

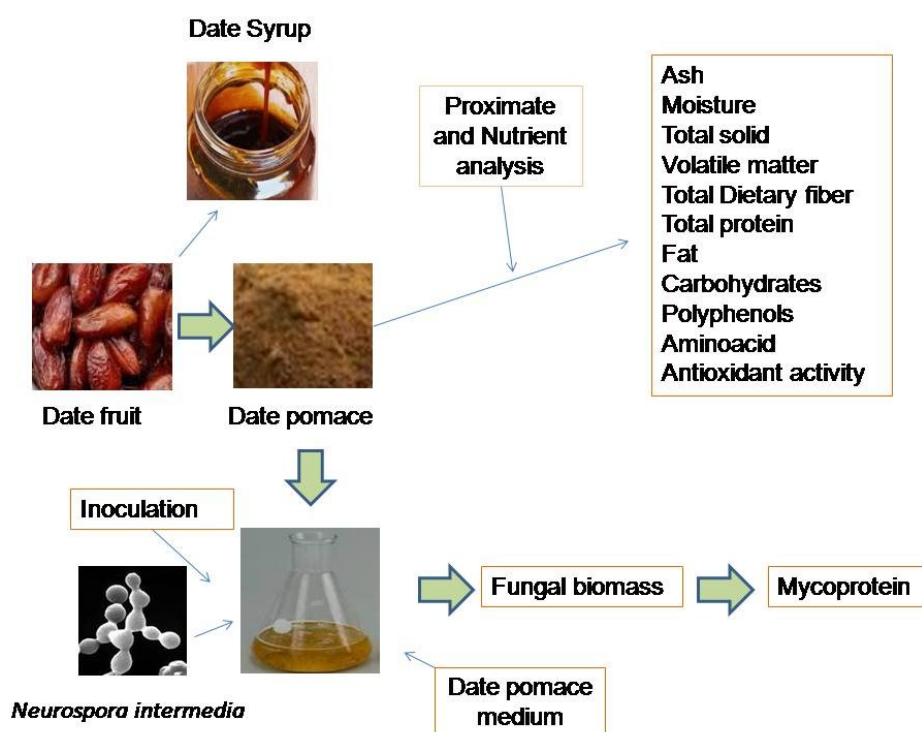
From Waste to Wealth: Valorizing Date Fruit Pomace for Enhanced Fungal Biomass and Mycoprotein from the Edible Fungus, *Neurospora intermedia*

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GRAPHICAL ABSTRACT



From Waste to Wealth: Valorizing Date Fruit Pomace for Enhanced Fungal Biomass and Mycoprotein from the Edible Fungus, *Neurospora intermedia*

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Waste valorization *via* edible fungus represents a novel transformative approach for single-cell protein production. The present study focused on the utilization of date fruit pomace for the cultivation of edible fungus *Neurospora intermedia*. A total of six date fruit varieties were used for this study: shalabi, barhi, khudri, sukkari, mabroom, and khalas. Chemical analysis revealed that date fruit pomace contained 1.6 ± 0.3 to $3.1 \pm 0.3\%$ ash, 6.4 ± 1.1 to $9.1 \pm 1.2\%$ moisture, 71.2 ± 1.1 to $91.2 \pm 0.9\%$ total solid content, a maximum of $27.3 \pm 0.2\%$ volatile matter, and $45.3 \pm 1.1\%$ total dietary fiber. The nutrient-rich barhi date variety was used as the substrate without any pretreatment for the bioconversion of protein-rich *N. intermedia* biomass. The optimum pH, temperature, and incubation time improved fungal biomass production ($p < 0.05$). The culture medium was statistically optimized *via* a central composite design. The optimized culture conditions improved the mycelial biomass 2-fold, and all the selected factors (pH, temperature, and incubation time) were significantly different ($p < 0.01$). The culture was filtered, and the fungal biomass was dried. The dried fungal biomass contained $17 \pm 2.1\%$ protein, $3.6 \pm 0.1\%$ carbohydrate, and $0.91 \pm 0.02\%$ fat.

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Keywords: Date fruit pomace; Valorization; Culture medium; Fungal biomass; Single-cell protein; Mycoprotein

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INTRODUCTION

The date palm family includes more than 200 genera and more than 2,500 species. The date palm (*Phoenix dactylifera* L.) is an important species, and more than 3,000 cultivars have been reported worldwide (Barakat and Alfheaid 2023). Date palm plays a critical role in meeting food security requirements, and this has led to its being called the “tree of life”. It provides fruit, construction material, and animal feed, and is used as an alternative medicine (Alu’datt *et al.* 2025). The global date production reached 9.7 million tons in 2024, and the major contributors are Saudi Arabia, Iran, Algeria, Iraq, Pakistan, Oman, Sudan, the United Arab Emirates, and Tunisia (FAOSTAT 2024). Date fruits are rich in carbohydrates, dietary fiber, vitamins, minerals, unsaturated fatty acids, and phytochemical compounds (Jaouhari *et al.* 2024). Currently, approximately 5000 date palm varieties are cultivated across the world and are classified on the basis of ripening time, fruit texture, and commercial value (Alu’datt *et al.* 2025).

Date fruits are consumed dry or fresh and are generally converted into value-added products such as pastes, syrups, jams, sugars, juices, jellies, and fermented products (Alam *et al.* 2023). The date palm processing industry generates many byproducts, especially pomace and seeds, which are often underutilized despite their functional properties. While a significant amount of harvested date fruit is consumed fresh, lower-quality date fruit are utilized in the food processing industry to produce products such as jelly, juice, jam, syrup, and powder (Al Farsi and Lee 2008). Overripening, transportation, rough handling, contamination, improper storage, and inadequate packing can cause >20% of production to be lost during the postharvesting process (Nancib *et al.* 2015). Date residues are used as animal feed and are rich in dietary fibers, carbohydrates, and phenolic compounds, making them preferable feedstocks for the conversion of value-added products (Rambabu *et al.* 2020).

Date fruit pomace is composed of a significant amount of residual sugars, and approximately 35% of soluble sugars are available in the form of fructose and glucose (Haris *et al.* 2023). Date fruit pomace is used as a substrate for the production of lactic acid (Haris *et al.* 2023), endopectinase (Bari *et al.* 2010), and single-cell proteins (Hashem *et al.* 2014). The bioconversion of agro-industrial residues into value-added products can save natural resources, minimize environmental pollution, and conserve natural ecosystems. The microbial conversion of agro-industrial residues is an alternative approach to produce industrially useful products.

Filamentous fungi is a group of microorganisms, including mushrooms, and moulds, are useful for the microbial recycling of biomass generated in the postharvesting industry. The large-scale heterologous production of animal protein is possible *via* fungi, thus reducing global greenhouse gas emission and land use (Humpenöder *et al.* 2022). Solid-state fermentation (SSF) utilizes solid wastes for the production of fermentable products and can reduce greenhouse gas emissions and land use (Arokiyaraj *et al.* 2025; Alarjani *et al.* 2024). This method requires minimal inputs and equipment. *Neurospora* is considered an important commercial filamentous fungus that has been extensively used for the production of value-added products *via* the bioconversion of agricultural waste. *N. intermedia* produces various enzymes that degrade cellulosic materials and produce sugar molecules; thus, it can be utilized for the production of essential amino acids, fats, and proteins.

It is hypothesized that *N. intermedia* can be cultured in SSF using date fruit pomace medium to improve nutrient profile. The excessive consumption of red meat is associated with an increased risk of various diseases (Qian *et al.* 2020). In recent years, research has focused on the production of microbial protein rather than insect protein, plant-based protein, or cultured meat (Gamarra-Castillo *et al.* 2022). Among these sources, microbial protein has gained increased interest from fungal species, especially filamentous fungi. Fungal strains, such as *Neurospora crassa* (Bartholomai *et al.* 2022), *Rhizopus oligosporus* (Furlan *et al.* 2024), and *Aspergillus oryzae* (Gamarra-Castillo *et al.* 2022), have been identified.

The optimum process parameters, such as medium pH, temperature, time, agitation, and inoculum, improve product formation. The optimum culture conditions varied with respect to the substrate, nutrient composition, and type of microbial strain. Hence, the optimization of growth parameters for the production of single-cell proteins is needed. The objective of the study is to analyze the nutrient profile and phytochemical compounds of date fruit pomaces. Then, barhi date fruit pomace was used as a low cost substrate for the production of mycoprotein using *Neurospora intermedia*.

