Food Research International 205 (2025) 115996

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

Harnessing oleaginous protist *Schizochytrium* for docosahexaenoic acid: Current technologies in sustainable production and food applications

Zongfan Peng [a], Liang Zhong [a], Yuqin Li [a,*], Siran Feng [b], Jinhua Mou [c], Yahui Miao [c], Carol Sze Ki Lin [c], Zhenyao Wang [b,*], Xuan Li [b]

[a] School of Chemical Engineering, Xiangtan University, Xiangtan, 411105, China
[b] Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Ultimo, NSW 2007, Australia
[c] School of Energy and Environment, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

ARTICLE INFO

Keywords:
Schizochytrium
Docosahexaenoic acid
Productivity
Function
Nutrition
Foods

ABSTRACT

Docosahexaenoic acid (DHA) exerts versatile roles in nutrition supplementation and numerous health disorders prevention. Global consumption demand for DHA has also been consistently increasing with enhanced health awareness. Oleaginous marine protist *Schizochytrium* is praised as a potential DHA source due to short growth cycle, convenient artificial culture, harmless to the human body, and easy manipulation of the DHA synthesis pathway. However, factors including strain performances, fermentation parameters, product harvest and extraction strategies, safety and stability maintenance, and also application limitations in health and functional properties affect the widespread adoption of *Schizochytrium* DHA products. This review provides a comprehensive summary of the current biotechnologies used for tackling factors affecting the *Schizochytrium* DHA production, with special focuses on *Schizochytrium* strain improvement technologies, fermentation optimization projects, DHA oil extraction strategies, safety evaluations and stability maintenance schemes, and DHA product application approaches in foods. Inspired by systematic literature investigations and recent advances, suggestive observations composed of improving strain with multiple breeding technologies, considering artificial intelligence and machine learning to optimize the fermentative process, introducing nanoparticles packing technology to improve oxidation stability of DHA products, covering up DHA odor defect with characteristic flavor foods, and employing synthetic biology to construct the structured lipids with DHA to exploit potential functions are formed. This review will give a guideline for exploring more *Schizochytrium* DHA and propelling the application development in food and health.

1. Introduction

Polyunsaturated fatty acids (PUFAs) are fatty acids with two or more unsaturated double bonds in the molecule and more than 20 carbon atoms. PUFAs can be categorized into four groups (i.e., n-3 PUFAs, n-6 PUFAs, n-7 PUFAs, and n-9 PUFAs) based on the position of the first double bond. Docosahexaenoic acid (DHA) as one of the typical n-3 PUFAs contains six double bonds in the acyl chain and the first double bond is located between the 3rd and 4th carbon atoms from the methyl end. Such special structure confers quite different properties on the fatty acid and possesses numerous structural and physiological functions for human beings (Zhang, Zhao, et al., 2024).

It is currently well-known that DHA exerts versatile roles in nutrition supplementation and promoting retinal nerve and brain development, strengthening immunity, and reducing the incidence rate of hypertension, heart attack, thrombosis, and stroke (Jiang et al., 2024; Liu et al., 2023; Xu et al., 2020) (Fig. 1a). Recent investigation has shown that the global market size for n-3 PUFAs valued at USD 3.1 billion in 2020, is projected to grow at a compound annual growth rate of 7.9 % from 2025 to 2030 (Kumari et al., 2023) (Fig. 1b). This indicates that global consumption demand for DHA has been consistently increasing with the enhanced health awareness and rising income levels. However, DHA is not produced de novo in the body and must be obtained from the diet or synthesized from α-linolenic acid (ALA). Currently, the available sources of DHA include oily fish, oleaginous plants, and marine protists (Fig. 1c). However, marine oily fish sources for DHA pose a risk of heavy metal contamination and are not suitable for vegetarians (Patel et al., 2021). Additionally, DHA derived from oily fish reduce by 10–58 % due to

* Corresponding authors.
E-mail addresses: yuqinli2004@xtu.edu.cn (Y. Li), zhenyao.wang-1@uts.edu.au (Z. Wang).

https://doi.org/10.1016/j.foodres.2025.115996
Received 16 September 2024; Received in revised form 17 January 2025; Accepted 10 February 2025
Available online 13 February 2025

global warming by 2100 (Colombo et al., 2020). Plant-based sources contain high proportions of ALA, but the conversion rate of ALA to DHA is only 0.5–9 % (Weylandt et al., 2015; Zhang et al., 2023) (Fig. 1d). Moreover, both oily fish and plant-based sources for DHA face challenges, such as long growth cycle, seasonal restrictions, and natural hazard (Chen et al., 2024).

Oleaginous marine protists such as *Crypthecodinium cohnii*, *Ulkenia amoeboida*, and *Schizochytrium* have recently been potential sources of DHA due to the capability of synthesizing high-quality DHA products with minimal nutritional requirements and without light exposure (Chi et al., 2022). Amongst these industrially-permissible DHA-producing species, *Schizochytrium* attracts much attention for its characteristics of high DHA content, short growth cycle, convenient artificial culture, harmless to the human body, and easy manipulation of lipid biosynthesis pathway and qualified as a sustainable DHA source (Bi et al., 2023; Ramos-Vega et al., 2018; Wang et al., 2021) (Fig. 1e). In particular, approval of *Schizochytrium* as a raw food material ensures the viability of developing DHA products by *Schizochytrium*. To further evidence the feasibility of *Schizochytrium* DHA, 480 literature data points are acquired from the Web of Science core collection, covering the period from 2010 to 2024, based on the search keywords "*Schizochytrium*" and "DHA". Fig. 1f depicts the evolution of keywords on *Schizochytrium* DHA in different periods. Investigators focus on fundamental research into *Schizochytrium* DHA production elevating methods before 2019. For instance, metabolic engineering, glycerol, cane molasses, culture-conditions, fed-batch, and lipid production embody a series of DHA production improvement technologies including species improvement, production cost reduction, and fermentation condition optimization. Synchronously, keywords such as health, toxicity, and safety are involved in the safety assessment of *Schizochytrium* DHA. Additionally, the keywords shift toward practical application and development in food and food feed-stocks from 2019 to 2024, with terms like *Atlantic salmon*, *Nile tilapia*, cows, and milk, indicating the application research of DHA has gradually attracted the interest of more researchers.

Further reflecting upon these published data find that the commercial of *Schizochytrium* DHA products still faces many obstacles due to poor DHA production performance of wild strains, expensive cultivation substrates, unmanageable fermentation conditions, environmentally hazardous chemical reagents for DHA extraction, imperfect safety certifications, and instability of DHA (Mu et al., 2022; Rodríguez-España et al., 2022; Sidari & Tofalo, 2019; Wang et al., 2023). Currently, some biotechnologies, strategies, or efforts have gradually evolved to species improvements and fermentation optimizations for high DHA productivity (Chen, Tong, Liu, et al., 2023), wastes utilization for reduced cultivation cost (Ma, Li, et al., 2023), bioreactor precise elements for fermentation control (Ding et al., 2022; Guo et al., 2017), supercritical CO_2 green extraction for DHA (Zinnai et al., 2016), safety evaluation extended to toxicity, genetic, biosafety, reproduction, and allergy (Thakur et al., 2024), microencapsulated applications in maintaining DHA stability (Chen, Wang, Zhang, et al., 2016). However, comprehensive summaries that cover the aforementioned entire process from *Schizochytrium* species to DHA products and final applications as well as integrating the advanced technologies into these procedures are scarce. For instance, Wang et al. (2021) and Chi et al. (2022) only mention the screening and improvement of natural species and fermentation optimization to enhance *Schizochytrium* DHA; Xu et al. (2020) mainly descript the utilizations of low-cost materials, stimulators, and the genetic engineering strategies to revolve the DHA yield of *Schizochytrium*. Synchronously, the downstream application technologies such as DHA oil extraction, bioavailability, and applications in biomedicine, food,

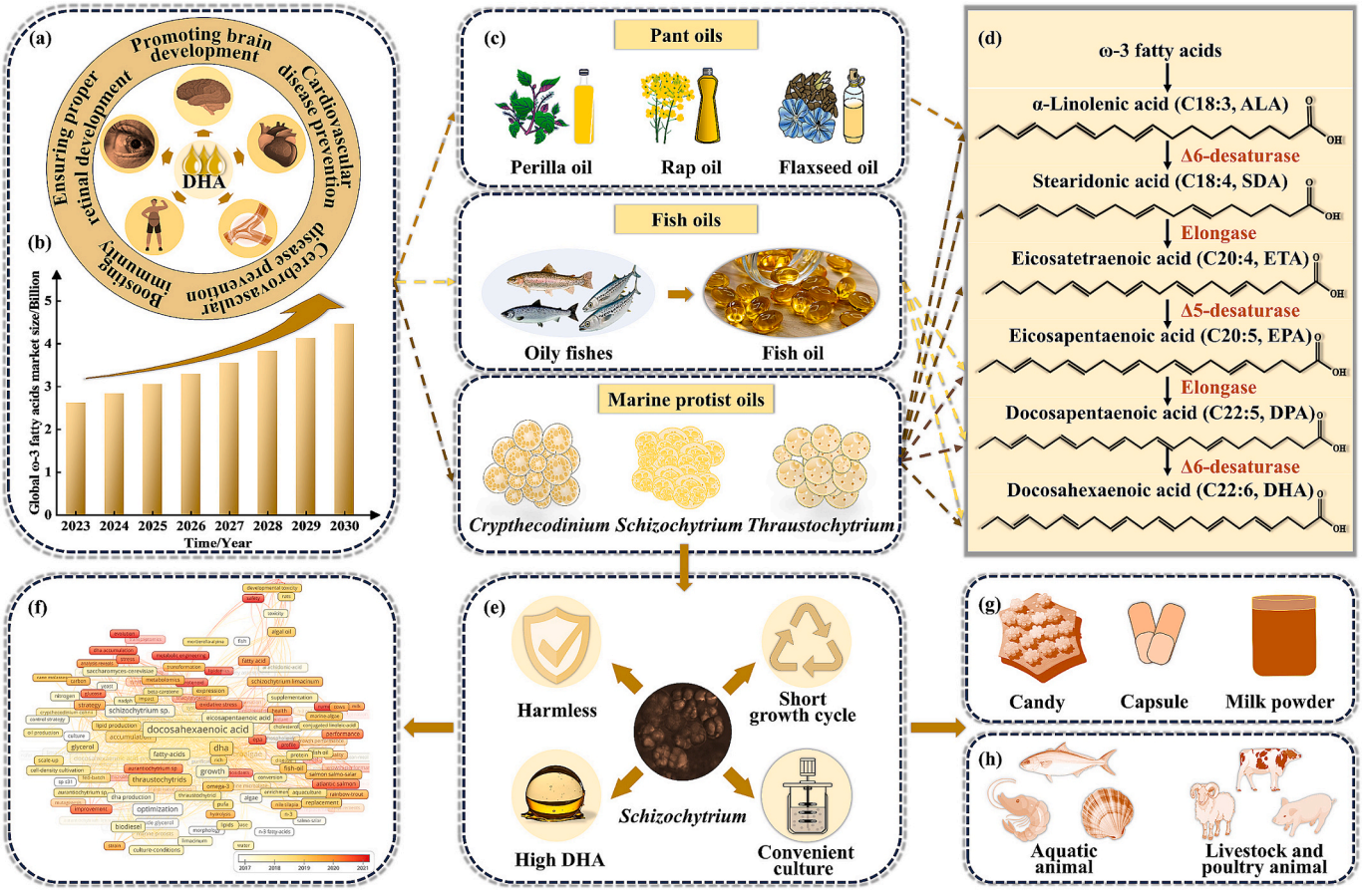

Fig. 1. Functions of docosahexaenoic acid (DHA) (a), ω-3 fatty acids requirements (b), DHA sources (c), α-linolenic acid (ALA) transformation DHA pathway (d), superior properties of *Schizochytrium* as DHA source (e), *Schizochytrium* hot spots (f), and *Schizochytrium* DHA products application in foods (g-h).

and feed fields are rarely summarized (Byreddy et al., 2016; Isa et al., 2022; Jeon et al., 2022). Therefore, a comprehensive literature review and analysis are essential to propel the broad-spectrum applications of *Schizochytrium* DHA.

This review summarizes and updates versatile biotechnologies for *Schizochytrium* DHA over the past few years with special attention to the enhancement of DHA productivity, DHA oil extraction, safety evaluations, and stability manipulations of *Schizochytrium* DHA products. Additionally, applications of *Schizochytrium* DHA products in foods and food raw materials, such as aquatic food raw material, livestock and poultry food, and also direct application in human food are discussed. In the final section, suggestive observations on the future perspectives are presented based on the current research trend and application status. The findings of this work ranging from species improvement and product applications can enrich the growing body of knowledge about production and application technologies in the *Schizochytrium* DHA industry while also highlighting the significance of realizing the large-scale industrialization of *Schizochytrium* dry biomass rich in DHA and DHA oil products to serve human health.

2. *Schizochytrium* species improvement for increasing DHA production

Some natural *Schizochytrium* species are usually isolated from mangrove, coastal, estuarine, and Iceland environments (Fig. 2). The DHA contents in TFAs of these natural species including *Schizochytrium* PKU#Mn4, *Schizochytrium* mangrovei FB1, FB3, and FB5 are reported to be ranging from 32.29 % to 44 %, which show a gap with the industrial DHA output requirement (Jiang et al., 2004; Liu et al., 2014) (Table 1). Additionally, constantly discovering new *Schizochytrium* species will result in expensive expenditure in the subsequent sequencing and species identification (Chi et al., 2022). Therefore, some breeding strategies such as adaptive laboratory evolution, mutagenesis, and genetic engineering are employed to improve the traits of the existing *Schizochytrium* species for further boosting DHA yield (Fig. 2). These strategies are described in detail below in Sections 2.1–2.3.

2.1. Adaptive laboratory evolution

Adaptive laboratory evolution strategies such as high temperature, high oxygen, and high salinity can strengthen the adaptive traits of wild *Schizochytrium* species in adverse environments consequently further improving the DHA (Hu et al., 2021; Sun et al., 2016; Sun, Ren, Bi, Ji, Zhao, & Huang, 2018). Hu et al. (2021) and Sun et al. (2016) find that the DHA yields of *Schizochytrium* sp. HX-308 treated with high temperature and high oxygen show 0.35-fold and 3.33-fold more than that of wild species, respectively. The high oxygen promotes DHA yield probably since that high oxygen adaptive evolution can enhance isocitrate dehydrogenase activity in the TCA cycle to crack more isocitric acid and generate more ATP for cell growth. Additionally, the high oxygen adaptive evolution weakens DHA sensitivity to oxygen, so the endpoint strain obtains higher DHA content in a higher oxygen supply (Sun et al., 2016). However, the DHA yield of the high temperature presents 8.5-fold more than that of high oxygen. This is on account of the increased carbon consumption rate and the decreased reactive oxygen species (ROS) to promote DHA biosynthesis under high temperature (Hu et al., 2021; Sun et al., 2016). Synchronously, Sun, Ren, Bi, Ji, Zhao, and Huang (2018) indicate that high salinity can also alleviate oxidative damage and up-regulate fatty acid synthase in *Schizochytrium* sp. HX-308. Such high salinity adaptation accumulates DHA yield of 9.52 g/L, representing 1.1- to 1.35-fold increase over the starting strain, high-oxygen adaptation, and high-temperature adaptation. However, many other environmental factors can also affect the DHA biosynthesis of *Schizochytrium* species. If multiple factors are implemented adaptive laboratory evolution in sequence, which not only has a long-time span but also affects DHA biosynthesis efficacy (Wang, Wang, et al., 2022;

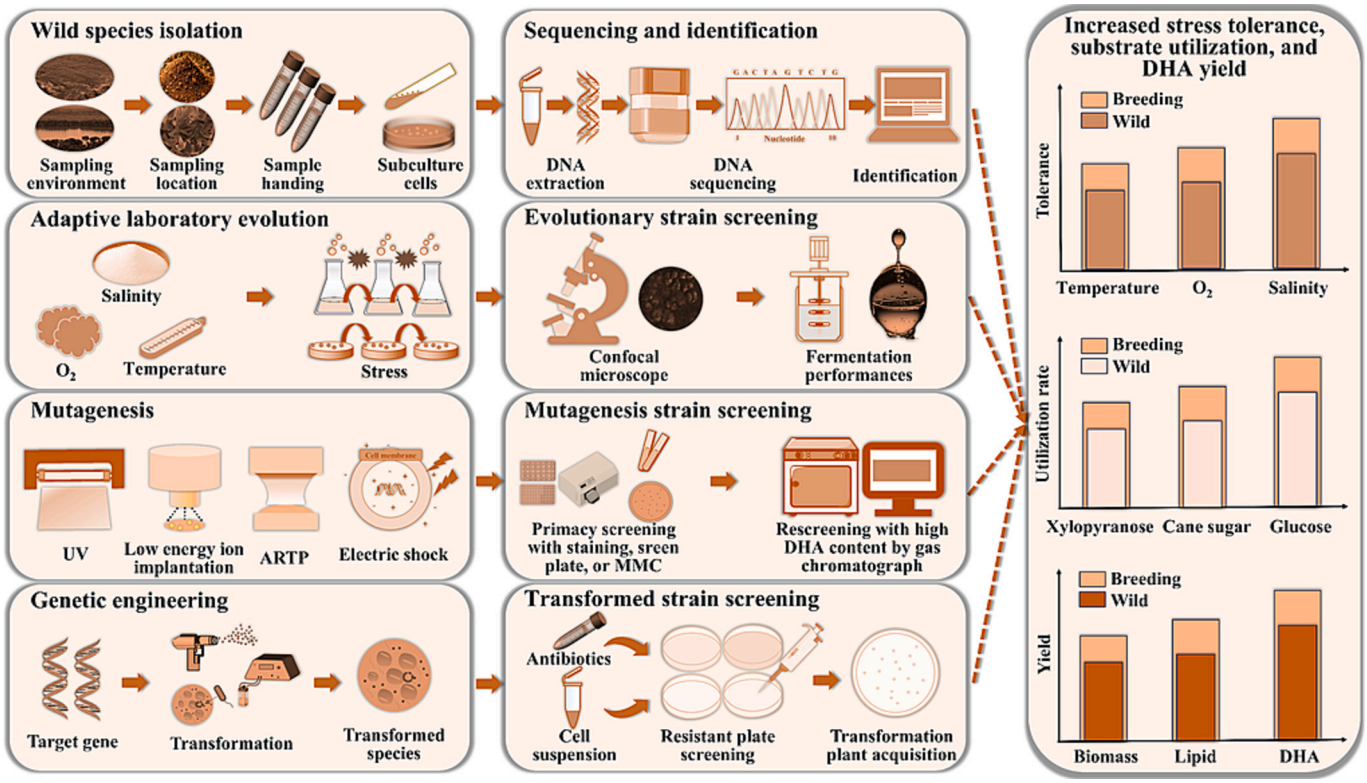

Fig. 2. *Schizochytrium* species improvement to enhance DHA with a variety of breeding techniques. UV, ARTP, and MMC represent ultra violet, atmospheric and room temperature plasma, microbial microdroplet culture. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Technologies in specie improvement to boost DHA yield of *Schizochytrium*.

