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Date Palm fronds as a promising feedstock to produce single-cell protein for the enrichment of animal feed

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Abstract

The growing global population represents a major challenge for decision-makers to provide food in a sustainable way. This situation requires serious efforts to find new and sustainable food sources by making better use of all available agricultural materials and reusing them to support global food supply chains. One of these sources is the date palm industry, which produces both primary products like dates and secondary by-products that are usually underutilized. Among these by-products is the large amount of date palm fronds, which are generated in high quantity during date production.

In this study, the focus was on increasing the benefit of Barhi date palm fronds, one of the most popular date varieties in Saudi Arabia. The goal was to extract complex sugars from the fronds and make them available as a carbon source for the growth of *Saccharomyces cerevisiae* yeast. The resultant increase in yeast biomass can be used to enrich animal feed with protein.

The sugars were released from the fronds by alkaline pretreatment using different concentrations of sodium hydroxide (NaOH), at temperatures between 60–100°C, and for treatment times of 30 to 60 minutes. The best sugar release was achieved at 2% NaOH concentration, 100°C, for 30 minutes using 1% ground raw material. The yeast successfully grew on the released sugars and showed an increase in dry weight, indicating high protein production. These results suggest that date palm fronds are a promising and sustainable source for single-cell protein (SCP) production.

Keywords: SCP; Fronds; Feed; Palm

1. Introduction

As the global population increases steadily and is expected to reach about 10 billion persons by 2050 (1) , there is an urgent need to meet its future food requirements. Livestock feed should afford a significant amount of protein to contribute to the buildup of tissue consumed later as meat by the public (2). Traditional plant crops such as soybean are normally used for this purpose. However, this is not sustainable for future needs due to limited land and water resources. Therefore, an alternative source of protein for feedstock is needed.

Single-cell proteins (SCP) are produced by fermentation of bio-products, such as plants, food processed materials and agricultural waste, by different microorganisms, such as fungi, algae, bacteria and yeast (2–9). The resultant organisms will contain high protein content, which can be applied as a supplement for animal feed. *Saccharomyces cerevisiae* yeast, is commonly used to produce SCP from various agricultural wastes (9) including potato peels (5), pineapples, bananas, apples (4), guava, cashew (6), wheat bran (10) oranges (7,11) and others.

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In addition to the agro-wastes mentioned, by-products from palm trees also offer potential as substrates for SCP production. There are two primary types of palm trees. The first is the oil palm tree (*Elaeis guineensis*), which originates from Africa and Southeast Asia, such as Indonesia and Malaysia. This type of palm tree is known for having 90% of its oil extracted from the mesocarp (12)

The second type is the date palm tree (*Phoenix dactylifera* and other species). Although it originates from Iraq, Saudi Arabia, Bahrain, and the United Arab Emirates, it is notably rich in sugars (13). According to Bourgis and colleagues (2011), a comparison using transcriptomic tools reveals significant differences in metabolites between the oil palm tree and the date palm tree, with the oil palm tree being less suited for carbohydrate accumulation. Research shows that syrup extracted from 35 samples of date palm fronds contain 66% carbohydrates, including glucose, fructose, and sucrose (14). Additionally, it has been found that the total carbohydrates in the waste leaf sheath of date palm fiber constitute approximately 73% of the dry weight, with glucose making up 45% of this amount (15)

The latest published data by the Saudi General Authority for Statistics reported cultivation of more than 37,160,827 palm trees for the production of dates in 2023(16), which make the kingdom of Saudi Arabia at the top list of the largest producer of fruiting dates globally. Cultivation of palm trees is an essential tradition in the Kingdom of Saudi Arabia (KSA), and nearly a third of dates produced around the world are of Saudi origin. This level of production, locally and globally, is usually accompanied by massive amounts of by-products as well as waste, including seeds of dates and palm tree leaves or fronds, which account for the bulk of solid wastes. Palm tree fronds contain complex sugars, particularly cellulose and hemicellulose (17). These complex sugars are made of basic units of the simple sugar glucose. Glucose can be readily used by microorganisms, such as *S. cerevisiae* yeast, as a nutritional component (8). The massive amounts of agricultural waste produced by the date farms in KSA present a potential use for these wastes to generate SCP. The produced SCP from fronds can be added to animal feed and will massively decrease feed imports and cultivation of high protein crops used as animal feed. This study aimed to test the feasibility of using date palm tree fronds to produce SCP by *S. cerevisiae* yeast.

2. Material and methods

All chemicals and reagents were purchased from Sigma-Aldrich.

