Journal of Bioscience and Biotechnology Discovery
Volume 8(2), pages 41-50, April 2023
Article Number: C83233BD4
ISSN: 2536-7064
https://doi.org/10.31248/JBBD2023.180
https://integrityresjournals.org/journal/JBBD
Full Length Research

Production of itaconic acid from sweet potato (*Ipomoea batata*) peel using naturally occurring fungi in solid state fermentation

Ajiboye, A. E.*, Aliyu, M. and Adedayo, M. R.

Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria.

*Corresponding author's Email: adeyinka.ajiboye@kwasu.edu.ng

Received 7th March 2023; Accepted 23rd April 2023

ABSTRACT: This study evaluates the potentials of sweet potato peel as a substrate for itaconic acid production in solid state fermentation using naturally occurring fungi. Sweet potato peel was analyzed for proximate composition using standard methods. Fungi were obtained from the peel by solid state fermentation and identified using microscopic and molecular methods. Fermentation for itaconic acid production was done using isolated fungus and *Aspergillus niger* (ATCC 16888) was used as control. Optimization of fermentation parameters was carried out using standard procedures. Assay for itaconic acid and total titratable acidity (TTA) were analyzed using standard methods. Reducing sugar was obtained using DNSA method. Proximate composition of the peel reveals carbohydrate content to be 79%, protein, 5% and ash, 4%. Fungus isolated was identified as *Aspergillus flavus* L-2482/2012. Highest itaconic acid production by *A. flavus* was 7.74±0.00 mg/ml on day 5 at 30 g substrate concentration. Using 2 ml of 1 x 10^7 spores/ml, *A. flavus* and *A. niger* produced 6.97±0.13 and 6.67±0.09 mg/ml respectively. Optimum temperature for itaconic acid production was 30°C for *A. niger* and *A. flavus.* TTA ranged between 0.04±0.00 to 0.21±0.01 and 0.05±0.00 to 0.26±0.00 mg/ml for *A. niger* and *A. flavus* respectively. The highest reducing sugar, 0.51±0.00 mg/ml was obtained at substrate concentration 40 mg/ml on day 5 by *A. flavus* while *A. niger* had reducing sugar value of 0.37±0.00 mg/ml. In conclusion, *A. flavus* L-2482/2012. has great potentials for itaconic acid production using sweet potato peel in solid state fermentation under optimized conditions.

Keywords: *Aspergillus flavus, Aspergillus niger,* itaconic acid, solid state fermentation, sweet potato peels.

INTRODUCTION

Itaconic acid is a naturally produced sustainable and non-toxic biochemical substitute for many chemically synthesized monomers in production of industrially important compounds (Ray *et al.*, 2017; Sriariyanun *et al*., 2019). Itaconic acid and its derivatives, owing to their easily polymerizable chemical structure with two carboxylic groups and a double bond, have the potential to be used as versatile building blocks for the manufacture of polymeric products like paints, adhesives, plastics, paper, resins, textiles, super-absorbent polymer, etc. (Kumar *et al.*, 2017; Sano *et al*., 2020). It is industrially produced in biorefineries through microbial fermentation of fungi.

The demand and production of environment-friendly products through biotechnological routes is tremendously increasing, and itaconic acid is no exception. Currently, itaconic acid is mostly chemically synthesized. The global itaconic market is majorly in Europe, America and Asia and it has been predicted to hit several millions of US dollars by the year 2026 (Global Market Insights, 2016; Market Data Forecast, 2021).

Though the itaconic acid market is attaining significant growth with the growing popularity of sustainable chemicals, usage of itaconic acid in products of day-to-day applications lags much behind petroleum-based products. Further development of the itaconic acid market for wide-scale production and consumption of its products as an economically viable replacement of petroleum-based products requires lowering its production cost to at least

$0.5/kg from the current cost of $1.5–2.0/kg (Klement and Büchs, 2013; Yang *et al*., 2019). Hence, there is a need to design microbial cell factories with efficient fermentation routes enabling high-level itaconic acid production on low-cost substrates. Such hyper-accumulating itaconic acid cell factories can be designed with the aid of synthetic biology, metabolic, evolutionary, and bioprocess engineering approaches (Bafana and Pandey, 2018).

The primary application of itaconic acid is in the polymer industry where it is employed as a co- monomer at a level of 1-5 % for certain products. Its derivatives are used in medicine and cosmetic preparation. Itaconic acid can react with acrylic and methacrylic acid or their esters which is widely employing to prepare resins used in emulsion coating, leather coating, coatings for car, refrigerators and other electrical appliances to improve adhesion, colour and weather resistance. In general, though several raw materials can be used; glucose, sucrose and xylose are preferred raw materials for itaconic acid fermentation, which are known to be utilized efficiently by most of the *Aspergillus* sp. (Meena *et al.,* 2010).

The sweet potato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the family Convolvulaceae. Its large, starchy, sweet-tasting, tuberous roots are an important root vegetable. The young leaves and shoots are sometimes eaten as greens of the approximately 50 genera and more than 1,000 species of Convolvulaceae, *Ipomoea batatas* is the only crop plant of major importance some others are used locally, but many are actually poisonous. Potatoes are full of starch which is a type of complex carbohydrate. It contains high carbohydrate content which is actually good as it takes a while for the body to breakdown. All types of potatoes provide complex carbohydrates in the form of starch and fibre (Coffman, 2011).

In Solid-State Fermentation (SSF), microorganisms grow on solid materials without the presence of free liquid (Bhargav *et al.,* 2008). The process occurs in absence or near absence of free water by employing a natural substrate or inert substrate as carbon source and solid support (Kumar *et al*., 2003). Currently, solid-state fermentation occupies a spectacular place as a substitute for submerged fermentation. When matched with fermentation through the submerged process, solid-state fermentation has better yield, lack of foam formation and compact design of reactors. Due to reduced water contents, the exposure for microbial contamination is very less (Pandey *et al*., 2000; Ramakrishnan *et al.,* 2020). Importantly, agricultural waste residues can be used as a source in solid-state fermentation.

