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Cell disruption of microalgae: advances and perspectives

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ABSTRACT: Microalgae are organisms whose biomass contains different biomolecules, such as carbohydrates, lipids, proteins, pigments, vitamins, minerals, and antioxidant compounds, with numerous industrial applications, highlighting the food, nutritional, cosmetic, pharmacological, and biofuel segments. However, access to these biomolecules in an integrated manner is often hampered due to the structural rigidity of their cell wall, requiring the application of a pre-treatment that promotes cell lysis. Various cell rupture techniques applied to microalgae biomass have been reported. These methods can be mechanical, especially ball milling techniques and high-pressure homogenization (HPH), or non-mechanical, such as chemical, thermal, and enzymatic procedures, each with advantages and disadvantages. Thus, this review addressed the different methods of cell disruption, listing their advantages and disadvantages, applications in cell biomass, and challenges.
Key words: homogenization, grinding, enzymes, chemical techniques, mechanical and non-mechanical methods.

Rompimento celular de microalgas: avanços e perspectivas

RESUMO: As microalgas são organismos cuja biomassa possui biomoléculas abundantes como carboidratos, lipídeos, proteínas, pigmentos, vitaminas, minerais e compostos antioxidantes, com inúmeras aplicações industriais com destaque para os segmentos de alimentos, nutricional, farmacológico, cosmético e de energia, na produção de biocombustíveis. Entretanto, o acesso de forma integra a essas biomoléculas pode ser dificultado pela rigidez estrutural de sua parede celular, sendo necessário a aplicação de um pré-tratamento que promova a lise celular. As diferentes técnicas de ruptura celular aplicáveis à biomassa microalgal descritas na literatura podem ser de natureza mecânica, com destaque para as técnicas de moagem em moinho de bolas e homogeneização à alta pressão (HAP) ou não mecânica (química, térmica e enzimática), cada uma com vantagens e desvantagens. Assim, o objetivo da presente revisão narrativa é descrever o uso de diferentes métodos para o rompimento de células de microalgas, com suas vantagens, desvantagens e seus desafios.
Palavras-chave: homogeneização, moagem, enzimas, métodos químicos, métodos mecânicos e não mecânicos.

INTRODUCTION

The search for new food sources is challenging for the food industry, given the United Nations' global population projection of nearly 9.7 billion people in 2050. Thus, the expected increase in demand for food, whether of animal or plant origin, is approximately 70% for the next 30 years (UNITED NATIONS, 2019). Therefore, the interest in sustainable and organic technologies for obtaining human foods supports scientific investigation of systems capable of producing large quantities of food materials rich in macronutrients and micronutrients. In this context, microalgae stand out among plant sources.

There is a great diversity of microalgal species, with over 40,000 identified among the estimated 100,000 species. It is estimated that microalgae produce 50% oxygen and fixate 50% carbon dioxide on Earth. They are classified according to the types of pigmentation, life cycle, morphology, and cell structure (HU et al., 2008). Microalgae are considered clean and sustainable sources of biocompounds with potential for various industrial applications because they *(1)* can be grown in a wide range of pH values, nutrient contents, and temperatures; *(2)* exhibit high productivity, up to 10 times higher than that of traditional crops; *(3)* are not affected by seasonality; *(4)* can recycle carbon dioxide from the atmosphere and thus minimize

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the associated environmental impacts; and *(5)* are efficient in carbon capture, revealing the possibility of cultivation in humid terrestrial, seawater, brackish or residual environments without the demand for the use of agricultural land (GROSSMANN et al., 2019).

The chemical composition of microalgae can be adjusted through different methods, conditions, and metabolic pathways permissible for cell growth because metabolite synthesis is due to the primary and secondary metabolism of microalgae. This behavior makes microalgae an alternative source of food or supplements, given the presence of lipids (12 to 51)%, protein (35 to 60)%, pigments (8 to 12)%, carbohydrates (17 to 25)%, minerals (5 to 25)%, and other constituents (SILVA et al., 2021). However, the biochemical and metabolic compositions of most species are unknown.

The recovery of intracellular target metabolites from microalgal biomass is due to the action of extracting agents, which must penetrate the breakdown cell walls; nevertheless, the resistance of the cell wall to disruption is a barrier that hinders the efficient extraction of intracellular components and can interfere with the accuracy of compound quantification (SCHÜLER et al., 2020). Consequently, efficient techniques are required for cell wall disruption (mechanical, chemical, and/or enzymatic) to release microalgal components safely. Complementary studies on metabolite extraction and quantification methods with preparative and analytical techniques that preserve their characteristics, such as green solvents and less aggressive extractive procedures, are also needed. Studies on the interaction between bioactive compounds can also improve the comprehension of biocompound extraction, as LEI et al. (2022) described. The authors: (1) evaluated the interaction mechanism and complexation among proteins, polysaccharides, and polyphenols in foods to predict the binding patterns and affinity and (2) also used the molecular docking technique to simulate the interactions between ligands and biomacromolecules.

The commercial production of microalgae in Brazil is incipient since only a few companies produce microalgal biomass, emphasizing *Chlorella* and the *Cyanobacterium* (Spirulina). The companies deal with specific niches within human and animal nutrition, cosmetics, and wastewater treatment segments. There is no information on large-scale biomass production to extract bioactive compounds for other applications. Microalgae cultivation, biomass concentration, and biomass processing are the three major steps for implementing a microalgae biorefinery (BHATTACHARYA & GOSWAMI, 2020). Thus, research is needed to develop and/or improve stages of production systems on a commercial scale, such as cell disruption, to offer new microalgae products and increase the number of commercially viable microalgal species.

In this context, the present narrative review evaluates cell disruption techniques for the subsequent extraction of microalgal intracellular constituents, such as pigments, proteins, lipids, and carbohydrates.

Microalgae

Algae are photosynthetic, microscopic organisms with simple biological structures that can be unicellular (microalgae) or pluricellular (macroalgae), with cell sizes between 2 μm and 200 μm. In general, macroalgae (a) are found attached to rocks and other aquatic structures; (b) are in free life and dispersed in humid terrestrial environments, marine and riverine ecosystems, and wastewater; (c) are grown in open systems (extensive and circular lagoons, raceway, tanks, and cascade arrangements), closed systems (tubular photobioreactors, flat plates, big bag systems, and columns), fermenters or hybrid systems, in batch, semi-continuous or continuous regimes, and with different cultivation modes; and (d) can be harvested by chemical, physical or biological processes (DE SOUZA LEITE et al., 2020).

Three cultivation modes can be used in cell growth, differing according to the energy types and carbon sources. Cell culture in autotrophic mode requires macro- and micronutrients, light energy, and carbon from CO_2 to accumulate intracellular metabolites or storage materials. The metabolite content ranges from 20% to 50% of the total biomass (CHISTI, 2008). In the heterotrophic mode, organic compounds are used as a source of energy and carbon to produce metabolites without light. In mixotrophic mode, *(i)* cells use both autotrophic and heterotrophic growth pathways for metabolite production; *(ii)* the energy sources can be light, organic, and inorganic compounds; and *(iii)* the carbon sources can be CO_2 and organic compounds (FRANCO et al., 2013). Strategies to optimize culture conditions should consider facilitating cell disruption, minimizing water evaporation, reducing contamination, favoring light absorption, and intensifying target compound production, among other approaches.

The industrial use of microalgal compounds also demands developing appropriate techniques for cell disruption of biomass, concentration, extraction, quantification, and conservation of metabolites. Table 1 shows the discrepancies in the metabolite contents for the same microalgal species, allowing us to infer the necessity of standardization of the

Table 1 - Chemical composition of different microalgal species.

Species	----------Composition (% dry matter)----------			References
	Proteins	Lipids	Carbohydrates	
Chlorella vulgaris	51-58	14-22	12-17	MARTINS et al. (2010)
Chlorella pyrenoidosa	57	2	26	GOUVEIA et al. (2008)
Dunaliela salina	57	6	32	GOUVEIA et al. (2008)
Euglena gracilis	39-61	22-38	14-18	BRUTON (2009) GOUVEIA et al. (2008)
Haematococcus pluvialis	10.2	40.7	33.6	BATISTA et al. (2013)
Porphyridium cruentum	28-39	9-14	40-57	BRUTON (2009) GOUVEIA et al. (2008)
Scenedesmus obliquus	50-56	12-14	10-17	SPOLAORE et al. (2006) CAI et al. (2013)
Scenedesmus dimorphus	8-18	16-40	21-52	BRUTON (2009) GOUVEIA et al. (2008)
Scenedesmus quadricauda	47	1-9	21-52	BRUTON (2009)

stages of microalgae production and the techniques of metabolite quantification.

Regarding the composition of microalgae, the lipid content is quite variable; some species, such as *Chlorella sp*., *Botryococcus braunii*, *Nannoclorophysis sp.*, *Neochloris oleobundas*, and *Dunaliella salina sp.*, tend to accumulate a greater amount of lipids and polyunsaturated fatty acids (arachidonic acid, docosahexaenoic acid, and eicosapentaenoic acid, among others) (HOSSAIN & MAHLIA, 2019).

