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Evaluating the potential of wild cocoyam (Caladium bicolor) for citric acid production in a submerged culture of *Aspergillus niger*

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Abstract

Background: Wild cocoyam (*Caladium bicolar*) is non-edible starchy material which is commonly known as 'Ede umuagbara' in the south-east region of Nigeria. Evaluating the potential of wild cocoyam for citric acid production was investigated using *Aspergillus niger* in a submerged culture.

Results: This study showed that wild cocoyam flour concentration, pretreatment of wild cocoyam, inoculums size, initial pH of wild cocoyam and incubation temperature of wild cocoyam medium had significant effect on the amount of citric acid produced from wild cocoyam ($p < 0.05$). Citric acid concentration increased as the concentration wild cocoyam increased up to 15% with the maximum citric acid concentration of 10.0 ± 0.251 g/l after 96 h of fermentation. Also citric acid concentration increased as the pretreatment time of wild cocoyam increased from 5 to 20 min. A 20 min pretreatment time at 121 °C was the optimum with maximum concentration of 14.0 ± 0.325 g/l citric acid after 96 h of fermentation. 15% inoculums was the optimum with the maximum concentration of 16.0 ± 0.431 g/l citric acid. However, the wild cocoyam initial pH value of 5.5 was the optimum for maximum citric acid concentration of 19.0 ± 0.316 after 96 h of fermentation. Moreover, citric acid concentration increased as the incubation temperature of wild cocoyam medium increased from 20 to 30 °C. Incubation of wild cocoyam medium at 30 °C was the optimum with the maximum citric acid concentration of 23.0 ± 0.432 g/l after 96 h of fermentation.

Conclusion: Therefore, the result revealed that wild cocoyam which is found plenty within the south-east region of Nigeria can be converted to citric acid if well harnessed.

Keywords: Temperature, Fermentation, Pretreatment, Wild cocoyam

Background

Citric acid, an intermediary of the tricarboxylic acid cycle, is one of the most vital commercially valuable products due to its commonly used mainly in food industry (70%), pharmaceuticals (12%), and others 18% (Makut and Ekeleme 2018). Due to its numerous applications, the volume of citric acid production by is continually increasing at high annual rate of 5% (Finogenova et al. 2005; Helen et al. 2014). The high demands in the market have increased the price of citric acid and its consumption rate is rising day by day due to its numerous applications (Makut and Ade-Ibijola 2012; Makut and Ekeleme 2018). Citric acid find its industrial application in such area as chelating agent, buffer, pH adjustment, laundry detergents, shampoos, cosmetics, enhanced oil recovery and chemical cleaning (Varsha 2015).

Most of the citric acid is manufactured through biological means, mainly through submerged fermentation of starch and sucrose based media (molasses) using *Aspergillus niger*. In recent years, various agricultural wastes residues and by-products have been investigated for their potential to be used as substrate for citric acid production (Makut and Ekeleme 2018).

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Aside the cost coming from the demand, the cost of the commodity chemical is high due to the high cost of substrate (raw materials). This present high percentage of citric acid total production cost, and has necessitated the search for cheap and easily available non edible starchy substrate for citric acid production.

Caladium bicolor is a wild cocoyam commonly known as 'Ede umuagbara' in the south-eastern part of Nigeria. They look like normal edible cocoyam (*Colocasia esculenta*). Wild cocoyam is not edible and found plenty in most of the bushes and farms within the region, which can be exploited for citric acid production. It is a starchy root tuber, which has enough starch that can be converted to citric acid. Therefore, this work was designed to evaluate the potential of wild cocoyam Caladium bicolor) for citric acid production in a submerged culture of *Aspergillus niger*.

Methods

Microorganism and culture maintenance

Aspergillus niger strain was obtained from Department of Microbiology University of Nigeria, Nsukka. The cultures were maintained on potato dextrose agar (PDA) slants at 40 C and sub-cultured at intervals.

Inoculum preparation

The inoculum was prepared according the method of Ezea et al. (2015), the spores of *Aspergillus niger* was harvested from potato dextrose Agar slant using a sterile solution of 0.01% Tween 80. The inoculation wire loop was used to dislodge the spores and to ensure proper mixing of the culture with the Tween 80. A 5 ml of 5 × 107 spores/ml was counted using haemocytometer.