Various microorganisms possess the ability to convert carbohydrate to high yield organic acids. However, fungi are the most widely used microorganisms in fermentation due to their ability to grow at low pH and moisture content; high tolerance for acids; ability to form hyphae which penetrates substrates and ability to produce various enzymes for the conversion of different wastes to useful products (Omojasola and Adeniran, 2014). Although itaconic acid production through submerged fermentation of sweet potato peel with *Aspergillus niger* and *Aspergillus terreus* have been reported (Omojasola and Adeniran, 2014), there is dearth of information on the production of itaconic acid through SSF of sweet potato peel with other fungi despite the advantages of SSF over submerged fermentation. This research therefore explored the possibility of producing itaconic acid from sweet potato peel through SSF with *Aspergillus flavus*.

MATERIALS AND METHODS

Collection of samples

Sweet potato (substrate) was purchased at Ipata market, Ilorin, Kwara State, Nigeria. It was put into a sterile polythene bag and immediately taken to the laboratory for analysis. The sample was identified at the Plant Biology Department, University of Ilorin, Nigeria as *Ipomoea batata* UILH/001/1326/2022. The sweet potatoes were washed to remove dirt, scraped to remove the peels and air-dried at room temperature (28±2°C), milled and kept in an air-tight container to prevent moisture absorption.

Proximate analysis of sweet potato peel

The proximate composition including moisture content, total ash, crude protein, fat and crude fiber of the ground sweet potato peels was determined according to AOAC (2019) standard methods.

Media preparation

Potato dextrose agar (PDA) and Czapek's Dox media were prepared according to the manufacturer's instructions, by weighing the required quantities (in grams) of powder and dissolved in an equivalent volume (ml) of distilled water in a sterilized glass. The media were homogenized completely on a hot plate, and sterilized at 121°C for 15 min.

Isolation of naturally occurring fungi from fermented sweet potato peel

Twenty (20) grams of sweet potato peel was put into sterile polythene bag and tied up for fermentation to take place for 21 days at 28±2°C. Afterwards, 1 g of fermented substrate was diluted with 9 ml of sterile distilled water in test tubes and dilutions were made up to 10^{-7}. An aliquot (0.1 ml) from each test tube was plated on Potato Dextrose Agar (PDA) containing 0.1 ml Streptomycin and incubated at 28°C for 7

days using the spread plate technique (Fawole and Oso, 2004). The fungi on the medium were sub cultured until pure cultures were obtained. The isolated fungi were characterized and identified macroscopically and microscopically according to Gilman (2001) methods. A typed strain of *Aspergillus niger* ATCC 16888 was obtained from the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos State, Nigeria. The culture was maintained on potato dextrose agar (PDA) slants and kept at 4°C prior to use.

Characterization and identification of fungal isolates

Colonial morphology

Physical features of the colony on the plates were used for the fungal identification according to Barnett and Hunter (1972). These include the colour of the colonies, colour of its reverse side, the height of the mycelia, and the pattern of growth.

Cellular characteristics

For microscopic identification, a drop of lactophenol cotton blue was placed on a clean glass slide. A loopful of the fungal growth was removed using a sterile wire loop and placed in the drop of lactophenol blue. It was covered with a coverslip and observed under the microscope using x10 and x40 objective lens (Barnett and Hunter, 1972; Onyeagba, 2004).

Molecular identification of fungal isolates

The fungi isolate was further identified through molecular study at the Inqaba Biotec West Africa Ltd (IBWA) Lab., Ibadan Nigeria and 18 S rRNA sequencing was employed for the identification of fungal strains which showed the maximum production of itaconic acid. Zhou *et al*. (2008) method was used for the isolation of fungal DNA and the amplification of DNA sequence was executed according to the method described by Gnanasekaran *et al*. (2018). Primers ITS4 - (5′ TCCTCCGCTTATTGATATGC 3′) and ITS1 – (5′TCCGTAGGTGAACCTG CGG 3′) were employed for DNA sequence magnification. The attained fungal specific sequences were compared with known database available in BLAST. Sequence alignment was accomplished by using Clustal omega. The resulted sequence alignment was used to construct a phylogenetic tree (Onyeagba, 2004; Wagner *et al.,* 2018).

Inoculum preparation

Spore suspensions of the isolates were prepared by adding 10 ml of sterilized distilled water containing 2 drops of 0.1% tween 80 to the sporulated 7 days old culture. A sterile wire-loop was used to dislodge the spore clusters under sterile conditions and then mixed thoroughly to prepare a uniform spore suspension. The number of spores were counted using Neubauer's counting chamber as described by Grigoryev (2013).

Preparation of bromine reagent

One milliliter of bromine, 3.00 g of potassium bromide, 1.87 g of potassium chloride, 48.50 ml of 1N hydrochloric acid, and 500 ml of water was used in the preparation of the bromine reagent as used by Friedkin (Friedkin,1945; Ahmed El-Imam *et al.,* 2013). Reagent was preserved in an amber reagent bottle.