Different species contain proteins with a diversity of amino acids, such as tryptophan, lysine, leucine, and arginine (*Porphyridium aerugineum*), arginine (*Tetraselmis chuii*), and leucine (*Nannoclorophysis granulata*) (TIBBETTS et al., 2015).

Microalgal carbohydrates are found intracellularly, mainly as cellulose and starch, which can perform various biological functions. They are classified into three groups: energy reserve polysaccharides, structural polysaccharides, and those responsible for cellular communication. *Tetraselmis sp*., *Isochrysis sp.*, *Porphyridium cruentum*, *Porphuridium purpureum*, *Chlorella sp.*, and *Rodella rediculata* are the most commonly used species to collect carbohydrates (HOSSAIN & MAHLIA, 2019).

Microalgae also have vitamins, minerals, antioxidants (phenolic compounds and tocopherols), chlorophyll, carotenoids (astaxanthin, beta-carotene, canthaxanthin, and lutein), xanthophyll and phycobilin (phycobiliprotein) (CANELI et al., 2022).

The viability of commercial use of microalgal biomass as a source of metabolites and bioactive compounds is also associated with efficient and low-cost cell disruption techniques. This condition explains the need to develop or adapt efficient cell wall disruption techniques (mechanical, chemical, and/or enzymatic) to release microalgae components safely.

Microalgal cell disruption for the extraction of intracellular compounds

The cell disruption of microalgae is considered a critical step in the processing of algal macromolecules (CARULLO et al., 2022; GÜNERKEN et al., 2015), allowing the access of extraction agents into the cells to separate metabolic contents such as carbohydrates, proteins, lipids, vitamins, and other minor compounds. The most significant factors determining cell wall strength are wall thickness, structure, and chemical composition, which confer unique features to the cells of different microalgal species. Thus, cell wall characteristics are crucial in defining the most effective disruption methods. The methods of cell disruption can be divided into two categories: mechanical (MCM) and non-mechanical (N-MCM) (LEE et al., 2017). The latter category comprises chemical, thermal, and enzymatic disruption techniques (Figure 1).

Cell disruption methods subject the cells to mechanical or non-mechanical stresses or a mixture of the two techniques. Combining at least two MCM techniques, two N-MCM techniques, or one MCN and one N-MCN technique can promote a high level of cell rupture and has been used to increase the efficiency and preservation of intracellular microalgal compounds.

Mechanical rupture methods are the most frequently used because they completely disintegrate the cell wall and do not depend on the specific chemical composition of the microalgae wall.

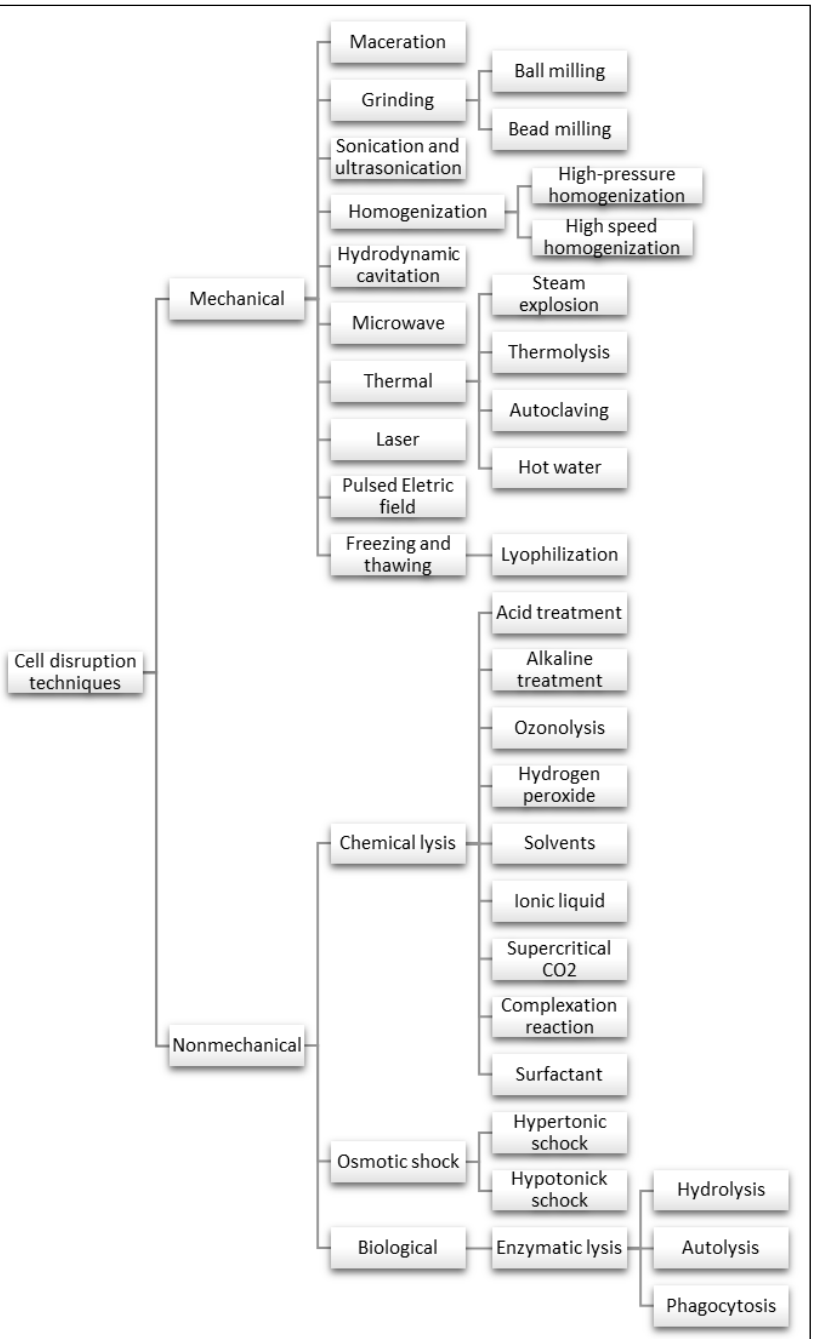

Figure 1 - Cell disruption methods.

However, mechanical disruption requires high energy consumption and expensive equipment. Conversely, non-mechanical methods, such as chemical and enzymatic techniques, do not depend on high investments in equipment but rather on the characteristics of cell walls. Thus, the choice of cell disruption technique will depend mainly on the microalgae's structural and morphological characteristics, the process's applicability and scalability, and energy viability.

Mechanical methods

Mechanical methods for cell wall rupture are based on the differences in pressure in the external and internal cell environments. This pressure

difference promotes cell disruption, creating high pressure on the fluid, grinding (high pressure on cells), or cavitation. Thus, high-pressure homogenization, grinding, ultrasonic, and microwaves (GOH et al., 2019) are the most generally used mechanical cell disruption methods for different microalgal species, as shown in table 2. High-pressure homogenization and ball mill grinding are broadly cited in the literature due to their high suitability for use on an industrial scale. These methods and the ultrasound and microwave techniques are detailed below.

High-pressure homogenization (HPH)

In this technique, cells are subjected to shear stresses that promote wall structure rupture, enabling the separation of intracellular compounds (ANGLES et al., 2017). Cell suspension flow occurs at high pressure on the cylindrical and narrow annular sections in the homogenizer valve. This flow generates shear stresses in the suspension particles, fractionating them into smaller, more homogeneous particles (GUL et al., 2017). HPH causes a nonselective release of intracellular constituents. It can generate *(1)* a high concentration of cellular residues, hindering the downstream separation step, and *(2)* an undesirable increase in temperature for heat-sensitive extracts (such as enzymes, lipids, pigments, and proteins).

HPH has the potential for industrial application in the rupture of microalgal cells due to its scalability, applicability in highly concentrated biomass, and efficacy in disaggregating rigid cell walls (LEE et al., 2017). The efficiency of HPH cell rupture is high despite the elevated energy demand for disruption (SPIDEN et al., 2013). The optimization of the HPF can improve the operational conditions of cell disruption and increase biocomposite recovery. Most HPH microalgal cell rupture studies were performed with up to 1,500 bar homogenization pressures,

Table 2 - Mechanical techniques of microalgae cell disruption.

