# Production of the economically important polyunsaturated fatty acids using *Rhizopus spp.* isolated from the uncultivated land of Yemen

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## ABSTRACT

*Zygomycetes* especially oleaginous fungi have a great ability to produce huge quantities of lipids high in economically important poly-unsaturated fatty acids. About 32 isolates were isolated from oleaginous zygotes. Six of the 32 were identified as *Rhizopus* spp. identified. Tested for production of high levels of lipids. 6 out of 32 have the ability to produce huge quantities of lipids. The *Rhizopus* sp. UC 13 strain was selected for further optimization as it produced 1.05 $gl^{-1}$ lipid with a lipid content of 39.21%. The production of lipids was increased using the Taguchi method which selected a number of factors at varying levels for the optimization process. Glucose, ammonium sulfate, temperature, pH and time directly affect lipogenesis. Saturated fatty acids (SFAs) were more prevalent than unsaturated fatty acids (USFAs) based on the GC-Mass profile, which also revealed that Caproic, Undecanoic, Tridecanoic, Palmitoleic, Heptadecanoic, Heneicosanoic, and Behenic were the most prevalent SFAs. Moreover, USFAs as Linolenic, Cis-11, 14, 17-Eicosatrienoic, Gamma-Linolenic, Cis-8, 11, 16-Docosadienoic and 14-Eicosatrienoic and Cis-13n were present in a small quantities. As a result, *Rhizopus* sp. UC 13 is regarded as a potential oleaginous filamentous fungus that can be applied in factories for the production of PUFAs.

**Keywords***:* poly-unsaturated fatty acids; *Rhizopus*; Yemen**.**

## INTRODUCTION

Lipids are an important for the functioning of all membranes and components of the cell, including the nervous system, the brain, the eyes, the heart, the adrenal system, and the thyroid. Lipids play a role in vascular wall maintenance, blood pressure regulation, and the formation of eicosanoids, a family of hormone-like compounds that control many organ systems (Tallima & El Ridi, 2018). Fats are the most energy-dense of all macronutrients; they play an important part in energy needs and the give ability to absorb fat-soluble vitamins A, D, and E in both humans and animals. Over the past few years, the public has become increasingly aware of the health benefits of good fat, which have been linked to various types of dietary fats (Savarino et al., 2021). The role of lipids in humans and animals is multifaceted. In physiology, lipids are essential structural elements of cell membranes, play a critical role in energy metabolism in many organisms, and are used in pharmaceuticals, nutraceutical products and food supplements (Okuyama et al., 2007).

Poly-unsaturated fatty acids with a long chain (PUFAs) have multiple carbon double bonds and 18+ atoms of carbon. PUFAs are classified based on the location of the first double bond, as calculated from their methyl end. For example, an omega-3 PUFA has a first double bond at location 3, as calculated from its methyl end. The symbol n is frequently used as a synonym for the symbol ω. For example, DHA, which is the abbreviation for all-Cis- 4,7, 10, 13, 16, 19-docosahemoic acid, may be denoted as Δ4(7, 10,13, 16, 19)-22:6, or n-22(6ω3), which indicates that the fatty acid has 22 atoms of carbon with 6 double bonds. The first double bond is found on the 3rd carbon atom (methyl end group) of the 3rd carbon. Other ambiguities connected with the omega-3 sequence are linolenic [18:3], stearidonic [18:5], moroctic [18:4], timnodonic [20:5], clupanodic [22:5] and cervonic [22:6], although not all of them are still used (Bharathiraja et al., 2017)**.**

The primary commercial source of 18 carbone atom PUFAs is derived from plant seed oils. Plants are unable to synthesize PUFAs higher than C18 due to a deficiency of the necessary enzymes in the cellular system (Rizzo et al., 2023). Long chain fatty acids, are mostly made from animals, eicosapentaenoic (EPA) and docosahexaenoic (DHA), are made from marine fish oil. The amount of these PUFAs in agricultural and animal products is usually low and varies depending on the time of year, climate, and where you live. Unfortunately, marine fish oil has some drawbacks, like bad taste and smell, high cholesterol, and a small amount of potentially toxic stuff that's hard to get rid of. So, if you're trying to meet the growing market demand for

PUFAs, you might run into some issues with the amount and quality of these conventional sources (Gladyshev & Sushchik, 2019). These drawbacks mean that we need to find new sources of polyunsaturated fatty acids (PUFAs) that are cheaper and better than the traditional ones. Microorganisms like bacteria, fungi, and algae have been found to contain a variety of PUFAs (Chen et al., 2023).

