1: Chemical composition of the corn cob employed.

Lignocellulose content (%) wt						
Glucose	Xylose	Arabinose	Lignin	C	N	C/N
31 ± 1	22.5 ± 0.4	7.8 ± 0.2	16.5 ± 0.2	47 ± 0.2	3.9 ± 0.3	12
Mineral content (%) wt						
K	Mg	Ca	Fe	Al	Na	Mn
1.19 ± 0.03	0.28 ± 0.01	0.062 ± 0.004	0.028 ± 0.001	0.017 ± 0.001	0.0149 ± 0.0002	0.0031 ± 0.0001

Note: the values provided for all sugars are expressed in terms of their anhydrous contents within the polymer.

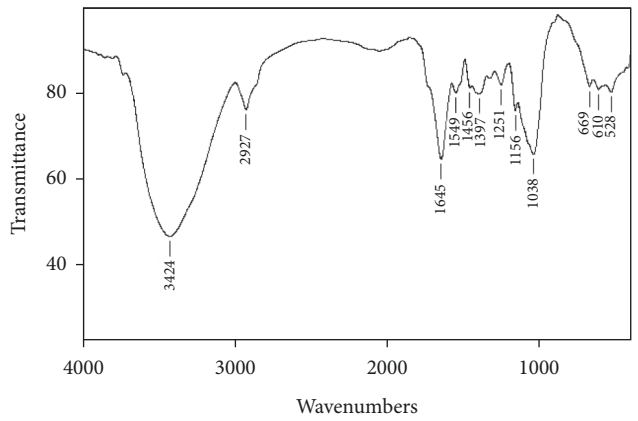

Figure 1: FTIR spectrum of the corn cob sample.

Table 2: GA_3 yields obtained from corn cob via SSF in the presence of *A. niger*.

Exp. no.	pH	Consistency (%)	GA_3 (g kg^{-1})
1	4	10	3.16
2	6	10	3.4
3	4	30	4.06
4	6	30	4.56
5	3.6	20	2.1
6	6.4	20	3.22
7	5	5.9	4.31
8	5	34.1	5.46
9	5	20	6.13
10	5	20	6.2
11	5	20	6.03

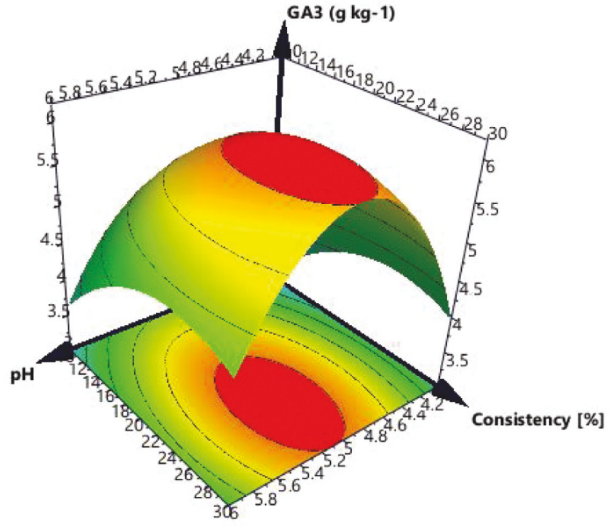

Figure 2: Response surfaces for the amount of GA_3 produced from corn cob upon variation in the pH and sample consistency (%).

Table 3: Analysis of variance (ANOVA) for the response surface quadratic model.

Source	DF	SS	MS (variance)	F	*P*
Total	11	234.37	21.31		
Constant	1	214.99	214.99		
Total corrected	10	19.38	1.94		
Regression	4	19.21	4.80	176.55	0.000, significant
Residual	6	0.16	0.03		
Lack of fit (model error)	4	0.15	0.04	5.09	0.171, not significant
Pure error (replicate error)	2	0.01	0.01		

Table 4: GA3 production via SSF using different by-products and microorganisms.

Substrate	GA_3 production (g kg^{-1})	Microorganism	Fermentation conditions	References
Corn cobs	6.1	*A. niger*	pH 5.1, 24% consistency, 30°C and 11 days	Current study
Citric pulp	7.6	*F. fujikuroi*	pH 5.5, 75% moisture, 29°C and 6 days	de Oliveira et al. [30]
Cajanus cajan pods	6.4–7.8	*F. proliferatum*	pH 4.8, 75–80% moisture, 29°C and 8–10 days	Satpute et al. [31]
Pea pods	5.7–6.4			
Corncobs	5.2–6.1			
Sorghum straw	4.1–5.5			
Citric pulp	5.9	*F. moniliforme*	pH 5–5.5, 75–80% moisture, 29°C and 3 days	Rodrigues et al. [9]
Coffee husk	0.493	*F. fujikuroi*	pH 5.3, 75% moisture, 29°C and 7 days	Machado et al. [32]
Wheat bran	3	*F. fujikuroi*	50% moisture, 28°C, and 10 days	Bandelier et al. [3]
Cassava	0.25	*F. fujikuroi*	pH 4.5, 70% moisture, 29°C and 35 h	Tomasini et al. [33]

maximum predicted GA_3 content was 6.2 ± 0.2 g·kg^{-1}. More specifically, the predicted values for the optimal extraction variables were a pH of 5.1 and a consistency of 24%. Importantly, the experimental GA_3 content obtained under these conditions was 6.1 ± 0.3 g·kg^{-1}, which agrees well with the predicted value.

It should also be noted that the level of GA_3 production obtained in this study using *A. niger* was similar to and in some cases higher, with previous reports describing the production of GA_3 from different substrates using *Fusarium* species under similar conditions (Table 4). For example, Machado et al. [32], Bandelier et al. [3], and Tomasini et al. [33] reported values of 0.49, 3, and 0.25 g·kg^{-1} of GA_3 production from coffee husk, wheat bran, and cassava after 6, 3, and 1.4 d, respectively, using *F. fujikuroi* as the main GA_3 production strain on an industrial level. Considering the current study, it should be noted that Sapute et al. [31] reported one of the few previous works related to the use of corn cob to obtain GA_3, wherein they obtained values of 5.2–6.1 g·kg^{-1} GA_3 via SSF with *F. proliferatum* after 10 d. In contrast to the previous study, the growth of GA_3 phytohormones from corn cob via SSF using the fungus *A. niger* presents great advantages because this microorganism is the most abundant mold found in the environment. In addition, it is easy to handle and can be used to ferment various by-products in high yields, and its strains can be improved to create industrial strains for use in commercial production [34].

4. Conclusions

The production of gibberellic acid (GA_3), a growth phytohormone, via the solid-state fermentation (SSF) of corn cob by *Aspergillus niger* is described in this study. This process is of particular interest, since corn cob is a low-cost agroindustrial by-product that is produced and discarded on a large scale every year. The optimal yield of GA_3 produced by *A. niger* upon variation in the pH and sample consistency was 6.1 g·kg^{-1}, which is among the highest values reported in the literature, thereby demonstrating that corn cobs can be used for the efficient production of GA_3 when combined with the most abundant mold found in the environment. The production of GA_3 from corn cob residues not only contributes to reducing the negative impact of agricultural by-products but also represents a new source of a key raw material for phytohormone production.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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