EXPERIMENTAL

Date Fruit Pomace Processing

Date fruit pomace is a residual product of date syrup processing industries. It consists of a mixture of a blend of date fruits and often a mixture of various fruit varieties. The date syrup processing steps included washing, depitting, homogenizing in water, heating at 90 °C for min, pressing, filtration, and evaporation. The major byproducts of date syrup are seeds and date fruit pomace. A total of six date fruit varieties were used for this study: shalabi, barhi (rutab), khudri (khudry), sukkari (sukkary), mabroom, and khalas. The date syrup was removed, and the date fruit pomace was washed with water six times. The soluble sugar was removed from the sample, and the washed date fruit pomace was dried at 60 °C for 12 h in a hot air oven. The dried pomace was subsequently ground to powder, packed and stored in a desiccator until further use.

Proximate Component Analysis

The proximate chemical composition of the date fruit pomace was evaluated *via* the AOAC method (1995). The fat content of each sample was analyzed *via* acid hydrolysis, followed by extraction with petroleum ether in a Soxhlet apparatus. The total protein content of each sample was tested according to the Kjeldahl procedure, and a conversion factor of 5.3 was used to convert nitrogen to crude protein. The total ash and solid contents were analyzed according to the methods of the National Renewable Energy Laboratory. To determine the total solid content, the sample was heated at 105 °C until a constant weight was reached. The ash content was determined by heating the sample at 600 °C for 4 h in a muffle furnace. The dried crucible was weighed, and the weight difference was calculated (Sluiter *et al.* 2008). The moisture content of the sample was estimated from the total solid content. The fixed carbon content of the sample and the volatile matter content were determined according to the ASTM D3175 (2020) method (Sait *et al.* 2012). To determine the volatile matter content, the crucible was filled with 2 g of date fruit pomace and heated in a furnace to 950 °C for 7 min. The crucible was cooled, and the volatile matter content was calculated. The sum of ash, moisture, and volatile matter was subtracted from the value of 100, and fixed carbon was determined.

Date Fruit Pomace Minerals

The mineral content of date fruit pomace was determined. Minerals, such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and sodium (Na) were detected as described previously by Heckman *et al.* (1971). Approximately 50 g of date fruit pomace was heated at 600 °C in a furnace for 4 h. Then, it was cooled and further boiled with 3 N HCl for 10 min. It was cooled, filtered, and used for mineral analysis. The mineral content was analyzed *via* inductively coupled plasma–mass spectrometry (Model ICP–MS 7900, Agilent Technologies, Waldbronn, Germany).

Analysis of Amino Acids

The amino acid content of date fruit pomace was tested as described by Alfaro-Viquez *et al.* (2018). The essential amino acid content of the date pomace was analyzed. The relative amino acid composition of date pomace was estimated by treating date fruit pomace with hydrogen peroxide, formic acid, and phenol and hydrogen peroxide (6 M). The amino acids were detected by photometric detection at 440 and 570 nm using ninhydrin reagent.

Total Phenolic Compound Analysis

The total phenolic content (TPC) of each sample was determined as described by Sobti *et al.* (2023) *via* Folin–Ciocalteu reagents. Briefly, 200 µL of sample was mixed with 800 µL of Folin–Ciocalteu reagent, and the volume was adjusted to 1.2 mL with double distilled water. The mixture was incubated for 10 min, and 1.8 mL of 10% sodium carbonate was added and further incubated for 5 min. To the blank, double distilled water was added. The absorbance of each sample was read at 765 nm *via* a UV–visible spectrophotometer (Evolution™ One/One Plus UV-Vis Spectrophotometers, Waltham, MA, USA). Gallic acid was prepared at various concentrations, and a calibration curve was plotted. The results are expressed as mg of gallic acid equivalent/100 g of dried date pomace.

Antioxidant Activity

The total antioxidant activity of the date pomace was determined *via* the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method (Alam *et al.* 2021). Briefly, the ABTS reagent was prepared by dissolving 200 mg of ABTS in 50 mL of double distilled water. It was mixed with 16.8 mg of potassium persulfate solution in 25 mL of double distilled water and kept in the dark overnight at room temperature (28 °C). The sample (0.1 mL) was mixed with 0.8 mL of ABTS reagent and incubated for 5 min, and the absorbance of the sample and standard were read at 734 nm *via* a UV–visible spectrophotometer. Gallic acid was prepared at various concentrations, and the results are expressed as mg gallic acid equivalent/100 g of dried date pomace (Sobti *et al.* 2023).

Fungal Maintenance and Culture

Neurospora intermedia (MTCC NO 877) was used for the production of mycoproteins from date pomace. The fungal strain was inoculated in potato dextrose agar (PDA) medium (Himedia, Mumbai, India) and incubated for 7 days at 30 °C, after which the plates were further stored at 4 °C until use. To prepare the inoculum, 10 mL of physiological saline suspension was flooded on PDA plates, and the spores were scraped aseptically *via* a sterile micropipette tip. The spore suspension was tested, and the concentration was maintained at 10⁶ spores/mL and used as inoculum.

Optimization of Culture Conditions for Determining Mycelial Biomass in Date Fruit Pomace

Barhi date fruit pomace was used as the culture medium for the optimized production of mycoprotein until otherwise stated. This date variety was selected because of rich of nutrients than other selected varieties. Process parameters, such as fermentation time (24 to 120 h), pH (4 to 6), and temperature (20 to 40 °C), were optimized by changing one factor at a time. To prepare the culture medium, 10% (w/v) barhi date fruit pomace was autoclaved, inoculated with 5% (v/v) inoculum. Then, it was incubated at 30 °C in an orbital shaker incubator at 150 rpm (Al-Ansari *et al.* 2020). The samples were withdrawn from the culture flask every 24 h, and the results are presented as the means ± SDs.

Fungal Biomass Analysis

The culture was withdrawn and filtered through Whatman paper, and the fungal biomass was separated from the culture. The fungal biomass was rinsed with double distilled water and dried at 60 °C overnight. The fungal biomass was calculated *via* the following Eq. 1:

$$\text{Fungal biomass} = \text{Total dry weight (g)} / \text{Total culture medium (L)} \quad (1)$$

The dried fungal biomass was homogenized with double distilled water. The total protein content of each sample was estimated using a nitrogen-to-protein conversion factor of 5.49 (Mariotti *et al.* 2008). The protein yield was determined *via* the following Eq. 2:

$$\text{Protein yield} = \text{Protein content (mg)} / \text{Dried substrate (g) in the culture medium} \quad (2)$$

Central Composite Design and Response Surface Methodology

A central composite design was used to optimize *N. intermedia* biomass production *via* the use of date fruit pomace medium as a low-cost substrate. To prepare the culture medium, 10% (w/v) date fruit pomace was autoclaved, inoculated with 5% (v/v) inoculum, and kept on a rotary shaker incubator at 150 rpm. The culture medium (50 mL) was prepared in a 100 mL Erlenmeyer flask. The other physiological factors were selected on the basis of the design of the experiments depicted in Table 1. Three independent factors (fermentation period, pH, and temperature) were tested, and the low and high levels are described in Table 1. These variables were selected based on preliminary experimental trials. The experiment was designed *via* Design Expert software (version 8.0.1), and the response Y (fungus biomass) was calculated. This experimental design is composed of a total of 20 experimental runs with six central points. Three experiments were performed, and the mean value was used for analysis (Vijayaraghavan *et al.* 2016).