There are many reasons why microbial oils are preferable to vegetable oils. Unlike vegetable oils, which are subject to seasonal fluctuations, microbial oils are not subject to seasonal fluctuations. They also have shorter production times and require less space and labor. Most importantly, they are not a substitute for the food industry. In addition, microbial oils can be produced from a wide range of carbon sources, which enhances the versatility and stability of a bio refinery. All in all, microbial oils are a sustainable and renewable alternative to vegetable oils that can be used as biofuel and biochemical feedstock (Caporusso et al., 2021). PUFAs play a significant part in brain, nerve, and eye function (Rey et al., 2019), neurodegenerative and psychiatric diseases (Bentsen, 2017), cardiovascular disease prevention (Yanai et al., 2018), infection (Djuricic & Calder, 2021), and cancer prevention (Zárate, 2017). According to the American Heart Association, oily fish is recommended for adults twice a week. Fish, especially oily species, have high EPA and DHA levels that have been shown to support cardioprotective effects. EPA has the primary human benefits of reducing risk of colorectal, breast, and pancreatic cancer, reducing plasma cholesterol, protecting against atherosclerosis and antiaggregatory properties, protecting against cardiovascular diseases, and essential for homeostasis (Phang et al., 2009). Fungi are characterized by their high growth rate, short life cycles, lack of light energy requirements, scalability, and utilizing a broad various sources of carbon such as lignocarbon biomass, agri-industrial residues, and wastewater (Chen et al., 2023).

The production of lipids from fungi appears to have been primarily focused on the genera *Zygomyctes* including *Cunningghamella, Cucor, Mucor, Mortierella* and *Rhizopus* (Tauk-Tornisielo et al., 2009). Factors such as incubation temperature, pH level at the start of the incubation, incubation time, carbon and nitrogen content were cited as the most important factors influencing lipid production in recent studies (Chiranjeevi & Venkata Mohan, 2016). Bandyopadhyay et al., (2003), found that 6 days is optimum for lipid production by *Rhizopus nigricans* SSSD-8. The objective of this study is to isolate and characterize poly-unsaturated fatty acids-producing *Rhizopus* spp. with optimization various process variables for poly-unsaturated fatty acids accumulation by method of Taguchi to obtain an oleaginous *Rhizopus* sp.

## MATERIALS AND METHODS

### Chemical and reagents.

Most of solvents and chemicals used were purchased from Sigma–Aldrich.

### Sampling and isolation of oleaginous Zygomycetes.

Soil samples were collected from various locations in the land of Yemen.1 g of each soil sample was individually suspended in 1 ml of sterile distilled water, serially diluted 10- to 2-fold, and plated onto Oxoid Malt Extract Agar (MEA). Plates were incubated at 30 °C for 3 days under controlled conditions. Single fungal colonies were isolated and transferred to new His MEA plates repeatedly until pure cultures were obtained. Pure cultures were grown on MEA slants and stored at 4°C until use (Suleiman et al., 2018).

### Media used and cultivation conditions.

Using MEA as the screening medium, the composition of the production medium (g/l): Glucose 100, yeast extract 10, adjusted to pH 5.4. A 10% (by volume) mycelial suspension of the isolated culture was inoculated into a 100 ml flask containing 25 ml of broth and incubated at 30°C for 7 days (Suleiman et al., 2018).

### Standard curve of lipid.

A standard lipid stock solution was prepared using 20 mg of commercial rapeseed oil in 10 mL chloroform. Place different amounts of the standard oil solution into an empty tube, depending on the concentration. The tube was kept at 60° C. for 10 minutes to evaporate the solvent, after which 100 µl of water was added. Samples were then prepared by the following sulfo-phospho-vanillin reaction method. Teflon-coated glass vials were used in all experiments (Mishra et al., 2014).

### Screening of oleaginous Zygomycetes.

Two techniques have been used to screen for lipid production in oleaginous zygotes. First, a qualitative analysis was performed using the dye binding method (Nile Red). The

fungal biomass was stored with 0.5 ml of PBS solution and 0.05 ml of Nile Red solution (25 µg Nile Red/1000 ml acetone) in the dark for 30 minutes. Photographs of the stained lipid bodies were subsequently taken with a fluorescence microscope (IX-70, Olympus, Tokyo, Japan) equipped with a CCD camera (U-CMT, Olympus, Tokyo, Japan). Quantitative analysis was then performed using sulfophosphovanillin and phosphovanillin reagents to quantify lipids according to (Mishra et al., 2014).

**Identification of fungal isolates.**

***Molecular and traditional identification of the isolated fungi.***

To identify fungal isolates cultured on potato dextrose agar, light and electron microscopy were used to observe morphological characteristics (colony color, textural appearance and diameter) and microscopic features (Desuoky et al., 2007). Rice field was used to identify isolated fungi at the molecular level. Primers used for amplification and sequencing of the gene encoding 18S rRNA were those described by (Suh & Nakase, 1995). PCR products were sequenced using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Sequences were analyzed using the BLAST (National Center for Biotechnology Information) program to identify the closest available database sequences.

**Taguchi method for optimizing lipid production.**

There are many factors that influence fungal lipid production. Temperature, pH, incubation time, carbon and nitrogen sources were tested for increased lipid production using Taguchi Design of Experiments (DOE) in software. The optimization process was performed in two stages. The first stage involves a preliminary step to select optimal carbon and nitrogen sources. Additionally, pH and incubation time were tested. In a second step, the effects of five factors on adipogenesis are tested (Suleiman et al., 2018).

