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Extraction of lipids from microalgae using classical and innovative approaches

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ABSTRACT

Microalgae, as a photosynthetic autotrophic organism, contain a variety of bioactive compounds, including lipids, proteins, polysaccharides, which have been applied in food, medicine, and fuel industries, among others. Microalgae are considered a good source of marine lipids due to their high content in unsaturated fatty acid (UFA) and can be used as a supplement/replacement for fish-based oil. The high concentration of docosahexaenoic (DHA) and eicosapentaenoic acids (EPA) in microalgae lipids, results in important physiological functions, such as antibacterial, anti-inflammatory, and immune regulation, being also a prerequisite for its development and application. In this paper, a variety of approaches for the extraction of lipids from microalgae were reviewed, including classical and innovative approaches, being the advantages and disadvantages of these methods emphasized. Further, the effects of microalgae lipids as high value bioactive compounds in human health and their use for several applications are dealt with, aiming using green(er) and effective methods to extract lipids from microalgae, as well as develop and extend their application potential.

1. Introduction

In recent years, the rapid growth of the global population has been accompanied by an increase in human demand for food, water, and various energy sources. With the continuous economic development, the sustainability of agriculture will be closely related to food safety in the next decades since more and more people pay attention to the impact of diet on health. For this, more functional foods are being developed to satisfy the people's needs. However, excessive agricultural production also threatens a sustainable development concerning climate and ecology. Therefore, it has become more urgent to reduce greenhouse gas emissions and ensure the sustainable development of resources under the premise of ensuring sufficient food supplies for the growing human population (Campi, Dueñas, & Fagiolo, 2021). Researchers have explored the bioactive compounds in a variety of animals and plants foods, which have been proven to have an impact on human health as components of functional foods (Görgüç, Gençdağ, & Yılmaz, 2020).

The ocean accounts for 70 % of the earth's surface and according to statistics, there are nearly 30,000 kinds of metabolites of marine organisms, which have become a treasure-house that can provide high-added-value compounds. The exploration and development of these compounds have laid the foundation for their application in for medicine, food, materials, and in other fields (Ali et al., 2021). Among these marine-derived bioactive compounds, the contribution of algae is about 30%.

Algae can be divided into macro- and microalgae, with macroalgae

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being usually termed seaweeds (Alvarez, Weyers, Goemann, Peyton, & Gardner, 2021). In Asian countries, such as Japan and Korea, macroalgae are already used as foods on a widespread basis. Compared with macroalgae and other marine organisms (fish, shellfish and so on), the utilization rate of microalgae is relatively low in food industry and it has development potential (Yamagata, 2021)

Microalgae are protist that can perform photosynthesis, including prokaryotic cyanobacteria and eukaryotic organisms such as green algae and grow mainly in fresh water and seawater (Mehariya, Goswami, Karthikeysan, & Verma, 2021). According to statistics, there are more than 1×10^5 to a 1×10^6 species of microalgae in nature that contain many macro and micro metabolites, including proteins, carbohydrates, lipids, phenols, and minerals. The bioactive compounds present in microalgae have been proven to have the potential to be used in medicine, food, bioenergy, and other fields (Jimenez-Lopez et al., 2021). Among them, the most abundant components are polysaccharides, such as carrageenan and fucoidans, which all show a variety of biological activities and are not degraded by enzymes in mammals. They are considered to be good dietary fibres, showing anti-tumour, anti-coagulation, and immune regulation effects (Carina, Sharma, Jaiswal, & Jaiswal, 2021). Proteins in microalgae are also present in a large proportion and the high content of amino acids provides another basis for application of microalgae. In addition, compounds such as phycocyanin in *spirulina* can be used as natural food colorants. The polyphenols of microalgae also make microalgae exhibit stronger antioxidant properties (Martelli, Folli, Visai, Daglia, & Ferrari, 2014). In the ocean, fish is the most important source of lipids and can be used as a source of high-quality lipids. The lipids content of microalgae is lower than in fish, but due to its high content of unsaturated fatty acids (UFAs), it is considered a potential source of marine lipids. Indeed, microalgae can be an alternative source of lipids and terpenes that are usually obtained from animals, such as whales and codfish, which raises increasing ethical and environmental issues.

The lipids in microalgae include polar lipids such as phospholipids and glycolipids, and neutral lipids such as triacylglycerol and UFAs. Polar lipids are mainly found in cell membranes and organelles whereas glycerol and UFAs are used for energy storage (Lupette & Benning, 2020). The type of microalgae, light, growth environment and temperature affect the lipids contents in microalgae. However, despite these variations, microalgae are still a source of high amount of polyunsaturated fatty acids (PUFAs) including docosahexaenoic (DHA) and eicosapentaenoic acid (EPA). These UFAs have also been proven to have antioxidant properties, prevent hypertension and showing immune regulation effects (Nascimento et al., 2020).

In addition to its high content in bioactive compounds, microalgae have other advantages that enable them to be widely used: a) microalgae grow in water, so they can save the land currently used for cultivation and relieve land pressure; b) compared with other crops, microalgae grow and reproduce extremely fast and can survive under harsher conditions; c) in agriculture production, microalgae can also be used to improve soil fertility by promoting soil nutrient cycling, which plays a positive role in crop growth (Alvarez et al., 2021). Overall, microalgae are regarded to have great normous potential further development and utilization.

Although algae resources are abundant, the current lipids extraction approaches are mostly based on animal-derived foods and for microalgae lipids still need to be further explored and summarized. Some high-efficiency and low-cost extraction methods have been explored to obtain and recover high-added value lipids in microalgae and greatly improve the utilization rate, which is also in line with the needs of sustainable development (Chen, Liu, Song, Sommerfeld, & Hu, 2020a).

This review aims providing an overview of classical and innovative approaches for lipids extraction from microalgae, based on existing studies, while aims to explore the effects of different approaches on its yield and biological activity, so as to develop its application potential.

2. Microalgae lipids-Extraction technology

Microalgae have thick-walled cell wall, and the release of their bioactive compounds is limited by the rigidity of the microalgae matrix. Therefore, appropriate pre-treatment and extraction methods should be selected to obtain lipids from microalgae and maintain their bioactivity becomes critically important. For a long time, some classical approaches, including Soxhlet extraction, Folch and Bligh-Dyer extraction have been used for lipids extraction (Fig. 1), but while these methods are usually simple to operate and low budget, what promotes their use, they also have disadvantages like large consumption of organic reagents and environmental pollution, in addition to the prolonged extraction times (several hours). In recent years, many innovative approaches have been explored for lipids extraction, such as supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) and others (Fig. 2). These innovative approaches overcome the shortcomings of classical approaches to a certain extent and at the same time, they are also beneficial to improve the extraction rate of lipids and maintain the bioactive activity of the extracts (Tables 1-2).

2.1. Traditional extraction approaches

2.1.1. Soxhlet extraction

Soxhlet extraction method was proposed as early as 1879 and was originally used to quantify the total lipids amount in milk (Soxhlet, 1879) and was further gradually promoted in the fields of food, pharmaceutical and other industries. Soxhlet extraction is a technique in which the sample is repeatedly in contact with the extractant during the extraction process, thereby increasing the yield of the extract. Although the traditional Soxhlet extraction has low cost and simple operation, it also has disadvantages such as long extraction time and large reagent consumption (Luque de Castro & García-Ayuso, 1998). In recent decades, based on the principle of Soxhlet extraction, some new methods have been used, such as high-pressure Soxhlet extraction, automatic Soxhlet extraction, microwave integrated assisted Soxhlet extraction technology and others (Luque de Castro & Priego-Capote, 2010).

As a solvent-based lipid extraction method, Soxhlet extraction is still one of the common methods for extracting lipids form microalgae. In order to evaluate the effect of Soxhlet extraction on the lipid́s recovery from microalgae, Ramluckan et al. (Ramluckan, Moodley, & Bux, 2014) studied 13 types of solvents with different polarities (including hexane, chloroform, toluene, acetone, methanol, etc.) on *Chlorella* sp. lipids extraction. When compared with other reagents, ethanol, chloroform, and hexane showed the higher extraction rates (≈10.78 %), and 3 h was the best extraction time. In addition, the mixture of chloroform: ethanol (1:1) also showed a high extraction rate with a value of about 11.76 %. Aravind et al. (Aravind, Barik, Ragupathi, & Vignesh, 2021) used *n*-hexane for *Spirulina* sp. lipid's extraction and found that completely dry and fine samples had a higher extraction rate, and the optimal extraction time was also 3 h. This may be because there is more contact between the smaller size of the *Spirulina* sp., and the solvent, thereby increasing the extraction rate. After many years of development, Soxhlet extraction has become a standard technique to measure the efficiency of lipid extraction as well as its shortcomings are gradually being overcome due to continuous development by researchers. However, there are still some disadvantages, including long extraction time, large reagent consumption and unfavourable environment.