Table 1. Experimental Design for Three Selected Variables

Factor	Variables	Units	Mean	Low Actual	High Actual
A	Fermentation period	h	60	24	96
B	Temperature	°C	30	25	35
C	pH		5	4	6

Statistical Analysis

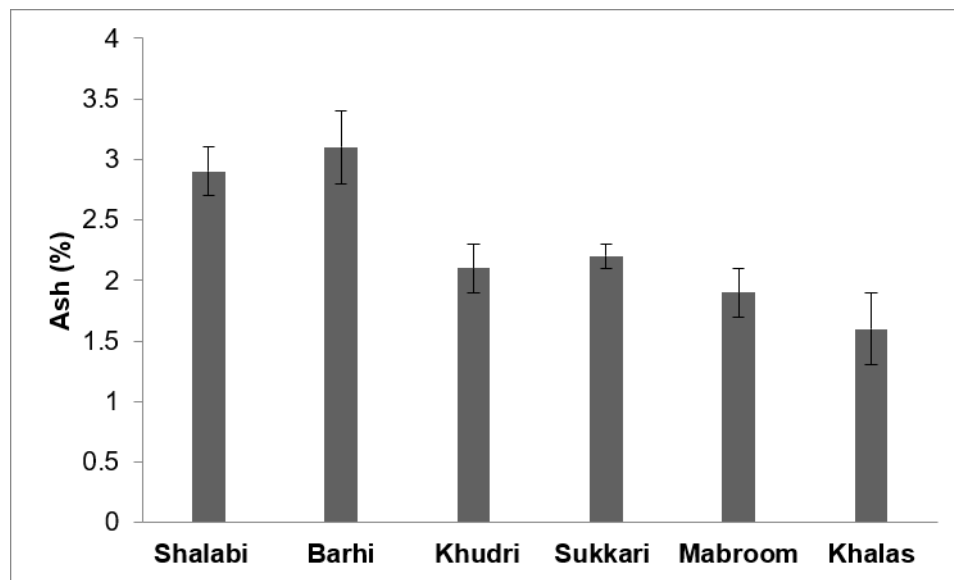
A triplicate experiment was performed, and the mean value was used for analysis. The collected data were analyzed *via* one-way analysis of variance (ANOVA), followed by Tukey's honest significant differences (HSD) test to determine the variations in fungal biomass and protein content. The result of a specific experimental condition was considered statistically significant if the p value was <0.01.

RESULTS AND DISCUSSION

Ash and Moisture Contents

Chemical analysis revealed that the ash content of date fruit pomace ranged from 1.6 ± 0.3 to $3.1 \pm 0.3\%$ (Fig. 1a). Among the selected varieties, barhi presented $3.1 \pm 0.3\%$ ash, followed by shalabi ($2.9 \pm 0.2\%$) and sukkari ($2.2 \pm 0.1\%$). The moisture content of the date pomace ranged from 6.4 ± 1.1 to $9.1 \pm 1.2\%$. The ash content observed in this study was lower than that reported previously (Fig. 1b). The ash content of date fruit pomace observed in this study was lower than that reported previously (4.1 g/100 g). The moisture content observed in this study was $9.1 \pm 1.2\%$, which was lower than that reported by Hariset *al.* (2023).

(a)



(b)

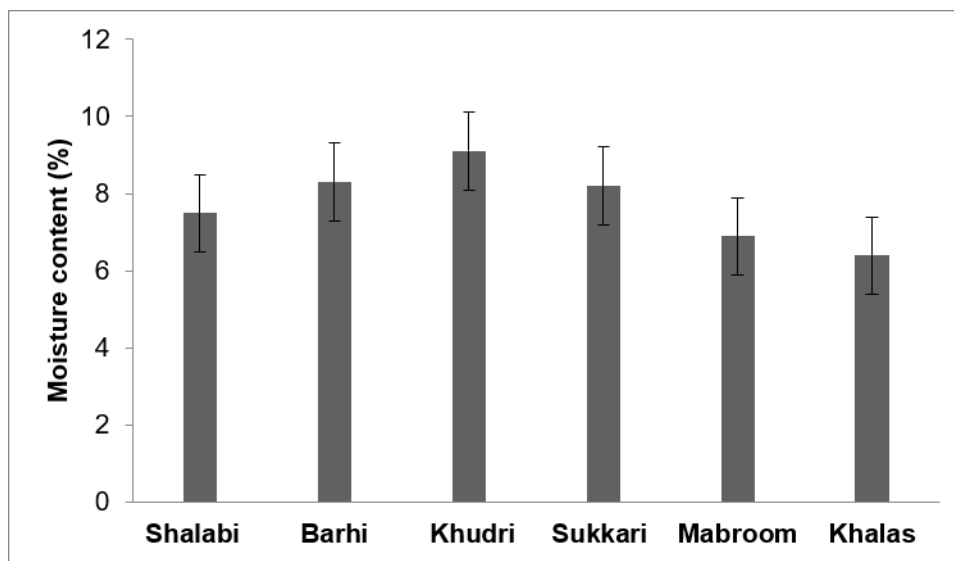


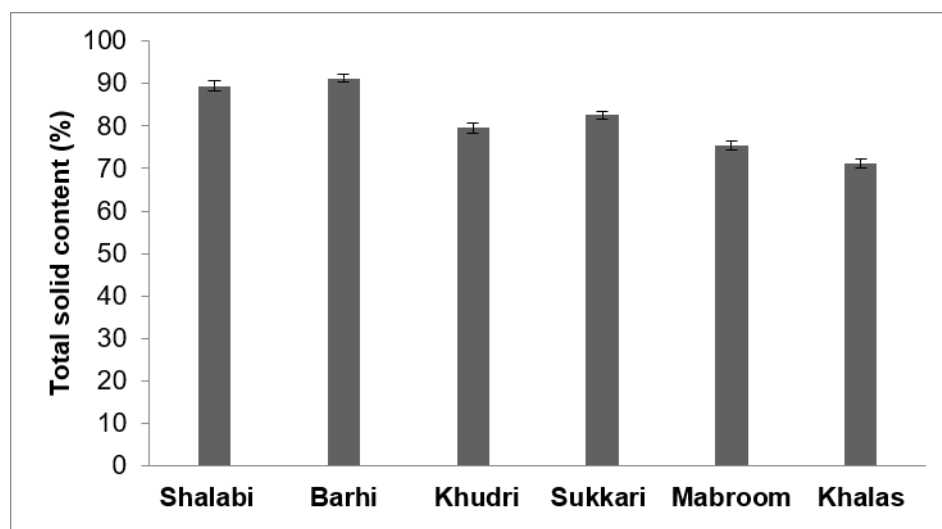
Fig. 1. Ash (a) and moisture (b) contents of date fruit pomace varieties

Total Solid Content and Volatile Matter

The total solid content was low ($71.2 \pm 1.1\%$) in the khalas variety fruit pomace, and it was greatest in barhi ($91.2 \pm 0.9\%$). The volatile matter content was greatest in khudri-type fruit pomace ($27.3 \pm 0.2\%$), followed by barhi ($25.2 \pm 0.2\%$), mabroom ($21.4 \pm 0.4\%$), and sukkari ($20.4 \pm 0.5\%$) (Fig. 2a). The volatile matter content ranged from $18.6 \pm 0.6\%$ to $27.3 \pm 0.2\%$ (Fig. 2b). The total solid content of date fruit pomace is a measure of the amount of substances remaining after complete evaporation. The present findings revealed that date palm byproducts are rich in essential nutrients and are required for human health due to the presence of reduced levels of fatty acids and cholesterol. The volatile

matter comprises various phytonutrients, such as amino acids, fatty acids, phenolics, carotenoids, and terpenoids. In date, volatile compounds, such as decanal, isopentyl alcohol, nonanal, limonene, hexanal, *n*-heptadecane, hexyl acetate, and β -ionone have been reported. The differences in volatile matter among date fruit varieties are based on the origin and ripening stage of the studied cultivar and the sensitivity of the analysis (El Arem *et al.* 2012).