Rupture techniques	Species	Operating conditions	References
High-pressure homogenization	*Chlorella vulgaris, Chlorella sorokiniana, Phaeodactylum tricornutum, Nannochloropsis*	10% w v^{-1} biomass 180 MPa, 22 °C	GROSSMANN et al., 2019
	Chlorella sorokiniana, Phaeodactylum tricornutum	5% w w^{-1} oil/biomass 1000 bar, 3 passes	EBERT et al., 2019
	Chlorella vulgaris	10% w v^{-1} biomass 150 MPa, 9 passes	DAI et al., 2020
HPH* and alkaline treatment	*Chlorella vulgaris*	1.3% w v^{-1} biomass 1.5 bar, 25 °C	URSU et al., 2014
Grinding (ball mill)	*Tetraselmis sp.*	Flow rate: 1.5 L min^{-1} 30 min, 20 °C	SCHWENZFEIER et al., 2011
	Parachlorella kessleri	Flow rate: 200 mL min^{-1} 20 °C	RIVERA et al., 2018
	Nannochloropsis oculate Porphyridium cruemtum	Flow rate: 48 to 200 mL min^{-1}, 8 m s^{-1}rotation speed, 0.375 - 2.15 mm ball diameter, 20 °C	MONTALESCOT et al., 2015
Sonication	*Chlorella vulgaris*	20% amplitude, 200 W, 5 pulses s^{-1}: on and off, 15 min	SANKARAN et al., 2018
	Scenedesmus obliquus	60% amplitude 20 KHz, 2 min	SILVA et al., 2021
Microwave	*Scenedesmus obliquus*	400 W, 10 min warm-up 0.25 h extraction	ZHOU et al., 2019
	Scnedesmus quadricauda	600 W, 8 min warm-up 3.5 h extraction	ONUMAEGBU et al., 2019
Hydrodynamic cavitation	*Chlorella pyrenoidosa*	1% w v^{-1} solids, 80% amplitude, 90 min cavitation	WAGHMARE et al., 2019
Pulsed electric field	*Haematococcus pluvialis*	10 to 80 pulses 5 min, 1 Hz	MARTÍNEZ et al., 2019
Thermal (Autoclaves)	*Synechocystis PCC 6803*	Autoclave: 15 min 121 °C (miminal)	SHENG. et al., 2012

*HPH: High-pressure homogenization.

requiring many passes of homogenization for a rigid wall of microalgae cells (GÜNERKEN et al., 2015). According to BERNAERTS et al. (2019), applying pressures greater than those used in HPH generates the ultra-high pressure homogenization (U-HPH) method, a more efficient rupture technique due to the decrease in homogenization passage number. The ability to accurately evaluate and quantify the degree of cell breakage is essential to understanding the phenomenon of rupture by HPH.

YAP et al. (2015) evaluated *Nannochloropsis sp*. cell disruption in a homogenizer with a single passage for lipid separation from biomass. Suspensions with 0.25%, 2.5%, and 25% w w^{-1} solids were used at 30 and 150 MPa pressures. The authors obtained lipid concentrations of up to 30% w w^{-1}. Furthermore, the lipid concentration did not significantly influence homogenization, and cell disruption depended on the homogenization pressure.

ANGLES et al. (2017) evaluated the disruption of *Nannochloropsis sp*. cells and their physiological and structural changes when subjected to growth conditions in the presence and absence of nitrogen. The microalgae suspensions were homogenized with pressures between 100 MPa and 270 MPa. Under both cultivation conditions, the rupture rate increased with increasing applied pressure levels. The authors reported changes in the structural rigidity of the microalgal cell wall, which modified the cell disruption behavior and energy consumption of the whole process.

GROSSMANN et al. (2018) evaluated the number of passes in HPH equipment necessary to promote the disruption of 4 distinct microalgal species, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Phaeodactylum tricornutum*, and *Nannochloropsis oceanica*. The differences in the structure and composition of the species' cell walls generated the following order of difficulty of rupture: *P. tricornutum* < *C. sorokiniana* < *C. vulgaris* < *N. oceanica*. According to the authors, the *Chlorella* cell wall, for example, consists of a very rigid polymer matrix, which generates a high resistance of cells to rupture induced by high pressure. The *Nannochloropsis* cells showed the highest resistance due to the highly thick cell wall compared to the other species studied. The cell's resistance to mechanical stress is also related to wall composition since the cell is formed by multiple layers containing an extremely resistant aliphatic polymer in the outermost layer. The *P. tricornutum* cell wall is very fragile because it contains small amounts of silica and breaks easily by intermediate mechanical stress due to the fusiform shape of its cells (EBERT et al., 2019; GROSSMANN et al., 2018). SERIVE et al. (2012) also reported that a ball mill easily disrupted *P. tricornutum* cells.

The optimization of the binomial parameters (pressure and the number of passes) in the HPH could offer promising results of cell rupture for the recovery of microalgae biocompounds.

Grinding

Grinding is a complex mechanical cell rupture technique in which the rotational movements of the mill spheres promote friction between the cells and the balls (GONG & BASSI, 2016). Consequently, the cells are broken. The mechanical shocks between cells and balls are influenced by parameters such as biomass feed rate and density, weight, and speed of the spheres. These parameters are dependent on the industrial design of the grinding chamber or agitator. Zinc is recommended for producing high-density beads, and glass is recommended for producing low-density beads. Zirconium is the most useful sphere material for beads in which cells with high viscosity should be broken, and glass is more suitable for those with low viscosity (WANG et al., 2020; GUNERKEN et al., 2015). The bead diameter and ball load conditions can define cell lysis efficacy.

SCHULLER et al. (2020) evaluated different solvents for carotenoid extraction from *Tetraselmis sp*. biomass by comparing the effectiveness of mechanical rupture by ball milling in wet and lyophilized biomasses. Acetone, methanol, and tetrahydrofuran (THF) solvents were used in the protocols. Cell rupture with glass beads in wet biomass assisted the extraction. Higher productivities of lutein (622 ± 40 µg g^{-1} of the aqueous dispersion) and β-carotene (618 ± 32 µg g^{-1} of the aqueous dispersion) were reported for the extraction method using THF.

RIVERA et al. (2018) evaluated the extraction and composition of lipids from *Parachlorella kessler* biomass after cell disruption using grinding. The microalgal biomass was pumped from a feed tank to a grinding chamber (600 mL capacity) with glass beads (1.30 mm in diameter) at a feed flow of 200 mL min^{-1} and a rotation speed of 8 m s^{-1}. The outlet temperature of the suspensions was maintained at 20 °C. Two biomass fractions were studied. The first fraction with a cell disruption level of 85% was obtained after three passes through the grinding chamber. The second fraction with a cell disruption level of 100% was obtained after five passages. The authors stated that despite the increase in lipid content in the second fraction, biomass grinding up to 100% favored the release of amphiphilic molecules, increasing the possibility of emulsification.

LIU et al. (2022) studied the recovery of lipids and water-soluble compounds from *Parachlorella kessler* biomass as affected by the microalgal biomass type, the grinding and centrifugation operational parameters, and the physicochemical characteristics of the granules. Cell rupture performed by ball milling was coupled with membrane centrifugation and microfiltration to separate microalgal compounds. The best treatment produced 23% w v^{-1} of the total lipids, 9% w v^{-1} of sugars, and 8% w v^{-1} of proteins.

Furthermore, AMORIM et al. (2020) used ball mill grinding in the mechanical disruption of dried microalgal biomass, highlighting the flexibility of grinding in cell rupture.

Ultrasonication

Among the mechanical methods, sonication uses sound waves to propagate pressure fluctuations, induce cavitation, and promote cell disruption. Since the technique does not involve high temperatures, it is promising for extracting thermolabile compounds such as proteins. It is also a suitable method for disrupting microalgae with a rigid cell wall, such as *Chlorella sp*., but it is not indicated for species with flexible walls (DO CARMO CESÁRIO et al., 2021; GOH et al., 2019).

The main mechanisms for cell rupture by ultrasound are the formation of bubbles with high pressure and consequent cavitation. Cavitation promotes the formation of mechanical shock waves with high shear stresses (GUNERKEN et al., 2015; LEE et al., 2017). Increasing the ultrasound power can optimize cell rupture by reducing the internal pressure and impacting bubble formation. The sonication frequency is a variable fully correlated with the characteristics of the studied species. The use of low cell biomass concentrations (1.5 g L^{-1} to 14 g L^{-1}) does not affect the rupture efficacy (KUROKAWA et al., 2016).

SAFI et al. (2015) evaluated the extraction of carotenoids in aqueous biomass, verifying that the hydrophilic compounds rapidly dissolved in the aqueous medium, whereas the dissolution of hydrophobic compounds (carotenoids and chlorophyll) did not occur. According to the authors, this may have occurred due to the high cell wall resistance of *Chlorella vulgaris*, making the cell rupture technique ineffective.

IDO et al. (2018) evaluated the action of several solvent mixtures in an ultrasound-assisted extraction process. The operational parameters studied were the resonance amplitude, n-hexane/isopropanol ratio, and reaction time. The procedures were performed continuously in pulse-free mode. Ultrasonication promoted cell lysis, and lipids (nonpolar and polar) were extracted with a mixture of n-hexane/isopropanol added previously to the system. The lipid yield was 26.66% under a 50 µm resonance amplitude, solvent ratio of 4:1 (v v^{-1}), and reaction time of 1.5 h. Finally, the authors verified that the extracted lipids have adequate parameters for biofuel processing.

Regarding the effect on bioactive compounds, some authors reported that cell disruption by ultrasound could generate slight heating when used at higher power, but since the exposure time is very short, the influence on these compounds is reduced (SILVA et al., 2021b; GILLE et al., 2016). For example, SILVA et al. (2021b) found that approximately 95% of *Scenedesmus obliquus* cells were disrupted after ultrasonication for 5 min, using an amplitude of 90% and a frequency of 20 kHz. GILLE et al. (2016) evaluated the effect of ultrasound cell disruption on the bioaccessibility of carotenoids (β-carotene and lutein) from *Chlamydomonas reinhardtii*, verifying that this method did not influence carotenoid bioaccessibility. SILVA et al. (2021b) studied ultrasound cell rupture for subsequent extraction of carotenoids and phenolic compounds of the microalga *S. obliquus*. These authors verified that the extracted compounds presented high antioxidant activity; therefore, the cell rupture method did not affect them. The literature reported that this cell disruption method also did not affect other thermosensitive compounds extracted from microalgal biomass, such as lipids, carbohydrates, and proteins (SILVA et al., 2021a; VIEIRA et al., 2021; LIMA et al., 2023).