2.1.2. Folch extraction

The Folch method was proposed in 1957 using a mixture of chloroform: methanol (2:1 *v/v*) to extract lipids from animal fat (Jordi, 1957). In this method, a mixture of chloroform and methanol are first used to extract the lipids, then water is added to achieve phase separation, and finally the extracted lipids can be obtained after rotary evaporation. This method does not need high temperature and/or high pressure during the

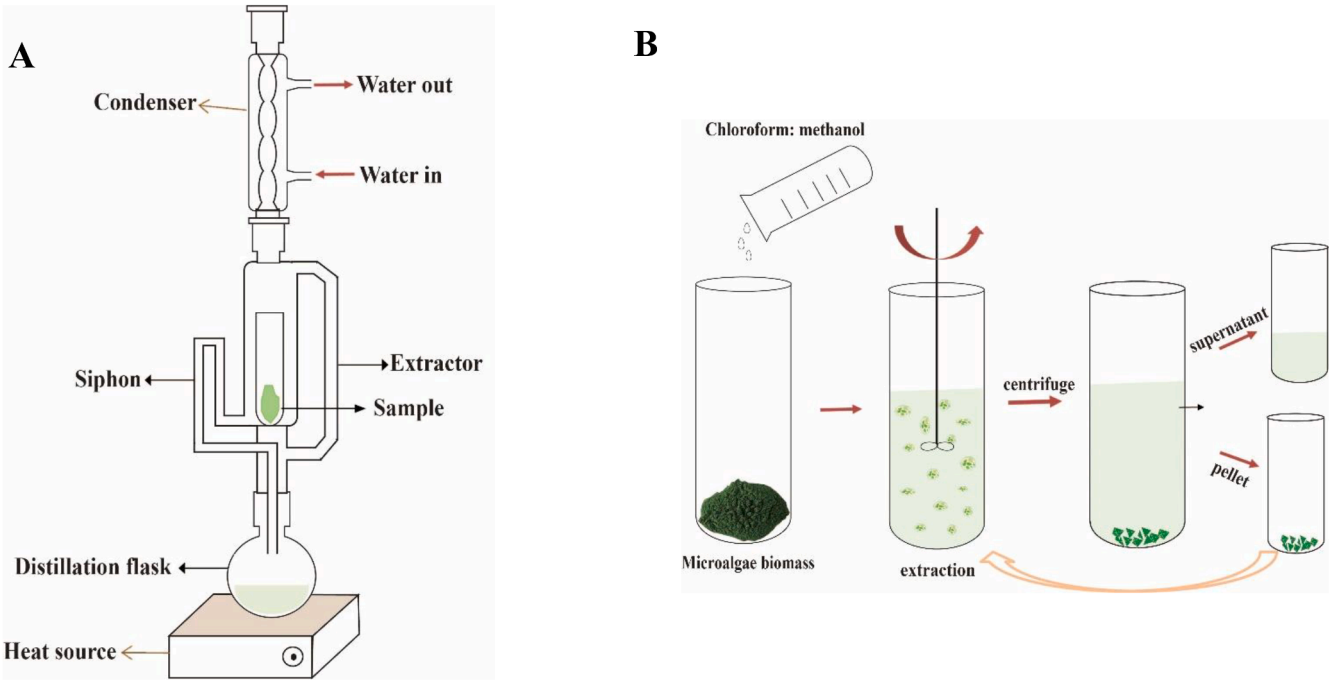

Fig. 1. Schematic diagram of classical approaches: A) Soxhlet extraction, B) Folch/Bligh-Dyer extraction.

extraction process and it is one of the reliable methods for lipids extraction.

As a classical method, Folch is often compared with Soxhlet extraction. For instance, Onay et al. (Onay, Sonmez, Oktem, & Yucel, 2016) evaluated the effects of various extraction methodologies (including Soxhlet, Folch and Bligh-Dyer, assisted by cell lyophilization, homogenization and ultrasound), on the lipids extraction of different thermoresistant microalgae (*Hindakia*, *Scenedesmus* and *Micractinium Species*). The three extraction methods showed different extraction rates on microalgae and among them Soxhlet proved to have a higher extraction rate for *Micractinium* spp., while Folch extraction was more suitable for *Scenedesmus spp*. In addition, when other assisted extraction technologies were applied, such as lyophilization and ultrasound, a higher lipids extraction rates were achieved.

Similarly, based on green solvents, Jesus et al. (Jesus et al., 2019) also compared the effects of Soxhlet, Folch and Bligh-Dyer on the extraction of *Chlorella pyrenoidosa* lipids. The results showed that when the solvent was a mixture of chloroform: methanol (2:1 *v/v*), Folch and Bligh-Dyer showed higher extraction rates, corresponded to 113.47 ± 7.58 and 115.05 ± 5.32 (mg lipids/g biomass) respectively (the fatty acid content was also higher than with other solvents). When traditional solvents were used (singly), Folch and Bligh-Dyer also showed high lipids extraction capabilities.

In order to achieve better extraction results, Kumari et al. (Kumari & Singh, 2019) improved Folch process by using a combination of different polar reagents (chloroform and methanol) the results showed that not only the extraction rate of lipids was improved, but also promoted the release of other components from microalgae. As a simple and fast extraction method, Folch can be used for lipids extraction of a large number of samples, but it uses more toxic reagents, which is harmful to human health and environment.

2.1.3. Bligh-Dyer extraction

The Bligh-Dyer extraction method is a method based on a two-phase solvent extraction and it can be considered as a variant of Folch (Bligh & Dyer, 1959) in which a mixture of chloroform–methanol-H_2O is used as extraction solvent. Furthermore, Bligh-Dyer can separate lipids from the chloroform phase while allowing proteins to be precipitated between different phases.

This methodology has been widely used in the extraction of microalgae lipids. Ellison et al. compared the effects of standard Bligh-Dyer and hexane (non-polar solvent) extraction on the extraction of total lipids of *Chlorella vulgaris/Cyanobacteria leptolyngbya* (Ellison, Overa, & Boldor, 2019). Compared with the extraction method using the non-polar solvent, the average lipids mass extracted by the Bligh-Dyer is twice that of hexane extraction, which makes it a promising method for lipids extraction. Dejoye Tanzi et al. (Dejoye Tanzi, Abert Vian, & Chemat, 2013) compared the effects of Soxhlet, Bligh-Dyer and a new technology called Simultaneous Distillation and Extraction Process (SDEP) on the extraction of two different microalgae (*Nannochloropsis oculata* and *Dunaliella salina*) lipids. The results showed that for *Nannochloropsis oculata*, SDEP-cymene and Bligh-Dyer methods have similar lipids extraction yields of 21.45 and 23.78 %, respectively, while for *Dunaliella salina*, SDEP-pinene and Bligh-Dyer provided the highest lipids extraction yields at 3.29 and 4.03 %. As well as in the previous study of Onay et al. (Onay et al., 2016), the extraction rate (52.5 % *w/w*) of *Chlorella vulgaris* lipids using the ultrasound-assisted Bligh-Dyer method was the highest compared with Soxhlet and Folch extraction. As a method close to Folch, Bligh-Dyer also uses toxic reagents, which generates a large amount of environmentally harmful waste. Therefore, waste recovery and cost need to be considered when it is used for large-scale extraction.

The above mentioned three lipids extraction methods commonly use toxic solvents such as methanol, chloroform, *n*-hexane, etc. Indeed, these solvents have negative impacts on both human health and environment and is in inconformity with the requirements for sustainable development. Therefore, in order to reduce the pollution caused by toxic reagents, some green extraction reagents are being explored to replace traditional toxic reagents (cyclopentyl methyl ether, 2-methyltetrahydrofuran, etc.), aiming to achieve a greener lipids extraction method (Jesus et al., 2019).

2.1.4. Other extraction techniques

Regarding traditional extraction processes, in addition to solvent-based extraction methods, mechanical-based methods can also be used in microalgae lipid's extraction, such as mechanical pressing, bead

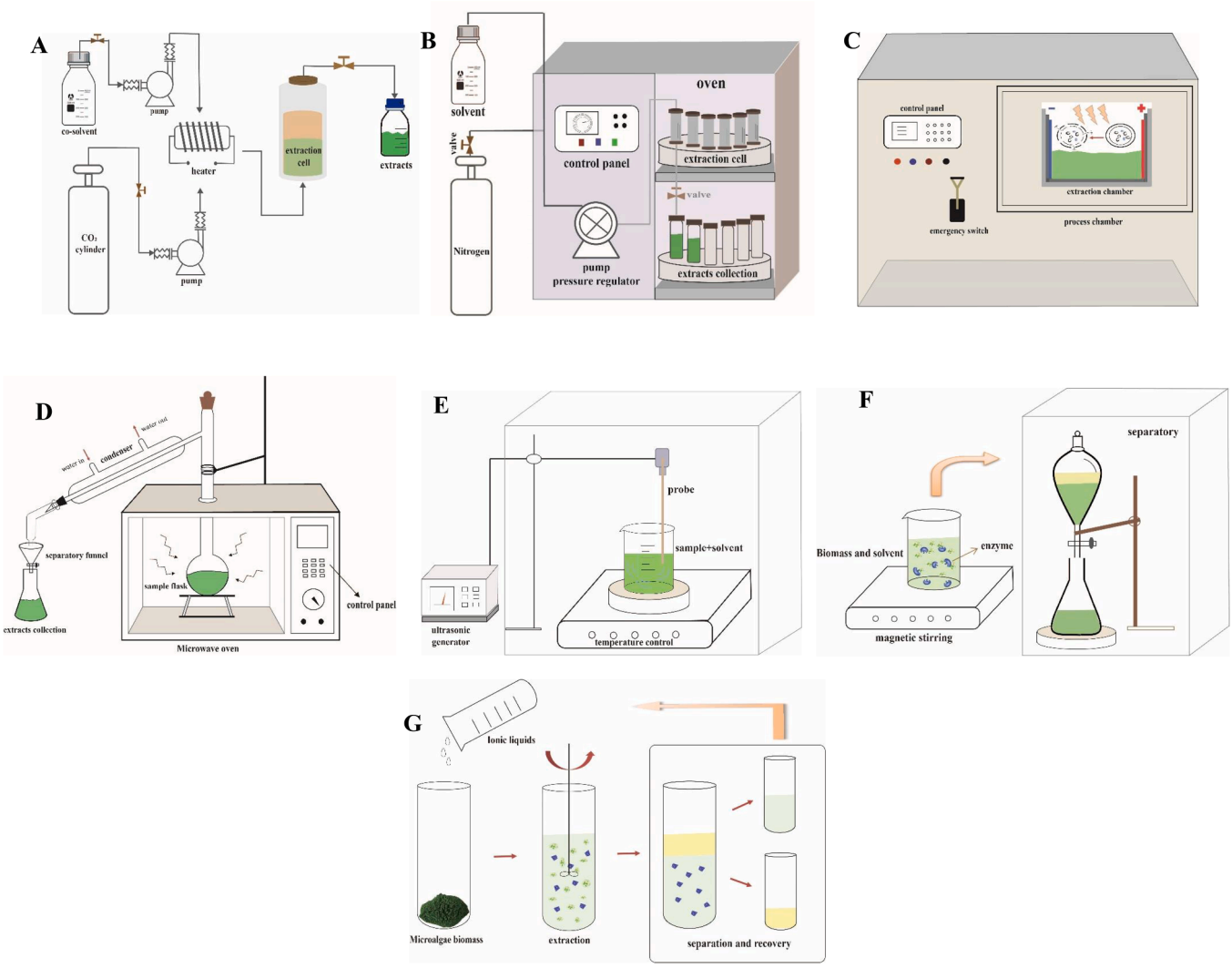

Fig. 2. Schematic diagram of innovative approaches: A) supercritical fluid extraction (SFE), B) pressurized liquid extraction (PLE), C) pulsed electric fields (PEF), D) microwave-assisted extraction (MAE), E) ultrasound-assisted extraction (UAE), F) enzyme-assisted extraction (EAE), G) ionic liquids (ILs).