(a)



(b)

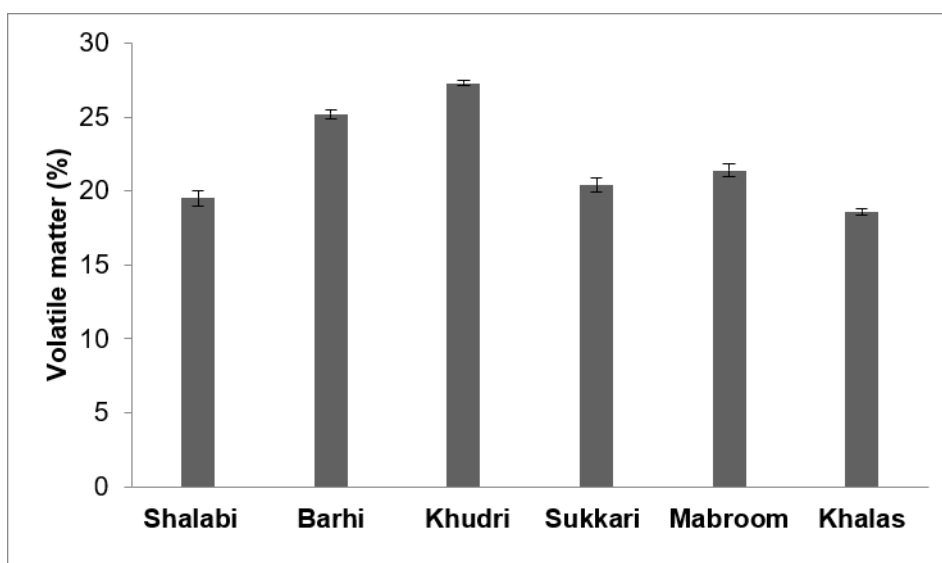


Fig. 2. Total solid content (a) and volatile matter content (b) of date fruit pomace varieties

Fixed Carbon

The fixed carbon content was greatest in barhi date pomace ($61.3 \pm 2.2\%$), followed by shalabi date pomace ($60.4 \pm 1.1\%$), sukkari ($59.5 \pm 2.1\%$), and khudri ($58.3 \pm 1.8\%$) (Fig. 3). The amount of fixed carbon content analyzed in this study is in agreement with previous studies reported for various types of biomass (Telmo *et al.* 2010). Fixed carbon

represents the carbon content of the material that remains after the volatile matter is completely expelled, while ash is the inorganic residue left after combustion step. Fixed carbon varies among different substances and can influence the calorific value of plant biomass (Racero-Galaraga *et al.* 2024). The fixed carbon content determined in this study was higher than the fixed carbon content of plant trunk (17.6%), and palm leaflets (10.5%) (Nasser *et al.* 2016).

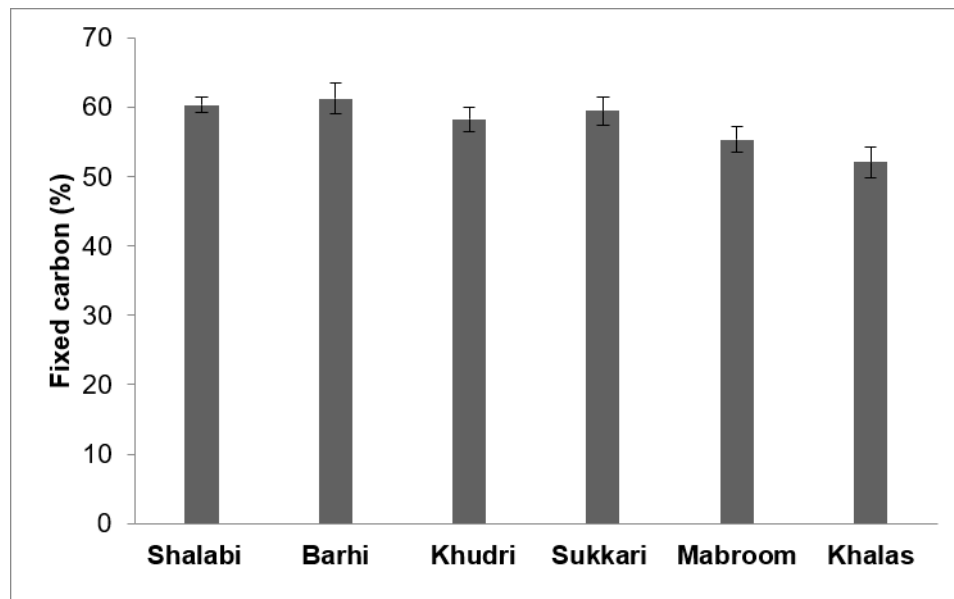


Fig. 3. Fixed carbon content of date fruit pomace of six date varieties

Mineral Components

Date fruit pomace is rich in natural minerals, and these minerals are required for human health. The amounts of various minerals, such as phosphorus, potassium, calcium, magnesium, iron, and sodium, are presented in Table 2. The results revealed that potassium, magnesium, and phosphorus were the major micronutrients in the date fruit pomace of the screened date varieties. The amount of potassium ranged from 302 ± 1.5 to 513 ± 2.5 mg/g 100, with a maximum of 513 ± 2.5 g (Shalabi). The potassium, calcium, and sodium contents were greatest in the khudri (71.5 ± 2.2 , 30.5 ± 1.5 , and 17.2 ± 1.1 mg/100 g, respectively). The mineral components observed in this study were consistent with those reported by Haris *et al.* (2023), and potassium was the major mineral (145.7 ppm), followed by calcium (46 ppm) and phosphorus (28.7 ppm). Minerals, such as phosphorus and potassium are involved in human cell regeneration, whereas calcium and magnesium are essential for the maintenance of bone health (Razzaque and Wimalawansa 2025). Iron is used for the production of hemoglobin; hence, due to the presence of these minerals, date fruit- and fruit-based products are considered healthy diets for humans (Habib and Ibrahim 2011).

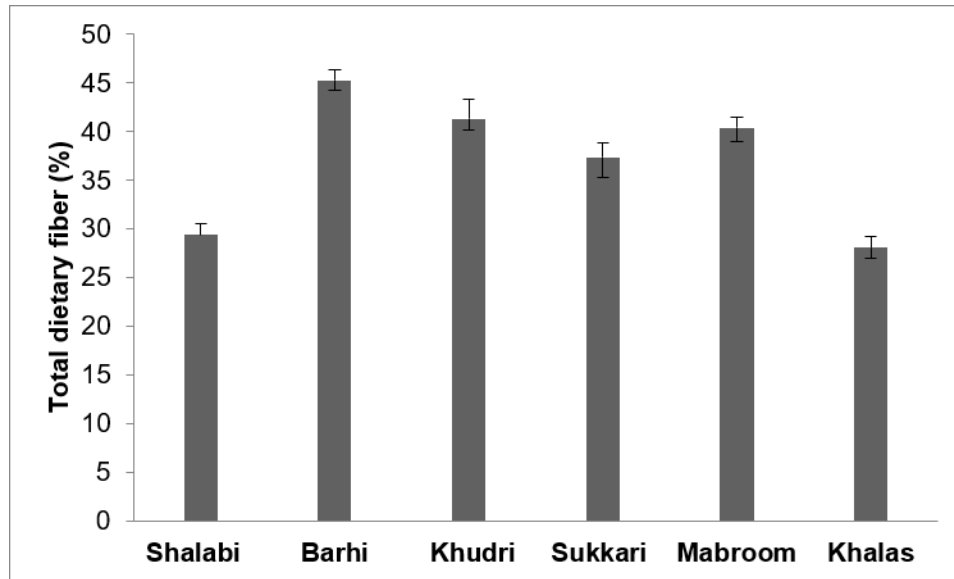
Table 2. Composition of Minerals in the Date fruit Pomace of the Six Date Varieties

Pomace	P ⁺	K ⁺	Ca ⁺	Mg ⁺	Fe ⁺	Na ⁺
Shalabi	62.5 ± 1.1	513 ± 2.5	29.4 ± 2.2	79.2 ± 1.1	1.4 ± 0.1	10.2 ± 1.2
Barhi	54.3 ± 2.1	414 ± 10.4	27.1 ± 1.9	84.5 ± 2.6	1.1 ± 0.2	8.4 ± 1.2
Khudri	71.5 ± 2.2	302 ± 1.5	30.5 ± 1.5	68.3 ± 1.4	1.5 ± 0.1	17.2 ± 1.1
Sukkari	40.3 ± 1.1	418 ± 2.4	25.1 ± 2.2	53.2 ± 2.2	0.6 ± 0.1	13.5 ± 1.2
Mabroom	51.3 ± 1.7	306 ± 1.5	18.4 ± 1.7	75.3 ± 6.3	1.6 ± 0.2	15.2 ± 0.9
Khalas	48.3 ± 1.2	429 ± 2.2	22.1 ± 2.2	61.4 ± 2.4	0.8 ± 0.2	11 ± 0.2

