Chapter 3:

Production, Characteristics, and Applications of Microbial-Derived Fats: Halal Issues

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This chapter "Microbial Fats" explores the rapid advancement of biotechnology and its application in producing fats and oils via microbial processes. This approach leverages fermentation and bioengineering techniques using diverse microorganisms, including algae, bacteria, yeast, and fungi, to accumulate oils, fatty acids, and triglycerides under specific cultivation conditions. Known as single-cell oils (SCOs), these microbial fats mirror plant oils in structure and can be tailored by adjusting growth conditions, offering promising alternatives for various industries such as food, cosmetics, pharmaceuticals, biofuel, and chemicals. The microbial production of oils has historical roots dating back to the 19th century, with renewed interest in their commercial potential due to their cost-effectiveness, reliance on non-arable feedstocks, and alignment with sustainable practices. Additionally, microbial fats, particularly Omega-3 fatty acids, are recognized for their nutritional benefits and increasing consumer demand as vegan sources of essential fatty acids. This chapter highlights the economic and environmental advantages of microbial fats, such as reduced land use and waste management synergy, while discussing the impact of microbial lipids on sustainable biofuel production, environmental conservation, and the circular economy. The chapter also addresses the importance of ensuring microbial fats' halal certification to meet global Muslim consumer demand. Ultimately, microbial fats represent a renewable, environmentally friendly alternative to conventional fats, though challenges remain in optimizing production, scalability, and cost-effectiveness.

1 Introduction

Recently, biotechnology has advanced quickly. The use of biotechnology for human needs, particularly the food business, was fairly widespread. Some traditional and contemporary biotechnological procedures require the employment of microbes in order to function. Through fermentation, specific microbes were added to traditional foods, including a variety of adaptable bacteria linked to numerous health-promoting end products, such as dietary fatty acids and naturally occurring fermenting microbial cells (Valentino, et al., 2024). According to available data, dietary fatty acid components either directly regulate genes through particular nuclear receptor binding or indirectly by altering regulatory transcription factors in a hormonally dependent manner. Microorganisms are additionally used in the DNA recombination process to create premium food items like GMOs (Genetically Modified Organisms).

The production of oil and fatty acid using microorganisms is feasible, it is through fermentation or bioengineering processes. Various microorganisms are recommended for this purpose, such as algae, bacteria, yeast, and fungi, that can accumulate oils, fats, fatty acids, and triglycerides under special cultivation conditions. In essence, microbial fat is oil produced from microbes, which has a long history going since the mid-1870s (Hopton & Woodbine, 1960; Ficinus, 1873; Nageli and Loew, 1878 and Ratledge, 2001). The produced oils and related materials are known as single-cell oils (SCOs), that are suitable for different applications (Ratledge, & Hopkins, 2006 and Ratledge, 1976), are created during the defatting process of yeasts cultured on hydrocarbons, and despite the fact that these lipids have intriguing features; it appears unlikely that they would be purposefully created elsewhere for cost-saving purposes, from the early 1980s, SCOs have gravitated more and more towards the most expensive materials. (Table 3.1), which may be

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directly compared to the oils and fats derived from plant oilseeds in terms of their chemical composition (Fig. 3.1).

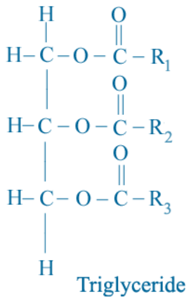

Figure 3.1: Chemical structure of a triacylglycerol (Yokochi et al., 1998)

Table 3.1: *Lipid content profiles (w/w) of some Oleaginous, Heterotrophic microorganisms as Sources of SCOsa* Ratledge (1997, 2001).