Table 1
Advantages and disadvantages of different extraction methods.

	Method	Advantages	Disadvantages
Traditional extraction methodologies	Soxhlet	Low cost; simple operation; high extraction rate.	Long extraction time, large reagent, and energy consumption.
	Folch	Fast, easy to handle large number of samples, the complete process is gentle.	Toxic reagents are used, which is harmful to human health and environment.
	Bligh-Dyer	Lipid extraction and separation can be achieved at the same time.	Extractive reagents are toxic and have few substitutes, the cost is high.
Emergent extraction methodologies	Super/subcritical fluids/ pressurized lipids extraction	High extraction efficiency, less use of toxic reagents and easy separation of lipids; protect bioactive compounds, reduce energy consumption and pollution.	It has selectivity to lipids of different polarity and the equipment is more expensive.
	Pulsed electric fields	The operation is simple and pollution-free; processing of large number of samples.	It is necessary to control the proper electric field strength. Electric field too high may adversely affect the extraction.
	Ultrasound-assisted extraction	The temperature in the process is low, and the energy required is less. High extraction rate can be achieved in a short time.	The intensity and time of ultrasound need to be controlled to avoid negative effects.
	Microwave-assisted extraction	Reduced extraction time and energy consumption, improve the extraction efficiency.	The polarity of the solvent has an impact on the extraction and is not suitable for treatment of heat sensitive substances.
	Ionic liquids	Low toxicity and high stability, with adjustable physical and chemical properties.	Possibility of pollution during synthesis.
	Enzyme-assisted extraction	Selective to substrate, pre-treatment can be completed at room temperature and pressure to reduce energy consumption.	The price of enzyme preparation is high, it is necessary to optimize the conditions to get the highest extraction rate.

Table 2
Application of various innovative extraction methods in the extraction of microalgae lipids.

Microalgae	Solvent	Conditions	Results	Reference
Supercritical carbon dioxide (SC-CO_2)				
Nannochloropsis oculata, *Chlorella vulgaris*	Ethanol (10 %, as cosolvent)	T: 50 °C P: 250/450/750 bar CO_2 flow: 25 g/min Solvent rate: 0.35 ~ 1.02 g/g·min^{-1} Ethanol flow rate: 1.9 g/min	The neutral lipids extraction rate of *C. vulgaris* was 97 %; the extraction rate of *N. oculate* was 83 %.	(Obeid et al., 2018)
Chlorella protothecoides		T: 70 °C P: 150 ~ 300 bar CO_2 flow: 75 CO_2 h^{-1} kg biomass^{-1}	Oil extraction yield was 21%.	(Viguera et al., 2016)
Schizochytrium sp.		T: 40/55 °C P: 25/40/55/70 MPa CO_2 flow: 10 kg/h	Compared with *n*-hexane extraction (Soxhlet extraction), SC-CO_2 has higher extraction rate, and the product has higher nutritional value (high ratios ω3/ω6 and DHA/EPA).	(Zinnai et al., 2016)
Scenedesmus obliquus, *Scenedesmus obtusiusculus*		T: 20 ~ 200 °C P: 7 ~ 80 MPa CO_2 flow: 10 kg/h CO_2 to biomass ratio: 20–200	The lipids extraction rate was higher than Bligh-Dyer method, and the recovery rate of lipids reached 92 % (*w*/*w*).	(Lorenzen et al., 2017)
Scenedesmus obliquus, *Chlorella protothecoides*, *Nannochloropsis salina*	Ethanol (5 %, co-solvent)	T: 45 °C P: 15/20/25/30 MPa CO_2 flow: 0.4 ± 0.05 kg/h	Compared with Soxhlet extraction (methanol: chloroform 2:1 was used as solvent), SC-CO_2 has higher extraction rate and is beneficial to the percentage of ω-3.	(Solana, Rizza, & Bertucco, 2014)
Subcritical fluid extraction				
Phaeodactylum tricornutum, *Nannochloropsis oculata*, *Porphyridium cruentum*	*n*-butane	T: 40 °C P: 15 bar Solvent flow: 3 mL/min	Compared with SC-CO_2, more PUFA (ω-3 and ω-6) can be obtained by subcritical *n*-butane extraction.	(Feller et al., 2018)
Nannochloropsis salina	water	T: 220/205 °C Time: 25 min	Compared with the classical method (Folch extraction), the extraction rate of lipids under subcritical water method is 70 %, with microwave assistance, the extraction rate can achieve 100 %. meanwhile, the energy was reduced by 2–8 times.	(Reddy et al., 2014)
T. obliquus	Dimethyl ether	T: 20 °C P: 0.55 MPa	Microwave, ultrasound, and heat assisted extraction of subcritical dimethyl ether can improve the extraction rate of lipid by changing the permeability of cell wall.	(Wang et al., 2021)
Pressurized fluid extraction (PLE, traded as accelerated solvent extraction, ASE)				
Scenedesmus sp., *Chlorella zofingiensis*, *Isochrysis galbana*	methanol/DMSO (9:1 *v*/*v*, 1 cycle) hexane/diethyl ether (1:1 *v*/*v*, 2 cycles)	T: 120 °C P: 1500 psi	Compared with classical method (Folch extraction), ASE has a higher extraction rate and reduces lipid oxidation.	(Chen et al., 2020b)
Nannochloropsis oculata	*n*-hexane, *n*-hexane/2-PrOH (2:1 vol%) ethanol (96 %)	T: 60 °C P: 10–12 MPa Time: 0.8 h	Among the three solvents, ethanol had the highest extraction rate (36 ± 4 mass%), while *n*-hexane had the lower extraction rate (6.1 ± 0.3 mass%).	(Pieber, Schober, & Mittelbach, 2012)
Pulsed electric field (PEF)-assisted extraction				
Ankistrodesmus falcatus	chloroform ethyl acetate	Pulse repetition frequency: 240 Hz Captured pulses: 24	PEF-assisted green solvent ethyl acetate extraction resulted in higher extraction rate of lipids than chloroform, which could be attributed to the fact that 90 % of cells were lysed under PEF.	(Zbinden et al., 2013)
Chlorella		Voltage: 35 kV Pulse repetition rate: 1 ~ 1 k Hz Square wave pulse: 2 ~ 99 μS	PEF treatment increased the extraction yield of lipids to 166 %.	(R. Zhang et al., 2021b)
Chlorella pyrenoidosa	hexane/ethanol	Voltage: 20 kV Pulse frequency: 150 Hz Pulse width: 0 ~ 10 μs	PEF pre-treatment leads to defects on the cell surface and release of intracellular water-soluble substances, increasing lipid extraction.	(Han et al., 2019)
Ultrasound-assisted extraction (UAE)				
Scenedesmus sp.	hexane	Ultrasonic power: 0 ~ 50 W Time interval: 0 ~ 5 s Time: 0 ~ 10 min	The optimal treatment condition was 20 W, 2 s time interval, 4 min treat time, the total lipid yield increased from 0.76 h/L to 1.31 g/L.	(Sivaramakrishnan & Incharoensakdi, 2019)
Nanoochloropsis sp.	hexane	Time: 1/3/5 min Intensity of ultrasonic: 20 kHz	High-intensity ultrasonication can increase lipids recovery by destroying microalgal cells, the yield is about 6.5 times higher than of high-pressure homogeneity.	(Yao et al., 2018)
Microwave-assisted extraction (MAE)				
Nannochloropsis sp., *Tetraselmis* sp.		Thermocouple: 65 °C Microwave radiation: 500 W	For the two species of microalgae, the maximum lipid extraction rates were: microwave-assisted	(Teo & Idris, 2014)

(*continued on next page*)

Table 2 (*continued*)

