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Review

Nutritional and Medicinal Properties of Microbial Oil

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Abstract: Plant and animal oils and fats currently dominate the edible oil market, but a new sustainable alternative of lipids from single-celled organisms has become advantageous in human nutrition and pharmacy. Single-cell oils (SCOs) are lipids biosynthesized and accumulated in the lipid bodies of oleaginous species of bacteria, yeasts, molds, and algae. The review has investigated SCOs' composition, with a detailed review of the described beneficial impact in medicine, cosmetics, pharmacy, and nutrition. Although microbial oil has been known for more than 100 years, it was not applied until the 21st century, when commercial SCO production for human use started and administrative regulations governing their use were completed. This article discusses the applications of SCOs, which can be easily found in microorganisms, in the pharmaceutical, cosmetic, and food industries. In addition, some aspects of 15- or 17-carbon-atom-long fatty acids were also pointed out. Furthermore, some challenges for heterotrophic single-cell oil synthesis and improvements in its extraction efficiency have also been concluded, which can further contribute to their broadened use in pharmacy, medicine, cosmetics, and food applications.

Keywords: essential fatty acids; single-cell oil; microbial oil; lipid source; nutrition

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1. Introduction

All microorganisms are composed of lipids, usually comprising around 6 to 8% (*w*/*w*) of their dry cell weight. Microorganisms producing more than 20% (*w*/*w*) of their dry cell weight as lipids are termed oleaginous [1,2]. To distinguish the lipids of single-celled organisms, or microorganisms, that are suitable for human consumption from the plant and animal oils and fats that currently dominate the edible oil market, the term "single-cell oils" (SCOs) was coined [1–3]. The manufacturers of microbial proteins believed that calling their products "single-cell proteins" (SCPs) would be a suitable way to refer to them without disclosing their sources, which include bacteria, yeasts, and fungi. The same reason stands behind the development of SCOs: to avoid outright disclosing the source of the oils, which the general public might find challenging to understand. The original intent of SCO was to denote the microbial fats, i.e., the triacylglycerol fraction of the total cell lipid, hence, it was expected to be equivalent to commercial plant and animal oils. However, the term has now been expanded to encompass lipids containing all fatty acids in a single cell. This includes algal lipids where triacylglycerols might not be the dominant fraction. Algal lipids comprise a complex array of other lipid types, including many glycosylated and sulfur-containing lipids associated with the photosynthetic apparatus of these organisms [1].

In recent years, the importance of omega-3 fatty acids (DHA—docosahexaenoic acid and EPA—eicosapentaenoic acid), essential for human health, has increased. The human body cannot synthesize omega-3, and it must be obtained through supplementation. Omega-3 supplements containing DHA and EPA have been found to reduce the risk of various heart conditions and neurodegenerative diseases like Alzheimer's [4]. Fish has traditionally been the primary source of omega-3, but due to the increasing human

population and the depletion of natural resources, sustainable alternatives are being explored. Microbial oils, derived from bacteria, yeast, fungi, and microalgae, are one such sustainable alternative [5]. High-yield microbial oleaginous yeasts are particularly promising for the future, due to their capacity for oil production. Compared to the production of omega-3 supplements from marine sources, microbial oil production is more cost-effective. Additionally, their long shelf life, vegetarian nature, and sustainability have led to increasing consumption. Microbial oils, first used in baby formulas to provide essential DHA for infant development, have been deemed to be generally recognized as safe (GRAS) by the FDA (Food and Drug Administration) [6], and, according to the EFSA (European Food Safety Authority), microbial oils are classified as novel foods. Currently, many companies produce microbial oils as dietary supplements, and they are expected to play an increasingly significant role in the market sector in the future. This study aims to investigate the impact of microbial oils on human health; the economy of their production; their importance in the pharmaceutical, cosmetic, and food industries; the regulations governing their use; and the exploration of the future applications of single-cell oils.

2. History and Development of Microbial Oil Manufacture

Microorganisms are a known source of animal feed and human foods. They are no stranger to the history of food, and they are used to ferment food products to improve their nutrition, taste, and texture. In the 20th century, microbial biomass became a source of nutritional food ingredients. Microbial oil can be an example of a new application of microorganisms [1,2]. The research on microbial lipids dates back to the 19th century. In the 1880s, Eugène Coubain discovered bacteria that can produce fatty acids [7]. According to databases of Scopus and Web of Science, the first recorded papers related to microbial oil were published in the 1940s. The first papers were related to bacterial lipids (Figure 1). The bacterial lipid metabolism had a significant impact on the understanding of the basic lipid metabolic pathways, enzyme mechanisms, and transcriptional regulation. The early work in the *Escherichia coli* system jump-started the investigation of fatty acid and phospholipid synthesis [8]. Although the study of individual phospholipids and their synthesis began in the 1920s in plants and then mammals, it was not until the early 1960s that Eugene Kennedy, using *E. coli*, initiated studies of the bacterial phospholipid metabolism at Harvard Medical School. In the 1970s and 1980s, most of the enzymes responsible for phospholipid biosynthesis were purified and identified, and, in the 1990s, the genes encoding those proteins were sequenced [9].

In 1895, a yeast termed *Torula pulcherrima* (now *Metschnikowia pulcherrima*) was discovered to produce an oil droplet, and, in 1915, the fungus *Endomyces vernalis* was demonstrated to produce up to 42% (*w*/*w*) lipid under nitrogen limitation [2,10]. The records describing the production of lipids from yeast date back to 1878 [11]. The first microbial oil was only marketed in 1985 by the company J & E Sturge (North Yorks, UK) and originated from the mold culture of *Mucor circinelloides* [1]. In the 1960s, yeast and molds became popular microbial lipid factories to be explored, and the study of lipids in cell biology ("lipidology") emerged with the first attempts to describe and understand the lipid composition of cells. The number of papers related to microbial oil doubled each decade from the 1960s to the 1990s (Figure 1). The involvement of the yeast *Saccharomyces cerevisiae*, which is a well-established experimental model organism, had proven to be valuable in understanding lipid synthesis and its regulation. Efforts have been made to understand the biochemistry of neutral lipid synthesis and its packaging and assembly into mature lipid droplets (metabolically highly active subcellular organelles present in all eucaryotic cells) [12,13]. During the 1980s, algae research gained significant importance regarding its potential as a source of oil. Researchers found out that certain types of algae, such as *Chlorella* and *Spirulina*, contain high levels of fatty acids, including omega-3. This discovery led to the exploration of using algae, as well as bacteria and fungi, to produce microbial oil with a high nutritional value [14].

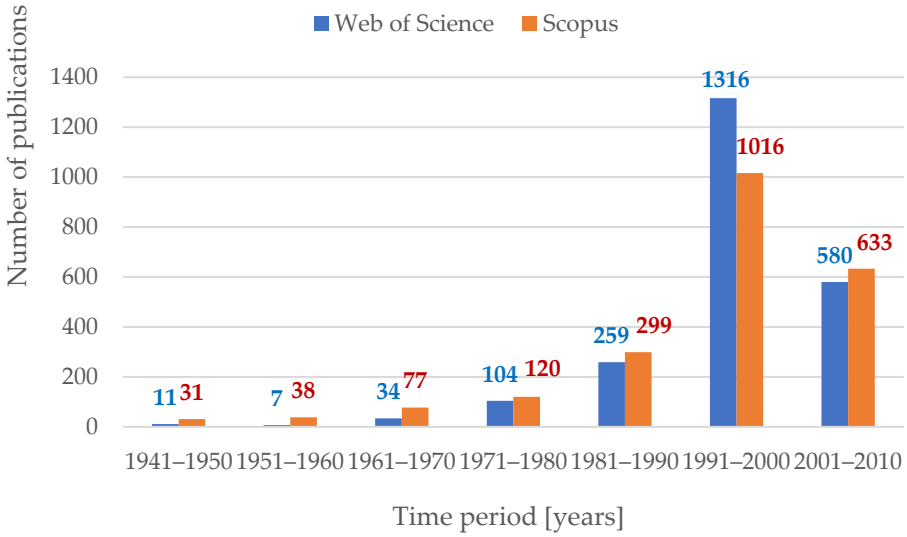

Figure 1. Period distribution of the number of publications on single-cell oil, microbial lipids, and bacterial lipids (Source: Scopus and Web of Science).

The rapid development of genetic engineering methods has made it possible to genetically manipulate microorganisms to increase the efficiency of lipid accumulation. For instance, yeast research on omega-3 fatty acids in the 1990s was a significant step for producing microbial oil with a higher nutritional value [15,16]. It can be seen from Figure 1 that, in the 2000s, the number of papers produced was more than five times higher than that found ten years before, and this number is still increasing now (Figure 1). The commercial production of yeast lipids in 2006 was developed by DuPont (Wilmington, NC, USA). The researchers genetically modified *Yarrowia lipolytica* to produce increased amounts of C20 fatty acids, mostly eicosapentaenoic acid (EPA, C20:5) [17]. The lipids were produced by CP Kelco (Atlanta, CA, USA) and sold in the USA as NewHarvest™ EPA oil for human consumption and Verlasso™ for animal feed. The oil gained GRAS (generally recognized as safe) status by the FDA, but the consumers criticized the product for hexane traces and being produced by GMOs (genetically modified organism) [2,18].

The company J & E Sturge (North Yorks, UK) was one of the first to extensively test the oil from *Mucor circinelloides* for any possible toxicity. The UK Advisory Committee on Novel Foods and Processes evaluated those results and approved the oil for sale in the UK in the late 1980s. Additional safety issues involved causing allergic reactions. Still, when assessing the safety of microbial oil, it is important to evaluate the production organism itself. Animal and plant pathogens are not equally considered. It should be noted that oils from *M. circinelloides, C. cohnii, Schizochytrium* sp., and *Mortierella alpina* were subjected by food authorities to acute oral studies, sub-chronic feeding, and their exposure to pregnant animals and carcinogenicity. The safety of SCOs was evaluated by regulatory authorities like the FDA in the USA, the EU "Regulations for novel foods and novel food ingredients," the FDR (Food and Drug Regulations) in Canada, and the FSC (Food Safety Code) in Australia [19].

In the 21st century, the increased interest in alternative energy sources and natural resources has contributed to the growing popularity of microbial oil as a biofuel feedstock. Microorganisms such as yeast and algae have become important candidates for biofuel production, because they can accumulate large amounts of fats that can be converted into liquid biofuels, such as biodiesel [20]. Research on microbial oil also focuses on its potential therapeutic applications, especially in the context of dietary supplementation of omega-3 fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and their effects on heart, brain, and nervous system health [21]. In the field of functional nutrition, microbial oil is used as a nutritionally enhanced food ingredient, especially in

products for people who require special diets, such as infants, children, and the elderly [22]. Some studies have shown the beneficial effects of these fatty acids on heart health, lowering triglyceride levels, and improving cognitive function [23]. Microbial oil, which is rich in fatty acids with anti-inflammatory properties, has revealed potential in the treatment of autoimmune diseases such as rheumatoid arthritis (RA) and psoriasis. Clinical studies have suggested that supplementation with omega-3 fatty acids may reduce the severity of inflammatory symptoms and improve the quality of life of patients with these conditions [23]. More medicinal and nutritional properties of SCOs are presented in the next chapters.

3. Microbial Oil Production

While edible and non-edible plant oils were the source of the first- and second-generation biodiesels, respectively, the greater use of microbes as an oil source gave rise to the third generation of biodiesels [24]. Microbial oils have a similar fatty acid composition to plant oils; therefore, they can be used as an alternative to them [25]. Microbial oils contain unsaturated fatty acids, which are highly beneficial for human health. Additionally, they can be used in the diets of vegan and vegetarian individuals. Studies have shown that the advantage of microbial oil production lies in the oleaginous microorganisms' ability to accumulate a high diversity of lipids in significant amounts with a low input (Figure 2). Microbial oil production occurs independently of climate conditions compared to plants. For example, many adverse conditions, such as the impact of poor climate conditions on plant growth, may affect the oil production from plants. However, such hindrances are minimal in microbial oil production. It requires no land area and ensures high efficiency [26].