**Cell dry mass determination and lipid extraction.**

Biomass production was determined by harvesting the cells by filtration and then drying them at 55-60°C overnight to obtain constant weight. The dried biomass was ground to a fine powder, and 1 g of dried mushroom powder was added to 40 ml of chloroform/methanol (2:1) the mixture was stirred in an orbital shaker at 20°C for 20 minutes, then filtered through Whatman 1 filter paper, then 0.9% sodium chloride solution was added. Lipid-containing solvents were separated, evaporated and lipids determined (Nisha et al., 2009).

**Methyl ester preparation and fatty acid analysis using gas chromatography-mass spectrometry (GC/MS)**.

The transesterification reaction was carried out using sulfuric acid as catalyst in a flask under the following conditions. 30 Methanol to oil molar ratio of 1, 160 rpm, reaction time of 5 hours, temperature of 55° C., and catalyst loading of 80% based on oil weight (Liu et al., 2004; Miao & Wu, 2006). The reaction mixture was cooled and allowed to stand in a separatory funnel until two layers formed. The upper FAME layer was separated with petroleum ether and the final FAME product was obtained by evaporating the ether from the solution. Fatty acid methyl esters were analyzed by GC/MS. This was run on an Agilent 6890N gas chromatograph equipped with an Agilent 5973 mass spectrometer at 70 eV (m/z 50-550; source 230 °C and quadruple 150 °C) in EI mode with an HP 5ms capillary column (30) connected. conducted with an inner diameter of 0.25 mm and a film thickness of 0.25 mm. J&W Scientific, USA). Helium carrier gas was maintained at a flow rate of 1.0 mL/min. The inlet temperature was maintained at 300°C and the oven was programmed at 150°C for 2 minutes, then ramped at 4°C/min to 300°C and held at 300°C for 20 minutes. Injection volume 1 mL, split ratio 50:1. Structure assignments were based on interpretation of fragmentation by mass spectrometry and confirmed by comparison of retention times and fragmentation patterns of authentic compounds and spectral data from Wiley and NIST libraries.

**Statistical analysis**

All experiments were performed in triplicate and statistical analyzes were performed with Mini-Tab software (version 17). Values are given as mean ± SD (standard deviation). A significance level of $p \leq 0.05$ was considered unless otherwise stated.

## RESULTS AND DISCUSSION

**Isolation and screening of fungal isolates**

Finding suitable microbial strains with fermentation capability is necessary for the industrial production of lignocellulose biomass biofuels. (Tsegaye et al., 2019). Fungal isolates

were found in 499 different soil samples. According to routine identification under a light microscope, 32 out of 122 are *Zygomycetes*. Zygomycete-related fungal isolates were chosen because of their capacity for accumulating lipids, encompassing genera *Mortierella* (Fakas et al., 2009a), *Cunninghamella* (Fakas et al., 2009b, Suleiman et al., 2018a), *Rhizopus* ( Suleiman et al., 2018b), *Mucor* (Lu et al., 2010).

All 32 using Nile red dye, selected isolates were qualitatively screened for their production of lipid, and as a result, 6 out of 32 fungal isolates were shown to be the higher producers of lipids. These 6 are after additional screening of isolates. Quantitative secondary tests for target isolates were conducted using sulfo-phospho vanillin (SPV). A technique for calculating the amount of lipids was produced by these isolates. The reagent for phospho-vanillin was made by first combining 0.6 g of vanillin with 10 ml of 100% ethanol, 90 ml of deionized water, and continuous stirring. The combination was then given 400 ml of strong phosphoric acid, and the resulting reagent was stored until use in the dark.

Just prior to each experiment, fresh phospho-vanillin reagent was made to ensure strong activity. 100 l of water with a known amount of biomass suspended in a known volume of liquid culture or collected by centrifugation at 4000 RPM for five minutes, was applied to the algal culture's SPV reaction for measuring lipids. 2 mL of concentrated (98%) $H_2SO_4$ was used to treat the sample. It was heated for 10 minutes at 100 $^{o}$C before cooling for 5 minutes in an ice bath. 5 mL of freshly made phospho-vanillin reagent was added, and the sample was then incubated for 15 min at 37 $^{o}$C using a 200-rpm shaker in an incubator. An absorbance reading at 530 nm was collected to calculate the quantity of fat contained (Mishra et al., 2014).

**Molecular and traditional identification of the isolated fungi.**

In addition to selecting the strongest oleaginous *Rhizopus* species, the amount of lipid produced by the selected fungal isolates was measured using canola oil as a benchmark. All six *Rhizopus* species that passed the lipid production test were categorised as oleaginous fungi. Table 1 demonstrates various *Rhizopus* species. Among other *Rhizopus* species, UC 13 has the highest total lipid production and lipid percentage; this isolate produced 1.05 gl-1and a lipid percentage of 39.32%. The 18S rDNA homology value of strain was determined using the alignment search tools at http://blast.ncbi.nlm.nih.gov.UC 13 showed similarity (99%) with *Rhizopus oryzae.*

The Gen Bank accession number for strain UC 13 nucleotide sequence OR072734, the isolates of *Rhizopus oryzae-JSK3, Rhizopus sp. JSK6, Rhizopus sp. JSK8, and Rhizopus sp. JSKp* (Sukrutha et al., 2014).