Total Dietary Fiber and Carbohydrates

The total dietary fiber content of the date fruit pomace was $45.3 \pm 1.1\%$ (Barhi) (Fig. 4a), which was consistent with the findings of Hariset *al.* (2023). The soluble sugar content was $34.2 \pm 1.1\%$ in the Khalas variety, which was similar to the findings of Hariset *al.* (2023). In fruits, dietary fiber has been reported, and these dietary fibers have significant bioactive properties due to the presence of carotenoids and flavonoids. This dietary fiber results in lower energy and improved phytic acid content (Quiles *et al.* 2018). Vegetable and fruit pomace have been widely used as fiber sources to supplement crackers, cookies, and various cereal-based food products (Sahni and Shere 2018). The proximate composition of date fruit pomace revealed high dietary fiber, which aligns with the findings of previous studies on date fruit pomace (Borchani *et al.* 2011; Sahni and Shere 2018). The increased dietary fiber content was characterized by a reduced moisture level and total protein content of $<10\%$, a decreased fat content, and a reduced amount of ash (Bchir *et al.* 2014). The carbohydrate content was high in the date fruit pomace (Fig. 4b). Majzoobi *et al.* (2019) reported that date press cake from ripe shahani date fruits grown in Iran contained 11.7% crude fiber and 79.1% carbohydrate. It has been previously reported that date press cake has lower protein, fat, and ash contents than blackberry and strawberry press cakes; moreover, it has higher ash, fat, and total protein contents than orange, apple, and pineapple press cakes do (Sudha *et al.* 2007; Kosmala *et al.* 2011; O'Shea *et al.* 2015). Date pomace consists of small quantities of residual sugars, and it has been previously dumped in landfills or used as animal feed. Haris *et al.* (2023) reported that date fruit pomace consists of 35% soluble sugars present in the form of fructose and glucose. These readily available soluble sugars make date fruit pomace a potential source for the production of microbial protein, and due to the presence of large quantities of soluble sugars, a pretreatment step may not be required for microbial fermentation *via* date seed pomace.

(a)



(b)

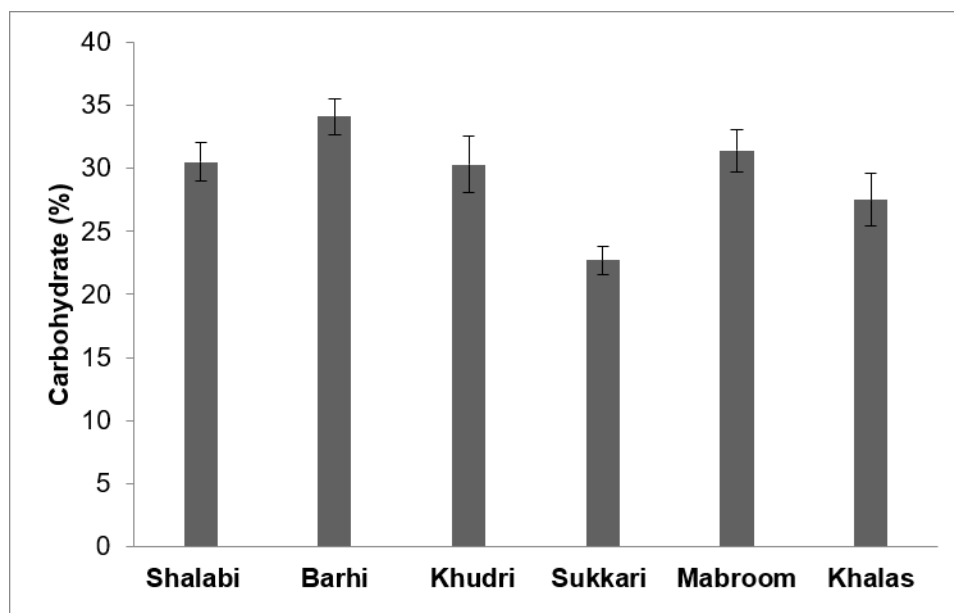


Fig. 4. Total dietary fiber (a) and carbohydrate (b) contents of date fruit pomace from the six date varieties

Amino Acid Composition of Date Fruit Pomace

In this study, the amino acid content of date fruit pomace was analyzed, and the compositions of various essential amino acids are depicted in Table 3. The table reveals that glutamine was the major amino acid found in the shalabi, barhi, sukkari, and khalas varieties. In addition, glycine and proline were detected in greater quantities in this study, and traces of valine and histidine were detected. These essential amino acids from date palm pomace are involved in the production of red blood cells. The prolonged consumption of dates reduces problems associated with malnutrition and serves as a building block of

proteins and various enzymes. The dried and fresh dates were rich in essential and nonessential amino acids (Al-Farsi *et al.* 2012). Glutamine is an essential amino acid (proline, aspartic acid, glycine, glutamine, valine, and histidine) (Rambabu *et al.* 2020), and it is a major amino acid in addition to other essential amino acids. Moreover, histidine was detected in very low quantities.

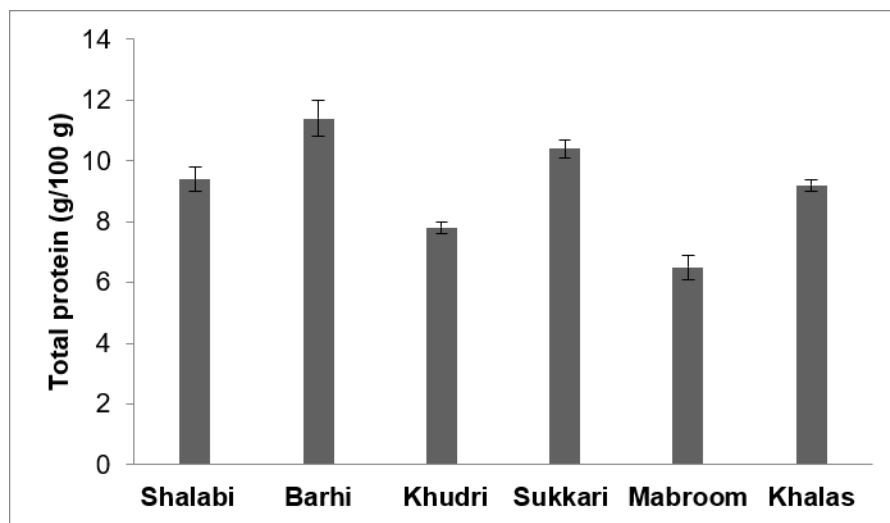
Table 3. Amino Acid Composition of Date Fruit Pomace

Pomace	Amino Acids (mg/100 g)					
	Aspartic Acid	Glutamine	Glycine	Histidine	Proline	Valine
Shalabi	129 ± 2.2	142 ± 1.2	80 ± 2.5	0.32 ± 0.03	49.4 ± 1.2	4.5 ± 0.22
Barhi	135.4 ± 1.8	178.2 ± 4.2	50.3 ± 1.1	0.52 ± 0.03	53.3 ± 0.2	6.1 ± 0.3
Khudri	90.1 ± 2.1	79.2 ± 1.2	40.2 ± 2.2	0.27 ± 0.01	50.1 ± 0.1	4.2 ± 0.2
Sukkari	112.4 ± 1.4	101.41.1	71.3 ± 1.1	0.22 ± 0.04	38.2 ± 0.2	3.3 ± 0.1
Mabroom	59.5 ± 2.2	69.2 ± 1.5	62.4 ± 1.9	0.38 ± 0.01	40.3 ± 0.1	1.9 ± 0.1
Khalas	100.2 ± 1.1	129 ± 2.4	49.3 ± 2.5	0.41 ± 0.01	33 ± 0.2	2.5 ± 0.1

Total Protein and Lipid Contents

Date pomace consists of increased amounts of protein ($11.4 \pm 0.6\%$) (barhi) and reduced amounts of fat ($0.72 \pm 0.1\%$) (mabroom) (Fig. 5a). This result was consistent with previous findings and reported the presence of 10.6% protein and 2.6% fat (Haris *et al.* 2023). Date fruit pomace is a byproduct of date fruit processing, and it consists of approximately 10% protein (Subhash *et al.* 2024). The protein content of the date pomace observed in this study was greater than the total protein content reported by Muñoz-Bas *et al.* (2025), and the fat content was lower than that reported earlier. The lipid content was lower in the date fruit pomace (Fig. 5b). The protein and fat contents of date fruit pomace are influenced by the proportion of seeds to flesh, date cultivar, agricultural conditions, ripening stage, and processing methods used to extract syrup (Okonkwo *et al.* 2025).

