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The production of gibberellic acid from shea nut shell (*Vitellaria paradoxa)* using *Fusarium moniliforme*

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ABSTRACT

The fungus Fusarium moniliforme was used to produce gibberellic acid from the shell of Shea nut (Vitellaria paradoxa). The shea nut shell was dried, sieved to remove the dirt and then blended. The shea nut shell was pre-treated with acid and steam. The powdered shell was then used as substrate in separate shake-flasks which contained mineral salts medium (MSM) and inoculum of Fusarium moniliforme for gibberellic acid production. The media contained the substrate, inoculum size 1.5%, substrate concentration 10% and pH 5.5 for 9 days; the result obtained at day1 was (0.10mg/ml) and at day 9 of the fermentation, the yield was (0.13mg/ml). In the production of this acid, the substrate concentration was varied from 2-12%, with the highest yield at 10% (1.60mg/ml); the inocula sizes were also varied from 0.5-2.5%, inoculum size 1.5% produced the highest yield (1.61mg/ml) and the pH was varied from 4.0-6.0, whereby pH 5.5 produced the highest yield (1.70mg/ml). These fermentation parameters were combined in a single optimization experiment. The result obtained from the optimization experiment gibberellic acid fermentation was 1.82mg/ml.

Keywords: Shea nut shell, *Fusarium moniliforme*, Gibberellic acid, proximate analysis, statistical analysis

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INTRODUCTION

Gibberellic acid was discovered in the United States, Scientist in the United States did not begin researching for gibberellins until after World War II. In 1950, John E. Mitchell of the Research Unit at Camp Dietrick, Maryland, began researching the fungus and gibberellins. Gibberellic acid (GA3), the most important gibberellin is primarily produced by submerged fermentation industrially. Gibberellic acid (GA3) refers to a group of compounds called gibberellins. Gibberellins are an important group of plant hormone.

The selected fruit (*Vitellaria paradoxa)* is a member of the Sapotaceae family, and is divided into two subspecies: *nilotica* and *paradoxa*. *V. paradoxa* subsp. *paradoxa*. The shea tree was originally brought to notice by Mungo Park, and named after him, *Butyrospermum parkii*. The botanical name for Shea tree is "*Vitellaria paradoxa"*, also classified as "*Butyrospermum parkii"* and '*B. paradoxa'*, commonly known as Nkuto, karité, Shea tree has a good commercial value however; it is not exploited to its full potential. Fruits obtained from Shea can be eaten either in their raw state or by cooking them. It is the nuts which hold greater commercial value than fruits because of the different products prepared from them [1]. *Vitellaria paradoxa* has become an important non-timber forest product on the international market. The products from *Vitellaria* are exported in one of two ways. Either the nuts themselves, after being roasted, are exported in bulk, or the nuts are processed into shea butter within the country of origin, and then exported [2]. Shea tree extract or oil obtained from seeds is used in the preparation of solid and creamy fat. It is mainly used for the purpose of skin moisturizing. However, Shea butter can also be used for edible purpose. The other applications of Shea tree extract are preparation of cooking oil, soaps and hair care products. The wood obtained from Shea trees is durable, strong, heavy and most importantly, termite resistant. Charcoal made

from the wood of Shea is of excellent quality. Extract of Shea nuts is also used in reducing inflammation that is associated with osteoporosis. One should also look for side effects if any, that result from cosmetics made of Shea butter. Testing should be done by applying a little amount on the skin [3]

The fruit consist of a sweat flesh pulp, which surround the nut and the shell, which houses the kernel. During the primary processing of the shea fruit, the shell is usually light brown colour, and resembles the shell of a Spanish chestnut .The shells were removed by crushing in a mortar and washed. The shell from the nut and the expressed kernel cake are generally left to go waste. Many physical, chemical and microbial pre-treatment methods for enhancing bioconversion of cellulosic materials have been reported [4].When a substrate is treated, delignification of the cellulolytic materials occur, that is cellulose open the structure and removes secondary interaction between glucose chains [4] and this makes the intake of nutrient easy for the microorganism by converting sugar to acids. Pre-treatment also helps to induce swelling of the whole cellulose fibres. This agent is able to break the hydrogen-bond network and made penetration into the crystalline areas easy.

Fusarium species are widely distributed in soil, subterranean and aerial plant parts, plant debris, and other organic substrates. *Fusarium* species are important plant pathogens causing various diseases such as crown rot, head blight, and scab on cereal grains [5] and they may occasionally cause infection in animals [6]. The widespread distribution of *Fusarium* species may be attributed to their ability to grow on a wide range of substrates and their efficient mechanisms for dispersal. *Fusarium moniliforme* have a very long history for being notoriously toxic. The objective of this study is to produce gibberellic acid from shea nut shell (*Vitellaria paradoxa*) using a fungus (*Fusarium moniliforme*).

