https://dx.doi.org/10.4314/ijs.v27i1.5

INULINASE PRODUCTION FROM *Aspergillus oryzae* OY-3 USING ONION PEEL AS AN ALTERNATIVE, LOW-COST SUBSTRATE

Oyedeji, O.*, Arowolo, T. B. and Oki, K. D.
Obafemi Awolowo University, Faculty of Science, Department of Microbiology, Ile-Ife, Nigeria.
*Corresponding Author's Email: ooyedeji@oauife.edu.ng
(Received: 25th February, 2025; Accepted: 25th March, 2025)

ABSTRACT

Inulinases have broad biotechnological applications in diverse industrial sectors such as the food, confectionary, pharmaceutical, and biofuel industries. This study was aimed at the production of inulinase from a filamentous fungus, under submerged fermentation conditions, using onion peel as a cheap, and renewable substrate. Nine strains of fungi were isolated from the soil of a Sugarcane Farmland in Obafemi Awolowo University, Ile-Ife, Nigeria, and screened for their relative inulinase production. The identity of the selected inulinase-producing fungus was presumptively determined by phenotypic method, and this was confirmed by molecular method through the sequencing of the internal transcribed spacer region of the ribosomal DNA. The agro-wastes onion, orange, pineapple, and plantain peels were collected from the Fruit Market and processing sites in Ile-Ife, Nigeria, and screened as suitable substrates for inulinase production from the selected fungus. The proximate analysis of the selected onion peel was carried out. The effect of each of the fermentation parameters incubation period, carbon sources, nitrogen sources, inoculum size, pH, and temperature on the enzyme production was also studied. The identity of the selected inulinase-producing fungus was confirmed by molecular method as *Aspergillus oryzae* OY-3. Of the agro-wastes screened, onion peel was observed to be the best substrate for the enzyme production from the fungus. The cultural parameters eliciting optimum inulinase production from the fungus were an incubation period of 72 h, initial pH, and incubation temperature of 4.0 and 30 °C, respectively. Others were a fungal spore inoculum size 0.5 mL, onion peel concentration 2.0% $^{w}/_{v}$, and nitrogen source peptone. This study has revealed the feasibility of the production of the industrially-important inulinase from *A. oryzae* OY-3, isolated from the soil of a Sugarcane Farmland, using onion peel as a low-cost, alternative substrate.

Keywords: *Aspergillus oryzae*, Fungi, Inulinase**,** Onion peel, Production, Submerged fermentation, Sugarcane farmland.

INTRODUCTION

Inulinases, also known as β-D-Fructanhydrolase or β-D-Fructohydrolase, are enzymes which catalyze the hydrolysis of the β-2,1-glycosidic bonds in inulin releasing various products such as fructose, glucose and inulooligosaccharides. They are an important group of carbohydrate-degrading enzymes belonging to the glycoside hydrolase (GH) family 32. They are classified into two classes based on their hydrolytic action on inulin – exoinulinases and endoinulinases (Kumar *et al.*, 2018). Exoinulinase [EC 3.2.1.80] hydrolyzes the terminal, non-reducing 2,1-linked ends of inulin with the resulting release of β-D-fructose sequentially. Endoinulinase [EC 3.2.1.7] breaks down the internal 2,1-glycosidic linkages in inulin to produce fructooligosaccharides of inulinooligosaccharides with variable chain lengths such as inulotriose, inulotetraose and inulopentaose (Singh and Singh, 2017). Apart from inulin, exoinulinases also catalyzes the hydrolysis of other substrates levan and sucrose. Inulinases occur across the biosphere, and are produced by plants, animals, and microorganisms. However, the microbial sources are preferred for commercial production of the enzyme owing to their ease of manipulation, shorter generation time, and the ability to produce copious amounts of relatively stable enzymes (Gracida-Rodriguez *et al.,* 2014). Several microorganisms including bacteria, yeasts, and filamentous fungi have been implicated in inulinase production. Prominent producers of the enzyme are found among the yeast genera *Klyveromyces* spp. and *Candida* spp., and the filamentous fungal genera *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., and *Rhizopus* spp. (Mohammed *et al.*, 2015; Karam *et al.*, 2018). The filamentous fungi are promising candidates for use in inulinase production because of their ability to grow on inexpensive substrates, and their production of enzymes with appreciable stability at high temperatures and low pH conditions (Nath

and Kango, 2022).

Inulinases have broad biotechnological applications and are used in the production of high fructose syrups and fructooligosaccharides which are important components of several functional foods, confectionery, and pharmaceutical formulations. Also, they are used industrially for the production of bioethanol, butanol, sorbitol, single-cell oil, single-cell protein, citric acid, gluconic acid, and lactic acid (Zhang *et al*., 2015; Ilgin *et al*., 2019).

Inulin is the primary substrate for inulinase activity. It is a water-soluble storage polysaccharide, belonging to a group of non-digestible carbohydrates called fructans. It is accumulated in the roots, bulbs, and tubers I different species of plants belonging to the families Amryllidaceae, Compositae, Liliaceae, and Gramineae (Chi *et al*., 2011) Such plants include Jerusalem artichoke, dandelion, chicory, leeks, garlic, asparagus, onion, banana, dahlia, wheat, rye, and barley (Mensink *et al*., 2015). Inulin is made up of β-D-2,1-fructosyl fructose linkages that are terminated by a sucrose molecule (Kango and Jain, 2011).

Despite the varied industrial utilization of inulinases, its comprehensive applications in the industries have been limited due to the high cost of production, mostly due to the input raw materials or substrate. Inulin and inulin-rich materials are used as raw materials in submerged fermentation for inulinase production (Singh and Chauhan, 2017). Enormous amounts of agro-wastes are generated annually worldwide, as a result of the various agro-industrial crop processing activities (Di Donato *et al*., 2011). These wastes, which are mostly of fruit and vegetables, are mostly generated from preliminary operations such as peelings and cuttings. Most times, the wastes are discarded, untreated, into the environment constituting pollution hazards. Onion (*Allium cepa* L.) is one of the main ingredients in the cooking of diverse human cultures, ranking second after tomatoes as an important food ingredient (Celano *et al*., 2021). The abundant production and processing of onion generate byproducts such as the peel which are mostly discarded into the environment. However, onion peel has been reported to contain a variety of nutrients such as carbohydrates, antioxidants, phenolic compounds, vitamins, and minerals which could serve as substrates for fungal growth and enzyme production (Dapper *et al.,* 2016). The increasing demands for inulinase production, high raw material costs, and environmental considerations have stimulated interest in the utilization of agro-wastes as low-cost substrates for the production of the enzyme. Several agro-wastes have been utilized for inulinase production such as sugarcane molasses (Treichel *et al*., 2009), wheat bran (Ali and Shahzadi, 2015), agave syrup (De-Oliveira *et al*., 2016), sugarcane stem (Das *et al*., 2020), and chicory root (Abdella *et al*., 2023).

The fermentation parameters such as carbon and nitrogen sources, pH, and temperature influence the enzyme yield in a fermentation process (El-Hadi *et al*., 2014). Therefore, the optimization of these parameters can lead to the achievement of high-level enzyme production.