Technologies	*Original Schizochytrium* species	Technology processes	Species selection principles	DHA outputs	References
Wild species isolation	*Schizochytrium*. PKU#Mn4	Separation and identification	Selecting strains with DHA production potential	DHA content reached to 44 % of TFAs	Liu et al. (2014)
	Schizochytrium mangrovei FB1, FB3, and FB5	Separation and identification	Selecting strains with DHA production potential	DHA content increased from 32.29 to 39.14 % of TFAs	Jiang et al. (2004)
Adaptive laboratory evolution	*Schizochytrium* sp. CCTCC M209059	High temperature stress	Increasing temperature to reduce production cost	DHA yield increased 4.33 times at 34 °C	Hu et al. (2021)
	Schizochytrium sp. HX-308	High oxygen stress	Producing more PUFAs and reduce oxidative injury	DHA yield increased by 34.83 %	Sun et al. (2016)
	Schizochytrium sp. HX-308	High salinity stress	Enhancing antioxidant system and lipid accumulation	DHA yield increased by 1.35 times	Sun et al. (2018)
	Schizochytrium sp. HX-308	Low temperature and high salinity stress	Increasing DHA accumulation and preventing lipid peroxidation	DHA yield increased by 57.52 %	Sun et al. (2018)
Mutagenesis	*Schizochytrium limacinum* B4D1	UV	Selecting mutants endowed with poor butanol tolerance	DHA content increased by 11 %	Li et al. (2017)
	Schizochytrium sp. ATCC20888	Low-energy ion implantation	Selecting mutants with more oil droplets	DHA content increased by 61 %	Fu et al. (2016)
	Schizochytrium limacinum B4D1	ARTP	Selecting mutants with high DHA	DHA yield increased by 25.51 %	Chen et al. (2022)
	Schizochytrium sp. PKU#Mn4	ARTP	Selecting mutants with clethodim	DHA content increased by 54.1 %	Liu et al. (2021)
	Schizochytrium sp. SR21	ARTP	Selecting mutant with microdroplet culture high throughput	DHA increased to 58.3 % of TFAs	Wang et al. (2023)
	Schizochytrium limacinum BCD	Electroporation	Selecting mutants with 40 μg/mL zeocin	DHA content increased by 95.51 %	Chen, Tong, Liu, et al. (2023)
Gene engineering	*Schizochytrium* sp. ATCC 20888	Electroporation	ACL and ACC overexpression to strengthen precursors supply	DHA yields increased by 23.3–48.8 %	Han et al. (2020)
	Schizochytrium sp. ATCC 20888	Electroporation	ACC and ACL overexpression and PEX10 knockout to strengthen precursors supply	DHA content increased by 72.1 %	Han et al. (2024)
	Schizochytrium sp. MYA1381	Electroporation	MAT overexpression to strengthen precursors supply	DHA content increased by 81.5 %	Li et al. (2018)
	Schizochytrium sp.H016	Electroporation	G6PD overexpression to strengthen NADPH supply	DHA content increased by 40.29 %	Feng et al. (2023)
	Schizochytrium sp. HX-308	Electroporation	ω-3 desaturase overexpression to promote DPA conversion DHA	DHA content increased to 49.23 %	Ren et al. (2015)
	Schizochytrium sp. HX-308	Agrobacterium transformation	PPTase and omega-3 FAD overexpressions to facilitate DPA conversion DHA	DHA content increased by 78.1 %	Li et al. (2023)
	Schizochytrium sp. ATCC20888	Electroporation	Fab R gene loss to remove DHA suppressor genes	DHA content increased by 46.5 %	Liu et al. (2024)
	Schizochytrium sp. ATCC20888	Electroporation	LipR gene loss to remove DHA suppressor genes	DHA content increased by 48 %	Han et al. (2022)
	Schizochytrium sp. HX-308 CCTCC M 209059	Agrobacterium transformation	Endogenous sucrose hydrolase overexpression to improve sucrose utilization	DHA yield reached 25.26 g/L	Ma, Zhang, et al. (2023)
	Schizochytrium sp. HX-308 CCTCC M 209059	Agrobacterium transformation	Isomerase and xylulose kinase overexpressions to improve xylose utilization	DHA increased 49.96 % of TFAs	Wang, Zhang, et al. (2022)

Zhang, Wu, & Meng, 2021). Subsequently, the synergetic adaption (e.g., high-salt coupling low-temperature) is developed to achieve a maximal biomass of 126.4 g/L and DHA of 38.12 g/L, which are 27.42 % and 57.52 % higher than the parental strain *Schizochytrium* sp. HX-308 (Sun, Ren, Bi, Ji, Zhao, Jiang, & Huang, 2018). The DHA yield with synergetic adaption represents 44 %–96 % higher than that of sole adaption such as high oxygen, high temperature, and high salinity. Such synergetic adaption strategies exert advantages for each condition such as the high salinity stimulates lipid accumulation and enhances the antioxidative defense systems and also the low temperature improves the PUFA content consequently increasing the DHA yield (Sun, Ren, Bi, Ji, Zhao, Jiang, & Huang, 2018). Although the synergetic adaption evolves relatively robust and promising mutants, the low-efficiency in generation and enrichment of genomic mutants is the main limitation in industrial applications (Wang, Wang, et al., 2022).

2.2. Mutagenesis

Atmospheric and room temperature plasma (ARTP) (Chen et al., 2022; Liu et al., 2021), low energy ion implantation (LEII) (Fu et al., 2016), and ultra violet (UV) (Li et al., 2017) can further increase the probability of gene mutagenesis of *Schizochytrium* species (Fig. 2 and Table 1). Some exemplifications are that the DHA yields of mutants of *Schizochytrium limacinum* B4D1, *Schizochytrium* sp. S31, and *Schizochytrium limacinum* B4D1 treated with LEII, UV, and ARTP are separately increased by 61 %, 11.2 %, and 25.51 % than that of the wild strain (Chen et al., 2022; Fu et al., 2016; Li et al., 2017). Interestingly, for the same *Schizochytrium limacinum* B4D1 strain, LEII may be an ideal strategy to achieve the high DHA content in mutants. However, the high operating cost limits widespread applications of LEII in the food industry (Fu et al., 2016). ARTP functions as a compromise strategy not only in operating cost but also in flexible operation and high-efficient mutation, but it needs to combine more effective screening methods to obtain mutagenic strains with high DHA (Liu et al., 2021). For instance, *Schizochytrium* sp. PKU#Mn4 mutant strain with up to 76 % DHA content was obtained by ARTP coupling clethodim-based screening method, representing 1.2- to 6.8-fold more than that of the aforementioned LEII (*Schizochytrium* sp. S31) and UV mutagenesis (*Schizochytrium limacinum* B4D1) (Liu et al., 2021). Further, the DHA yield in *Schizochytrium* sp. SR21 mutant treated with ARTP coupling microbial microdroplet

culture (MMC) high-throughput screening method is increased by 83.2 % in comparison with wild strain, even outperforms 7.2 %–72 % than LEII, UV, ARTP coupling iodoacetic acid and clethodim-based strategies (Wang et al., 2023). Moreover, a recent report indicates that *Schizochytrium limacinum* BCD treated with electric shock can accumulate DHA by 95.51 % than wild species (Chen, Tong, Liu, et al., 2023). This further increases the possibilities of obtaining mutants with the high DHA content for industrial application. However, more efforts need to be devoted to promoting the applications of electroinduced mutagenesis in the DHA production of *Schizochytrium*. Therefore, ARTP is still the main mutagenesis strategy to obtain more robust and promising mutant strains with high DHA content in the industry at present.

2.3. Genetic engineering

Genetic engineering strategy in enhancing DHA yield of *Schizochytrium* mainly focuses on manipulating genes responsible for enhancing substrates supply, increasing the reducing power, promoting docosapentaenoic acid (DPA) conversion to DHA, and also blocking competitive pathways (Feng et al., 2023; Han et al., 2024; Liu et al., 2024; Ren et al., 2015). As illustrated in Fig. 3, manipulating genes such as ATP-citrate lyase (ACL), acetyl-CoA carboxylase (ACC), acetyl-CoA acyltransferase (AT), and malonyl-CoA: ACP transacylase (MAT) can effectively accumulate key substrates (e.g., acetyl-coenzyme A, malonyl-coenzyme A, malonyl-ACP, and enol acyl-ACP) to promote DHA biosynthesis. Han et al. (2020) find that over-expressions of ACL and ACC significantly enhance the DHA in transgenic species by 23.3 % and 41.9 % compared to wild *Schizochytrium* sp. S31 strain. Except for substrates supply, DHA biosynthesis also requires nicotinamide adenine dinucleotide phosphate (NADPH) reducing force to reduce the acetyl group to the acyl chain due to the high reducibility of PUFAs (Feng et al., 2023). Whereas the over-expression of endogenous glucose-6-phosphate dehydrogenase in *Schizochytrium* sp. H016 enhances the NADPH to result in DHA increment by 46.51 % (Feng et al., 2023). Further, an interesting case is that the increments in precursors and NADPH and the reduction in β-oxidation by multiple genes (e.g., malic enzyme (ME) and β-oxidation enzyme) manipulations in transgenic strain dramatically enhances DHA by 17.5 %–72.1 % than that of wild *Schizochytrium* sp. S31 strain and transgenic strain by sole gene manipulation (Han et al., 2024). This shows consistence with the report of Han et al. (2020) where the DHA content (48.8 %) in transgenic *Schizochytrium* sp. S31 by co-expression of ACL and ACC genes is higher than that of transformants by sole ACL (23.3 %) or ACC (41.9 %). These results indicate that the DHA content of multigene-manipulated transformant strain is higher than single gene manipulation. Certainly, a special case is that the overexpression of the sole MAT gene in *Schizochytrium* sp. MYA1381 also promotes an increment in DHA content by 81.5 % compared with wild strain (Li et al., 2018). This may be due to that MAT as a key gene directly regulates the dominant polyketide synthase (PKS) pathway for DHA synthesis (Li et al., 2018) (Fig. 3).

Actually, as indicated in Fig. 3, regulating the elongating-desaturase (e.g., Δ4-desaturase, Δ17-desaturase, and Δ5-elongase) in the FAS pathway can also achieve DHA biosynthesis (Yan et al., 2024). However, DHA biosynthesis from linoleic acid (LA, C18:2) usually experiences multiple steps, which inevitably triggers excessive accumulation of intermediate fatty acids such as DPA and γ-linolenic acid (GLA) in the FAS pathway. A recent report indicates that when the key genes responsible for DHA biosynthesis show up-regulations, only 5.6 % DHA is accumulated but intermediates are accumulated abundantly with 11.7 % LA, 30.1 % GLA, and 18.3 % DPA (Yan et al., 2024). Whereas the PKS as the more efficient pathway to biosynthesize DHA generates fewer intermediates and thus is the more efficient DHA synthesis pathway in many species. For instance, the biosynthetic gene clusters encoding polyketide synthase-like PUFA synthase from myxobacteria in the PKS pathway are overexpressed to achieve the DHA titer of 350 mg/L and account for 16.8 % in TFAs, which is 3.0-fold more that of FAS pathway. However, there are few reports on the DHA biosynthesis by regulating genes in the FAS pathway in *Schizochytrium*. This does not hinder the further investigations on key genes responsible for DHA biosynthesis in FAS pathway in *Schizochytrium* since future advanced genetic

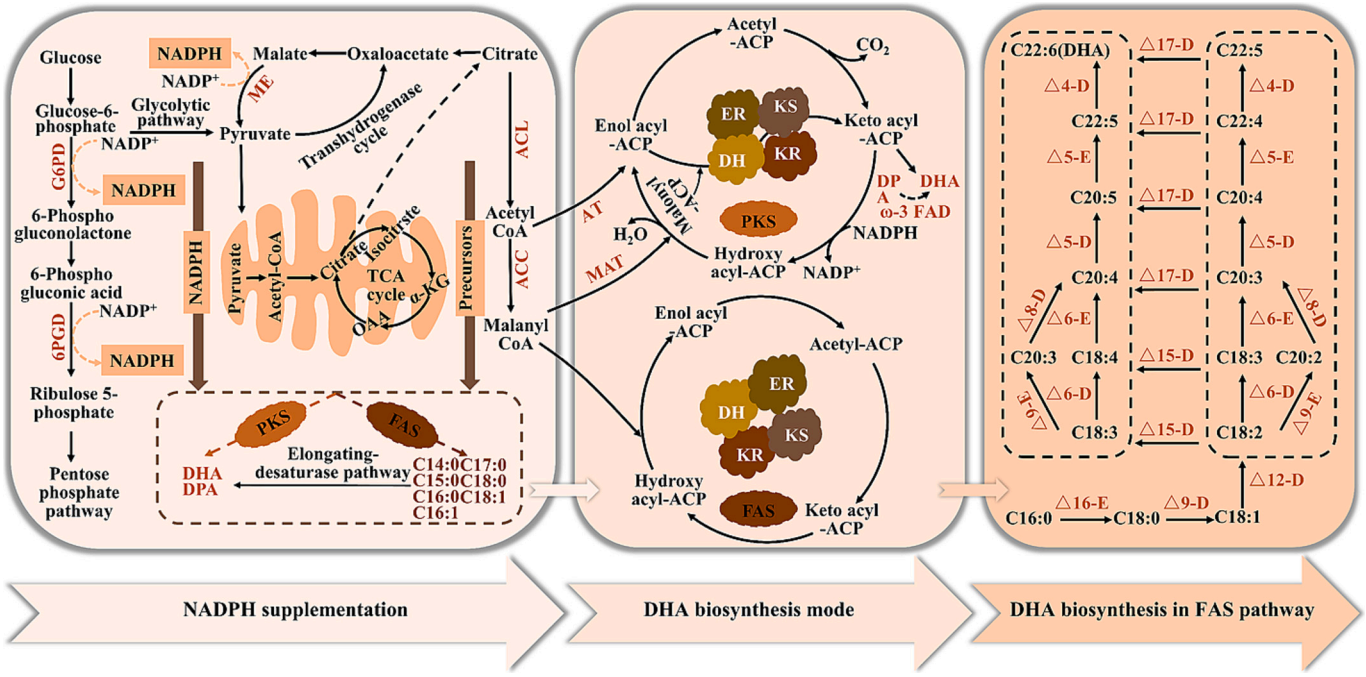

Fig. 3. The metabolic and biosynthesis modes of DHA in *Schizochytrium* cells. ACL, ACC, MAT, PKS, FAS, ω-3 FAD, G6PD, 6PGD, ME, NADPH, DPA, ER, DH, KS, and KR separately represent ATP-citrate lyase, acetyl-CoA carboxylase, and malonyl-CoA: ACP transacylase, polyketide synthase, fatty acid synthase, ω-3 fatty acid desaturase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme, nicotinamide adenine dinucleotide phosphate, docosapentaenoic acid, enoyl reductase, dehydratase, β-ketoacyl synthase, and ketoacyl reductase. Δ4-D, Δ5-D, Δ6-D, Δ8-D, Δ9-D, Δ12-D, Δ15-D, and Δ17-D separately represented Δ4-, Δ5-, Δ6-, Δ8-, Δ9-, Δ12-, Δ15-, and Δ17-desaturase. Δ5-E, Δ6-E, Δ9-E, and Δ16-E indicated Δ5-, Δ6-, Δ9-, and Δ16-elongase.

manipulations can achieve high DHA titers by using the elongating-desaturase in FAS.

Additionally, manipulating genes to block DPA biosynthesis in the PKS pathway is also a promising strategy for boosting the DHA yield of *Schizochytrium*. For instance, the genetically-modified *Schizochytrium* sp. HX-308 strains with ω-3 desaturase or ω-3 fatty acid desaturase (ω-3 FAD) overexpression separately achieve 49.23 % and 49.5 % increment in DHA content by converting 3 % DPA into DHA (Li et al., 2023; Ren et al., 2015); Li et al. (2023) report that co-overexpression of phosphopantetheinyl transferase and ω-3 FAD in *Schizochytrium* sp. HX-308 further converts DPA into DHA and the final DHA yield increases by 78.1 %. An interesting case is that whether manipulating the genes responsible for the substrates and NADPH generation or the genes in preventing DPA conversion, the synchronous operations of multiple genes are more effective in promoting DHA of transgenic *Schizochytrium*. It is worth mentioning that the other competitive pathway such as phospholipid synthesis derived from DHA is also performed inhibition investigation. Zhang, Cui, et al. (2024) find that phospholipase gene knockout increases the DHA content by 13.3 % without affecting the cell growth of the transgenic *Schizochytrium limacinum* SR21 strain. However, the promotion efficiency of DHA yield is far less than that of multiple genes operation in the DPA competitive pathway. Additionally, the recent strategies also focus on over-expressions of genes (e.g., sucrose hydrolase, xylose isomerase, and xylulose kinase) responsible for energy (e.g., propionic acid, xylose, and sucrose) utilization, which increase the DHA contents of transgenic by 30.4 %–42.69 % in comparison with the wild *Schizochytrium* sp. HX-308 strain (Ma,Zhang, et al., 2023; Wang, Zhang, et al., 2022). Although gene engineering technology can directly achieve the DHA output, the species obtained by gene manipulation are often defined as transgenic species and are controversial in practical applications. The corresponding national policies are clarified in the future to further guide the industrial production of transformed *Schizochytrium* DHA.