Microalgae	Solvent	Conditions	Results	Reference
			Hara-Radin extraction (8.19 %) and microwave-assisted Folch extraction (8.45 %).	
Chlorella PY-ZU1	chloroform:methanol (1:1 *v/v*)	T: 80 ~ 120 °C Time: 5/10 min	Microalgae were treated at 80 °C for 26 min, and the cell walls were destroyed at the maximum curvature. The increase in microwave temperature also increases the degree of damage to the cell wall. In addition, the microwave electromagnetic effect also led to the increase of short chain and saturated fatty acid in the extract.	(Cheng et al., 2013)
Ionic liquids extraction (ILs)				
Chlorella sorokiniana, Nannochloropsis salina, Galdieria sulphuraria	1-butyl-3-methylimidazolium hydrogen sulphate [BMIM][HSO_4]	[BMIM][HSO_4]-microwave: T: 120 °C Power: 800 W Time: 10 ~ 60 min [BMIM][HSO_4]-ultrasound: T: 120 °C Power: 800 W Time: 60 min	Compared with the classical Bligh-Dyer method, [BMIM][HSO_4]-microwave extraction could improve the lipid extraction rate to 1990 %, 370 % and 1170 %. The addition of [BMIM][HSO_4] makes the lipids easier to extract.	(Pan et al., 2016)
Nannochloropsis oculata, Chlorella salina	Ten carboxylate protic ionic liquids (PILs) with lactam and ammonium cations	PIL-sonication treatment: Intensity of ultrasonic: 20 kHz Power: 100 W Time: 1 min	Hexanoate and formate PILs exhibited enhanced lipids recovery, PILs also showed an inhibitory effect on lipase.	(Mukund et al., 2019)
Enzyme-assisted extraction				
Chlamydomonas reinhardtii	lysozyme, collagenase, trypsin, autolysin		Enzymatic pre-treatment can degrade the cell walls of microalgae, thereby facilitating the extraction of lipids and proteins from biomass.	(Sierra et al., 2017)
Photoelectrochemical system				
Chlorella	hexane, isopropanol	Cathode: P-Pd Photoelectric anode: 0.5 M, 1 M, and 1.5 M nitrogen (N)-doped TiO2 nanotube Anode electrolyte: Na_2SO_4	The •OH produced by the photochemical system attacks the cell walls and membranes of microalgae, facilitating lipid extraction.	(Wu et al., 2021)
Osmotic shock				
Chlamydomonas reinhardtii	methanol: *n*-hexane (10:0, 7:3, 4:6 *v/v*)	T: 20 °C Speed: 20 ~ 25 rpm (1 d)	After osmotic shock treatment, lipids recovery of microalgae biomass can be increased by more than three times.	(Yoo et al., 2012)

beating (also known as bead milling) and homogenization.

Extracting lipids from raw materials by pressing and compressing is an ancient and simple method. Chemical reagents are unnecessary in this method, instead, uniaxial high pressure is used directly to rupture the cell walls of biomass to extract lipids. In this method choose a suitable pressure for lipids extraction is a key factor because excessive pressure or heat production will reduce the extraction rate of lipids and have a negative impact on the quality of the extracts. Compared with solvent extraction, the extraction efficiency of pressing is lower, which accounts about 75 % of solvent extraction (Topare et al., 2011). Bead beating is also an assisted lipids extraction method, which allows the sample cells to be destroyed when passing through high-speed moving beads. The density of the beads determines the final state of the sample being processed but a large amount of energy needs to be consumed during the processing, which increases production costs (Günerken et al., 2015). Similar to bead beating, homogenization is also an effective method to rupture microalgae cell walls. The high-energy bubbles produced by homogeneous cavitation can cause cell rupture, which is a simple and rapid method for cell destruction. Mulchandani et al. (Mulchandani & Kar, 2015) used high-pressure homogenization to extract lipids from *Chlorella saccharophila*. Compared with the chloroform–methanol solvent extraction method, the lipids recovery rate of high-pressure homogenization was nearly 89.91 ± 3.69 % (*w/w*). The main disadvantage of this method is that the considerable temperature increase caused by homogenization, which will destroy/alter bioactive compounds in the biomass. However, with the improvement and development of technology, now these technologies are mostly used for the pre-treatment of microalgae, and they need to be combined with other technologies to achieve better extraction results.

2.2. Innovative approaches

2.2.1. Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) has been proven to be one of the effective methods for extracting lipids. Supercritical fluids mainly refer to compounds in a critical state, where its pressure and temperature are above the critical point (Nagappan et al., 2019). The unique intermediate characteristics make supercritical fluids to have transportability properties like gases and the solubility like liquids and several. As a kind of non-toxic solvent for lipids extraction, several supercritical fluids have the characteristics of environmental-friendly solvents.

Substances that can be used as supercritical extractants include carbon dioxide (CO_2), methanol, ammonia, and others. Among them, supercritical carbon dioxide (SC-CO_2) is the most widely used, because of low costs and non-toxicity and low critical temperature and pressure, which can avoid the degradation of heat-sensitive compounds during the extraction process and reduce operation cost (Bhargavi, Nageswara Rao, & Renganathan, 2018). In addition, under normal temperature and pressure, CO_2 is gaseous and can be separated directly from the extracts easily, thus disregarding the need of solvent separation (by distillation, etc.).

In terms of microalgae lipids extraction, SC-CO_2 can be used as an

alternative to organic solvent extraction to increase the extraction rate. Obeid et al. (Obeid et al., 2018) used SC-CO_2 combined with ethanol as co-solvent to extract neutral lipids from freeze-dried *Nannochloropsis oculata* and *Chlorella vulgaris*. Under the optimal condition, the lipids extraction rates of *Nannochloropsis oculata* and *Chlorella vulgaris* were 83 % and 97 %, respectively. Similarly, Viguera et al. (Viguera, Marti, Masca, Prieto, & Calvo, 2016) also optimized the conditions for SC-CO_2 extraction of lipids from microalgae (*Chlorella protothecoides*), showing the highest lipids extraction rate at 300 bars and 70 °C. In addition to improving the extraction rate, SC-CO_2 also affects the fatty acids composition in the extract. Zinnai et al. (Zinnai, Sanmartin, Taglieri, Andrich, & Venturi, 2016) used SC-CO_2 and *n*-hexane to extract lipids from *Schizochytrium* sp. and the long-chain PUFAs were analysed. Compared with *n*-hexane, the lipids obtained by SC-CO_2 extraction have higher nutritional value, which is mainly represented by a high ratio of ω-3/ω-6 and DHA/EPA. Lorenzen et al. (Lorenzen et al., 2017) compared SC-CO_2 and Bligh-Dyer extraction to extract and purify lipids from *Scenedesmus obliquus* and *Scenedesmus obtusiusculus* biomass and found that at 12 MPa (120 bar) and 20 °C, the lipids extraction rate can reach 92 %, indicating the potential application of SC-CO_2 technology for industrial use, due to the high extraction rate and mild operational conditions (12 MPa and 20 °C). Because of the non-polarity of CO_2, it is easier for SC-CO_2 to extract neutral lipids that are not combined with polar lipids. In order to increase extraction rate and improve the solubility of more polar lipids, some co-solvents can be applied to the extraction process. For example, the above cited work of Obeid et al. (Obeid et al., 2018) used ethanol as co-solvent to increase the extraction rate. De Melo et al. (De Melo et al., 2020) also used the ethanol as the co-solvent and set different processing conditions to recover lipids from *Aurantiochytrium* sp. biomass. Under the optimal extraction conditions, the DHA content in the extract is about 3.5 times that of the ordinary fish oil. Anyway, still high-priced equipment limits the large-scale use of SC-CO_2 to a certain extent.

2.2.2. Pressurized liquid extraction (PLE)

Pressurized Liquid Extraction (PLE), also known as Accelerated Solvent Extraction (ASE), is a technology to extract solid or semi-solid samples using the combination of temperature and pressure, which can better retain bioactive compounds and shorten the extraction time as well as has been widely studied (Ruiz-Domínguez et al., 2021). As a clean and green solvent, water is often used for subcritical extraction, since when water is heated over the boiling point and below the critical point, as well as the pressure is controlled to keep it in a liquid state, water reaches a state that is called a subcritical state. In this state, the polarity and dielectric constant of water change, with potential to achieve a possible better extraction effect. The use of water as a solvent is also called subcritical water extraction (Wani, 2021). Reddy et al. (Reddy et al., 2014) used subcritical water and microwave-assisted subcritical water to extract lipids from wet *Nannochloropsis salina* algal biomass. The results showed that the extraction efficiency of subcritical water is much higher than that of the traditional Folch method, about 70 % *vs* 30 %. In addition, when using microwave assistance, all lipids can be extracted, and the energy consumption is reduced by 2 ~ 8 times. In addition to water, several other solvents are also used in subcritical extraction, such as dimethyl ether, *n*-butane, propane and others (Wang, Oshita, Takaoka, & Shiota, 2021). Wang et al. (2021) used subcritical dimethyl ether, a solvent that is not harmful to the environment, to extract lipids from *T. obliquus*. Microwave and ultrasounds are sometimes combined with subcritical extraction enhance cells disruption to facilitate extraction. The results showed that, compared with the Bligh-Dyer extraction, subcritical dimethyl ether can effectively extract lipids from microalgae, and the assisted extraction techniques such as microwave or ultrasound can enhance the permeability of the cell wall, thereby improving the extraction efficiency. Nowadays, some bio-based solvents are also used in PLE to replace traditional reagents because of their green and degradable properties. Golmakani et al. (Golmakani, Mendiola, Rezaei, & Ibáñez, 2014) used limonene, ethanol, and hexane as solvents to extract lipids from a variety of microalgae (*Spirulina*, *Phormidium*, *Anabaena* and *Stigeoclonium*) using PLE, and the fatty acids composition was also analysed. The results showed that the extraction of *Spirulina* lipids with limonene:ethanol (1:1, *v/v*) resulted in the highest extraction rate and the higher content of omega-3 fatty acids. Similarly, Ruiz-Domínguez et al. (Ruiz-Domínguez et al., 2021) used ethanol, water and limonene extracts as solvents and designed response surface experiments to explore the effect of PLE on the extraction rate of bioactive compounds from *Geitlerinema* sp. When water and ethanol were used as solvents, the extraction rate of phycobiliproteins is improved, while ethanol: limonene extract is more effective for the recovery of lipids and methyl palmitate. The application of these bio-based solvents makes the extracts easier to be applied to the food and pharmaceutical industries.