**Increasing lipid production by Taguchi method**

***Preliminary optimization procedure***

Numerous factors, including as pH, temperature, carbon supply, and incubation time, were used in this method. The lipid synthesis process was optimised using Taguchi design (Enshaeieh et al., 2014;Chiranjeevi & Venkata, 2016). L9, the most effective carbon and nitrogen source for the most powerful fungus's production of lipids, was found using an array of Taguchi designs, This experiment demonstrates that among various carbon and nitrogen sources, glucose with ammonium sulphate gives the maximum quantity of lipid, as seen in figure 2. but most previous studies showed glucose and yeast extract are the best for lipid production by many genera of *mucorales* as *Mortierella* sp., *Mucor* sp. and *Cunninghamella* sp. (Rocky et al., 2011;Ling et al., 2016). Different levels of pH and temperature were used, and figure 5 shows pH 5 and temperature 30 better for lipid production. Figure 5 shows that lipid production is at its best between 1 and 7 days. Following the application of interactions between various components and their levels to obtain a high quantity of lipids, the main optimisation step is what follows all these optimal conditions.

**Identification of fatty acids (saponifiable matters) by gas chromatography mass spectroscopy**

A particular class of fatty acid ester called fatty acid methyl esters (FAME) is produced by trans esterifying lipids with methanol. The FAME composition revealed greater unsaturated to saturated fatty acid ratios under Taguchi design-optimized settings. Using the gas chromatography mass spectrometry (GC-MS) technique, the saponifiable matter (fatty acids) contents of *Rhizopus sp.* UC 13 were calculated. The relative percentage of each component was calculated in table (3) and figure (6). The obtained results revealed the presence of 7

saturated fatty acids namely, Tridecanoic, Caproic, Undecanoi, Palmetoleic, Heptadecanoic, Heneicosanoic and Behenic, several unknown and 5 unsaturated fatty acids namely, Linolenic, Gamma-Linolenic, Cis-8,11,14-Eicosatrienoic, Cis-11,14,17-Eicosatrienoic, and Cis-13,16-Docosadienoic.

It was clear from table (12) and figure 4 that Cis-11, 14,17-Eicosatrienoic (12.70%) and Undecanoic (5.30%) presented the higher percentages of fatty acids in *Rhizopus sp.* UC 13, respectively. Gamma-Linolenic (0.45%) and Palmetoleic (0.42%) were the lowest percentages of fatty acids in *Rhizopus sp.* UC 13. The results revealed that the largest concentration of saturated fatty acids was found in the lipid contents, which explains the findings of the fundamental chemical characteristics of the lipids of *Rhizopus* sp**.** UC 13.

In addition to docosahexaenoic acid (DHA-C22: 6), a fatty acid important for health because it is very nutritive, the vast majority of lipids produced in the fungal biomass were mostly represented by palmitic (C16:0), oleic (C18:1), and linoleic (C18: 2) fatty acids (Silveira et al., 2010). Also, Fakas et al., (2008**)** detected Cunninghamella echinulata produced oleic (C18:1), palmitic (C16:0), stearic (C18:0), linoleic (C18:2), and gamma-linolenic (C18:3) fatty acids in the biomass produced when it was grown in Potato Dextrose Agar (PDA) medium containing glucose, whey concentrate, and hydrolyzed tomato residue, as well as commercial corn. Musa et al., (2015) revealed that palmitic acid is a bioactive antimicrobial agent; it does exhibit anti-oxidant qualities and can protect rats from atherosclerosis. It does not raise cholesterol in the body if it is combined with linoleic acid. Because the human body cannot synthesize linoleic acid, which has the molecular name cis, cis-9, 12-octadecadienoic acid and is one of the shortest-chained Omega- 6 fatty acids (C18:2), it is regarded as an essential fatty acid. A family of polyunsaturated fatty acids known as omega-6 fatty acids contains both pro- and anti-inflammatory properties (Scorletti & Byrne, 2013). Gamma-linolenic acid (GLA) is produced in the body from linoleic acid. Arachidonic acid (AA) can then result from additional deterioration. In fact, GLA might lessen inflammation. According to some research, ingesting gamma linolenic acid (GLA) for six months or longer may help diabetics with neuropathy have fewer sensations of nerve discomfort. GLA might work better for those with good blood sugar control than for those with poor blood sugar control (De Lorgeril & Salen, 2012). These earlier studies provide evidence for the beneficial roles played by linoleic and palmitic acids in the examined biological impacts of the plants.