3. *Schizochytrium* fermentation optimizations for increasing DHA production

Schizochytrium specie improvement provides a batch of mutants with excellent performances in producing DHA. However, the subsequent applications of wild *Schizochytrium* or mutants require a high-quality fermentation process since it can determine the further DHA yield performance and production cost (Wu et al., 2005). Therefore, the factors (e.g., nutrient substrates, wastewater/wastes, chemical regulators, conditions control, and fermentation modes) affecting fermentation quality are described in Sections 3.1–3.5 and summarized in Fig. 4.

3.1. Nutrient substrates

3.1.1. Carbon sources

Carbon sources can form carbon skeletons in the lipid pathway and promote DHA accumulation in microorganisms (Chen, Zhou, Zhang, et al., 2016). However, the different carbon sources (e.g., glucose, fructose, and mannose) have disparate effects on DHA biosynthesis. Wu et al. (2005) indicate that the DHA yield of *Schizochytrium* sp. S31 with glucose (0.31 g/L) and fructose (0.25 g/L) are significantly higher than lactose, maltose, sucrose, and soluble starch. Similar results are observed in the report of Ding et al. (2022) where the DHA yields in *Schizochytrium* sp. I-F-9 by glucose (7.0 g/L) and glycerol (6.0 g/L) exceed sucrose (0.2 g/L) and maltose (0.1 g/L). The aforementioned results indicate glucose and glycerol may be the most indulgent in promoting DHA accumulation in *Schizochytrium* (Kujawska et al., 2021b). This may be due to that glucose and glycerol can directly integrate into glycolysis to provide energy and acetyl-CoA precursor while other carbon sources into glycometabolic process by collateral branch pathways consequently the DHA biosynthesis efficiency is compromised (Chen, Zhou, Zhang, et al., 2016). However, a recent report find that the lag growth phase is terminated at 72 h and the

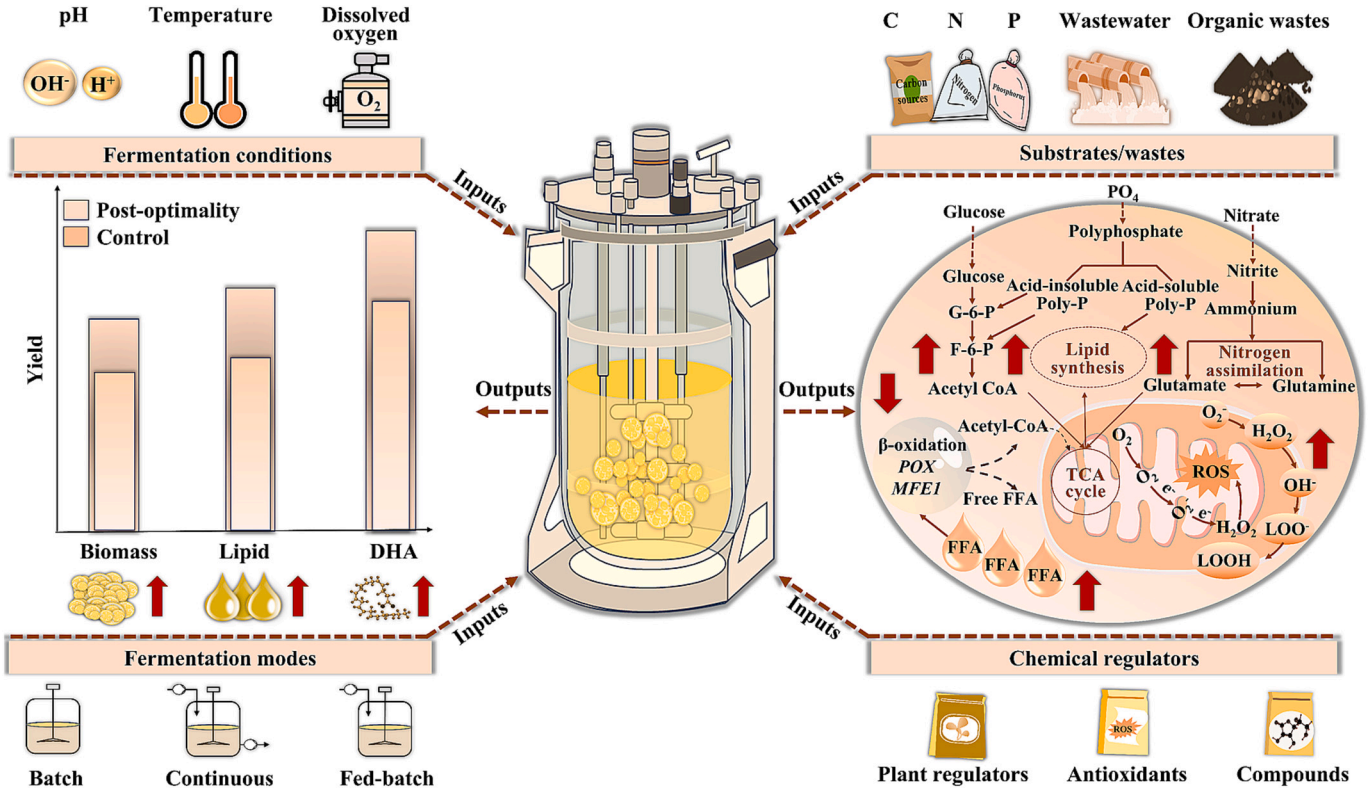

Fig. 4. Fermentation factors influencing and enhancing DHA production by *Schizochytrium*. DHA, FFA, ROS, G-6-P, F-6-P, POX, MFE, and TCA separately represent docosahexaenoic acid, free fatty acid, reactive oxygen species, glucose-6-phosphate, fructose-6-phosphate, proline oxidase, multifunctional enzyme, and tricarboxylic acid.

highest biomass at 180 h with glycerol, these periods exhibit prominent postponement in comparison with glucose at 30 and 146 h (Li et al., 2021). This suggests that glucose can effectively shorten the fermentation time course compared to glycerol. This is mainly due to that the activities of glycerol kinase and glycerol-3-phosphate dehydrogenase were inhibited by the reduced available oxygen during the cultivation process to influence the glycerol assimilation efficiency of *Schizochytrium* (Li et al., 2015). Certainly, glycerol carbon source exerts a cost advantage over glucose. Therefore, in light of the performances of glucose and glycerol, some investigators also attempt to simultaneously use both as fermentative carbon sources to further enhance DHA accumulation in *Schizochytrium* (Li et al., 2015). The DHA productivity with glucose and glycerol reaches 32.36 g/L and separately shows 4.62- and 5.93-fold more than that of sole glucose and glycerol in *Schizochytrium limacinum* SR21 (Li et al., 2015). Such high DHA yield is not only from the functions of respective carbon sources but also generates a positive synergistic effect of the mixed carbon sources (Li et al., 2021).

3.1.2. Nitrogen sources

Actually, nitrogen source also plays an important role in accumulating DHA and the different types of nitrogen sources possess discrepant effects on DHA biosynthesis efficiency (Jiang et al., 2017). The current investigations indicate that *Schizochytrium* is able to take advantage of organic nitrogen and can also utilize inorganic nitrogen to accumulate biomass and synthesize DHA (Ding et al., 2022). Wu et al. (2005) separately use tryptone, peptone, yeast extract, urea, monosodium glutamate, sodium nitrate, and ammonium chloride to achieve the DHA yield ranging from 0.08 g/L to 0.31 g/L in *Schizochytrium* sp. S31. Yeast extract may be the beneficial fermentation nitrogen source since the DHA yield represents 1.07- to 3.9-fold that of other nitrogen sources. Whereas a recent investigation shows that peptone is the most effective nitrogen source for DHA accumulation in *Schizochytrium* sp. I-F-9 even the DHA yield (6.22 g/L) represents 19.81-fold more than that of yeast extract (Ding et al., 2022). This discrepancy may be due to the different medium components, different nitrogen sources, and different *Schizochytrium* strains, the wild strain in Wu et al. (2005) and the engineering strains with superior shape in Ding et al. (2022). Additionally, nitrogen supplementation strategies are also crucial in stimulating DHA accumulation in *Schizochytrium*. A majority of literature gives such results that the DHA content doubled or several times increased by nitrogen-limitation or nitrogen-deficiency induction (Chen et al., 2024). For instance, Jia et al. (2024) use nitrogen-limiting conditions (1 g/L peptone and 1 g/L yeast extract) to achieve a maximum DHA level by 106.7 mg/L/h in *Schizochytrium* sp. S31, which is 58.1 % greater than that of the control group (10 g/L yeast extract and 2 g/L peptone). However, the nitrogen concentration is not always the lower the better. Ju et al. (2020) and Jiang et al. (2017) further find that 10–20 g/L yeast extract and 10–20 g/L monosodium glutamate instead of 5 g/L yeast extract and 5 g/L monosodium glutamate stimulates DHA accumulation and prevents cell lysis of *Schizochytrium* sp. ABC101 and *Schizochytrium* sp. ATCC 20888, the DHA yield is increased to 12.20 g/L and 19.50 g/L with this potential strategy. Thus, nitrogen type and concentration controls are crucial steps for *Schizochytrium* response to the fermentation process to accumulate DHA.

3.1.3. Phosphorus sources

Except for carbon and nitrogen substrates, phosphorus is also an essential element in fermenting *Schizochytrium* to produce DHA. Although phosphorus source types are less reported for *Schizochytrum* DHA production, the current evidence is that the phosphate limitation strategy seems to be more conducive for *Schizochytrium* DHA (Ren et al., 2013; Sun et al., 2014). As the findings in the report of Ren et al. (2013) where the maximum DHA productivity (148.3 mg/L/h) of *Schizochytrium* sp. HX-308 with 0.1 g/L KH_2PO_4 treatment is higher than the DHA productivity (86.1 mg/L/h) by 4 g/L KH_2PO_4. Not only in terms of DHA yield, the glucose and glutamate consumption rates (separately 2.0 g/L/h and 0.63 g/L/h) under 0.1 g/L KH_2PO_4 are also higher than 4.0 g/L KH_2PO_4 condition (separately 1.58 g/L/h and 0.47 g/L/h). Additionally, Sun et al. (2014) indicate that the phosphorus limitation strategy can also shorten the fermentation time of *Schizochytrium* sp. HX-308 from 56 h to 44 h and the achieved DHA productivity (291 mg/L/h) is 1.42-fold more than that of the phosphate-repletion condition. This may be due to that phosphate-limitation can maintain higher activities of ME and G6PD better than phosphate-repletion mode. This higher activity of enzymes provides more NADPH separately at the early and late stages of fermentation consequently promoting DHA production enhancement (Sun et al., 2014).

3.1.4. Salinity

Marine *Schizochytrium* usually requires a relatively high concentration of salt to meet with the growth and DHA biosynthesis except for carbon, nitrogen, and phosphorus. The previous investigation of Zhu et al. (2007) supports such fact that the relatively-high sea salt ranging from 1.8 % to 3.6 % significantly promotes the oil content from 41.34 % to 48.97 % whereas sea salt reduces from 1.8 % to 0 % decreases the oil content from 48.97 % to 30.55 % in *Schizochytrium limacinum* OUC88. Although the relatively-high salinity can induce the high DHA accumulation in *Schizochytrium*, the introduction of a large number of chloride ions inevitably leads to corrosion of the metal fermentation tank and the high downstream water treatment costs (Dong et al., 2023). Subsequently, total salinity reduction or sulfate substitutions are used to mitigate such situations. For instance, Chen, Zhou, Zhu, et al. (2016) find that 1 % sodium sulfate substitution to 2 % sea salt can achieve the increments of *Schizochytrium* sp. S056 in biomass from 34.76 to 38.93 g/L and the DHA yield by 20.27 %. However, such superior performances usually need osmoregulator supplementation to support the osmotic pressure requirements for *Schizochytrium*, which undoubtedly results in the complexity of the fermentation process. Further, Dong et al. (2023) adopt a hypo-salinity (6 g/L sea salt) stress strategy to boost DHA content in TFAs from 14.45 % to 18.28 %, representing 1.27-fold more than that of high salinity (20 g/L) in *Schizochytrium* sp. S31. The elevated DHA in *Schizochytrium* response to hypo-salinity stress may be due to the up-regulations of NADPH, acetyl-CoA, and inositol to provide energy and precursors, the down-regulation of intracellular ROS level to prevent the oxidation of PUFAs, and also the increased contents of necessary components such as glycerol osmoregulator and cell wall polysaccharides to support the osmotic pressure requirements (Dong et al., 2023). These discoveries can be valuable in developing possible low-salt processes for *Schizochytrium* fermentation to produce DHA. However, the most direct direction is to combine the aforementioned breeding technologies in Section 2 to obtain the hypo-salinity tolerant *Schizochytrium* species to produce DHA under low-salt fermentation.

3.2. Wastewater and wastes

3.2.1. Wastes as carbon sources

The aforementioned elaborations support that commercial nutrient matrixes such as glucose, yeast powder, and peptone are superior fermentation substrates for *Schizochytrium* DHA. However, the high-cost artificial substrates can limit the large-scale industrial progress for *Schizochytrium* DHA (Zhao et al., 2022). Using wastewater/wastes to produce lipids can further optimize and reduce the costs associated with fermentation medium, especially regarding nutrients as well as achieve a win-win for evading waste environmental pollution and resource recycling (Li et al., 2024). Actually, some industrial and agricultural wastes rich in nutrients have been treated as premium carbon components to serve *Schizochytrium* DHA biosynthesis. And the waste utilization can be judged from the outputs that yacon tuber enzymatic hydrolysate (YTH), bagasse hydrolysate, sugarcane molasses, crude glycerin, sweet sorghum stem juice, and starch dioscorea zingiberensis tuber hydrolysate as fermentative substrates effectively enhance the DHA yield of *Schizochytrium* sp. (Kujawska et al., 2021a, 2021b).

Thereinto, the maximum DHA yield of 10.85 g/L is achieved by *Schizochytrium* sp. S31 treated with a YTH carbon source, which is 28.67 % and 32.05 % higher than that of glucose and fructose (Zhao et al., 2022). This may be due to that YTH compared with glucose and fructose significantly enhances the activities of key lipogenic enzymes such as ACL, G6PD and ME, and therefore provides more acetyl-CoA and NADPH to promote DHA accumulation (Zhao et al., 2022). Additionally, abundant antioxidant phenolic compounds present in YTH obviously reduce the intracellular ROS accumulation and alleviate PUFAs peroxidation consequently contributing to DHA yield.

3.2.2. Wastes as nitrogen sources

Synchronously, some investigations find that wastewaters including alanine mother liquor, tofu whey wastewater, and ammonia wastewater treatment can also boost the DHA yields of *Schizochytrium* sp. strain HX-308, *Schizochytrium* sp. CCTCC M 209059, and *Schizochytrium* sp. S31 ranging from 0.24 g/L to 46.4 g/L (Chen, Yang, He, et al., 2023; Ma, Li, et al., 2023; Wang et al., 2020). Even the engineered *Schizochytrium* sp. CCTCC M 209059 strain with methylmalonyl-CoA mutase overexpression obtains the lipid yield by 64.4 g/L with propionate wastewater treatment (Ma, Li, et al., 2023). These achieved DHA yields are 7.46- to 149.68-fold more than that of traditional nitrogen nutrient matrixes such as yeast powder and peptone in Section 3.1. Not only the high-yielding DHA treated with wastes, the use of extra carbon and nitrogen source is avoided due to the rich nutrients in wastes and the total DHA cost is approximately <1/3 of that of traditional media (Wang et al., 2020). Further, some investigations find that the DHA yields by *Schizochytrium* can also receive effective increments under the combinations of different wastes (Song et al., 2015; Wang, Tian, et al., 2022; Yin, Zhu, et al., 2019). For instance, algae-residue and cane molasses result in a final DHA yield of 15.22 g/L in *Schizochytrium* sp. HX-308 (Yin, Zhang, et al., 2019); saline wastewater, tofu whey wastewater, and crude glycerol reach a DHA yield of 2.65 g/L in *Schizochytrium* sp. S31 (Wang, Tian, et al., 2022); and using maize starch hydrolysate and soybean meal hydrolysate increases the DHA yield of *Schizochytrium limacinum* OUC88 to 20.7 g/ L (Song et al., 2015). Although the current DHA yields by the combinations of different wastes are comparable and even slightly lower than that of single types of wastes, the combinations of multiple wastes can provide a great variety of nutrients and further avoid the supplementation of other components. However, the potential mechanisms in promoting DHA by the combinative wastes, the troublesome pretreatment procedures of multiple wastes, and the safety of *Schizochytrium* DHA produced with the wastes need to be on the agenda.