2.1. Collection, processing and preparation of fronds

Barhi variety fronds were collected from a date farm in Makkah, KSA. Fronds were incubated at 60°C for 5 days until dried completely. Dried leaves of fronds were chopped by hand and cutting tools to small pieces. Chopped leaves were grounded by a home grinding machine to a fine material then sieved through 0.5mm steel mesh sieve.

2.2. Extraction and measurement of total sugars

Total sugars were extracted by following the protocol of Mirsiaghi et al. (18) with minor modifications. Briefly, 100 mg of ground frond material was mixed with 1 ml of 72% H_2SO_4 in glass test tube and incubated at 30°C for 1 hour. The mixture was transferred to a glass beaker and 26.4 ml distilled water was added to dilute the acid to a concentration of 4%, followed by heating at 121°C in an autoclave for 1 hour. Once cooled down, the pH was neutralized by NaOH. The resultant content was spun down in a centrifuge at 10,000 rpm to separate the supernatant (containing dissolved sugars) from other residues. An aliquot of the supernatant was taken for total carbohydrate analysis while the rest was stored in the fridge until further analysis.

Total carbohydrates were estimated by 3,5-dinitrosalicylic acid (DNS) as described earlier Miller (19) with modifications by King et al. (20). Briefly, glucose stock solutions at different concentrations (0, 0.25, 0.5, 1, 1.5, 2 and 5 mg/ml) were prepared in distilled water. The stocks and samples (extracted supernatants) were mixed with the DNS reagent, followed by heating at 95°C for 20 minutes. After cooling, the developed color was measured by a spectrophotometer at 540 nm. The measurements from the glucose stocks were used to plot a calibration curve, which was used to determine the concentration of the samples as depicted below.

2.3. Thermal and chemical treatment of ground frond material

To identify the optimal treatment for releasing constituent carbohydrates from palm fronds, ground frond material was suspended in different concentrations of NaOH (0, 0.5, 0.75, 1 or 2%) in distilled water. For all treatments, the concentration of ground fronds was 1% (w/v). Mixtures were incubated in a water bath set to 60, 80 and 100°C for 30, 45 and 60 minutes. Samples were collected from each time point at each concentration of NaOH, followed by centrifugation at 10,000 rpm. The total amount of released sugars were assayed in the supernatant by anthrone reagent

(21) as follows. Different glucose stocks (0-1 mg/ml) were prepared in distilled water and used to plot a calibration curve. A 100 µl aliquot from each stock was mixed in glass test tube with 1 ml anthrone reagent (0.2 g dissolved in 100 ml H_2SO_4 and chilled for at least 2 hours before use), followed by incubation at 100°C for 16 minutes. The developed color ranged from light green for low glucose concentrations to very dark green for high concentrations. After cooling at room temperature, the developed color was measured by a spectrophotometer at 630 nm. The calibration curve was used to estimate the unknown concentrations of released glucose from different treatments of ground frond material.

2.4. Selection of the optimal treatment for producing single-cell protein by yeast

Data analysis showed that treatment of fronds with 2% NaOH at 100°C for 30 minutes was the optimal for releasing sugars from fronds. A volume of 300 ml of 1% ground frond material in 2% NaOH was incubated at 100°C for 30 minutes. After cooling, the solution was filtered by filter paper to remove debris. Alkalinity of the solution was lowered to pH 5.5 by diluted H_2SO_4, followed by further filtration. The solution was autoclaved at 121°C for 15 min, followed by inoculation by yeast, *Saccharomyces cerevisiae*. To assess the effect of extracted sugars on the growth of yeast, 5% (w/v) of dried yeast was suspended in sterile water and used as negative control. Samples and control flasks were incubated on an orbital shaker 150rpm at 28°C and volumes of 5 ml were collected regularly in pre-weighted glass test tubes then dried in an oven to measure the produced mass of yeast after incubation.

2.5. Yeast Maintenance and Culture

Saccharomyces cerevisiae, was utilized in this study. A 1% (w/v) suspension of lyophilized *S. cerevisiae* was prepared aseptically in sterile distilled water. Using an inoculating loop, the suspension was streaked onto Yeast Extract Malt Extract Peptone Dextrose (YMPD) agar plates, which contained the following composition per liter: yeast extract (3 g), malt extract (3 g), peptone (5 g), D-glucose (10 g), and agar (15 g). The plates were then incubated at 28°C for 5-7 days to allow for the development of individual colonies. Once developed, the yeast colonies were maintained on YMPD slant agar at 4°C and subcultured onto fresh YMPD agar every two weeks.