Contrarily, when incubation time was extended by an additional 3 days at 15OC, SFAs were decreased to 47.82%, but USFAs were drastically increased to 51.45%, entirely altering FAME percentages and producing new PUFAs. Broughton,(2011) showed that incubation at 15°C resulted in the highest unsaturation index value, indicating that temperature affects the lipid composition of fungi and that lower temperatures may raise lipid unsaturation levels. Two omega-6 PUFAs, linolenic acid (2.79%) and linoleic acid (0.10%), also emerge at low temperatures. C18:3 n6 was discovered to be made by *Rhizopus* sp., and it produced 4.37 mg/g of dry substrate (Conti et al., 2001).

## CONCLUSION

The findings of this research indicated that the *Rhizopus oryzae* isolate is an important fungi for the accumulation of lipids, especially poly-unsaturated fatty acids (PUFAs). In the current research, we found that method of Taguchi is considered superior in process of optimization, especially production of lipids, and demonstrated that lipid formation is significantly influenced by nitrogen, carbon source, and temperature. Furthermore, using this method, the optimum conditions of 39 % lipid content and 1.69 gl-1 lipid yield in *Rhizopus* oryzae at 30 °C, pH 5 for 5 days were determined. As a result, *Rhizopus* sp. UC 13 is regarded as a potential oleaginous filamentous fungus that can be applied in factories for the production of PUFAs.

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**Table 1:** Screening of *Rhizopus spp.* for lipid production using SPV method:

| *Rhizopus* sp. | Isolates Code | Dry biomass (g/l) | Total lipid (g/l) | Lipid (%) |
|---|---|---|---|---|
| 1 | CD | 1.79 ± 0.13 | 0.51 ± 0.07 | 28.49±0.14 |
| 2 | CR | 1.46 ± 0.11 | 0.48 ± 0.02 | 32.87±0.62 |
| 3 | UC | 2.67 ± 0.09 | 0.83 ± 0.05 | 31.09±0.32 |
| 4 | CQ | 3.15 ± 0.12 | 0.97± 0.03 | 31.09±0.32 |
| 5 | UC 13 | 2.67 ± 0.08 | 1.05 ± 0.06 | 39.32±0.67 |
| 6 | UC | 1.78 ± 0.12 | 0.47 ± 0.02 | 29.40±0.33 |

**Table 2:** Optimum conditions for lipid production by *Rhizopus* sp. UC 13:

| Conditions | Best Conditions |
|---|---|
| Incubation time | 5 days |
| pH | 5 |
| Temperature | 30°c |
| Carbon source | Glucose |
| Nitrogen source | Amm. Sulphate |

**Table 3:** GC-MS profile of fatty acid obtains from *Rhizopus sp*:

| Peak No. | Common name | Chemical name | Number of carbon atom | Type | RT | Area % |
|---|---|---|---|---|---|---|
| 1 | Unknown | - | - | VV | 0.702 | 3.61 |
| 2 | Unknown | - | - | BV | 1.433 | 1.03 |
| 3 | Unknown | - | - | VV | 1.934 | 0.74 |
| 4 | Caproic | Hexanoic acid | 6:0 | VB | 2.005 | 0.93 |
| 5 | Unknown | - | - | BB | 2.380 | 1.73 |
| 6 | Undecanoic | Undecanoic acid | 11:0 | BV | 4.011 | 5.30 |
| 7 | Unknown | - | - | VB | 5.061 | 1.48 |
| 8 | Unknown | - | - | BB | 5.589 | 0.44 |
| 9 | Unknown | - | - | VV | 5.927 | 0.72 |
| 10 | Tridecanoic | Tridecanoic acid | 13:0 | VB | 6.182 | 1.02 |
| 11 | Unknown | - | - | BV | 9.298 | 0.97 |
| 12 | Unknown | - | - | BV | 9.298 | 0.97 |
| 13 | Palmitoleic | Palmitoleic acid | 16:0 | BV | 10.050 | 0.42 |
| 14 | Heptadecanoic | Heptadecanoic acid | 17:0 | VB | 11.152 | 1.49 |
| 15 | Unknown | - | - | BB | 11.952 | 11.13 |
| 16 | Unknown | - | - | VV | 12.608 | 1.22 |
| 17 | Unknown | - | - | BB | 12.906 | 1.03 |
| 18 | Linolenic | (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid | 18:2 | BB | 18.217 | 1.38 |
| 19 | Gamma-Linolenic | (6Z,9Z,12Z)-octadeca-6,9,12-trienoic acid | 18:2 | VV | 18.773 | 0.45 |
| 20 | Unknown | - | - | VB | 19.653 | 1.09 |
| 21 | Unknown | - | - | VV | 20.239 | 1.09 |
| 22 | Unknown | - | - | BV | 25.096 | 1.09 |
| 23 | Cis-11,14,17-Eicosatrienoic | (11Z,14E,17E)-icosa-11,14,17-trienoic acid | 21:3 | VV | 25.274 | 12.70 |
| 24 | Unknown | - | - | VV | 26.058 | 1.08 |
| 25 | Unknown | - | - | BV | 26.544 | 5.72 |
| 26 | Unknown | - | - | VV | 26.571 | 0.83 |
| 27 | Cis-8,11,14-Eicosatrienoic | (8Z,11Z,14Z) -(113C)henicosa-8,11,14-trienoic acid | 21:3 | VB | 26.588 | 1.49 |
| 28 | Unknown | - | - | BV | 30.345 | 2.88 |
| 29 | Heneicosanoic | Heneicosanoic acid | 21.0 | VB | 30.532 | 2.50 |
| 30 | Behenic | Methyl docosanoate | 22.0 | BV | 31.329 | 5.21 |
| 31 | Unknown | - | - | BV | 31.796 | 7.49 |
| 32 | Unknown | - | - | VV | 31.884 | 1.18 |
| 33 | Unknown | - | - | VB | 31.914 | 0.88 |
| 34 | Unknown | - | - | VV | 31.954 | 0.44 |
| 35 | Unknown | - | - | VV | 31.995 | 1.51 |
| 36 | Unknown | - | - | VV | 32.022 | 2.39 |
| 37 | Unknown | - | - | VB | 32.083 | 0.49 |

