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Production of gluconic acid from sweet potato peels using naturally occurring fungi by submerged fermentation

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Abstract

The oxidation of glucose produces gluconic acid, a significant organic acid. The aim of this study was to produce gluconic acid from sweet potato peels by submerged fermentation. Isolation and identification of fungi were done using standard microbiological methods. Proximate analysis of substrate and screening of fungal isolates for gluconic acid production was done using standard procedures. Gluconic acid yields were determined using High Performance Liquid Chromatography. A standard gluconic acid producer, *Aspergillus niger* ATCC 10577, was used as control. A sum of six different fungal species were isolated and identified. They included *Aspergillus niger*, *Aspergillus flavus*, *Penicillum* sp., *Cladosporium* sp., *Rhizopus stolonifer* and *Aspergillus terreus*. Proximate composition of the sweet potato peels showed percentage carbohydrate of 20.81 ± 0.07, percentage moisture of 64.02 ± 0.27. Screening for gluconic acid production showed that *Aspergillus niger* had the highest zone of clearance and identified as *Aspergillus niger* UFMGCB 14248. Our data further showed that gluconic acid concentrations (mg/ml) was highest at substrate concentration 50 g/L, carbon source starch, incubation day 7 and pH 6 for both *Aspergillus niger* UFMGCB 14248 and *Aspergillus niger* ATCC 10577. The findings showed that the fungal isolates used in this study were good gluconic acid producers.

Keywords: High Performance Liquid Chromatography, Gluconic Acid, *Aspergillus niger,* Sweet Potato Peels.

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INTRODUCTION

Gluconic acid has the chemical formula $C_6H_{12}O_7$ and is an organic molecule; a straight forward dehydrogenation reaction facilitated by glucose oxidase produces gluconic acid from glucose. Gluconic acid creates the gluconate ion in an aqueous solution with a neutral pH. Gluconates refers to the salts of gluconic acid because such molecules develop from the oxidation of glucose (Mao, 2016). A moderate organic acid is gluconic acid that has attracted considerable interest because of its wide range of commercial applications in the pharmaceutical, animal feed, textile, and leather industries (Mao, 2016). It has also been used widely in the beverage and food industries as additives for improvement of edible products (Lian *et al*., 2022) and its derivatives have been employed in medicine, textile and construction industries amongst others (Ma *et al*., 2022). In order to decrease setting time, boost strength, and improve water resistance, gluconic acid is also used as an ingredient in cement. Gluconic acid, a moderate organic acid that is non-corrosive, non-volatile, non-toxic, and gives many foods like wine and fruit juice a delightful sour flavor. Plants, fruits, and other foods like rice, meat, dairy products, wine (up to 0.25%), honey (up to 1%), and vinegar are all rich sources of gluconic acid. Gluconic acid can also be used as a chelator in producing and improving casein-based beverages as suggested by Choi and Zhong (2020). Gluconic acid is produced by microorganisms which include bacteria such as *Pseudomonas ovalis, Acetobacter methanolicus, Zymomonas mobilis, Acetobacter diazotrophicus, Gluconobacter oxydans, Gluconobacter suboxydans, Azospirillum brasiliens* fungi such as *Aspergillus niger, Penicillium funiculosum, P. variabile, P. amagasakiense* (Rodriguez *et al*., 2004) and yeasts such as *Aureobasidium pullulans* formerly known as *Dematium* or *Pullularia pullulans* (Anastassiadis *et al.,* 2005).

In order to achieve sustainable development in the word today, conversion of wastes to wealth has become paramount. Transformation of these wastes to value added products through fermentation is gaining grounds in the industry. El Sheikha and Ray (2022) reviewed the bioprocessing of horticultural wastes into innovative bioproducts such as antibiotics, enzymes, bioethanol, bioactive compounds and organic acids,

Sweet potato peels generated by industrial processes constitute a lot of garbage. These wastes are discarded and constitute nuisance to the environment. The peels are not utilized for human consumption and rarely used for manures or feeding animals (Akoetey *et al*., 2017).

The peels are usually utilized for fertilizer or as animal feed. In order to meet food needs, fight poverty, and improve food security, sweet potatoes play a number of crucial roles in the world food system. (El-Sheikha and Ray, 2010). Conversion of horticultural wastes to value added products can reduce effectively the cost of operation and drastically reduce environmental pollution. (El-Sheikha and Ray, 2022).