Pretreatments of wild cocoyam flour

Wild cocoyam (*Caladium bicolor*) was obtained from a bush and farm land at Nsukka in Enugu State of Nigeria. The wild cocoyam was peeled, sundried, ground and sieved into fine powder (flour) using Muslim cloth. The flour was thermally pretreated to gelatinize the starch by suspending in 100 ml of the basal nutrient medium. The sample was pretreated with an autoclave at 121 °C for 15 min.

Submerged fermentation of wild cocoyam flour

Submerged fermentation was carried out in a modified method Ezea et al. (2015), in 250 ml foam-plugged Erlenmeyer flask. A 10 g wild cocoyam tuber flour was weighed using DENVER digital weighing balance, Model: MXX-123 USA and suspended in 100 ml nutrient medium containing NH4NO_3, 2 g/l; KH_2PO_4, 0.2 g/l; $ZnSO_4 \cdot 7H_2O$, 0.01 g/l; $Fe(SO_4)_2 \cdot 7H_2O$, 0.01 g/l and $MgSo_4 \cdot 7H_2O$, 0.5 g/l before pretreatment. The sample was inoculated with 5 ml of *Aspergillus niger* spores and incubation at 30 °C for 144 h under rotary incubator shaker (model: VWR International by B. Bran Scientific & Instrument Company England) at 225 rotations per minutes.

Effect of wild cocoyam flour concentration on citric acid production

Effect of wild cocoyam flour concentration on citric acid production was carried by suspending different percentage of wild cocoyam flour ranging from 5 to 25% in 100 ml nutrient medium into 250 ml foam-plugged Erlenmeyer flask and incubated under rotary incubator shaker (model: VWR International by B. Bran Scientific & Instrument Company England) at 225 rotations per minutes (rpm) for 144 h.

Effect of wild cocoyam pretreatment time on citric acid production

The wild cocoyam flour was thermally pretreated to gelatinize the starch by suspending 10 g in 100 ml of the basal nutrient medium. The suspension was pretreated in an autoclave at 1210C for 5 min, 10 min, 15 min, 20 min, 25 min, and 30 min. Thereafter the medium was allowed to cool before inoculation and incubated for 144 h in a rotary shaker at 225 rpm (model: VWR International by B. Bran Scientific & Instrument Company England).

Effect of inoculums size on citric acid production from wild cocoyam

The effect of inoculums size on citric acid production from wild cocoyam was carried out by inoculating different inoculums concentrations from 5 to 25% into 250 ml foam-plugged Erlenmeyer flask and incubated under rotary incubator shaker (model: VWR International by B. Bran Scientific & Instrument Company England) at 225 rotations per minutes (rpm) for 144 h.

Effect of initial pH on citric acid production from wild cocoyam

The effect of initial pH on citric acid production from wild cocoyam was carried out by adjusting the pH to 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 using 0.1 M HCl and 0.1 M NaOH before Pretreatment.

Effect of incubation temperature on citric acid production from wild cocoyam

The effect of incubation temperature on citric acid production form wild cocoyam by *Aspergillus niger* was carried under rotary incubator shaker (model: VWR International by B. Bran Scientific & Instrument Company England) at 225 rotations per minutes, under the following temperature 200 C, 250 C, 300 C, 350 C and 400 C for 144 h.

Analytical techniques

Citric acid was estimated using pyridine acetic anhydride method as reported by Marrier and Boulet (1958). A 1 ml of diluted culture filtrate along with 1.30 ml of pyridine was added in the test tube and swirled briskly. Then 5.70 ml of acetic anhydride was added in the test tube. The test tube was placed in a water bath at 32 °C for 30 min. The absorbance was measured on a Spectrophotometer 722S B. Bran Scientific and Instrument Company, England at 420 nm against the blank and the citric acids of the samples were estimated with reference standard. The pH of the sample was determined using digital pH meter (DENVER Instrument, Model: UB-10058245 ultraBASIC USA).

Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) and the means were separated using the least significant difference.