Itaconic acid production by solid state fermentation

Fermentation was carried out by taking 10 g of substrate in 500 ml conical flasks, to which 30-50 ml of the nutrient solution of the following composition (g/L), glucose 40.0 g/L; $(NH_4)_2SO_4$, 2.36 g/L; KH_2PO_4, 1.0 g/L; $MgSO_4$, 0.5 g/L; $CaCl_2$, 0.13 g/L; NaCl, 0.074 mg/L; $CuSO_4.5H_2O$, 0.2 mg/L; $FeSO_4.7H_2O$, 0.01 mg/L, and $ZnSO_4.7H_2O$, 1.3 mg/L in distilled water (Rafi *et al.,* 2014) were added. The contents were sterilized by autoclaving at 121ºC for 20 min. After sterilization, the spore suspension containing $1x10^7$ spores/ml of *Aspergillus niger* and *Aspergillus flavus* were added as inoculum in solid state fermentation process. The fermentation was carried out at different temperature (30 and 35ºC), inoculum concentration (1 – 3 ml), substrate concentration (30, 40 and 50 mg/ml) and incubation period (1 – 9 days) changing one variable and keeping all others constant to obtain the optimum conditions for the production of itaconic acid. All the experiments were conducted in triplicates and the average values were recorded. After fermentation, 1 g of the fermented substrate was mixed with 50 ml distilled water in a conical flask, mixed thoroughly, allowed to stand for 30 min before filtering. The filtrate was used to determine the itaconic acid concentration, total titratable acidity and reducing sugar.

Extraction and assay for itaconic acid

The suspended material and fungi biomass were separated by centrifugation at 4000 rpm for 10 min. The clarified supernatant was filtered with filter paper and used as crude itaconic acid. The itaconic acid concentration was measured by colorimetric method at 385 nm (Hartford, 1962). An authenticated itaconic acid standard was used to quantify itaconic acid (Rafi *et al.,* 2014). Into a 3 ml cuvette, 0.3 ml bromine reagent was made up to 1.0 ml with distilled water before HCl (pH 1.2) was added to make

up to 3.0 ml and left for 15 min. In another 3 ml Beckman cuvette, 0.3 ml bromine reagent, sample (1.0 ml) and HCl (pH 1.2) were added to make it 3.0 ml volume. After 15 min, the change in optical density was read at 385 nm, wavelength of maximum absorption of bromine reagent was also read. Reading was repeated in 20 min to ascertain that reaction is completed (Friedkin,1945).

Determination of reducing sugars

The reducing substance (sugar) obtained due to the enzymatic reaction were estimated by dinitro salicylic acid (DNS) method. A spectrophotometer was used for measuring the absorbance. Reducing sugar contents were determined by taking a 1.0 ml diluted solution (1 ml sample in 100 ml distilled water) with 3.0 ml of DNS reagent in a test tube. Blank containing 1.0 ml of distilled water and 3.0 ml of DNS were run parallel. The tubes were heated in a boiling water bath for 5 min. After cooling the tubes at room temperature, 1 ml 40% Rochelle salt solution were added in each, and absorbance were noted at 510 nm using a spectrophotometer. Sugar concentration was determined from the standard curve of glucose (Miller, 1959).

Determination of total titratable acidity (TTA)

The total acidity of the culture filtrates was determined by titration against standard alkaline solution 0.1N NaOH (Ahmed *et al.,* 2015) using phenolphthalein as an indicator.

Statistical analysis

The data were statistically processed to estimate the mean ± standard deviation (SD) and using the one-way analysis of variance (ANOVA). All data were analyzed according to the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL). P value of < 0.01 was statistically significance.

RESULTS

Proximate composition of sweet potato peel

The proximate composition of sweet potato peel obtained are carbohydrate content (79%), lipid (3.1%), crude fibre (2.50%), crude ash (4.68) and crude protein (5.23%) as shown in Figure 1.

Identification of fungal isolate from fermented sweet potato peel

Two fungal isolates, namely F_1 had black spot, white edges, large and flat. It was identified as *Aspergillus niger*, while the F_2 having greenish, white mycelia, large and flat was identified as *Aspergillus flavus* as shown in Table 1.

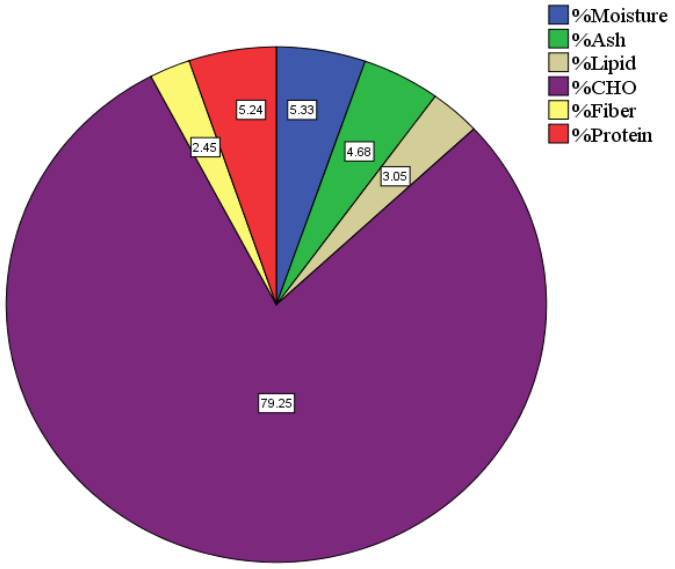

Figure 1. Proximate composition of sweet potato peel.

Molecular identification of fungal isolate

The fungal isolate from fermented sweet potato peel was molecularly identified as *Aspergillus flavus* L-2482/2012 as shown in Figure 2.

Reducing sugar of fermented sweet potato peel using different substrate concentration

The highest reducing sugar 0.51±0.00 mg/ml was obtained at 40 g substrate concentration on day 5 using *A. flavus* while the lowest value 0.05±0.00 mg/ml was obtained on day 1 using the different substrate concentrations. While *A. niger* having the highest value of 0.37±0.00 mg/ml using 40 g on day 5 and the lowest was obtained at day 1 (Table 2).