(a)



(b)

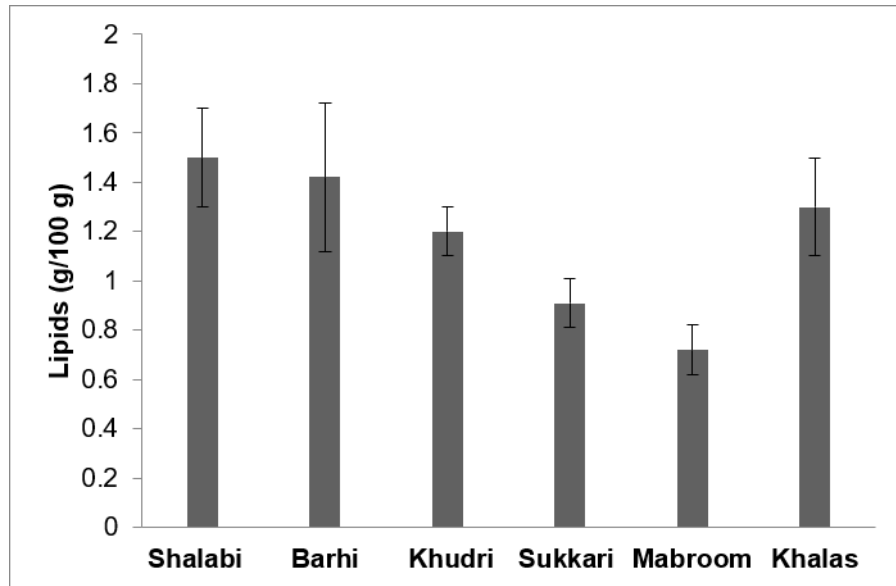
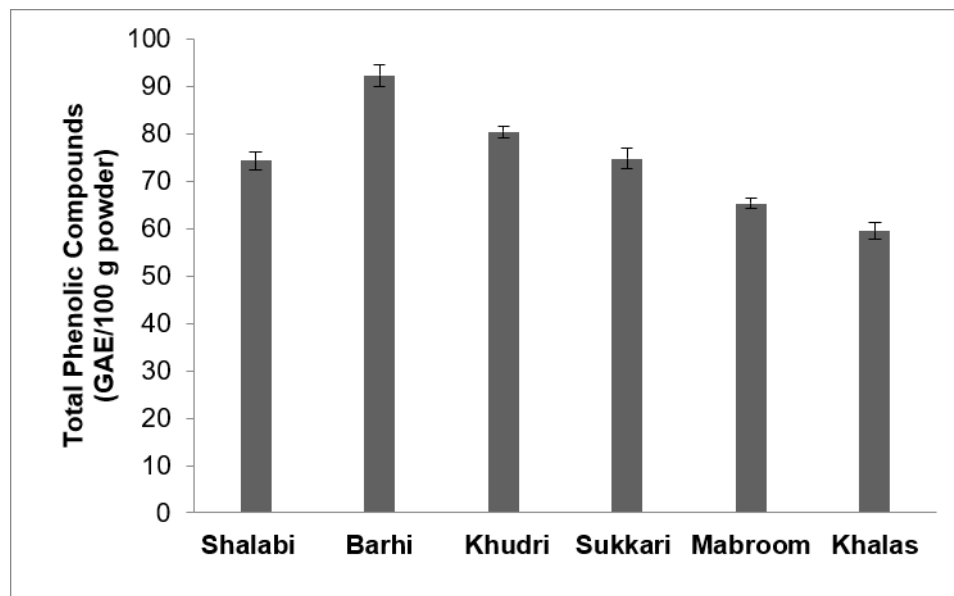


Fig. 5. Total protein (a) and lipid (b) contents of date fruit pomace from six date fruit varieties

Total Phenolic Content and Antioxidant Activity of Date Fruit Pomace

Date fruit pomace is characterized by increased amounts of total phenolic compounds. The total phenolic compounds ranged from 59.5 ± 1.7 (khalas) to 92.3 ± 2.2 mg GAE/100 g powder (Barhi) (Fig. 6a). The total antioxidant activity was greatest in the barhi variety (53.1 ± 1.5 mg GAE/100 g powder) because of the increased amount of total phenolic compounds. The total antioxidant activity ranged from $38.2 \pm 1.5\%$ to 53.1 ± 1.5 mg GAE/100 g (Fig. 6b). The total antioxidant activity of pomace varies widely and was 151 mg/100 g (Al-Janah and Al-Mudhafr 2021), with 170 to 260 mg gallic acid equivalents (Haris *et al.* 2023). Total phenolic content was high in barhi pomace which increased antioxidant activity in this pomace than other pomaces. The antioxidant activity observed in this study was consistent with previous results. The antioxidant activity of date fruit pomace varies in terms of phenolic, flavonoid, sterol, quinine, and anthocyanin contents (Cheng *et al.* 2020). Recently, date pomace has been used to improve the functional properties by microbial fermentation. Date pomace was fermented with lactic acid bacteria and total phenolics (441.83 ± 2.3 mg QE/100 g) and flavonoids (441.83 ± 2.3 mg QE/100 g) were higher (Barathikannan *et al.* 2025).

(a)



(b)

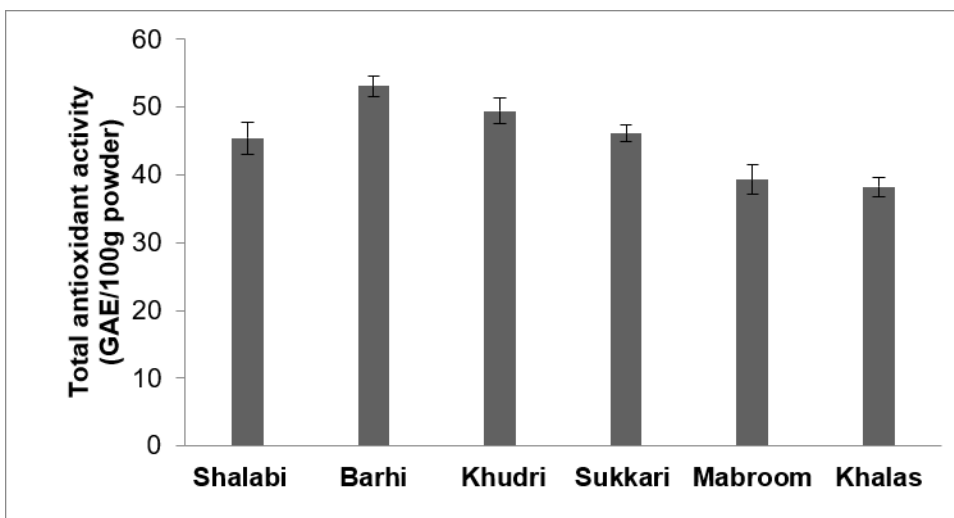


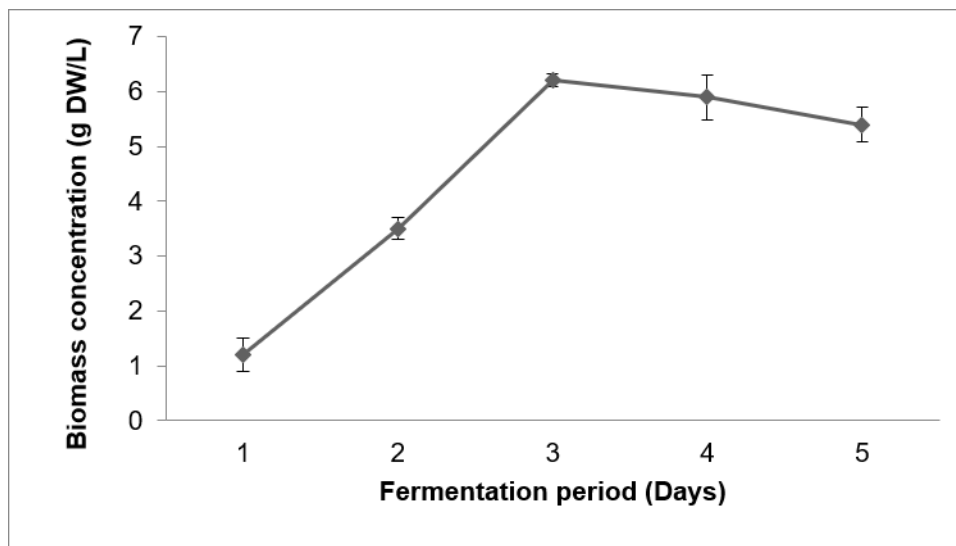
Fig. 6. Total phenolic content (a) and antioxidant activity (b) of the date fruit pomace of the six date varieties