Ultrasonication application is still restricted to the laboratory scale despite presenting relevant results in the rupture of microalgae cells with a complex structure. Therefore, its use for commercial purposes is restricted to the recovery of specific biocompounds with high added value.

Microwaves

Rapid and uniform heating of biomasses is inherent in unit operations assisted by microwaves, such as cell rupture. In the microwave technique, heat moves from the external medium to the inside of the cell. The presence of polar molecules, such as water, contributes to the rapid absorption of energy by the cells. Accordingly, the internal pressure increases, promoting lysis in the cell and facilitating the release of components in the extraction solvent, especially lipids (TIGRINE-KORDJANI et al., 2011). Thus, for water molecules, thermal energy is produced by the friction of the water molecules inside the cells, water evaporates, and the cell is disrupted due to the pressure of water vapor on the cell wall. Since heating favors the extraction of lipids from microalgae and

may reduce extraction time and energy consumption, microwaves are a technique tested for microalgal lipid extraction (GOH et al., 2019).

ONUMAEGBU et al. (2019) used microwave pre-treatment to extract lipids from *Scenedesmus quadricauda*. The microwave method was effective in cell disruption and was associated with the system's energy increase. According to the authors, the efficacy of cell rupture decreased after a specific time of pretreatment. The best lipid yield was obtained under 600 W of power, 8 min of heating time, and 3.5 h of extraction.

ZHOU et al. (2019) evaluated the optimization of lipid separation from *S. obliquus* by comparing two extraction methods: the first used biomass heating in a water bath, and the second was assisted by microwaving. The operational parameters described as ideal were 130 °C, extraction time 0.25 h, 3:2 solvent ratio of n-hexane:isopropanol, and 50:1 (mL g^{-1}) cosolvent:biomass phase ratio. Microwave-assisted extraction recovered 88.25% of lipids and 95.88% of fatty acid methyl esters, higher than those obtained in the first method. Scanning microscopic analysis (SEM) indicated that cell rupture facilitated solvent penetration in the cells.

Microwaves have been applied for cell disruption of microalgae because they can make extracting and recovering lipids easier. Nevertheless, microwaves can degrade thermolabile compounds due to the heating intensity. Additionally, using microwaves or ultrasound on an industrial scale is still unfeasible due to the difficulty in extracting biocompounds from denser media, affecting the process's scalability.

Pulsed electric field (PEF)

The permeabilization of wall membranes of different biological systems can be achieved by using PEF. The working principle of the PEF treatment is based on the application of short electric pulses (from a few nanoseconds to a few milliseconds) of high voltage (0.1 to 80 kV cm^{-1}) to the product between two electrodes (WANG et al., 2023). A critical electrical potential across the cell membrane is induced by applying high-intensity electric field pulse discharges that promote membrane permeabilization (electroporation phenomenon). This behavior can change the cell properties due to the appearance of pores in the membranes, increasing their permeability and promoting cytoplasmic dissolution (ZHOU et al., 2022). The basic PEF device setup typically includes an electrical pulse generator, a treatment chamber, and electrodes, with the electrical pulse placed between or across two electrodes (NALIYADHARA et al., 2022).

GAO et al. (2022) evaluated the PEF and ultrasonic treatments to enhance the extraction of selenium-enriched tea polysaccharides (Se-TPS) from selenium-enriched green tea leaves. The authors reported that PEF + aqueous extraction (solvent extraction) was optimal for extracting Se−TPS. PEF differs from pulsed ultrasound because PEF uses an electrical effect (power) to promote cell permeabilization, and pulsed ultrasound uses a mechanical effect (ultrasound wave).

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BENSALEM et al. (2020) researched the effects of electrical or mechanical constraints on the cell wall and membrane structure of *C. reinhardtii* using a combination of microscopic tools. Electroporation was considered irreversible at 7 kV cm^{-1} with 10 µs of led pulses. The electrical treatment combined with mechanical compression breaks the cell wall structure.

PEF extraction of substances is a non-thermal technology studied on a laboratory scale for recovering microalgal biomolecules due to its cleaning, safety, and high-efficiency advantages (GATEAU et al., 2021). WANG et al. (2023) evaluated the effects of PEF (3 kV cm^{-1}, 44 pulses, 99 kJ kg^{-1}), solvent [water and 50% dimethylsulfoxide (DMSO)], and extraction time (0, 10, 20, 30, 60, 90, 120, and 180 min) on the separation of biomolecules from *Chlorella*. At 120 min of extraction, more proteins and polyphenols were obtained using water as the extraction solvent, while more chlorophyll a and b and total carotenoids were obtained using 50% DMSO as the solvent. The analysis of *Chlorella* microstructure under a fluorescence microscope shows cells disrupted or damaged after PEF treatment, indicating that the electroporation phenomenon occurred during the PEF treatment and enhanced biomolecule recovery.

CARULLO et al. (2022) evaluated the cell rupture behavior using PEF and more intense treatments, such as HPH or ball milling. The PEF technology promoted higher efficiency in extracting pigments and chlorophyll from *Chlorella vulgaris* and lower efficiency in protein extraction. According to the authors, the pores formed in the microalgal cell membrane during PEF treatment are not large enough to release high molecular mass proteins. Therefore, these proteins remain trapped inside the cell or are limited to the cell wall. Thus, in cascade operation, combining PEF and HPH methods allowed the efficient extraction of biomolecules from C. vulgaris and the selective separation of distinct classes of compounds. The combined PEF-HPH technologies compared to

HPH treatment show *(1)* superior extraction yields of carbohydrate and lipid, *(2)* greater purity of the extracts, and *(3)* extracted biocompounds with improved quality due to the shorter biomass processing time with consequent shorter time of exposure to the heat generated in the HPH stage. Although, PEF is still restricted to the laboratory scale, its application can promote better disruption and extraction results when combined with other methods.

Non-mechanical methods

Non-mechanical disruption methods promote cell wall rupture due to changes in membrane permeability and cellular appearance caused by the action of chemicals, enzymatic agents, or heat. Several techniques for non-mechanical cell disruption of different microalgal species are depicted in table 3. The most used are the chemical and enzymatic methods.

Chemical methods

Chemical compounds such as solvents, acids, alkalis, hypochlorites, and surfactants interact with cell wall components, promoting cellular rupture. Acids and organic solvents stand out among the agents for chemical cell breakage (SIERRA et al., 2017; WANG et al., 2020). Chemical compounds have greater accessibility and lower acquisition costs than mechanical techniques for cell disruption. However, applying chemicals randomly in cell disruption can generate environmental problems and contamination since the usable chemicals are seldom classified as safe (WANG et al., 2020).

Acid treatment

Cell disruption using acid can be performed with organic acids, inorganic acids, and ionic liquids. Organic acids are less toxic and have greater

Table 3 - Non-mechanical rupture techniques.

-------Rupture techniques-------		Species	Operational conditions	References
Chemical	Acid	*Scenedesmus sp.* *Chlorella sp.* *Ankistrosdemus sp.* *Micromonas sp.* *Chlamydomonas sp.*	0 to 1.5 M H_2S0_4 40 to 120 min hydrolysis 23 to 90 °C	CASTRO et al., 2015
		Spirulina plantesis	0.5 to 5.5 M H_2S0_4 30 to 120 min hydrolysis 25 to 100 °C	DUONGBIA et al., 2019
		Haematococcus pluvialis	0.5 to 4.5 M HCL 3.2 to 16.8 min hydrolysis 56 to 84 °C	VECHIO et al., 2021
	Alkaline	*Chlorococcum infusionum*	0.75% w v^{-1} NaOH 30 min hydrolysis; 120 °C	HARUN et al., 2011
	Surfactant	*Nannochloropsis* sp.	Surfactant concentration: 60 to 500 mg L^{-1} Reaction time: 1 to 6 h 25 to 50 °C	WU et al., 2017
		Chlorella sorokiniana sp.	Surfactant concentration: 288.37 to 647 g mol^{-1} Surface charge: -49.9 to 61.5 mV *CMC: 0.2 to 14.4 mM	TAGHAVIJELOUDAR et al., 2021
Biological	Enzymatic	*Chlorella pyrenoidosa*	10% w v^{-1} biomass ** Cellulase: 200 U g^{-1} 200 mL mixture	ZHANG et al., 2020
		Schizochytrium sp.	1:10 ratio of biomass:hemicellulase Reaction time: 48 h; 55 °C	HAC ISA et al., 2021
	Enzymatic	*Chlorella vulgaris*	$1.5 \cdot 10^{-4}$ to $2 \cdot 10^{-1}$ mg mL^{-1} enzyme poll Reaction time: 24 h; 37 °C	CANELLI et al., 2021
---------Osmotic shock--------		*Chaetoceros muelleri*	1:5, biomass:water ratio	GONZÁLES-GONZÁLES et al., 2021

*Critical micelle concentration; **Amount to catalyze the transformation of 1 µmol of substrate per minute.

biodegradability than ionic liquids and inorganic acids. Nevertheless, inorganic acids are efficient in breaking microalgae cells and can act as catalysts, allowing higher yields of lipids, carbohydrates, and proteins when used at low temperatures and short reaction times. Temperatures between 110 and 150 °C and acid concentrations ranging from 1 to 1.5% (w v^{-1}) are operational conditions appropriate for the acidic rupture of cells (LEE & HAN, 2015).