Total titratable acidity of fermented sweet potato peel using different substrate concentration by *Aspergillus flavus* and *Aspergillus niger*

Substrate concentration of 40 g sweet potato peel fermented with *A. flavus* for 5 days had the highest concentration (0.26 mg/ml) of TTA and 50 g substrate fermented with *A. flavus* at day 1 had the lowest concentration (0.04 mg/ml). While 40 g substrate fermented with *A. niger* for 7 days had the highest concentration (0.21 mg/ml) of TTA and 30 mg/ml substrate fermented with *A. niger*at day 1 had the lowest concentration (0.04 mg/ml) as shown in Table 3.

Table 1. Morphological characterization of fungal isolates.

Fungal isolates	Macroscopic and microscopic description	Probable organisms identified
F_1	Black spore on the surface and usually whitish underneath, large, it has a branching hypha.	*Aspergillus niger*
F_2	Yellowish green, large, whitish mycelia. It has aerial hypae bearing conidiophores, which are colorless, thick walled, rough and bearing vesicles.	*Aspergillus flavus*

Keys: F_1–F_2=Fungal Isolates.

Figure 2. Phylogenetic tree constructed by Neighbor-Joining method derived from analysis of the 18S rRNA gene sequences of native isolates and related sequences obtained from NCBI.Numbers in the parenthesis represent Genbank Accession Numbers.

Itaconic acid content of fermented sweet potato peel using different substrate concentration by *Aspergillus flavus* and *Aspergillus niger*

The highest itaconic acid concentration by *Aspergillus flavus* was 6.45±0.00 mg/ml using 40 g on day 5 while the lowest concentration (0.09±0.01 mg/ml) was observed at day 1 with 50 g. Also, the highest itaconic acid concentration by *Aspergillus niger* was 8.96±0.01 mg/ml using 40 g on day 5 while the lowest concentration (0.08±0.01 mg/ml) was observed at day 1 as seen in Table 4.

Reducing sugar of fermented sweet potato peel using different inoculum size by *Aspergillus flavus* and *Aspergillus niger*

The highest reducing sugar (0.63±0.00 mg/ml) was obtained with 2 ml inoculum size using 40 g substrate fermented by *Aspergillus flavus* on day 5. The lowest reducing sugar (0.05±0.00 mg/ml) was obtained at day 1, across the different inoculum sizes. While fermenting with *Aspergillus niger*, the highest concentration (0.41±0.00 mg/ml) was obtained with 2 ml inoculum size at day 5 and

Table 2. Reducing sugar content during fermentation by *Aspergillus flavus* and *Aspergillus niger* at different substrate concentration.

Substrate conc.(g)	Fungal Isolates	Fermentation period (Days)/Reducing sugar (mg/ml)				
		1	3	5	7	9
30	AN	0.05±0.00^{a}	0.13±0.00^{a}	0.22±0.00^{e}	0.19±0.00^{d}	0.11±0.00^{c}
	AF	0.05±0.00^{a}	0.13±0.00^{a}	0.24±0.00^{d}	0.20±0.00^{c}	0.11±0.00^{c}
40	AN	0.05±0.00^{a}	0.13±0.00^{a}	0.37±0.00^{b}	0.23±0.00^{b}	0.15±0.00^{b}
	AF	0.05±0.00^{a}	0.14±0.00^{a}	0.51±0.00^{a}	0.25±0.00^{a}	0.18±0.00^{a}
50	AN	0.05±0.00^{a}	0.13±0.00^{a}	0.27±0.00^{c}	0.21±0.00^{b}	0.11±0.00^{c}
	AF	0.05±0.00^{a}	0.13±0.00^{a}	0.22±0.00^{e}	0.23±0.00^{b}	0.14±0.00^{b}

Values are means of duplicate reading ± standard deviation of reducing sugar of sweet potato peels for itaconic acid production. Values with the different superscripts in the column are significantly different ($p<0.05$). **Keys:** AN - *Aspergillus niger* ATCC 16888; AF - *Aspergillus flavus* strain L-2482/2012.

Table 3. Total titratable acidity during fermentation by *Aspergillus flavus* and *Aspergillus niger* at different substrate concentration.

Substrate conc.(g)	Fungal	Fermentation period (Days)TTA (mg/ml)				
		1	3	5	7	9
30	AN	0.04±0.00^{c}	0.14±0.01^{d}	0.14±0.00^{cd}	0.20±0.00^{a}	0.18±0.00^{a}
	AF	0.05±0.00^{ab}	0.16±0.00^{cd}	0.20±0.01^{b}	0.20±0.02^{a}	0.20±0.01^{a}
40	AN	0.05±0.00^{bc}	0.19±0.00^{bc}	0.20±0.00^{b}	0.20±0.01^{a}	0.21±0.01^{a}
	AF	0.07±0.00^{a}	0.25±0.01^{a}	0.26±0.00^{a}	0.20±0.00^{a}	0.20±0.01^{a}
50	AN	0.05±0.00^{ab}	0.14±0.00^{d}	0.12±0.00^{d}	0.18±0.01^{a}	0.17±0.01^{a}
	AF	0.04±0.00^{c}	0.21±0.00^{b}	0.17±0.00^{c}	0.18±0.01^{a}	0.18±0.01^{a}

Values in the table are means of two replicate readings and standard deviations. Values within the column having different superscripts are significantly different ($p<0.05$). **Key:** AN - *Aspergillus niger* ATCC 16888; AF - *Aspergillus flavus* strain L-2482/2012.

Table 4. Itaconic acid content during fermentation by *Aspergillus flavus* and *Aspergillus niger* at different substrate concentration.