Results

Effect of wild cocoyam flour concentration on citric acid production

Figure 1 shows the effect of different concentrations of wild cocoyam flour on citric acid production. Citric acid concentration increased as the concentration of wild cocoyam increased up to 15% with the maximum citric acid concentration of 10.0 ± 0.251 g/l after 96 h of fermentation.

Effect of wild cocoyam pretreatment time on citric acid production

Citric acid concentration increased as the pretreatment time of wild cocoyam increased from 5 to 20 min at the pretreatment temperature of 121 °C with maximum concentration of 14.0 ± 0.325 g/l after 96 h of fermentation (Fig. 2). There was a decreased on citric acid concentration from 25 to 30 min.

Effect of inoculum size on citric acid production from wild cocoyam flour

The effect of different inoculums size on citric acid production from wild cocoyam was investigated. Citric acid concentration increased as the percentage inoculums increased from 5 to 15%. 15% inoculums was the optimum with the maximum concentration of 16.0 ± 0.431 g/l citric acid (Fig. 3). There was a decreased on citric acid concentration from 20 to 25% inoculums.

Effect of initial pH on citric acid production from cocoyam

Figure 4 shows the effect of initial pH of wild cocoyam flour on citric acid production. Citric acid concentration increased as the pH increased from 3.5 to 5.5. A pH value of 5.5 was the optimum for maximum citric acid concentration of 19.0 ± 0.316 g/l after 96 h of fermentation. Citric acid concentration decreased from pH 6.5 to pH 8.5 (Fig. 4).