During storage, a lot of raw foods decompose and produce wastes that could pollute the environment. Utilization of these waste materials can transform them from being waste into prospective suppliers while also aiding in the reduction of environmental degradation on the one hand and the creation of products with additional value on the other. The present study was aimed at screening fungal isolates to ascertain the best gluconic acid producers and to study the effect of various fermentation parameters on their production of gluconic acid.

MATERIALS AND METHODS

Sample collection

The soil sample and the substrate, sweet potato peels, were collected from Ilorin metropolis. The soil was collected at a depth of 15 inches from the top using a sterile spatula from a single location. The sweet potato peels and the soil sample were taken in sterile sample bags and labeled appropriately before being immediately transported to the Microbiology laboratory of Kwara State University.

Preparation and pre-treatment of substrate

Sweet potato peels were cleaned by washing with sterile distilled water and air-dried to reduce its moisture content. After drying, it was chopped into small pieces of uniform sizes, pulverized into a powdered form and sieved using a fine mesh of 0.05 mm in diameter, and stored in an air-tight sample container until further use.

Proximate analysis of sweet potato peels

Proximate analysis of the sweet potato peels was carried out to evaluate the carbohydrate content; proximate analysis was done on the sweet potato peels using the Association of Official Analytical Chemists' method (AOAC, 2000). The total carbohydrate was determined by differential method i.e., by subtracting total protein, moisture content, ash, lipid, and fiber from 100.

Thus: Carbohydrate (%) = (100- (moisture (%) +ash (%) + fat (%) + protein (%) + fiber (%)).

Isolation of fungi

Fungi were isolated from soil sample and fermented sweet potato peels using serial dilution, dilutions were made up to the fifth test tube (10^{-5}). An aliquot of 0.1 ml was taken from each test tube of soil and substrate suspension. The dilutions were plated on Potato Dextrose Agar (PDA) containing 0.1ml of streptomycin and incubated at 28 °C for 7 days using the spread plate technique (Fawole and Oso, 2004). The fungi cultures grown on the medium were subcultured repeatedly until pure cultures were obtained and then transferred on to PDA slants and maintained at 4^{o}C for further use. A typed strain, *A. niger* ATCC 10577 was obtained from the Federal Institute of Industrial Research, Oshodi, (FIIRO), Lagos state. This was used as the control and was compared with the fungal isolate from the soil sample for gluconic acid production. The control fungus was maintained on potato dextrose agar slants and kept at 4 °C until to further use.

Microscopic and molecular identification of fungal isolates

fungal isolates were identified by macro and microscopic characteristics using methods described by Duncan (2017). The fungi spore suspensions were prepared by suspending the spores on the slant in 10 ml of sterilized saline (Grigoryev, 2013). The fungal isolate producing maximum clear zone after screening was used for gluconic acid production and identified molecularly (Shamala *et al*., 2014).

Screening of the fungal cultures for gluconic acid production

The fungal isolates were screened for gluconic acid production on calcium carbonate plates. The media used for the preparation of calcium carbonate plates consisted of, glucose, 10 g; $(NH_4)_2HPO_4$, 0.4 g; $MgS0_4.7H_2O$, 0.2 g; KH_2PO_4, 0.2 g; $CaCO_3$ 50 g and agar 20 g dissolved in one liter of double distilled water and autoclaved at 15 Ib for 15 min. $CaCO_3$ was sterilized separately and added to the medium just before plating. The medium was inoculated with 1ml of spore suspension followed by incubation at 30 ^{o}C for 5 days (Makwin *et al*., 2021).

Fermentation technique

Gluconic acid fermentation were carried out by submerged fermentation in 250 ml cotton wool plugged Erlenmeyer flasks with 100 ml of fermentation media of Czapek's Dox broth consisting of (g/L) sucrose 30.0, $NaNO_3$ 3.0, KH_2PO_4 1.0, $MgSO_4.7H_2O$ 0.5, KCl 0.5 and $FeSO_4.7H_2O$ 0.01 having pH 6.0. The medium was modified by substituting sucrose with thirty grams of powdered sweet potato peels which was dispensed into an Erlenmeyer flask. The medium was autoclaved at 121°C for 15 mins. After cooling to room temperature, the flasks were inoculated with 1 ml of fungal spore suspension containing $2.0x10^5$ spores/ml, following incubation at 30 ^{0}C on a rotary shaker (200 rpm) (Ashraf *et al*., 2014).