Type of microb	Lipid (% w/w)
Yeasts	
Cryptococcus curvatus	58
Lipomyces starkeyi	63
Rhodosporidium toruloides	66
Rhodotorula glutinis	72
Rhodotorula graminis	36
Yarrowia lipolytica	36
Molds	
Entomophthora coronate	43
Cunninghamella japonica	60
Mortierella alpin	50
Mucor circinelloides	25
Pythium ultimum	48
Algae (grown heterotrophically)	
Crypthecodinium cohnii	40
Schizochytrium limacinum[b]	50
Thraustochytrium aureum	15

2 Types of microbial fats

Microbial lipids have identical triacylglycerol structure as plant oils and the same distribution of fatty acids at the three positions of glycerol: saturated fatty acids are generally excluded from the central (sn-2) carbon atom. Moreover, microbs can be improved by genetic modications much more easily than higher organisms. Therefore, microbial lipids have attracted considerable interest during the past decade as well as other microbial metabolites (Akoh, 2017).

An important characteristic of microbial fat production is the possibility of changing the composition of microbial fatty acids by altering the microbial growth in the environment. Although microbial fat production is not commercial, it is possible to make it economical by increasing production by selecting a suitable microorganism under an optimum condition, especially since microbial fatty acids are above all other polyunsaturated fatty acids (Ghazani, & Marangoni, 2022).

Cryptococcus curvatus, an oleaginous yeast strain was observed to grow on sweet sorghum syrup derived from sorghum juice. The overall lipid productivity was 4.0 g/L-day. Through use of microwave-assisted lipid extraction, 78.1% of available lipid was extracted out of *C. curvatus* cells in 4 min using methanol as the only solvent. This study demonstrated that fermentation of *C. curvatus* using sorghum syrup will result in high lipid yield. (Cui & Liang, 2015).

3 Production, Uses and Commercial Importance of Microbial Fats

This Chapter aims to present overviews and covers the related research progress about different oleaginous microorganisms producing oils, and the prospects of such microbial oils and their exploitation in various sectors such as food, cosmetics, pharmaceuticals, biofuel, and chemicals production are also discussed.

Throughout the majority of the 20th century, there have been discussions about using microbial oils as commercial oils and fats sources. During both world wars, Germany made serious efforts to create processes that would produce useful quantities of oils and fats for a nation without access to significant supplies of such commodities. Somewhat surprisingly, from roughly 1920 to 1945, Germany made significant progress in the identification of appropriate lipid-producing species (Bernhauer, 1943; Bernhauer and Rauch, 1948; Hesse, 1949). The interest in the potential commercial benefits of creating microbial oils was evident until the end of the 1950s in other nations, such as the United States and the United Kingdom (Colin Ratledge & James P. Wynn, 2002), although microbial fats and oils offer several advantages over conventional methods. Firstly, it can be achieved using a wide range of feedstocks, including agricultural waste, industrial by-products, and non-arable land, reducing reliance on traditional crops. This is also in line with the driving principles of the circular economy, according to which residues can be utilized for novel, innovative products. This chapter's objective of the production of microbial fats is to draw attention to the most well-known oleaginous yeast, mold & mold genera by highlighting their oleaginous properties in addition to a variety of other traits, including their ability to use inexpensive carbon sources, to withstand the effects of inhibitory compounds, and to have fatty acid compositions that are commercially advantageous, due to their enormous demand for fatty materials in the food, medical, oleochemical, and biofuel industries. Some concerns were developed in the early 1980s for the promising production of a cocoa butter equivalent (CBE) fat using yeasts (Colin Ratledge, 2010; Moreton, 1988; Smit et al., 1992). In the context of describing the numerous tactics effectively used to increase the synthesis of lipid and lipid-derived metabolites. There has recently been a significant increase in interest in studies pertaining to various aspects of microbial lipid synthesis, which is one of the top subjects in pertinent research disciplines.

As suggestive evidence in accordance with the extraordinary oleaginicity of these organisms, a substantial amount of in silico data obtained on the lipid build-up in some oleaginous yeasts have been meticulously curated. The various genetic components used by these yeasts to carry out these techniques have undergone meticulous examination, highlighting the main categories of recently discovered and synthetically created promoters, transcription terminators, and selection markers. A tonne of sophisticated genetic toolboxes and methods have been applied in oleaginous yeasts recently, encouraging homologous recombination, genome editing, DNA assembly, and transformation with astounding efficacy. They can hasten and efficiently direct the rational design of system-wide metabolic engineering strategies that identify the primary targets for creating suitable yeast strains for industrial use (Atrayee C & Mrinal K. Maiti 2021). Lipid accumulation by the so-called "oleaginous microorganisms" can produce more than 20% w/w of oil in dry biomass and is controlled by a variety of factors, including the pH of the medium, the temperature of the incubation chamber, the availability of nutrients, and the C/N (carbon/nitrogen) ratio, adjusting the optimum environment and finding the cheapest substrate to enable lipid fermentation by oleaginous microorganisms have a significant impact on the bioprocess for producing lipids. This research required a lot of effort up until this point (Carsanba et al., 2017).