Based on its composition, we hypothesized that onion peel could be a good substrate for fungal growth, and inulinase production. Therefore, this study aimed to study the effect of fermentation parameters on extracellular inulinase production from a fungus isolated from the soil of sugarcane farmland, using onion peel as a cheap and renewable substrate, under submerged fermentation conditions.

MATERIAL AND METHODS

Isolation of fungi and culture maintenance

Fungal strains were isolated from the soil samples collected from the Sugarcane Farmland at the Obafemi Awolowo University Farm, after carrying out serial dilutions. The dilutions were inoculated on sterile potato dextrose agar (PDA) in Petri plates. Upon incubation, each morphologically distinct colony was successively subcultured on fresh Potato dextrose agar medium until a pure culture was obtained. The pure fungal colonies were maintained on PDA slants and stored at a temperature of 4 °C.

Screening of fungal isolates for inulinase production

The isolates were screened for their relative

inulinase production under submerged fermentation conditions, in a medium with inulin 2.0% ($^{w}/_{v}$) as the only carbon source. The fungal strain exhibiting the most appreciable enzyme production, as indicated by the inulinolytic activity in the medium, was then selected for further studies and maintained on potato dextrose agar (Fluka, St. Louis, Mo, USA) slants at a temperature of 4 ºC.

Characterization and identification of selected inulinolytic fungus

Phenotypic characterization and identification of selected fungus

The selected inulinolytic fungus was characterized phenotypically by observing the macroscopic features on plates such as surface colour, texture, and diameter. Also, the microscopic characteristics were observed using a compound binocular microscope to view a lactophenol cotton blue-stained slide mounted with a small portion of the fungal mycelium. The spore type and shape, sporangia type, and hyphae structure were observed, recorded, and compared with standard fungal identification references (Gaddeya *et al*., 2012).

Molecular characterization and identification of selected fungus

The identity of the selected fungus was confirmed by molecular method. The total fungal genomic DNA was extracted from a 72-hour-old culture using the Zymo Research (ZR) Fungal/Bacterial DNA Miniprep™ kit (ZYMO RESEARCH, USA). Primers ITS 1 (5'-TCC GTA GGT GAA CCT GCG-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') were used to amplify the ribosomal gene (White *et al*. 1990). Amplification was carried out in a thermal cycler (Applied Biosystems) with reaction conditions: initial denaturation at 94 °C for 5 min, 36 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, elongation at 72 °C for 45 s, and followed by a final elongation step at 72 °C for 7 min and hold temperature at 10 °C. The amplified fragments were sequenced using a Genetic Analyzer 3130xl Sequencer (Applied Biosystems).

Collection and preparation of agro-wastes

The agro-wastes onion (*Allium cepa* L.), orange (*Citrus sinensis* L.), pineapple (*Ananas comosus*), and plantain (*Musa paradisiaca* L.) peels were obtained locally from the Fruit Market and processing sites in Ile-Ife, Nigeria. They were shredded and prepared by exhaustive washing with distilled water, dried at 60 °C for 48 h, and thereafter milled and sieved into 0.5 mm particle sizes. They were stored in clean, dried, airtight containers, maintained at a temperature of 4 °C (Ahmed *et al*., 2016).

Screening of agro-wastes as substrates for inulinase production

The different agro-wastes were screened for their suitability as a substrate for fungal growth, and inulinase production, under submerged fermentation conditions. They were each incorporated into the fermentation medium, as the sole carbon source, at 2.0% ($^{w}/_{v}$) concentration to replace the primary substrate inulin. After inoculation with the standardized fungal spore suspension (5.0 x 10^5 spores/mL), the flasks were incubated at 30 °C for 96 h. At the end of the incubation, the medium was filtered and the supernatant was assayed for inulinase activity. The agricultural waste that produced the highest activity was selected for use as substrate in the enzyme production process.

Proximate analysis of onion peel

The chemical and nutritional composition of the selected agro-waste onion peel was determined by using the method described by AOAC (2005). The components analyzed in the peel, in percentage (%), were the total carbohydrate, crude protein, lipid, crude fibre, moisture, and ash content.

Submerged fermentation for inulinase production

The fermentation medium for inulinase production was prepared inside Erlenmeyer flasks (250 mL) and contained 100 mL of medium composed of $NaNO_3$ (3.0 g), MgSO4.7H_2O (0.5 g), KH_2PO_4 (1.0 g), KCl (0.5 g), FeSO4.7H_2O (0.01 g), all dissolved in 100 ml distilled water. Onion peel (2.0 g) was added as the substrate (carbon source). The initial pH of the medium was adjusted to 5.0 and the culture medium was inoculated with standardized fungal spore suspension (5×10^5 spores/mL) and incubated at 30 ºC for 5 days. After incubation, the cultures

were filtered through glass fibre filter (Whatman GF/A), and the culture filtrate was used to estimate inulinase activity. Fermentations were carried out in triplicates.

Assay for inulinase activity

The dinitro salicylic acid (DNSA) assay method, described by Miller (1959), was used to determine the inulinase activity. The enzyme reaction mixture contained 0.1 mL enzyme extract and 0.1 mL 1.0% ($^{w}/_{v}$) inulin in sodium acetate buffer, pH 5.0, and incubated at 50 °C for 15 min. The enzyme reaction was terminated by adding a 2.0 mL dinitro salicylic acid (DNSA) reagent, and the mixture boiled for 5 min. The test tubes were allowed to cool down and the absorbance was read at 540 nm using the spectrophotometer. One unit of inulinase activity was defined as the amount of enzyme that produced 1.0 µmol of fructose per milliliter per minute under the assay conditions.

Effect of cultural parameters on inulinase production from selected fungus

Effect of incubation period on fungal growth and inulinase production

The effect of the incubation period on fungal growth and inulinase production in the fermentation medium was determined. The fermentation medium was inoculated with the standardized spore suspension of the elected fungus, and incubated at 30 °C for 120 h. At 24-hour intervals, the flasks were sampled, and the inulinase activity was assayed. Also, the fungal mycelial dry weights obtained after culture filtration, and drying at 70 °C, were estimated by using the weighing balance.

Effect of pH on production of inulinase

The effect of pH on inulinase production was determined by adjusting the pH of the fermentation medium to different levels 3.0 to 8.0. Each flask, containing medium adjusted to a specific pH level, was inoculated with standardized spore suspension (5 x 10^5 spore/mL) and incubated at 30 °C for 120 h. After incubation, the cultures were then filtered through the glass fibre filter paper, and the filtrate was assayed for enzyme activity.

Effect of temperature on the production of inulinase

The effect of temperature on the production of inulinase was studied by varying the incubation temperature of the fermentation culture from 25 to 60 °C. After incubation for 120 h, culture extract was assayed for enzyme activity.

Effect of inoculum size on inulinase production

Different fungal spore inoculum sizes (0.25, 0.5, 0.75, and 1.0 mL) were used in the fermentation medium. After incubation at 30 °C for 72 h, the culture extract was obtained and the enzyme activity was determined.

Effect of carbon sources on the production of inulinase

Different carbon sources (glucose, fructose, sucrose, lactose, maltose, lactose, starch, inulin, and onion peel), at 2.0% ($^{w}/_{v}$), were investigated for their effect on inulinase production from the fungus. Incubation was at 30 °C for 72 h.