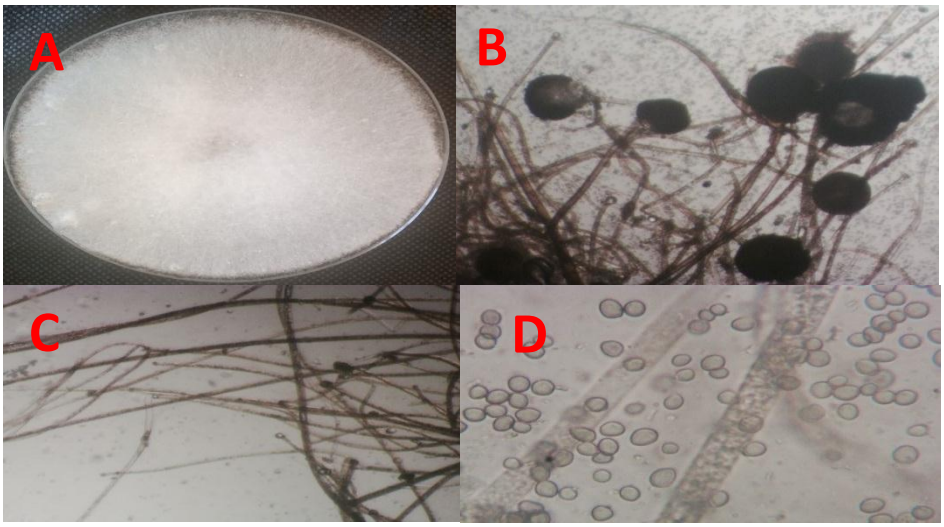


**Figure 1:**shows *Rhizopus* sp. UC 13.pictures (A) surface growth on MEA. (B) Sporangium and sporangiophore under light microscope. (C) Rhizoids under light microscope. (D) Spores and hyphae under light microscope.


**Figure 2:** Phylogenetic tree of gene sequences of the *Rhizopus oryzae* isolate with the sequences retrieved from NCB Gene Bank site.