In recent years, there has been a significant increase in research efforts to advance microbial lipid technology as a sustainable source of oil. The aim is to use it to replace unsustainable oils, such as palm oil, and to create advanced biofuels. However, the commercialization of this new technology is facing some challenges, such as scalability and economic and ecological sustainability. The production costs of the lipids are still high, and consumers are reluctant to accept GMO-derived oil [2,3]. Some ideas to help overcome these challenges will be discussed in the upcoming section.

Bacterial cells face two major limitations when it comes to producing lipids. They tend to have lower biomass yields compared to other groups, resulting in fewer lipids per gram of biomass. Additionally, lipid extraction can be difficult, due to their adhesion to cell membranes [27]. Yeasts are also used in the research on the biosynthesis of microbial oil. Oleaginous yeasts include representatives of such species as *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *Trichosporon oleaginosus*, *Lipomyces starkeyi*, and *Y. lipolytica*, the last of which being considered a model organism for studying the mechanisms involved in lipid metabolism covering lipid uptake, their storage, and their deposition or mobilization [27–29]. As previously mentioned, the biosynthesis of microbial lipids can take place in two different biochemical pathways, i.e., de novo and ex novo, which involve the synthesis of fatty acid precursors from glucose or the uptake of fatty substances from the environment and their accumulation in lipid bodies, respectively; therefore, the fatty acid composition is largely dependent on the substrate used [30]. The wide range of substrates used, often waste ones, allows for the simultaneous management of industrial waste and the production of valuable metabolites [31,32].

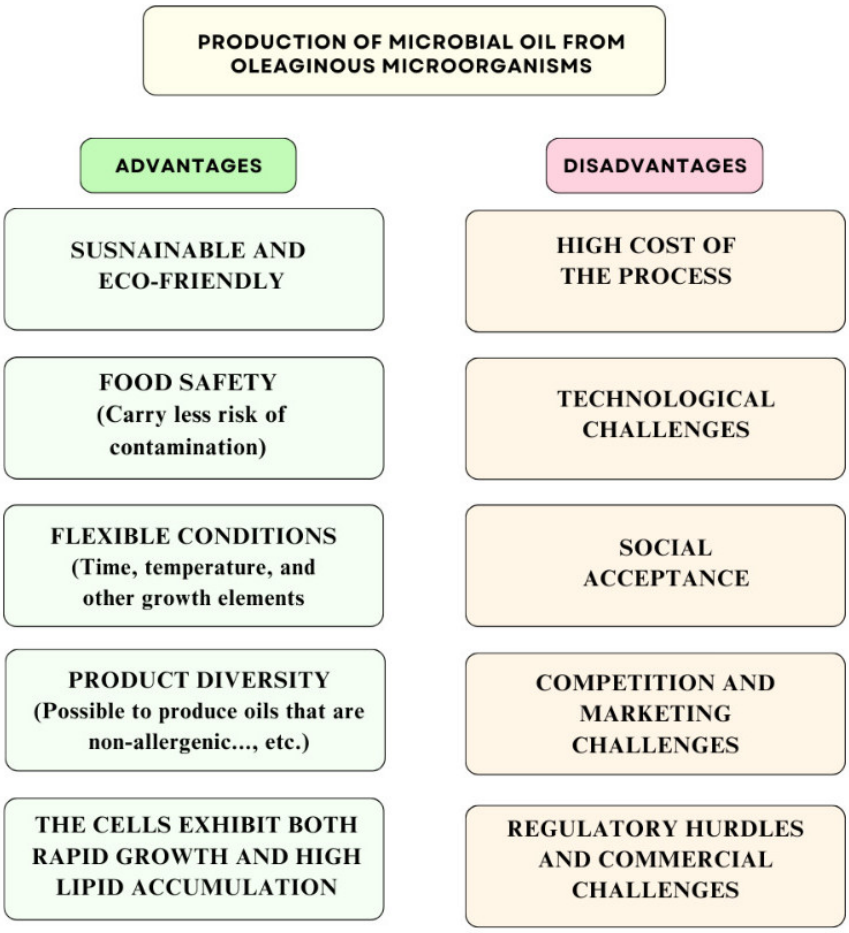

Figure 2. Advantages and disadvantages of microbial oil production.

Filamentous fungi cannot be ignored when discussing oleaginous microorganisms. At least two fungal cell oils have been commercialized so far, i.e., microbial oil from the culture of *M. circinelloides* with a high level of γ-linolenic acid and arachidonic-rich oil from *M. alpina* [27,33].

In contrast to bacteria and fungi, microalgae are autotrophic microorganisms and convert sunlight and CO_2 into biomass with the simultaneous biosynthesis of lipids, pigments, and carbohydrates [34]. Typical subjects to research on microbial oil biosynthesis are the following algae species: *Schizochytrium* sp., *Nannochloropsis oceanica*, *Chlamydomonas reinhardtii*, *Crypthecodinium cohnii*, *Chlorella vulgaris*, and *Dunaliella salina* [35].

There are two stages in the production of SCOs by oleaginous microorganisms: biomass growth and lipid accumulation. The first stage involves a nutrient-rich medium that supports cell proliferation and biomass growth [26]. Oil or storage lipid accumulation occurs under nutrient-limited conditions. The fermentation techniques used for yeast SCO production often control the C:N ratio by limiting the amount of nitrogen and providing excess carbon (often in the form of glucose or some other sugar substrate). Without available nitrogen, the metabolism shifts to triacylglycerol (TAG) synthesis for energy storage until favorable growing conditions return [36]. Nitrogen limitation has been found to profoundly influence the lipid accumulation in yeasts, mainly because of a shift in its metabolic flux leading to the cessation of growth and triggering lipid accumulation [16,37,38]. It is noteworthy that phosphorus limitation may further improve the efficiency of

microbial lipid biosynthesis, which was proved by Wierzchowska et al. [32]. Still, some balance needs to be achieved, as the simultaneous limitation of both phosphorus and nitrogen sources promotes lipid accumulation in cells, creating unfavorable conditions for biomass growth.

Remarkably, oleaginous and non-oleaginous organisms do not differ in the biosynthesis pathways of lipids; however, there is a need for a continuous supply of the triacylglycerol building block—acetyl-CoA—and NADPH production. The continuous production of acetyl-CoA in oleaginous microorganisms is achieved by a cascade of enzyme reactions triggered by a nutrient limitation, among which ATP-citrate lyase seems unique. It cleaves the acetyl-CoA from the citrate in the cytosol and disrupts the Krebs cycle. The malic enzyme and the pentose phosphate pathway are crucial to the NADPH supply [37,39]. De novo and ex novo synthesis are the two available routes for lipid accumulation in oleaginous yeast cells. The above-mentioned mechanisms are connected to de novo lipid biosynthesis, where fatty acid precursors, such as acetyl-CoA and malonyl-CoA, are generated and incorporated into lipid storage biosynthesis. For ex novo synthesis, the lipid accumulation is initiated independently from nitrogen availability in the hydrophobic medium, and it is generated simultaneously with cell growth [30,40].

It is necessary to alter a microorganism's metabolic pathways to cause lipid buildup by preventing cell division beyond a particular point. Although it is not always the case, nitrogen limiting is the recommended approach. However, the culture medium also requires an abundant supply of carbon, which is typically provided by glucose, though alternative carbohydrate feedstocks may be utilized if they are more affordable [41]. Usually, nitrogen sulfate or yeast extracts are applied as a nitrogen source, but more varied carbon sources such as glycerol, monosaccharides, lignocellulose, or oily wastes could also be used. SCO can be produced using different modes of cultures, including SmF (submerged) and SSF (solid-state fermentation). Oleaginous microorganisms can be cultivated in batch, fed-batch, and continuous cultures. Usually, flask cultures are less effective than stirred-tank bioreactors [32,39].

The volumetric productivity practically obtainable in the yeast lipid fermentation process is generally limited (less than 100 g/L). Therefore, the industrial production processes tend to involve the processing of significant volumes of culture broth to harvest cell mass and recover lipids [38]. Extensive studies have been conducted on improving the heterotrophic lipid productivity of yeast species like *R. toruloides*, *R. glutinis,* and *Y. lipolytica* [42]. For engineered *Y. lipolytica* yeast strains, it is possible to achieve 1.2 g/L/h productivity in a glucose-based medium [43]. Several papers have detailed pilot-scale cultivations of oleaginous yeast in up to 300-L bioreactors, but still, there is no clear indication of whether a heterotrophic process is economically feasible. On the other hand, autotrophic microalgae can produce lipids whose price can amount to USD 1.7 to USD 5.9, according to some techno-economic studies, but they can hardly compete with plant oils whose process varies between USD 0.5 and USD 1.9 [42]. SCO costs impact the upstream costs of the raw materials, the mid-stream bioreactor-associated costs, and the downstream costs related to cell disruption and intracellular oil recovery [44]. Karamerou et al. [42] applied techno-economic modeling to determine the minimum cost possible for a microbial palm oil substitute. They described three key areas of research that can lead to SCO commercialization. These are designing the continuous process methodology, a new product design that uses whole cells instead of extracted oil, and a process where the lipids are produced alongside low-molecular-bulk chemicals. The recent trends in research on microbial oils are also related to the homologous or heterologous expression of enzymes involved in elongating and desaturating fatty acids in order to increase the content of PUFA in storage lipids [45].

4. Extraction of Microbial Oil

The reality of using SCOs is far from being achieved, due to the high cost of their recovery from biomass. The costs can be lowered by the usage of a waste carbon source in

the culture medium and by a reduction in the operational costs of SCO extraction, which may be accomplished by mechanical methods, chemical and enzymatic methods, or a combination of them [46,47]. Based on the current literature, there is no extraction method that is 100% effective in yielding oils derived from microorganisms. Still, there is a lot of research describing examples of their use.

Different biomass treatments (wet, oven-dried, and freeze-dried) can be applied before cell lysis. It is claimed that dry biomass extraction is more efficient than wet biomass extraction for lipid recovery in solvent-based extraction processes. A decreased amount of water can maximize the mass transfer and decrease the formation of emulsion. However, the biomass drying step before the extraction is economically costly for large-scale applications [48,49]. A study conducted by Willis et al. [49] showed that adopting wet extraction practices reduces the energy required for extraction by almost 60%, providing further evidence of its energy-saving benefits. The most effective solvent historically used to extract oil from wet materials at ambient conditions is chloroform, as shown in Folch's and Bligh and Dyer's methods. Chloroform cannot be used for commercial use, because it is highly toxic and carcinogenic [49]. The safest and, nowadays, most popular way to ensure that all of the cellular lipids are extracted is to employ a ternary solvent composition including a polar as well as a non-polar solvent [50]. Recently, an alternative, green solvent, cyclopentyl methyl ether (CPME), was successfully used in biphasic systems of CPME:water:alcohol to extract the lipids from the wet cells of the oleaginous yeast *Lipomyces starkeyi* [51]. Breil et al. [50] used the greener solvent pair ethyl acetate/ethanol with wet sample yeast *Y. lipolytica*, followed by the addition of water and ethyl acetate 1:2 (*v*/*v*), for the separation of aqueous and organic phases. Among the 41 tested solvents, isoamyl acetate was selected as the most appropriate "green" solvent, maximizing the lipid extraction compared to *n*-hexane for *Y. lipolytica* in the studies provided by Imatoukene et al. [52].