2.2.3. Pulsed electric field (PEF)

As a nonthermal processing method, pulsed electric field (PEF) has been widely used in the food industry, including the extraction of bioactive compounds, food preservation and drying (Barba et al., 2015). The main principle of PEF is the "electroporation" theory, that is, high voltage PEF is applied to treat the sample during a short period of time, which causes the cell membrane to rupture and form temporary/permanent holes (pores). In this way, the nutritional content and sensory quality of the food will be retained to a greater extent. The application of PEF may cause changes in the conductivity and rigidity of the microalgae cell wall, thereby increasing the extraction rate (Gómez et al., 2019). In Silve's et al. (Silve et al., 2018) research, the effects of PEF-assisted processing combined with ethanol-hexane blends solvent extraction on the lipid extraction from *Auxenochlorella protothecoides* were explored. The results showed that the PEF treatment may lead to cell permeabilization, thereby causing the release of intracellular ions, increasing the conductivity of the sample and the lipids recovery rate could reach 90 %. Moreover, Nile red staining was applied to verify that the cell structure is still intact under the PEF treatment. Zhang et al. (Zhang et al., 2021a) also used PEF to extract *Chlorella* lipids and explored the relationship between lipids extraction and cell breakdown under PEF treatment. Han et al. (Han et al., 2019) also explored the effect of PEF-assisted treatment on the extraction of *Chlorella* lipids, with the results showing that PEF-assisted Bligh-Dyer extraction can effectively enhance the lipids extraction rate of *Chlorella pyrenoidosa*, which was 12 % higher than ultrasonic pre-treatment. The PEF-assisted technology causes surface defects in the cell wall and promotes the contact between the extraction solvent and the lipids to increase the extraction rate. In general, PEF is used to extract microalgae lipids mainly by increasing the permeability of the cell wall to increase the extraction rate, and it can process a large amount of sample, since it can operate, and usually operates in continuous, and it is considered a low-polluting extraction technology.

2.2.4. Ultrasound-assisted extraction (UAE)

Ultrasounds are mechanical waves that propagates through compression and sparseness in a media. In the process of lipid-assisted extraction technology, it mainly destroys cells through cavitation and acoustic effects (Saini & Keum, 2018). The solvent generates bubbles under the action of ultrasonic waves, which burst due to the reaction force when contacting the cells and generate local shock waves to destroy the cells, resulting in an increase in the lipids extraction rate (Sallet et al., 2019). Compared with classical methods, UAE is simple to operate, requires less energy and is a fast and effective assisted extraction technology (Li, Gao, Liu, Zhang, & Zhang, 2018). There are both reports on the use of high-power and low-power ultrasound to extract lipids from microalgae. Sivaramakrishnan et al. (Sivaramakrishnan & Incharoensakdi, 2019) used low-power ultrasound-assisted hexane to extract lipids from *Scenedesmus* sp. biomass. Under optimized conditions, the lipids yield increased from 0.76 to 1.31 g/L. In addition to

increasing the yield of lipids, the assistance of ultrasound also increased the release rate of carotenoids and oxygen, resulting in higher photosynthesis efficiency. Yao et al. (Yao, Mettu, Law, Ashokkumar, & Martin, 2018) also confirmed that high-power ultrasound can destroy *Nannochloropsis* sp. cells. The results showed that when the treatment time is within 1 to 3 min, the cell rupture increases with time, and the rupture effect on the cells decreases after the treatment time exceeds 5 min, which effectively increases the lipid yield in a short time, from 11 to 70 %. Araujo et al. (Araujo et al., 2013) also confirmed that UAE can improve the extraction rate of microalgae lipids, by using ultrasound combined with five methods including Bligh-Dyer, Folch and Soxhlet to extract lipids from *Chlorella vulgaris*. The results showed that the extraction rate of lipids under ultrasound Bligh-Dyer (52.5 %) was higher than other methods, and this might be due to the increased weakness of the cell wall promoted by ultrasounds, leading to cell rupture. Additionally, Pez et al. (Pez, Rech, Damasceno, Marczak, & Domeneghini, 2017) used UAE with ethanol to extract lipids and carotenoids from *Heterochlorella luteoviridis* and found that when the ultrasound intensity is 40 ~ 80 % and the ethanol concentration is 60 ~ 75 %, the lipids extraction only increases with the increase of ethanol concentration, which was an expected result, as a higher ethanol content should dissolve higher amounts of lipids, thus increasing the extraction rate. It can be inferred that the intensity and frequency of ultrasound will affect the extraction rate, although a proper selection of the extraction solvent is of upmost importance.

2.2.5. Microwave-assisted extraction (MAE)

Microwave mainly refers to non-contact electromagnetic radiation with an energy of 0.3 ~ 3000 GHz. In the process of microwave treatment, heat will be transferred from the inside to the outside through the medium to heat the entire samples. When microwave is used to extract bioactive compounds, the pressure rises after the cells absorb energy and the cell wall is destroyed so that the bioactive compounds can be extracted into the solvent (Paré et al., 1997). Solvents of different polarities have different microwave absorption capabilities, and strong polar solvents have better microwave absorption capabilities (Virot, Tomao, Ginies, Visinoni, & Chemat, 2008). MAE has been used to extract a variety of bioactive compounds from microalgae, such as polysaccharides and lipids (Yao et al., 2018; Zhao, Zhang, & Zhou, 2019) and it can significantly increase the yield of lipids, but the most suitable method for different microalgae is microalgae dependent. For example, microwave assisted Folch extraction can significantly increase the lipids recovery rate of *Nannochloropsis* sp. (Teo & Idris, 2014). Moreover, the extraction time and temperature can have an important influence on the microwave extraction effect. Cheng et al. (Cheng et al., 2013) used the microwave-assisted Bligh-Dyer method to extract lipids from microalgae and explored the effects of processing conditions on the extraction rate. When the microwave processing temperature is within 80 ~ 120 °C, the increase of temperature leads to the increase of the fractal dimension of cell, which also reflects the increase in the degree of damage to the cell wall. On the other hand, as the processing time increases, the pore size on the cell wall gradually increases, making lipids extraction easier to achieve. Many studies have used MAE to extract biologically active substances from microalgae, but considering its working principle, the biomass is heated under microwave action to cause cell wall rupture, so the disadvantage of this method is that it is not suitable for the extraction of heat-sensitive compounds.

2.2.6. Ionic liquids extraction (ILs)

ILs are a low-melting salt with anions and cations (generally below 100 °C), some of which can be liquid at room temperature. By selecting different ions solvents for specific purposes can we obtain it, which makes ionic liquids customizable (in tailor made way) and leads to a large number of ILs, about 10^6 types currently (Skoronski, Fernandes, Malaret, & Hallett, 2020). Common cations in ionic liquids, such as alkylammonium, alkylnitride, etc., have asymmetry that restricts the accumulation of ILs crystals, which leads to the generation of low melting-point solvents (Prusty, Pradhan, & Mishra, 2021). In general, ILs shows high stability and non-flammability. Moreover, because ILs have good solubility for lignocellulose, one of the main components of cell walls, ILs are potential good disruptors of the cell wall, thus being also potential to extract lipids from microalgae, since they can be useful to overcome the obstacles of the hard and thick cell walls of microalgae during the extraction process (Kilpeläinen et al., 2007).

Pan et al. used ILs-assisted ([BMIM]HSO_4) microwave/ultrasound to extract lipids from different microalgae and compared with traditional methods, microwave- ILs can increase the lipid extraction rate of *Chlorella sorokiniana*, and that of *Nannochloropsis salina* by 10-fold. ILs can effectively promote the extraction of microalgae lipids without affecting fatty acids composition (Pan et al., 2016). Mukund et al. (Mukund, Pratap, Ramesh, Krishnamurthi, & Mathur, 2019) also used ILs to pre-treat two types of microalgae (*Nannochloropsis oculata.* and *Chlorella salina*) and recover lipids, using ten different ILs, with Butyrolactm hexanoate showing strong cell disruption ability, being able to destroy about 84 % of cells, and the lipids recovery rate for the *Nannochloropsis oculata* and *Chlorella salina* reached 134.9 % and 85.4 %, respectively, compared with Bligh-Dyer method. In addition, ILs also inhibits enzyme activity inhibition and prevent lipolysis during wet extractions. However, not all ILs are green and non-toxic, while some ILs may be accompanied by the production of toxic substances during the synthesis process, which may affect the environment.

2.2.7. Enzyme-assisted extraction

As aforementioned, breaking the cell wall of microalgae is the core step in the extraction of its biologically active ingredients. Therefore, in order to improve the extraction rate, researchers have adopted a variety of methods to destroy the cell wall of microalgae (such as PEF, UAE, bead milling, etc.), but this usually requires the use of expensive equipment (Günerken et al., 2015). To overcome these difficulties, some biochemical methods have been used to improve the extraction rate, such as enzyme-assisted extraction. Compared with other mechanical treatments, enzyme-assisted cell destruction has a higher substrate selectivity and can be carried out under normal pressure and low temperature, effectively reducing the energy consumption (Zhang et al., 2018). Enzyme-assisted extraction is also considered to be a method that can be explored to replace classical methods to obtain a variety of bioactive compounds from microalgae, as normally these enzymes target the microalgae cell walls. Previous studies have shown that the use of enzymes to extract lipids from microalgae not only improve the extraction rate, but also facilitate the fractionation of lipids (Alavijeh, Karimi, Wijffels, van den Berg, & Eppink, 2020). He et al. (He et al., 2020) used four different hydrolases (cellulase, hemicellulase, papain and pectinase) to disrupt *Nannochloropsis* cells and combined the third phase partitioning to extract lipids. The results showed that comparing the extraction effects of four hydrolytic enzymes on microalgae lipids, the extraction rate after cellulase treatment was higher, and the combination of the four enzymes increased the extraction rate of microalgae lipids by 2-fold. Generally, enzyme-assisted extraction combined with other extraction methods can achieve better extraction results. Alavijeh et al. (Alavijeh et al., 2020) combined bead milling and enzymatic hydrolysis to separate and extract lipids, proteins, and carbohydrates from *Chlorella vulgaris*. The results showed that the lipid extraction rates of bead milling, and enzymatic hydrolysis were as high as 75 and 88%, respectively, and the recovery rates of protein and carbohydrates were also improved. Moreover, the combined use of the two extraction methods could successfully separate different biologically active compounds and minimize losses and shorten the extraction time. Similarly, Sierra et al. (Sierra, Dixon, & Wilken, 2017) combined the Bligh-Dyer method with enzymatic hydrolysis to extract lipids and proteins from *Chlamydomonas reinhardtii*, and found that enzymatic pre-treatment can degrade the cell walls of microalgae, thereby facilitating the extraction of lipids and proteins.