Optimization for gluconic acid production

To find out how effectively sweet potato peels produced gluconic acid under the best conditions, the fermentation conditions were varied. The fermentation conditions varied were: substrate concentrations (10g - 50g), pH (2, 4, 6, 8 and 10), incubation days (1, 3, 5, 7, 9 and 11 days), and carbon sources (starch, glucose, sucrose and lactose). These conditions were varied by changing one variable and keeping all others constant. Optimal conditions obtained from the variations were later combined into a single fermentation to optimize the yield of the gluconic acid.

Assay for gluconic acid

The suspended material and fungi biomass were separated by centrifugation at 4000 rpm for 10 minutes. The supernatant was further clarified by filtering through a filter paper. The gluconic acid produced was determined quantitatively by high

performance liquid chromatographic analysis (Singh *et al*., 2001).

Statistical analysis

The data obtained were statistically processed to estimate the mean ± standard deviation (SD) using the one-way analysis of variance (ANOVA). All data were analyzed using the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL). A $P < 0.05$ was considered to be statistically significant.

Results

Proximate analysis of sweet potato peels

The results obtained showed percentage moisture 64.02 ± 0.27, percentage ash 3.24 ±0.16, percentage carbohydrate 20.81 ± 0.07, calorific value 402.02 ±1.20, percentage lipid 0.31 ± 0.01, percentage crude fibre 0.31 ± 0.01 and crude protein 2.55 ± 0.04 presented in Table 1.

Fungi enumeration and identification

A total of six (6) distinct fungi namely *Aspergillus niger, Aspergillus flavus, Penicillum* sp*, Cladosporium* sp*, Rhizopus stolonifer and Aspergillus terreus* were isolated and tentatively identified as presented in Table 2.

Screening of fungal isolates for gluconic acid production

Aspergillus niger showed the highest zone of clearance 6.70 ± 0.30^{d} mm, *Penicillum* sp, showed the lowest zone of clearance 0.80 ± 0.30^{b} while *Aspergillus flavus, Cladosporium* sp. and *Rhizopus stolonifer* showed no clearance zones. The result is presented in Table 3.

Molecular identification of fungal isolate

Blasting of the fungi genomic DNA sequence confirmed the fungi isolate to be *Aspergillus niger* UFMGCB 14248 as shown in Figure 1.

Effect of incubation days on gluconic acid production

At different incubation days, the highest gluconic acid concentration was observed at day 7 with *Aspergillus niger* UFMGCB 14248 having 18.23±0.08 mg/ml while control *Aspergillus niger* ATCC 10577 had 63.12 ± 0.17 mg/ml. This is presented in Figure: 2**.**

Effect of substrate concentrations on gluconic acid production

The results obtained revealed that the highest concentration of gluconic acid produced by *Aspergillus niger* UFMGCB 14248 and control *Aspergillus niger* ATCC 10577 was at substrate concentration 50 g/l with 69.01 ± 0.11 mg/ml and 70.67 ± 0.08 mg/ml respectively. This is presented in Figure: 3.

Table 1: Proximate composition of sweet potato peels

Parameters	Values (%)
Moisture	64.02 ± 0.27
Ash	3.24 ±0.16
Carbohydrate	20.81± 0.07
Calorific value (Kj/100g)	402.02 ±1.20
Lipid	0.31 ± 0.01
Crude fibre	9.04 ±0.04
Crude protein	2.55 ± 0.04

Table 2: Characteristics of fungal isolates

Fungal Isolates	Macroscopic and Microscopic Description	Probable Organisms
F_1	It appears black in color powdery and reverse is yellow. It has smooth colored conidiophore and the conidia are in chains.	*Aspergillus niger*
F_2	It is yellowish green in color powdery and flat. It has aerial hyphae bearing conidiophores, which are colorless, thick-walled, rough and bearing vesicles.	*Aspergillus flavus*
F_3	It appears white in color and reverse is pale yellow; grow flat and cottony in texture. It has septate hyaline hyphae, branched conidiophores with conidia which appear round in shape.	*Penicillium sp.*
F_4	It appears powdery, green to black color and the reverse is black. It has septate hyphae, pigmented conidiophores and conidia are in chains.	*Cladosporium sp.*
F_5	It is deeply cottony white in color becomes grey on aged and reverse is pale white. It has non septate hypae, sporangiophores are brown in color.	*Rhizopus stolonifer*
F_6	It appears as brown color, reverse is yellow, grow flat on the plate. It has septate hyphae and hyaline; conidiophores are smooth-walled and have small conidia.	*Aspergillus terreus*