In recent years, several companies and research institutions have made significant progress in the development of microbial fats and oils. The lipases category represents less than 10% of the global enzymes market, with a broad range of industrial applications: detergents, oil processing, food processing, and pharmaceutical end-users (Guerrand, 2017).

Carsanba et al., (2018) reported on the production of oils and fats by oleaginous microorganisms, emphasizing the potential of the non-conventional yeast Yarrowia lipolytica. Their research findings were telling that lipid accumulation by the so-called "oleaginous microorganisms" can generate more than 20% w/w of oil in dry biomass and is governed by a plethora of parameters, such as medium pH, incubation temperature, nutrient limitation, and carbon/nitrogen ratio, which drastically affects the lipid production bioprocess.

The commercial importance of microbial lipids is due to the high demand of these fatty materials in food, medical, oleochemical and biofuel industries, which is one of the top research topics nowadays.

4 Microbial lipids in food

In food industry, Guerrand, (2017), reported that microbial lipids have diverse applications across various industries. They can be used as substitutes for plant and animal-derived fats and oils in food products, such as margarine, spreads, and baked goods. Their composition and physical properties can be tailored to meet specific functional requirements. Microbial oils are also being explored as sources of omega-3 fatty acids, essential for human health. (Gunstone, 2006).

The Omega−3 is the highest commercialized microbial lipids product, consumer interest and demand are rapidly increasing due to an increase in the incidence of chronic diseases as natural dietary sources of n−3 PUFAs (Table 3.2). Omega−3 (n−3) polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) acid are well known to protect against numerous metabolic disorders. Microbial sources of Omega−3 are Thraustochytrids and Microalgae are currently used for the commercial production of vegan EPA and DHA, which is now considering the nutritional and commercial importance of n−3 PUFAs, (Ramesh Kumar Saini, 2021); Adarme-Vega, et al., (2012); Wells, M.L., et al., (2017). The heterotrophic fungus-like clade of *Stramenopiles* known as *throustochytrids* is a commercially significant source of dietary EPA and DHA, (Leyland, B. et al., 2017).

Fed-batch fermentation is used to produce DHA, DHA can accumulate in *Aurantiochytrium limacinum SR2* up to 48.51% of the total FAs with high productivity (32.36 g/L and 337.1 mg/L/h) (Li, J. et al., 2015). In flask and bioreactor, batch fermentation is used for *Schizochytrium limacinum SR21* that produced DHA concentrations of 45.54 and 67.76% of total lipids, respectively (Patel, A. et al., 2020), glycerol is employed as a carbon source (Scott, S.D. et al., 2011).

On the other hand, the primary source of VLC-PUFAs for fish, zooplankton, and other multicellular creatures is microalgae (Wells, M.L., et al., 2017). Microalgae like *Nannochloropsis spp*., which have high total lipid content (up to 37–60% of dry weight), can collect up to 37.8% EPA. (Ma, X.-N. et al., 2016) (Table 3.3). Moreover, microalgae are abundant in necessary amino acids, lipids (fucosterol and β-sitosterol), polysaccharides (alginate and β-glucans in brown algae), vitamins (A, E, B1, B2, B6, and B12), and minerals (Wells, M.L., et al., 2017).