Screening of Culture Conditions for Biomass Production

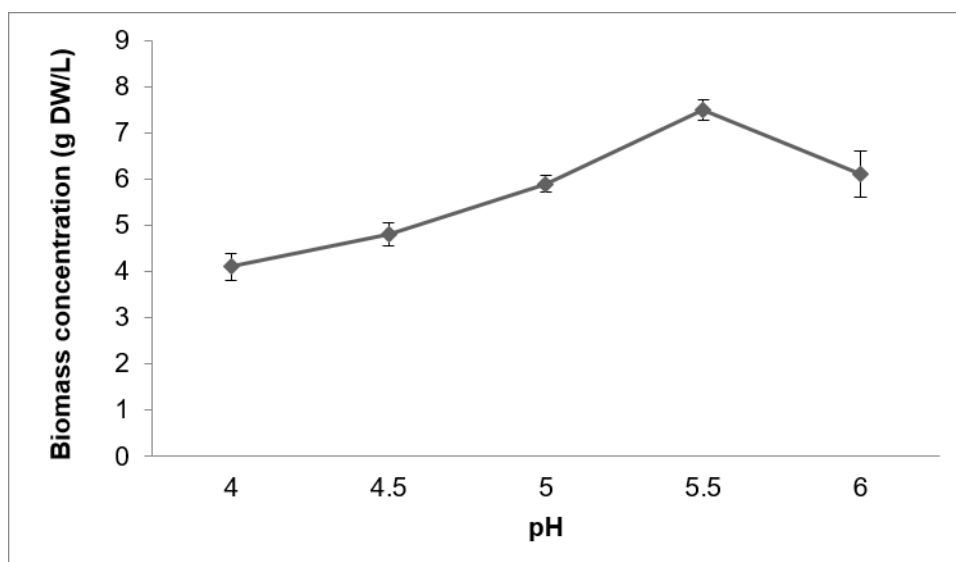
In this study, barhi date fruit pomace was used as the substrate for optimization studies. The effect of the fermentation period on biomass production was evaluated, and the results are depicted in Fig. 7. Biomass production was high after 72 h of cultivation, with a maximum biomass concentration of 6.2 ± 0.12 g/L (Fig. 7a). A shorter or longer fermentation period affects the biomass yield (El-Sheikh *et al.* 2020). The present findings were consistent with those of a previous report, and a 72-h incubation time improved the biomass yield (Karimi *et al.* 2019). *Rhizopus oryzae* utilizes fish industry byproducts, and the fungal biomass yield reaches a maximum after a 72 h incubation period (Sar *et al.* 2020). In this study, the culture medium pH had a major impact on the biomass yield (Fig.

7b). At pH 5.5, the biomass yield was 7.5 ± 0.22 g/L. Moreover, the biomass yield decreased at higher and lower pH values than pH 5.5.

(a)



(b)



(c)

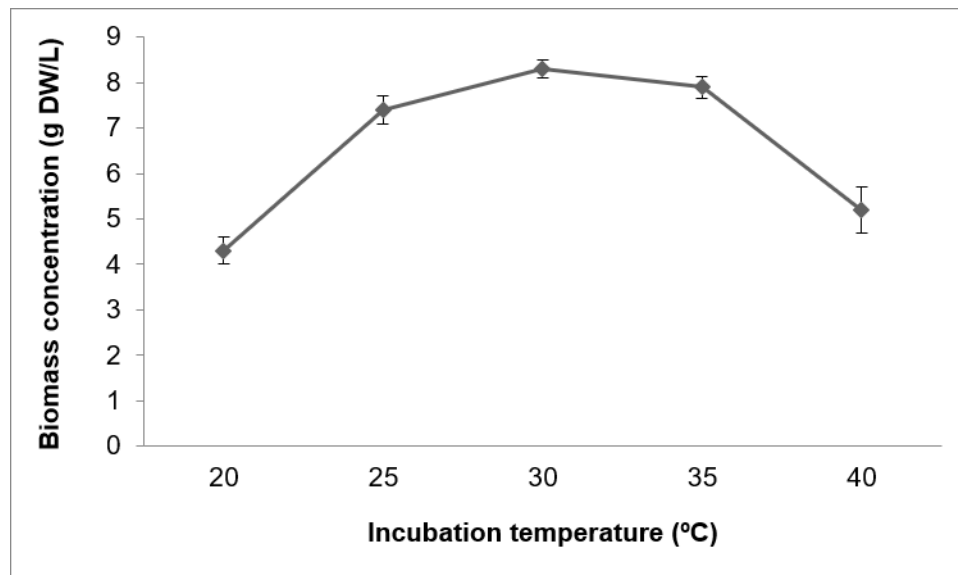


Fig. 7. Effects of fermentation period (a), pH (b), and incubation temperature (c) on *N. intermedia* biomass production

The decreased yield was attributed to the minimal microbial activity at lower (<4.5) and higher pH values (Akram *et al.* 2015). In the pH range of 3.0 to 4.0, pellet formation is greater (Nair *et al.* 2015) in *N. intermedia*. *N. intermedia* is considered a mesophilic fungal strain, and the effect of temperature on biomass production was evaluated. Figure 7c reveals that biomass production reached a maximum at 30 °C (8.3 ± 0.2 g/L). The results of the present study are consistent with earlier reports on biomass yield in *N. intermedia*, and maximum biomass production was achieved at 30 °C in baker's yeast wastewater medium (Hashemi *et al.* 2021). The incubation temperature affects *N. intermedia* biomass, nutrient absorption, and enzyme activity (Abbasi and Samaei 2019). At lower temperatures, the growth rate of *N. intermedia* was lower, possibly due to the reduced potential for enzymatic activity, which are cellular processes that reduce metabolic processes. In addition, nutrient absorption is affected at relatively cold incubation temperatures because of reduced membrane permeability and enzyme activity. In contrast, increased temperature affects cellular function and biomass growth by affecting the lipid layer, cellular proteins, denatured proteins, and DNA and affecting membrane integrity (Araújo *et al.* 2019).

Statistical Optimization of Fungal Biomass Production Using Date Fruit Pomace Medium by Fungal Fermentation

Statistically optimized culture media improved fungal biomass production in submerged fermentation *via* date fruit pomace media (Arokiyaraj *et al.* 2025). The optimal culture conditions for improving biomass yield were analyzed. As shown in Table 4, the fermentation period, pH, and temperature effectively influenced biomass production. Biomass production ranged from 3.3 to 18.8 g/L, and biomass production was more than twofold greater than that of the unoptimized culture medium. The model F test result was 37.58, and the p-value was <0.05, indicating that the fitted quadratic regression model was statistically significant ($p < 0.05$).

In this study, A, B, C, AB, AC, A², B², and C² were significant model terms (Table 5). The insignificant lack of fit of the model implied that the designed model was well fitted. The R-squared value of this model was 0.971, and the adjusted R-squared value was 0.945. This analysis revealed that 94.5% of the variation observed in the biomass could be attributed to the fitted second-order polynomial equation. The coefficient estimate was positive for the fermentation period (2.66), temperature (2.37), and pH (2.3). The predicted response was 19.4 g/L, which was 18.4 g/L. The observed and predicted values were similar, and the model was validated.