3.3. Chemical regulators

Except for the virtues of traditional fermentative substrates and wastes in enhancing DHA productivity, the utilization of chemical regulators (e.g., antioxidants, plant regulators, and organic compounds) also strengthens DHA enrichment in a different regulatory manner (Chen, Yang, Tong, et al., 2023). Currently, antioxidants such as sesamol, ascorbic acid, and proanthocyanidins can reduce intracellular ROS levels and prevent PUFAs oxidation consequently ensuring DHA product (Fig. 4). For instance, the DHA yield of *Schizochytrium* sp. H016 treated with 1 mM sesamol is increased by 78.30 % compared to the control (Bao et al., 2022); the utilization of ascorbic acid and proanthocyanidins strengthen the antioxidant capacity of *Schizochytrium* sp. HX-308, reduces ROS levels, and eventually increases DHA by 30.44 % and 53.4 % (Ren et al., 2017); further, Zhang, Chen, et al. (2021) evaluate the effects of seven antioxidants (ascorbic acid, alpha-tocopherol, tea extract, melatonin, mannitol, sesamol, and butylated hydroxy-toluene) on lipid accumulation and find that the interaction of mannitol and ascorbate can significantly reduce ROS levels and increase PUFAs accumulation to 1.45 g/L in *Schizochytrium* sp. PKU#Mn4.

However, some organic compounds such as inositol, norflurazon, and malic acid enhance DHA accumulation in different ways (Liu et al., 2019). The basic modes are to manipulate metabolic pathways and key genes responsible for DHA biosynthesis or enhance endogenous antioxidant capacity. Concretely, norflurazon induction enhances the antioxidant system and attenuates the competitive pathways (e.g., terpenoids, several amino acids, and TCA cycle to lead a lower ROS level and drive the carbon flux toward FA synthesis) (Bi et al., 2023). With 100 μM norflurazon supplementation, the DHA content of *Schizochytrium* sp. S31 increased 29.3 % in comparison with controls. Whereas *p*-aminobenzoic acid (*p*-ABA) can promote glycolysis, weaken the TCA cycle, and increase NADPH generation by upregulating the pentose phosphate pathway (PPP) to redirect the metabolic flux into lipid biosynthesis. *Schizochytrium limacinum* SR21 treated with 200 mg/L *p*-ABA enhance the DHA yield by 33.28 % compared with control (Li et al., 2019). Malic acid manipulation mainly causes the increased expression of genes involved in NADPH and acetyl-CoA metabolism, the TCA cycle, oxidative phosphorylation, nitrogen metabolism, and vitamin B6 metabolism to lead to an increase in the influx throughputs of fatty acids. Such malate manipulation directly increases the DHA content by 22 % during the fed-batch culture of *Schizochytrium* sp. FJU-512 (Zhang et al., 2022). The elaborations in this Section also indicate that the identified metabolic pathways or biomarkers can provide important guidelines for engineering *Schizochytrium* with high DHA content in the future.

Additionally, some plant regulators such as naphthoxyacetic acid (NAA), jasmonic acid (JA), and 6-benzylaminopurine (6-BA) by sole manipulation or combination of several regulators can also effectively contribute to DHA biosynthesis (Mehta et al., 2023). The results indicate that the effects of NAA or JA on lipid content are dose-dependent. 2.0 mg/L of NAA or 20 mg/L of JA treatment can increase the lipid contents of *Schizochytrium* sp. S31 by 11.16 % and 12.71 % in comparison with controls. Whereas the combination of 2 mg/L naphthoxyacetic acid (BNOA) and 20 mg/L JA further increases the lipid content of *Schizochytrium* sp. S31 from 11.16 % to 16.79 % (Wang et al., 2018). A similar phenomenon is also found in the report of Mehta et al. (2023) where the cooperative effect of selected concentrations (10 mg/L 6-BA and 200 mg/L sesamol) further increase the DHA productivities of *Schizochytrium* sp. MTCC5890 to 3.2 g/L/d, representing 69 % more than that of the individual addition of 6-BA or sesamol. Notably, these results provide valuable clues that multiple regulators manipulation is more beneficial for DHA accumulation than that of sole regulators. This may be due to that multiple regulators synergetic manipulation induce unsaturated fatty acids synthesis, which in turn increases cell membrane fluidity to accelerate the substrate consumption in the medium consequently providing more precursors and energy for DHA accumulation (Mehta et al., 2023). Additionally, the detailed molecular mechanisms of DHA biosynthesis in *Schizochytrium* mediated by plant regulators can be elucidated by appropriate techniques such as deep RNA sequencing. Once the differentially expressed genes or pathways of *Schizochytrium* after treated with phytohormones are revealed, synthetic biology approaches can be utilized to develop suitable chassis for DHA production (Wang et al., 2018).

3.4. Fermentation conditions

Fermentation conditions such as temperature, pH, and dissolved oxygen (DO) level are also crucial elements in influencing cell growth and DHA formation in *Schizochytrium* (Zeng et al., 2011). However, the deepening investigations find that fermentation temperature values for the highest biomass and the optimal DHA accumulation are completely-inconsistent. As observed in the report of Zeng et al. (2011) where the biomass of *Schizochytrium* sp. HX-308 increases from 62.20 g/L at 20 °C to 84.40 g/L at 30 °C and the DHA content ranges from 52 % to 36.62 % at 30 °C whereas the DHA content maintains at 48.05–51.28 % at 20 °C. The results demonstrate that this strain has a relatively higher biomass as temperature rises but has a preference for DHA synthesis at the relatively-low temperature. Similar patterns are also observed in terms

of pH and DO (Shafiq et al., 2020; Sun, Geng, Ren, Ji, Hao, Chen, & Huang, 2018). For instance, the highest biomass is achieved by 97.11 g/L at an initial pH of 7.0 while a higher DHA content of 27.39 % at pH 5.0 for *Schizochytrium* sp. HX-308 (Yin, Zhang, et al., 2019); the maximal biomass and DHA content reach 62.63 g/L and 18.69 % separately at pH 7.0 and 5.0 for *Schizochytrium* sp. AB-610 (Zhao et al., 2017); additionally, *Schizochytrium* sp. HX-308 performs the maximum biomass improvement by 49 % under adequate oxygen supply with 400 rpm agitation speed and shows the highest DHA content by 46 % under oxygen supply with 300 rpm agitation speed (Bi et al., 2018). These phenomena may be attributed to the fact that different fermentative conditions respectively target important metabolic activities which are responsible for either the growth or DHA biosynthesis, thereby achieving the optimal biomass or DHA yield (Bi et al., 2018).

However, many investigations usually use unchanging temperature, pH, and DO to cover the whole fermentation process, which ultimately results in a low DHA yield (Guo et al., 2017; Yin, Zhang, et al., 2019). Subsequently, some researchers propose fermentation condition phase controls the initial optimal cell growth condition followed by the superior DHA biosynthesis environment. Zeng et al. (2011) adopt temperature phase control by switching the temperature from 30 °C to 20 °C to achieve 5.11 g/L of DHA in *Schizochytrium* sp. HX-308, representing 28 % more than that of the uncontrolled temperature group; using the two-stage pH control strategy, Yin, Zhang, et al. (2019) and Zhao et al. (2017) promote the DHA yield of *Schizochytrium* sp. HX-308 to 25.85 g/L and 11.44 g/L, which show 19.7 % and 22.2 % higher than that of the uncontrolled pH group; additionally, Guo et al. (2017) employ a three-stage oxygen manipulation strategy to increase the DHA yield of *Schizochytrium* sp. HX-308 by 44.3 g/L, representing 83.77 % more than that of control. Such significant enhancement of DHA yield response to fermentation phase control lies in the up-regulation of genes (e.g., hexokinase and phosphofructokinase) responsible for PPP and branched-chain amino acid degradation and also down-regulation of FAS and ME to provide more intermediates into PKS pathway (Hu et al., 2020). Meanwhile, such phase control strategy also reduces intracellular ROS accumulation to prevent lipids from oxidizing consequently achieving the maximum DHA yield of *Schizochytrium* (Jia et al., 2023) (Fig. 4). Even though the stage manipulations of fermentation course achieve the breakthrough in DHA productivity, such strategy needs extra energy and cost input to ensure the fermentative system within the appropriate condition range for each stage. For instance, in large-scale outdoor reactors, the radiation from the sun is intense and additional cooling systems are required to prevent overheating when low-temperature manipulation is performed (Hu et al., 2021).

3.5. Fermentation modes

3.5.1. Batch fermentation mode

Fermentative optimization of substrates and conditions gives a guarantee for the high-density growth of *Schizochytrium* (Chi et al., 2022). However, further increments in DHA also need the involvement of the applicable fermentation modes (Fig. 4). A recent investigation finds that traditional batch fermentation mode performs the characteristics of inhibiting the growth and DHA biosynthesis of *Schizochytrium* due to one-time supplementation of the highly-concentrated substrates (Qu et al., 2013). The typical case is that the DHA yield of *Schizochytrium* sp. HX-308 is increased from 4.33 g/L to 8.84 g/L as one-time supplementation glucose at 60 to 100 g/L whereas the DHA productivity is decreased to 8.34 g/L with one-time supplementation glucose at 120 g/L. This inhibitory effect can be explained as that the high-concentration glucose increases the intracellular ATP concentration and causes enzyme biosynthesis repression consequently resulting in slower metabolization of energy source (Qu et al., 2013).

3.5.2. Fed-batch fermentation mode

In comparison with the batch fermentation mode, fed-batch fermentation can realize nutrient supplementation at a certain time interval and achieve a further increase in DHA production. Therefore, some investigators adopt the fed-batch fermentation mode as the first-line selection for *Schizochytrium* DHA industrialization. Some exemplifications include 1) DHA yield of *Schizochytrium* sp. S31 is increased from 5.39 g/L to 12.24 g/L with fed-batch fermentation (Chang et al., 2020); 2) the control of the total sugar concentration to approximate 20 g/L at a constant feeding rate under fed-batch fermentation mode promotes the ultimate DHA yield of *Schizochytrium* sp. HX-308 to 25.25 g/L (Ma, Zhang, et al., 2023); 3) the fed-batch fermentation by replacing part of the mature culture with fresh medium not only saves seed culture, inoculation, and sterilization time between each fermentation cycle but also results in DHA productivity of *Schizochytrium* sp. HX-308 by 132.8 mg/L/h (Qu et al., 2013). However, the fluctuations of the fermentation system caused by substrate supplementation need to be monitored in the whole fermentation process to ensure the stability of the fermentation parameters.

3.5.3. Continuous fermentation mode

Further, the continuous fermentation mode possesses the potential to evade the growth inhibitory effects and feed fluctuations caused by batch and fed-batch modes since constant fresh media supplementation substitutes the mature culture liquid (Henley, 2019). However, Ethier et al. (2011) find that the achieved biomass (11.78 g/L) and DHA yield (1.74 g/L) of *Schizochytrium limacinum* SR21with continuous fermentation mode are less than that of batch (18.04 g/L and 3.07 g/L) and fed-batch (37.9 g/L and 6.56 g/L) fermentation modes. Whereas the similar conclusions are also found in the previous report where the achieved biomass (8.0 g/L) and DHA yield (40 mg/L/h) of *Schizochytrium* sp. strain G13/2S show 1.875-fold and 1.25-fold less than that of batch fermentation with glucose and glutamate as supplements (Ganuza & Izquierdo, 2007). Such continuous fermentation needs the precision fermentation element investment and is easily contaminated by miscellaneous bacteria. Additionally, the other limitations of continuous fermentation mode are reflected in algae that tend to float on the surface, sink to the bottom, or adhere to surfaces that are unsuitable to perform continuous culture (Henley, 2019). Therefore, it is premature to dismiss continuous fermentation mode as impracticable or to assert it as suitable or superior.

4. *Schizochytrium* dry biomass preparation and DHA extraction

4.1. Schizochytrium cells harvest

The upstream species improvement and fermentation optimization strategies fulfill the high biomass and DHA yield for *Schizochytrium* (Chi et al., 2022). The downstream applications of *Schizochytrium* DHA products involve cell harvest, dry biomass preparation, and DHA oil extraction. The most common harvesting methods are categorized into centrifugation, sedimentation, flotation, or filtration (Zhu et al., 2024). Actually, biomass harvesting is the initial challenge for products since harvesting manipulation contributes to circa 20 to 30 % of the total cost of production. Whereas high-cost energy consumption such as centrifugation consumes about 8 kWh/m^3 is a major limiting factor in harvesting manipulation. Currently, few studies or reviews comprehensively summary the biomass harvesting strategies of *Schizochytrium*, but technological challenges do not prevent studies from *Schizochytrium* culture to low-cost cell harvest since these manipulations have already been well addressed in other microorganisms species. For instance, de Souza Celente et al. (2024) propose a two-step separation strategy, which comprises concentrating the biomass with coagulation/flocculation, gravity sedimentation, flotation, or electrical-based methods to about 2–7 % of total suspended solids (TSS) followed by dewatering with filtration or centrifugation to further concentrate algae slurry to 15–25 % of TSS; another two scenarios composed of the first centrifugation and the second membrane filtration for harvesting

biomass separately reduce 52 % and 45 % in the total cost of ownership and energy (de Souza Celente et al., 2024); additionally, the dissolved air floatation combined with pH modulation achieve >90 % recovery efficiency for cell biomass (de Souza Celente et al., 2024). The aforementioned achievements can provide consults in improving the centrifugation harvest strategy for *Schizochytrium* biomass. Additionally, the appropriate method also considers the characteristics of *Schizochytrium* and target products.

4.2. Schizochytrium cells desiccation and disruption

Once the *Schizochytrium* cells are efficiently harvested, the subsequent stage is to perform desiccation for the wet cells to obtain dry biomass. Currently, wet cell treatment is commonly divided into spray drying, oven/air-drying, and freeze-drying (Sales et al., 2022). Although the energy cost is outputted, freeze-drying is considered a promising strategy for *Schizochytrium* dry biomass preparation since it can better maintain cellular integrity and intracellular components (Min et al., 2022). On the other hand, the cell wall of *Schizochytrium* is mainly composed of cellulose and is very durable and difficult to break (Lin et al., 2018). Whereas the cell wall breaking directly determines the DHA extraction efficiency (Nagappan et al., 2019). A series of cell disruption methods including physical (e.g., microwave, ultrasonic, and homogenization), chemical (e.g., acid heat and alkali), and enzymatic hydrolysis are widely reported (Byreddy et al., 2016). Amongst these strategies, cell disruption with the enzymatic method might be a promising way since it can avoid the need for mechanical equipment and energy input by physical disruption as well as the FAs destruction by chemical disruption. A previous report indicates that the oil extraction rate reached 63 % under the optimal cell wall-breaking efficiency with enzyme hydrolysis, which shows 1.47- to 2.86-fold improvements than that of acid digestion and solvent digestion (Lin et al., 2018). Further, hemicellulase used for cell disruption of *Schizochytrium* sp. achieves the most effective cell wall-breaking than that of acid treatment with HCl, osmotic shock, and ultrasonic homogenizer (Isa et al., 2022). And such a cell wall-breaking method with enzyme hydrolysis is high-efficiency, environmentally-friendly, non-toxic, and industrial-scale feasible and can be used as a recommendation in future studies.

4.3. Schizochytrium DHA extraction

Subsequently, multifarious extraction and purification strategies are developed to acquire DHA. Traditional organic reagents (e.g., hexane, chloroform, butanol, ethanol, and methanol) play important roles in DHA extraction due to the advantages of high extraction efficiency and low peroxide value (Isa et al., 2022). However, the used reagents for DHA extraction are usually flammable or toxic and exists safety hazards. The utilization of organic reagents may also negatively affect the nutritional and functional properties of the extracted DHA and human health (Nagappan et al., 2019). Additionally, centrifugation and solvent extract account for >90 % of energy consumption for *Schizochytrium* products and chemical disruption easily triggers the FAs destruction (de Souza Celente et al., 2024; Isa et al., 2022). Supercritical fluid extraction (SFE) is a widely considered green extraction technology and has been employed for DHA (Zinnai et al., 2016). A recent report found that the lipid extraction efficiency of *Schizochytrium* sp. can reach 76 % by supercritical fluid extraction technology and 17.51 g DHA/100 g dry biomass was obtained (Rodríguez-España et al., 2022). Additionally, SFE extraction technology is comparable with *n*-hexane in the theoretical process yield and the FA composition of the extracts but the extraction rate is much faster than that of *n*-hexane. For instance, when working under the hardest SFE conditions (55 °C and 70 MPa), the rate at the beginning of the extraction is about 20-fold higher than that measured when performing the extraction with *n*-hexane (Zinnai et al., 2016). SFE also offers a negligible environmental impact than *n*-hexane. Therefore, SFE strategy seems to be suitable for DHA extraction even if more generalization information are needed.

4.4. Schizochytrium DHA purification

Generally, the extracted oil contains other impurities and targets DHA to require further enrichment and purification. Some researchers propose urea adduct formation, low-temperature solvent crystallization, molecular distillation, and preparative high-performance liquid chromatography (HPLC) to increase DHA purity (Oh et al., 2020). Amongst these strategies, the urea adduct formation method is the most cost-effective, but it can produce carcinogenic alkyl carbamates and also perform relatively-lower DHA purification efficiency (Oh et al., 2020). Another low-temperature solvent crystallization can increase DHA purity to 62.46 % by optimizing fractionation temperature, solvent type, and free fatty acids/solvent ratio. However, the disadvantage of this method is that the operation process may be more cumbersome due to multiple parameter optimizations (Mu et al., 2016). Molecular distillation can further achieve the DHA enrichment ratio of 92.98 %, but this strategy for impurities removal requires high temperature under vacuum, which creates the risk of oxidation, polymerization, and trans-isomers of n-3 FAs (Zhang et al., 2013). These indicate that the chromatographic method especially HPLC may be suitable for obtaining pharmaceutical levels of high-purity DHA. For instance, Oh et al. (2020) optimize the HPLC process to result in the purity of DHA by 98.5 % and is further scaled up to a preparative column to achieve a 99.0 % DHA fraction. However, no consensus as to which strategy is preferable in the industrial production process. The safer, greener, and high-efficiency technology should be developed for high-quality DHA to approach people's nutrition and health.