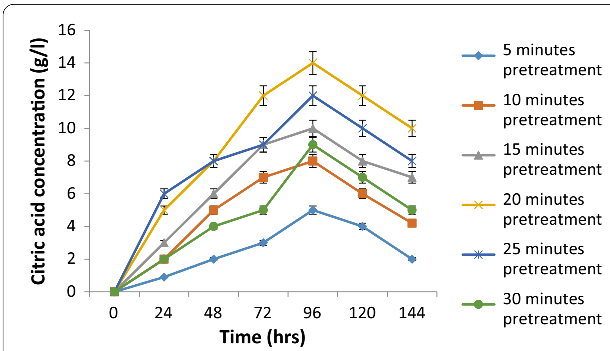

Fig. 2 Effect of wild cocoyam pretreatment on citric acid production

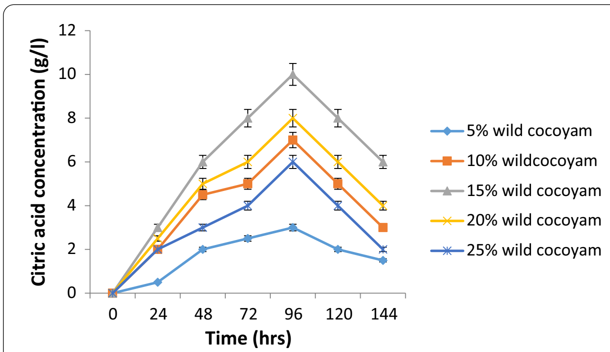

Fig. 1 Effect of wild cocoyam flour concentration on citric acid production

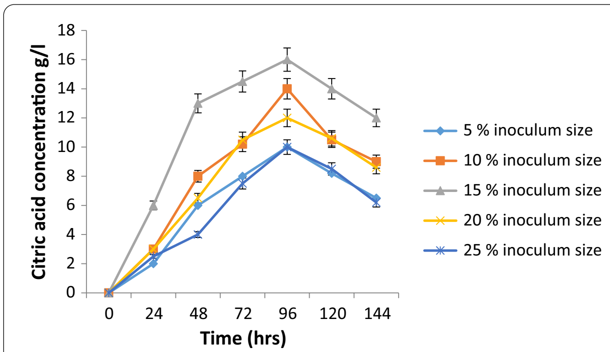

Fig. 3 Effect of inoculum size on citric acid production from wild cocoyam flour

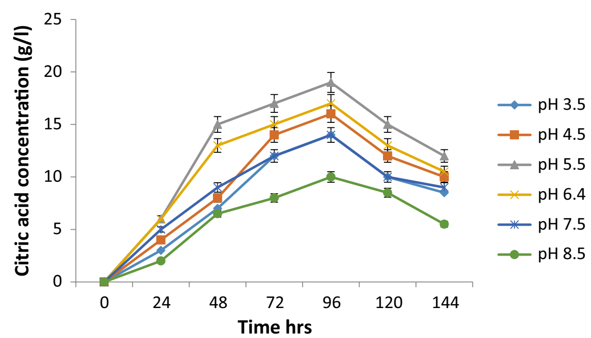

Fig. 4 Effect of initial pH on citric acid production from wild cocoyam

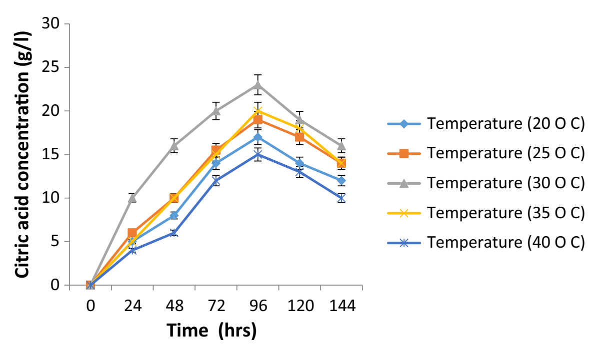

Fig. 5 Effect of incubation temperature on citric acid production from wild cocoyam

Effect of incubation temperature on citric acid production from wild cocoyam

The effect of different temperature on citric acid production from wild cocoyam flour was investigated. Citric acid concentration increased as the temperature increased from 20 to 30 °C. Incubation of the wild cocoyam medium at 30 °C was the optimum with the maximum citric acid concentration of 23.0 ± 0.432 g/l. Further increased in temperature up to 35 °C and 40 °C led to decrease in citric acid concentration (Fig. 5).

Discussion

Effect of wild cocoyam flour on citric acid production

Citric acid concentration increased as the concentration of wild cocoyam increased up to 15% with the maximum citric acid concentration of 10.0 ± 0.251 g/l after 96 h of fermentation. Citric acid has been shown to be viable with many cheep starchy raw materials. In this research work, different concentrations of starchy wild cocoyam flour produced different concentrations of citric acid. This is in agreement with Ezea et al. (2021) who reported citric acid production in a simple solid state culture of *Aspergillus niger* using some starchy substrate, rice grain, maize and cassava flour. Kudzai et al. (2016) reported potato and rice starch extract during citric acid production by *Aspergillus niger* using different substrates. Ezea et al. (2015) reported on utilization of cassava pulp wastes for citric acid production during biological production of citric acid in submerged culture of *Aspergillus niger*. Lodhi et al. (2001) reported citric acid production from waste bread using *Aspergillus niger*. Xue et al. (20121) reported that *Aspergillus niger* exhibited a reduction of the total sugar content and reducing sugar from starch while the final citric acid production was significantly increased. This result suggested that starchy substrates may be good raw material for citric acid synthesis because of conversion of starch to simple sugar by *Aapergillus niger* during hydrolysis and fermentation which later enter the krebs cycle where citric acid is produced as an intermediate.

Effect of wild cocoyam pretreatment time on citric acid production

Citric acid concentration increased as the pretreatment time of wild cocoyam increased from 5 to 20 min at the pretreatment temperature of 121 °C with maximum concentration of 14.0 ± 0.325 g/l after 96 h of fermentation. Though there is scanty information on the variation of substrate pretreatment and time on citric acid production. Adudu et al. (2019) reported pretreatment of corn stalk using oven at 60 °C for 2 h during production of citric acid from corn stalk through submerged fermentation by *Aspergillus niger*. Torrado et al. (2011) reported substrate pretreatment at 100 °C for 1 h using autoclave during citric acid production from orange peel wastes by solid state fermentation. Ali et al. (2002) used combined pretreatment of 1% HCl and temperature at 30 °C for 1 h during Biosynthesis of citric acid from single and cop culture based fermentation technology using agro-wastes. This suggested that different substrates have different pretreatment temperature and time. Pretreatment of lignocellulosic biomass and starchy raw materials prior to hydrolysis and fermentation process is a mandatory step to improve feedstock biodegradability and metabolites production. At high temperature more starch are released from the tubers which may trigger overproduction of citric acid. Also with high temperature P, Mn, Fe and Zn content of the tubers are markedly decreased which may equally trigger overproduction of citric acid.