Key: F_1 –F_6 = Fungal isolates

Table 3: Screening of Fungal Isolates for Gluconic Acid Production

Fungal Isolates	Zone of Clearance (mm)
Aspergillus niger	6.70 ± 0.30^{d}
Aspergillus flavus	0.00 ± 0.00^{a}
Penicillum sp	0.80 ± 0.30^{b}
Cladosporium sp	0.00 ± 0.00^{a}
Rhizopus stolonifer	0.00 ± 0.00^{a}
Aspergillus terreus	1.90 ± 0.30^{c}

Values are mean of duplicate readings and standard error of mean of screening of isolate for gluconic acid production and Values on the same column with different alphabets are significantly different at $P < 0.05$.

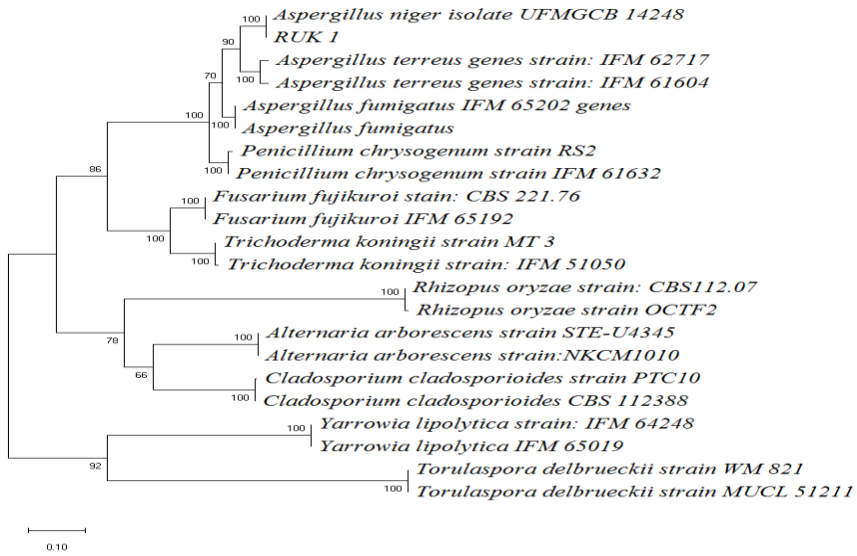

Figure 1: Phylogenetic tree of *Aspergillus niger* UFMGCB 14248

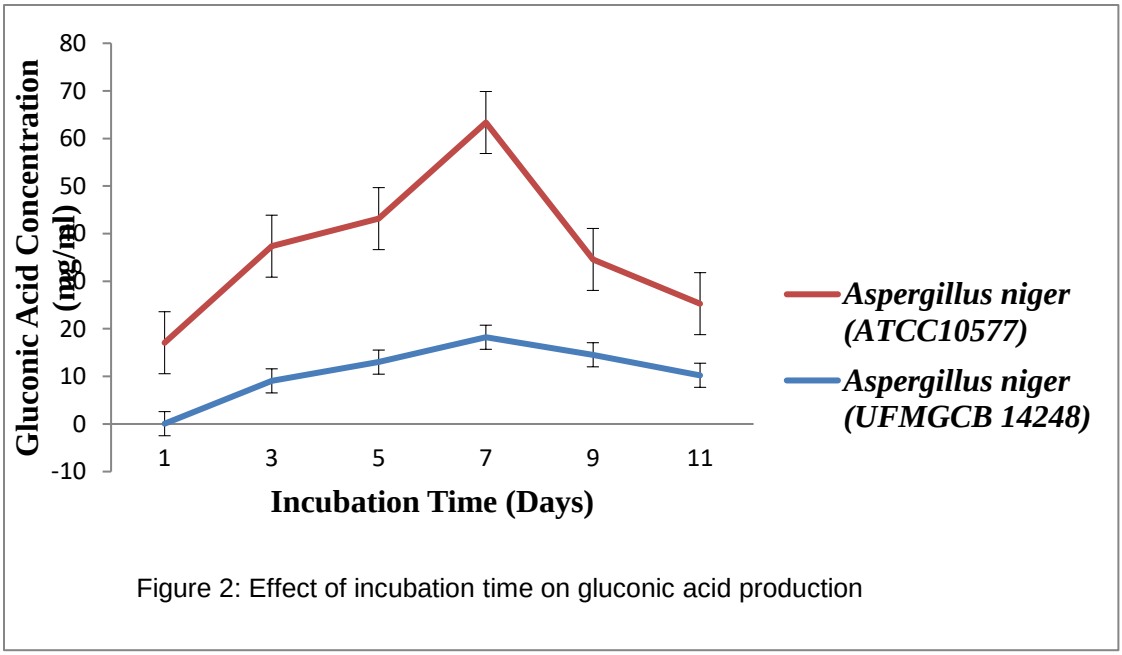

Figure 2: Effect of incubation time on gluconic acid production

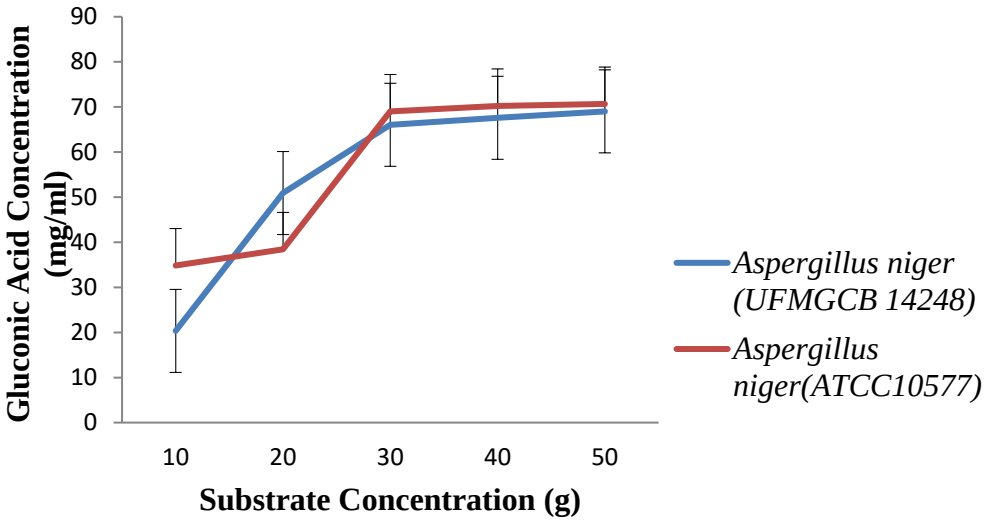

Figure 3: Effect of substrate concentration on gluconic acid production

Effect of Carbon Sources on Gluconic Acid Production

The highest concentration of Gluconic acid 16.78 ± 0.13 mg/ml was obtained by *Aspergillus niger* UFMGCB 14248 while control *Aspergillus niger* 10577 produced 13.45 ± 0.27 mg/ml when starch was used as the carbon source. This is presented in Figure: 4.

Effect of pH on gluconic acid production

The highest concentration of gluconic acid 28.32 ± 0.08 mg/ml was obtained by *Aspergillus niger* ATCC 10577 and *Aspergillus niger* UFMGCB 14248 had 21.01 ± 0.13 mg/ml at pH 6 as presented in Figure 5.

Gluconic acid production using the optimized fermentation parameters

Using the optimized fermentation parameters with highest gluconic acid yield, *Aspergillus niger* UFMGCB 14248 produced gluconic acid of 69.89 ± 0.21[a] mg/ml while the control, *Aspergillus niger* ATCC 10577, produced gluconic acid with a yield of 75.54 ± 0.06[a] mg/ml as shown in Table 4.

DISCUSSION

Despite the fact that gluconic acid can be produced in many different ways, microbial fermentation is still the method of choice. This is because other methods are more expensive and less effective than fermentation with *Aspergillus niger* as being the most commonly used microorganism (Ramachandran *et al*., 2006). This is unexpected because fungi are crucial to the breakdown of the majority of organic and inorganic wastes in the environment, and it is consistent with earlier research by Nimkar *et al.* (2010). In recent times, conversion of agricultural wastes to useful products has been by microorganisms through their enzymatic property (Manikandan *et al*., 2022).

