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Original article

Bioconversion of Libyan Date Palm Kernels into Glucose Using Trichoderma reesei Enzymatic Hydrolysis and Process Optimisation

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Abstract
The date palm (*Phoenix dactylifera* L.) is recognised as one of the largest evergreen fruit plants, often found in subtropical, dry, and semi-dry areas. It grows widely throughout diverse regions of Libya, thanks to its good economic and dietary value. Date palm cultivation is sizeable in the United States of America, supported by the availability of suitable soil and irrigation water. There are about 7 million date palm trees in Libya, of which no more than 5 million are fruit-bearing, with an annual fruit production of about 70,500 heaps. The primary regions wherein these trees develop are the central, southern, and coastal regions; studies have indicated that the central region is the most suitable area for date palm cultivation in Libya. The date pit, also known as the seed, is a solid, rectangular-shaped body, tapered at both ends, and located in the middle of the fruit. It weighs between 0.5 and 3 grams, measures 12–20 mm in length, and makes up 10–15% of the whole weight of the fruit. Experiments have proven the viability of date pits at the side of other by means of-merchandise from date-processing centres as raw materials for various finished and semi-finished business products. Date pits are composed largely of hemicellulose, which may be transformed into dextrose via the procedures of acid or enzymatic hydrolysis. They comprise 14.1% reducing sugars and 15.89% total sugars. Date pits are used in animal feed, and the oil extracted from them is applied in several industries, which includes cleaning soap manufacturing. Furthermore, this oil is appropriate for human consumption and possesses more oxidative stability than most other vegetable oils.
Keywords: Trichoderma sp.; Solid State Fermentation; Libyan Date Palm Kernels; Glucose Production.

Introduction

Date pits are a by-product of the date fruit. Their production has seen a notable increase, coinciding with the expansion of date palm cultivation across various regions in Libya. The processes of sorting and processing dates generate pits and other agricultural residues that can be utilised rather than discarded. Libya's date production reached approximately 187,039 tons [1]. Recent studies have explored the potential of repurposing these residues, particularly the pits, to derive beneficial components and create value-added products [2]. These local resources have gained significant importance recently, with research highlighting variations among different date varieties—specifically regarding their composition, including moisture and protein content [3]. Date pits account for 10–15% of the weight of date fruits and contain 5.9–6.7% protein, 2.43–4.65% carbohydrates, and 22.5–80.2% dietary fibre [4]. It is possible to extract cellulose, hemicellulose, and lignin from them. The fungus *Trichoderma reesei* is a significant producer of cellulolytic enzymes, the production of which is influenced by cultivation conditions [5].

Beta-glucosidases are essential for completing the conversion of cellulose degradation products into glucose; their addition enhances the release of glucose from cellulose [6]. The production of glucose from date pits using enzymes derived from *Trichoderma reesei* has reached 32.31 g/L, achieved through the fermentation of date pits using a co-culture [7]. This study aims to investigate and evaluate the efficiency of *Trichoderma reesei*, laying the groundwork for future research specifically concerning Libyan date waste. Date pits, in particular, represent a promising resource for sustainable bioprocessing, capable of transforming local agricultural waste into high-value glucose.

Methodology

A commercial isolate of the fungus *Trichoderma reesei* with the potential to produce the enzyme cellulase was obtained. The culture medium consisted of a mixture of 155 mL of V8 vegetable juice, 4 grams of agar, and 75 mL of distilled water. The components were thoroughly mixed, and the pH was adjusted to 5.00. The solution was then heated to 122°C for 20 minutes. After allowing the medium to cool to room temperature, it was poured into Petri dishes. *Trichoderma reesei* was inoculated onto the prepared medium and incubated for 6 days at 27°C (culture maintenance), with 7 hours of daily light exposure. The fungus was observed to have grown across the entire surface of the plates. A small piece (1.0 mm^3) was then taken from the culture and transferred to new Petri dishes containing the previously prepared growth medium.

Preparation of date palm kernel samples

Fresh date palm kernel samples were obtained from a palm plantation. These fresh samples were divided into two equal portions; the first portion was placed directly into containers and stored in a refrigerator at 4°C for later use. The thoroughly dried date palm kernels were ground and sieved using a mechanical shaker equipped with sieves to obtain particles with a size of 250 micrometres for use in subsequent evaluations.