Effect of inoculum size on citric acid production from wild cocoyam flour

The effect of different inoculum size on citric acid production from wild cocoyam was investigated. Citric acid concentration increased as the percentage inoculum increased from 5 to 15%. The maximum concentration of 16.0 ± 0.431 g/l citric acid was produced with 15% inoculums. This is in agreement with Auta et al. (2014) who reported the effect of vegetative inoculum size (1–5%) on citric acid production during citric acid production by *Aspergillus niger* cultivated on *Parkia biglobosa* fruit pulp. They reported 3% inoculums size as the optimum for maximum citric acid production. Citric acid production increased up to 3% inoculums. The same trend was in accordance with the findings of Ali et al. (2016) who reported an increased in citric acid concentration as the inoculum increased up to certain level during production of citric acid by *Aspergillus niger* using cane molasses in a stirred fermentor. This present work suggested that despite following the same trend on citric acid production, there is a variation on the percentage of inoculums, suggesting that different strain of *Aspergillus niger* has different inoculum size depending on the substrate or raw material used.

Effect of initial pH on citric acid production from wild cocoyam

Citric acid concentration increased as the pH increased from 3.5 to 5.5. A pH value of 5.5 was the optimum for maximum citric acid concentration of 19.0 ± 0.316 g/l after 96 h of fermentation. This in accordance with Ezea et al. (2021) who reported pH 5.5 as the optimum for citric acid production during biological production of citric acid in solid state culture of *Aspergillus niger.* Moataza (2002) reported that citric acid production increased with increase in the initial pH of the substrate with optimum at pH 6.5. The results implied that pH is an important factor that may affect citric acid production. Different strains, depending on the substrate have different pH optimum for citric acid production.

Effect of incubation temperature on citric acid production from wild cocoyam

The effect of different temperature on citric acid production from wild cocoyam flour was investigated. Citric acid concentration increased as the temperature increased from 20 to 30 °C. Further increased in temperature up to 35 °C and 40 °C led to decrease in citric acid concentration. This result is in agreement with Okareh et al. (2016) who reported incubation temperature of 30 °C as the optimum for the maximum citric acid production from solid state fermentation of sugarcane waste using *Aspergillus niger* and indigenous sugarcane microflora. Ezea et al. (2015) reported 30 °C as the optimum for maximum citric acid production during biological production of citric acid in submerged culture of *Aspergillus niger* using cassava pulp wastes. Also in agreement to this result is the work of Torrato et al. (2011) who reported 30 °C as the optimum for the maximum production of citric acid using orange peel wastes. Chergui et al. (2021) reported incubation temperature during optimization of citric acid production by *Aspergillus niger* using downgrade Algerian date varieties. Contrary, Auta et al. (2014) found incubation temperature of 55 °C as optimum for the maximum citric acid production using *parkia biglobosa* fruit pulp. These results suggested that citric acid production may vary on the incubation temperature requirement depending on the substrate and likely the microbial strain used.

Conclusions

wild cocoyam tubers have the potential to be used as substrate in the production of citric acid by *Aspergillus niger* in a submerged culture. Its concentrations have a positive significant effect on the citric acid production. Pretreatment of wild cocoyam, inoculums size of *Aspergillus niger*, initial pH of wild cocoyam and wild cocoyam incubation temperature had significant positive effect on citric acid production. Development of technologies for processing of wild cocoyam tubers into a useful metabolites will guarantee market and prices and reduce the cost of citric acid production. This study has demonstrated that wild cocoyam, which is sufficiently found in the Eastern region of Nigeria and many tropical countries can be used in citric acid production.

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Authors' contributions
IBE drafted the proposal, participated in laboratory work and writing of the manuscript; EE-participated in laboratory and statistical analysis of the work. Both authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
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