5. Safety and stability of *Schizochytrium* DHA products

5.1. Safety assessments

The safety of *Schizochytrium* DHA products can guarantee further applications in improving the quality of animal-origin food raw materials and strengthening the nutritional function of foods (Sidari & Tofalo, 2019). Currently, *Schizochytriu*m dry biomass rich in DHA has been licensed as animal feed or feed additive in aquaculture and animal husbandry in many countries (Sidari & Tofalo, 2019; Thakur et al., 2024). This indicates that *Schizochytriu*m dry biomasses are safe and guarantee the source. Whereas the applications of *Schizochytrium* DHA oil as edible additives, supplements, or fortifications in foods focus evaluations on toxicity, genetics, biosafety, reproduction, and allergy (Qiu et al., 2021; Turck et al., 2020; Turck et al., 2021a, 2021b). The assessments present beneficial effects, including 1) it is safe under the proposed service conditions by marine biotoxins level, acute, subchronic, genotoxic, and allergenicity symptom indexes (Qiu et al., 2021; Turck et al., 2020; Turck et al., 2021b); 2) there are no observed adverse effects on gene mutations, clinical signs, body weight, food consumption, and also reproductive performances (Falk et al., 2017; Fedorova-Dahms et al., 2011); 3) it does not affect estrus cycles, litter size, sex ratio, offspring viability indices, and the physical development of animals (Hammond et al., 2002; Hammond, Mayhew, Holson, et al., 2001; Hammond, Mayhew, Robinson, et al., 2001); 4) it also does not produce significant changes in physical, physiological, biochemical, hematological, and histopathological parameters in any of the doses used in the 28-day or 90-day repeated toxicity study in rats (Lewis et al., 2016); 5) it is also safe as food supplements (e.g., infant formula) at the maximum intake level of 1 g DHA/day with 28-day dietary study by using Sprague–Dawley suckling rats (Qiu et al., 2021; Turck et al., 2021a). Further, the European Food Safety Authority's Nutrition, Novel Foods and Food Allergens (NDA) Group also indicate that DHA oils from several *Schizochytrium* species (i.e., FCC-3204, CABIO-A-2, and WZU477) are safety and can be included in infants, adults, pregnant women, and children formula (Thakur et al., 2024; Turck et al., 2023). These evaluations built

great confidence in promoting the further applications of *Schizochytrium* DHA products in the food field.

5.2. Stability maintenance

The stability maintenance of *Schizochytrium* DHA oil is another overriding concern since DHA is extremely susceptible to oxidation consequently affecting oil quality and applications (Perez-Velazquez et al., 2019; Shen et al., 2021; Wang, Ossemond, et al., 2022). Recent investigations showed that microencapsulation technology can preserve the stability of DHA oil to a great extent (Chen, Wang, Zhang, et al., 2016). For instance, the peroxide values of DHA-rich oil and DHA-rich oil encapsulated with casein, glucose, and lactose hybrid material are 52.57 meq/kg and 50 meq/kg during storage separately at 4 °C and 45 °C, confirming that the microcapsule products exhibit efficient heat-resistance and protect oil quality (Chen, Wang, Zhang, et al., 2016); the DHA oil microcapsules encapsulated with the mixture of maltodextrin and alginate are exposed to a temperature increase (80 °C) before recording the fourier transform infrared spectroscopy spectra after 24 and 48 h, the spectra of the maltodextrin/alginate do not change even after 48 h of exposure, suggesting that the DHA oil is not degraded and can be well preserved (Arevalo-Gallegos et al., 2023); further, the peroxide value of the encapsulated DHA oil ranging from 0.73 mmol/kg to 7.27 mmol/kg during storage from 0 d to 35 d is far below the free oil, indicating that the copolymer of chitosan and modified starch yield more oxidative stable microcapsules (Mu et al., 2022). Such oxidation inhibition performances are also superior to that of the aforementioned hybrid material composed of casein, glucose, and lactose as well as the

Table 2
Applications of *Schizochytrium*-based DHA products in foods and raw-food material.

	Application object	Addition strategies	Foods and raw-food material quality improvement	References
Aquatic products	Atlantic salmon	3–9 % *Szhizochytrium* DHA oil substitute to fish oil	95–98 % increase in apparent digestibility of DHA	Tibbetts et al. (2020)
	European sea bass	5–10 % *Schizochytrium* powder supplementation into dietary	0.43- to 0.85-fold increase in DHA content in fish fillets	Terova et al. (2021)
	Hybrid striped bass	10–50 % *Schizochytrium* powder substitute to fish oil	1.29-fold increase in DHA content in whole-body of fish	Perez-Velazquez et al. (2019)
	Sablefish	4–12 % *Schizochytrium* powder substitute to fish oil	1.13-fold increase in DHA content in fish fillets	Neylan et al. (2024)
	Gilthead seabream	50 % *Schizochytrium* powder substitute to fish oil	36.64–95.42 % increase in n-6 PUFA in muscle	Karapanagiotidis et al. (2022)
	Nile tilapia	30 g/kg *Schizochytrium* powder supplementation into diets	3.38 % increase in DHA content in fillets	Jorge et al. (2022)
	Atlantic salmon	29.83 % *Schizochytrium* powder supplementation into diets	n-3 LC-PUFA content increase in fillet	Hart et al. (2021)
	Gilthead seabream	33 % *Schizochytrium* power substitute to fish oil	0.7 g increase in DHA content in 100 g filet	Ferreira et al. (2022)
	Gilthead sea bream	6 % *Schizochytrium* powder supplementation into diets	0.92- to 1.95-fol increase in DHA content in larvae	Carvalho et al. (2022)
	Female rainbow trout	6.9 % *Schizochytrium* powder supplementation into diets	Maintaining reproductive performance, egg quality, and fry survival	Cardona et al. (2022)
	Nile tilapia	0.5 % *Schizochytrium* powder supplementation into diet	2.14-fold increase in DHA content in muscle	Ibrahim et al. (2022)
Livestock and poultry	Milk calves	5–40 g *Schizochytrium* powder addition per calf per day	Improving growth performance and antioxidant capacity	La et al. (2021)
	Ewes	20–40 g *Schizochytrium* powder/eve/day	1.23 % increase in DHA content in milk	Zisis et al. (2022)
	Female goats	15–35 g *Schizochytrium* powder/female goat/day	4.32–5.93 times increase in DHA content in milk	Zhu et al. (2022)
	Cows	50–150 g *Schizochytrium* powder per cow per day	0.37 g DHA in 100 g milk fat	Till et al. (2019)
	Milk goats	10 g *Schizochytrium* powder/goat/day	0.32 g DHA in 100 g milk	Pajor et al. (2021)
	Goats	5 g *Schizochytrium* powder addition per goat per day	0.24 g DHA in 100 g milk and cheese	Pajor et al. (2023)
	Goats	20–60 g *Schizochytrium* powder/goat/day	Inhibiting methanogens in rumen fluid	Mavrommatis, Sotirakoglou, Skliros, et al. (2021)
	Milk goats	20–40 g *Schizochytrium* powder supplementation	1.35 % increase in DHA content in milk	Mavrommatis, Sotirakoglou, Kamilaris, and Tsiplakou (2021)
	Goats	10 g *Schizochytrium* powder/goat/day	0.18 and 0.34 g DHA and rumenic fatty acid content in 100 g cheese	Bodnár et al. (2023)
	Laying hens	2% *Schizochytrium* powder supplementation into diets	n-6/n-3 fatty acids ratio decrease from 11.7 to 3.61	Wang et al. (2017)
	Laying hens	0.5–1.5 % *Schizochytrium* powder supplementation into diets	n-6/n-3 PUFAs ratio decrease from 4.63 to 3.58 in egg yolks	Kralik et al. (2020)
	Broilers	2% *Schizochytrium* powder supplementation into diets	n-6/n-3 PUFA ratio decrease by 68 %	Jeon et al. (2022)
	Pigs	5.0–7.0 % *Schizochytrium* powder supplementation into diets	2.0-fold increase in lipid containing ≥5 double bonds in pig muscles	Dannenberger et al. (2022)
Foods	Healthy participants	200 mg/capsule *Schizochytrium* DHA oil	Human plasma DHA levels by algal oil capsules surpass fish oil	Ryan and Symington (2015)
	Pork loins	1.46 mg *Schizochytrium* DHA oil/1 g fresh pork loins	n-6/n-3 PUFA ratio increase from 5:1 to 1.7:1 in pork	Meadus et al. (2013)
	Bread	50 mg *Schizochytrium* DHA oil/slice of bread	Imbue bread with PUFAs and better crumb texture	Serna-Saldivar et al. (2006)
	Vegetable puree	80 mg *Schizochytrium* powder/100 g vegetable puree	80 mg /100 g n-3-LC-PUFA in vegetable puree	Gheysen et al. (2020)
	Dry fermented sausages	15–25 % *Schizochytrium* oil substitution to pork backfat	1.30 g/100 g product of DHA in sausages	Valencia et al. (2007)

mixture of maltodextrin and alginate. This may be ascribed to that the cationic characteristic of chitosan facilitates it to chelate pro-oxidant metal ions and the emulsifying property of modified starch helps induce a high microencapsulation efficiency of the microcapsules. Further, the combination of chitosan with modified starch produces a thick layer that can protect the oil droplets against oxidation during storage (Mu et al., 2022). Totally, the microencapsulation manipulation of DHA oil may be a promising strategy in improving oxidation stability and extending the quality guarantee period, which will be beneficial for fortified food formulations. However, it is also necessary to further explore new-style, low-cost, and high-efficient microencapsulated wall materials.

6. Applications of *Schizochytrium* DHA products in foods and food raw material

6.1. Improving aquatic food raw material quality

Schizochytrium dry biomass rich in DHA as potential substitutes to traditional aquatic feed fish oil, fishmeal, and vegetable oils not only can increase PUFAs level in tissues but also bring positive effects in antioxidant ability, immune response, disease resistance, and the fish seedlings quality (Ibrahim et al., 2022; Neylan et al., 2024) (Table 2). *Schizochytrium* supplementation into diets of female rainbow trout generates beneficial results: 1) the fry survival shows 0.48-fold upregulation; 2) eggs exhibit greater integrity illustrated by fewer white eggs after hydration to indicate better overall egg quality; 3) the cholesterol and long-chain PUFAs are enhanced in the progeny of female rainbow trout (Cardona et al., 2022). For hybrid striped bass, the percent weight gain increased from 209.5 to 235.3 %, the largest protein efficiency ratio and protein retention reach 2.70 g/g and 53.1 %, and the DHA progressively increases from 5.8 % to 13.3 % of FA in whole-body of fish fed the diet with 30 % *Schizochytrium* biomass replacement (Perez-Velazquez et al., 2019). Additionally, as summarized in Table 2, *Schizochytrium* powder as a supplement to the diet also promotes growth performances, meat quality, and DHA enrichment in tissues of juvenile Atlantic salmon, European sea bass, sablefish, Nile tilapia, Atlantic salmon, and gilthead sea bream. Even the DHA level in TFAs of gilthead sea bream larvae treated with *Schizochytrium* powder is increased to 26.78 %, which is 1.95-fold higher than that of control (Carvalho et al., 2022). These aquatic animals fed with *Schizochytrium* dry biomass rich in DHA are high-quality food resources to ultimately benefit consumers.

6.2. Improving livestock and poultry food raw material quality

Schizchytrium products are also superior feed supplements in establishing favorable influences on immunity and anti-inflammation of livestock and poultry (La et al., 2021; Mavrommatis, Sotirakoglou, Skliros, et al., 2021; Pajor et al., 2021) (Table 2). An investigation finds that a diet supplemented with *Schizchytrium* dry biomass rich in DHA reduces mean somatic cell count and udder pathogens by 5.34 log cells/mL and 10 % to greatly improve the udder health of goats (Pajor et al., 2021). While in milk calves, the propionate in the rumen is decreased from 22.46 to 17.92 mmol/L as well as the activities of catalase and glutathione peroxidase are increased from 4.16 to 11.52 U/mL and 139.33 to 180.18 mmol/L to enhance antioxidant capacity (La et al., 2021). Moreover, *Schizchytrium* dry biomass rich in DHA supplementation promotes PUFAs and flavor enrichment in tissues, meat, milk, and eggs of livestock and poultry. For instance, *Schizochytrium* supplementation into the diet of livestock and poultry, the DHA increased by 1.4 % in chicken thigh meat, an almost 2.0-fold higher number of lipid species containing $\geq$5 double bonds in pig muscles, and an 11.4-fold increment in DHA content in male Qaidamford cattle are observed (Dannenberger et al., 2022; Xu et al., 2021). Not only that, the contents of DHA in goat's milk, milk of ewes, goat cheese, and cow cheese separately reach 0.43 %, 1.06 %, 0.24 g/100 g of FA, and 0.29 g/100 g FA, which show increasing rates of 0.0 % to 158 % in comparison without *Schizochytrium* supplementation groups (Pajor et al., 2023; Till et al., 2019; Zhu et al., 2022; Zisis et al., 2022). *Schizochytrium* supplementation also enhances the DHA content in egg yolks without adverse effects on production performance and egg quality of hens or laying hens (Wang et al., 2017). In terms of flavor enrichment, the sensory scores of sustained juiciness, flavor intensity, and sustained tenderness are 5.94–6.50 in male Qaidamford cattle by *Schizochytrium* supplementation, which is significantly higher than that of control (4.94–5.63) (Xu et al., 2021). Collectively, *Schizochchytrium* DHA product supplementation can achieve health performances and DHA enrichment and provide humans with high-quality animal-derived foods and multiple feasible DHA sources.

6.3. Endowing foods with multiple nutrition and functionality

Schizochytrium DHA products can also impart foods with some particular nutritional and functional properties (Gheysen et al., 2020). When pork loin with 3.1 % DHA injection experiences cooking, the DHA content increases from 1.16 to 1.46 mg, this meets over half the adult human recommended daily requirements for DHA and reduces plasma triglycerides (Meadus et al., 2013). Using 25 % DHA oil as an emulsion instead of pork backfat supplies 1.34 g DHA/100 g sausage in dry-fermented sausage products, which is notably higher than 0.04 g/100 g sausage of control (Valencia et al., 2007). Moreover, DHA oil is fused by gel or microcapsule and supplemented into yogurt to present the average peroxide and p-anisidine values of 6.83 mmol O_2/kg of oil and 117.95 abs/g, which not only are comparable to 7.17 mmol O_2/kg of oil and 118.85 abs/g with tocopherol as antioxidant but also give superior nutritional values (Zhang & Akoh, 2023). These encouraging findings can further guide the incorporation of *Schizochytrium* DHA products into foods. For example, the fortified bread containing 25 mg or 50 mg DHA/slice with *Schizochytrium* biomass supplementation and the breads show better crumb texture, adequate baking properties, high storage stability, sensory acceptability, and nutritional value (Serna-Saldivar et al., 2006); vegetable purees can reach a concentration of 80 mg n-3-LC-PUFA/100 g puree by *Schizochytrium* biomass, which provides another way to intensify DHA supplementation (Gheysen et al., 2020). The other applications of *Schizochytrium* DHA oil are specific in soy protein bars, processed vegetable drinks, hard and soft candies, non-dairy and cream powder substitutes, jams and jellies, milk and flavored milk, and soy milk (Sidari & Tofalo, 2019). Additionally, *Schizochytrium* DHA oil can be developed as capsules to fortify DHA requirement for people (Ryan & Symington, 2015). However, it needs to concern the stability and odor of foods caused by *Schizchytrium* dry biomass rich in DHA or DHA oil supplementation. The applicable food processing and preservation technologies should also participate in the utilization of *Schizchytrium* DHA oil.

7. Concluding remarks and future perspectives

Schizochytrium DHA offers new dimensions to meet demand as a nutritional and functional additive in food and health products. Concerted research has strengthened the understanding of the entire process from yield improvements to product application of *Schizochytrium* DHA. However, challenges such as unstable productivity, low extraction efficiency, sensitivity to oxidation, and potential applications still need further contributions or efforts to shorten the gap between *Schizochytrium* DHA and commercial reality. Therefore, the following suggestive observations are formed: 1) Developing the combinations of multiple breeding technologies (e.g., gene engineering and mutagenesis) to evade sole induction strategy DHA accumulation amount limits and improve DHA biosynthesis performances of the *Schizochytrium* strain. Furthermore, breeding technologies coupling waste utilization on one hand can improve the utilization efficacy of wastes and achieve resource recycling on the other hand save *Schizochytrium* DHA cost; 2)

Recent strides in artificial intelligence and machine learning can participate in strain selection and fermentative parameters optimization of *Schizochytrium* DHA production. For instance, artificial intelligence aids in potential *Schizochytrium* strain selection and fermentative nutrient optimization whilst machine learning models enhance fermentative process efficiency and nutrient utilization consequently enhancing the strain performances and fermentation process sustainability; 3) Anchoring crucial genes or transcription factors responsible for preventing DHA oxidation as well as targeting genes or metabolic pathways responsible for characteristic flavor substances biosynthesis in *Schizochytrium*, and further combining gene and metabolic engineering collectively manipulate these factors to boost DHA yield and circumvent the odor defect of *Schizochytrium* products. Another attempt is to cover up the odor defect of DHA oil by combining foods with different flavor characteristics; 4) Except for DHA oil parceled by traditional gel and microcapsule, the other forms such as nanoparticles, liposome encapsulation, and porous polymer can be tested in *Schizochytrium* DHA oil to further improve oxidation stability and utilization efficiency; 5) Constructing the new-type medium- and long-chain structured lipids with *Schizochytrium* DHA oil by synthetic biology means can exploit some unknown nutrition and functions to further promote application values in food (e.g., infant powder) and medicine (e.g., pancreatic lipase deficiency, bile salt deficiency cardiovascular, and cerebrovascular diseases) fields.