Effect of different concentrations of onion peel on inulinase production

The fermentation medium was supplemented with various concentrations of onion peels (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% (w/v). At the end of incubation, enzyme activity was determined.

Effect of nitrogen sources on the production of inulinase

Different nitrogen sources were investigated for their effects on inulinase production by supplementing the fermentation medium with each of the following: ($NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, peptone, yeast extract, and urea). They were added at 3.0% ($^{w}/_{v}$) concentrations.

Statistical analysis

All the experiments were carried out in triplicates. The data were subjected to statistical analysis for determination of means and standard deviation, using SPSS version 16.

RESULTS

Isolation of fungi and screening for inulinase production

Nine fungal strains were isolated from samples collected from the soil of a Sugarcane Farmland in Obafemi Awolowo University, Ile-Ife, Nigeria. The nine fungal isolates were screened for their inulinase production, under submerged fermentation conditions. The fungal strain OY-3 exhibited the highest inulinase-production ability and was therefore selected for further studies (Table 1).

Table 1: Screening of fungal isolates for inulinase production.

S/N	Isolate code	Inulinase activity (U/mL)
1	OY-2	29.60 ± 0.47
2	OY-3	45.43 ± 0.56
3	OY-4	25.60 ± 0.57
4	OY-5	17.77 ± 0.35
5	OY-6	26.19 ± 0.47
6	OY-7	16.24 ± 0.41
7	OY-8	11.11 ± 0.58
8	OY-9	30.42 ± 0.57
9	OY-10	40.37 ± 0.69

Values are means of triplicate determinations ± standard deviation

Identification of selected fungus

The study fungus was identified by phenotypic method as a strain of *Aspergillus* sp. by observing its morphological characteristics on potato dextrose agar plates and its microscopic characteristics on a lactophenol cotton blue-stained slide mounted with a small portion of the fungal mycelium (Table 2). The identity of the fungus was confirmed by molecular method by sequencing the ITS region of the ribosomal DNA. The 600 bp fragment of the sequence showed 100% identity with other *A. oryzae* strains. This sequence was deposited in the GenBank of the National Center for Biotechnology Information (NCBI) under accession number PV069384.1. The study strain was therefore designated as *A. oryzae* OY-3. A phylogenetic tree was constructed based on the alignment of the sequences from the ribosomal genes from some *Aspergillus* species. Phylogenetic analysis revealed a close relationship between this species and other fungal species in the GenBank that have been implicated in inulinase production (Figure 1).

Table 2: Phenotypic characterization of inulinolytic fungal isolate.

Morphological characteristics	Microscopic characteristics	Probable identity
Fluffy, yellowish-brown growth with flat surface and lighter margin	Long conidiophores. Large, rough-walled, and radiate conidial head. Conidial globose	*Aspergillus* sp.

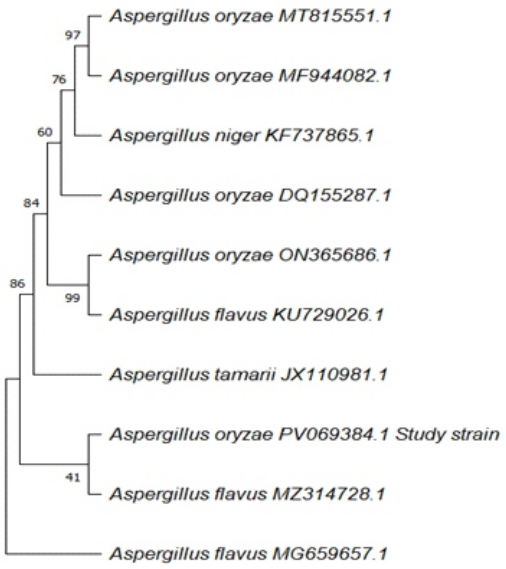

Figure 1: Phylogenetic relationship between *Aspergillus oryzae* OY-3 and other inulinase-producing *Aspergillus* strains in the Genbank. GenBank accession numbers are included.

Screening of agro-wastes as substrates for inulinase production from fungus

Inulinase production was observed with the use of all the agro-wastes, individually, as substrates for fermentation. However, onion peel was observed to be the best substrate for inulinase production from *A. oryzae* OY-3, with inulinase activity of 38.25 ± 0.85 U/mL, followed by the use of pineapple peel (33.90 ± 0.83 U/mL) as substrate (Figure 2).

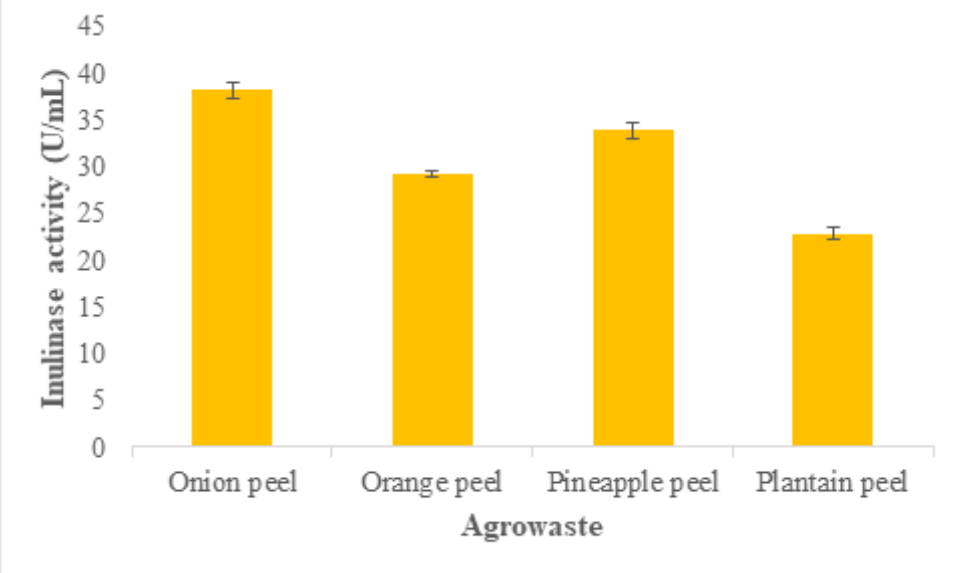

Figure 2: Screening of agro-wastes as substrate for inulinase production from *A. oryzae* OY-3.

Proximate analysis of onion peel

The percentage composition of the chemical and nutritional components of onion peel is presented in Table 3. The onion peel contained 57.30 ± 0.12% total carbohydrate, 1.53 ± 0.26% crude protein, 2.55 ± 0.52% lipid, 19.94 ± 0.88% crude fibre, 13.47 ± 0.55% moisture, and 6.20 ± 0.35% ash contents (Table 3).

Table 3: Proximate analysis of onion peel.

Nutrient	Percentage composition (%)
Ash	6.20 ± 0.35
Moisture	13.47 ± 0.55
Fibre	19.94 ± 0.88
Lipid	2.55 ± 0.52
Crude protein	1.53 ± 0.26
Total carbohydrate	57.30 ± 0.12

Values are means of triplicate determinations ± standard deviation

Effect of cultural parameters on inulinase production

Effect of incubation period on the fungal growth and inulinase production

The inulinase production from the fungus increased with the incubation period reaching a maximum at 72 h (46.44 ± 0.84 U/mL). Beyond this period, the enzyme production declined to 3.39 ± 0.74 U/mL at the end of the 120 h fermentation period (Figure 3). The fungal growth increased with the incubation period, in direct proportion to inulinase production, reaching a peak at 96 h (Figure 3).