Obviously, the assistance of enzymatic hydrolysis can significantly increase the lipid extraction rate, but enzymes have specific reaction conditions and therefore, it is very important to select suitable enzymes and control enzymatic hydrolysis conditions to promote the destruction of microalgae cell walls and increase the extraction rate.

2.2.8. Other methods

In addition to the above methods, other new technologies have also been explored for the extraction of microalgae lipids. For example, based on the principle of photoelectron-chemistry, Wu et al. (Wu et al., 2021) constructed a photoelectric system that uses TiO_2-based photoanode and UV–Vis light to generate •OH at a phosphate-palladium cathode, which was used to extract *Chlorella* lipids. After the pretreatment of the photoelectric system, the extraction rate of *Chlorella* lipids can reach 96 %, which is higher than that of samples without pretreatment. It also can be considered as an environmentally friendly, low-energy processing method. Similarly, osmotic shock treatment has also been explored for the extraction of microalgae lipids. For example, Yoo et al. (Yoo, Park, Kim, Choi, & Yang, 2012) used NaCl to adjust the intensity of solvent osmotic shock to extract lipids from *Chlamydomonas reinhardtii* biomass and found that osmotic shock can double the lipids recovery rate, indicating that it is also a promising technology in microalgae lipid extraction.

In general, choosing the appropriate cell disruption process and extraction method can increase the extraction rate, and the ingenious combination of different technologies can also show great application value in the extraction of microalgae lipids.

3. Bioactivity of microalgae lipids

As one of the main producers of marine lipids, the high content of (PUFAs, especially EPA and DHA, is believed to have a variety of biological activities, including antibacterial, antifungal, anti-inflammatory, alleviating cardiovascular diseases and modulating gut microbiome (Fig. 3). In addition, microalgae also contain other essential fatty acids, such as linoleic acid, linolenic acid, arachidonic acid, etc., which also endow the microalgae lipids with more functions, so that the microalgae can be used in animal feed, biodiesel and so on (Das, 2018).

3.1. Antimicrobial activity

The antimicrobial activity of PUFAs has been well known and PUFAs are used as an antibacterial food additive, like having an inhibitory effect on the growth of *Staphylococcus*, *Streptococcus*, *Bacillus*, etc. The main contribution to these antibacterial activities is linoleic, linolenic, and oleic acids, while some fatty acids derivatives may also have the potential to exert antibacterial activity (Kabara, Swieczkowski, Conley, & Truant, 1972; C. J. Zheng et al., 2005). Considering that microalgae can be used as a natural source of fatty acids, its antibacterial activities has also been explored and the antibacterial effects of microalgae lipids and their extracts against a variety of Gram-positive and negative bacteria have been reported (Table 3).

As early as 1996, scientists isolated a mixture of fatty acids from algae and found that it showed inhibitory effects on both Gram-positive and Gram-negative bacteria (Dellar, Cole, & Waterman, 1996). On this basis, some microalgae with high fatty acids content have been studied. Najdenski et al. (Najdenski et al., 2013) selected nine species of blue-algae (cyanobacteria) and explored the antibacterial and antifungal activities of their intracellular and extracellular compounds, including polysaccharides, fatty acids, phycocyanin, etc. In terms of lipids and fatty acids, a variety of microalgae (*Aphanizomenon flos-aquae*, *Scenedesmus obliquus* and *Rhodella violacea*) have shown an inhibitory effect on *Streptococcus pyogenes* and *Staphylococcus aureus*, while the lipids of *Trachidiscus minutus* and *Nostoc* sp. only had an inhibitory effect on

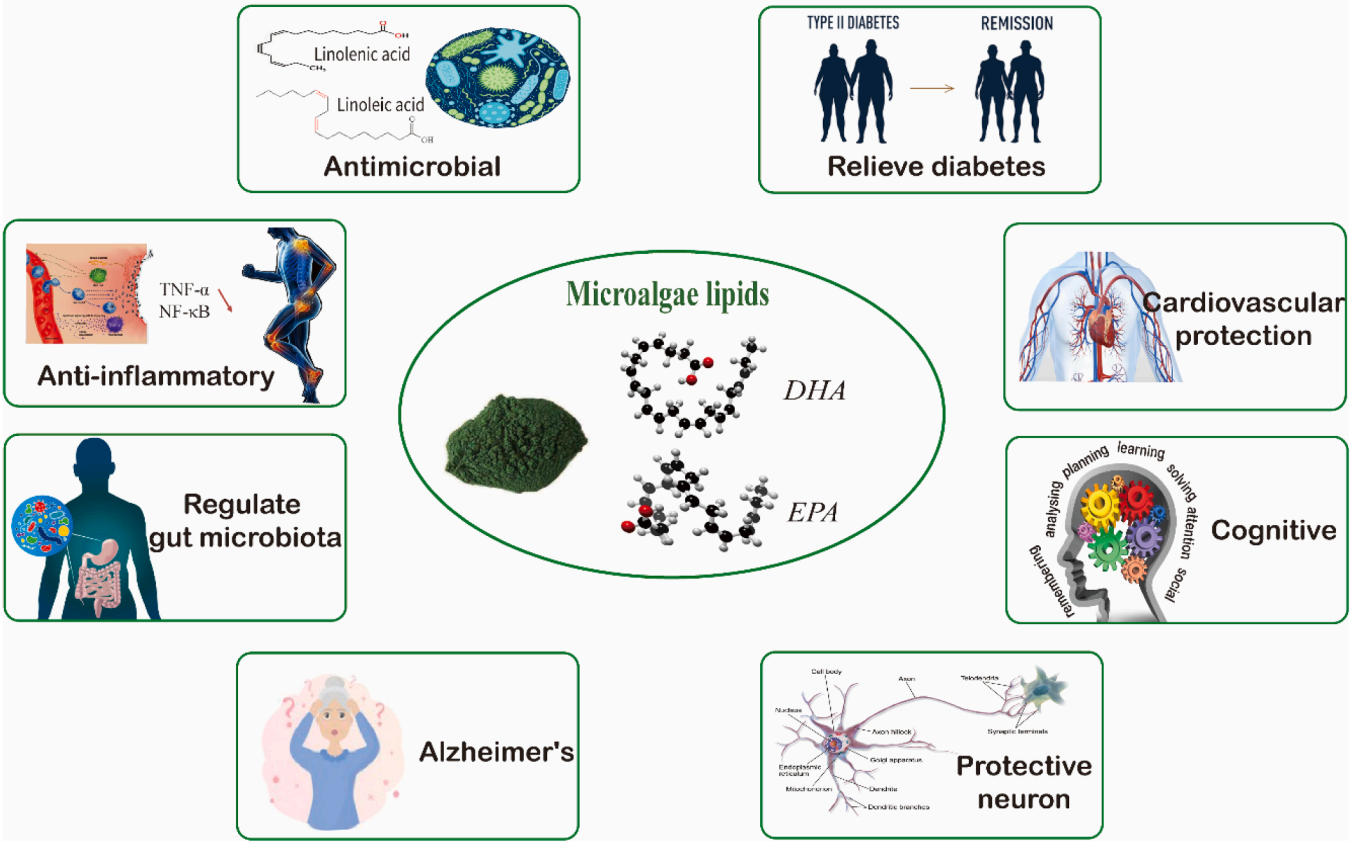

Fig. 3. Bioactivity of microalgae lipids.

Table 3
Studies on antibacterial activity of microalgae lipids.

Microalgae	Active compounds	Bacteria	Reference
Phaeodactylum tricornutum	EPA (20:5)	*Bacillus cereus, Staphylococcus epidermidis Staphylococcus aureus, Listonella anguillarum*	(Desbois et al., 2009)
Rhodella maculata, Phaeodactylum tricornutum, Boekelovia hooglandii, Goniochloris sculpta, and Chloridella simplex	Capric acid (10:0), palmitoleic acid (16:1), γ-linolenic acid [18:3 (n-6)], arachidonic acid [20:4 (n-6)], and docosadienoic acid [22:2 (n-6)]	*Escherichia coli Staphylococcus aureus*	(Ruffell et al., 2016)
Scenedesmus intermedius	Fatty acid methyl esters	*Escherichia coli Pseudomonas aeruginosa*	(Davoodbasha et al., 2018)
Fischerella sp.	γ-linolenic acid [18:3 (n-6)]	*Staphylococcus aureus Escherichia coli, Salmonella typhi (local strain) Pseudomonas aeruginosa Enterobacter aerogenes*	(Asthana et al., 2006)
Aphanizomenon flos-aquae, Scenedesmus obliquus, Rhodella violacea	Intracellular extract (fatty acid)	*Streptococcus pyogenes*	(Najdenski et al., 2013)
Chlorella sp., *Coelastrella* sp., *rviolacea*		*Staphylococcus aureus*	
Dunaliella salina	Hexane, methanol, and DCM extracts	Micrococcus luteus *Escherichia coli* Pseudomonas aeruginosa	(Yavuz, Murat, & Meltem, 2014)
	Ethanol extracts Methanol, and ethanol extracts	Candida albicans Vibrio alginolyticus,	
Phaeodactylum tricornutum Bohlin	EPA, secondary algal metabolites	Vibrio vulnificus, Vibrio parahaemolyticus Vibrio cholerae	(Wang et al., 2018)

