Algal Derived Functional Lipids and their Role in Promoting Health

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Abstract: Algae are organisms with an enormous biodiversity and contain manifold fatty acid molecular structures and ratios. This chapter presents information about the structure and distribution of fatty acids in algae whether microalgae or macroalgae. The parameters affecting lipid and fatty acid contents as well as distribution are also discussed. Methods for isolation and purification of fatty acids from algae to obtain highly pure fatty acid or fatty acid fractions with good overall yields are presented. Algal fatty acids are known as sources of bioactive compounds for health promotion and wellness. Lipid fractions and fatty acids act as actives or additives for cosmeceutical applications. For health promotion, preventive effects of n-3 polyunsaturated fatty acids on cardiovascular disease, metabolic syndrome and cancer are discussed. The role of n-3 fatty acids in cancer therapy and antiviral activities of lipids are also exposed. Furthermore, potential commercial application domains and concepts are discussed in this chapter.

Key words: fatty acids; microalgae; macroalgae; seaweed; human health; cosmetic; purification; cancer; cardiovascular disease; metabolic syndrome

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1. Introduction

Lipids are fatty or waxy organic compounds soluble in non-polar solvents and include fats, waxes, sterols, fat-soluble vitamins, glycerolipids, phospholipids or glycolipids among others (Holdt & Kraan, 2011). The main roles of lipids comprise energy storage (Reeds, Brzezinski, Coury, Graham, & Petty, 1999), cell signaling and structural composition of intracellular and cellular membranes (Thompson, 1996). Study of the lipidome consists generally in a total lipid content determination and in a fatty acid (FA) profiling. Eukaryotic algae comprise more than 50,000 species (Guiry & Guiry, 2019) and include microalgae (phytoplankton) and macroalgae (seaweed) separated according to the genomic and phenotypic classification. This chapter reviews both microalgae and macroalgae including, but not limited to Chlorophyta, Rhodophyta, Ochrophyta, Chrysophyta, Dinophyta, Prymnesiophyta and Bacillariophyceae (diatoms).

Algal FA account for a wide molecular variety and are studied for their potential nutritional benefits and their capacity to act as chemotaxonomic markers. Moreover, the algal FA content and profile can be affected or even controlled by external parameters such as light, nutrient availability, osmotic stress, temperature or presence of pollutants (Bhaskar, Miyashita, & Hosokawa, 2004; Colombo et al., 2006; Floreto, Hirata, Yamasaki, & Castro, 1994a, 1994b; Floreto, Teshima, & Ishikawa, 1996; Hotimchenko, 2002; Khotimchenko & Levchenko, 1997; McCauley, Meyer, Winberg, & Skropeta, 2016; Mishra, Temelli, Ooraikul, Shacklock, & Craigie, 1993; Schmid, Guihéneuf, & Stengel, 2014).

This chapter reviews data dealing with FA composition, isolation and purification of FA, health properties of FA and potential commercial applications of FA whether they are from microalgae or macroalgae.

2. Types and structures of fatty acids from algae

FA possess a carboxylic group linked to an aliphatic chain that may be straight or branched, saturated or unsaturated and with a length ranging from 4 to 28 carbons whether with an odd or even number of carbons. FA are divided into families according to the degree of unsaturation: Saturated Fatty Acids (SFA; **Figure 1**) with no double bond, Monounsaturated Fatty Acids (MUFA; **Figure 1**) with one double bond and Polyunsaturated Fatty Acids (PUFA; **Figure 1**) with two or more double bonds. For MUFA and PUFA, the location of the first double bond (X) from the methyl end is indicated with the n-X or ω-X number (**Figure 1**). This numbering defines the n-6 (or ω-6) and n-3 (or ω-3) well-known classes of fatty acids (**Figure 1**). For example, the eicosapentaenoic acid (EPA), also called C20:4n-3 indicates that this acid is a 20 carbon long fatty acid with 4 double bonds and the first one is located at three carbons from the methyl end (**Figure 1**).