The operational conditions of the acid treatment must be adapted following the target biomolecule characteristics. The acid agent concentrations can be adjusted as a function of the time and temperature of the process to avoid the degradation of some biomolecules, especially pigments, antioxidants, and proteins (NITSO et al., 2020). In general, treatment with dilute acid is an up-and-coming and effective alternative for the recovery of lipids and carbohydrates from microalgae. Acid treatment is cheaper than enzymatic treatments.

WANG et al. (2016) evaluated the use of hydrochloric acid and formic acid in the disruption of *Chlorella protothecoides* cells for lipid extraction. The microalgae were treated with acids in an acid:dry biomass ratio (w w^{-1}) of 1.5:1 for hydrochloric acid and 6.4:1 for formic acid. Formic acid disrupted the microalgae cells in aqueous suspensions only when it was supplemented with a small amount of hydrochloric acid.

RIZZA et al. (2017) studied the acid hydrolysis of *Desmodesmus sp*. biomass to maximize the extraction efficiency of sugar for bioethanol production. The authors reported optimal operational conditions: biomass concentrations of 10% (w w^{-1}) and 2% H_2SO_4 (v v^{-1}), and heating at 120 °C for 30 min. Under these conditions, 9% of the total sugar content was released.

VECHIO et al. (2021) analyzed multiple parameters of cell rupture of wet *Haematococcus pluvialis* biomass to maximize the recovery of astaxanthin by solid-liquid extraction. Astaxanthin was recovered at the highest rate (99 ± 0.48)% under the operational conditions of 71 °C, 17 min of acid hydrolysis, and [HCl] = 3.7 N. Despite the good astaxanthin recovery, the treatment did not cause total cell rupture, allowing the solvent to penetrate only by microperforations in the cell wall, according to the authors.

Remarkably, the purification of extracts using large volumes of solvents makes acid treatment in large-scale production challenging. The optimization of the time and temperature of extraction is necessary to avoid degradation of the target biocompounds. Unlike acids and some organic solvents, another chemical agent to break cells can be used in large-scale production to address this limitation, i.e., surfactants.

Surfactants

Surfactants interact with phospholipids in the cell wall, promote cell rupture, and synchronously enable the release of biomolecules (HUANG & KIM, 2013). Surfactants are an economically and environmentally viable alternative for microalgal cell breaking when the harvesting conditions of feedstock are unfavorable.

LAI et al. (2018) evaluated the recovery of lipids and pigments from the cyanobacterium *Synechocystis sp* PCC 6803. The cationic surfactant hexadecyltrimethylammonium bromide (CTAB) was more effective in breaking the cells and recovering the pigments than dodecyl trimethylammonium bromide (DTAB). The latter was more effective in recovering lipids than CTAB. According to the authors, the higher number of carbons of the alkyl group of CTAB than DTAB influenced the extraction efficiency of the target metabolites. Thus, the concentration and type of surfactant used should be specifically directed to the target biomolecule.

TAGHAVIJELOUDAR et al. (2021) studied the effect of different surfactants on the harvest and extraction of exopolysaccharides (EPS) from *Chlorella sorokiniana sp*. The non-ionic surfactant Triton X-100 was utilized to optimize EPS extraction from the biomass. The pH adjustment reduced the flocculant amount for EPS release. According to the authors, all surfactants efficiently separated EPS from the biomass. The efficiency order of EPS release was Triton X-100 > SDS > CTAB > DTAB. Triton X-100 and SDS represent non-ionic and anionic surfactants, respectively, whereas the CTAB and DTAB surfactants correspond to cationic surfactants.

Among the different chemical agents, surfactants fit cell disruption due to their economic viability, low harm to environmental issues, and suitability for use on a large scale.

Enzymatic methods

Enzymatic hydrolysis is a non-mechanical disruption technique with the advantage of the sustainability of the biological enzyme for cell breaking. Nonetheless, hydrolysis efficiency with individual enzymes is low, requiring a pool of enzymes. Furthermore, the mechanisms of microalgal cell wall rupture need to be evaluated in depth because studies on cell disruption from enzymes are mostly centered on the effect of process conditions, enzyme amount, temperature, and pH on disruption

efficiency. For example, an enzymatic blend of xylanase, pectinase, snailase, lysozyme, and trypsin promoted higher hydrolysis than using the enzymes individually (ZHANG et al., 2022).

ZHANG et al. (2020) applied cellulase to hydrolyze *Chlorella pyrenoidosa* biomass. The authors reported *(a)* dry biomass yields of lipids of 16.89% to 23.65% and proteins of 32.30% to 42.16%, *(b)* a satisfactory degree of unsaturation of the fatty acids for biodiesel production, and *(c)* the utilization of the hydrolyzed biomass as a carbon source for the mixotrophic cultivation of different microalgal species; thus, the hydrolyzed biomass is an alternative carbon source economically viable for use in microalgae growth.

ZHANG et al. (2022) evaluated the combination of different enzymes in the pre-treatment of *Scenedesmus obliquus* for a more effective recovery of lipids. The best treatment corresponded to the combination (mg of enzyme/g of dry biomass) of pectinase (10 mg g^{-1}), cellulase (20 mg g^{-1}), and xylanase (14 mg g^{-1}). Microscopic scanning was used to check the enzymes' action in the tri-laminary layers of the cell wall, indicating that enzymatic hydrolysis was an effective disruption technique to recover lipids from microalgae.

SIERRA et al. (2017) used autolysin, a cell wall-degrading protease from *Chlamydomonas reinhardtii*, as a pretreatment to break the *C. reinhardtii* cell walls because only the residues rich in proline in the cell membranes are substrates suitable for degradation. Thus, recoverable bioproducts such as proteins, lipids, and pigments remain intact. The authors noted that the action of this enzymatic rupture method can preserve valuable bioproducts while allowing high levels of cell rupture, as autolysin has specific sites of action.

SOUZA et al. (2020) tested different cell disruption methods for *Chlorella sorokiniana*, including enzymatic rupture. The action of the cellulase enzyme was ineffective in cell rupture and did not contribute to the increase in the starch content. However, the authors reported a higher release of biocompounds by combining enzyme procedures and vibratory grinding. Thus, enzymatic cell breaking required pre-treatment to increase the rupture or an enzyme pool to enhance the disruption efficiency. SIERRA et al. (2017) also proposed aqueous enzymatic-assisted extraction with solvent to increase cell wall disruption.

CONCLUSION

Microalgae have many biomolecules that perform numerous biological functions; however, integral access to these components is limited due to the rigid nature of the cell walls. Among the mechanical methods, the ball milling technique was promising mainly due to its better efficacy and possible use in larger production scales. The optimization of the pressure and number of passes in the HPH could offer promising results of cell rupture aiming the recovery of microalgae biocompounds. However, mechanical processes release much energy in the heat form and must be coupled to a cooling system to avoid the degradation of thermolabile compounds such as proteins and pigments.

Non-mechanical methods include using chemical reagents or enzymes to interact with the microalgal cell membranes and break the cell walls. Consequently, the intercellular components can be transferred to the ruptured biomass suspension. It is worth emphasizing the proper selection of chemical reagents or enzymes for effective disruption and to determine ideal process conditions. Conversely, non-mechanical methods, which are less expensive than mechanical methods, may present toxicity and degradability in thermolabile compounds, which would be a reason to reduce the use of these compounds in cell rupture.

High-pressure homogenization with higher working pressures, such as ultra-high-pressure homogenization, and fewer passes are also desirable in microalgal cell disruption. Such conditions can be easily implemented in industrial plants, mainly because it is a known technique used in industry for processing dairy products. Moreover, studies using combinations of cell disruption techniques that can be implemented in the same processing line should be expanded, aiming to increase the yield of biocompound extraction, contributing to the value of the microalgae production chain and the extraction of co-products.

Overall, implementing cell disruption associated with energy efficiency and selective extraction of biocompounds from microalgae remains largely unexplored. Therefore, future research should focus on designing combined methods of cell disruption and selective extraction of compounds, providing economically viable and functional processes. More research must be carried out to optimize the extraction of biocompounds without causing their degradation. In addition, the mechanism of interaction of bioactive compounds under the treatment of innovative technologies should be further investigated.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest and do not have any financial or personal association with institutions, teams, or individuals who could improperly affect the paper.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

REFERENCES

AMORIM, M. L. et al. Microalgae proteins: production, separation, isolation, quantification, and application in food and feed. **Critical Reviews in Food Science and Nutrition**, v.61, n.12, p.1976-2002, 2021. Available from: <https://www.tandfonline.com/doi/full/10.1080/10408398.2020.1768046>. Accessed: Jun. 05, 2022. doi: 10.1080/10408398.2020.1768046.