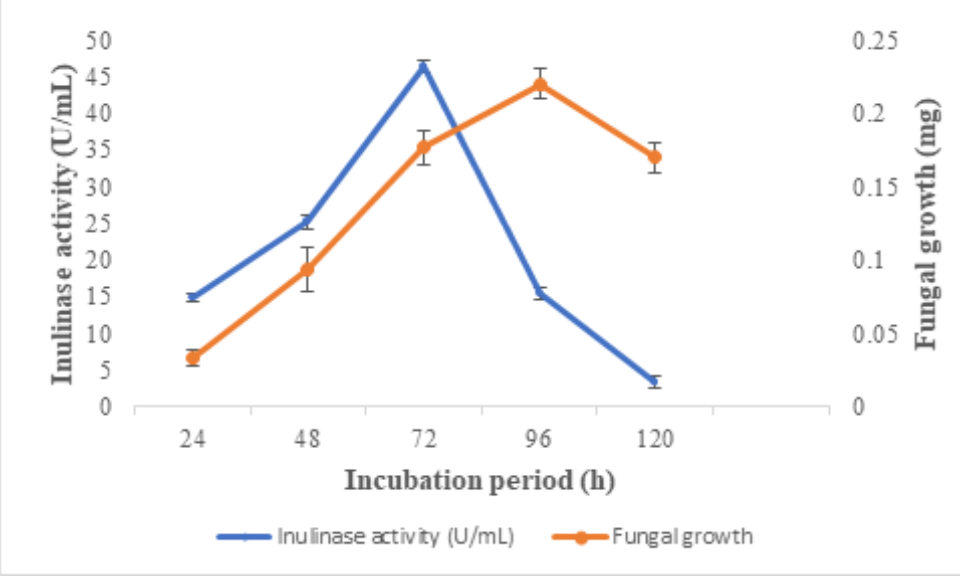

Figure 3: Effect of incubation period on fungal growth and inulinase production at 30 ºC and pH 4.0. Each value represents the mean of triplicate determinations standard deviation

Effect of pH on inulinase production from fungus
Aspergillus oryzae OY-3 produced the maximum level of inulinase at pH 4.0 (36.58 ± 0.60 U/mL). A gradual decrease was observed with increasing pH levels reaching the minimum value of 4.35 ± 0.36 U/mL at pH 8.0 (Figure 4).

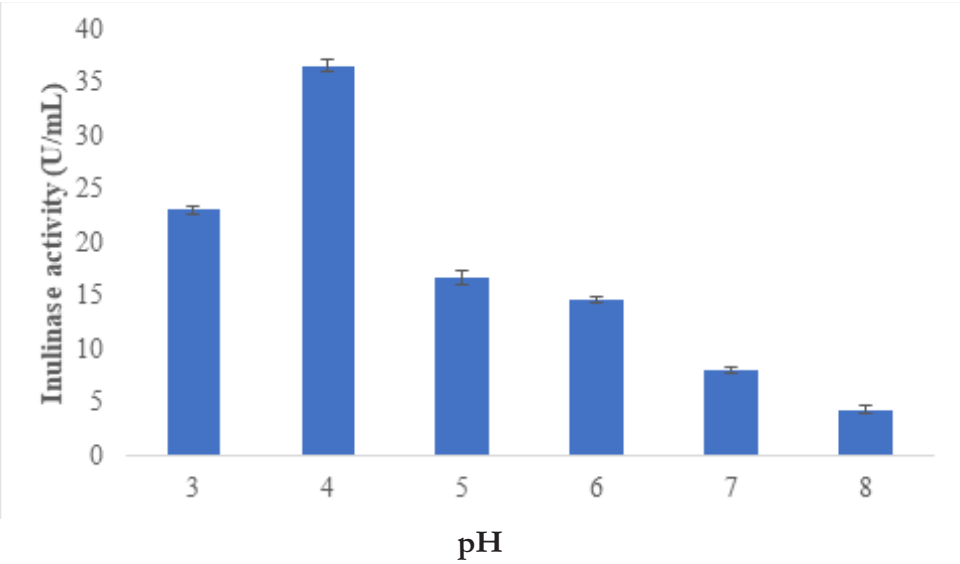

Figure 4: Effect of pH on inulinase production from *A. oryzae* OY-3 for 72 h at 30 °C. Each value represents the mean of triplicate determinations standard deviation

Effect of temperature on inulinase production
The maximum level of inulinase production from *A. oryzae* OY-3 was at 30 °C (40.21 ± 0.0.56 U/mL). As the incubation temperature increased beyond this level, enzyme production decreased. The minimum level of inulinase production was at 50 °C with inulinase activity 16.62 ± 0.51 °C (Figure 5).

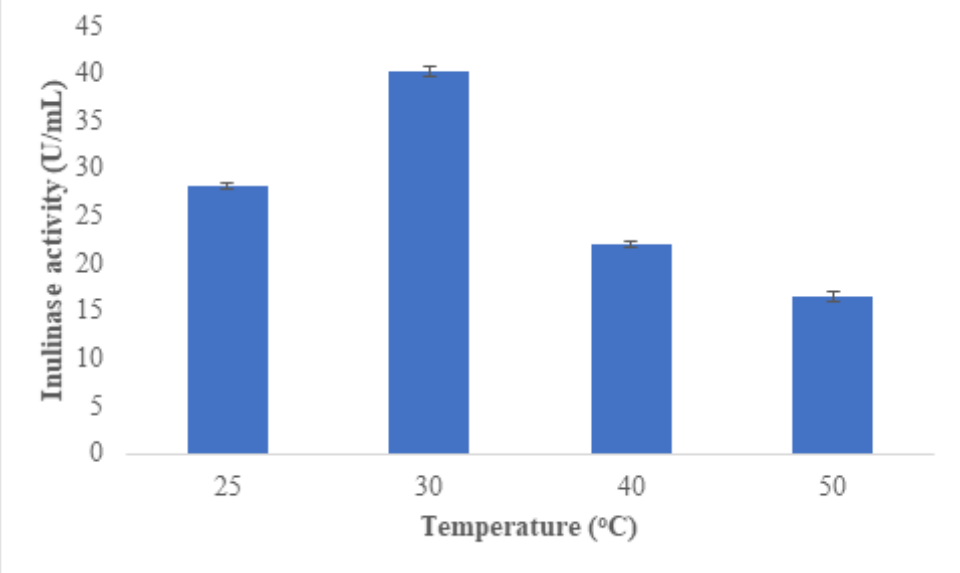

Figure 5: Effect of temperature on inulinase production from *A. oryzae* OY-3 for 72 h using onion peel as substrate.

Effect of inoculum size on inulinase production from fungus

Inulinase production from *A. oryzae* OY-3 was maximum when the fungal spore inoculum size 0.5 mL was used in the fermentation medium (44.21 ± 1.01 U/mL). Inulinase production decreased with a further increase in the fungal spore inoculum size (Figure 6).