The process of extracting lipids from oleaginous yeasts is often hindered by the resistance of the cell wall, the limited accessibility of the lipids, and the difficulty of mass transfer. The cost-effectiveness of cell disruption is related to energy consumption, the time to obtain the reasonable effect, the consumables, the product quality, and the required labor intensity [53,54]. Alternative pre-treatments could be freezing/defrosting, cold drying, bead milling, and microwave treatment. These methods can help to make the lipid structure more accessible to the solvents, thereby reducing the biggest limitation of the process—the diffusion of the solvent into the raw material [54]. Timotheo et al. [47] evaluated liquid nitrogen pre-treated biomass and maceration, followed by ultrasonication extraction, as the treatment with the highest percentage of disrupted cells and the highest oil yield of *Y. lipolytica* QU21 and *Meyerozyma guilliermondii* BI281A. Some investigations point out that the lipid yield decreased with increasing pressure, and low pressure (200 MPa) collapsed the cells, while high pressure (400 MPa) created protrusions on the cell wall and the cell fragments spread into the environment [55]. High-pressure homogenization (HPH) fragments cells via shear stress, cavitation, turbulence, and friction [56], and has recently been extensively investigated for oil recovery in the wet biomass of *Y. lipolytica* yeast. The authors of those studies showed that a pressure of 1500 bar and five passes, provided by mixing using a high-speed disperser, allows maximum cell disruption, comparable to the total oil recovery reached with the dry route when the yeast biomass is lyophilized and subjected to *n*-hexane extraction [57]. Other alternative techniques based on cell electroporation— high-pulsed electric fields (HPEF), high-voltage electrical discharges (HVED), mechanical expression (ME), and moderate-pulsed electric fields—assist the mechanical expression (MPEF-ME) seem to be less efficient. HPH, ME, and MPEF-ME induced changes in the content of some fatty acids [58,59]. What should be mentioned is that many authors increase lipid recovery by using a combination of techniques, e.g., bead milling and HPH [52]. Finally, the softest method of cell disintegration is ultrasound (US)-based technology related to the cavitation phenomena, which is the formation of vapor bubbles and their implosion near the cell surface and free radical formation. The majority of investigations with the US deal with the laboratory level, and the

power used varies even up to 2800 W [59]. Usually, the highest lipid contents are achieved using ultrasound-assisted extraction coupled with Folch's, bead milling, or Soxhlet's techniques [60]. Some nonmechanical methods, e.g., acid and base digestions or osmotic shock, have been also tested [40].

A small number of papers deal with supercritical carbon dioxide (SC-CO_2) extraction. Milanesio et al. [61] used ethanol-macerated yeast cells of *Y. lipolytica*, resulting in 15 mg of yeast oil per g of solvent used. The results showed that oil recovery is more efficient when adding a pretreatment to the extraction procedure, and none of the techniques tested were efficient in recovering all of the oil contained in the yeast [57,61].

The main methods used to disrupt microalgae cells on a large scale are bead milling, high-speed homogenization, and high-pressure homogenization; however, ultrasounds, microwave treatments, enzymatic lysis, and pulsed electric field have the potential for scale-up and have been already applied for lipid extraction from *Chlorella vulgaris* (enzymatic lysis [62]) and *Nannochloropsis* sp. (HPH and HSH) [63,64]. Microwave treatment has been especially dedicated to lipid recovery and investigated for *Chlorella* sp., *Noctoc* sp., *Synechocytis, Scenedesmus obliquus,* and *Botryococcus* [65]. The challenges for designing high-yield oil extraction methods from algae are highly variable ranging for the cell wall composition. The bottlenecks cited in the literature due to the high energy demand are still downstream processing in the microalgae commercial applications in biorefineries [53,65]. It is also claimed that algae must be dried (rotary or spray-dried) prior to being fed into a press to obtain the lipids [66].

Some authors claim that a better understanding of the cell wall composition and knowledge of the cell wall chemistry would provide information to aid in the design of better protocols for oil extraction, including genetic engineering techniques. Cell wall modification displays a potentially promising approach to improve both the harvesting of oleaginous yeast biomass and cell susceptibility to lysis. The target genes for further modifications are glucanase, chitinase, and cross-linking enzymes expressed in the cell wall of the oleaginous strain [38]. A mutant of *Trichosporon* has been recently isolated, possessing a modified cell wall, which acquired the ability to generate cell aggregates that are more easily separable from the culture broth by centrifugation [67].

To sum up, many papers claim that the novel extraction processes of oil from wet biomass or the use of emerging technologies can considerably reduce the energy required for drying; however, the majority of them are limited to a small laboratory scale, a need for unavailable commercial-scale instruments, or are still not as good as conventional solvent extraction. The most promising seems to be the application of ultrasounds and high-pressure techniques, as the most difficult barrier to overcome in microbial oil recovery is the cell wall and the cell membrane. Developing scalable and economically attractive methods of cell disruption that preserve the cell constituents to a high extent is still needed.

5. Composition and Properties of Microbial Oil

All microorganisms are capable of synthesizing lipids, but only those referred to as oleaginous can accumulate significant amounts of them. Single-cell oils, or so-called microbial oils, are lipids produced by microorganisms, including bacteria, yeasts, molds, and algae [33,68–74]. Their properties and composition depend entirely on the microorganisms involved in their biosynthesis. The fatty acid composition of microbial oils typically consists of mainly palmitic and oleic fatty acids, with other common acids like myristic, palmitoleic, stearic, and linoleic, as well as α- and γ-linolenic acids, while the oils obtained from algae may be a source of other polyunsaturated fatty acids, like EPA (eicosapentaenoic acid, C20:5) and DHA (docosahexaenoic acid, C22:6) [27].

Bacteria belonging to the *Rhodococcus* genus are one of the most frequently studied prokaryotes capable of synthesizing lipids [75]. According to the data shown in Table 1, it can be seen that *R. opacus*, as well as *R. jostii* and *R. rhodochrous*, have the potential to produce microbial oil with an unusual fatty acid composition. In the studies of Chu et al. [68]

and Silva et al. [69], the bacteria of the *Rhodococcus* genus produced significant amounts of odd-chain fatty acids, where both saturated and unsaturated odd-chain acids were achieved. The most common fatty acids in these papers were pentadecanoic (C15:0), heptadecanoic (C17:0), and heptadecenoic (C17:1) acids. In the case of *R. opacus* PD630, which grew on a mixture of glucose and 1-propanol, about 48% of the cell dry weight consisted of microbial lipids, whereas approximately 69% of the obtained fatty acids were those with an odd number of carbon atoms in the molecule [68].

A wide range of substrates can be converted in nitrogen-limited conditions by *Rhodococcus* strains into triacylglycerols. Silva et al. [69] confirmed such possibilities using gluconate, benzoate, hexadecane, and even naphthalene and naphthyl-1-dodecanoate. During the cultivation with gluconate and benzoate as the main carbon sources, *Rhodococcus* sp. 602 was able also to biosynthesize polyhydroxyalkanoate, i.e., 3-hydroxybutyrate and 3-hydroxyvalerate, which may find its application in the polymer industry. Moreover, the capability of metabolizing and converting hydrocarbons into lipids in nitrogen-deficient conditions may be significant in its potential use in the bioremediation processes of pollutant-contaminated soils [68,69].

Yeasts are used for the biosynthesis of microbial oil from waste substrates such as waste cooking oils, palm oil or olive oil wastewaters, fish waste, pork lard, and other animal-based fats [31,32]. For example, a culture of *Y. lipolytica* W29 in a medium with eucalyptus bark hydrolysate with a high concentration of glucose and xylose allows for the production of lipids-rich biomass. A biosynthesis yield of 26% (5.6 g/L) can be achieved, and the lipids consist of more than 85% of unsaturated fatty acids, including oleic acid (48.4%), palmitoleic (20.2%), linoleic (17.1%), and palmitic acids (14.3%) [71].

In the case of using hydrophobic substrates, Miranda et al. [70] evaluated the possibility of using hexadecane as the main carbon source in the culture of *Y. lipolytica* CBS 2075. The authors confirmed that, in yeast cells, alkanes are oxidized to fatty alcohols. Then, through aldehydes, finally become fatty acids, which, in favorable culture conditions, are stored in the lipid bodies as triacylglycerols and steryl esters. As *Y. lipolytica* yeasts are strictly aerobic, the agitation rate and, thus, the dissolved oxygen in the medium was crucial for the highest lipid accumulation. Moreover, the low ratio of C/N is also considered a limiting factor in microbial oil synthesis, and the authors also observed that nitrogen-deficient media are suitable for the outstanding yield of microbial oil syntheses. Regarding the microbial oil composition, it was found that such oil contains palmitic and palmitoleic acids (saturated and unsaturated fatty acids with 16 carbons), as well as oleic and linoleic acids, which are commonly present in microbial oils [70].

Interestingly, wild-type yeasts can also be a source of long-chain polyunsaturated fatty acids, such as EPA and DHA. Fabiszewska et al. [76] demonstrated the ex novo microbial oil synthesis approach with the use of waste fish oil in the culture of *Y. lipolytica* KKP 379. The yeasts selectively accumulated fatty acids from the substrate, and the biomass contained about 23% of lipids, which was the source of the docosahexaenoic (C22:6), erucic (C22:1), eicosapentaenoic (C20:5), and eicosenic (C20:1) acids.

In addition, taking a closer look at other yeast species, the profile of the fatty acids present in the microbial oils of these yeasts resembles known oils and fats that occur in nature, which are used in various industries. What comes to the forefront is the microbial oil of *Trichosporon oleaginosus* (syn. *Apiotrichum curvatum* or *Cryptococcus curvatus*), whose composition resembles that of cocoa butter [77]. The production of cocoa-butter-like oils from yeasts has a significant advantage over cocoa butter, as it is not influenced by weather, water shortages, or climate change [78].

Table 1. Comparison of the fatty acid compositions of single-cell oils produced by bacteria, yeast, fungi, and algae.

	Microorganism							
Fatty Acid (%)	***Rhodococcus opacus* PD630 [68]**	***Rhodo-coccus* sp. 602 [69]**	***Y. lipolytica* CBS 2075 [70]**	***Y. lipolytica* W29 [71]**	***Tricho-sporon* sp. F1-2 [72]**	***Mortierella alpina* CCF2861 [33]**	***Schizo-chytrium* sp. T18 [73]**	***Nannochloropsis oceanica* 0011NN [74]**
C14:0	0.9	3.4	-	-	0.4	0.8	11.2	5.6
C15:0	17.8	6.2	-	-	-	-	1.6	0.2
C15:1	0.9	-	-	-	-	-	-	-
C16:0	13.2	30.7	41	14.3	18.1	14.5	26.5	14.1
C16:1	4.5	11.3	34	20.2	0.8	0.1	4.5	33.4
C17:0	19.1	10.3	-	-	-	-	0.4	0.1
C17:1	30.4	11.3	-	-	-	-	-	0.1
C18:0	3	11.9	-	-	5.3	5.6	1	0.9
C18:1	8.6	14.9	16.9	48.4	59.1	13.7	4.1	3.2
C18:2	1.2	-	7.8	17.1	13.7	23.8	0.4	2.4
C18:3 *n*-3	-	-	-	-	1.2	2.3	0.1	-
C18:3 *n*-6	-	-	-	-	-	0.5	-	0.1
C20:3 *n*-6	-	-	-	-	-	2.5	-	0.6
C20:4 *n*-3	-	-	-	-	-	-	0.5	-
C20:4 *n*-6	-	-	-	-	-	26.4	-	9.5
C20:5 *n*-3	-	-	-	-	-	-	0.8	28.9
C22:6 *n*-3	-	-	-	-	-	-	40.7	-
C24:0	-	-	-	-	0.7	-	-	-
Others *	0.5	0	0.3	0	0.9	9.8	8.2	0.9
SFA	54.5	62.5	41	14.3	24.9	20.9	40.7	21.1
MUFA	44.4	37.5	50.9	68.6	60.4	13.8	8.6	36.7
PUFA	1.2	0	7.8	17.1	14.9	55.5	42.8	41.5
UFA	45.6	37.5	58.7	85.7	75.3	69.3	51.4	78.2
OCFA	68.7	27.8	0	0	0	0	2	0.4
%CDW	48	64.9	27.3	26	58.9	13.8	ND	26.1
Substrate	Glucose and 1-propanol	Sodium benzoate	Hexadecane	Eucalyptus bark hydrolysate	Sucrose	Cornmeal	ND	Autotrophic conditions

* Others—oils not mentioned by the authors or those whose percentage content was very low. Abbreviations: SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids; UFA—unsaturated fatty acids; OCFA—odd-chain fatty acids; %CDW—microbial oil in cell dry weight; ND—no data.