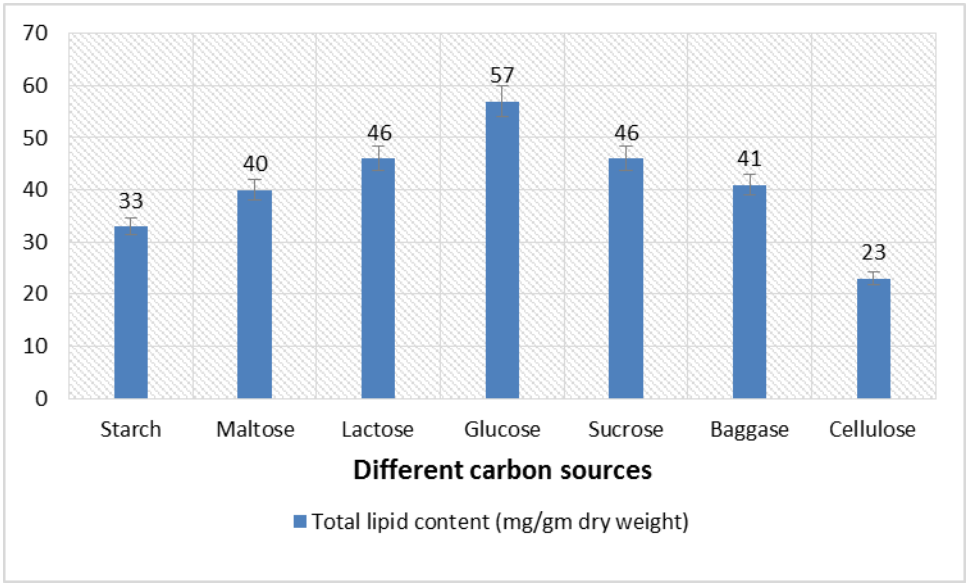


**Figure 3:** Effect of different carbon sources on lipid production by *Rhizopus* sp. UC 13.

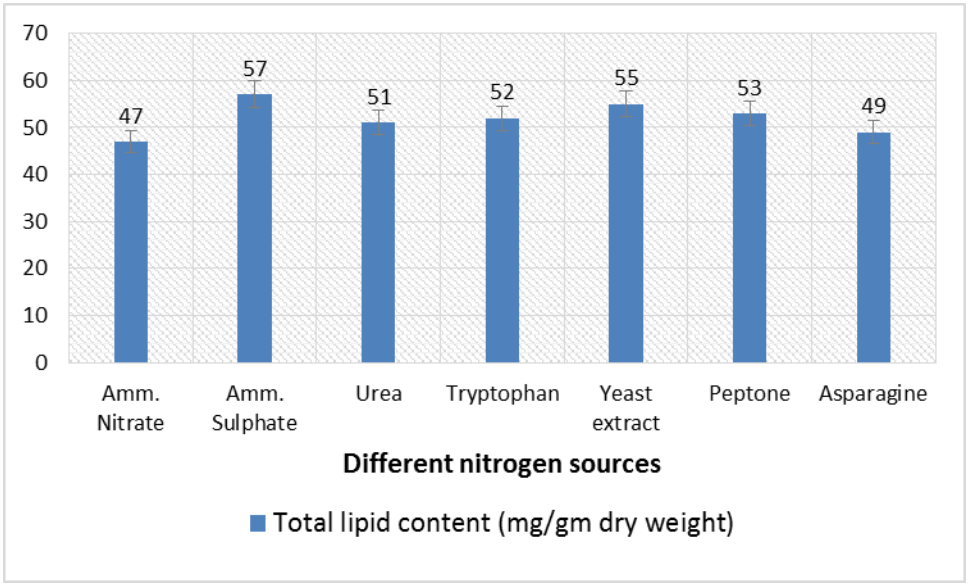


**Figure 4:** Effect of different nitrogen sources on lipid production by *Rhizopus* sp. UC 13.

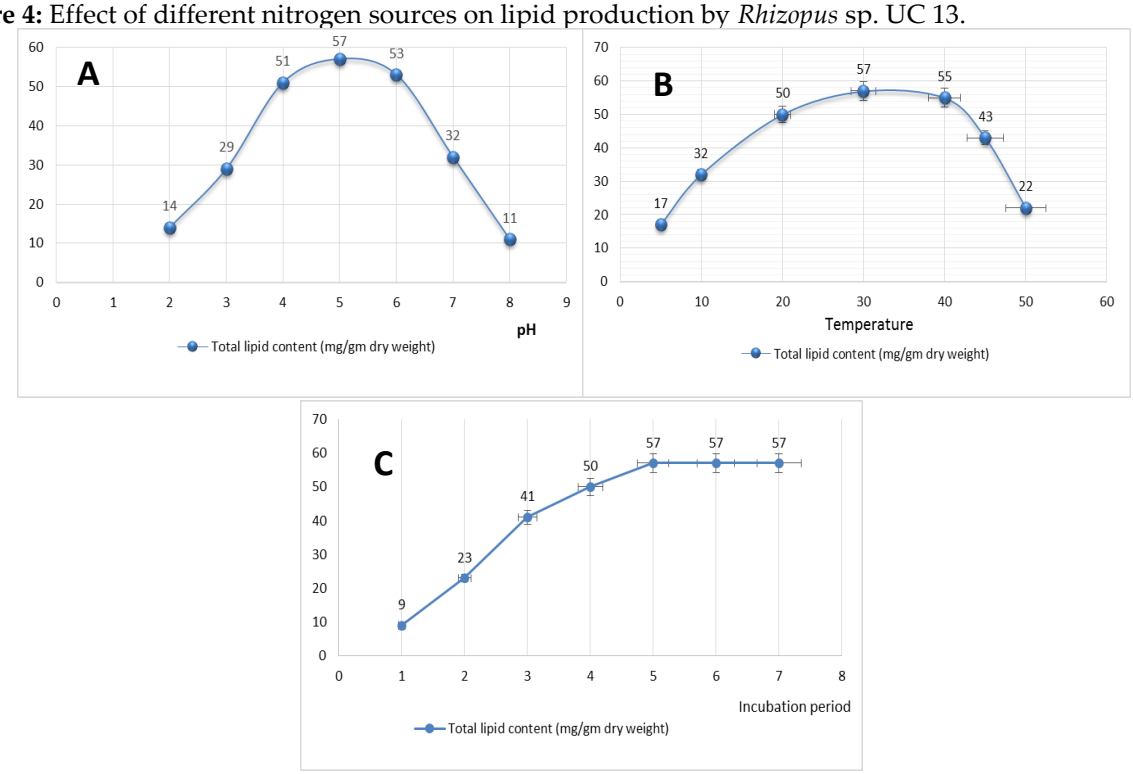


**Figure 5:** Effect of different **(A)** pH levels **(B)** temperature **(C)** incubation periods on lipid production by *Rhizopus* sp. UC13.

