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# The production of citric acid from shea nut shell (*Vitellaria paradoxa)* using *Aspergillus niger*

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**ABSTRACT**

*The fungus Aspergillus niger was used to produce citric acid from the (shell) of Shea nut (Vitellaria paradoxa). The shea nut shell was dried, sieved to remove the dirt and then blended. The waste was pre-treated with acid and steam. The powdered was then used as substrate in separate shake-flasks which contained mineral salts medium (MSM) and inoculum of Aspergillus niger for citric acid production. Fermentations were carried out in flasks containing the MSM, waste substrate at pH 5.0, 5% substrate concentration, 1.5% inoculum size and at 29±1$^0$C for 5 days; the result obtained at day1 was (0.012mg/ml) and at day5 of the fermentation, the yield was (0.020mg/ml) for the citric acid production by Aspergillus niger. These fermentation parameters were combined in a single optimization experiment. The result obtained from the optimization experiment for citric acid was 0.028mg/ml.*

**Keywords**: Shea nut shell, *Aspergillus niger*, Citric acid, proximate analysis, statistical analysis

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## INTRODUCTION

The shea tree 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. Pre-treatment of Shea nut shell, 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.

*Aspergillus niger* is a fungus and one of the most common species of the genus *Aspergillus*. *Aspergillus niger* is cultured for the industrial production of many substances Various strains of *Aspergillus niger* are used in the industrial preparation of citric acid (E330) and gluconic acid (E574) and have been assessed as acceptable for daily intake by the World Health Organization. *Aspergillus niger* fermentation is "generally recognized as safe" (GRAS) by the United States Food and Drug Administration. *Aspergillus niger* has been a very important microbe used in the field of biotechnology.

The production of citric acid by *Aspergillus niger* is one of the most commercially utilized examples of fungal overflow metabolism. Many microorganisms such as fungi and bacteria can produce citric acid. The various fungi, which have been found to accumulate citric acid in their culture media, include strains of *Aspergillus niger, A. awamori, Penicillium restrictum, Trichoderma viride, Mucor piriformis* and *Yarrowia lipolytica* [5]. But *Aspergillus niger* remained the organism of choice for the production of citric acid. In submerged fermentor, either purified compressed air or oxygen along with agitation is used [6]. The objective of this study is to produce Citric acid from Shea nut shell (*Vitellaria paradoxa)* using a fungus (*Aspergillus niger*)

## 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 $80^0$C 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. *Aspergillus niger* was collected from Department of Microbiology, University of Ilorin, Ilorin, while pure citric acid used as standard was purchased from East Anglia chemicals, Hadleigh Ipswich Suffolk. The physio-chemical properties of this substrate were taken. Mary mandel's mineral salts solution was used along with different Carbon and Nitrogen sources which contained the following: Ammonium sulphate (0.25%) Di-hydrogen potassium sulphate (0.10%), Magnesium sulphate (0.02%) and also inocula of the organism mentioned. Citric acid was estimated colorimetrically, using pyridine-acetic anhydride method as reported by Marrier and Boulet (1958).A volume of 1ml of the diluted culture filtrate along with 1.30ml of pyridine was added in the test tube and swirled briskly. Then 5.70ml of acetic anhydride was added to the test tube. The test tube was placed in a water bath at $32^0$C for 30minutes. The absorbance was measured on a spectrophotometer (420nm) and citric acid contents of the sample were estimated. Samples were withdrawn from the culture at 24-hour intervals for a period of 5 days and they were assayed for citric acid.

## RESULTS AND DISCUSSION

The result for the proximate parameters of the shea nut shell analysed were: 9.28$\pm$0.01% Ash, 12.04$\pm$0.01% Moisture content, 27.37$\pm$0.01% Crude fibre, 7.37$\pm$0.01g Crude protein, 7.39$\pm$0.02 % fat and 0.03$\pm$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 use933d might have a reasonable quantity of mineral element. The ash content is always a rough measure of the organic mineral elements in the sample.