In recent years, there has been an increase in interest in the use of genetic engineering methods to modify various species of yeast, directing them to the production of microbial oils with a high content of selected fatty acids, such as the following: γ-linolenic acid by the expression of Δ6-fatty acid desaturase gene [79]; α-linolenic acid through the expression of *F. moniliforme* Δ12/ω3 desaturase [80]; conjugated linoleic acids via the elimination of β-oxidation; the deletion of DAG acyltransferases and the overexpression of the Δ12-desaturase gene in *Y. lipolytica* [81]; and increased levels of α-linolenic or unusual fatty acids, i.e., eicosatrienoic (C20:3) and eicosadienoic (C20:2) acids, by the transformation of *Trichosporon oleaginosus* ATCC 20,509 [82]; as well as the production of EPA- and DHA-rich oil in *Y. lipolytica* by the expression of several desaturase and elongase genes [83,84]. The engineered strains of *Y. lipolytica* were also harnessed for the biosynthesis of a human milk fat substitute by Bhutada et al. [85]. Milk fat is characterized by the presence of

palmitic acid in the *sn-2* position in the glycerol backbone. The authors have expressed lysophosphatidic acid acyltransferases with palmitoyl-Coenzyme A specificity (*LPAAT2*) from *Chlamydomonas reinhardtii* and obtained a mutant capable of producing a high titer of lipids in the dry cell weight, with a significant content of C16:0 fatty acid in the *sn-2* position in triacylglycerols with the potential application as an ingredient in infant formulas [85].

Several advantages of filamentous fungi over the other groups of microorganisms have led to the introduction of such oils into the market—there were high accumulation rates of up to 80% and significant unsaturation of the produced oils abundant in essential fatty acids, like the aforementioned ALA (alpha-linolenic acid), GLA (gamma-linolenic acid), ARA (arachidonic acid), etc., (Table 1) [27,30,33].

Finally, the last, but not least, group of well-known single-celled organisms capable of producing polyunsaturated fatty acids is algae. It has been found that the profile of the fatty acids in the SCOs of microalgae is species-dependent, and, according to Table 1, for instance, the microbial oil from *Schizochytrium* sp. T18, which served as a feed ingredient for Atlantic salmon (*Salmo salar*), consisted of 40.7% of DHA, 26.5% of palmitic acid, and 11.2% of myristic acid. Subsequently, after feeding the salmon the microbial oil, high levels of DHA were found in their muscles and livers, in comparison to those of the control diets [73]. In another study, Couto et al. [74] compared the outdoor and indoor cultures of *N. oceanica* and *N. limnetica*. The microalgae of the *Nannochloropsis* genus are known for EPA and polar lipid production. The authors confirmed that, for both species, the outdoor environments and, thus, the exposure to the variable natural radiation and temperatures, led to the higher final content of EPA in the microbial oils.

6. Applications of Microbial Oils in Cosmetics, Pharmacy, and Medicine

The scientific community is interested not only in the production of odd-chain fatty acids, but also in their potential applications. Research on the biological activity of acids with 15 and 17 carbon atoms in their molecules has been carried out for over 20 years. Both cis-9-heptadecenoic and its 6-methyl derivative produced by *Pseudozyma flocculosa* exhibit antifungal activity against *Cladosporium cucumerinum* and *Botrytis cinerea*, which are fungal plant pathogens. Interestingly, heptadecenoic acid primarily increase the fungal membrane permeability and cause cytoplasmic disintegration. As the authors pointed out, due to the difficulties in the extraction and isolation of odd-chain fatty acids from *P. flocculosa*, a chemical procedure for their synthesis was elaborated [86,87].

In addition, some of the derivatives of odd-chain fatty acids, such as methyl heptadecanoate, are commonly used as internal standards for quantitative analysis, like the evaluation of the fatty acid composition through gas chromatography [28]. In addition, pentadecanoic acid serves as a marker for the intake of milk fat and other dairy foods, where it turns out that the consumption of butter and the total milk fat intake are positively correlated with the levels of C15:0 in serum cholesterol esters; moreover, an inverse association between diabetes risk and this odd-chain fatty acid in a multiethnic cohort study was observed [88–90]. Other studies have confirmed the usefulness of pentadecanoic acid as a biomarker in non-alcoholic steatohepatitis; in addition, the authors have confirmed, throughout in vivo studies on mice, that the deficit of C15:0 in methionine and a choline-deficient diet are associated with liver injury through inflammation and an increased aspartate aminotransferase (AST) level [91]. Finally, according to recent research, pentadecanoic acid has shown cytotoxic effects in the human breast carcinoma MCF-7 stem-like cell line. It has been found to be a novel JAK2/STAT3 signaling pathway inhibitor, which is also responsible for cell cycle arrest and apoptosis in the mentioned cell line. These findings suggest that pentadecanoic acid may have potential therapeutic benefits in treating breast cancer [92].

Lipids play a vital role in personal care products, providing functional and performance benefits to cosmetic formulations. The careful selection and use of different lipid types can optimize the efficacy and sustainability of cosmetic products. Oils with a high

content of linoleic, α-linolenic, and γ-linolenic acids are successfully used in cosmetics. They are classified as emollients, due to their moisturizing, softening, and smoothing properties. It has been confirmed that the lack of linoleic acid in the diet leads to skin abnormalities such as dryness and inflammation [93]. Such oils act as a base for creams, emulsions, cosmetic milks, etc., and may be treated as a solvent for many of the biologically active substances found in cosmetics, like vitamins A, D, and E or pigments [94,95]. Unsaturated fatty acids are often crucial remedies in skincare and the treatment of dermatoses, such as atopic dermatitis (eczema). Linolenic acids both regenerate the damaged lipid barrier of the epidermis and limit water loss [96]. Maintaining the structure and function of the outer layer of skin, known as the stratum corneum, is strictly dependent on fatty acids. The lipids present in the intercellular matrix of the stratum corneum help to keep the layer cohesive, protect the skin from harmful substances, and prevent water loss. Therefore, the deficiency of fatty acids and lipids can significantly affect vascular fragility, weaken the immune system, and interfere with the blood clotting process [94].

Taking into account the source of triacylglycerols, vegetable oils do not contain arachidonic acid (ARA), but fungi can easily produce it. In the human body, arachidonic acid in its esterified form is essential for the maintenance and function of cell membranes. It serves as a precursor in the biosynthesis of eicosanoids, which are signaling molecules, such as prostaglandins and leukotrienes. These molecules are considered proinflammatory and are responsible for stimulating the immune response and triggering oxidative stress [97,98].

Eicosapentaenoic and docosahexaenoic acids have 20 and 22 carbon atoms in their chain, respectively, and 5 and 6 unsaturated double bonds in their molecule, respectively. Their main source is algae, and the research conducted using EPA and DHA has confirmed their positive effect on the human body. First of all, lipids are abundantly present in the brain, making up more than 50% of the brain's dry weight, and, moreover, they have a unique ability to cross the blood–brain barrier, allowing them to readily access the brain [99]. The specific roles of DHA and EPA highlight the critical importance of lipids for proper brain health and cognition. Furthermore, their consumption helps to reduce the risk of cardiovascular diseases, osteoporosis, and inflammatory diseases. Additionally, EPA and DHA are involved in lowering the blood cholesterol levels, thereby reducing the risk of heart disease. A deficiency of these acids may have an impact on cell death, cognitive function, and brain disorders, as well as the prevention and treatment of Alzheimer's disease and other neurodegenerative diseases [99–101].

Over the past few years, numerous research teams have conducted cohort studies, meta-analyses, and systematic reviews that explore the link between the intake of polyunsaturated omega-3 fatty acids and neurodegenerative conditions, such as Alzheimer's and Parkinson's diseases and various forms of dementia, as well as cognitive impairment and decline [102–105]. Wei et al. [102] observed that long-term users of omega-3 supplements have a 64% reduced risk of developing Alzheimer's disease. Additionally, they found that the intake of DHA could lower the risk of dementia and cognitive decline by up to 20%. They also noted that, for each 0.1 g/day increase in DHA or EPA intake, the risk of cognitive decline lowers by 8–9.9%. Yamagata [103] analyzed the role of DHA in preventing vascular dementia induced by ischemic stroke. This study found that DHA and its metabolites, which are produced in cerebral vascular endothelial cells, have antioxidative and anti-inflammatory properties. These properties lead to a decrease in the production of the Aβ-42 (amyloid beta) peptide, which in turn may prevent the onset of the disease. Furthermore, preclinical studies have also been carried out on well-established animal models to verify the hypothesis that polyunsaturated fatty acids (PUFA—polyunsaturated fatty acids) have beneficial effects. These studies showed that DHA improved the performance of rodents in memory tests and cognition, provided neuroprotection, and reduced inflammation [104].

Lipid nanoparticles, which are useful in pharmaceuticals and, thus, in drug delivery, are composed of both solid and liquid lipids. The choice of lipids is influenced by various

factors, including drug solubility, production costs, and stability. Lipids, with their ability to act as excellent solvents for poorly soluble drugs, as well as their taste-masking and non-toxic properties, have potential in the field. Different types of oils and fats have been applied in recent years in the formulation of lipid nanoparticles. However, novel formulations should explore oils, such as those derived from oleaginous microbial organisms, to meet the high demand for sustainable production [99].

7. Applications of Microbial Oil in the Food Industry and Nutrition

In today's market, the use of omega-3, particularly in formulations such as infant food, has experienced significant growth, due to its inclusion of DHA. Hence, a growing importance is placed on the production of DHA-rich PUFAs for the safe use of formulas, especially those produced for infants. The production of microbial oils is sustainable and replicable, due to their high yield and low nutrient requirements. Various microorganisms, such as yeasts and algae, are used in microbial oil production. The most common ones are the algae *Ulkenia* sp. and *Schizochytrium* sp.; the mold *M. alpin*a; and the yeast *Y. lipolytica*, which has a very high oil production efficiency. Research on lipid production from microorganisms has been ongoing since the 19th century. However, lipid production by microorganisms for the food industry began in the mid-1970s. Today, microbial oils, which are considered novel foods, are widely used in many different food products. Microbial oils were first used in infant formulas. For example, microbial oils produced by *Schizochytrium* sp. are used in breakfast cereals and dairy products other than milk-based beverages. Microbial oils produced by *Ulkenia* sp. are used in various food products, such as bakery products, cereal bars, and soft drinks [106].

The studies conducted from the past to the present have shown that microorganisms can accumulate lipids over 20% of their dry weight. Therefore, lipid production from single-celled organisms began as an alternative for the food industry. The diversity of microorganisms and their ability to accumulate lipids in high amounts make microbial oil production a significantly advantageous method [26].

In the assessment of food safety, one of the key factors is tolerability. Tolerability can be defined as the absence of adverse events or increased sensitivity when consuming a food product. Generally, the following three processes are considered in food safety evaluation: risk assessment, risk management and regulation, and risk communication [107]. Organizations like the FDA and EFSA play significant roles in examining and regulating food components. For instance, in the United States, the FDA handles various tasks related to the assessment of new foods and their components. This may involve submitting a petition to the FDA or making a GRAS determination for a formal pre-market review [108]. Microbial oils' safety has been evaluated and discussed over the last decade, with products like DHASCO and ARASCO having their safety determined through both clinical and nonclinical studies. These oils have subsequently been commercially marketed for use in over 75 infant formulas [109]. The evaluation of the safety of food components relies on an assumption of their potential to cause harm. In this regard, the FDA's RedBook serves as a crucial resource for food safety assessment. In the United States, the FDA follows two paths for determining food safety, as follows: the food additive petition process, where the responsibility lies with the FDA to assess and approve the safety; and the GRAS process, where the responsibility lies with the manufacturer. Many new foods, including SCOs, often follow the GRAS process for marketing in the United States [110]. Table 2 summarizes some commercial SCO products and the medicinal and nutritional claims declared by the companies [19,39,107,111–119].

Table 2. Commercial SCO products and their medicinal and nutritional properties.