Substrate conc.(g)	Fungal Isolates	Fermentation period (Days)/Itaconic Acid (mg/ml)				
		1	3	5	7	9
30	AN	0.08±0.01^{c}	5.59±0.01^{b}	6.83±0.05^{d}	0.86±0.04^{b}	0.48±0.04^{b}
	AF	0.10±0.01^{d}	5.46±0.15^{b}	7.74±0.02^{c}	1.72±0.26^{a}	0.26±0.03^{b}
40	AN	0.11±0.00^{a}	5.12±0.18^{b}	8.96±0.01^{a}	0.43±0.08^{bc}	0.36±0.09^{b}
	AF	0.10±0.00^{ab}	6.10±0.43^{ab}	6.45±0.00^{e}	0.32±0.15^{bc}	0.82±0.47^{ab}
50	AN	0.09±0.00^{bc}	7.08±0.46^{a}	8.12±0.02^{b}	0.20±0.020^{c}	1.49±0.03^{a}
	AF	0.09±0.01^{bc}	5.97±0.34^{a}	0.09±0.01^{f}	0.53±0.22^{bc}	0.40±0.11^{b}

Values are means of duplicate reading± Standard Deviation. Values with the different supper script on the column are significantly different ($p < 0.05$). **Key:** AN - *Aspergillus niger* ATCC 16888; AF - *Aspergillus flavus* strain L-2482/2012.

the lowest concentration (0.04±0.00 mg/ml) was observed at day 1 with 2 ml inoculum size as seen in Table 5.

Total titratable acidity of fermented sweet potato peel using different inoculum size by *Aspergillus flavus* and *Aspergillus niger*

As revealed in the results, 2 ml inoculum size of *A. flavus* fermented 40 g potato peel (substrate) at 5 days to give the highest concentration of TTA (0.27±0.00 mg/ml). The lowest concentration (0.04±0.00 mg/ml) was observed at day 1 using 3 ml inoculum of *A. flavus*. While *Aspergillus niger* had the highest concentration (0.20±0.00 mg/ml) with 1 ml inoculum size at day 5 and the lowest concentration (0.05±0.00 mg/ml) was at day 1 using 1 ml inoculum size (Table 6).

Table 5. Reducing sugar content during fermentation by *Aspergillus flavus* and *Aspergillus niger* at different inoculum size.

Inoculum size(ml)	Fungal	Fermentation period (Days)/Reducing sugar (mg/ml)		
		1	3	5
1	AN	0.05±0.00^{a}	0.13±0.00^{c}	0.27±0.00^{e}
	AF	0.05±0.00^{a}	0.14±0.00^{b}	0.51±0.00^{b}
2	AN	0.04±0.00^{a}	0.18±0.00^{a}	0.41±0.00^{c}
	AF	0.05±0.00^{a}	0.17±0.00^{a}	0.63±0.00^{a}
3	AN	0.04±0.00^{a}	0.12±0.00^{d}	0.35±0.01^{d}
	AF	0.05±0.00^{a}	0.14±0.00^{b}	0.51±0.00^{b}

Values are means of duplicate readings ± standard deviations. Values with different superscripts in each column are significantly different ($p < 0.05$). Substrate concentration – 40mg/ml.Key: AN - *Aspergillus niger*ATCC 16888; AF - *Aspergillus flavus* strain L-2482/2012.

Table 6. Total titratable acidity during fermentation by *Aspergillus flavus* and *Aspergillus niger* at different inoculum size.

Inoculum size(ml)	Fungal Isolates	Fermentation Period (Days)/TTA (mg/ml)		
		1	3	5
1	AN	0.05±0.00^{c}	0.19±0.00^{b}	0.20±0.01^{b}
	AF	0.07±0.00^{a}	0.25±0.01^{a}	0.26±0.01^{a}
2	AN	0.06±0.00^{b}	0.19±0.00^{b}	0.20±0.00^{b}
	AF	0.07±0.00^{a}	0.24±0.01^{b}	0.27±0.00^{a}
3	AN	0.05±0.00^{c}	0.16±0.00^{c}	0.14±0.01^{c}
	AF	0.04±0.00^{d}	0.20±0.00^{b}	0.19±0.00^{b}

Values are means of duplicate readings ± standard deviations. Values with the different superscripts on the column are significantly different ($p < 0.05$). Substrate concentration – 40 mg/ml.**Key:** AN - *Aspergillus niger*ATCC 16888; AF - *Aspergillus flavus*. strain L-2482/2012.

Table 7. Itaconic acid content during fermentation by *Aspergillus flavus* and *Aspergillus niger* at different inoculum size.

Inoculum size(ml)	Fungal isolates	Fermentation period (days)/Itaconic acid (mg/ml)		
		1	3	5
1	AN	0.11±0.00^{ab}	5.12±0.17^{b}	8.96±0.02^{a}
	AF	0.10±0.00^{b}	6.10±0.43^{a}	6.46±0.01^{cd}
2	AN	0.11±0.01^{ab}	4.53±0.20^{b}	6.67±0.09^{c}
	AF	0.12±0.00^{a}	5.01±0.11^{b}	6.97±0.13^{b}
3	AN	0.10±0.00^{ab}	4.76±0.07^{b}	5.45±0.13^{e}
	AF	0.09±0.00^{b}	4.94±0.10^{b}	6.38±0.11^{d}

Substrate concentration – 40 mg. Values are means of duplicate readings ± standard deviations. Values with the different superscript in the column are significantly different ($p < 0.05$).**Key:** AN - *Aspergillus niger* strain ATCC 16888; AF - *Aspergillus flavus* strain L-2482/2012.

Itaconic acid content of fermented sweet potato peel using different inoculum size by *Aspergillus flavus* and *Aspergillus niger*

The results shows that 2 ml inoculum size of *A. flavus* fermented 40 g potato peel (substrate) at day 5 had the highest itaconic acid concentration of (6.97±0.13 mg/ml), while the lowest concentration (0.09±0.00 mg/ml) was observed with 3 ml inoculum size at day 1. *Aspergillus niger*had the highest concentration (8.96±0.02 mg/ml) of itaconic acid at day 5 with 1ml inoculum size and the lowest itaconic acid concentration (0.10±0.00 mg/ml) was at day 1 using 3 ml inoculum size as shown in Table 7.