MATERIALS AND METHODS

The shea nuts were collected from Ido-Ota, Idofian, Kwara state. The fleshy part of a ripe shea fruit was depulped, washed, parboiled and dried. This was cracked so as to separate the shell from the kernel. The shell was sieved in order to remove the dirt. The substrate was later oven-dried at 80^0C for two days, after which the substrate was ground in a grinding machine and was kept in an air-tight container with some bags of silica gel in order to avoid moisture until when needed. *Fusarium moniliforme* was collected from Department of Food Science, Federal University of Technology, Akure while pure gibberellic acid used as standard was purchased from Lab-tech chemicals, India. The pysico-chemical properties of this substrate were taken. A composition of basal medium for Gibberellic acid production; the medium contained the following: glucose 20 g l^{-1}, corn steep liquor 25 g l^{-1}, Ammonium sulphate $(NH_4)_2SO_4$, 3.3 g l^{-1}, Di- hydrogen potassium phosphate (KH_2PO_4), 0.5gl^{-1}, Potassium sulphate (K_2SO_4), 0.2 g l^{-1} with the inocula of the organism mentioned. Gibberellic acid was estimated colorimetrically, using Hydrochloric acid and Ethanol as reported by [7]. One ml of absolute ethanol was introduced into a test tube containing 1ml of the sample. HCL, 3.75 M, was added up to make to 10 ml and then vigorously mixed for 10 s. The absorbance of the resulting solution was measured at 600 nm and recorded. A calibration graph was then obtained by using standard GA3 solutions prepared by dissolving pure GA3 in absolute alcohol and diluted to 100 ml in a volumetric flask with absolute alcohol. Samples were withdrawn from the culture at 24-hour intervals for a period of 9 days and they were assayed for Gibberellic acid production. The objective of this study is to produce Gibberellic acid from Shea nut shell (*Vitellaria paradoxa)* using a fungus (*Fusarium moniliforme*)

RESULTS AND DISCUSSION

The result for the proximate parameters of the shea nut shell analyzed were: 9.28+0.01% Ash, 12.04+0.01% Moisture content, 27.37+0.01% Crude fibre, 7.37+0.01g Crude protein, 7.39+0.02 % fat and 0.03+0.01 mg/g Sugar. The result of proximate composition of the waste used (shea nut shell) showed an appreciable amount of protein content. The high value of the ash content in this sample shows that the waste used might have a reasonable quantity of mineral element. The ash content is always a rough measure of the organic mineral elements in the sample.

In determining the optimal conditions for gibberellic acid production there was a high yield of this acid at day 7 of the fermentation (1.70mg/ml) which was presented in Table 1, this findings was supported by [8].Moreover, Table 2 showed the effect of varying pH values. It has been reported that pH 5.5 produced the optimal yield of Gibberellic acid and this result support the findings of [9] who deduced that the optimal yield of this acid is mostly observed between the pH of 5.0 to 5.8.The substrate concentration for the production of Gibberellic acid was varied from 2%-12% which was showed in Table 3, the maximum yield observed was obtained at substrate concentration 10%. Among the factors that determine morphology and the general course of fungal fermentation, the type and size of inoculum is of prime important and also in this study, (Table 4) showed the effect of varying inocula sizes (0.5-2.5%) on the production of this acid by *Fusarium moniliforme*. Optimum production was obtained with 1.5% of inoculum size and this finding is supported by [9]. Gibberellic acid production under optimized conditions was

1.82mg/ml using *Fusarium moniliforme* (Table 5).The results obtained from the combined optimization experiment were however higher than when standard conditions were used. In conclusion, the shea nut shell which is usually considered as waste. Considering proximate analysis and ability to produce gibberellic acid could be of economic important.

Table 1: Effect of varying time on Gibberellic acid production by *Fusarium moniliforme* using treated shell

Days	Gibberellic acid (mg/ml)
1	$0.10 + 0.01^{ab}$
2	0.18+ 0.03
3	$0.53 + 0.01^{b}$
4	$0.65+ 0.07^{c}$
5	$0.25+ 0.01^{a}$
6	$0.30+ 0.01^{b}$
7	$1.70 + 0.02^{c}$
8	0.40 + 0.01
9	0.13 + 0.01

(Substrate concentration: 10%, pH 5.5, Temp: $29+1^{0}$C and inoculum size 1.5%) Values represented in the table are means and standard deviation (n=3), All groups are compared to each other at $p<\alpha = 0.05$. Values with different superscripts are statically different.

Table 2: Effect of varying pH on Gibberellic acid production by *Fusarium moniliforme* using treated shell

Fermentation Period (Days)	pH				
	4.0	4.5	5.0	5.5	6.0
1	$0.05+0.01^{ab}$	$0.05+0.00^{a}$	$0.18+0.03^{b}$	$0.10+0.01^{ab}$	$0.10+0.03^{b}$
2	0.03+0.00	0.18+0.05	0.28+0.02	0.18+0.03	0.13+0.02
3	$0.20+0.01^{a}$	$0.05+0.02^{a}$	$0.05+0.01^{a}$	$0.53+0.01^{b}$	$0.10+0.01^{a}$
4	$0.13+0.00^{b}$	$0.25+0.05^{b}$	$0.05+0.01^{a}$	$0.65+0.07^{c}$	$0.28+0.02^{b}$
5	$0.10+0.01^{a}$	$0.30+0.02^{a}$	$0.10+0.02^{a}$	$0.25+0.01^{a}$	$0.13+0.00^{a}$
6	$0.25+0.02^{b}$	$0.30+0.02^{b}$	$0.18+0.01^{b}$	$0.30+0.01^{b}$	$0.05+0.01^{a}$
7	$0.70+0.02^{b}$	$0.30+0.01^{a}$	$0.48+0.02^{b}$	$1.70+0.02^{c}$	$0.75+0.01^{b}$