Product	Company	Microorganism	Properties Declared by the Company	References
DHASCO	Martek Biosciences Corporation	*C. cohnii*	DHA is an omega-3 fatty acid that is important for heart health and brain development.	[111]
ARASCO	Dsm-Firmenich	*M. alpina*	Due to the arachidonic acid it contains, it provides hormonal balance. It contributes to the production of hormones, such as prostaglandins, in the body. It is effective in developing nervous system and brain health. Arachidonic acid plays a role in the growth and development of babies especially.	[112]
DHASCO-S	Martek Biosciences	*Schizochytrium* sp.	It supports brain development during infancy, protects retina health, and regulates heart rhythm and improves blood pressure.	[113]
DHASCO®-B	Dsm-Firmenich	*Schizochytrium* sp.	It is beneficial for eye and retina health. Its anti-inflammatory properties reduce inflammation in the body.	[114]
DHA45	DSM Nutritional P.	*Ulkenia* sp.	The fatty acids found in the retina, testicles, and sperm help the regular functioning of these organs/cells and the functioning of hormones.	[107,115]
SUNTGA40S (CABIO)	Cargill, Wuhan Alking Bioengineering DSM	*M. alpina*	It was developed for use in baby nutrition. It is rich in omega-6 fatty acids. It plays a role in baby development and eye health.	[19,116]
Neuromins	Martek Biosciences	*C. cohnii*	It contributes to baby brain development, especially during pregnancy and breastfeeding. It supports eye health and reduces the risk of eye diseases. It supports the development of the immune system.	[117]
Life's DHA	DSM	*C. cohnii*	It increases brain and mind development, visual brain development, and mental attention. It helps to reduce the risk of premature birth.	[39,118]
Life's ARA	DSM	*Y. lipolytica*	It is found naturally in breast milk. It supports the development of the baby's brain. It supports the immune system, blood circulation and vascular function, and bone formation.	[119]

Microbial oils require flexibility in determining their safety, due to the uniqueness of the source organisms used in their production. It is crucial to characterize the physical and chemical properties of the produced microbial oil for food safety purposes [120]. Particularly, examining minor components is highly important. The guidelines established by organizations such as the FDA and EFSA should be followed to evaluate the safety and quality of microbial oils. For example, the toxicity testing guidelines from the FDA are considered in determining the safety of foods. Similar tests should be conducted on microbial oils to ensure their safety [107].

The safety of single-cell oils (SCO) is assessed by examining the safety of their components. SCOs typically consist of fatty acids esterified with glycerol and may contain small amounts of other lipid classes. The fatty acids in commercialized SCOs are found in normal human diets or the metabolites of fatty acids. The sterols in SCOs are found in traditional food sources like animal fat, vegetable oil, and human milk. The history of the safe use of individual fatty acids and sterols is further enhanced by their abundance in foods; their small consumption amounts; the extensive information on their absorption, distribution, metabolism, and excretion in mammalian species; and the published safety information on specific compounds [108].

The research indicates that ARA (omega-6 fatty acid) is an important component in infant formulas and plays a significant role in growth and development. The literature reviews suggest that a daily intake of up to 1.5 g of ARA generally does not have adverse effects in healthy adults, but its effects in diseased individuals are uncertain. Some studies suggest that supplemental ARA intake does not affect the blood pressure, serum lipid, or glucose levels [39]. However, concerns have been raised that high doses of ARA may increase platelet activation and potential pro-thrombotic effects. DHASCO, DHASCO-S, DHA45-oil, SUNTGA40S, and ARASCO (Table 2) are produced through algal fermentation and do not contain any fish components [121]. The FDA considers these types of oils as "highly refined oils," and does not associate them with allergic reactions. Generally, edible oils can be derived from major food allergens, such as soybeans and peanuts, and may sometimes contain protein. The FDA states that "the consumption of highly refined oils derived from major food allergens by individuals allergic to the source food does not appear to be associated with allergic reactions" [107]. As an example, David Kyle, of the Martek Corporation, began research on the fermentative production of DHA from the microorganism *Crypthecodinium cohnii* in 1992. The oil produced, known as DHASCOTM, was granted generally recognized as safe (GRAS) status by the FDA in the United States in 2002. Since then, following this status being granted by the FDA in 2002, the microbial oil has been permitted for use in baby formulas in America [26].

8. Future Perspectives

In the production of functional foods, which has been actively researched in recent years, demands have increased for people to have a healthier and better life. One of these demands is the uptake of supplemental omega-3 fatty acids, which cannot be synthesized by the human body and must be taken daily. In general, on average, the omega-3 supplements obtained from fish that a person should consume daily is 500 mg/day. Today, the average annual demand for fish oil is around 1 million tons. However, most of the fish oil produced is mainly used in the production of feed for salmon [26]. Only a small proportion is used as fish oil for human consumption. According to the data analyzed in recent years, it has been observed that fish production has become increasingly stable, and no increase has been achieved. In 2014, people consumed a total of approximately 282,000 tons of fish oil. This consumption is thought to increase to 711,000 in 2025 in response to the care that people show towards their nutrition and their desire to obtain enough nutrients in their diets. In response to this increasing consumption, there has been a trend towards microbial-derived oils, as opposed to fish oil, as a source of omega-3 [19]. For this reason, there is a large deficit in the production of this oil for human consumption. These deficits have led to the search for new alternative sources.

Many reasons have supported the research and development of new alternatives, such as the exposure of the fish used in oil production to heavy metals in the marine environment and the difficulty of extraction, the long and costly process of obtaining oil from fish, and the fact that it is not efficient or sustainable [26]. In addition, some of the properties of fish oils may disturb consumers during use. For example, the distinct odor of fish oils affects the sensory quality. As a result of the negative effects of lipid peroxidation, the shelf life of fish oils is shortened. Non-vegetarian fish-based oils have been a challenging situation for vegetarian individuals. Innovations have been made to produce clean, non-animal-derived, sustainable oils rich in DHA and various components [16]. Attention has been paid to the high omega-3 ratio in the feeds used. It is important that this pattern remains constant and sustainable [16].

Omega-3 supplements are used in many areas, such as functional foods, pharmaceuticals, infant formulas, and animal feeds. Since the consumption of microbial oil has increased, the functional food sector is the area where it is used the most and where will be used in the future. Microbial oils containing omega-3 and omega-6 fatty acids with a high ARA and DHA content for the development of babies who cannot be fed breast milk are also used in the baby food sector, and their use is increasing rapidly day by day.

Omega-3 fatty acids, which also play an important role in human nutrition, are now used as supplements in diets. In addition, some microbial oils have started to be added to foods such as soft drinks and breakfast cereals. The sector of omega-3 fats has expanded, especially due to the addition of DHA to infant formulas, which is important for infant development. The use of oils produced from microorganisms in the food industry began in the mid-1970s. Microbial oils, which were then used as an alternative for the food industry, are similar to vegetable and animal fats. The advantage of microbial oil is that it has many varieties and has a high lipid deposition efficiency. It is more advantageous than other source oils, due to its rapid growth and low cost. It can be produced independently of climatic conditions. It provides high productivity in less space. The area covered is less than that required for oils produced traditionally [1,19]. When discussing the autotrophic production cost of algae, it can be considered as lower budget compared to the oils produced in the traditional way. Today, with the increasing use of microbial oil, large companies in the food sector produce and sell oils obtained by using many different yeast and fungal sources as supplements [122]. It is important that the smell, taste, and color of the microbial oils produced are more attractive to consumers than those of the oils obtained from fish. In addition, the demand is increasing rapidly with the increasing consumption of omega-3 fatty acids. Lipids with nutraceutical properties are very important for the food industry, both today and in the future. The alternatives produced to meet the need for omega-3 are sustainable by minimizing environmental and natural damage [123].

Omega-3 fatty acids are of great importance for human health. In particular, they play a role in brain and eye health, hormonal balance, psychological disorders, cardiovascular and Alzheimer's diseases, supporting the immune system, and especially in the growth and development of infants. Microbial oils, usually taken as supplements to support human nutrition, are increasingly used in a wider range of food sectors. After first being used in baby food, they have been added to many foods and have an important place in the pharmaceutical and cosmetic sectors. They are of great importance for the future, due to their sustainability, efficient oil content, and high content of omega-3 fatty acids, such as DHA and ARA, not to mention that lipids are the main constituents of food, and, in the era of the green evolution, we can expect that microbial-based food products can be the future of human nutrition. Still, the challenge is to lower the costs of heterotrophic single-cell oil synthesis and improve its extraction efficiency, which can further contribute to its broadened use in pharmacy, cosmetics, and food applications.

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References

1. Ratledge, C. Microbial Oils: An Introductory Overview of Current Status and Prospects. *Ocl* **2013**, *20*, D602. https://doi.org/10.1051/ocl/2013029.
2. Abeln, F.; Chuck, C.J. The History, State of the Art and Future Prospects for Oleaginous Yeast Research. *Microb. Cell Fact.* **2021**, *20*, 221. https://doi.org/10.1186/s12934-021-01712-1.
3. Graham, A.E.; Ledesma-Amaro, R. The Microbial Food Revolution. *Nat. Commun.* **2023**, *14*, 2231. https://doi.org/10.1038/s41467-023-37891-1.
4. Salem, N., Jr.; Eggersdorfer, M. Is the World Supply of Omega-3 Fatty Acids Adequate for Optimal Human Nutrition? *Curr. Opin. Clin. Nutr. Metab. Care* **2015**, *18*, 147–154. https://doi.org/10.1097/mco.0000000000000145.
5. Leong, W.-H.; Lim, J.-W.; Lam, M.-K.; Uemura, Y.; Ho, Y.-C. Third Generation Biofuels: A Nutritional Perspective in Enhancing Microbial Lipid Production. *Renew. Sustain. Energy Rev.* **2018**, *91*, 950–961. https://doi.org/10.1016/j.rser.2018.04.066.
6. Spök, A.; Arvanitakis, G.; McClung, G. Status of Microbial Based Cleaning Products in Statutory Regulations and Ecolabelling in Europe, the USA, and Canada. *Food Chem. Toxicol.* **2018**, *116*, 10–19. https://doi.org/10.1016/j.fct.2017.12.057.
7. Coubain, E. Recherches Sur Les Matières Grasses de l'état Normal et Pathologique. *Arch. Physiol. Norm. Pathol.* **1885**, *7*, 239–300.