ANGLES, E. et al. Wet lipid extraction from the microalga Nannochloropsis sp. disruption, physiological effects and solvent screening. **Algal Research**, v.21, p.27–34, 2017. Available from: <https://www.sciencedirect.com/science/article/pii/S2211926416306221>. Accessed: Nov. 20, 2021. doi: 10.1016/j.algal.2016.11.005.

BATISTA, A. P. et al. Comparison of microalgal biomass profiles as novel functional ingredient for food products. **Algal Research**, v.2, n.2, p.164-173, 2013. Available from: <https://www.sciencedirect.com/science/article/pii/S2211926413000210>. Accessed: Jul. 06, 2022. doi: 10.1016/j.algal.2013.01.004.

BERNAERTS, T. M. et al. The potential of microalgae and their biopolymers as structuring ingredients in food: A review. **Biotechnology Advances**, v.37, n.8, e107419, 2019. Available from: <https://www.sciencedirect.com/science/article/pii/S0734975019301193>. Accessed: Oct. 23, 2021. doi: 10.1016/j.biotechadv.2019.107419.

BENSALEM, S. et al. Impact of pulsed electric fields and mechanical compressions on the permeability and structure of Chlamydomonas reinhardtii cells. **Scientific Reports**, v.10, n.1, p.1-11, 2020. Available from: <https://www.researchgate.net/publication/339268590>. Accessed: May, 11, 2022. doi: 10.1038/s41598-020-59404-6.

BHATTACHARYA, M.; GOSWAMI, S. Microalgae – A green multiproduct biorefinery for future industrial prospects. **Biocatalysis and Agricultural Biotechnology**, v.25, e10151080, 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S1878818119318699>. Accessed: Feb. 19, 2022. doi: 101580. 10.1016/j.bcab.2020.101580.

BRUTON, T. **A review of the potential of marine algae as a source of biofuel in Ireland**. Dublin: Sustainable Energy Ireland, 2009. 88p. (Technical Report). Available from: <http://cdn.technologynetworks.com/TN/Resources/PDF/algae%20report%2004%202009.pdf>. Accessed: Jul. 06, 2022.

CAI, T. et al. Nutrient recovery from wastewater streams by microalgae: status and prospects. **Renewable and Sustainable Energy Reviews**, v.19, p.360–369, 2013. Available from: <https://www.sciencedirect.com/science/article/pii/S1364032112006429>. Accessed: Apr. 10, 2022. doi: 10.1016/j.rser.2012.11.030. doi: 10.1016/j.rser.2012.11.030.

CANELLI, G. et al. Tailored enzymatic treatment of *Chlorella vulgaris* cell wall leads to effective disruption while preserving oxidative stability. **LWT - Food Science and Technology**, v.143, e111157, 2021. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0023643821003108>. Accessed: Dec. 17, 2021. doi: 10.1016/j.lwt.2021.111157.

CASTRO, A. Y. et al. Optimization of wastewater microalgae saccharification using dilute acid hydrolysis for acetone, butanol, and ethanol fermentation. **Applied Energy**, v.140, n.15, p.14-19, 2015. Available from: <https://www.sciencedirect.com/science/article/pii/S0306261914012148>. Accessed: Sep. 18, 2021. doi: 10.1016/j.apenergy.2014.11.045.

CARULLO, D. et al. Application of pulsed electric fields and high-pressure homogenization in biorefinery cascade of *C. vulgaris* microalgae. **Foods**, v.11, n.3, p.1-16, 2022. Available from: <https://pubmed.ncbi.nlm.nih.gov/35159621/>. Accessed: Mar. 29, 2022. doi: 10.3390/foods11030471.

CHISTI, Y. Biodiesel from microalgae beats bioethanol. **Trends in Biotechnology**, v.26, n.3, p.126-131, 2008. Available from: <https://pubmed.ncbi.nlm.nih.gov/34643788/>. Accessed: Feb. 10, 2022. doi: 10.1016/j.tibtech.2007.12.002.

DAI, L. et al. Emulsifying properties of acid-hydrolyzed insoluble protein fraction from *Chlorella protothecoides*: Formation and storage stability of emulsions. **Food Hydrocolloid**, v.108, e105954, 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S0268005X19330413>. Accessed: May, 10, 2021. doi: 10.1016/j.foodhyd.2020.105954.

DE SOUZA LEITE, L. et al. Microalgae harvesting from wastewater by pH modulation and flotation: assessing and optimizing operational parameters. **Journal of Environmental Management**, v.254, e109825, 2020. Available from: <https://pubmed.ncbi.nlm.nih.gov/31733467/>. Accessed: Sep. 10, 2021. doi: 10.1016/j.jenvman.2019.109825.

DO CARMO CESÁRIO, C. et al. Biochemical and morphological characterization of freshwater microalga *Tetradesmus obliquus* (Chlorophyta: Chlorophyceae). **Protoplasma**, p.1-12, 2021. Available from: <https://www.sciencedirect.com/science/article/pii/S0167779908000218>. Accessed: Jan. 23, 2022. doi: 10.1007/s00709-021-01712-3.

DUONGBIA, N. et al. Acidic hydrolysis performance and hydrolyzed lipid characterizations of wet *Spirulina platensis*. **Biomass Conversion and Biorefinery**, v.9, p.305-319, 2019. Available from: <https://link.springer.com/article/10.1007/s13399-018-0350-6>. Accessed: Mar. 30, 2022. doi: 10.1007/s13399-018-0350-6.

EBERT, S. et al. Emulsifying properties of water-soluble proteins extracted from the microalgae: *Chlorella sorokiniana* and *Phaeodactylum tricornutum*. **Food & Function**, v.10, n.2, p.754–64, 2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/30667441/>. Accessed: Dec. 20, 2021. doi: 10.1039/c8fo02197j.

FRANCO, A. L. C. et al. Biodiesel de microalgas: avanços e desafios. **Química Nova**, v.36, n.3, p.437-448, 2013. Available from: <https://www.scielo.br/j/qn/a/tgQT4yYWsDmdJJ7J86Y49JL/?lang=pt>.

Accessed: Oct. 27, 2021. doi: 10.1590/S0100-40422013000300015.

GAO, W. et al. Polysaccharides in selenium-enriched tea: Extraction performance under innovative technologies and antioxidant activities. **Foods**, v.11, n.17, 2545, 2022. Available from: <https://www.mdpi.com/2304-8158/11/17/2545>. Accessed: Jul. 18, 2023. doi: 10.3390/foods11172545.

GATEAU, H. et al. Application of pulsed electric fields for the biocompatible extraction of proteins from the microalga *Haematococcus pluvialis*. **Bioelectrochemistry**, v.137, p.107588, 2021. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1567539419308333?via%3Dihub>. Accessed: May, 11, 2022. doi: 10.1016/j.bioelechem.2020.107588.

GILLE, A. et al. Bioaccessibility of carotenoids from *Chlorella vulgaris* and Chlamydomonas reinhardtii. **International Journal of Food Sciences and Nutrition**, v.67, n.5, p.507–513, Jul. 3, 2016. Available from: <https://www.tandfonline.com/doi/abs/10.1080/09637486.2016.1181158>. Accessed: Jul. 18, 2023. doi: 10.1080/09637486.2016.1181158.

GOH, B. H. H. et al. Sustainability of direct biodiesel synthesis from microalgae biomass: A critical review. **Renewable and Sustainable Energy Reviews**, v.107, p.59–74, 2019. Available from: <https://www.sciencedirect.com/science/article/pii/S1364032119301054>. Accessed: Nov. 17, 2021. doi: 10.1016/j.rser.2019.02.012.

GONG, M., BASSI, A. Carotenoids from microalgae: a review of recent developments. **Biotechnology Advances**, v.34, n.8, p.1396–1412, 2016. Available from: <https://pubmed.ncbi.nlm.nih.gov/27816618/>. Accessed: Apr. 04, 2022. doi: 10.1016/j.biotechadv.2016.10.005.

GONZÁLES-GONZÁLES, L. M. et al. Osmotic shock pretreatment of *Chaetoceros muelleri* wet biomass enhanced solvent-free lipid extraction and biogas production. **Algal Research**, v.54, e10217, 2021. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S2211926420310456>. Accessed: Apr. 05, 2022. doi: 10.1016/j.algal.2020.102177.

GOUVEIA, L. et al. Microalgae in novel food product. In: KONSTANTINOS, N.; PAPADOPOULOS, P. P. **Food Chemistry Research Developments**. New York: Nova Science Publishers Inc., 2008. Chap.2, p.1-37.

GROSSMANN, L. et al. Production of protein-rich extracts from disrupted microalgae cells: Impact of solvent treatment and lyophilization. **Algal Research**, v.36, p.67–76, 2018. Available from: <https://www.sciencedirect.com/science/article/pii/S2211926418303175>. Accessed: Nov. 23, 2021. doi: 10.1016/j.algal.2018.09.011.

GROSSMANN, L. et al. Solubility of extracted proteins from *Chlorella sorokiniana*, *Phaeodactylum tricornutum*, and *Nannochloropsis oceanica*: Impact of pH-value. **LWT - Food Science and Technology**, v.105, p.408–416, 2019. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0023643819300519?via%3Dihub>. Accessed: Mar. 11, 2022. doi: 10.1016/j.lwt.2019.01.040.