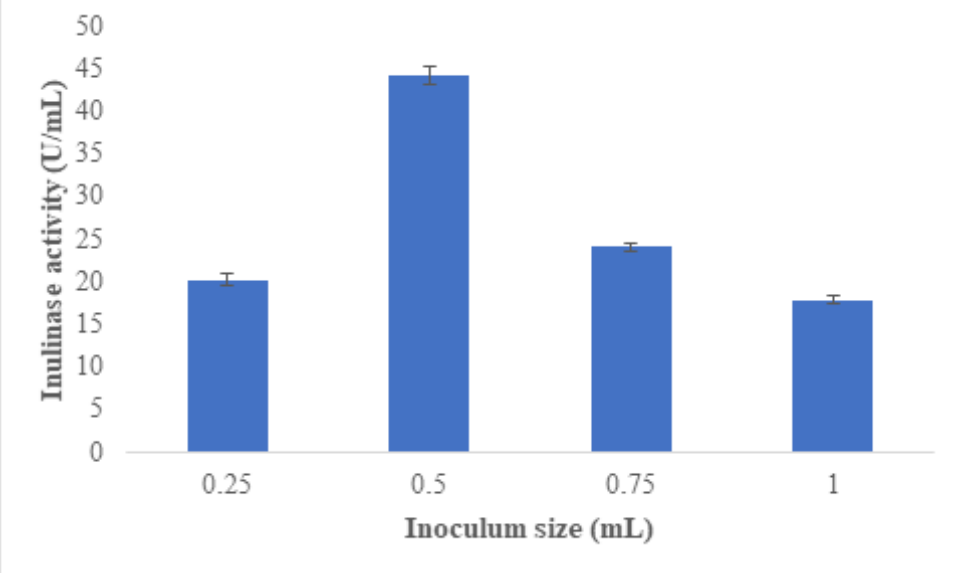

Figure 6: Effect of inoculum concentration on inulinase production from *A. oryzae* OY-3 for 72 h at 30 °C and pH 4.0

Effect of carbon sources on inulinase production

Maximum inulinase production from *A. oryzae* OY-3 was observed with inulin as the carbon source in the fermentation medium (37.67 ± 0.58 U/mL). This was followed by onion peel (30.00 ± 0.56 U/mL) as the carbon source. Minimal levels of enzyme production occurred with the use of glucose (6.50 ± 0.48 U/mL), and fructose (5.84 ± 0.45 U/mL) as carbon sources (Figure 7).

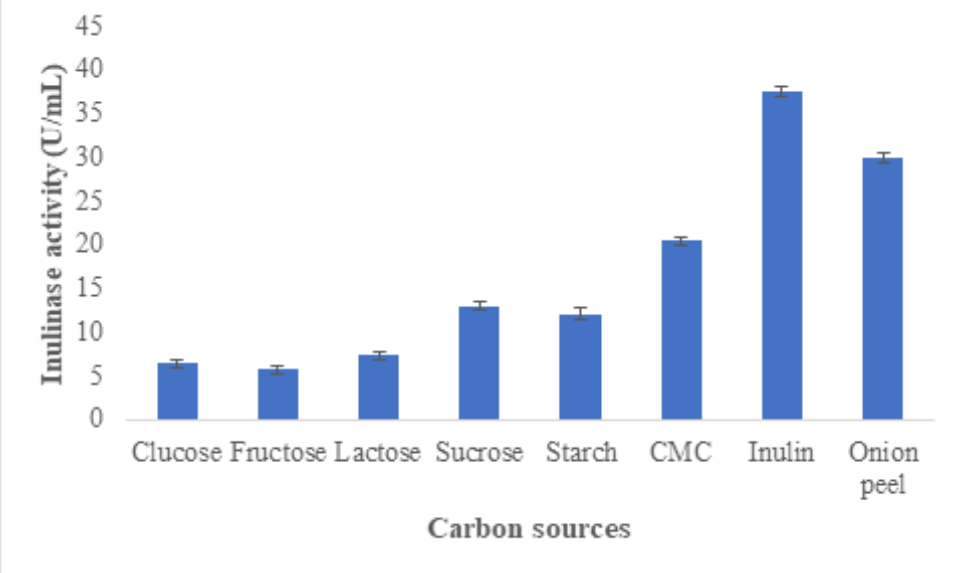

Figure 7: Effect of different carbon sources on inulinase production from *A. oryzae* OY-3 for 72 h at 30 °C and pH 4.0.

Effect of onion peel concentrations on inulinase production from fungus

Inulinase production from the fungus increased with an increase in onion peel concentration from 0.5% ($^{w}/_{v}$) to (2.0% ($^{w}/_{v}$) when it reached the maximum level (48.00 ± 0.46 U/mL). Inulinase production then declined as the onion peel concentration increased to 2.5% ($^{w}/_{v}$) (40.08 ± 0.41 U/mL) (Figure 8).

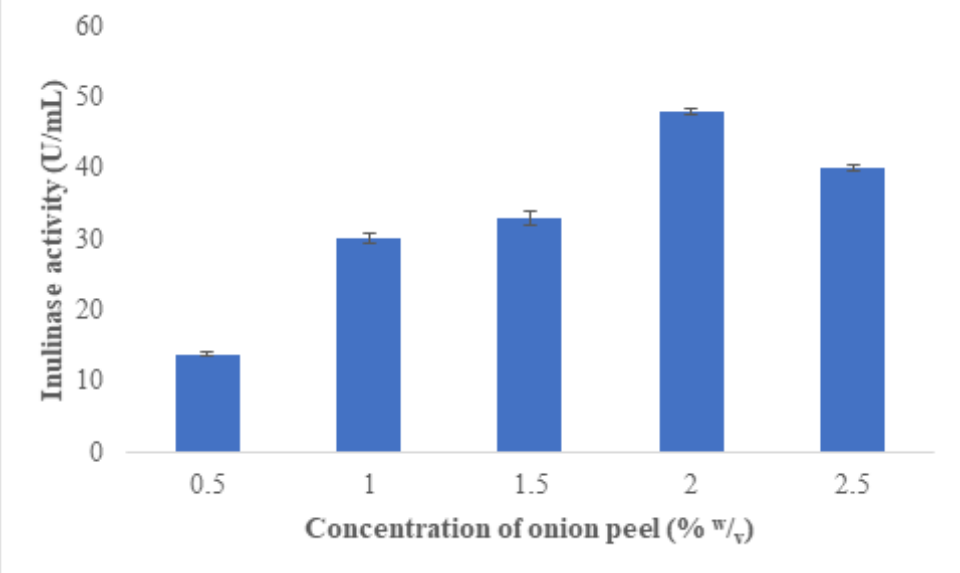

Figure 8: Effect of onion peel concentration on inulinase production from *A. oryzae* OY-3 for 72 h at 30 °C and pH 4.0

Effect of nitrogen sources on inulinase production from fungus

Peptone was the best nitrogen source for inulinase production from *A. oryzae* OY-3 (22.42 ± 0.44 U/mL), followed by urea (19.27 ± 0.59 U/mL). The minimum enzyme production was observed using ammonium sulphate as a nitrogen source (Figure 9).