8. Parsons, J.B.; Rock, C.O. Bacterial Lipids: Metabolism and Membrane Homeostasis. *Prog. Lipid Res.* **2013**, *52*, 249–276. https://doi.org/10.1016/j.plipres.2013.02.002.
9. Dowhan, W. A Retrospective: Use of *Escherichia coli* as a Vehicle to Study Phospholipid Synthesis and Function. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2013**, *1831*, 471–494. https://doi.org/10.1016/j.bbalip.2012.08.007.
10. Lindner, P. Das Problem Der Biologischen Fettbildung Und Fettgewinnung. *Angew. Chem. Weinh. Bergstr. Ger.* **1922**, *35*, 110–114. https://doi.org/10.1002/ange.19220351903.
11. Nägeli, C.; Loew, O. Ueber Die Chemische Zusammensetzung Der Hefe. *Justus Liebigs Ann. Chem.* **1878**, *193*, 322–348. https://doi.org/10.1002/jlac.18781930204.
12. Santos, A.X.S.; Riezman, H. Yeast as a Model System for Studying Lipid Homeostasis and Function. *FEBS Lett.* **2012**, *586*, 2858–2867. https://doi.org/10.1016/j.febslet.2012.07.033.
13. Radulovic, M.; Knittelfelder, O.; Cristobal-Sarramian, A.; Kolb, D.; Wolinski, H.; Kohlwein, S.D. The Emergence of Lipid Droplets in Yeast: Current Status and Experimental Approaches. *Curr. Genet.* **2013**, *59*, 231–242. https://doi.org/10.1007/s00294-013-0407-9.
14. Becker, E.W. Micro-Algae as a Source of Protein. *Biotechnol. Adv.* **2007**, *25*, 207–210. https://doi.org/10.1016/j.biotechadv.2006.11.002.
15. Schena, M.; Marucco, D. Enhanced Lipid Biosynthesis in Yeasts by Metabolic Engineering. *J. Biol. Chem.* **1975**, *250*, 6816–6821.
16. Ratledge, C.; Cohen, Z. Microbial and Algal Oils: Do They Have a Future for Biodiesel or as Commodity Oils? *Lipid Technol.* **2008**, *20*, 155–160. https://doi.org/10.1002/lite.200800044.
17. Xue, Z.; Sharpe, P.L.; Hong, S.-P.; Yadav, N.S.; Xie, D.; Short, D.R.; Damude, H.G.; Rupert, R.A.; Seip, J.E.; Wang, J.; et al. Production of Omega-3 Eicosapentaenoic Acid by Metabolic Engineering of *Yarrowia lipolytica*. *Nat. Biotechnol.* **2013**, *31*, 734–740. https://doi.org/10.1038/nbt.2622.
18. Xie, D.; Jackson, E.N.; Zhu, Q. Sustainable Source of Omega-3 Eicosapentaenoic Acid from Metabolically Engineered *Yarrowia lipolytica*: From Fundamental Research to Commercial Production. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 1599–1610. https://doi.org/10.1007/s00253-014-6318-y.
19. Ratledge, C. Microbial Production of Polyunsaturated Fatty Acids in Nutraceuticals. In *Microbial Production of Food İngredients, Enzymes and Nutraceuticals*; McNeil, B., Archer, D., Giavasis, I.; Harvey, L. Woodhead Publishing: Thorston, UK, 2013.
20. Sitepu, I.R.; Garay, L.A.; Sestric, R.; Levin, D.; Block, D.E. Exploring Fatty Acid Production in Engineered Strains of *Yarrowia lipolytica*. *Metab. Eng.* **2014**, *25*, 107–115.
21. Kris-Etherton, P.M.; Harris, W.S.; Appel, L.J.; American Heart Association. Nutrition Committee Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. *Circulation* **2002**, *106*, 2747–2757. https://doi.org/10.1161/01.cir.0000038493.65177.94.
22. Swanson, D.; Block, R.; Mousa, S.A. Omega-3 Fatty Acids EPA and DHA: Health Benefits throughout Life. *Adv. Nutr.* **2012**, *3*, 1–7. https://doi.org/10.3945/an.111.000893.
23. Calder, P.C. Marine Omega-3 Fatty Acids and Inflammatory Processes: Effects, Mechanisms and Clinical Relevance. *Biochim. Biophys. Acta* **2015**, *1851*, 469–484. https://doi.org/10.1016j.bbalip.2014.08.010.
24. Lee, R.A.; Lavoie, J.-M. From First- to Third-Generation Biofuels: Challenges of Producing a Commodity from a Biomass of Increasing Complexity. *Anim. Front.* **2013**, *3*, 6–11. https://doi.org/10.2527/af.2013-0010.
25. Bharathiraja, B.; Sridharan, S.; Sowmya, V.; Yuvaraj, D.; Praveenkumar, R. Microbial Oil—A Plausible Alternate Resource for Food and Fuel Application. *Bioresour. Technol.* **2017**, *233*, 423–432. https://doi.org/10.1016/j.biortech.2017.03.006.
26. Finco, A.M.d.O.; Mamani, L.D.G.; Carvalho, J.C. de; de Melo Pereira, G.V.; Thomaz-Soccol, V.; Soccol, C.R. Technological Trends and Market Perspectives for Production of Microbial Oils Rich in Omega-3. *Crit. Rev. Biotechnol.* **2017**, *37*, 656–671. https://doi.org/10.1080/07388551.2016.1213221.
27. Sun, H.; Gao, Z.; Zhang, L.; Wang, X.; Gao, M.; Wang, Q. A Comprehensive Review on Microbial Lipid Production from Wastes: Research Updates and Tendencies. *Environ. Sci. Pollut. Res. Int.* **2023**, *30*, 79654–79675. https://doi.org/10.1007/s11356-023-28123-6.
28. Fabiszewska, A.; Paplińska-Goryca, M.; Misiukiewicz-Stępień, P.; Wołoszynowska, M.; Nowak, D.; Zieniuk, B. Expression Profile of Selected Genes Involved in Storage Lipid Synthesis in a Model Oleaginous Yeast Species *Yarrowia lipolytica*. *Int. J. Mol. Sci.* **2022**, *23*, 1041. https://doi.org/10.3390/ijms23031041.
29. Beopoulos, A.; Chardot, T.; Nicaud, J.-M. *Yarrowia lipolytica*: A Model and a Tool to Understand the Mechanisms Implicated in Lipid Accumulation. *Biochimie* **2009**, *91*, 692–696. https://doi.org/10.1016/j.biochi.2009.02.004.
30. Papanikolaou, S.; Aggelis, G. Lipids of Oleaginous Yeasts. Part I: Biochemistry of Single Cell Oil Production. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 1031–1051. https://doi.org/10.1002/ejlt.201100014.
31. Wierzchowska, K.; Zieniuk, B.; Nowak, D.; Fabiszewska, A. Phosphorus and Nitrogen Limitation as a Part of the Strategy to Stimulate Microbial Lipid Biosynthesis. *Appl. Sci.* **2021**, *11*, 11819. https://doi.org/10.3390/app112411819.
32. Wierzchowska, K.; Zieniuk, B.; Fabiszewska, A. Use of Non-Conventional Yeast *Yarrowia lipolytica* in Treatment or Upgradation of Hydrophobic Industry Wastes. *Waste Biomass Valorization* **2022**, *13*, 757–779. https://doi.org/10.1007/s12649-021-01516-9.
33. Slaný, O.; Klempová, T.; Shapaval, V.; Zimmermann, B.; Kohler, A.; Čertík, M. Biotransformation of Animal Fat-by Products into ARA-Enriched Fermented Bioproducts by Solid-State Fermentation of *Mortierella alpina*. *J. Fungi* **2020**, *6*, 236. https://doi.org/10.3390/jof6040236.

34. Javed, M.U.; Mukhtar, H.; Zieniuk, B.; Rashid, U. Algal-Based Hollow Fiber Membrane Bioreactors for Efficient Wastewater Treatment: A Comprehensive Review. *Fermentation* **2024**, *10*, 131. https://doi.org/10.3390/fermentation10030131.
35. Sun, X.-M.; Ren, L.-J.; Zhao, Q.-Y.; Ji, X.-J.; Huang, H. Microalgae for the Production of Lipid and Carotenoids: A Review with Focus on Stress Regulation and Adaptation. *Biotechnol. Biofuels* **2018**, *11*, 272. https://doi.org/10.1186/s13068-018-1275-9.
36. Ratledge, C. Fatty Acid Biosynthesis in Microorganisms Being Used for Single Cell Oil Production. *Biochimie* **2004**, *86*, 807–815. https://doi.org/10.1016/j.biochi.2004.09.017.
37. Wynn, J.P.; Ratledge, C.; Hamid, A.A.; Li, Y. Biochemical Events Leading to the Diversion of Carbon into Storage Lipids in the Oleaginous Fungi *Mucor circinelloides* and *Mortierella alpina*. *Microbiology* **2001**, *147*, 2857–2864. https://doi.org/10.1099/00221287-147-10-2857.
38. Khot, M.; Raut, G.; Ghosh, D.; Alarcón-Vivero, M.; Contreras, D.; Ravikumar, A. Lipid Recovery from Oleaginous Yeasts: Perspectives and Challenges for Industrial Applications. *Fuel* **2020**, *259*, 116292. https://doi.org/10.1016/j.fuel.2019.116292.
39. Ochsenreither, K.; Glück, C.; Stressler, T.; Fischer, L.; Syldatk, C. Production Strategies and Applications of Microbial Single Cell Oils. *Front. Microbiol.* **2016**, *7*, 1539. https://doi.org/10.3389/fmicb.2016.01539.
40. Zainuddin, M.F.; Fai, C.K.; Ariff, A.B.; Rios-Solis, L.; Halim, M. Current Pretreatment/Cell Disruption and Extraction Methods Used to Improve Intracellular Lipid Recovery from Oleaginous Yeasts. *Microorganisms* **2021**, *9*, 251. https://doi.org/10.3390/microorganisms9020251.
41. Ratledge, C.; Lippmeier, C. Microbial production of fatty acids. In *Fatty Acids*; AOCS Press:, Urbana, Ilinois, USA, 2017; pp. 237–278.
42. Karamerou, E.E.; Parsons, S.; McManus, M.C.; Chuck, C.J. Using Techno-Economic Modelling to Determine the Minimum Cost Possible for a Microbial Palm Oil Substitute. *Biotechnol. Biofuels* **2021**, *14*, 57. https://doi.org/10.1186/s13068-021-01911-3.
43. Qiao, K.; Wasylenko, T.M.; Zhou, K.; Xu, P.; Stephanopoulos, G. Lipid Production in *Yarrowia lipolytica* Is Maximized by Engineering Cytosolic Redox Metabolism. *Nat. Biotechnol.* **2017**, *35*, 173–177. https://doi.org/10.1038/nbt.3763.
44. Yousuf, A.; Khan, M.R.; Islam, M.A.; Wahid, Z.A.; Pirozzi, D. Technical Difficulties and Solutions of Direct Transesterification Process of Microbial Oil for Biodiesel Synthesis. *Biotechnol. Lett.* **2017**, *39*, 13–23. https://doi.org/10.1007/s10529-016-2217-x.
45. Bellou, S.; Triantaphyllidou, I.-E.; Aggeli, D.; Elazzazy, A.M.; Baeshen, M.N.; Aggelis, G. Microbial Oils as Food Additives: Recent Approaches for Improving Microbial Oil Production and Its Polyunsaturated Fatty Acid Content. *Curr. Opin. Biotechnol.* **2016**, *37*, 24–35. https://doi.org/10.1016/j.copbio.2015.09.005.
46. Fabiszewska, A.; Pakulska, A.; Zieniuk, B.; Wierzchowska, K.; Jasinska, K.; Małajowicz, J.; Nowak, D. Unconventional Extraction Methods of Oleaginous Yeast Cell Pretreatment and Disruption. *Appl. Sci.* **2023**, *13*, 13135. https://doi.org/ 10.3390/app132413135.
47. Timotheo, C.A.; Fabricio, M.F.; Ayub, M.A.Z.; Valente, P. Evaluation of Cell Disruption Methods in the Oleaginous Yeasts *Yarrowia lipolytica* QU21 and *Meyerozyma guilliermondii* BI281A for Microbial Oil Extraction. *An. Acad. Bras. Ciências* **2023**, *95*, e20191256. https://doi.org/10.1590/0001-3765202320191256.
48. Dong, T.; Knoshaug, E.P.; Pienkos, P.T.; Laurens, L.M.L. Lipid Recovery from Wet Oleaginous Microbial Biomass for Biofuel Production: A Critical Review. *Appl. Energy* **2016**, *177*, 879–895. https://doi.org/10.1016/j.apenergy.2016.06.002.
49. Willis, R.M.; McCurdy, A.T.; Ogborn, M.K.; Wahlen, B.D.; Quinn, J.C.; Pease, L.F., III; Seefeldt, L.C. Improving Energetics of Triacylglyceride Extraction from Wet Oleaginous Microbes. *Bioresour. Technol.* **2014**, *167*, 416–424. https://doi.org/10.1016/j.biortech.2014.06.013.
50. Breil, C.; Abert Vian, M.; Zemb, T.; Kunz, W.; Chemat, F. "Bligh and Dyer" and Folch Methods for Solid–Liquid-Liquid Extraction of Lipids from Microorganisms. Comprehension of Solvatation Mechanisms and towards Substitution with Alternative Solvents. *Int. J. Mol. Sci.* **2017**, *18*, 708. https://doi.org/10.3390/ijms18040708.
51. Probst, K.V.; Wales, M.D.; Rezac, M.E.; Vadlani, P.V. Evaluation of Green Solvents: Oil Extraction from Oleaginous Yeast *Lipomyces starkeyi* Using Cyclopentyl Methyl Ether (CPME). *Biotechnol. Prog.* **2017**, *33*, 1096–1103. https://doi.org/10.1002/btpr.2473.
52. Imatoukene, N.; Koubaa, M.; Perdrix, E.; Benali, M.; Vorobiev, E. Combination of Cell Disruption Technologies for Lipid Recovery from Dry and Wet Biomass of *Yarrowia lipolytica* and Using Green Solvents. *Process Biochem.* **2020**, *90*, 139–147. https://doi.org/10.1016/j.procbio.2019.11.011.
53. D'Hondt, E.; Martín-Juárez, J.; Bolado, S.; Kasperoviciene, J.; Koreiviene, J.; Sulcius, S.; Elst, K.; Bastiaens, L. Cell Disruption Technologies. In *Microalgae-Based Biofuels and Bioproducts*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 133–154.
54. Meullemiestre, A.; Breil, C.; Abert-Vian, M.; Chemat, F. Microwave, Ultrasound, Thermal Treatments, and Bead Milling as Intensification Techniques for Extraction of Lipids from Oleaginous *Yarrowia lipolytica* Yeast for a Biojetfuel Application. *Bioresour. Technol.* **2016**, *211*, 190–199. https://doi.org/10.1016/j.biortech.2016.03.040.
55. Tuhanioglu, A.; Alpas, H.; Cekmecelioglu, D. High Hydrostatic Pressure-assisted Extraction of Lipids from *Lipomyces starkeyi* Biomass. *J. Food Sci.* **2022**, *87*, 5029–5041. https://doi.org/10.1111/1750-3841.16347.
56. Tribst, A.A.L.; Franchi, M.A.; Cristianini, M. Ultra-High Pressure Homogenization Treatment Combined with Lysozyme for Controlling Lactobacillus Brevis Contamination in Model System. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 265–271. https://doi.org/10.1016/j.ifset.2007.07.012.
57. Drévillon, L.; Koubaa, M.; Vorobiev, E. Lipid Extraction from *Yarrowia lipolytica* Biomass Using High-Pressure Homogenization. *Biomass Bioenergy* **2018**, *115*, 143–150. https://doi.org/10.1016/j.biombioe.2018.04.014.
58. Drévillon, L.; Koubaa, M.; Nicaud, J.-M.; Vorobiev, E. Cell Disruption Pre-Treatments towards an Effective Recovery of Oil from *Yarrowia lipolytica* Oleaginous Yeast. *Biomass Bioenergy* **2019**, *128*, 105320. https://doi.org/10.1016/j.biombioe.2019.105320.