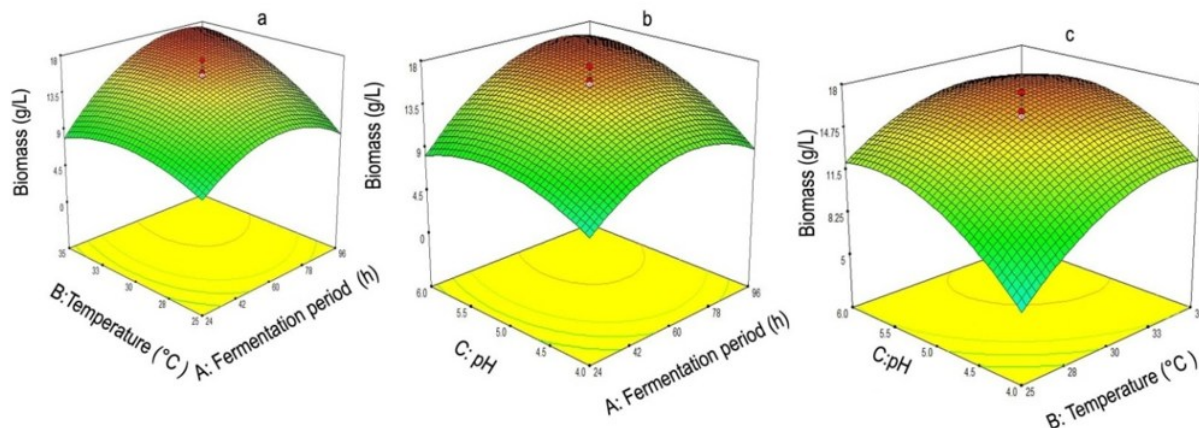
As evidenced by the response surface graph (3D), the response curves are indicative of the effects of various combinations of fermentation period, pH, and temperature on fungal biomass production (Fig. 8). Previous studies have revealed that optimizing culture medium components can effectively improve biomass and product yield. For example, Vijayaraghavan *et al.* (2017) used response surface methodology for the production of bacterial fibrinolytic enzymes from cow dung substrates, and the selected variables were moisture, sucrose, and MgSO₄, and twofold yields were reported. Bijiet *et al.* (2016) used cuttle fish waste for the production of enzymes from *Bacillus cereus* in solid-state fermentation.

Table 4. Experimental Results of Central Composite Design for the Optimization of Fungus Biomass Production

Runs	Fermentation Period (h)	Temperature (°C)	pH	Biomass (g/L)
1	24	25	4	3.3
2	96	25	4	2.15
3	24	35	4	7.1
4	96	35	4	11.3
5	24	25	6	8.3
6	96	25	6	10.4
7	24	35	6	6.47
8	96	35	6	18.8
9	0	30	5	0
10	120.5	30	5	11
11	60	21.5	5	5.54
12	60	38.4	5	13.2
13	60	30	3.3	5.3
14	60	30	6.6	11.98
15	60	30	5	15
16	60	30	5	16
17	60	30	5	15.3
18	60	30	5	17.4
19	60	30	5	15.5
20	60	30	5	15.6

Table 5. ANOVA of the Central Composite Design for the Production of Fungal Biomass

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F
Model	551.2224	9	61.24693	37.58285	<0.0001
A-Fermentation period	96.18496	1	96.18496	59.02182	<0.0001
B-Temperature	76.87892	1	76.87892	47.17508	<0.0001
C-pH	71.9856	1	71.9856	44.17241	<0.0001
AB	30.34205	1	30.34205	18.61874	0.0015
AC	16.18805	1	16.18805	9.933446	0.0103
BC	5.08805	1	5.08805	3.122172	0.1077
A ²	166.9642	1	166.9642	102.4539	<0.0001
B ²	59.26785	1	59.26785	36.36843	0.0001
C ²	75.31684	1	75.31684	46.21655	<0.0001
Residual	16.29651	10	1.629651		
Lack of Fit	12.67651	5	2.535302	3.501798	0.0976
Pure Error	3.62	5	0.724		
Cor Total	567.5189	19			

**Fig. 8.** 3D response surface graphs of the effects of the variables (fermentation period, pH, and temperature) on fungal biomass production by *N. intermedia* in date fruit pomace medium

Mycoprotein Fermentation

The edible fungus *N. intermedia* was cultivated in a 250-mL Erlenmeyer flask containing 150 mL of 10% date pomace medium under optimized culture conditions. The culture was filtered, and the fungal biomass was dried. The dried fungal biomass contained $17 \pm 2.1\%$ protein, $3.6 \pm 0.1\%$ carbohydrate, and $0.91 \pm 0.02\%$ fat. Figure 9 shows the nutrient composition of fungal biomass cultured in optimized media. The protein content of *N. intermedia* improved in the optimized culture medium. These include the agitation time, pH of the medium, and temperature. For certain biomasses, pretreatment procedures often improve the nutrient profile. The optimum temperature (30 °C), agitation speed (100to200 rpm), inoculum (1 to 5%), and incubation time (3 to 5 days) improved the biomass components (Starzyńska-Janiszewska *et al.* 2016; Gmoser *et al.* 2018). Dai *et*

al.(2025) used a soy whey substrate to produce mycoprotein from *Neurospora media*, and the optimized medium containing okara improved the mycoprotein yield. Filamentous fungi are promising alternatives to animal and plant proteins because of their high protein content, rapid biomass accumulation, and potential to utilize inexpensive agro-industrial residues. *Neurospora intermedia* can be cultivated *via* solid-state fermentation in low-cost media, and low-cost media improve the total protein content and essential mineral, vitamin, and essential amino acid contents (Gmoser *et al.* 2020). Because of its high protein content and decreased protein content, mycoprotein is considered an alternative to animal protein. Agro-industrial residues, including date, seaweed, pea byproducts, and pineapple peels, have been utilized as substrates for the production of mycoproteins (Majumder *et al.* 2024). *Neurospora intermedia* is a protein-rich fungus, and cultivation of this fungus *via* agro-industrial residues for its growth has the potential to generate sustainable waste of protein-rich biomass for food applications (Maini Rekdal *et al.* 2023).

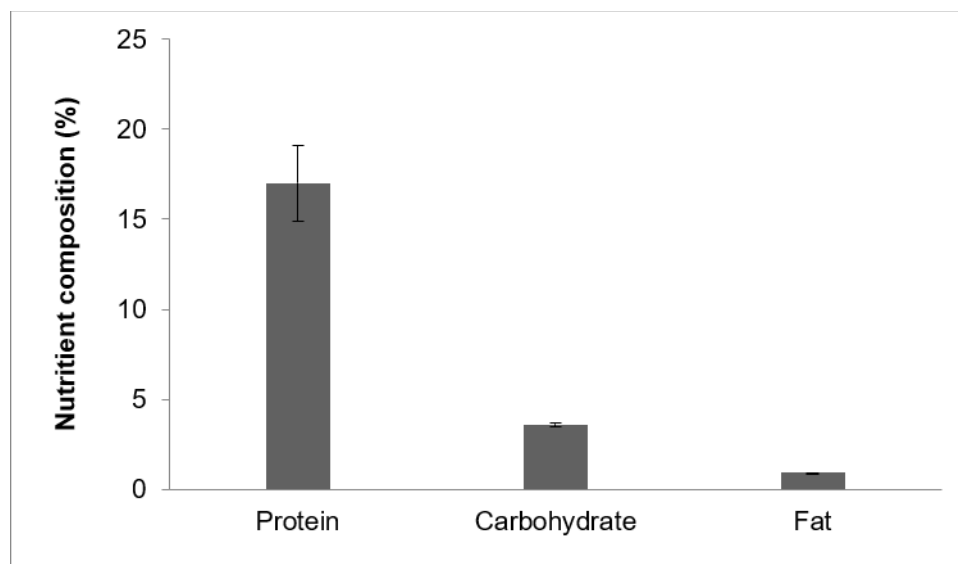


Fig. 9. Nutritional composition of *Neurospora* biomass cultivated in date pomace medium

CONCLUSIONS

1. Proximate analysis of the date fruit pomace of the six date varieties (shalabi, barhi, khudri, sukkari, mabroom, and khalas) showed that all six date varieties exhibited high dietary fiber, carbohydrates, minerals, and essential amino acids increased.
2. Barhi date fruit pomace was used as the culture medium for the optimized production of mycoprotein. The culture medium was optimized *via* response surface methodology, and the biomass was increased 2-fold. The dried fungal biomass contained $17 \pm 2.1\%$ protein, $3.6 \pm 0.1\%$ carbohydrate, and only $0.91 \pm 0.02\%$ fat.
3. Mycoprotein is a sustainable alternative to animal protein because of its rich amino acid profile and good digestibility of nutrient components. Fruit pomace was used as an inexpensive source of carbon and energy for the production of mycoprotein by the edible fungus *Neurospora intermedia*.

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Conflict of Interest

The authors do not have any conflict of interest in publication of this research article.

Use of Generative AI

Authors did not use any AI tools in the preparation of text, and data analysis.

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