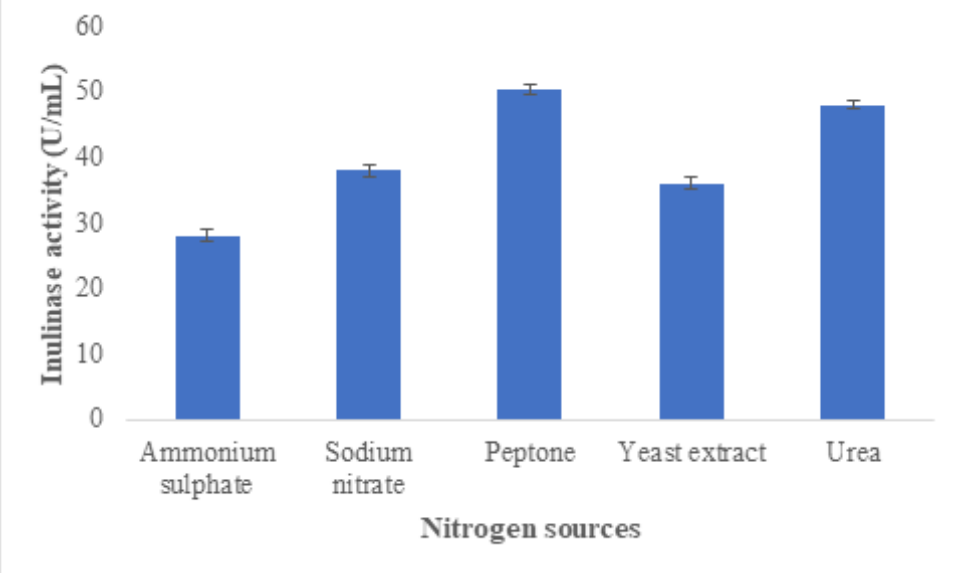

Figure 9: Effect of nitrogen sources on inulinase production from *A. oryzae* OY-3 for 72 h at 30 °C and pH 4.0

DISCUSSION

The search for new microbial strains is an important step in fermentation processes as it allows for the discovery of new organisms with better potential for producing the target product. Nine fungal strains were isolated from the soil of a Sugarcane Farmland in Obafemi Awolowo University, Ile-Ife, Nigeria. They were screened for their relative inulinase production abilities, under submerged fermentation conditions. The strain OY-3 was observed to exhibit the most appreciable inulinase production and was therefore selected for further study. The presumptive identification of the fungus by phenotypic method revealed it to be *Aspergillus* sp. OY-3. The identity of the fungus was confirmed by molecular method, and it was assigned as *Aspergillus oryzae* OY-3. The sequence was deposited to the GenBank with the accession number PV069384. *Aspergillus oryzae* has been described as an important biotechnological tool in the production of enzymes with potential applications in various industries (Ghoson *et al.*, 2021). The remarkable secretion machinery of the fungus enables it to secrete copious amounts of heterologous proteins into the culture medium (He *et al.*, 2014; Ghoson *et al.*, 2021). Several strains of *Aspergillus* species have been implicated in inulinase production such as *A. tamarii* U4 (Garuba and Onilude, 2020), *A. niger* (Ilgin *et al.*, 2020), *A. flavus* (Das *et al.*, 2019), *A. oryzae* (Ali and Shahzadi, 2015) and *A. oryzae* NRRL 2217 (Abdella *et al.*, 2023).

Onion peel is generated in large amounts as a result of onion bulb processing for use as an ingredient in food (Ly *et al.*, 2005). The peel is usually discarded into the environment where it constitutes environmental pollution hazards. The utilization of the peel for the production of value-added products such as industrial enzymes, will be desirable from the points of view of low-cost production, and environmental waste management. The proximate analysis of the onion peel revealed it to be abundant in chemical nutrients such as carbohydrates, lipids, protein, minerals, and water. Other researchers have reported the peel to possess high quantities of moisture, and nutrients such as carbohydrates, minerals, vitamins, and other compounds (Ifesan, 2017; Kumar *et al.*, 2022). These constituents will promote the growth of microorganisms resulting in the production of a significant yield of useful enzymes (Bennet *et al.*, 2002). Several agro-wastes have successfully been utilized as cheap substrates for inulinase production from *Aspergillus* species such as the use of wheat bran (Chen *et al.*, 2011; Ali and Shahzadi, 2015), banana peel, (Narayanan *et al.*, 2013), and chicory (Saber and El-Naggar *et al.*, 2009). The differences in the concentrations of the constituent nutrient molecules in the different agro-wastes give rise to differences in

their suitability as substrates for microbial growth and enzyme production. The agro-waste onion peel was observed to be the best substrate for inulinase production from the selected fungus. This could be as a result of the nutrient compounds in the agro-waste being in a relatively better form for utilization by the fungus for growth and enzyme production. The use of onion peel as a renewable substrate for microbial inulinase production is of significant interest from the point of view of low-cost enzyme production, and environmental pollution management.

Inulinase production from the test fungus was influenced by several parameters such as the incubation period. The enzyme production increased with an increase in the incubation period and fungal growth until it reached the maximum level at 72 h fermentation period. Thereafter, the enzyme yield declined probably due to the depletion of available nutrients in the medium, competition, and or the accumulation of toxic by-products of metabolism over time during the fermentation (Sindhu et al., 2009). This could also be as a result of enzyme denaturation. Similar results were obtained by Kumar *et al.* (2005) and Singh *et al.* (2018) who reported the optimum incubation period for inulinase production as 72 h from *Aspergillus niger* AUP19 and *Mucor circinelloides* BGPUP-9, respectively. The fungal growth was observed to increase further beyond the peak period of enzyme production up till 96 h. This could be as a result of the availability of nutrients in the medium, and the fungus still being at the exponential stage of growth.

The pH of 4.0 was optimum for inulinase production from *A. oryzae* OY-3 which is consistent with the report of a study conducted by Sharma *et al* (2016). However, Saryono *et al.* (2015) reported the pH of 5.0 as being optimum for inulinase production from the endophytic fungus *Aspergillus clavatus* (BB5). Extreme pH values negatively impact inulinase production, possibly due to undesirable changes in enzyme conformation or altered enzyme-substrate interactions (Sharma *et al.* 2016).

The optimum incubation temperature for inulinase production from the fungus was 30 °C. This is similar to the reported production of inulinase from a fungal species isolated from rotten garlic samples (Surti and Mhatre, 2021). Deviations from the optimum temperature level can affect enzyme stability and substrate binding, leading to the overall reduction of enzyme yield.

Inulin was observed to be the best carbon source for inulinase production from the fungus followed by onion peel. Inulin has been reported to be the best substrate for inulinase production from different fungal strains (Kalra and Kumari, 2017; Surti and Mhatre, 2021). Inulin, the primary substrate of inulinase, likely promotes enzyme production by inducing the expression of the relevant microbial genes (Singh *et al.*, 2019). The use of glucose and fructose as carbon sources only produced a minimal yield of the enzyme which could result from the catabolite repression of the enzyme production.

Peptone was observed to be the most suitable nitrogen source for inulinase production from *A. oryzae* OY-3. However, the use of yeast extract, as a nitrogen source, led to the maximum level of inulinase production from a strain of *Aspergillus niger* (Kalra and Kumari, 2017). Organic nitrogen sources are reported to be more suitable inducers of fungal growth and enzyme production than inorganic nitrogen sources (Arotupin and Ogunmolu, 2012). The hydrolysis of the organic nitrogen probably led to the release of the constituent mineral nutrients and growth factors in forms more easily metabolized by the fungus.