Table 1 shows the effect of varying time from day 1 to day 5 which deduced that at day 4 of the fermentation, the highest yield of citric acid production was produced (0.026mg/ml) and this could be possibly due to the ability of the organism to utilize the amount of sugar present for the production of the acid at that period of time.

Table 2 shows the effect of varying substrate concentration on citric acid production using treated shell from 1%-7% by the organism increases until it reached its optimum at 5% concentration on day 4 of the fermentation (0.025mg/ml) and this leads to the nutrients which may adversely affect the cell concentration [7].

Table 3 shows the effect of varying pH which was studied and maximum yield anhydrous citric acid was obtained when initial pH of the fermentation medium was kept at 5.0, decrease in pH caused reduction in citric acid production (Table 3), This finding is in agreement with [8]. It might be due toxic released during mycelium growth of fermentation. It has been reported that pH 5.0 produced high yield of Citric acid out of other pH values, this may also be because most microbes grow best at pH near neutrality but fungi grows under acidic condition. Table 4

shows the effect of varying inocula size from 0.5% to 2.5%, maximal yield was obtained with 1.5% of inoculum size (0.015mg/ml). Increase in mycelia formation in the medium causes a reduction in the yield of citric acid; this is in agreement with Delgado and Liao, (1997). Table 5 showed the Citric acid production under optimized conditions using *Aspergillus niger* was 0.028mg/ml 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 citric acid could be of economic important.

**Table 1: Effect of varying time on Citric acid production by *A. niger* using treated Shea nut shell**

| Time (Days) | Citric acid (mg/ml) |
|---|---|
| 1 | $0.012\pm0.01^{b}$ |
| 2 | $0.017\pm0.01^{b}$ |
| 3 | $0.006\pm0.00^{a}$ |
| 4 | $0.026\pm0.00^{d}$ |
| 5 | $0.020\pm0.00^{c}$ |

*(Substrate concentration 5%, Temp: $29\pm1^{0}$C, inoculum size: 1.5%, pH 5.0) 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 statistically different.*

**TABLE 2: Effect of varying Substrate conc. on citric acid production by *A. niger* using treated shell**

| Fermentation Period (Days) | Substrate Concentration (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | 0.002±0.00 | 0.006±0.00 | 0.015±0.01 | 0.011±0.00 | 0.012±0.00 | 0.015±0.00 | 0.012±0.00 |
| 2 | $0.015\pm0.00^{a}$ | $0.017\pm0.00^{b}$ | $0.016\pm0.00^{c}$ | $0.018\pm0.00^{d}$ | $0.017\pm0.00^{b}$ | $0.010\pm0.00^{c}$ | $0.013\pm0.00^{a}$ |
| 3 | $0.005\pm0.00^{a}$ | $0.005\pm0.00^{a}$ | $0.011\pm0.00^{c}$ | $0.006\pm0.00^{b}$ | $0.006\pm0.00^{b}$ | $0.006\pm0.00^{ab}$ | $0.006\pm0.00^{b}$ |
| 4 | $0.005\pm0.00^{a}$ | $0.002\pm0.00^{a}$ | $0.002\pm0.00^{a}$ | $0.007\pm0.00^{c}$ | $0.025\pm0.00^{e}$ | $0.004\pm0.00^{a}$ | $0.006\pm0.00^{b}$ |

*(Temp: $29\pm1^{0}$C, inoculum size: 1.5%, pH 5.0 and Time: 4days) 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 statistically different.*