2.6. Yeast inoculum preparation for treated fronds

A single colony of *S. cerevisiae* grown on YMPD agar was aseptically transferred to a conical flask containing 50 mL of YMPD broth. The flask was then incubated on an orbital shaker at 150 rpm for 24 hours at 28°C, allowing the yeast to reach the log phase of growth. Subsequently, 1.5 mL aliquots of the yeast broth were transferred into microfuge tubes and centrifuged at 12,000 rpm for 5 minutes. The supernatant was discarded, and the pelleted biomass was washed aseptically twice with sterile distilled water to remove excess sugars and salts.

2.7. Determining the suitability of alkali-treated palm fronds for growing *S. cerevisiae*

A conical flask containing 50 mL of sterilized, NaOH-treated palm fronds was inoculated in triplicate with washed *S. cerevisiae* inoculum at an optical density of 0.1 OD600, maintaining the final volume at 50 mL. In parallel, YMPD broth flasks were used as positive controls, while flasks containing only sterile distilled water served as negative controls. All control flasks were inoculated in the same manner as the flasks containing treated palm fronds. The flasks were transferred to an orbital shaker at 150 rpm and incubated at 28°C. Growth was monitored regularly by assaying the developed dry weight over time. At predetermined intervals, 1 mL of the cultivated yeast was withdrawn aseptically and transferred into pre-weighed dry glass tubes. The samples were dried at 60°C in an oven until a constant weight was achieved. The net dried weight was calculated by subtracting the weight of the dried tubes from their initial weight.

2.8. Statistical analysis

All measurements were performed in triplicate. The presented results here are the mean of triplicates ± standard error of the mean (SEM) produced by GraphPad Prism version 8.02 for Windows (GraphPad Software, La Jolla, California, USA).

3. Results and discussion

3.1. Total sugars in ground fronds

The total amount of sugars in ground fronds was 1.71 mg/ml. As the starting amount of frond material was 100 mg, the concentration of starting material was 3.33 mg/ml, translating to a concentration of 51.4% w/w. This means more than a half of the component of frond is sugar. This makes the fronds of date palm tree a promising feedstock for industries based on carbohydrates extraction, such as SCP. Although this finding is related to fronds of the specific species used in this study (Barhi), it is in line with the previous studies those analyzed the metabolite content of date palm trees

components and found that carbohydrates are ranged from nearly 49 up to 65 %. Palm tree is considered as lignocellulosic materials, where those materials contained mainly of cellulose (28-51%), hemicellulose (8-31%) and lignin (12-44%) (15,22,23). As long as those materials entrapped monomers of glucose, they can be utilized by *S. cerevisiae* once liberated successfully for producing SCP.

3.2. The effect of different treatments on releasing sugars from frond material

The obtained results demonstrated a general increasing trend in the release of sugars across all treatments with varying concentrations (Figure 1–3). The highest amount of released sugars among all treatments (p <0.05) was observed under the condition of 2% NaOH for 30 minutes at 100°C (Figure1).

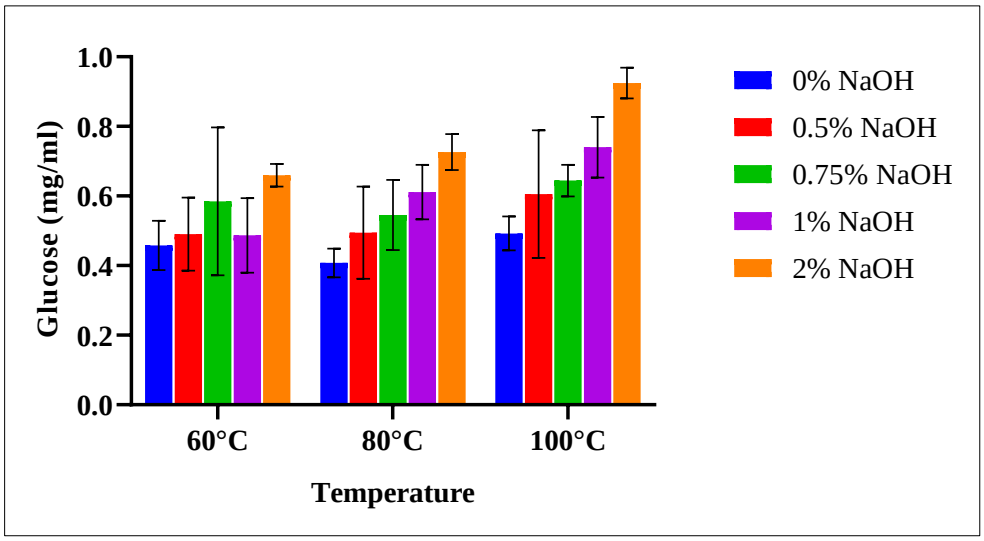

Figure 1 Amount of released sugars (mg/mL), determined using a glucose standard curve, from 1% (w/v) ground fronds of date palm treated with different NaOH concentrations for 30 minutes. Each data point represents the mean ± SEM of three replicates

Furthermore, sugar release increased proportionally (p <0.05) with rising NaOH concentration. However, when the residence time was extended; the amount of released sugars slightly decreased when the treatment duration was increased from 30 minutes (Figure1) to 45 and 60 minutes (Figure2 and3, respectively).