Microalgae are more prolific than other potential sources such as bacteria, fungi, fish, and transgenic plants because of their quick reproduction and harvesting time. Moreover, n-3 PUFAs accumulate in large amounts under stress circumstances such as salinity, temperature, UV radiation, nutritional deprivation, and pH due to their high energy content and capacity to maintain membrane fluidity (Paliwal, C. et al., 2017). In particular, low-temperature stress is crucial for PUFA formation because PUFA (mostly EPA and DHA) support life in low-temperature environments by preserving the fluidity of the membrane. By changing the late log phase growth culture in *Nannochloropsis spp*., low temperature (10 °C) and low light enhanced EPA generation by 3.4-fold. In *Phaeodactylum tricornutum*, high urea concentration (0.01 M) and phosphate depletion (Mitra, M. et al., 2015). High CO_2 levels (0.15%), and low temperatures (25 °C -15 °C) can increase EPA accumulation by 45.0, 38.6, 73, and 18%, respectively (Cui, Y. et al., 2019)

Table 3.2: *Commercial products of Microencapsulated vegan n−3 fatty acids-based.*

Company	Ingredient Brand	Major n−3 Fatty Acids
FrieslandCampina N.V. (Amersfoort, Netherlands)	Vana®-Sana algae DHA 11 IF	Microalgal derived DHA
Algarithm Ingredients, Inc. (Saskatoon, Saskatchewan)	Betamega³	Microalgal oil powder (120 mg DHA/g)
	Gamma³	Microalgal DHA emulsions (400 mg DHA/g emulsion)
Cubiq Foods (Granollers. Barcelona)	Go!Mega3®	Microalgal DHA+EPA (2% w/w)
Seanova (Finistère, Brittany)	Algal DHA powder H100	100 mg/g DHA from Schizochytrium sp.
	Chia powder-125	60 mg/g ALA from chia seeds
	Chia powder-435	55 mg/g ALA from chia seeds

Source: Company websites.

Table 3.3: *The major commercially available Microalgae dietary products vegan n−3 PUFA-based*

EPA	*Microalgae Nannochloropsis* sp.	Dietary supplement	Jemmax Nutraceuticals Co. https://jemmaxnutraceuticals.com/
DHA	DHASCO-B® nutritional oil and powder, and life'sDHA® vegetarian capsules from microalgae	Dietary supplement	DSM and Firmenich Co. https://www.dsm-firmenich.com/en/home.html
	Vegan Omega 3 DHA capsules from *Thraustochytrid Schizochytrium* sp.	Dietary supplement	OMVITS Co. https://omvits.com/?srsltid=AfmBOoqRSwsEojtqeLEDB3AdyMwI5u_GCr3FD68kJprEaLbenxikIthb Naturopathica Co. https://naturopathica.com.au/

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Additionally, several fungi have been investigated as a source of dietary essential fatty acids since they contain unique oils. *Mucor javanicus* and *Mucor isabellana*, are important fungi that produce dietary polyunsaturated fatty acids of dietary importance, including γ-linoleic acid (contains primrose oil, which helps women suffering from premenstrual tension). The substrate used for γ-linoleic acid production in large is potato-paste dextrose. A strain of *Mortierella alpina* may also synthesize icosapentaenoic acid, which primarily occurs in fish oils, with yields of up to 20% of the total fatty acid content.

Additionally, yeasts have the potential to serve as a commercial source of fats similar to cocoa butter. The useful triglyceride oil produced by *Cryptococcus curvatus,* the oleaginous yeast that is grown on some wastes like whey using the lactose constituent, has been intensively studied for the production of cocoa butter equivalent or single cell oil that has a considerable industrial interest (Hassan et al. 1995, Moon et al. 1978, Ykema 1989)

Moreover, as *Saccharomyces cerevisiae* or *Candida utilis*, *Rhodotorula* and *Lipomyces* produce lipids in the form of triacylglycerol (Rattray, 1989). Fatty acid synthesis by yeast is expected to become an economically practical idea.

5 Microbial Lipids in Environmental Impact

Production of microbial lipids also reduces the environmental impact associated with land use change and deforestation. Furthermore, microbial fermentation processes can be optimized to enhance lipid production efficiency, leading to higher yields and cost-effectiveness. Oleaginous microorganisms act as factories that can grow on various carbon substrates like agri-food streams, municipal wastes, industrial wastes, etc., thus making them suitable players in the bio-based economy by producing lipids known for having numerous applications. Dar et al., (2024), reported update knowledge of microbial lipid production from low-cost organic wastes with an emphasis on lipogenesis in oleaginous microorganisms.