(Substrate concentration: 10%, Time: (7days), Temp: $29+1^{0}$C and inoculum size 1.5%) Values represented in the table are means and standard deviation (n=3), All groups are compared to each other at $p<\alpha = 0.05$. Values with different superscripts are statically different.

Table 3: Effect of Substrate concentration on Gibberellic acid production by *Fusarium moniliforme* using treated shell

Fermentation Period (Days)	Substrate Concentration (%)					
	2.0	4.0	6.0	8.0	10.0	12.0
1	$0.09+0.01^{a}$	$0.10+0.03^{a}$	$0.08+0.02^{a}$	$0.07+0.00^{a}$	$0.18+0.01^{b}$	$0.10+0.02^{a}$
2	$0.15+0.02^{ab}$	$0.13+0.01^{a}$	$0.11+0.01^{a}$	$0.19+0.00^{b}$	$0.37+0.07^{d}$	$0.09+0.00^{a}$
3	$0.18+0.00^{b}$	$0.17+0.02^{b}$	$0.10+0.00^{a}$	$0.11+0.01^{a}$	$0.56+0.03^{e}$	$0.13+0.01^{a}$
4	$0.14+0.04^{a}$	$0.24+0.02^{c}$	$0.21+0.02^{c}$	$0.24+0.03^{c}$	$0.64+0.04^{e}$	$0.19+0.01^{b}$
5	$0.23+0.03^{c}$	$0.28+0.01^{c}$	$0.16+0.01^{b}$	$0.35+0.02^{d}$	$1.44+0.04^{f}$	$0.21+0.01^{b}$
6	$0.26+0.02^{c}$	$0.22+0.01^{c}$	$0.21+0.04^{c}$	$0.33+0.01^{d}$	$1.47+0.03^{f}$	$0.24+0.03^{b}$
7	$0.28+0.01^{c}$	$0.30+0.01^{d}$	$0.35+0.02^{d}$	$0.50+0.02^{e}$	$1.60+0.01^{f}$	$0.28+0.01^{c}$

(Time: 7days, pH 5.5, Temp: $29+1^{0}$C and inoculum size 1.5%) Values represented in the table are means and standard deviation (n=3), All groups are compared to each other at $p<\alpha = 0.05$. Values with different superscripts are statically different.

Table 4: Effect of Inocula size on Gibberellic acid production by *Fusarium moniliforme* using treated shell

Fermentation Period (Days)	Inocula size (%				
	0.5	1.0	1.5	2.0	2.5
1	$0.12+0.03^{a}$	$0.09+0.01^{a}$	$0.19+0.01^{b}$	$0.08+0.00^{a}$	$0.11+0.01^{a}$
2	$0.22+0.03^{c}$	$0.18+0.02^{b}$	$0.38+0.04^{d}$	$0.14+0.02^{a}$	$0.07+0.00^{a}$
3	$0.35+0.04^{d}$	$0.13+0.01^{a}$	$0.45+0.02^{d}$	$0.24+0.03^{c}$	$0.16+0.02^{b}$
4	$0.32+0.03^{d}$	$0.21+0.04^{c}$	$0.73+0.01^{e}$	$0.26+0.01^{c}$	$0.10+0.03^{a}$
5	$0.19+0.01^{b}$	$0.15+0.02^{b}$	$0.89+0.03^{e}$	$0.28+0.01^{c}$	$0.21+0.00^{c}$
6	$0.43+0.03^{d}$	$0.28+0.01^{c}$	$1.53+0.04^{e}$	$0.32+0.03^{d}$	$0.25+0.02^{c}$
7	$0.50+0.02^{de}$	$0.30+0.01^{d}$	$1.61+0.01^{e}$	$0.53+0.01^{d}$	$0.28+0.00^{c}$

(Substrate concentration: 10%, pH 5.5, Temp: 29+1^{0}C and Time: 7days) Values represented in the table are means and standard deviation (n=3), All groups are compared to each other at $p<\alpha = 0.05$. Values with different superscripts are statically different.

Table 5: Optimization Experiment for Gibberellic acid production at optimal pH, subs. Conc. and inoculum size by *Fusarium moniliforme*

Fermentation Period (Days)	Concentration (mg/ml) Gibberellic acid
1	0.13+ 0.01^a
2	0.21+ 0.01^b
3	0.56+ 0.07^e
4	0.68+ 0.03^f
5	0.29+ 0.06^c
6	0.37+ 0.01^d
7	1.82+ 0.02^g

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