**TABLE 3: Effect of varying pH on citric acid production by *A. niger* using treated shell**

| Fermentation Period (Days) | pH | | | | |
|---|---|---|---|---|---|
| | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 |
| 1 | $0.001\pm0.00^{a}$ | $0.003\pm0.00^{a}$ | $0.006\pm0.00^{b}$ | $0.004\pm0.00^{a}$ | $0.008\pm0.00^{b}$ |
| 2 | $0.002\pm0.00^{a}$ | $0.001\pm0.00^{a}$ | $0.008\pm0.00^{c}$ | $0.005\pm0.00^{b}$ | $0.010\pm0.00^{c}$ |
| 3 | $0.001\pm0.00^{a}$ | $0.002\pm0.00^{a}$ | $0.009\pm0.00^{c}$ | $0.007\pm0.00^{c}$ | $0.009\pm0.00^{c}$ |
| 4 | $0.001\pm0.00^{a}$ | $0.002\pm0.00^{a}$ | $0.015\pm0.00^{d}$ | $0.008\pm0.00^{c}$ | $0.011\pm0.00^{c}$ |

*(Time: 4days, Substrate concentration: 5%, Temp: $29\pm1^{0}$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 statistically different.*

**TABLE 4: Effect of varying inoculum size on citric acid production by *A. niger* using treated shell**

| Fermentation Period (Days) | Inocula size (%) | | | | |
|---|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 |
| 1 | $0.003\pm0.00^{a}$ | $0.002\pm0.00^{a}$ | $0.005\pm0.00^{a}$ | $0.001\pm0.00^{a}$ | $0.002\pm0.00^{a}$ |
| 2 | $0.005\pm0.00^{a}$ | $0.004\pm0.00^{a}$ | $0.003\pm0.00^{a}$ | $0.004\pm0.00^{a}$ | $0.004\pm0.00^{a}$ |
| 3 | $0.006\pm0.00^{b}$ | $0.007\pm0.00^{b}$ | $0.011\pm0.00^{c}$ | $0.002\pm0.00^{a}$ | $0.003\pm0.00^{a}$ |
| 4 | $0.007\pm0.00^{b}$ | $0.009\pm0.00^{b}$ | $0.015\pm0.00^{d}$ | $0.010\pm0.00^{c}$ | $0.006\pm0.00^{b}$ |

*(Time: 4days, Substrate concentration: 5%, Temp: $29\pm1^{0}$C and pH 5.0) 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 statistically different.*

**Table 5: Optimization Experiment for Citric acid production at optimal pH, substrate Conc. and inoculum size by *Aspergillus niger***

| Fermentation Period (Days) | Concentration (mg/ml) Citric acid |
|---|---|
| 1 | 0.014± 0.01 |
| 2 | 0.019± 0.01 |
| 3 | 0.017± 0.00 |
| 4 | 0.028± 0.01 |

## REFERENCES

[1] Blench, R. Trees on the march: The dispersal of economic trees in the prehistory of West-Central Africa. ODI, Safa Conference, Cambridge, U.K., May 26, **2000**. pp. 13.
[2] Boffa, J.M., Taonda, S., Dickey, J., Knudson, D., *Agroforestry Systems* **2000** 49, 153–175.
[3] Collinson, C., Zewdie-Bosuener A. Shea butter markets: Their implications for Ghanaian shea butter processors and exporters. Project A0772, **1999** Report No. 2403. 20.

[4] Tang, L.G., D.N.S. Hon, Y.Q. Pan sh Zhu, Z. Wang and Z.Z. Wang, *J. Applied Polymer* **1996** *Sci., 59: 483-488*.
[5] Arzumanov, T.E., Shishkanova, N.V., Finogenova, T.V., *Applied Microbiology and Biotechnology*, **2000** vol. 53, no. 5, p. 525-529
[6] Moreira, M.T., Sanroman, A., Feijoo, G., Lema, J.M. *Enzyme and Microbial Technology*, **1996** vol. 19, no. 4, p. 261-266**...**
[7] Omojasola, P.F., Jilani, O.P. : *Research Journal of Microbiology*, **2009** 4(2): 67-74
[8] Shankarananda,V.S., Losane, B.K.. *World J MicrobiolBiotechnol*, **1994** 10 165-168.