CRediT authorship contribution statement

Zongfan Peng: Writing – original draft, Visualization, Investigation. **Liang Zhong:** Software, Formal analysis, Data curation. **Yuqin Li:** Writing – review & editing, Supervision, Conceptualization. **Siran Feng:** Writing – review & editing. **Jinhua Mou:** Writing – review & editing. **Yahui Miao:** Writing – review & editing. **Carol Sze Ki Lin:** Writing – review & editing. **Zhenyao Wang:** Writing – review & editing, Supervision, Data curation. **Xuan Li:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work is supported by the Natural Science Foundation of Hunan Province (2024JJ7548) and the National Natural Science Foundation of China (21676228).

Data availability

Data will be made available on request.

References

Arevalo-Gallegos, A., Cuellar-Bermudez, S. P., Melchor-Martinez, E. M., Iqbal, H. M. N., & Parra-Saldivar, R. (2023). Comparison of alginate mixtures as wall materials of *Schizochytrium* oil microcapsules formed by coaxial electrospray. *Polymers, 15*. https://doi.org/10.3390/polym15122756. Article 2756.

Bao, Z. D., Zhu, Y. M., Feng, Y. M., Zhang, K., Zhang, M., Wang, Z. K., & Yu, L. J. (2022). Enhancement of lipid accumulation and docosahexaenoic acid synthesis in *Schizochytrium* sp. H016 by exogenous supplementation of sesamol. *Bioresource Technology, 345*, Article 126527. https://doi.org/10.1016/j.biortech.2021.126527

Bi, Y. L., Guo, P. F., Zeng, L., Dong, L., Chen, L., & Zhang, W. W. (2023). Deciphering and regulating carotenoid synthesis using norflurazon to enhance fatty acid synthesis in oleaginous marine protist *Schizochytrium* sp. S31. *Industrial Crops and Products, 202*, Article 116880. https://doi.org/10.1016/j.indcrop.2023.116880

Bi, Z. Q., Ren, L. J., Hu, X. C., Sun, X. M., Zhu, S. Y., Ji, X. J., & Huang, H. (2018). Transcriptome and gene expression analysis of docosahexaenoic acid producer *Schizochytrium* sp. under different oxygen supply conditions. *Biotechnology for Biofuels, 11*. https://doi.org/10.1186/s13068-018-1250-5. Article 249.

Bodnár, Á., Egerszegi, I., Póti, P., Kuchtik, J., & Pajor, F. (2023). Influence of marine algae (*Schizochytrium* sp.) supplementation, ripening and vacuum packaging on goat cheese composition and fatty acid profile. *Small Ruminant Research, 226*, Article 107058. https://doi.org/10.1016/j.smallrumres.2023.107058

Byreddy, A. R., Barrow, C. J., & Puri, M. (2016). Bead milling for lipid recovery from thraustochytrid cells and selective hydrolysis of *Schizochytrium* DT3 oil using lipase. *Bioresource Technology, 200*, 464–469. https://doi.org/10.1016/j.biortech.2015.10.019

Cardona, E., Segret, E., Cachelou, Y., Vanderesse, T., Larroquet, L., Hermann, A., Surget, A., Corraze, G., Cachelou, F., Bobe, J., & Skiba-Cassy, S. (2022). Effect of micro-algae *Schizochytrium* sp. supplementation in plant diet on reproduction of female rainbow trout (*Oncorhynchus mykiss*): Maternal programming impact of progeny. *Journal of Animal Science and Biotechnology, 13*. https://doi.org/10.1186/s40104-022-00680-9. Article 33.

Carvalho, M., Marotta, B., Xu, H. L., Geraert, P. A., Kaushik, S., Montero, D., & Izquierdo, M. (2022). Complete replacement of fish oil by three microalgal products rich in n-3 long-chain polyunsaturated fatty acids in early weaning microdiets for gilthead sea bream (*Sparus aurata*). *Aquaculture, 558*, Article 738354. https://doi.org/10.1016/j.aquaculture.2022.738354

Chang, M., Zhang, T., Guo, X., Liu, Y., Liu, R. J., Jin, Q. Z., & Wang, X. G. (2020). Optimization of cultivation conditions for efficient production of carotenoid-rich DHA oil by *Schizochytrium* sp. S31. *Process Biochemistry, 94*, 190–197. https://doi.org/10.1016/j.procbio.2020.04.007

Chen, D., Chen, J., Dai, R. C., Zheng, X. H., Han, Y. Y., Chen, Y. Q., & Xue, T. (2024). Integration analysis of ATAC-seq and RNA-seq provides insight into fatty acid biosynthesis in *Schizochytrium limacinum* under nitrogen limitation stress. *BMC Genomics, 25*. https://doi.org/10.1186/s12864-024-10043-5. Article 141.

Chen, L. M., Liu, X. M., Li, C. F., Li, H. C., Chen, W. X., & Li, D. M. (2022). Transcriptome analyses reveal the DHA enhancement mechanism in *Schizochytrium limacinum* LD11 mutant. *Algal Research, 67*, Article 102868. https://doi.org/10.1016/j.algal.2022.102868

Chen, L. M., Tong, S., Liu, W. Q., Zhang, Y., Khalid, H., Long, L. C., … Chen, G. Y. (2023). Electroporation-induced mutation and transcriptome analysis for high DHA production in *Schizochytrium limacinum* GCD2032. *Algal Research, 76*, Article 103297. https://doi.org/10.1016/j.algal.2023.103297

Chen, W., Zhou, P. P., Zhang, M., Zhu, Y. M., Wang, X. P., Luo, X. A., … Yu, L. J. (2016). Transcriptome analysis reveals that up-regulation of the fatty acid synthase gene promotes the accumulation of docosahexaenoic acid in *Schizochytrium* sp. S056 when glycerol is used. *Algal Research, 15*, 83–92. https://doi.org/10.1016/j.algal.2016.02.007

Chen, W., Zhou, P. P., Zhu, Y. M., Xie, C., Ma, L., Wang, X. P., … Yu, L. J. (2016). Improvement in the docosahexaenoic acid production of *Schizochytrium* sp. S056 by replacement of sea salt. *Bioprocess and Biosystems Engineering, 39*, 315–321. https://doi.org/10.1007/s00449-015-1517-1

Chen, W. X., Wang, H. J., Zhang, K., Gao, F., Chen, S. L., & Li, D. M. (2016). Physicochemical properties and storage stability of microencapsulated DHA-rich oil with different wall materials. *Applied Biochemistry and Biotechnology, 179*, 1129–1142. https://doi.org/10.1007/s12010-016-2054-3

Chen, Z. L., Yang, L. H., He, S. J., Du, Y. H., & Guo, D. S. (2023). Development of a green fermentation strategy with resource cycle for the docosahexaenoic acid production by *Schizochytrium* sp. *Bioresource Technology, 385*, Article 129434. https://doi.org/10.1016/j.biortech.2023.129434

Chen, Z. L., Yang, L. H., Tong, L. L., Wang, Y., Liu, M. Z., & Guo, D. S. (2023). Improvement of lipid and terpenoid yield in thraustochytrids using chemical regulators: A review. *Biotechnology and Bioprocess Engineering, 28*, 720–733. https://doi.org/10.1007/s12257-023-0086-4

Chi, G. X., Xu, Y. Y., Cao, X. Y., Li, Z. P., Cao, M. F., Chisti, Y., & He, N. (2022). Production of polyunsaturated fatty acids by *Schizochytrium* (*Aurantiochytrium*) spp. *Biotechnology Advances, 55*, Article 107897. https://doi.org/10.1016/j.biotechadv.2021.107897

Colombo, S. M., Rodgers, T. F. M., Diamond, M. L., Bazinet, R. P., & Arts, M. T. (2020). Projected declines in global DHA availability for human consumption as a result of global warming. *Ambio, 49*, 865–880. https://doi.org/10.1007/s13280-019-01234-6

Dannenberger, D., Eggert, A., Kalbe, C., Woitalla, A., & Schwudke, D. (2022). Are *n*-3 PUFAs from microalgae incorporated into membrane and storage lipids in pig muscle tissues?-A lipidomic approach. *ACS Omega, 7*, 24785–24794. https://doi.org/10.1021/acsomega.2c02476

Ding, J., Fu, Z. L., Zhu, Y. K., He, J. H., Ma, L., & Bu, D. P. (2022). Enhancing docosahexaenoic acid production of *Schizochytrium* sp. by optimizing fermentation using central composite design. *BMC Biotechnology, 22*. https://doi.org/10.1186/s12896-022-00769-z. Article 39.

Dong, L., Wang, F. Z., Chen, L., & Zhang, W. W. (2023). Metabolomic analysis reveals the responses of docosahexaenoic-acid-producing *Schizochytrium* under hyposalinity conditions. *Algal Research, 70*, Article 102987. https://doi.org/10.1016/j.algal.2023.102987

Ethier, S., Woisard, K., Vaughan, D., & Wen, Z. Y. (2011). Continuous culture of the microalgae *Schizochytrium limacinum* on biodiesel-derived crude glycerol for producing docosahexaenoic acid. *Bioresource Technology, 102*, 88–93. https://doi.org/10.1016/j.biortech.2010.05.021

Falk, M. C., Zheng, X. H., Chen, D. L., Jiang, Y., Liu, Z. S., & Lewis, K. D. (2017). Developmental and reproductive toxicological evaluation of arachidonic acid (ARA)-rich oil and docosahexaenoic acid (DHA)-rich oil. *Food and Chemical Toxicology, 103*, 270–278. https://doi.org/10.1016/j.fct.2017.03.011

Fedorova-Dahms, I., Marone, P. A., Bauter, M., & Ryan, A. S. (2011). Safety evaluation of DHA-rich algal oil from *Schizochytrium* sp. *Food and Chemical Toxicology, 49*, 3310–3318. https://doi.org/10.1016/j.fct.2011.08.024

Feng, Y. M., Zhu, Y. M., Bao, Z. D., Wang, B. H., Liu, T. T., Wang, H. H., … Yu, L. J. (2023). Construction of glucose-6-phosphate dehydrogenase overexpression strain of *Schizochytrium* sp. H016 to improve docosahexaenoic acid production. *Marine Drugs, 21*. https://doi.org/10.3390/md21010017. Article 17.

Ferreira, M., Ribeiro, P. C., Ribeiro, L., Barata, M., Domingues, V. F., Sousa, S., … Valente, L. M. P. (2022). Biofortified diets containing algae and selenised yeast: Effects on growth performance, nutrient utilization, and tissue composition of gilthead seabream (*Sparus aurata*). *Frontiers in Physiology, 12*, Article 812884. https://doi.org/10.3389/fphys.2021.812884

Fu, J., Chen, T., Lu, H., Lin, Y. F., Xie, X. L., Tian, H., … He, D. P. (2016). Enhancement of docosahexaenoic acid production by low-energy ion implantation coupled with screening method based on Sudan black B staining in *Schizochytrium* sp. *Bioresource Technology, 221*, 405–411. https://doi.org/10.1016/j.biortech.2016.09.058

Ganuza, E., & Izquierdo, M. S. (2007). Lipid accumulation in *Schizochytrium* G13/2S produced in continuous culture. *Applied Microbiology and Biotechnology, 76*, 985–990. https://doi.org/10.1007/s00253-007-1019-4

Gheysen, L., Durnez, N., Devaere, J., Bernaerts, T., Van Loey, A., De Cooman, L., & Foubert, I. (2020). Oxidative stability of vegetable purees enriched with n-3-LC-PUFA microalgal biomass: Impact of type of vegetable. *International Journal of Food Science and Technology, 55*, 751–759. https://doi.org/10.1111/ijfs.14378

Guo, D. S., Ji, X. J., Ren, L. J., Li, G. L., & Huang, H. (2017). Improving docosahexaenoic acid production by *Schizochytrium* sp using a newly designed high-oxygen-supply bioreactor. *AICHE Journal, 63*, 4278–4286. https://doi.org/10.1002/aic.15783

Hammond, B. G., Mayhew, D. A., Holson, J. F., Nemec, M. D., Mast, R. W., & Sander, W. J. (2001). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp.: II. Developmental toxicity evaluation in rats and rabbits. *Regulatory Toxicology and Pharmacology, 33*, 205–217. https://doi.org/10.1006/rtph.2001.1459

Hammond, B. G., Mayhew, D. A., Kier, L. D., Mast, R. W., & Sander, W. J. (2002). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp.: IV. Mutagenicity studies. *Regulatory Toxicology and Pharmacology, 35*, 255–265. https://doi.org/10.1006/rtph.2002.1535

Hammond, B. G., Mayhew, D. A., Robinson, K., Mast, R. W., & Sander, W. J. (2001). Safety assessment of DHA-rich microalgae from *Schizochytrium* sp.: III. Single-generation rat reproduction study. *Regulatory Toxicology and Pharmacology, 33*, 356–362. https://doi.org/10.1006/rtph.2001.1477

Han, X., Liu, Y. N., & Chen, Z. (2022). Zinc finger protein LipR represses docosahexaenoic acid and lipid biosynthesis in *Schizochytrium* sp. *Applied and Environmental Microbiology, 88*. https://doi.org/10.1128/aem.02063-21. Article e02063-21.

Han, X., Liu, Y. N., Yuan, Y. N., & Chen, Z. (2024). Metabolic engineering of *Schizochytrium* sp. for superior docosahexaenoic acid production. *Algal Research, 77*, Article 103355. https://doi.org/10.1016/j.algal.2023.103355

Han, X., Zhao, Z. N., Wen, Y., & Chen, Z. (2020). Enhancement of docosahexaenoic acid production by overexpression of ATP-citrate lyase and acetyl-CoA carboxylase in *Schizochytrium* sp. *Biotechnology for Biofuels, 13*. https://doi.org/10.1186/s13068-020-01767-z. Article 131.

Hart, B., Schurr, R., Narendranath, N., Kuehnle, A., & Colombo, S. M. (2021). Digestibility of *Schizochytrium* sp. whole cell biomass by Atlantic salmon (*Salmo salar*). *Aquaculture, 533*, Article 736156. https://doi.org/10.1016/j.aquaculture.2020.736156

Henley, W. J. (2019). The past, present and future of algal continuous cultures in basic research and commercial applications. *Algal Research, 43*, Article 101636. https://doi.org/10.1016/j.algal.2019.101636

Hu, F., Clevenger, A. L., Zheng, P., Huang, Q. Y., & Wang, Z. K. (2020). Low-temperature effects on docosahexaenoic acid biosynthesis in *Schizochytrium* sp. TIO01 and its proposed underlying mechanism. *Biotechnology for Biofuels, 13*. https://doi.org/10.1186/s13068-020-01811-y. Article 172.

Hu, X. C., Tang, X. Y., Bi, Z. Q., Zhao, Q. Y., & Ren, L. J. (2021). Adaptive evolution of microalgae *Schizochytrium* sp. under high temperature for efficient production of docosahexaeonic acid. *Algal Research, 54*, Article 102212. https://doi.org/10.1016/j.algal.2021.102212

Ibrahim, D., Abd El-Hamid, M. I., Al-Zaban, M. I., ElHady, M., El-Azzouny, M. M., ElFeky, T. M., … Omar, A. E. (2022). Impacts of fortifying nile tilapia (*Oreochromis niloticus*) diet with different strains of microalgae on its performance, fillet quality and disease resistance to *Aeromonas hydrophila* considering the interplay between antioxidant and inflammatory response. *Antioxidants, 11*. https://doi.org/10.3390/antiox11112181. Article 2181.

Isa, M. H., Metin, C., Ercan, E., & Alparslan, Y. (2022). Effect of different cell disruption methods on lipid yield of *Schizochytrium* sp. *Journal of the American Oil Chemists Society, 99*, 129–139. https://doi.org/10.1002/aocs.12551

Jeon, J. J., Kim, H. J., Kang, H. K., Kim, C. H., Kim, H. S., Hong, E. C., … Kim, S. H. (2022). Effects of dietary thraustochytrid *Schizochytrium* sp. and other omega-3 sources on growth performance, carcass characteristics, and meat quality of broilers. *Animals, 12*. https://doi.org/10.3390/ani12091166. Article 1166.