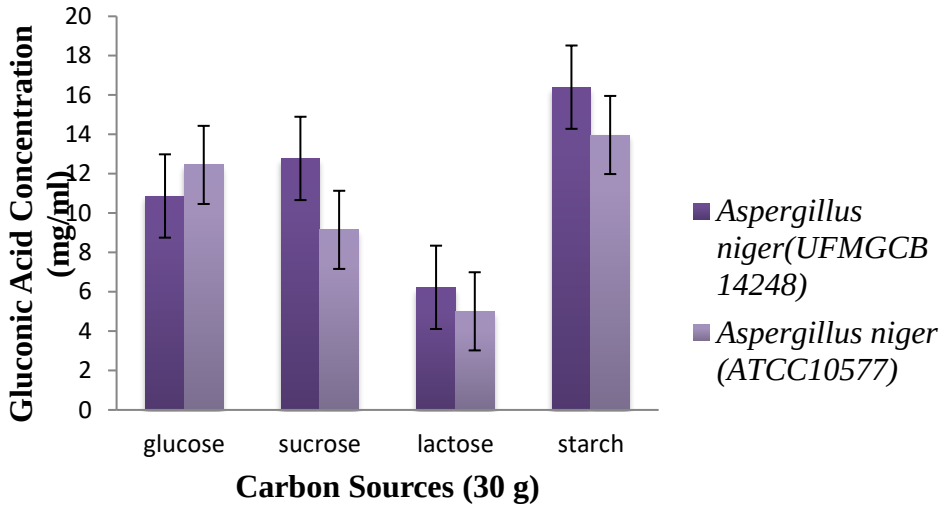

Figure 4: Effect of carbon sources on gluconic acid production

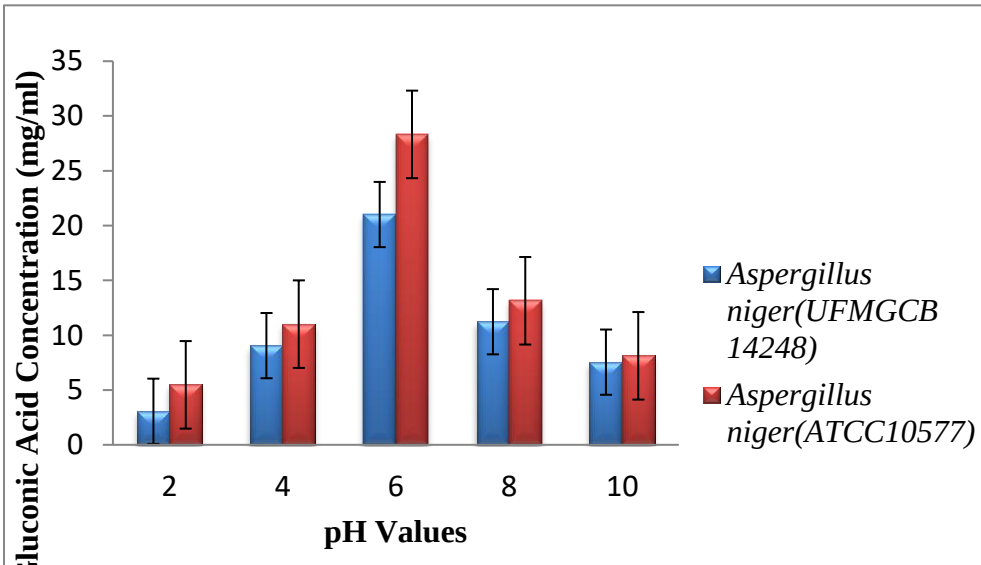

Figure 5: Effect of pH on the gluconic acid production

Table 4: Gluconic acid produced from fermented sweet potato peels using optimized parameters

Fungal isolate	Gluconic acid concentration (mg/ml
Aspergillus niger (UFMGCB 14248)	69.89 ± 0.21[a]
Aspergillus niger (ATCC10577)	75.54 ± 0.06[a]

Values are mean of duplicate readings and standard error of mean and values on the same column with same alphabets is not significantly different at P < 0.05.
Optimized Fermentation Parameters: Temperature 30 ^{o}C, pH 6.0, Substrate concentration, 50g, Starch 30 g, Incubation period, Day 7.