Microorganisms are flexible and able to grow on different side-stream carbon sources, such as those deriving from agri-food wastes that may affect the environment if not suitably utilized. The agri-food industry annually produces huge amounts of crop residues and wastes. The suitable management of these products is important to increase the sustainability of agro-industrial production by optimizing the entire value if residues can become feedstocks for novel processes. (Caporusso, Capece & De Bari, 2021). Ensuring the sustainability and safety of microbial lipid production systems is crucial to avoid potential environmental risks.

6 Biofuel

The problem of the industrial utilization of food plant oils has become more urgent with the development of global biodiesel production. Oils and fats of vegetable and animal origin have been the most important renewable feedstock of the chemical industry in the past and in the present (Biermann, et al., 2011). Introducing and cultivating more new oil plants or other new sources containing/producing fatty acids with interesting and desired properties for chemical utilization will be important.

Microbial fats and oils also have potential applications in the production of biofuels, such as biodiesel and aviation fuels. The high energy content and compatibility with existing infrastructure make them attractive alternatives to fossil fuels that will save an enormous amount of capital required to replace the current infrastructure to accommodate biofuels with properties significantly different from petroleum-based fuels (Lee et al., 2008). Moreover, microbial lipid production can be integrated with waste treatment processes, enabling simultaneous waste management and biofuel production.

Recently, much attention has been paid to the development of microbial lipids. These microbial lipids have gained significant attention as potential alternatives to traditional plant and animal-derived fats and oils, especially in the context of sustainable and renewable resources. Biodiesel has become more attractive recently because of its environmental benefits and the fact that it is made from renewable resources. Compared to other plant oils, microbial oils have many advantages, such as a short life cycle, less labour required, less affection by venue, season, and climate, and easier to scale up (Patel et al., 2020). With the rapid expansion of biodiesel, microbial oils might become one of the potential oil feedstocks for biodiesel production in the future.

7 Halal Issue

A food is considered Halal if it adheres to the strict guidelines outlined in Islamic law. All foods are deemed Halal unless they fall under the category of Haram, which are clearly specified in the Qur'an. Ensuring the sustainability and safety of microbial lipid production systems is crucial. These systems must be manufactured in full compliance with Islamic law, as attested by Halal certification. Halal certification is essential in the food, cosmetics, and pharmaceutical industries to mitigate potential health risks and reassure Muslim consumers that their religious precepts have been respected. It also ensures that high standards of product hygiene and safety are met to address the growing demand for Halal products in global markets.

Any potential health risks may render the product unsafe, making it doubtful or non-Toyyib (pure and wholesome) for human consumption.

Indonesia, as a country with a majority Muslim population, places significant emphasis on the Halal status of food products. This is especially important for biotechnology foods, which utilise microorganisms and must be carefully managed to ensure compliance with Halal standards. It is vital that all production processes are carefully monitored to avoid contamination with non-Halal materials (Hayyun Durrotul Faridah & Silvi Kurnia Sari, 2019).

8 Conclusion

Overall, microbial fats and oils present a sustainable and renewable alternative to conventional fats and oils, with a wide range of applications in food, cosmetics, pharmaceuticals, biofuels, and other industries. Ongoing research and development efforts are focused on improving production efficiency, scalability, and cost-effectiveness, making microbial lipids a viable, Halal, and environmentally friendly option for the future. While microbial fats and oils hold significant promise, several challenges remain. These include the economics of large-scale production, optimization of lipid yields, and the development of efficient downstream processing techniques for lipid extraction. Additionally, ensuring the sustainability and safety of microbial lipid production systems is essential to prevent potential environmental and health risks. However, microorganisms have many advantages over plants and animals such as higher growth rate, higher lipid content, less sensitivity to seasonal or climatic changes, and possibility of production in small areas. Moreover, oil yield and composition of the oil can be developed or modied by easily by adjusting cultural conditions.

9 Publisher's Note

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