Jia, L. Q., Li, T. Y., Wang, R. Y., Ma, M. Y., & Yang, Z. Q. (2024). Enhancing docosahexaenoic acid production from *Schizochytrium* sp. by using waste *Pichia pastoris* as nitrogen source based on two-stage feeding control. *Bioresource Technology, 403*, Article 130891. https://doi.org/10.1016/j.biortech.2024.130891

Jia, L. Q., Li, T. Y., Yang, Z. Q., He, T., Ding, J., Li, T., & Huang, A. G. (2023). Eliminating the accumulation of reactive oxygen species through periodic hypoxic stress control for effective DHA production by *Schizochytrium* sp. *Chemical Engineering Science, 280*, Article 119040. https://doi.org/10.1016/j.ces.2023.119040

Jiang, M. S., Hu, Z. J., Huang, Y. X., Chen, X. D., & Wu, P. (2024). Impact of wall materials and DHA sources on the release, digestion and absorption of DHA microcapsules: Advancements, challenges and future directions. *Food Research International, 191*, Article 114646. https://doi.org/10.1016/j.foodres.2024.114646

Jiang, X., Zhang, J., Zhao, J., Gao, Z. Q., Zhang, C. Z., & Chen, M. (2017). Regulation of lipid accumulation in *Schizochytrium* sp ATCC 20888 in response to different nitrogen sources. *European Journal of Lipid Science and Technology, 119*, Article 1700025. https://doi.org/10.1002/ejlt.201700025

Jiang, Y., Fan, K. W., Wong, R. T.-Y., & Chen, F. (2004). Fatty acid composition and squalene content of the marine microalga *Schizochytrium mangrovei*. *Journal of Agricultural and Food Chemistry, 52*, 1196–1200. https://doi.org/10.1021/jf035004c

Jorge, T. B. F., Moura, G. D., Ribeiro, V., Jr., Donzele, J. L., Pedreira, M. M., Sousa, T. V., & Lanna, E. A. T. (2022). Effects of dietary supplementation time with *Schizochytrium* microalgae meal on growth, meat quality and fatty acid composition of Nile tilapia. *Aquaculture Research, 53*, 528–543. https://doi.org/10.1111/are.15597

Ju, J. H., Ko, D. J., Heo, S. Y., Lee, J. J., Kim, Y. M., Lee, B. S., … Oh, B. R. (2020). Regulation of lipid accumulation using nitrogen for microalgae lipid production in *Schizochytrium* sp. ABC101. *Renewable Energy, 153*, 580–587. https://doi.org/10.1016/j.renene.2020.02.047

Karapanagiotidis, I. T., Metsoviti, M. N., Gkalogianni, E. Z., Psofakis, P., Asimaki, A., Katsoulas, N., … Zarkadas, I. (2022). The effects of replacing fishmeal by *Chlorella vulgaris* and fish oil by *Schizochytrium* sp. and *Microchloropsis gaditana* blend on growth performance, feed efficiency, muscle fatty acid composition and liver histology of gilthead seabream (*Sparus aurata*). *Aquaculture, 561*, Article 738709. https://doi.org/10.1016/j.aquaculture.2022.738709

Kralik, Z., Kralik, G., Grcevic, M., Hanzek, D., & Margeta, P. (2020). Microalgae *Schizochytrium limacinum* as an alternative to fish oil in enriching table eggs with n-3 polyunsaturated fatty acids. *Journal of the Science of Food and Agriculture, 100*, 587–594. https://doi.org/10.1002/jsfa.10052

Kujawska, N., Talbierz, S., Debowski, M., Kazimierowicz, J., & Zielinski, M. (2021a). Cultivation method effect on *Schizochytrium* sp. biomass growth and docosahexaenoic acid (DHA) production with the use of waste glycerol as a source of organic carbon. *Energies, 14*. https://doi.org/10.3390/en14102952. Article 2952.

Kujawska, N., Talbierz, S., Debowski, M., Kazimierowicz, J., & Zielinski, M. (2021b). Optimizing docosahexaenoic acid (DHA) production by *Schizochytrium* sp. grown on waste glycerol. *Energies, 14*. https://doi.org/10.3390/en14061685. Article 1685.

Kumari, A., Pabbi, S., & Tyagi, A. (2023). Recent advances in enhancing the production of long chain omega-3 fatty acids in microalgae. *Critical Reviews in Food Science and Nutrition, 1-19*. https://doi.org/10.1080/10408398.2023.2226720

La, A. L. T. Z., Pierce, K. M., Liu, W. H., Gao, S. T., Bu, D. P., & Ma, L. (2021). Supplementation with *Schizochytrium* sp. enhances growth performance and antioxidant capability of dairy calves before weaning. *Animal Feed Science and Technology, 271*, Article 114779. https://doi.org/10.1016/j.anifeedsci.2020.114779

Lewis, K. D., Huang, W. F., Zheng, X. H., Jiang, Y., Feldman, R. S., & Falk, M. C. (2016). Toxicological evaluation of arachidonic acid (ARA)-rich oil and docosahexaenoic acid (DHA)-rich oil. *Food and Chemical Toxicology, 96*, 133–144. https://doi.org/10.1016/j.fct.2016.07.026

Li, D. M., Zhang, K., Chen, L. M., Ding, M. X., Zhao, M. L., & Chen, S. L. (2017). Selection of *Schizochytrium limacinum* mutants based on butanol tolerance. *Electronic Journal of Biotechnology, 30*, 58–63. https://doi.org/10.1016/j.ejbt.2017.08.009

Li, J., Liu, R. J., Chang, G. F., Li, X. Y., Chang, M., Liu, Y. F., … Wang, X. G. (2015). A strategy for the highly efficient production of docosahexaenoic acid by *Aurantiochytrium limacinum* SR21 using glucose and glycerol as the mixed carbon sources. *Bioresource Technology, 177*, 51–57. https://doi.org/10.1016/j.biortech.2014.11.046

Li, J., Zheng, Y., Yang, W. Q., Wei, Z. Y., Xu, Y. S., Zhang, Z. X., … Sun, X. M. (2023). Enhancing the accumulation of lipid and docosahexaenoic acid in *Schizochytrium* sp. by co-overexpression of phosphopantetheinyl transferase and ω-3 fatty acid desaturase. *Biotechnology Journal, 18*, Article e2300314. https://doi.org/10.1002/biot.202300314

Li, Y. Q., Meng, X., Wang, Z. Y., Lin, X., Xu, Y., Mou, J. H., … Li, X. (2024). Utilization of tofu wastewater and *Nannochloropsis oceanica* for eutrophication mitigation and eicosapentaenoic acid valorization: Advancing carbon neutrality and resource recycling. *Chemical Engineering Journal, 493*, Article 152706. https://doi.org/10.1016/j.cej.2024.152706

Li, Z. P., Ling, X. P., Zhou, H., Meng, T., Zeng, J. J., Hang, W., … He, N. (2019). Screening chemical modulators of benzoic acid derivatives to improve lipid accumulation in *Schizochytrium limacinum* SR21 with metabolomics analysis. *Biotechnology for Biofuels, 12*. https://doi.org/10.1186/s13068-019-1552-2. Article 209.

Li, Z. P., Meng, T., Hang, W., Cao, X. Y., Ni, H., Shi, Y. Y., … He, N. (2021). Regulation of glucose and glycerol for production of docosahexaenoic acid in *Schizochytrium limacinum* SR21 with metabolomics analysis. *Algal Research, 58*, Article 102415. https://doi.org/10.1016/j.algal.2021.102415

Li, Z. P., Meng, T., Ling, X. P., Li, J., Zheng, C. Q., Shi, Y. Y., … He, N. (2018). Overexpression of malonyl-CoA: ACP transacylase in *Schizochytrium* sp to improve polyunsaturated fatty acid production. *Journal of Agricultural and Food Chemistry, 66*, 5382–5391. https://doi.org/10.1021/acs.jafc.8b01026

Lin, Y. F., Xie, X. L., Yuan, B., Fu, J., Liu, L. Y., Tian, H., … He, D. P. (2018). Optimization of enzymatic cell disruption for improving lipid extraction from *Schizochytrium* sp through response surface methodology. *Journal of Oleo Science, 67*, 215–224. https://doi.org/10.5650/jos.ess17166

Liu, L., Bai, M. H., Zhang, S., Li, J. T., Liu, X. H., Sen, B., & Wang, G. Y. (2021). ARTP mutagenesis of *Schizochytrium* sp. PKU#Mn4 and clethodim-based mutant screening for enhanced docosahexaenoic acid accumulation. *Marine Drugs, 19*. https://doi.org/10.3390/md19100564. Article 564.

Liu, X. Y., Zhang, X. Y., Ding, L. X., Jin, H. B., Chen, N., Huang, X., … Cai, Z. X. (2023). Natural egg yolk emulsion as wall material to encapsulate DHA by two-stage homogenization: Emulsion stability, rheology analysis and powder properties. *Food Research International, 167*, Article 112658. https://doi.org/10.1016/j.foodres.2023.112658

Liu, Y., Singh, P., Sun, Y., Luan, S. J., & Wang, G. Y. (2014). Culturable diversity and biochemical features of thraustochytrids from coastal waters of southern China. *Applied Microbiology and Biotechnology, 98*, 3241–3255. https://doi.org/10.1007/s00253-013-5391-y

Liu, Y. N., Han, X., Dai, Y. J., & Chen, Z. (2024). bZIP transcription factor FabR: Redox-dependent mechanism controlling docosahexaenoic acid biosynthesis and H_2O_2 stress response in *Schizochytrium* sp. *Free Radical Biology and Medicine, 210*, 246–257. https://doi.org/10.1016/j.freeradbiomed.2023.11.027

Liu, Z. X., You, S., Tang, B. P., Wang, B., Sheng, S., Wu, F. A., & Wang, J. (2019). Inositol as a new enhancer for improving lipid production and accumulation in *Schizochytrium* sp. SR21. *Environmental Science and Pollution Research, 26*, 35497–35508. https://doi.org/10.1007/s11356-019-06056-3

Ma, W., Li, X., Zhang, F., Zhang, Z. Y., Yang, W. Q., Huang, P. W., … Sun, X. M. (2023). Enhancing the biomass and docosahexaenoic acid-rich lipid accumulation of *Schizochytrium* sp. in propionate wastewater. *Biotechnology Journal, 18*, Article e2300052. https://doi.org/10.1002/biot.202300052

Ma, W., Zhang, Z. Y., Yang, W. Q., Huang, P. W., Gu, Y., Sun, X. M., & Huang, H. (2023). Enhanced docosahexaenoic acid production from cane molasses by engineered and adaptively evolved *Schizochytrium* sp. *Bioresource Technology, 376*, Article 128833. https://doi.org/10.1016/j.biortech.2023.128833

Mavrommatis, A., Sotirakoglou, K., Kamilaris, C., & Tsiplakou, E. (2021). Effects of inclusion of *Schizochytrium* spp. and forage-to-concentrate ratios on goats' milk quality and oxidative status. *Foods, 10*. https://doi.org/10.3390/foods10061322. Article 1322.

Mavrommatis, A., Sotirakoglou, K., Skliros, D., Flemetakis, E., & Tsiplakou, E. (2021). Dose and time response of dietary supplementation with *Schizochytrium* sp. on the abundances of several microorganisms in the rumen liquid of dairy goats. *Livestock Science, 247*, Article 104489. https://doi.org/10.1016/j.livsci.2021.104489

Meadus, W. J., Turner, T. D., Dugan, M. E. R., Aalhus, J. L., Duff, P., Rolland, D., … Gibson, L. L. (2013). Fortification of pork loins with docosahexaenoic acid (DHA) and its effect on flavour. *Journal of Animal Science and Biotechnology, 4*. https://doi.org/10.1186/2049-1891-4-46. Article 46.

Mehta, P., Rani, R., Gupta, R., Mathur, A., & Ramakumar, S. S. V. (2023). Simultaneous production of high-value lipids in *Schizochytrium* sp. by synergism of chemical modulators. *Applied Microbiology and Biotechnology, 107*, 6135–6149. https://doi.org/10.1007/s00253-023-12698-8

Min, K. H., Kim, D. H., Ki, M. R., & Pack, S. P. (2022). Recent progress in flocculation, dewatering, and drying technologies for microalgae utilization: Scalable and low-cost harvesting process development. *Bioresource Technology, 344*, Article 126404. https://doi.org/10.1016/j.biortech.2021.126404

Mu, H. Y., Song, Z. X., Wang, X., Wang, D. D., Zheng, X. Q., & Li, X. D. (2022). Microencapsulation of algae oil by complex coacervation of chitosan and modified starch: Characterization and oxidative stability. *International Journal of Biological Macromolecules, 194*, 66–73. https://doi.org/10.1016/j.ijbiomac.2021.11.168

Mu, H. Y., Zhang, H. J., Li, Y., Zhang, Y., Wang, X. S., Jin, Q. Z., & Wang, X. G. (2016). Enrichment of DPAn-6 and DHA from *Schizochytrium sp.* oil by low-temperature solvent crystallization. *Industrial & Engineering Chemistry Research, 55*, 737–746. https://doi.org/10.1021/acs.iecr.5b03766

Nagappan, S., Devendran, S., Tsai, P. C., Dinakaran, S., Dahms, H. U., & Ponnusamy, V. K. (2019). Passive cell disruption lipid extraction methods of microalgae for biofuel production – A review. *Fuel, 252*, 699–709. https://doi.org/10.1016/j.fuel.2019.04.092

Neylan, K. A., Johnson, R. B., Barrows, F. T., Marancik, D. P., Hamilton, S. L., & Gardner, L. D. (2024). Evaluating a microalga (*Schizochytrium* sp.) as an alternative to fish oil in fish-free feeds for sablefish (*Anoplopoma fimbria*). *Aquaculture, 578*, Article 740000. https://doi.org/10.1016/j.aquaculture.2023.740000

Oh, C. E., Kim, G. J., Park, S. J., Choi, S., Park, M. J., Lee, O. M., … Son, H. J. (2020). Purification of high purity docosahexaenoic acid from *Schizochytrium* sp. SH103 using preparative-scale HPLC. *Applied Biological Chemistry, 63*. https://doi.org/10.1186/s13765-020-00542-w. Article 56.

Pajor, F., Egerszegi, I., Szucs, A., Póti, P., & Bodnár, A. (2021). Effect of marine algae supplementation on somatic cell count, prevalence of udder pathogens, and fatty acid profile of dairy goats' milk. *Animals, 11*. https://doi.org/10.3390/ani11041097. Article 1097.

Pajor, F., Várkonyi, D., Dalmadi, I., Pásztorné-Huszár, K., Egerszegi, I., Penksza, K., Póti, P., & Bodnár, A. (2023). Changes in chemical composition and fatty acid profile of milk and cheese and sensory profile of milk via supplementation of goats' diet with marine algae. *Animals, 13*. https://doi.org/10.3390/ani13132152. Article 2152.

Patel, A., Karageorgou, D., Katapodis, P., Sharma, A., Rova, U., Christakopoulos, P., & Matsakas, L. (2021). Bioprospecting of thraustochytrids for omega-3 fatty acids: A sustainable approach to reduce dependency on animal sources. *Trends in Food Science & Technology, 115*, 433–444. https://doi.org/10.1016/j.tifs.2021.06.044

Perez-Velazquez, M., Gatlin, D. M., González-Félix, M. L., García-Ortega, A., de Cruz, C. R., Juárez-Gómez, M. L., & Chen, K. Q. (2019). Effect of fishmeal and fish oil replacement by algal meals on biological performance and fatty acid profile of hybrid striped bass (*Morone crhysops* ♀ × *M. Saxatilis* ♂). *Aquaculture, 507*, 83–90. https://doi.org/10.1016/j.aquaculture.2019.04.011

Qiu, C. Y., He, Y. J., Huang, Z. C., Qiu, W. S., Huang, J., Wang, M. Z., & Chen, B. L. (2021). Biosafety evaluation of *Nannochloropsis oculata* and *Schizochytrium* sp. oils as novel human milk fat substitutes. *Food & Function, 12*, 2972–2984. https://doi.org/10.1039/d0fo03000g

Qu, L., Ren, L. J., Sun, G. N., Ji, X. J., Nie, Z. K., & Huang, H. (2013). Batch, fed-batch and repeated fed-batch fermentation processes of the marine thraustochytrid *Schizochytrium* sp. for producing docosahexaenoic acid. *Bioprocess and Biosystems Engineering, 36*, 1905–1912. https://doi.org/10.1007/s00449-013-0966-7

Ramos-Vega, A., Rosales-Mendoza, S., Bañuelos-Hernández, B., & Angulo, C. (2018). Prospects on the use of *Schizochytrium* sp to develop oral vaccines. *Frontiers in Microbiology, 9*. https://doi.org/10.3389/fmicb.2018.02506. Article 2506.