Streptococcus pyogenes. In addition, the lipids isolated from *Chlorella* sp., *Coelastrella* sp. and *Rhodellales violacea* showed obvious inhibitory effects on *Staphylococcus aureus*. Asthana et al. (Asthana, Srivastava, Kayastha, Nath, & Singh, 2006) extracted γ-linolenic acid (GLA) from the *Fischerella* sp. and found that it is active against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*. Among them, the effect on *Staphylococcus aureus* was the most significant, followed by *Salmonella typhi* and showing a lower inhibitory effect on *Enterobacter aerogenes*, which also reflects the difference in the effect of fatty acids on different bacteria. The potential of microalgae fatty acids as antibiotics was also evaluated. Ruffell et al. (Ruffell, Müller, & McConkey, 2016) explored the effects of 29 fatty acids present in different types of microalgae on *Escherichia coli* and *Staphylococcus aureus*. Five fatty acids including palmitic and γ-linolenic acid showed great antibacterial activity. Since fatty acids content are affected by growth conditions, the results can be used as basis for microalgae cultivation and screening, so that high-concentration and high-quality fatty acids under specific growth condition can we promote it, becoming a good source of antibiotics. Davoodbasha et al. (Davoodbasha, Edachery, Nooruddin, & Lee, 2018) further explored the high content of palmitic acid (C16:0) fatty acid methyl esters in *Scenedesmus* sp. and determined its antimicrobial abilities against a variety of bacteria and fungi. The fatty acid methyl esters showed higher inhibitory activities against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) compared with Gram-positive bacteria, with the lowest inhibitory concentration being about 12 ~ 24 μg mL^{-1}. Furthermore, *Phaeodactylum tricornutum*, *Chlamydomonas reinhardtii*, *Anabaena*, etc. have also been shown to have antimicrobial properties against *Staphylococcus aureus*, *Salmonella typhimurium* and other bacteria (Desbois, Mearns-spragg, & Smith, 2009; Svircev, Cetojevic-simin, Simeunovic, & Karaman, 2008).

Due to the increased use of antibiotics, drug resistance of bacteria has increased and the search for new natural antimicrobial compounds from nature has attracted the attention of researchers, and in this context, microalgae lipids and fatty acids are considered to have great development potential because their antibacterial abilities.

3.2. Anti-inflammatory activity

Inflammation is one of the common reactions of the human innate immune system, which can protect the body from damage by parasites, microorganisms, or viruses. When the body is injured by inflammation, uncontrolled inflammation in the body may be linked to the occurrence of some diseases, including cardiovascular disease, intestinal inflammation, obesity, diabetes and so on. If there is no inflammatory at all, the body's immune system will be weakened, so it is very important to maintain a balance between inflammatory and anti-inflammatory processes (Liu, 2021). Research has indicate that omega-3 fatty acids related lipids have anti-inflammatory effects and omega-6 fatty acid have the opposite effect, which can be attributed to the products of lipids metabolism (Kumar et al., 2019). In addition, high concentrations of EPA and DHA have also been extensively studied for their anti-inflammatory effects (Itariu et al., 2012). As one of the main sources of PUFAs in the marine, microalgae are being explored for their anti-inflammatory potential (Table 4).

In order to explore the influence of microalgae lipids on inflammation, Sibi (Sibi, 2015) explored the inhibitory effects of six *Chlorella* lipid extracts on inflammation, inhibition of pro-inflammatory cytokines and tumor necrosis factor-α (TNF-α) produced by human peripheral blood mononuclear cells. It was also evaluated the extract's inhibition of lipase and active oxygen to explore the anti-inflammatory mechanism. The inhibitory range of various lipid extracts on TNF-α was about 58.39 ~ 78.67 %, with *Chlorella vulgaris* showed the lowest inhibitory concentration of about 10 μg/mL, followed by *Chlorella ellipsoidea*, *Chlorella protothecoides* and *Chlorella pyrenoidosa* with about 20 μg/mL. At the same time, *Chlorella* ellipsoidea, *Chlorella* vulgaris and *Chlorella* protothecoides showed also high lipase inhibitory activity (58.9 ~ 61.73 %). Further analysis of fatty acid methyl esters revealed that of the 19 fatty acids in the extract, 14 were C14 to C24 unsaturated fatty acids. The results showed that the lipid extract of *Chlorella* can inhibit the activity of Propionibacterium acnes by inhibiting the activity of lipase, thereby alleviating the inflammatory disease acne vulgaris. Furthermore, oxylipins produced by the oxidation of fatty acids have also been found to have the effect of relieving inflammation. Ávila-Román et al. (Ávila-Román, Talero, de los Reyes, García-Mauriño, & Motilva, 2018) explored the *in vitro* anti-inflammatory mechanism of the oxylipins in *Chlamydomonas debaryana* and *Nannochloropsis gaditana*, since oxylipins can reduce the production of pro-inflammatory factors in THP-1 macrophages and HT-29 colon cells, thereby acting as an activator of PPAR-γ to inhibit the activation of the NF-κB pathway, showing the potential in the treatment of inflammatory diseases. The EPA and DHA abundant in microalgae can inhibit the pro-inflammatory factors and change the phospholipid composition of the cell membrane, reducing the expression of inflammatory genes and other potential anti-inflammatory capabilities.

Table 4
Effects of microalgae lipids on health.

Microalgae	Model	Active compounds	Dosage	Result	Reference
Anti-inflammatory					
Chlorella	Human peripheral blood mononuclear cells	Lipid extracts	10 ~ 20 μg/mL	It can alleviate the acne vulgaris by inhibiting the activity of Propionibacterium acnes.	(Sibi, 2015)
Chlamydomonas debaryana, Nannochloropsis gaditana	THP-1 macrophages and HT-29 colon cells	Bioactive molecules generated by the oxidation of fatty acids	100 μM	Inhibition of the activation of NF-κB pathway, reduce the production of inflammatory markers and contribute to the regression of acute inflammation.	(Ávila-Román et al., 2018)
Anti-diabetes					
Chlorella pyrenoidosa, Spirulina platensis	High-fat, high-sucrose mice	Ethanol extracts	150 mg/kg per day	Work against hypoglycaemia and regulate gut microbiota	(Wan et al., 2019)
Spirulina platensis	Mice	Ethanol extracts	150 mg/kg per day	Reduces blood lipid, regulate gut microbiota and relieves diabetes	(Li et al., 2018)
Isochrysis galbana, Nannochloropsis oculata	Mice	Fatty acids	5 μg/5 mg/50 mg	Increased low density lipoproteins and decreased high density lipoproteins in healthy/diabetic mice.	(Nuño et al., 2013)
Cardiovascular disease					
Odontella aurita	Mice	PUFAs	12 % (*w/w*)	Reduces platelet aggregation and oxidative stress, as well as prevents CVD.	(Haimeur et al., 2016)
Spirulina	Washed rabbit platelets	Lipid extracts		Exhibit inhibitory effect on PAF and thrombin, showing anti-thrombotic properties.	(Koukouraki et al., 2020)
Microalgae	Persons without coronary heart disease	DHA supplementation	~1.68 g/d	Decreased serum TG and increase HDL/LDL-cholesterol in persons without coronary heart disease	(Bernstein, Ding, Willett, & Rimm, 2012)
Others					
Nannochloropsis, Schizochytrium	Mice	EPA and DHA	1.8/2.4 %	It is beneficial for the protection of neurons.	(Lopes et al., 2017)
Botryococcus braunii, Nannochloropsis oculata	Human neuroblastoma cell line (SH-SY5Y)	Organic reagent extract		Protect SH-SY5Y cells from H2O2-induced cytotoxicity.	(Custódio et al., 2015)
Turbinaria ornata	Human colon cancer cells (HT-29)	Hexadecanoic acid (HA)		The extract exhibits potential antioxidant and anticancer activity	(Bharath et al., 2021)

3.3. Regulating gut microbiota and alleviating diabetes

Diabetes is a metabolic disease with high morbidity and mortality. The distinctive feature of diabetes is the high blood glucose levels, so the control of these levels has become an important part of the treatment of diabetes (Wang et al., 2021). With the improvement of people's living standards and the extension of life expectancy, diabetes has gradually become a disease with increasing prevalence around the world and at the present, diet combined with medicine treatment are effective methods to alleviate the diabetes (Kgosidialwa et al., 2015). However, medicine treatment will more and less bring certain side effects, so the development of healthier products that can be introduced in the diet has also attracted people's attention. Previous studies have shown that the gut microbiota may be related to the occurrence of various metabolic syndromes, including diabetes. Therefore, it is possible to regulate the structure of the gut microbiota through diet to alleviate the occurrence of diabetes (Arora et al., 2021).