59. Koubaa, M.; Imatoukene, N.; Drévillon, L.; Vorobiev, E. Current Insights in Yeast Cell Disruption Technologies for Oil Recovery: A Review. *Chem. Eng. Process.* **2020**, *150*, 107868. https://doi.org/10.1016/j.cep.2020.107868.
60. Jeevan Kumar, S.P.; Banerjee, R. Enhanced Lipid Extraction from Oleaginous Yeast Biomass Using Ultrasound Assisted Extraction: A Greener and Scalable Process. *Ultrason. Sonochem.* **2019**, *52*, 25–32. https://doi.org/10.1016/j.ultsonch.2018.08.003.
61. Milanesio, J.; Hegel, P.; Medina-González, Y.; Camy, S.; Condoret, J.-S. Extraction of Lipids from *Yarrowia lipolytica*. *J. Chem. Technol. Biotechnol.* **2013**, *88*, 378–387. https://doi.org/10.1002/jctb.3840.
62. Zheng, H.; Yin, J.; Gao, Z.; Huang, H.; Ji, X.; Dou, C. Disruption of Chlorella Vulgaris Cells for the Release of Biodiesel-Producing Lipids: A Comparison of Grinding, Ultrasonication, Bead Milling, Enzymatic Lysis, and Microwaves. *Appl. Biochem. Biotechnol.* **2011**, *164*, 1215–1224. https://doi.org/10.1007/s12010-011-9207-1.
63. Samarasinghe, N.; Fernando, S.; Lacey, R.; Faulkner, W.B. Algal Cell Rupture Using High Pressure Homogenization as a Prelude to Oil Extraction. *Renew. Energy* **2012**, *48*, 300–308. https://doi.org/10.1016/j.renene.2012.04.039.
64. Balasubramanian, R.K.; Yen Doan, T.T.; Obbard, J.P. Factors Affecting Cellular Lipid Extraction from Marine Microalgae. *Chem. Eng. J.* **2013**, *215–216*, 929–936. https://doi.org/10.1016/j.cej.2012.11.063.
65. Günerken, E.; D'Hondt, E.; Eppink, M.H.M.; Garcia-Gonzalez, L.; Elst, K.; Wijffels, R.H. Cell Disruption for Microalgae Biorefineries. *Biotechnol. Adv.* **2015**, *33*, 243–260. https://doi.org/10.1016/j.biotechadv.2015.01.008.
66. Show, K.-Y.; Lee, D.-J.; Tay, J.-H.; Lee, T.-M.; Chang, J.-S. Microalgal Drying and Cell Disruption—Recent Advances. *Bioresour. Technol.* **2015**, *184*, 258–266. https://doi.org/10.1016/j.biortech.2014.10.139.
67. Mehta, V.; Nayyar, C.; Gulati, N.; Singla, N.; Rai, S.; Chandar, J. A Comprehensive Review of Trichosporon Spp.: An Invasive and Emerging Fungus. *Cureus* **2021**, *13*, e17345. https://doi.org/10.7759/cureus.17345. .
68. Chu, M.-Y.; Zhang, L.-S.; Lou, W.-Y.; Zong, M.-H.; Tang, Y.-Q.; Yang, J.-G. Preparation and Characterization of Oil Rich in Odd Chain Fatty Acids from *Rhodococcus opacus* PD630. *J. Am. Oil Chem. Soc.* **2020**, *97*, 25–33. https://doi.org/10.1002/aocs.12304.
69. Silva, R.A.; Grossi, V.; Olivera, N.L.; Alvarez, H.M. Characterization of Indigenous *Rhodococcus* sp. 602, a Strain Able to Accumulate Triacylglycerides from Naphthyl Compounds under Nitrogen-Starved Conditions. *Res. Microbiol.* **2010**, *161*, 198–207. https://doi.org/10.1016/j.resmic.2010.01.007.
70. Miranda, S.M.; Lopes, M.; Belo, I. Exploring the Use of Hexadecane by *Yarrowia lipolytica*: Effect of Dissolved Oxygen and Medium Supplementation. *J. Biotechnol.* **2024**, *380*, 29–37. https://doi.org/10.1016/j.jbiotec.2023.12.006.
71. Dias, B.; Lopes, M.; Fernandes, H.; Marques, S.; Gírio, F.; Belo, I. Biomass and Microbial Lipids Production by *Yarrowia lipolytica* W29 from Eucalyptus Bark Hydrolysate. *Renew. Energy* **2024**, *224*, 120173. https://doi.org/10.1016/j.renene.2024.120173.
72. Cao, X.; Pan, Y.; Wei, W.; Yuan, T.; Wang, S.; Xiang, L.; Yuan, Y. Single Cell Oil Production by *Trichosporon* sp.: Effects of Fermentation Conditions on Fatty Acid Composition and Applications in Synthesis of Structured Triacylglycerols. *Lebenson. Wiss. Technol.* **2021**, *148*, 111691. https://doi.org/10.1016/j.lwt.2021.111691.
73. Wei, M.; Parrish, C.C.; Guerra, N.I.; Armenta, R.E.; Colombo, S.M. Extracted Microbial Oil from a Novel *Schizochytrium* sp. (T18) as a Sustainable High DHA Source for Atlantic Salmon Feed: Impacts on Growth and Tissue Lipids. *Aquaculture* **2021**, *534*, 736249. https://doi.org/10.1016/j.aquaculture.2020.736249.
74. Couto, D.; Conde, T.A.; Melo, T.; Neves, B.; Costa, M.; Cunha, P.; Guerra, I.; Correia, N.; Silva, J.T.; Pereira, H.; et al. Effects of Outdoor and Indoor Cultivation on the Polar Lipid Composition and Antioxidant Activity of *Nannochloropsis oceanica* and *Nannochloropsis limnetica*: A Lipidomics Perspective. *Algal Res.* **2022**, *64*, 102718. https://doi.org/10.1016/j.algal.2022.102718.
75. Alvarez, H.M.; Hernández, M.A.; Lanfranconi, M.P.; Silva, R.A.; Villalba, M.S. *Rhodococcus* as Biofactories for Microbial Oil Production. *Molecules* **2021**, *26*, 4871. https://doi.org/10.3390/molecules26164871.
76. Fabiszewska, A.U.; Zieniuk, B.; Kozłowska, M.; Mazurczak-Zieniuk, P.M.; Wołoszynowska, M.; Misiukiewicz-Stępień, P.; Nowak, D. Studies on Upgradation of Waste Fish Oil to Lipid-Rich Yeast Biomass in *Yarrowia lipolytica* Batch Cultures. *Foods* **2021**, *10*, 436. https://doi.org/10.3390/foods10020436.
77. Bracharz, F.; Beukhout, T.; Mehlmer, N.; Brück, T. Opportunities and Challenges in the Development of *Cutaneotrichosporon oleaginosus* ATCC 20509 as a New Cell Factory for Custom Tailored Microbial Oils. *Microb. Cell Fact.* **2017**, *16*, 178. https://doi.org/10.1186/s12934-017-0791-9.
78. Szczepańska, P.; Hapeta, P.; Lazar, Z. Advances in Production of High-Value Lipids by Oleaginous Yeasts. *Crit. Rev. Biotechnol.* **2022**, *42*, 1–22. https://doi.org/10.1080/07388551.2021.1922353.
79. Sun, M.-L.; Madzak, C.; Liu, H.-H.; Song, P.; Ren, L.-J.; Huang, H.; Ji, X.-J. Engineering *Yarrowia lipolytica* for Efficient γ-Linolenic Acid Production. *Biochem. Eng. J.* **2017**, *117*, 172–180. https://doi.org/10.1016/j.bej.2016.10.014.
80. Damude, H.G.; Zhang, H.; Farrall, L.; Ripp, K.G.; Tomb, J.-F.; Hollerbach, D.; Yadav, N.S. Identification of Bifunctional Δ12/Ω3 Fatty Acid Desaturases for Improving the Ratio of Ω3 to Ω6 Fatty Acids in Microbes and Plants. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 9446–9451. https://doi.org/10.1073/pnas.0511079103.
81. Imatoukene, N.; Verbeke, J.; Beopoulos, A.; Idrissi Taghki, A.; Thomasset, B.; Sarde, C.-O.; Nonus, M.; Nicaud, J.-M. A Metabolic Engineering Strategy for Producing Conjugated Linoleic Acids Using the Oleaginous Yeast *Yarrowia lipolytica*. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 4605–4616. https://doi.org/10.1007/s00253-017-8240-6.
82. Görner, C.; Redai, V.; Bracharz, F.; Schrepfer, P.; Garbe, D.; Brück, T. Genetic Engineering and Production of Modified Fatty Acids by the Non-Conventional Oleaginous Yeast *Trichosporon oleaginosus* ATCC 20509. *Green Chem.* **2016**, *18*, 2037–2046. https://doi.org/10.1039/c5gc01767j.
83. Palyzová, A.; Spížek, J.; Vítová, M.; Řezanka, T. Biosynthesis of Polyunsaturated Fatty Acids by Metabolic Engineering of Yeast *Yarrowia lipolytica*. In *Bioactive Natural Products*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 197–223, ISBN 9780323910996.