GUL, O. et al. Effect of high pressure homogenization (HPH) on microstructure and rheological properties of hazelnut milk. **Innovative Food Science & Emerging Technologies**, v.41, p.411-420, 2017. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1466856417305222>. Accessed: Feb. 15, 2022. doi: 10.1016/j.ifset.2017.05.002.

GÜNERKEN, E. et al. Cell disruption for microalgae biorefineries. **Biotechnology Advances**, v.33, n.2, p.243–260, 2015. Available from: <https://www.sciencedirect.com/science/article/pii/S074397501500021X>. Accessed: Nov. 23, 2021. doi: 10.1016/j.biotechadv.2015.01.008.

HAC ISA, M. C. et al. Effect of different cell disruption methods on lipid yield of *Schizochytrium sp*. **Journal of the American Oil Chemists` Society**, v.99, n.2, p.129-139, 2021. Available from: <https://www.authorea.com/users/420143/articles/526520-effect-of-different-cell-disruption-methods-on-lipid-yield-of-schizochytrium-sp>. Accessed: Apr. 09, 2022. doi: 10.1002/aocs.12551.

HARUN, R. et al. 2011. Exploring alkaline pretreatment of microalgal biomass for bioethanol production. **Applied Energy**, v.88, n.10, p.3464-3467, 2011. Available from: <https://www.sciencedirect.com/science/article/pii/S1364032109002664>. Accessed: Apr. 03, 2022. doi: 10.1016/j.apenergy.2010.10.048.

HOSSAIN, N.; MAHLIA, T. M. I. Progress in physicochemical parameters of microalgae cultivation for biofuel production. **Critical Reviews in Biotechnology**, v.39, n.6, p.835-859, 2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/31185749/>. Accessed: Feb. 06, 2022. doi: 10.1080/07388551.2019.1624945.

HU, Q. et al. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. **The Plant Journal**, v.54, n.4, p.621-39, 2008. Available from: <https://asu.pure.elsevier.com/en/publications/microalgal-triacylglycerols-as-feedstocks-for-biofuel-production>. Accessed: Sep. 19, 2021. doi: 10.1111/j.1365-313X.2008.03492.x.

HUANG, W.; KIM, J. Cationic surfactant-based method for simultaneous harvesting and cell disruption of microalgal biomass. **Bioresource Technology**, v.149, p.579–581, 2013. Available from: <https://www.sciencedirect.com/science/article/pii/S0960852413015277>. Accessed: Oct. 29, 2021. doi: 10.1016/j.biortech.2013.09.095.

IDO, A. L. et al. Application of central composite design in the optimization of lipid yield from *Scenedesmus obliquus* microalgae by ultrasound-assisted solvent extraction. **Energy**, v.157, n.15, p.949-956, 2018. Available from: <https://www.sciencedirect.com/science/article/pii/S0360544218307941>. Accessed: Apr. 07, 2022. doi: 10.1016/j.energy.2018.04.171.

KUROKAWA, M. et al. Effect of sonication frequency on the disruption of algae. **Ultrasonics Sonochemistry**, v.31, p.157–162, 2016. Available from: <https://www.sciencedirect.com/science/article/pii/S1350417715301048#:~:text=The%20results%20showed%20that%20reduction,and%204.3%20MHz%20for%20C>. Accessed: Apr. 09, 2022. doi: 10.1016/j.ultsonch.2015.12.011.

LAI, Y. S. et al. Cell disruption by cationic surfactants affects bioproduct recovery from *Synechocystis* sp. PCC 6803. **Algal Research**, v.34, p.250-255, 2018. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S2211926418302868>. Accessed: Apr. 02, 2022. doi: 10.1016/j.algal.2018.08.010.

LEE, S. Y. et al. Cell disruption and lipid extraction for microalgal biorefineries: A review. **Bioresource Technology**, v.244, n.2, p.1317–1328, 2017. Available from: <https://www.sciencedirect.com/science/article/pii/S0960852417309252>. Accessed: Oct. 16, 2021. doi: 10.1016/j.biortech.2017.06.038.

LEE, I.; HAN, J-I. Hydrothermal-acid treatment for effectual extraction of eicosapentaenoic acid (EPA)-abundant lipids from *Nannochloropsis salina*. **Bioresource Technology**, v.191, p.1–6,

2015. Available from: <https://pubmed.ncbi.nlm.nih.gov/25966023/>. Accessed: Apr. 07, 2022. doi: 10.1016/j.biortech.2015.04.124.

LEI, D. et al. Complexation of soybean protein isolate with β-glucan and myricetin: Different affinity on 7S and 11S globulin by QCM-D and molecular simulation analysis. **Food Chemistry**: X, v.15, 100426, 2022. Available from: <https://www.sciencedirect.com/science/article/pii/S2590157522002243>. Accessed: Jul. 17, 2023. doi: 10.1016/j.fochx.2022.100426.

LIMA, V. S. et al. Stabilization of oil–water emulsions with protein concentrates from the microalga *Tetradesmus obliquus*. **Journal of Food Science and Technology**, v.60, n.2, p.797–808, 2023. Available from: <https://link.springer.com/article/10.1007/s13197-023-05666-7>. Accessed: Feb. 13, 2023. doi: 10.1007/s13197-023-05666-7.

LIU, S. et al. Coupling bead-milling and microfiltration for the recovery of lipids and proteins from *Parachlorella kessleri*: Impact of the cell disruption conditions on the separation performances. **Separation and Purification Technology**, v.287, n.15, e120570, 2022. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1383586622001307>. Accessed: Apr. 02, 2022. doi: 10.1016/j.seppur.2022.120570.

MARTÍNEZ, J. M. et al. Use of pulsed electric field permeabilization to extract astaxanthin from the nordic microalgae *Haematococcus pluvialis*. **Bioresource Technology**, v.289, p.e121694, 2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/31254897/>. Accessed: Dec. 16, 2021. doi: 10.1016/j.biortech.2019.121694.

MARTINS, A. et al. Microalgae for biodiesel production and other applications: a review. **Renewable Sustainable Energy Reviews**, v.14, n.1, p.217–232, 2010. Available from: <https://www.sciencedirect.com/science/article/pii/S1364032109001646>. Accessed: Apr. 08, 2022. doi: 10.1016/j.rser.2009.07.020.

MONTALESCOT, V. et al. Optimization of bead milling parameters for the cell disruption of microalgae: process modeling and application to *Porphyridium Cruentum* and *Nannochloropsis Oculata*. **Bioresource Technology**, v.196, p.339–346, 2015. Available from: <https://www.sciencedirect.com/science/article/pii/S0960852415010378>. Accessed: Sep. 18, 2021. doi: 10.1016/j.biortech.2015.07.075.

NALIYADHARA, N. et al. Pulsed electric field (PEF): Avant-garde extraction escalation technology in food industry. **Trends in Food Science & Technology**, v.122, p.238-255, 2022. Available from: <https://www.sciencedirect.com/science/article/pii/S092422442200067X>. Accessed: May, 11, 2022. doi: 10.1016/j.tifs.2022.02.019.

NITSOS, C. et al. Current and novel approaches to downstream processing of microalgae: A review. **Biotechnology Advances**, v.45, 107650, 2020. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S073497502030152X?via%3Dihub>. Accessed: Apr. 06, 2023. doi: 10.1016/j.biotechadv.2020.107650.

ONUMAEGBU, C. et al. Modelling and optimization of wet microalgae *Scenedesmus quadricauda* lipid extraction using microwave pretreatment method and response surface methodology. **Renewable Energy**, v.132, p.1323-1331, 2019. Available from: <https://www.sciencedirect.com/science/article/pii/S0960148118310711>. Accessed: Apr. 03, 2022. doi: 10.1016/j.renene.2018.09.008.

RIVERA, E. C. et al. Mechanical cell disruption of *Parachlorella kessleri* microalgae: impact on lipid fraction composition. **Bioresource Technology**, v.256, p.77–85, 2018. Available from: <https://hal.archives-ouvertes.fr/hal-01823864>. Accessed: Apr. 06, 2022. doi: 10.1016/j.biortech.2018.01.148.

RIZZA, L. S. et al. Bioprospecting for native microalgae as an alternative source of sugars for the production of bioethanol. **Algal Research**, v.22, p.140–147, 2017. Available from: <https://www.sciencedirect.com/science/article/pii/S2211926416308268>. Accessed: Sep. 15, 2022. doi: 10.1016/j.algal.2016.12.021.

SAFI, C. et al. Understanding the effect of cell disruption methods on the diffusion of *Chlorella vulgaris* proteins and pigments in the aqueous phase. **Algal Research**, v.8, p.61–68, 2015. Available from: <https://www.sciencedirect.com/science/article/pii/S2211926415000132>. Accessed: Apr. 17, 2022. doi: 10.1016/j.algal.2015.01.002.

SANKARAN, R. et al. Extraction of proteins from microalgae using integrated method of sugaring-out assisted liquid biphasic flotation (LBF) and ultrasound. **Ultrasonics Sonochemistry**, v.48, p.231–239, 2018. Available from: <https://www.sciencedirect.com/science/article/pii/S1350417718300944>. Accessed: Feb. 21, 2022. doi: 10.1016/j.ultsonch.2018.06.002.