Marine-derived PUFAs have been applied to a variety of disease models due to their bioactivity, among them, microalgae as an important source of fatty acids and others bioactive compounds. These compounds are popular in the food and pharmaceutical industries (Jia, Heng, Yang, & Gao, 2014). Wan et al. (Wan et al., 2019) explored the anti-diabetes activity of PUFAs in two common microalgae (*Chlorella pyrenoidosa* and *Spirulina platensis*), with ethanol extracts of microalgae being used to feed mice on the high-fat, high-sucrose diet. After 8 weeks, DNA from the cecum of the mice was collected and extracted, and 16S rRNA sequencing was performed, with the results showing that both microalgae extracts can supplement and maintain the beneficial bacteria in the intestines, including *Oscillibacter*, *Parasutterella*, and *Ruminococcus*, and reduce the abundance of *Blautia* and *Turicibacter*. At the same time, *Chlorella pyrenoidosa* showed more obvious effects than *Spirulina platensis* in the fight against hypoglycaemia in mice. Based on the correlation analysis between blood glucose level and intestinal flora, it is speculated that *Ruminococcus* may be a bacteria related to the regulation of diabetes, so it can provide a supplement for further exploration of the treatment of diabetes and the regulation of the mechanism of lowering blood glucose. Similarly, studies carried out by Li et al. (Li et al., 2018) with *Spirulina platensis*, also explored the effects of PUFAs on the regulation of gut microbiota and lipid metabolism in high-fat mice. In order to alleviate metabolic disorders and reduce blood lipids, a 95 % ethanol extract was fed to mice and the protective effect of the extract on the liver was evaluated. RT-PCR analysis of liver DNA found that the extract increased the abundance of beneficial bacteria (including *Prevotella* and *Alloprevotella*) which were positively correlated with low-density-lipoprotein cholesterol levels, triglycerides, etc., and the decrease of the abundance of microbes such as *Romboutsia* and *Clostridium XVIII*, which were negatively correlated with the serum high-density-lipoprotein cholesterol levels. The extract had regulating effects on the patient's gut microbiota to alleviate diabetes and shown the potential as functional foods. An increased number of studies have shown that biologically active compounds derived from microalgae, including PUFAs can alleviate and treat diabetes by regulating the structure of the gut microbiota of patients.

3.4. Impact on cardiovascular disease

Cardiovascular disease (CVD) has become one the main causes of damage to health in non-communicable diseases, hypertension, high cholesterol, and oxidative stress and may also lead to coronary heart disease and atherosclerosis (Krijger et al., 2021). Among them, hypertension is widely concerned as a controllable risk factor in CVD and its impact on health. Diet and lifestyle will all influence the occurrence of CVD, at the same time, medication has long been used to relieve chronic diseases such as CVD. Considering the possible side effects of long-term

medication, the role of some natural active ingredients in alleviating CVD is being explored (Ejike et al., 2017).

Researcher have confirmed that PUFAs, including EPA and DHA, as dietary supplements also show certain advantages in the prevention of CVD in addition to anti-inflammatory activity. Several studies on fish oils with high PUFAs content in marine sources and showed that EPA and DHA can effectively lower the incidence of CVD and play a certain protective effect on the cardiovascular system (Yamagata, 2020). Meanwhile, microalgae as one of the effective alternative sources of PUFAs, in which high content of PUFAs and other high-value compounds have shown great development potential in human health. Haimeur et al. (Haimeur et al., 2016) compared the effects of fish oil with rich in omega-3 PUFA and microalgae oil on CVD. Fish oil and freeze-dried *Odontella aurita* were fed to mice on a high-fat diet. After 8 weeks, the plasma insulin, tissue lipids, platelet activity were analysed. The results showed that although the addition of microalgae and fish oil can reduce high-fat levels in mice with serum lipid levels and insulinemia, *Odontella aurita* showed a more pronounced effect in preventing lipid denaturation and in the reduction of triglyceride levels, showing a great biological effect, which may be due to microalgae with high PUFAs content and other bioactive compounds. In addition, the lipid extracts of microalgae can also affect the formation of thrombus. Koukouraki et al. (Koukouraki, Tsoupras, Sotiroudis, Demopoulos, & Sotiroudis, 2020) explored the relationship between *Spirulina* lipid extracts and thrombosis through the effects of platelet activating factor (PAF) and thrombin, and found that the lipid extracts of *Spirulina* exhibit a strong inhibitory effect on PAF and thrombin, showing anti-thrombotic properties. Microalgae oil can be considered as a substitute of fish oil as a supplement for dietary nutrition.

3.5. Other bioactivities

In addition to the above-mentioned bioactivities that have been investigated, microalgae lipids have also been found to have an impact in other ways and are being further studied. Long-chain PUFAs, especially EPA are associated with the occurrence of some neurological diseases, such as Alzheimer's disease, cognitive decline, depression, and other diseases. Because microalgae are rich in DHA and EPA, it has been found that it can alleviate the imbalance of plasma metabolites in mice, and has a protective effect on the plasma lipid profile, which is beneficial to the structure and functions of neurons (Lopes et al., 2017). The highly PUFAs extracts of microalgae showed also inhibitory effects on human colon cancer cells (HT-29 cells) and may be developed into a potential anti-cancer material in the future (Bharath, Perinbam, Devanesan, AlSalhi, & Saravanan, 2021). Compared with the waste of resources and fishy smell that may occur during the processing of fish, microalgae can be regarded as a good substitute for PUFAs due to its large yield and fast growth, which has great development value.

4. Application potential of microalgae lipids

4.1. Food and medicine industry

Due to changes in modern lifestyles, some high-caloric or unhealthy foods are consumed by people, which has led to a series of health problems, such as diabetes and heart disease mentioned above (Sathasivam, Radhakrishnan, Hashem, & Abd, 2019). As a source of high-value bioactive compounds, microalgae can be used to fortify human diet, to obtain more nutrition and functional foods, especially because PUFAs in microalgae have high nutritional value. PUFAs are used as dietary supplements in health products, beverages, jam, pasta products and other daily foods with no or low content of PUFAs (Lafarga, 2019; Robertson et al., 2016). Foods containing high-value fatty acids can reduce the risk of some diseases by adjust cholesterol and other indicators, and can also reduce the accumulation of toxins and heavy metals that may be due to the consumption of fish products (Shahidi & Ambigaipalan, 2018). In addition to PUFAs, microalgae also contain many other bioactive compounds, such as protein, minerals, polysaccharides, which also contribute to the increase of nutrients that have potential to be added to food products (Paula, Gouveia, Bandarra, Franco, & Raymundo, 2013).

4.2. Biofuel

The extensive use of traditional fossil fuels produces harmful substances while also polluting the environment, leading to global warming. Obtaining new plant biofuels from some renewable raw materials to replace part of fossil fuels can reduce the release of CO_2 during the combustion process and reduce the damage to the environment (Chhandama & Satyan, 2021; Xue et al., 2021). At present, traditional plants or crops such as corn and rapeseed can be used as biofuels, but most of them also can be used as edible oils. Microalgae not only have faster growth and reproduction ability, but also have strong lipid production and accumulation ability of inedible triglycerides that can be converted into biofuels, making it possible for microalgae to be used as a biofuel (Xue et al., 2021). The advantage of microalgae biofuels is that it will not have a negative impact on arable land, but can also alleviate the problem of energy shortage to a certain extent, which has broad prospects (Peng, Fu, Chu, Wang, & Qi, 2020).

4.3. Aquaculture and animal feed

The bioactivity of PUFAs present in microalgae has been explored and found to have a positive effect on the health of animals. Furthermore, microalgae products have also been explored to be applied to animals and aquaculture in different forms as feed. Common microalgae used to feed include: *Spirulina*, *Chlorella*, *Lobosphaera incisa*, *Isochrysis*, *Schizochytrium* sp., *Phaeodactylum*, *Nannochloropsis* and others (Lu, Li, Xiao, & Liu, 2021; Sathasivam et al., 2019). Feeds rich in PUFAs can improve the ratio of fatty acids in meat products while improving the immunity of animals, further affecting the flavour or mouthfeel of the products (Medeiros et al., 2020).

4.4. Cosmetic products

In addition to above, PUFAs-rich microalgae are also used in the cosmetic industry. The PUFAs contained in microalgae provide a variety of potential benefits to the products due to their biological activity. For example, PUFAs has antioxidant properties and can be used in anti-aging and sunscreen products; microalgae rich in PUFAs have also anti-inflammatory effects and eliminates inflammation and regulate the balance of water and oil (Ying et al., 2020). At present, products containing microalgae are produced for skin care, hair care and water supplies (Lourdes, Carmen, & Jose, 2017).

5. Conclusions and future perspectives

Over the past few decades, concerns about food safety and agricultural sustainability have increased and microalgae have gained interest as natural substances rich in a variety of bioactive compounds, including lipids, proteins, amino acids, and polysaccharides. Among them, lipids are a good replacement for fish oil because of its high PUFAs content. Microalgae lipids extraction has been studied more specifically considering the effects on the cell walls. This paper summarizes the techniques and assisted techniques of microalgae lipids' extraction in order to provide more comprehensive information about the topic, and to identify opportunities and challenges to overcome. Classical extraction techniques (Soxhlet, Folch, Bligh-Dyer, etc.) have been widely used and fully explored. Although most of them are simple to operate, they also cause damage to the environment due to the extensive use of toxic reagents and the extraction time is long. Therefore, it is necessary to explore efficient and environmentally friendlier methods. A series of

innovative techniques have been applied to the extraction of microalgae lipids, such as SFE, PLE, PEF, UAE, MAE, ILs, enzyme-assisted extraction and others. These methods have proven to have positive effects in improving extraction rate and reducing pollution. As a valuable bioactive compounds, microalgae lipids have also been found to have physiological functions such as antimicrobial and anti-inflammatory effects and the capability to alleviating diabetes and cardiovascular diseases. Based on the bioactivity of microalgae lipids, its applications in various industries are also summarized, including in food, medicine, cosmetics, animal feed, biofuels, and other industries. As an important source of PUFAs in the ocean, microalgae have attracted more and more attention of researchers. Therefore, is of great interest to explore appropriate techniques to improve the extraction rate and reduce the cost on the premise of ensuring the bioactive abilities of lipids, so that microalgae can be better applied to industries. In the future, the application of microalgae on a larger scale is worth expecting. In addition to combining existing technologies and develop new technologies, it is also necessary to further explore the use of bio-based non-toxic extractants in the extraction process. This makes it easier for the target components to be extracted to be applied, as well as recycling of the remaining material after extraction. Moreover, algae biomass fractionation, in the scope of algae biorefinery approach for high-value compounds extraction, is expected to boost in the next years.

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.

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