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Solid-state fermentation (SSF) of palm kernels using Trichoderma reesei fungi

Solid-state fermentation was conducted using fungi on 6 grams of palm kernel substrate, with the addition of 2 mL of Mandel's medium. Mandel's medium was formulated with the following additives (g/L): urea 0.4, peptone 0.85, yeast 0.25, ammonium sulfate ($(NH_4)_2SO_4$) 1.4, potassium dihydrogen phosphate (KH_2PO_4) 2.0, calcium chloride ($CaCl_2$) 0.3, and magnesium sulfate ($MgSO_4 \bullet 7H_2O$) 0.3. Additionally, trace elements (mg/L) included ferrous sulfate ($FeSO_4 \bullet 7H_2O$) 5, manganese sulfate ($MnSO_4 \bullet 4H_2O$) 1.6, zinc sulfate ($ZnSO_4 \bullet 7H_2O$) 1.4, and cobalt chloride ($CoCl_2 \bullet 6H_2O$) 20 [8]. The subculture medium and trace elements were sterilised in glass flasks using an autoclave set at 123°C for 15 minutes. Once the culture medium had cooled to room temperature, a specific quantity of the sterilised trace elements was added to it. The substrate was also sterilised in an autoclave at 123°C for 15 minutes and then left to cool to room temperature; subsequently, the palm kernel substrates were inoculated with fungal suspensions containing a concentration of 3×10^8 spores/mL. A control sample was also prepared in the same manner as the other samples, but without the addition of any fungi. All samples, including the controls, were kept in an incubator for 6 days at the temperatures evaluated (20, 30, and 40°C; see Figure 2) [9].

Measurement of Glucose Concentration

To measure the glucose concentration in the culture medium, a stock glucose solution with a concentration of 10 mg/mL was prepared (approximately 10 mL). From this stock solution, a series of dilutions was prepared with distilled water to obtain glucose standards of 20, 40, 60, 80, and 100 µg/mL, following standard laboratory protocols. The phenol–sulfuric acid method was applied in the same way to each standard and to the culture sample: a 0.5 mL aliquot was drawn with a pipette and placed into a separate flask, and 0.5 mL of 5% phenol was added, followed by 0.5 mL of concentrated sulfuric acid (H_2SO_4). The mixtures were left for 10 minutes to allow the reaction to occur, shaken to ensure thorough mixing, and then left for a further 30 minutes for the colour to develop. Finally, the absorbance of the coloured mixture was measured at a wavelength of 490 nm using a spectrophotometer.

Results

As shown in the scanning electron microscopy (SEM) photographs in Figure 1, colonies of the fungus *Trichoderma reesei* blanketed the surface of palm kernel substrates, both those containing oil and those from which the oil was eliminated following a 6-day incubation period at 30°C, with 7 hours of daily light exposure. Notably, the conidiophores (spore-bearing structures) appeared thicker on the oil-free substrates. This indicates that the culture conditions were favourable for fungal growth, although substrates with a lower oil content appeared to better support such growth. A particle size of 250 µm was selected for the palm kernel cake (PKC) substrates, based on preliminary experiments demonstrating that this size allows for the maximum recovery of residual oil during the supercritical fluid extraction (SFE) process.



Figure 1. Scanning electron microscopy (SEM) images of a Trichoderma reesei isolate grown on palm kernel-based substrates, following 6 days of incubation at 30°C with 7 hours of daily light exposure.

Figure 2 illustrates the effect of incubation temperature, pH, and fermentation time in solid-state fermentation (SSF) on the glucose concentration produced by *Trichoderma sp.* fungal cultures when using palm kernels. Increasing the incubation temperature from 20°C to 30°C increased glucose concentration from 16.5 to over 18.5 µg/mL; conversely, raising the temperature further to 40°C led to a decrease in glucose concentration, reaching levels similar to those recorded at 20°C (below 15 µg/mL). At a pH of 3, glucose concentrations were recorded at approximately 18.4 µg/mL. Increasing the pH of the incubation medium to 5 resulted in an incredible rise in glucose concentration to about 21.2 µg/mL; however, further increasing the pH led to a decline in glucose concentration, returning it to 18 µg/mL. Meanwhile, altering the incubation length from 4 to 8 hours, and later to 12 hours, resulted in substantial adjustments in the glucose concentration within the medium. However, increasing the incubation time from 12 to 16 hours ended in a sharp and significant decrease in glucose concentration, from about 19.3 to 18 µg/mL.