Sweet potato peels were used as substrate for gluconic acid production. The proximate composition revealed that the carbohydrate, protein and fat content etc. was high enough to serve as good source of carbon and energy for gluconic acid production. The results from this analysis were similar to that of Makut *et al*. (2021). The small differences observed may be due to soil fertility or to climate differences.

The screening of the distinct fungal isolates revealed that *Aspergillus niger* had the highest zone of clearance compared to other fungal isolates. This is similar to the report of Sharma *et al*. (2015) that *Aspergillus niger* is the most common producer of gluconic acid. It is also in agreement with the reports of Ma *et al*. (2022) that *Aspergillus* spp are the most frequently used fungi employed for gluconic acid production

The *Aspergillus niger* strain producing the highest zone of clearance was confirmed to be *Aspergillus niger* UFMGCB 14248. At different incubation days, the highest production of gluconic acid was observed at day 7 for both *Aspergillus niger* UFMGCB 14248 and control *Aspergillus niger* ATCC 10577. From our study, the active fungal growth began after day 2 of fermentation and was accompanied by increasing levels of gluconic acid production. The kinetics of growth and gluconic acid production has been studied by Znad *et al*. (2004) and his observation supported our results.

The highest gluconic acid concentration produced by both *Aspergillus niger* UFMGCB 14248 and control *Aspergillus niger* ATCC 10577 was at substrate concentration 50 g/l this could be as a result of the amount of substrate, cause the gluconic acid produced was increasing with increase in substrate concentration. One of the most relevant factors that affects the efficiency of fermentative processes in the production of organic acids is substrate concentration. Others include inoculum size, carbon and nitrogen sources and enzymes amongst others (Šelo, *et al*., 2021).

Makut *et al***.** (2021) reported highest gluconic acid production at different substrate concentration by *Aspergillus niger*, starch produced the highest concentration of gluconic acid by both *Aspergillus niger* UFMGCB 14248 and control *Aspergillus niger* ATCC 10577, this might be as a result of starch been a polysaccharide sugar. Sharma *et al*. (2015) contradicted this when high gluconic acid concentration was reported from glucose and sucrose by *Aspergillus niger*. When producing organic acids or bio-acids with fungi or other microorganisms, pH influence is a crucial factor. Both isolates produced the highest levels of gluconic acid at pH 6. This is in support with Makut *et al*. **(**2021) who reported over 50 % yield of gluconic acid at pH range from 5 to 7. The best yield was at pH 6.0. However, the acid yield above and below this pH was poor. The best combined parameters, there was no significant difference between the concentration of gluconic acid produced by both the control *Aspergillus niger* ATCC 10577and *Aspergillus niger* UFMGCB 14248 that means *Aspergillus niger* UFMGCB 14248 can compete favorably with the control in the production of gluconic acid.

CONCLUSION

In accordance with the results of this study, it was observed that the presence of suitable physical parameters and nutritional requirements during fermentation determines the extent to which good yields of the intended products will be produced. Therefore, it can be concluded from this report that the experimental *Aspergillus niger* UFMGCB 14248 and *Aspergillus niger* ATCC 10577 can be

used for large-scale production of gluconic acid when grown on an optimized biological process. However further studies should be done on other fermentation parameters such as Effect of nitrogen sources, temperature e.t.c. that can lead to better yield of gluconic acid and the use of improved strain of *Aspergillus niger* UFMGCB 14248 for better production of gluconic acid.

Agricultural wastes such as sweet potato peel should be used as a natural substrate for the production of gluconic acid, as a means of converting waste into wealth. This will reduce environmental pollution and the fungus, *Aspergillus niger* UFMGCB 14248 can be employed in the industry for gluconic acid production.

Conflict of Interest

Authors have no conflict of interest to declare**.**

Author Contributions

Adeyinka Elizabeth Ajiboye designed the study, provided suggestions, revised and edited the manuscript on the production of gluconic acid from sweet potato peels by naturally occurring fungi. Rukayat Olaitan Said carried out the research, analyzed and interpreted the results and also drafted the manuscript.

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