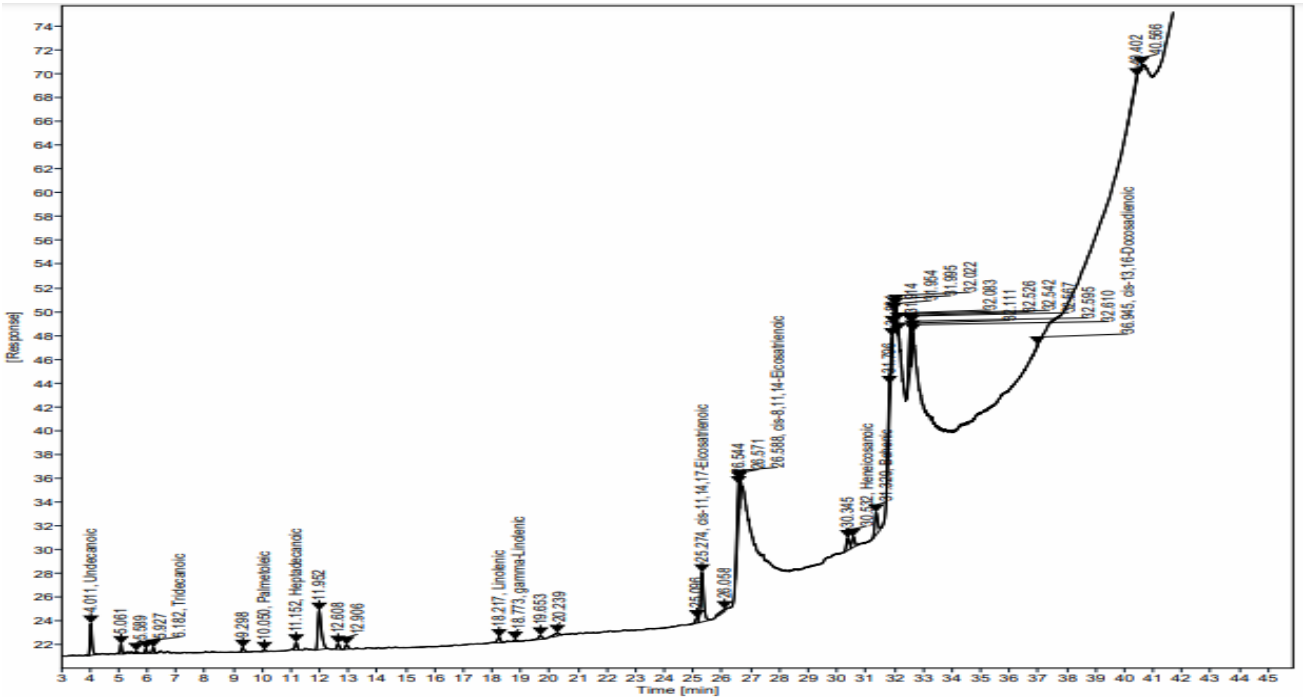


**Figure 6:** GC-MS spectral chromatogram of *Rhizopus sp*. UC 13:

## إنتاج الأحماض الدهنية غير المشبعة ذات الأهمية الاقتصادية باستخدام *عفن الخبز* المعزول من أرض اليمن غير المزروعة

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**الملخص العربى**

الفطريات الزيجوتية خاصة الفطريات الزيتية لها قدرة كبيرة على إنتاج كميات ضخمة من الدهون الغنية بالأحماض الدهنية المتعددة غير المشبعة المهمة اقتصاديًا. تم عزل ما مجموعه 32 عزلة من الفطريات الزيجوتية الزيتية. تم تحديد ستة من 32 على أنهم سلالة *عفن الخبز* تم اختبارهم لإنتاج مستويات عالية من الدهون. 6 من 32 لديهم القدرة على إنتاج كميات هائلة من الدهون. تم اختيار سلالة غ م 13 لمزيد من التحسين حيث أنتجت 1.05 جم 1 ليبيد مع محتوى دهني بنسبة 39.21٪. تمت زيادة إنتاج الدهون. تم استخدام طريقة تاغوشي التي اختارت عددًا من العوامل على مستويات مختلفة لعملية التحسين. يؤثر الجلوكوز وكبريتات الأمونيوم ودرجة الحرارة ودرجة الحموضة والوقت بشكل مباشر على تكوين الدهون. كانت الأحماض الدهنية المشبعة أكثر انتشارًا من الأحماض الدهنية غير المشبعة وفقًا لتحليل كروماتوجرافيا الغاز الفائق الدقة ، والذي كشف أيضًا وجود بعض الاحماض الدهنية الغير مشبعة بكميات صغيرة. نتيجة لذلك ، فإن فطر *عفن الخبز* غ م 13 محل الدراسة يعتبرمن الفطريات الخيطية الزيتية المحتملة التي يمكن تطبيقها في المصانع لإنتاج بالأحماض الدهنية المتعددة غير المشبعة.

**الكلمات الاسترشادية:** الأحماض الدهنية المتعددة غير المشبعة، *عفن الخبز*، اليمن.