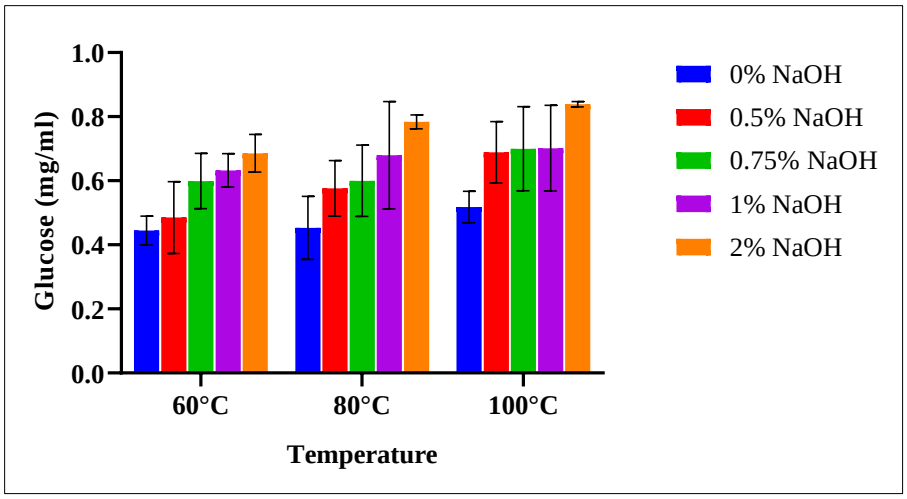

Figure 2 Amount of released sugars (mg/mL), determined using a glucose standard curve, from 1% (w/v) ground fronds of date palm treated with different NaOH concentrations for 45 minutes. Each data point represents the mean ± SEM of three replicates

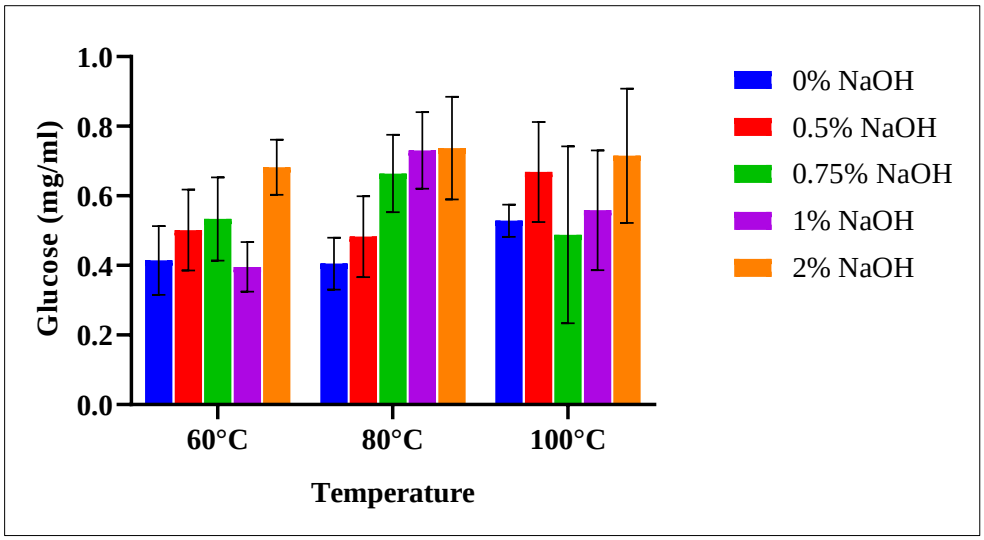

Figure 3 Amount of released sugars (mg/mL), determined using a glucose standard curve, from 1% (w/v) ground fronds of date palm treated with different NaOH concentrations for 60 minutes. Each data point represents the mean ± SEM of three replicates

This reduction may be attributed to the potential degradation of carbohydrates caused by prolonged exposure to NaOH at elevated temperatures. Previous studies support these observations. For example, extended pretreatment of wheat straw was shown to reduce the yield of released reducing sugars due to the dissolution of cellulose and hemicellulose components (24). Similarly, excessive residence time during NaOH treatment of sugarcane bagasse at 121°C led to a decline in hemicellulose content, suggesting possible carbohydrate degradation (25). Based on current findings, there is a critical importance of optimizing NaOH concentration, temperature, and pretreatment duration. Such optimization is essential for achieving effective delignification while minimizing carbohydrate losses in future studies involving the pretreatment of date palm fronds.