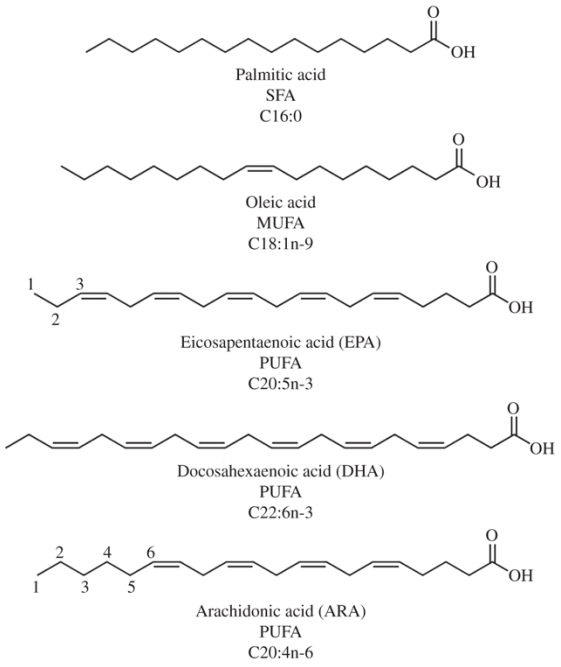

Figure 1: Chemical structure examples of Saturated Fatty Acid (SFA), Monounsaturated Fatty Acid (MUFA) and Polyunsaturated Fatty Acids (PUFA).

Most of the double bonds of PUFA are non-conjugated with a methylene group between double bonds. However, non-methylene-interrupted FA (NMI) with more than one methylene group between double bonds (**Figure 2**) are detected in macroalgae (Jamieson & Reid, 1972; Khotimchenko, 1991; Ratnayake & Ackman, 1979). FA with conjugated double bonds are also found in the FA profiles of algae (**Figure 2**) (Bhaskar, Kinami, et al., 2004; Mikhailova et al., 1995).

FA can also be branched (Kendel et al., 2015; Lang, Hodac, Friedl, & Feussner, 2011) or contain functional groups as hydroxyl (Gelin, Volkman, De Leeuw, & Sinninghe Damsté, 1997; Kendel, Barnathan, Fleurence, Rabesaotra, & Wielgosz-Collin, 2013; Kendel et al., 2015), halogen (Dembitsky & Srebnik, 2002; Kornprobst, 2014), keto (Eltgroth, Watwood, & Wolfe, 2005) or epoxy groups (Lang et al., 2011) (**Figure 3**).

Oxylipins have also been identified in algae (**Figure 4**). They are oxidized FA formed by enzyme or chemical oxidation (Kumari, Kumar, Reddy, & Jha, 2013). This family contains hydroxyl, oxo, epoxy FA, polyunsaturated aldehydes, prostaglandins, leukotriene or polycyclic FA (Barofsky & Pohnert, 2007; Bernart, Whatley, & Gerwick, 1993; Bouarab, 2004; D'Ippolito et al., 2005, 2004; Fontana et al., 2007).