Reducing sugar of fermented sweet potato peel at different temperatures by *Aspergillus flavus* and *Aspergillus niger*

The highest reducing sugar (0.51±0.00 mg/ml) was obtained at temperature of 30°C using *A. flavus* and a substrate concentration of 40 g at day 5 while the lowest

Table 8. Reducing sugar content during fermentation by *Aspergillus flavus* and *Aspergillus niger* at different temperatures.

Temperature (°C)	Fungal isolates	Fermentation period (days)/reducing sugar (mg/ml)		
		1	3	5
30	AN	0.06±0.00^{a}	0.13±0.00^{b}	0.37±0.00^{b}
	AF	0.05±0.00^{a}	0.14±0.00^{a}	0.51±0.00^{a}
35	AN	0.05±0.00^{a}	0.09±0.00^{d}	0.24±0.01^{c}
	AF	0.04±0.00^{a}	0.10±0.00^{c}	0.34±0.02^{b}

Values are means of duplicate readings ± standard deviations. Values with the different superscripts in the column are significantly different (p <0.05). Substrate concentration – 40 mg/ml. **Key:** AN - *Aspergillus niger* strain ATCC 16888; AF - *Aspergillus flavus* strain L-2482/2012.

Table 9. Total titratable acidity during fermentation by *Aspergillus flavus* and *Aspergillus niger* at different temperature.

Temperature (°C)	Fungal isolates	Fermentation period (Days)/TTA (mg/ml)		
		1	3	5
30	AN	0.05±0.00^{b}	0.19±0.01^{b}	0.20±0.00^{b}
	AF	0.07±0.00^{a}	0.25±0.01^{a}	0.26±0.01^{a}
35	AN	0.07±0.01^{a}	0.18±0.02^{b}	0.19±0.01^{b}
	AF	0.07±0.00^{a}	0.17±0.01b^{b}	0.20±0.01^{b}

Values are means of duplicate readings ± standard deviations. Values with different superscripts in the column are significantly different (p < 0.05). Substrate concentration – 40 mg/ml. **Key:** AN - *Aspergillus niger* strain ATCC 16888*;* AF - *Aspergillus flavus* strain L-2482/2012.

Table 10. Itaconic acid content during fermentation by *Aspergillus flavus* and *Aspergillus niger* at different temperature.

Temperature (°C)	Fungal isolates	Fermentation period (Days)/Itaconic Acid (mg/ml)		
		1	3	5
30	AN	0.11±0.00 ^{a}	5.12±0.018^{b}	8.96±0.02^{a}
	AF	0.10±0.00 ^{a}	6.10±0.43^{a}	6.46±0.01^{b}
35	AN	0.10±0.01 ^{a}	1.71±1.02^{c}	5.52±0.17^{c}
	AF	0.07±0.01^{b}	3.01±0.10^{c}	5.37±0.08^{c}

Values presented are means of duplicate readings ± standard deviations. Values with different superscripts in the column are significantly different (p < 0.05). Substrate concentration – 40 mg/ml.**Key:** AN - *Aspergillus niger* strain ATCC 16888; AF - *Aspergillus flavu*s strain L-2482/2012.

reducing sugar (0.04±0.00 mg/ml) was obtained at temperature of 35°C, day 1. Using *Aspergillus niger*, the highest reducing sugar (0.37±0.00 mg/ml) was obtained at 30°C, day 5 while the lowest reducing sugar (0.05±0.00 mg/ml) was obtained at 35°C, day 1 (Table 8).

Total titratable acidity of fermented sweet potato peel at different temperatures by *Aspergillus flavus* and *Aspergillus niger*

At temperature, 30°C using *A. flavus,* the highest TTA concentration (0.26±0.01 mg/ml) was obtained on day 5 while the lowest concentration (0.07±0.00 mg/ml) was obtained at day 1 at both temperatures. Using *A. niger,* the highest TTA (0.20±0.01 mg/ml) was obtained at 30°C, day 5 while the lowest concentration (0.05±0.00 mg/ml) was at day 1 (Table 9).

Itaconic acid content of fermented sweet potato peel at different temperature by *Aspergillus flavus* and *Aspergillus niger*

The highest itaconic acid content (6.46±0.01 mg/ml) was obtained using *A. flavus*at 30°C, day 5 while using *A. niger,* the highest concentration was (8.96±0.02 mg/ml) at 30°C, day 5 (Table 10).

DISCUSSION

Itaconic acid due to its wide applications remains profoundly demanding (Ajiboye *et al.,* 2018). Sweet potato peels despite the reports of its rich polysaccharides, has been underutilized in the production of highly valuable organic acid like itaconic acid. This study therefore, investigated the production of itaconic acid from sweet

potato peels as substrate using *Aspergillus niger* and *Aspergillus flavus* in a solid-state fermentation. The high percentages of carbohydrates and protein (carbon and nitrogen sources, respectively) observed in sweet potato peels in this study demonstrated its suitability as good substrate candidate for microorganisms to thrive and for the production of organic acids. This corroborates the findings of Omojasola and Adeniran (2014), where sweet potato peel recorded high carbohydrate content as a great hot spot for microbial activities. High carbohydrate content that is easily metabolized is essentially required as a substrate for fungi in the fermentation process for optimum production of itaconic acids (organic acids) (Max *et al*., 2010; Ajiboye *et al*. 2018).

Furthermore, the presence of appreciable amount of carbohydrate and other nutrients in sweet potato peel of this study make it susceptible to microbial contamination and hence the probable identification of *A. niger* and *A. flavus*. Moreover, naturally occurring fungi, *Aspergillus flavus* growing on fermented sweet potato peel have the potential of producing itaconic acid which can be used in industries.