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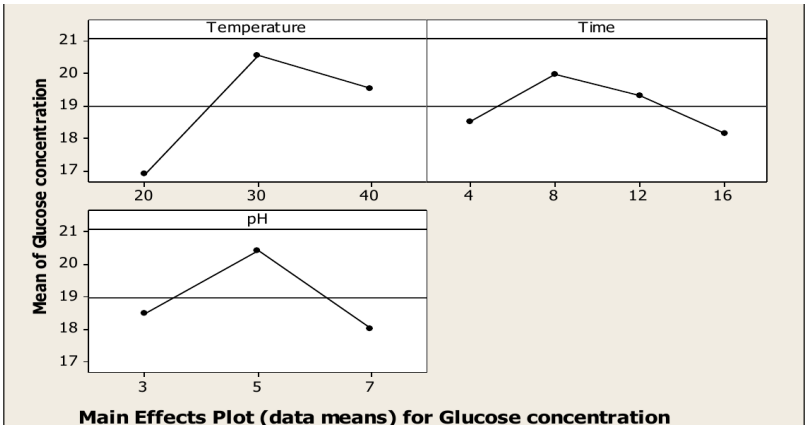

Figure 2. Effect of incubation temperature, time, and pH on the solid-state fermentation (SSF) of a palm kernel substrate using a Trichoderma reesei isolate, in terms of glucose concentration (µg/mL)

Discussion

It was found that colonies of the fungus *Trichoderma reesei* completely blanketed the surface of palm kernel substrates after 6 days of incubation at 30°C, the most reliable temperature amongst the ones tested, because it yielded the highest glucose concentration under a daily 7-hour light exposure [10]. Furthermore, *Trichoderma reesei* has been shown to produce cellulase and xylanase enzymes all through solid-state fermentation (SSF) the usage of coconut residues and wheat bran [11]. These studies addressed the manufacturing of cellulase and xylanase enzymes by way of *Trichoderma reesei* using lignocellulosic substrates through solid-state fermentation (SSF).

It was discovered that the optimum temperature for fungal growth is 28°C, which additionally yields the best cellulase enzyme activity. Furthermore, in a study examining the physiological characteristics of *Trichoderma reesei* isolated from oyster mushrooms (*Pleurotus* spp.), Al-Qassab et al. [12] demonstrated that the optimal temperature for the growth of *Trichoderma reesei* ranges between 27°C and 30°C. This aligns with the conditions observed in the present study, supporting the conclusion that the selection of 30°C, one of the incubation temperatures tested in this experiment, falls within the acceptable range for optimal fungal growth. Temperature is a critical factor in the growth of fungi, particularly those of the genus *Trichoderma reesei*. Studies on the effect of various temperatures on the mycelial growth of this genus have shown that growth increases with higher temperatures, peaking at 30°C, whereas no growth was observed at 15°C [13]. Furthermore, as cited via [14], optimising moisture supply during solid-state fermentation using *Trichoderma reesei* RUT-C30 resulted in a 1.7-fold increase in CMCase activity and a 2.3-fold increase in FPase activity in comparison to the traditional system. The pH level additionally plays a crucial position in fungal growth [12]; *Trichoderma reesei* fungi select conditions starting from acidic to impartial (pH 5–7) for optimal growth.

Typically, glucose is produced with the aid of *Trichoderma reesei* fungi through the acid or enzymatic hydrolysis of starch present in the substrates upon which fungi grow. In many other elements of the arena, glucose is by and large produced via the enzymatic hydrolysis of cereal starch [15]. In another study [16], soil-associated microorganisms, the fungus *Aspergillus niger* and the bacterium *Streptomyces* sp., were used to provide the enzymes glucoamylase and glucoisomerase, respectively. These have been used to transform cassava starch into a syrup containing glucose and fructose, thereby highlighting the potential of microorganisms in sugar manufacturing. Date pits offer vast capacity if utilised optimally for the commercial manufacturing of glucose and other sugars. As an agricultural resource, they are distinctly promising for the manufacturing of biochemical compounds and sugars, given their lignocellulosic composition, which includes cellulose, hemicellulose, and lignin. Al-Saidi et al. [17] explored a powerful technique for generating glucose through solid-state fermentation (SSF) using the fungus *Trichoderma reesei* for the bioprocessing of date pits.

Conclusions

The results of the contemporary observational study show the promising capability of the use of Libyan date pits for bioconversion strategies and glucose manufacturing, employing the fungus *Trichoderma sp*. The findings indicate that an incubation temperature of 30°C is the optimal condition among those tested; at this temperature, the fungal colonies were able to cover the entire surface of the substrate within six days, coinciding with the highest recorded glucose concentration.

Conflict of interest. Nil

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