Ren, L. J., Feng, Y., Li, J., Qu, L., & Huang, H. (2013). Impact of phosphate concentration on docosahexaenoic acid production and related enzyme activities in fermentation of *Schizochytrium* sp. *Bioprocess and Biosystems Engineering, 36*, 1177–1183. https://doi.org/10.1007/s00449-012-0844-8

Ren, L. J., Sun, X. M., Ji, X. J., Chen, S. L., Guo, D. S., & Huang, H. (2017). Enhancement of docosahexaenoic acid synthesis by manipulation of antioxidant capacity and prevention of oxidative damage in *Schizochytrium* sp. *Bioresource Technology, 223*, 141–148. https://doi.org/10.1016/j.biortech.2016.10.040

Ren, L. J., Zhuang, X. Y., Chen, S. L., Ji, X. J., & Huang, H. (2015). Introduction of ω-3 desaturase obviously changed the fatty acid profile and sterol content of *Schizochytrium* sp. *Journal of Agricultural and Food Chemistry, 63*, 9770–9776. https://doi.org/10.1021/acs.jafc.5b04238

Rodríguez-España, M., Mendoza-Sánchez, L. G., Magallón-Servín, P., Salgado-Cervantes, M. A., Acosta-Osorio, A. A., & García, H. S. (2022). Supercritical fluid extraction of lipids rich in DHA from *Schizochytrium* sp. *Journal of Supercritical Fluids, 179*, Article 105391. https://doi.org/10.1016/j.supflu.2021.105391

Ryan, L., & Symington, A. M. (2015). Algal-oil supplements are a viable alternative to fish-oil supplements in terms of docosahexaenoic acid (22,6n-3; DHA). *Processings of the Nutrition Society, 19*, 852–858. https://doi.org/10.1016/j.jff.2014.06.023

Sales, R., Lopes, R. G., Derner, R. B., & Tsuzuki, M. Y. (2022). Concentrated microalgal biomass as a substitute for fresh microalgae produced on site at hatcheries. *Aquaculture Research, 53*, 5771–5786. https://doi.org/10.1111/are.16072

Serna-Saldivar, S. O., Zorrilla, R., De La Parra, C., Stagnitti, G., & Abril, R. (2006). Effect of DHA containing oils and powders on baking performance and quality of white pan bread. *Plant Foods for Human Nutrition, 61*, 121–129. https://doi.org/10.1007/s11130-006-0009-5

Shafiq, M., Zeb, L., Cui, G. N., Jawad, M., & Chi, Z. Y. (2020). High-density pH-auxostat fed-batch culture of *Schizochytrium limacinum* SR21 with acetic acid as a carbon source. *Applied Biochemistry and Biotechnology, 192*, 1163–1175. https://doi.org/10.1007/s12010-020-03396-6

Shen, Y., Guo, C., Lu, T., Ding, X. Y., Zhao, M. T., Zhang, M., … Zhou, D. Y. (2021). Effects of gallic acid alkyl esters and their combinations with other antioxidants on oxidative stability of DHA algae oil. *Food Research International, 143*, Article 110280. https://doi.org/10.1016/j.foodres.2021.110280

Sidari, R., & Tofalo, R. (2019). A comprehensive overview on microalgal-fortified/based food and beverages. *Food Reviews International, 35*, 778–805. https://doi.org/10.1080/87559129.2019.1608557

Song, X. J., Zang, X. N., & Zhang, X. C. (2015). Production of high docosahexaenoic acid by *Schizochytrium* sp. using low-cost raw materials from food industry. *Journal of Oleo Science, 64*, 197–204. https://doi.org/10.5650/jos.ess14164

de Souza Celente, G., de Cassia de Souza Schneider, R., Medianeira Rizzetti, T., Lobo, E. A., & Sui, Y. X. (2024). Using wastewater as a cultivation alternative for microalga *Dunaliella salina*: Potentials and challenges. *Science of the Total Environment, 911*, Article 168812. https://doi.org/10.1016/j.scitotenv.2023.168812

Sun, L. N., Ren, L. J., Zhuang, X. Y., Ji, X. J., Yan, J. C., & Huang, H. (2014). Differential effects of nutrient limitations on biochemical constituents and docosahexaenoic acid production of *Schizochytrium* sp. *Bioresource Technology, 159*, 199–206. https://doi.org/10.1016/j.biortech.2014.02.106

Sun, X. M., Geng, L. J., Ren, L. J., Ji, X. J., Hao, N., Chen, K. Q., & Huang, H. (2018). Influence of oxygen on the biosynthesis of polyunsaturated fatty acids in microalgae. *Bioresource Technology, 250*, 868–876. https://doi.org/10.1016/j.biortech.2017.11.005

Sun, X. M., Ren, L. J., Bi, Z. Q., Ji, X. J., Zhao, Q. Y., & Huang, H. (2018). Adaptive evolution of microalgae *Schizochytrium* sp. under high salinity stress to alleviate oxidative damage and improve lipid biosynthesis. *Bioresource Technology, 267*, 438–444. https://doi.org/10.1016/j.biortech.2018.07.079

Sun, X. M., Ren, L. J., Bi, Z. Q., Ji, X. J., Zhao, Q. Y., Jiang, L., & Huang, H. (2018). Development of a cooperative two-factor adaptive-evolution method to enhance lipid production and prevent lipid peroxidation in *Schizochytrium* sp. *Biotechnology for Biofuels, 11*. https://doi.org/10.1186/s13068-018-1065-4. Article 65.

Sun, X. M., Ren, L. J., Ji, X. J., Chen, S. L., Guo, D. S., & Huang, H. (2016). Adaptive evolution of *Schizochytrium* sp by continuous high oxygen stimulations to enhance docosahexaenoic acid synthesis. *Bioresource Technology, 211*, 374–381. https://doi.org/10.1016/j.biortech.2016.03.093

Terova, G., Moroni, F., Antonini, M., Bertacchi, S., Pesciaroli, C., Branduardi, P., … Rimoldi, S. (2021). Using glycerol to produce european sea bass feed with oleaginous microbial biomass: Effects on growth performance, filet fatty acid profile, and FADS2 gene expression. Frontiers in marine. *Science, 8*, Article 715078. https://doi.org/10.3389/fmars.2021.715078

Thakur, S., Singh, H., Sharma, S., Kaur, M., Singh, A., Kaur, A., & Jain, S. K. (2024). Pre-clinical and cellular safety assessment of oral administered DHA rich microalgae oil from *Schizochytrium* sp. (strain ATCC-20889): Acute, sub-chronic and genotoxicity. *Drug and Chemical Toxicology*, 1–13. https://doi.org/10.1080/01480545.2024.2308835

Tibbetts, S. M., Scaife, M. A., & Armenta, R. E. (2020). Apparent digestibility of proximate nutrients, energy and fatty acids in nutritionally-balanced diets with partial or complete replacement of dietary fish oil with microbial oil from a novel *Schizochytrium* sp. (T18) by juvenile Atlantic salmon (*Salmo salar* L.). *Aquaculture, 520*, Article 735003. https://doi.org/10.1016/j.aquaculture.2020.735003

Till, B. E., Huntington, J. A., Posri, W., Early, R., Taylor-Pickard, J., & Sinclair, L. A. (2019). Influence of rate of inclusion of microalgae on the sensory characteristics and fatty acid composition of cheese and performance of dairy cows. *Journal of Dairy Science, 102*, 10934–10946. https://doi.org/10.3168/jds.2019-16391

Turck, D., Bohn, T., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Maciuk, A., … Knutsen, H. K. (2023). Safety of oil from *Schizochytrium* sp (strain CABIO-A-2) for use in infant and follow-on formula as a novel food pursuant to Regulation (EU) 2015/2283 *EFSA Journal, 21*. https://doi.org/10.2903/j.efsa.2023.8415. Article e8415.

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., … Knutsen, H. K. (2020). Safety of *Schizochytrium* sp. oil as a novel food pursuant to regulation (EU) 2015/2283. *EFSA Journal, 18*. https://doi.org/10.2903/j.efsa.2020.6242. Article 6242.

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., … Knutsen, H. K. (2021a). Safety of oil from *Schizochytrium limacinum* (strain FCC-3204) for use in food supplements as a novel food pursuant to regulation (EU) 2015/2283. *EFSA Journal, 19*. https://doi.org/10.2903/j.efsa.2021.6345. Article 6345.

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., … Knutsen, H. K. (2021b). Safety of oil from *Schizochytrium limacinum* (strain FCC-3204) for use in infant and follow-on formula as a novel food pursuant to regulation (EU) 2015/2283. *EFSA Journal, 19*. https://doi.org/10.2903/j.efsa.2021.6344. Article 6344.

Valencia, I., Ansorena, D., & Astiasarán, I. (2007). Development of dry fermented sausages rich in docosahexaenoic acid with oil from the microalgae *Schizochytrium* sp.: Influence on nutritional properties, sensorial quality and oxidation stability. *Food Chemistry, 104*, 1087–1096. https://doi.org/10.1016/j.foodchem.2007.01.021

Wang, H., Zhang, H. J., Wang, X. C., Wu, S. G., Wang, J., Xu, L., & Qi, G. H. (2017). Dietary choline and phospholipid supplementation enhanced docosahexaenoic acid enrichment in egg yolk of laying hens fed a 2% *Schizochytrium* powder-added diet. *Poultry Science, 96*, 2786–2794. https://doi.org/10.3382/ps/pex095

Wang, J., Ossemond, J., Jardin, J., Briard-Bion, V., Henry, G., Le Gouar, Y., … Pédrono, F. (2022). Encapsulation of DHA oil with heat-denatured whey protein in Pickering emulsion improves its bioaccessibility. *Food Research International, 162*, Article 112112. https://doi.org/10.1016/j.foodres.2022.112112

Wang, J., Wang, Y. X., Wu, Y. J., Fan, Y. W., Zhu, C. L., Fu, X. D., … Mou, H. J. (2022). Application of microalgal stress responses in industrial microalgal production systems. *Marine Drugs, 20*. https://doi.org/10.3390/md20010030. Article 30.

Wang, K., Sun, T., Cui, J. Y., Liu, L. S., Bi, Y. Q., Pei, G. S., … Zhang, W. W. (2018). Screening of chemical modulators for lipid accumulation in *Schizochytrium* sp. S31. *Bioresource Technology, 260*, 124–129. https://doi.org/10.1016/j.biortech.2018.03.104

Wang, L. R., Zhang, Z. X., Nong, F. T., Li, J., Huang, P. W., Ma, W., … Sun, X. M. (2022). Engineering the xylose metabolism in *Schizochytrium* sp. to improve the utilization of lignocellulose. *Biotechnology for Biofuels and Bioproducts, 15*. https://doi.org/10.1186/s13068-022-02215-w. Article 114.

Wang, Q., Han, W., Jin, W. B., Gao, S. H., & Zhou, X. (2021). Docosahexaenoic acid production by *Schizochytrium* sp.: Review and prospect. *Food Biotechnology, 35*, 111–135. https://doi.org/10.1080/08905436.2021.1908900

Wang, Q., Jin, W. B., Han, W., Song, K., Chen, Y. D., Chen, C., … Zhou, X. (2023). Enhancement of DHA production from *Aurantiochytrium* sp. by atmospheric and room temperature plasma mutagenesis aided with microbial microdroplet culture screening. *Biomass Conversion and Biorefinery, 13*, 16807–16818. https://doi.org/10.1007/s13399-021-02147-9

Wang, S. K., Tian, Y. T., Dai, Y. R., Wang, D., Liu, K. C., & Cui, Y. H. (2022). Development of an alternative medium via completely replaces the medium components by mixed wastewater and crude glycerol for efficient production of docosahexaenoic acid by *Schizochytrium* sp. *Chemosphere, 291*, Article 132868. https://doi.org/10.1016/j.chemosphere.2021.132868

Wang, S. K., Wang, X., Tian, Y. T., & Cui, Y. H. (2020). Nutrient recovery from tofu whey wastewater for the economical production of docosahexaenoic acid by *Schizochytrium* sp. S31. *Science of the Total Environment, 710*, Article 136448. https://doi.org/10.1016/j.scitotenv.2019.136448

Weylandt, K. H., Serini, S., Chen, Y. Q., Su, H. M., Lim, K., Cittadini, A., & Calviello, G. (2015). Omega-3 polyunsaturated fatty acids: The way forward in times of mixed evidence. *BioMed Research International, 2015*, Article 143109. https://doi.org/10.1155/2015/143109

Wu, S. T., Yu, S. T., & Lin, L. P. (2005). Effect of culture conditions on docosahexaenoic acid production by *Schizochytrium* sp. S31. *Process Biochemistry, 40*, 3103–3108. https://doi.org/10.1016/j.procbio.2005.03.007

Xu, C. C., Zhang, S., Sun, B. Z., Xie, P., Liu, X. C., Chang, L., … Zhang, S. S. (2021). Dietary supplementation with microalgae (*Schizochytrium* sp.) improves the antioxidant status, fatty acids profiles and volatile compounds of beef. *Animals, 11*. https://doi.org/10.3390/ani11123517. Article 3517.

Xu, X. D., Huang, C. Y., Xu, Z. X., Xu, H. X., Wang, Z., & Yu, X. J. (2020). The strategies to reduce cost and improve productivity in DHA production by *Aurantiochytrium* sp.: From biochemical to genetic respects. *Applied Microbiology and Biotechnology, 104*, 9433–9447. https://doi.org/10.1007/s00253-020-10927-y

Yan, C. X., Zhang, Y., Yang, W. Q., Ma, W., Sun, X. M., & Huang, H. (2024). Universal and unique strategies for the production of polyunsaturated fatty acids in industrial oleaginous microorganisms. *Biotechnology Advances, 70*, Article 108298. https://doi.org/10.1016/j.biotechadv.2023.108298

Yin, F. W., Zhang, Y. T., Jiang, J. Y., Guo, D. S., Gao, S., & Gao, Z. (2019). Efficient docosahexaenoic acid production by *Schizochytrium* sp. via a two-phase pH control strategy using ammonia and citric acid as pH regulators. *Process Biochemistry, 77*, 1–7. https://doi.org/10.1016/j.procbio.2018.11.013

Yin, F. W., Zhu, S. Y., Guo, D. S., Ren, L. J., Ji, X. J., Huang, H., & Gao, Z. (2019). Development of a strategy for the production of docosahexaenoic acid by *Schizochytrium* sp. from cane molasses and algae-residue. *Bioresource Technology, 271*, 118–124. https://doi.org/10.1016/j.biortech.2018.09.114

Zeng, Y., Ji, X. J., Lian, M., Ren, L. J., Jin, L. J., Ouyang, P. K., & Huang, H. (2011). Development of a temperature shift strategy for efficient docosahexaenoic acid production by a marine fungoid protist, *Schizochytrium* sp. HX-308. *Applied Biochemistry and Biotechnology, 164*, 249–255. https://doi.org/10.1007/s12010-010-9131-9

Zhang, B., Wu, J. Y., & Meng, F. P. (2021). Adaptive laboratory evolution of microalgae: A review of the regulation of growth, stress resistance, metabolic processes, and biodegradation of pollutants. *Frontiers in Microbiology, 12*, Article 737248. https://doi.org/10.3389/fmicb.2021.737248

Zhang, G. Y., Liu, J., & Liu, Y. F. (2013). Concentration of omega-3 polyunsaturated fatty acids from oil of *Schizochytrium limacinum* by molecular distillation: Optimization of technological conditions. *Industrial & Engineering Chemistry Research, 52*, 3918–3925. https://doi.org/10.1021/ie3020044

Zhang, H. Q., Zhao, X. Y., Zhang, J. X., Liu, L. P., & Liu, J. J. (2024). Overview of docosahexaenoic acid (DHA) biosynthesis by pathway, high-yield mechanism, and metabolic engineering strategies. *Food Reviews International*, 1–39. https://doi.org/10.1080/87559129.2024.2423767

Zhang, M. L., Gao, Y. L., Yu, C., Wang, J., Weng, K. X., Li, Q., … Li, L. (2022). Transcriptome analysis of malate-induced *Schizochytrium* sp. FJU-512 reveals a novel pathway for biosynthesis of docosahexaenoic acid with enhanced expression of genes responsible for acetyl-CoA and NADPH accumulation. *Frontiers in Microbiology, 13*, Article 1006138. https://doi.org/10.3389/fmicb.2022.1006138

Zhang, S., Chen, X. H., Sen, B., Bai, M. H., He, Y. D., & Wang, G. Y. (2021). Exogenous antioxidants improve the accumulation of saturated and polyunsaturated fatty acids in *Schizochytrium* sp. PKU#Mn4. *Marine Drugs, 19*. https://doi.org/10.3390/md19100559. Article 559.

Zhang, S. Y., & Akoh, C. C. (2023). Combining antioxidants and processing techniques to improve oxidative stability of a *Schizochytrium* algal oil ingredient with application in yogurt. *Food Chemistry, 417*, Article 135835. https://doi.org/10.1016/j.foodchem.2023.135835

Zhang, Y. Q., Kalpio, M., Tao, L. W., Haraldsson, G. G., Guðmundsson, H. G., Fang, X. R., … Yang, B. R. (2023). Metabolic fate of DHA from regio- and stereospecific positions of triacylglycerols in a long-term feeding trial in rats. *Food Research International, 174*, Article 113626. https://doi.org/10.1016/j.foodres.2023.113626

Zhang, Y. T., Cui, X. W., Lin, S. Z., Lu, T., Li, H., Lu, Y. H., … Ling, X. P. (2024). Knockout of a *PLD* gene in *Schizochytrium limacinum* SR21 enhances docosahexaenoic acid accumulation by modulation of the phospholipid profile. *Biotechnology for Biofuels and Bioproducts, 17*. https://doi.org/10.1186/s13068-024-02465-w. Article 16.

Zhao, B., Li, Y. F., Mbifile, M. D., Li, C. L., Yang, H. L., & Wang, W. (2017). Improvement of docosahexaenoic acid fermentation from *Schizochytrium* sp AB-610 by staged pH control based on cell morphological changes. *Engineering in Life Sciences, 17*, 981–988. https://doi.org/10.1002/elsc.201600249

Zhao, G. F., Zhao, J., Zhang, X. R., Wang, S., Fu, D. M., & Chen, M. (2022). Yacon (*Smallanthus sonchifolius*) tuber: A novel and promising feedstock for enhanced high-value docosahexaenoic acid production by *Schizochytrium* sp. *Industrial Crops and Products, 188*, Article 115597. https://doi.org/10.1016/j.indcrop.2022.115597

Zhu, H. Q., Wang, X. D., Zhang, W. Y., Zhang, Y. M., Zhang, S. W., Pang, X. Y., … Lv, J. P. (2022). Dietary *Schizochytrium* microalgae affect the fatty acid profile of goat milk: Quantification of docosahexaenoic acid (DHA) and its distribution at sn-2 position. *Foods, 11*. https://doi.org/10.3390/foods11142087. Article 2087.

Zhu, J. Y., Wakisaka, M., Omura, T., Yang, Z. F., Yin, Y. Q., & Fang, W. M. (2024). Advances in industrial harvesting techniques for edible microalgae: Recent insights into sustainable, efficient methods and future directions. *Journal of Cleaner Production, 436*, Article 140626. https://doi.org/10.1016/j.jclepro.2024.140626

Zhu, L. Y., Zhang, X. C., Ji, L., Song, X. J., & Kuang, C. H. (2007). Changes of lipid content and fatty acid composition of *Schizochytrium limacinum* in response to different temperatures and salinities. *Process Biochemistry, 42*, 210–214. https://doi.org/10.1016/j.procbio.2006.08.002

Zinnai, A., Sanmartin, C., Taglieri, I., Andrich, G., & Venturi, F. (2016). Supercritical fluid extraction from microalgae with high content of LC-PUFAs. A case of study: Sc-CO_2 oil extraction from *Schizochytrium* sp. *Journal of Supercritical Fluids, 116*, 126–131. https://doi.org/10.1016/j.supflu.2016.05.011

Zisis, F., Kyriakaki, P., Satolias, F. F., Mavrommatis, A., Simitzis, P. E., Pappas, A. C., … Tsiplakou, E. (2022). The effect of dietary inclusion of microalgae *Schizochytrium* spp. on ewes' milk quality and oxidative status. *Foods, 11*. https://doi.org/10.3390/foods11192950. Article 2950.