The results of Ahmed El-Imam *et al.* (2013), who showed a reduction in reducing sugar concentration during the manufacture of itaconic acid using Jatropha oilseed cake, do not agree with the observed increasing concentration of reducing sugar with an increased substrate concentration in the current investigation. Fungi (*A. niger* and *A. flavus*) via amylolitic action convert polysaccharides into sugars (reducing and non-reducing sugars) during fermentation, accounting for the rise in reducing sugar content up until day 5. This process may also consequently produce an elevated level of itaconic acid (Petruccioli *et al*., 1999; Omojasola and Adeniran, 2014). The findings of Ahmed El-Imam *et al.* (2013) and Ajiboye *et al*. (2018), who worked on Jatropha oilseed cake and Date pulp (*Phoenix dactylifera* L.), respectively, are similar in that they found itaconic acid concentration increased with an increase in substrate concentration with a maximum yield at 40 g. The noticeable decline might be explained by the fungi consuming all of the necessary nutrients. The titratable acidity (TTA) followed the same trend as expected and peaked at a substrate concentration of 40 g.

The highest concentration of reducing sugar observed on day 5 of fermentation with 2 ml *A. flavus* could be due to the inoculum size. Lower inoculum size results in lower number of cells in the fermentation medium thereby needing a longer time to grow to the level required to utilize enough of the substrate so as to give the desired product (Saxena and Singh, 2011). During the lag phase of microbial growth, low amount of complex carbohydrates is hydrolyzed to simple sugars due to adjustment to the new environment. The results suggest that *A. flavus* and *A. niger* were in the exponential phase of growth by day 5 hence breaking down more of the polysaccharide to yield more reducing sugar and itaconic acid. This is in accordance with the observation of Mohit *et al.* (2011) during the production of ethanol by *Saccharomyces cerevisiae* using *Jatropha curcas* seed cake, where the rate of consumption of glucose is lower in the first 4 days of fermentation, but picks up in later days. Ahmed El-Imam *et al*. (2013) also noticed residual glucose increase in the first two days before the concentration began to decline.

The maximum yield of itaconic acid observed on day 5 of fermentation with 2 ml inoculum of *A. flavus* is similar to the findings of Ajiboye *et al*. (2018) who worked on Date pulp (*Phoenix dactylifera* L.) and observed a maximum yield on day 6 of fermentation with 2 ml inoculum of *A. niger*. However, there was a decrease in 3 ml which was the highest concentration. This may be due to high spore density thus leading to rapid consumption of available nutrients leaving limited nutrients for utilization for the production of the itaconic acid.

Expectedly, the titratable acidity (TTA) followed the same pattern and was highest at day 5 of fermentation with 2 ml *A. flavus* inoculum. During fermentation, the quantity of sugars fermented by microorganisms determines the concentration of the acid produced. The high concentration of itaconic acid on day 5 could be responsible for the high TTA on the same day.

In this study, volumetric productivity of itaconic acid was found to be temperature-dependent. This is because external temperature has a significant effect on the cell growth metabolism and thereby the production of itaconic acid. *A. flavus* were found to grow optimally at 30°C which is an average temperature at which fungi grow best. However, itaconic acid production can continue with an increase in temperature up to 35 °C which is in line with the findings of Sudarkodi *et al.* (2012).

Conclusion

From the study, sweet potato peel has demonstrated a promising substrate for the production of Itaconic acid using *Aspergillus flavus* isolated from the natural fermentation of sweet potato peel. Production of high quantity of itaconic acid from sweet potato peel was enhanced through optimization parameters such as temperature, substrate concentration and inoculum size.

Recommendation

In line with the findings of this study, it is recommended that production of itaconic acid from sweet potato peel using *Aspergillus. flavus* be employed in the industries. Further studies on improving the strain of *A. flavus* for better itaconic acid yield is recommended as well.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

REFERENCES

Ahmed El-Imam, A. M, Kazeem, M. O., Odebisi, M. B., & Abidoye, A. O. (2013). Production of itaconic acid from Jatropha curcas seed cake by Aspergillus terreus. *Notulae Scientia Biologicae*, *5*(1), 57-61.

Ahmed, A. S., Farag, S. S., Hassan, I. A., & Botros, H. W. (2015). Production of gluconic acid by using some irradiated microorganisms. *Journal of Radiation Research and Applied Sciences*, *8*(3), 374-380.

Ajiboye, A. E., Adedayo, M. R., Babatunde, S. K., Odaibo, D. A., Ajuwon, I. B., & Ekanem, H. I. (2018). Itaconic acid production from Date palm (Phoenix Dactylifera L) using fungi in solid state fermentation. *Covenant Journal of Physical and Life Sciences*, *6*(1), 20-35.

AOAC (2019). *Official Methods of Analysis* (20th Edition). Association of Official Analytical Chemists, Washington, DC., USA.

Bafana, R., & Pandey, R. A. (2018). New approaches for itaconic acid production: Bottlenecks and possible remedies. *Critical Reviews in Biotechnology*, *38*(1), 68-82.

Barnett, H. L., & Hunter, B. B. (1972). *Illustrated Genera of Imperfect Fungi.* Burgess Publishing Company, Minneapolis. Pp: 62-63.

Bhargav, S., Panda, B. P., Ali, M., & Javed, S. (2008). Solid-state fermentation: an overview. *Chemical and Biochemical Engineering Quarterly*, *22*(1), 49-70.

Coffman, M. A. (2011). Carbohydrates in potato starch. *Life Strong Foundation, 16*:1-3.

Fawole, M. Q., & Oso, B. A. (2004). *Laboratory manual of microbiology*. Spectrum Books, Limited, Ibadan.