84. Zhu, Q.; Xue, Z.; Yadav, N.; Damude, H.; Pollak, D.W.; Rupert, R.; Seip, J.; Hollerbach, D.; Macool, D.; Zhang, H.; et al. Metabolic Engineering of an Oleaginous Yeast for the Production of Omega-3 Fatty Acids. In *Single Cell Oils*; Elsevier: Amsterdam, The Netherlands, 2010; pp. 51–73.
85. Bhutada, G.; Menard, G.; Bhunia, R.K.; Hapeta, P.P.; Ledesma-Amaro, R.; Eastmond, P.J. Production of Human Milk Fat Substitute by Engineered Strains of *Yarrowia Lipolytica*. *Metab. Eng. Commun.* **2022**, *14*, e00192. https://doi.org/10.1016/j.mec.2022.e00192.
86. Avis, T.J.; Boulanger, R.R.; Bélanger, R.R. Synthesis and Biological Characterization of (*Z*)-9-Heptadecenoic and (*Z*)-6-Methyl-9-Heptadecenoic Acids: Fatty Acids with Antibiotic Activity Produced by *Pseudozyma flocculosa*. *J. Chem. Ecol.* **2000**, *26*, 987–1000. https://doi.org/10.1023/a:1005464326573.
87. Avis, T.J.; Bélanger, R.R. Specificity and Mode of Action of the Antifungal Fatty Acid *Cis*-9-Heptadecenoic Acid Produced by *Pseudozyma flocculosa*. *Appl. Environ. Microbiol.* **2001**, *67*, 956–960. https://doi.org/10.1128/aem.67.2.956-960.2001.
88. Smedman, A.E.M.; Gustafsson, I.-B.; Berglund, L.G.T.; Vessby, B.O.H. Pentadecanoic Acid in Serum as a Marker for Intake of Milk Fat: Relations between Intake of Milk Fat and Metabolic Risk Factors. *Am. J. Clin. Nutr.* **1999**, *69*, 22–29. https://doi.org/10.1093/ajcn/69.1.22.
89. Santaren, I.D.; Watkins, S.M.; Liese, A.D.; Wagenknecht, L.E.; Rewers, M.J.; Haffner, S.M.; Lorenzo, C.; Hanley, A.J. Serum Pentadecanoic Acid (15:0), a Short-Term Marker of Dairy Food Intake, Is Inversely Associated with Incident Type 2 Diabetes and Its Underlying Disorders. *Am. J. Clin. Nutr.* **2014**, *100*, 1532–1540. https://doi.org/10.3945/ajcn.114.092544.
90. Jenkins, B.; West, J.A.; Koulman, A. A Review of Odd-Chain Fatty Acid Metabolism and the Role of Pentadecanoic Acid (C15:0) and Heptadecanoic Acid (C17:0) in Health and Disease. *Molecules* **2015**, *20*, 2425–2444. https://doi.org/10.3390/molecules20022425.
91. Yoo, W.; Gjuka, D.; Stevenson, H.L.; Song, X.; Shen, H.; Yoo, S.Y.; Wang, J.; Fallon, M.; Ioannou, G.N.; Harrison, S.A.; et al. Fatty Acids in Non-Alcoholic Steatohepatitis: Focus on Pentadecanoic Acid. *PLoS ONE* **2017**, *12*, e0189965. https://doi.org/10.1371/journal.pone.0189965.
92. To, N.B.; Nguyen, Y.T.-K.; Moon, J.Y.; Ediriweera, M.K.; Cho, S.K. Pentadecanoic Acid, an Odd-Chain Fatty Acid, Suppresses the Stemness of MCF-7/SC Human Breast Cancer Stem-Like Cells through JAK2/STAT3 Signaling. *Nutrients* **2020**, *12*, 1663. https://doi.org/10.3390/nu12061663.
93. Poljšak, N.; Kočevar Glavač, N. Vegetable Butters and Oils as Therapeutically and Cosmetically Active Ingredients for Dermal Use: A Review of Clinical Studies. *Front. Pharmacol.* **2022**, *13*, 868461. https://doi.org/10.3389/fphar.2022.868461.
94. Ahmad, A.; Ahsan, H. Lipid-Based Formulations in Cosmeceuticals and Biopharmaceuticals. *Biomed. Dermatol.* **2020**, *4*, 12. https://doi.org/10.1186/s41702-020-00062-9.
95. De Luca, M.; Pappalardo, I.; Limongi, A.R.; Viviano, E.; Radice, R.P.; Todisco, S.; Martelli, G.; Infantino, V.; Vassallo, A. Lipids from Microalgae for Cosmetic Applications. *Cosmetics* **2021**, *8*, 52. https://doi.org/10.3390/cosmetics8020052.
96. Olejnik, A.; Gornowicz-Porowska, J.; Jenerowicz, D.; Polańska, A.; Dobrzyńska, M.; Przysławski, J.; Sansone, A.; Ferreri, C. Fatty Acids Profile and the Relevance of Membranes as the Target of Nutrition-Based Strategies in Atopic Dermatitis: A Narrative Review. *Nutrients* **2023**, *15*, 3857. https://doi.org/10.3390/nu15173857.
97. Zhang, Y.; Liu, Y.; Sun, J.; Zhang, W.; Guo, Z.; Ma, Q. Arachidonic Acid Metabolism in Health and Disease. *MedComm* **2023**, *4*, e363. https://doi.org/10.1002/mco2.363.
98. Jang, Y.; Kim, M.; Hwang, S.W. Molecular Mechanisms Underlying the Actions of Arachidonic Acid-Derived Prostaglandins on Peripheral Nociception. *J. Neuroinflammation* **2020**, *17*, 30. https://doi.org/10.1186/s12974-020-1703-1.
99. Alhattab, M.; Moorthy, L.S.; Patel, D.; Franco, C.M.M.; Puri, M. Oleaginous Microbial Lipids' Potential in the Prevention and Treatment of Neurological Disorders. *Mar. Drugs* **2024**, *22*, 80. https://doi.org/10.3390/md22020080.
100. Barbalace, M.C.; Malaguti, M.; Giusti, L.; Lucacchini, A.; Hrelia, S.; Angeloni, C. Anti-Inflammatory Activities of Marine Algae in Neurodegenerative Diseases. *Int. J. Mol. Sci.* **2019**, *20*, 3061. https://doi.org/10.3390/ijms20123061.
101. Kousparou, C.; Fyrilla, M.; Stephanou, A.; Patrikios, I. DHA/EPA (Omega-3) and LA/GLA (Omega-6) as Bioactive Molecules in Neurodegenerative Diseases. *Int. J. Mol. Sci.* **2023**, *24*, 10717. https://doi.org/10.3390/ijms241310717.
102. Wei, B.-Z.; Li, L.; Dong, C.-W.; Tan, C.-C.; Xu, W. The Relationship of Omega-3 Fatty Acids with Dementia and Cognitive Decline: Evidence from Prospective Cohort Studies of Supplementation, Dietary Intake, and Blood Markers. *Am. J. Clin. Nutr.* **2023**, *117*, 1096–1109. https://doi.org/10.1016/j.ajcnut.2023.04.001.
103. Yamagata, K. Docosahexaenoic Acid Inhibits Ischemic Stroke to Reduce Vascular Dementia and Alzheimer's Disease. *Prostaglandins Other Lipid Mediat.* **2023**, *167*, 106733. https://doi.org/10.1016/j.prostaglandins.2023.106733.
104. Kerdiles, O.; Layé, S.; Calon, F. Omega-3 Polyunsaturated Fatty Acids and Brain Health: Preclinical Evidence for the Prevention of Neurodegenerative Diseases. *Trends Food Sci. Technol.* **2017**, *69*, 203–213. https://doi.org/10.1016/j.tifs.2017.09.003.
105. Barbaresko, J.; Lellmann, A.W.; Schmidt, A.; Lehmann, A.; Amini, A.M.; Egert, S.; Schlesinger, S.; Nöthlings, U. Dietary Factors and Neurodegenerative Disorders: An Umbrella Review of Meta-Analyses of Prospective Studies. *Adv. Nutr.* **2020**, *11*, 1161–1173. https://doi.org/10.1093/advances/nmaa053.
106. Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 Establishing the Union List of Novel Foods in Accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on Novel Foods. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R2470 (accessed on 6 April 2024).
107. Ryan, A.S.; Zeller, S.; Nelson, E.B. Safety Evaluation of Single Cell Oils and the Regulatory Requirements for Use as Food Ingredients. In *Single Cell Oils*; Elsevier: Amsterdam, The Netherlands, 2010; pp. 317–350.

108. Jackson-Davis, A.; White, S.; Kassama, L.S.; Coleman, S.; Shaw, A.; Mendonca, A.; Cooper, B.; Thomas-Popo, E.; Gordon, K.; London, L. A Review of Regulatory Standards and Advances in Essential Oils as Antimicrobials in Foods. *J. Food Prot.* **2023**, *86*, 100025. https://doi.org/10.1016/j.jfp.2022.100025.
109. Palumbo, M.; Harris, L.J. Microbiological food safety of olive oil: A review of the literature. In *US Davis Olive Center Report*; University of California: Davis, CA, USA 2011.
110. Aloui, H.; Khwaldia, K. Natural Antimicrobial Edible Coatings for Microbial Safety and Food Quality Enhancement. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 1080–1103. https://doi.org/10.1111/1541-4337.12226.
111. Fidler, N.; Sauerwald, T.; Demmelmair, H.; Pohl, A.; Koletzko, B. Oxidation of an Oil Rich in Docosahexaenoic Acid Compared to Linoleic Acid in Lactating Women. *Ann. Nutr. Metab.* **1999**, *43*, 339–345. https://doi.org/10.1159/000012802.
112. Dhasco And Arasco Oıls As Sources Of Long-Chaın Polyunsaturated Fatty Acıds In Infant Formula A Safety Assessment Technıcal Report Available online: https://www.foodstandards.gov.au/sites/default/files/publications/Documents/DHASCO%20and%20ARASCO%20in%20infant%20formula.pdf (accessed on 20 April 2024).
113. Arterburn, L.M.; Oken, H.A.; Hoffman, J.P.; Bailey-Hall, E.; Chung, G.; Rom, D.; Hamersley, J.; McCarthy, D. Bioequivalence of Docosahexaenoic Acid from Different Algal Oils in Capsules and in a DHA-fortified Food. *Lipids* **2007**, *42*, 1011–1024. https://doi.org/10.1007/s11745-007-3098-5.
114. Yeiser, M.; Harris, C.L.; Kirchoff, A.L.; Patterson, A.C.; Wampler, J.L.; Zissman, E.N.; Berseth, C.L. Growth and Tolerance of Infants Fed Formula with a New Algal Source of Docosahexaenoic Acid: Double-Blind, Randomized, Controlled Trial. *Prostaglandins Leukot. Essent. Fat. Acids* **2016**, *115*, 89–96. https://doi.org/10.1016/j.plefa.2016.09.001.
115. Kroes, R.; Renwick, A.G.; Feron, V.; Galli, C.L.; Gibney, M.; Greim, H.; Guy, R.H.; Lhuguenot, J.C.; van de Sandt, J.J.M. Application of the Threshold of Toxicological Concern (TTC) to the Safety Evaluation of Cosmetic Ingredients. *Food Chem. Toxicol.* **2007**, *45*, 2533–2562. https://doi.org/10.1016/j.fct.2007.06.021.
116. Tyburczy, C.; Brenna, M.E.; DeMari, J.A.; Kothapalli, K.S.D.; Blank, B.S.; Valentine, H.; McDonough, S.P.; Banavara, D.; Diersen-Schade, D.A.; Brenna, J.T. Evaluation of Bioequivalency and Toxicological Effects of Three Sources of Arachidonic Acid (ARA) in Domestic Piglets. *Food Chem. Toxicol.* **2011**, *49*, 2320–2327. https://doi.org/016/j.fct.2011.06.033.
117. Villalta, M.; Estévez, A.; Bransden, M.P. Arachidonic Acid Enriched Live Prey Induces Albinism in Senegal Sole (*Solea senegalensis*) Larvae. *Aquaculture* **2005**, *245*, 193–209. https://doi.org/10.1016/j.aquaculture.2004.11.035.
118. Nutritional Lipids. Available online: https://www.dsm.com/human-nutrition/en/products/nutritional-lipids.html (accessed on 20 April 2024).
119. Carsanba, E.; Papanikolaou, S.; Erten, H. Production of Oils and Fats by Oleaginous Microorganisms with an Emphasis given to the Potential of the Nonconventional Yeast *Yarrowia lipolytica*. *Crit. Rev. Biotechnol.* **2018**, *38*, 1230–1243. https://doi.org/10.1080/07388551.2018.1472065.
120. Béligon, V.; Christophe, G.; Fontanille, P.; Larroche, C. Microbial Lipids as Potential Source to Food Supplements. *Curr. Opin. Food Sci.* **2016**, *7*, 35–42, . https://doi.org/10.1016/j.cofs.2015.10.002.
121. Sabikhi, L.; Sathish Kumar, M.H. Fatty Acid Profile of Unconventional Oilseeds. In *Advances in Food and Nutrition Research*; Elsevier: Amsterdam, The Netherlands, 2012; Volume 67; pp. 141–184.
122. Ahmad, F.B.; Zhang, Z.; Doherty, W.O.S.; O'Hara, I.M. The Prospect of Microbial Oil Production and Applications from Oil Palm Biomass. *Biochem. Eng. J.* **2019**, *143*, 9–23. https://doi.org/10.1016/j.bej.2018.12.003.
123. Lourdes, R.S.; Cheng, S.Y.; Chew, K.W.; Ma, Z.; Show, P.L. Prospects of Microbial Enhanced Oil Recovery: Mechanisms and Environmental Sustainability. *Sustain. Energy Technol. Assess.* **2022**, *53*, 102527. https://doi.org/10.1016/j.seta.2022.102527.