SCHÜLER, L. M. et al. Improvement of carotenoid extraction from a recently isolated, robust microalga, *Tetraselmis sp*. CTP4 (Chlorophyta). **Bioprocess and Biosystems Engineering**, v.43, p.785–796, 2020. Available from: <https://link.springer.com/article/10.1007/s00449-019-02273-9>. Accessed: Feb. 25, 2022. doi: 10.1007/s00449-019-02273-9.

SCHWENZFEIER, A. et al. Isolation and characterization of soluble protein from the green microalgae *Tetraselmis sp*. **Bioresource Technology**, v.102, n.19, p.9121–9127, 2011. Available from: <https://pubmed.ncbi.nlm.nih.gov/21831634/>. Accessed: Oct. 25, 2021. doi: 10.1016/j.biortech.2011.07.046.

SERIVE, B. et al. Selection and optimization of a method for efficient metabolites extraction frommicroalgae. **Bioresource Technology**, v.124, p.311-320. Available from: <https://www.sciencedirect.com/science/article/pii/S0960852412011595>. Accessed: Mar. 02, 2021. doi: 10.1016/j.biortech.2012.07.105.

SHENG, J. et al. Disruption of *Synechocystis* PCC 6803 for lipid extraction. **Water Science and Technology**, v.65, p.567-73, 2012. Available from: <https://pubmed.ncbi.nlm.nih.gov/22258690/>. Accessed: Apr. 07, 2022. doi: 10.2166/wst.2012.879.

SIERRA, L. S. et al. Enzymatic cell disruption of the microalgae *Chlamydomonas reinhardtii* for lipid and protein extraction. **Algal Research**, v.25, p.149–159, 2017. Available from: <https://www.sciencedirect.com/science/article/pii/S2211926417303752>. Accessed: Dec. 10, 2021. doi: 10.1016/j.algal.2017.04.004.

SILVA, M. E. T. et al. *Scenedesmus obliquus* protein concentrate: a sustainable alternative emulsifier for the food industry. **Algal Research**, v.59, e102468, 2021a. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S2211926421002873>. Accessed: Dec. 18, 2021. doi: 10.1016/j.algal.2021.102468.

SILVA, M. E. T. DA et al. Microalga *Scenedesmus obliquus*: extraction of bioactive compounds and antioxidant activity. **Revista Ciência Agronômica**, v.52, n.2, 2021b. Available from: <http://ccarevista.ufc.br/seer/index.php/ccarevista/article/view/6848/2005>. Accessed: Oct. 19, 2021. doi: 10.5935/1806-6690.20210036.

SOUZA, M. F. et al. Effect of milling and enzymatic hydrolysis in the production of glucose from starch-rich *Chlorella sorokiniana* biomass.

Algal Research, v.50, p.e101961, 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S2211926419300025>. Accessed: Apr. 05, 2022. doi: 10.1016/j.algal.2020.101961.

SPIDEN, E. M. et al. Quantitative evaluation of the ease of rupture of industrially promising microalgae by high pressure homogenization. **Bioresource Technology**, v.140, p.165-71, 2013. Available from: <https://pubmed.ncbi.nlm.nih.gov/23688668/>. Accessed: Dec. 15, 2021. doi: 10.1016/j.biortech.2013.04.074.

SPOLAORE, P. et al. Commercial applications of microalgae. **Journal of Bioscience and Bioengineering**, v.101, n.2, p.87–96, 2006. Available from: <https://www.sciencedirect.com/science/article/pii/S1389172306705497>. Accessed: Apr. 07, 2022. doi: 10.1263/jbb.101.87.

TAGHAVIJELOUDAR, M. et al. Simultaneous harvesting and extracellular polymeric substances extrusion of microalgae using surfactant: Promoting surfactant-assisted flocculation through pH adjustment. **Bioresource Technology**, v.319, e124224, 2021. Available from: <https://www.sciencedirect.com/science/article/pii/S096085242031498X>. Accessed: Apr. 07, 2022. doi: 10.1016/j.biortech.2020.124224.

TIBBETTS, S. M. et al. Nutritional evaluation of whole and lipid-extracted biomass of the microalga *Scenedesmus sp*. AMDD isolated in Saskatchewan, Canada for animal feeds: proximate, amino acid, fatty acid, carotenoid and elemental composition. **Current Biotechnology**, v.4, p.530 p.546, 2015. Available from: <https://www.ingentaconnect.com/content/ben/cbiot/2015/00000004/00000004/art00015#expand/collapse>. Accessed: Feb. 15, 2021. doi: 10.2174/2211550104666150827201854.

TIGRINE-KORDJANI, N. et al. Contribution of microwave accelerated distillation in the extraction of the essential oil of *Zygophyllum album* L. **Phytochemical Analysis**, v.22, n.1, p.1-9. Available from: <https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/pca.1236>. Accessed: Mar. 29, 2022. doi: 10.1002/pca.1236.

UNITED NATIONS. **WPP - World population prospects: the 2019 revision**. New York: United Nations Population Division, 2019. (Technical Report). Available from: <https://population.un.org/wpp/>. Accessed: Mar. 29, 2022.

URSU, A. V. et al. Extraction, fractionation and functional properties of proteins from the microalgae *Chlorella vulgaris*. **Bioresource Technology**, v.157, p.134–139, 2014. Available from: <https://www.sciencedirect.com/science/article/pii/S0960852414000960>. Accessed: Oct. 19, 2021. doi: 10.1016/j.biortech.2014.01.071.

VIEIRA, B. B. et al. Optimized extraction of neutral carbohydrates, crude lipids and photosynthetic pigments from the wet biomass of the microalga *Scenedesmus obliquus* BR003. **Separation and Purification Technology**, v.269, n.12, 2021. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1383586621004238>. Accessed: Oct. 19, 2021. doi: 10.1016/j.seppur.2021.118711.

VECHIO, H. et al. A new approach on astaxanthin extraction via acid hydrolysis of wet *Haematococcus pluvialis* biomass. **Journal of Applied Phycology**, v.33, n.5, p.2957-2966, 2021. Available from: <https://link.springer.com/article/10.1007/s10811-021-02495-z>. Accessed: Apr. 10, 2022. doi: 10.1007/s10811-021-02495-z.

WAGHMARE, A. et al. Hydrodynamic cavitation for energy efficient and scalable process of microalgae cell disruption. **Algal Research**, v.40, e101496, 2019. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S2211926418306490>. Accessed: Apr. 07, 2022. doi: 10.1016/j.algal.2019.101496.

WANG, M. et al. Algal cell lysis by bacteria: A review and comparison to conventional methods. **Algal Research**, v.46, e101794, 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S2211926419311403>. Accessed: Apr. 02, 2022. doi: 10.1016/j.algal.2020.101794.

WANG, M. et al. Pulsed electric field (PEF) recovery of biomolecules from *Chlorella*: Extract efficiency, nutrient relative value, and algae morphology analysis. **Food Chemistry**, v.404, p.134615, 2023. Available from: <https://www.sciencedirect.com/science/article/pii/S0308814622025778>. Accessed: May, 11, 2022. doi: 10.1016/j.foodchem.2022.134615.

WU, C. et al. Surfactants assist in lipid extraction from wet *Nannochloropsis sp*. **Bioresource Technology**, v.243, p.793-799, 2017. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0960852417310969?via%3Dihub>. Accessed: Apr. 02, 2022. doi: 10.1016/j.biort ech.2017.07.010.

YAP, B. H. et al. Energy evaluation of algal cell disruption by high pressure homogenization. **Bioresource Technology**, v.184, p.280-285, 2015. Available from: <https://www.sciencedirect.com/science/article/pii/S0960852414016514>. Accessed: Oct. 27, 2021. doi: 10.1016/j.biortech.2014.11.049.

ZHANG, J. et al. Utilization of enzymatic cell disruption hydrolysate of *Chlorella pyrenoidosa* as potential carbon source in algae mixotrophic cultivation. **Algal Research**, v.45, e101730, 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S2211926419302723>. Accessed: Apr. 05, 2022. doi: 10.1016/j.algal.2019.101730.

ZHANG, Y. et al. Assessment of enzyme addition strategies on the enhancement of lipid yield from microalgae. **Biochemical Engineering Journal**, 177, e108198, 2022. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1369703X21002746>. Accessed: Apr. 06, 2022. doi: 10.1016/j.bej.2021.108198.

ZHOU, X. et al. Optimization of microwave assisted lipid extraction from microalga *Scenedesmus obliquus* grown on municipal wastewater. **Journal of Cleaner Production**, v.221, n.1, p.502-508, 2019. Available from: <https://www.sciencedirect.com/science/article/pii/S0959652619306651>. Accessed: Apr. 07, 2022. doi: 10.1016/j.jclepro.2019.02.260.

ZHOU, L. et al. Bioactive compounds in microalgae and their potential health benefits. **Food Bioscience**, p.101932, 2022. Available from: <https://www.sciencedirect.com/science/article/pii/S2212429222003923?via%3Dihub>. Accessed: May, 11, 2022. doi: 10.1016/j.fbio.2022.101932.