Friedkin, M. (1945). Determination of itaconic acid in fermentation liquors. *Industrial & Engineering Chemistry Analytical Edition*, *17*(10), 637-638.

Gilman, J. C. (2001). *A manual of soil fungi*. Oxford & IBH Publishing Corporation, New Delhi, India.

Global Market Insights, (2016). Itaconic acid market. Retrieved 26th May 2021 from https://www.gminsights.com/industry-analysis/itaconic-acid-market.

Gnanasekaran, R., Dhandapani, B., Gopinath, K. P., & Iyyappan, J. (2018). Synthesis of itaconic acid from agricultural waste using novel Aspergillus niveus. *Preparative Biochemistry and Biotechnology*, *48*(7), 605-609.

Grigoryev, Y. (2013). Cell counting with a hemocytometer: Easy as 1,2,3. Retrieved from https://bitesizebio.com/13687/cell-counting-with-a-hemocytometer-easy-as-1-2-3/.

Klement, T., & Büchs, J. (2013). Itaconic acid–a biotechnological process in change. *Bioresource Technology*, *135*, 422-431.

Kumar, D., Jain, V. K., Shanker, G., & Srivastava, A. (2003). Citric acid production by solid state fermentation using sugarcane bagasse. *Process Biochemistry*, *38*(12), 1731-1738.

Kumar, S., Krishnan, S., Samal, S. K., Mohanty, S., & Nayak, S. K. (2017). Itaconic acid used as a versatile building block for the synthesis of renewable resource-based resins and polyesters for future prospective: a review. *Polymer International*, *66*(10), 1349-1363.

Market Data Forecast (2021). Itaconic Acid Market. Retrieved 26th May 2021 from https://www.marketdataforecast.com/market-reports/itaconic-acid-market.

Max, B., Salgado, J. M., Rodríguez, N., Cortés, S., Converti, A., & Domínguez, J. M. (2010). Biotechnological production of citric acid. *Brazilian journal of Microbiology*, *41*(4), 862-875.

Meena, V., Sumanjali, A., Dwarka, K., Subburathinam, K. M., & Sambasiva Rao, K. R. S. (2010). Production of itaconic acid through submerged fermentation employing different species of Aspergillus. *Rasayan J Chem*, *3*(1), 100-109.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426-428.

Mohit, S. M., Chandrashekhar, B., Tanushree, C., & Kanwal, S. (2011). Production of bio-ethanol from Jatropha oilseed cakes via dilute acid hydrolysis and fermentation by Saccharomyces cerevisiae. *International Journal of Biotechnology Applications*, *3*(1), 41-47.

Omojasola, P. F., & Adeniran, E. A. (2014). The production of itaconic acid from sweet potato peel using *Aspergillus niger* and *Aspergillus terreus*. *Albanian Journal of Agricultural Sciences*, *13*(4), 72-77.

Onyeagba, R. A. (2004). *Laboratory guide for microbiology* (1st edition). Crystal Publishers, Okigwe, Imo State, Nigeria. Pp. 95-117.

Pandey, A., Soccol, C. R., & Mitchell, D. (2000). New developments in solid state fermentation: I-bioprocesses and products. *Process biochemistry*, *35*(10), 1153-1169.

Petruccioli, M., Pulci, V., & Federici, F. (1999). Itaconic acid production by Aspergillus terreus on raw starchy materials. *Letters in Applied Microbiology*, *28*(4), 309-312.

Rafi, M. M., Hanumanthu, M. G., Rao, D. M., & Venkateswarlu, K. (2014). Production of itaconic acid by Ustilago maydis from agro wastes in solid state fermentation. *Journal of Biological Science and Biotechnology, 3*(2), 163-168.

Raja, H. A., Miller, A. N., Pearce, C. J., & Oberlies, N. H. (2017). Fungal identification using molecular tools: a primer for the natural products research community. *Journal of Natural Products*, *80*(3), 756-770.

Ramakrishnan, G., Dhandapani, B., Krishnamoorthy, S., Dhithya, V., & Palaniyappan, H. (2020). Next-generation itaconic acid production using novel *Aspergillus japonicas* from *Citrullus lanatus* rind through solid-state fermentation. *Bioresource Technology Reports*, *11*, 100544.

Ray, P., Smith, C., Simon, G. P., & Saito, K. (2017). Renewable green platform chemicals for polymers. *Molecules*, *22*(3), 376.

Sano, M., Tanaka, T., Ohara, H., & Aso, Y. (2020). Itaconic acid derivatives: structure, function, biosynthesis, and perspectives. *Applied Microbiology and Biotechnology*, *104*, 9041-9051.

Saxena, R., & Singh, R. (2011). Amylase production by solid-state fermentation of agro-industrial wastes using *Bacillus sp*. *Brazilian Journal of Microbiology*, *42*, 1334-1342.

Sriariyanun, M., Heitz, J. H., Yasurin, P., Asavasanti, S., & Tantayotai, P. (2019). Itaconic acid: A promising and sustainable platform chemical? *Applied Science and Engineering Progress*, *12*(2), 75-82.

Sudarkodi, C., Subha, K., Kanimozhi, K., & Panneerselvam, A. (2012). Optimization and production of itaconic acid using Aspergillus flavus. *Advances in Applied Science Research*, *3*(2), 1126-1131.

Wagner, K., Springer, B., Pires, V. P., & Keller, P. M. (2018). Molecular detection of fungal pathogens in clinical specimens by 18S rDNA high-throughput screening in comparison to ITS PCR and culture. *Scientific reports*, *8,* Article number 6964.

Yang, J., Xu, H., Jiang, J., Zhang, N., Xie, J., Wei, M., & Zhao, J. (2019). Production of itaconic acid through microbiological fermentation of inexpensive materials. *Journal of Bioresources and Bioproducts*, *4*(3), 135-142.