The fungal spore inoculum size of 0.5 mL resulted in the maximum level of inulinase production from the fungus. The use of an appropriate inoculum size is of prime consideration in fermentation studies for the achievement of maximum enzyme production (Singh and Bhermi, 2008). Higher levels of inoculum size can result in increased competition, rapid depletion of nutrients, and subsequent reduction in enzyme production. On the other hand, lower levels of inoculum size may result in a longer lag phase of growth and insufficient biomass, limiting enzyme synthesis (Singh and Bhermi, 2008).

CONCLUSION

Inulinase has broad biotechnological applications in diverse industrial sectors such as the food,

confectionery, pharmaceutical, and biofuel. *Aspergillus oryzae* OY-3, isolated from the soil of a Sugarcane Farmland in Obafemi Awolowo University, Ile-Ife, Nigeria, was observed to be highly inulinolytic with efficient inulinase-production ability. The agro-waste onion peel was used as a cheap, and renewable substrate for the low-cost production of the industrially-important enzyme from the fungus, under submerged fermentation conditions. The cultural conditions eliciting optimum *A. oryzae* OY-3 inulinase production were an incubation period of 72 h, initial pH 4.0, incubation temperature 30 ^{o}C, inoculum size 0.5 mL/100 mL, and onion peel concentration (2.0% $^{w}/_{v}$) and peptone as the nitrogen source. This study has demonstrated inulinase production from a locally isolated fungus *A. oryzae* OY-3 using onion peel as an alternative, low-cost substrate, and the effectual management of a pollution source in the environment.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Oyedeji O.: Conceptualization; Supervision; investigation; methodology; data curation; formal analysis; manuscript preparation. Oki, K. D.: Methodology, data curation; formal analysis; resources; manuscript preparation. Arowolo, T. B.: Methodology; data curation; formal analysis; resources; manuscript preparation.

ACKNOWLEDGEMENTS

Authors appreciate the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria, for the provision of the research environment and use of the available technical assistance and facilities for the study.

REFERENCES

Abdella, A., Al-Saman, M., Abou-Elazm, F. I., El-Far, S. W. (2023). *Rhizopus oryzae* inulinase Production and characterization with application in chicory root saccharification. *Microbiology Research*, 14:297-315.

Ahmed, I., Zia, M. A., Azhar, M., Akram, Z., Naveed, I., Nowrouzi, A. (2016). Bioprocessing of Citrus waste peel for induced pectinase production by *Aspergillus niger*, its purification and characterization. *Journal of Radiation Research and Applied Science*, 9(2):148-154.

Ali, S., Shahzadi, H. (2015). Nutritional optimization for improved exo-inulinase production from *Aspergillus oryzae* for high fructose syrup preparations. *International Journal of Current Microbiology and Applied Sciences*, 4:618-631.

AOAC (2005) Official methods of analysis of the Association of Official Analytical Chemists (18th Edition). AOAC: Gaithersburg, Md., USA (2005).

Arotupin, D. J., Ogunmolu, F. E. (2012). Screening of fungal isolates from Nigerian tar sand deposit in Ondo state for novel biocatalysts. *Journal of Biological Sciences*, 12(1):57-61.

Bennet, J. W., Wunch, K. G., Faison, B. O. (2002). Use of Fungi in Bioremediation. In: Manual of Environmental Microbiology, 2nd Edition, Hurst, C. J., Crawford, R. L., Garland, J. L., Lipson, D. A., Mills, A. L., Stetzenbach, L. D. (Eds.). ASM Press, Washington DC. pp. 960-971.

Celano, R., Docimo, T., Piccinelli, A. L., Gazzerro, P., Tucci, M., Di Sanzo, R., Carabetta, S., Campone, L., Russo, M., Rastrelli, L. (2021). Onion peel: Turning a food waste into a resource. *Antioxidants*, 10:304.

Chen, H. Q., Chen, X. M., Chen, T. X., Xu, X. M., Jin, Z. Y. (2011). Optimization of solid-state medium for the production of inulinase by *Aspergillus ficuum* JNSP 5-06 using response surface methodology. *Carbohydrate Polymers*, 86:249-254.

Chi, Z. M., Zhang, T., Cao, T. S., Liu, X. Y., Cui, W., Zhao, C. H. (2011). Biotechnological potential of inulin for bioprocesses. *Bioresource Technology*, 102:4295-4303.

Dapper, T. B., Arfelli, V. C., Henn, C., Simoes, M. R., Dos Santos, M. F., Della Tore, C. L., Da Conceicao Silva, J. L., De Cassia Garcia Simao, R., Kadowaki, M. K. (2016). B-fructofu-ranosidase production by *Aspergillus versicolor* isolated from Atlantic Forest and grown on apple pomace. *African Journal of Microbiology Research*, 10(25):938-948

Das, D., Bhat, R., Salvaraj, R. (2020). Optimization of inulinase production by a newly isolated *Penicillium amphipolaria* strain using solid-state fermentation of hardy sugarcane stems. *Biocatalysis and Agricultural Biotechnology*, 30:101875.

Das, D., Selvaraj, R., Bhat, M. R. (2019). Optimization of inulinase production by a newly isolated strain *Aspergillus flavus* var. *flavus* by solid state fermentation of *Saccharum arundinaceum*. *Biocatalysis and Agricultural Biotechnology*, 22:101363.

De Oliveira, L. P. A., De Oliveira, T. F., Contiero, J., Cazetta, M. L. (2016). Agave syrup as a substrate for inulinase production by *Kluyveromyces marxianus* NRRL Y-7571. *Acta Scientiarum. Biological Sciences*, 38(3):283-289.

Di Donato, P. Fiorentino, G., Anzelmo, G., Tommonaro, G., Nicolaus, B., Poli, A. (2011). Re-use of vegetable wastes as cheap substrates for extremophile biomass production. *Waste Biomass Valorization*, 2:103-111.

El-Hadi, A. A., El-Nour, S. A., Hammad, A., Kamel, Z. and Anwar, M. (2014). Optimization of cultural and nutritional conditions for carboxymethylcellulase production by *Aspergillus hortai*. *Journal of Radiation Research and Applied Sciences*, 7(2014):23-28.

Gaddeya, G., Niharika, P. S., Bharathi, P., Kumar, K. P. R. (2012). Isolation and identification of soil microflora in different crops field at Salur Mandal. *Advance Applied Science Research*, 3:2020-2026.

Garuba, E. O., Onilude, A. A. (2020). Exoinulinase production by a catabolite repression-resistant Mutant thermophilic *Aspergillus tamariii* -U4 in solid state fermentation. *Biotechnology Journal International*, 24:21-31.

Ghoson, M. D., Faten, A. M., Waill, A. E. (2021). The ancient koji mold (*Aspergillus oryzae*) as a modern biotechnological too. *Bioresources and Bioprocessing*, 8:52.

Gracida-Rodriguez, M. A., Goncalves, H. B., Furriel, R. D., Jorge, J. A. and Guimaraes, L. H. S. (2014). Characterization of the co-purified invertase and beta-glucosidase of a multifunctional extract from *Aspergillus terreus*. *World Journal of Microbiology and Biotechnology*, 30:1501-1510.