3.3. Yeast growth on released sugars from frond material and protein amount

The released sterilized sugars was used to assess its suitability as feedstock to grow yeast as a source of protein. Figure 4 shows the mass of yeast grown on released frond sugars. This increase in mass seems to continue as long as sugar was still available for use by the yeast.

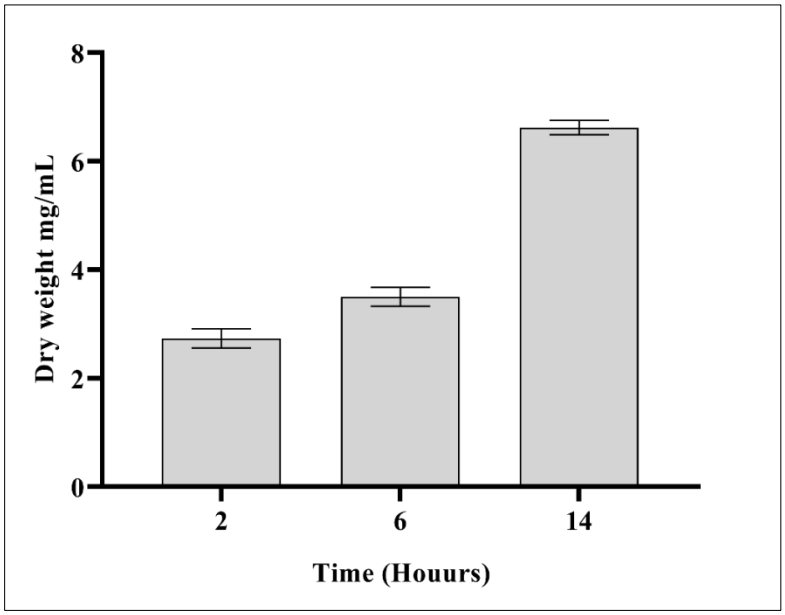

Figure 4 Dry mass (mg/ml) of produced *S. cerevisae* grown on sugars released from treated palm fronds. Each data point represents the mean ± SEM of three replicates

The dry weight measurement is a basic indicator for biomass production, and the increase in this parameter suggests that the yeast was metabolically active and able to multiply during the incubation period. The growth could be attributed to the availability of fermentable sugars like glucose, fructose, or sucrose which are often present in plant-derived hydrolysates (26). Dry weight is commonly used as a parameter in metabolic studies to estimate the overall cellular content in microorganisms, including *Saccharomyces cerevisiae*. The total amount of proteins in *S. cerevisiae* is a significant fraction of the accumulated metabolites, as proteins are integral to the cellular structure and function. This makes dry weight a reliable surrogate for quantifying protein levels when measuring overall biomass in yeast cultures.

Several studies have indicated that proteins, being one of the major components of cellular biomass, represent a substantial portion of the accumulated metabolites in *S. cerevisiae*. Proteins account for approximately 45-60% of the total dry weight in yeast cells which confirms that the total protein content is closely correlated with the total dry weight measurements, making it an effective method for estimating protein accumulation without requiring direct protein quantification methods (27–29).

However, it is important to mention that no total protein quantification was performed in this study. Measuring the total protein could provide more understanding about the quality of yeast biomass, since protein content is often considered as a nutritional and functional parameter especially in industrial applications (30). In future work, including such biochemical measurements could enhance the interpretation of yeast growth performance on palm frond sugar medium

4. Conclusion

In this study, various alkaline pretreatment conditions using NaOH were applied to release entrapped sugars from date palm fronds. These sugars were intended for utilization by *Saccharomyces cerevisiae* to produce single-cell protein (SCP). The released sugars obtained from the fronds of palm tree was used as a carbon source to cultivate *S. cerevisiae*. The results showed that the dry weight of yeast biomass increased gradually along the time course. This increase indicates that the sugar content present in palm frond extract can support the growth of *S. cerevisiae*. The resulting biomass can be lyophilized and further processed to be used as a supplement in animal feed, with the aim of enhancing the protein content of dietary formulations.

Overall, the results are promising and suggest that palm fronds can be converted into a valuable resource such as SCP, contributing to waste valorization and sustainable bioprocessing.

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