He, M., Wu, D., Wu, J., Chen, J. (2014). Enhanced expression of endoinulinase in *Aspergillus niger* by codon optimization in *Pichia pastoris* and its application in inulooligosaccharides production. *Journal of Industrial Microbiology and Biotechnology*, 41:105-114.

Ifesan, B. O. T. (2017). Chemical composition of onion (*Allium cepa*) peel and its ability to serve as a preservative in cooked beef. *International Journal of Science and Research Methodology*, 7(4):1-10.

Ilgin, M., Germec, M., Turhan, I. (2019). Inulinase production and mathematic modelling from carob extract by using *Aspergillus niger*. *Biotechnology Progress*. https://doi.org/10.1002/btpr.2919.

Ilgın, M., Germec, M., Turhan, I. (2020). Statistical and kinetic modeling of *Aspergillus niger* inulinase fermentation from carob extract and its partial concentration. *Industrial Crops and Products*, 156:112866.

Kalra, K., Kumari, R. (2017). Isolation and production of inulinase from banana peel by using *Aspergillus niger* under submerged fermentation. *International Journal of Scientific Research Publications*, 7(3):454-461.

Kango, N., Jain, S. C. (2011). Production and properties of microbial inulinases: Recent Advances. *Food Biotechnology*, 25:165-212.

Karam, E. A., Kansoh, A. L., Moharam, M. E., Hassan, M. E., Kansoh, A. L. (2018). Immobilization of inulinase produced by *Rhizopus oligosporous* NRRL 2549 for continuous fructose Kproduction. *Journal of Material and Environmental Science*, 9(8):2315-2321.

Kumar, G. P., Kunamneni, A., Prabhakar, T., Ellaiah, P. (2005). Optimization of process parameters for the production of inulinase from a newly isolated *Aspergillus niger* AUP19. *World Journal of Microbiology and Biotechnology*, 21:1359-1361.

Kumar, V., Satnanayana, C. S. (2018). Inulinases: Industrial biocatalysts for the production of biologically active oligosaccharides. In: Pandey, C, L., Soccol, C. R. (Eds), *Current Developments in Biotechnology and Engineering: Industrial Biotechnology and Commodity Products*. p 123-141.

Kumar, M., Barbhai, M. D., Hassan, M., Punia, S., Dhumal, S., Radha, R. N., Chandran, D., Pandiselvam, R., Kothakota, A., Tomar, M., Santankar, V., Senapathy, M., Anitha, T., Dey, A., Sayed, A. A. S., Gadallah, F. M., Amarowicz, R., Mekhemar, M. (2022). Onion (*Allium cepa* L.) peels: A review on bioactive compounds and biomedical activities. *Biomedicine and Pharmacotherapy*, 146:112498.

Ly, T. N., Hazama, C., Shimoyamada, M., Ando, H., Kato, K., Yamauchi, R. (2005). Antioxidative compounds from the outer scales of Onion. *Journal of Agriculture and Food Chemistry*, 53:8183-8189.

Mensink, M. A., Frijlink, H. W., van der Voort Maarschalk K., Hinrichs, W. L. J. (2015). Inulin, a flexible oligosaccharide I: review of its physicochemical characteristics. *Carbohydrate Polymer*, 130:405-419.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31:426-442.

Mohammed, S. A., Sabah, H. A., Moharam, M. E., Foda, M. S., Fahmy, A. S. (2015). Chracteterzation of two thermostable inulinases from *Rhizopus oligosporous* NRRL 2710. *Genetic Engineering and Biotechnology*, 13:65-69.

Narayanan, M., Srinivasan, B., Gayathiri, A., Ayyadurai, A., Mani, A. (2013). Studies on the optimization and characterization for the biosynthesis of inulinase under solid-state fermentation. *International Journal of Chemical Technology Research*, 5(1):376-384.

Nath, S., Kango, N. (2022). Recent developments in industrial mycozymes: A Current Appraisal. *Mycology*, 13:81-105.

Saber, W. I. A., El-Naggar, N. E. (2009). Optimization of fermentation conditions for the biosynthesis of inulinase by the new source, *Aspergillus tamarii,* and hydrolysis of some inulin-containing agro-wastes. *Biotechnology*, 8:425-433.

Saryono, C. A. M., Ardhi, A., Pratiwi, N. W. (2015). Production, purification and characterization of inulinase from dahlia rizhosphere-isolated *Aspergillus clavatus*, *Journal of Chemical and Pharmaceutical Research*, 7(9S):165-176.

Sharma, V., Chandel, A. K., Singh, O. V. (2016). Role of nutritional and environmental factors in the production of inulinase: a review. *Food Technology and Biotechnology*, 54(4):375-391.

Sindhu, R., Suprabha, G. N., Shashidhar, S. (2009). Optimization of process parameters for the production of amylase from *Penicillium janthinellum* (NCIM 4960) under solid state fermentation. *African Journal of Microbiology Research*, 3(9):498-503.

Singh, R. S., Bhermi, H. K. (2008). Production of extracellular exoinulinase from *Kluyveromyces marxianus* YS-1 using root tubers of *Asparagus officinalis*. *Bioresources Technology*, 99(15): 7418-7423.

Singh, R. S., Chauhan, K. (2017). Inulinase production from a new inulinase, *Penicillium oxalicum* BGPUP-4. *Biocatalysis and Agricultural Biotechnology*, 9:1-10.

Singh, R. S., Chauhan, K., Kaur, R., Kaur, R. (2018). Inulinase production in shake flask fermentations from *Mucor circinelloides* BGPUP-9. *Journal of Applied Biology and Biotechnology*, 606:18-25.

Singh, N., Raiv, V., Gupta, M. (2019). Enhanced production of inulinase from *Aspergillus niger* using response surface methodology and its application in fructose production. *Biocatalysis and Agricultural Biotechnology*, 20:101217.

Singh, R. S., Singh, R. P. (2017). Inulinases. In: Current developments in Biotechnology and Bioengineering: Production, Isolation, and Purification of Industrial Products. Elsevier B. V. Amsterdam, Netherlands. p423-446.

Surti, A., Mhatre, S. (2021). Optimization of inulinase production by a fungal species isolated from rotten garlic samples. *Journal of Applied Biotechnological Reports*, 8(2):164-171.

Treichel, H., Mazutti, M. A., Maugeri, F., Rodrigues, M. I. (2009). Use of a sequential study of experimental design to optimize the inulinases production in a batch bioreactor. *Journal of Industrial Microbiology and Biotechnology*, 36(7):895-900.

White, T. J., Bruns, T. D., Lee, S. B., Taylor, J. W., Innis, M. A., Gelfand, D. H. and Sninsky, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenets. In: PCR Protocols: A Guide to Methods and Applications, (Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. eds). pp. 315-322.

Zhang, L. L., Tan, M. J., Liu, G. L., Chi, Z., Wang, G. Y., Chi, Z. M. (2015). Cloning and characterization of an inulinase gene from the marine yeast *Candida membranifaciens* subsp. *flavirogenie* W14-3 and its expression in *Saccharomyces* sp. WO for ethanol production. *Molecular Biotechnology*, 57:337-347.