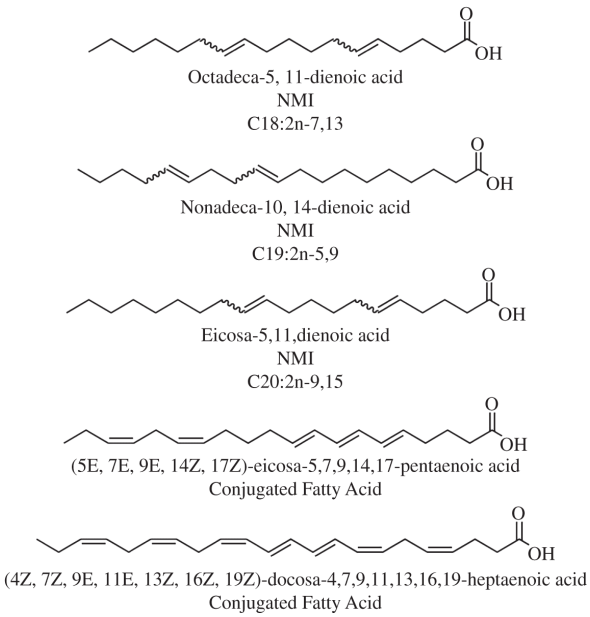

Figure 2: Chemical structure of non-methylene-interrupted fatty acids (NMI) and conjugated fatty acid identified in algae. C18:2n-7,13 is identified in *Cladophora rupestris* (Chlorophyta, *Chladophorales*) (Ratnayake & Ackman, 1979); C19:2n-5,9 in *Grateloupia turuturu* (Rhodophyta, *Halymeniales*) (Kendel et al., 2013) and C20:2n-9,15 in *Sargassum* species (Ochrophyta, *Fucales*) (Khotimchenko, 1991). The conjugated fatty acid (5E,7E,9E, 14Z, 17Z)-eicosa-5,7,9,14,17-pentaenoic acid is found in *Acanthophora spicifera* (Rhodophyta, *Ceramiales*) (Bhaskar, Kinami, et al., 2004) and (4Z, 7Z, 9E, 11E, 13Z, 16Z, 19Z)-docosa-4,7,9,11,13,16,19-heptaenoic acid in *Anadyomene stellata* (Chlorophyta, *Chladophorales*) (Mikhailova et al., 1995).

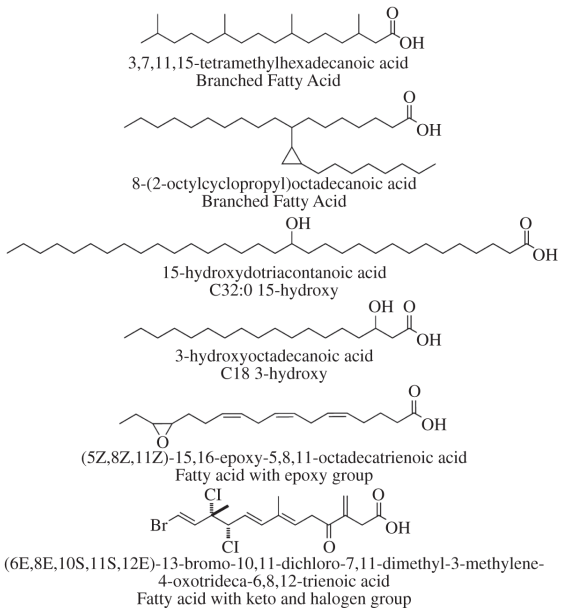

Figure 3: Branched and functionalized fatty acids. 3,7,11,15-tetramethylhexadecanoic acid is identified in *Heterococcus fuornensis* (Ochrophyta, *Tribonematales*) (Lang et al., 2011); 8-(2-octylcyclopropyl)octadecanoic acid in *Chlamydomonas zebra* (Chlorophyta, *Chlamydomonadales*) (Lang et al., 2011), 15-hydroxydotriacontanoic acid in *Nannochloropsis sp.* (Ochrophyta, *Eustigmatales*) (Gelin et al., 1997); 3-hydroxyoctadecanoic acid in *Solieria chordalis* (Rhodophyta, *Gigartinales*) (Kendel et al., 2015); (5Z,8Z,11Z)-15,16-epoxy-5,8,11-octadecatrienoic acid in *Chlamydomyxa montana* (Ochrophyta, *Chlamydomyxales*) (Lang et al., 2011) and (6E,8E,10S,11S,12E)-13-bromo-10,11-dichloro-7,11-dimethyl-3-methylene-4-oxotrideca-6,8,12-trienoic acid in *Plocamium cartilagineum* (Rhodophyta, *Plocamiales*) (Dembitsky & Srebnik, 2002).

FA are rarely free in biological matrix. They are linked by an ester bond to a glycerol moiety forming an acylglycerol (mono-, di- or tri- depending on the quantity of FA for each glycerol), to a sugar (simple, complex or sulfated) forming a glycolipid or to a phosphatidylglycerol group then forming a phospholipid. Thus, FA are present in the three lipid classes, *i.e.*, neutral lipids, glycolipids and phospholipids. Triacylglycerols (TAG) have storage and energy functions in algal cells (Khotimchenko & Yakovleva, 2004; MacDougall, McNichol, McGinn, O'Leary, & Melanson, 2011; Thompson, 1996). Glycolipids play an important role for energy supply and cell protection against stresses by stabilizing the membrane bilayer and acting as markers for cellular recognition (Boudière et al., 2014; Holdt & Kraan, 2011). Phospholipids act as transport materials for membrane structure preservation (Holdt & Kraan, 2011).

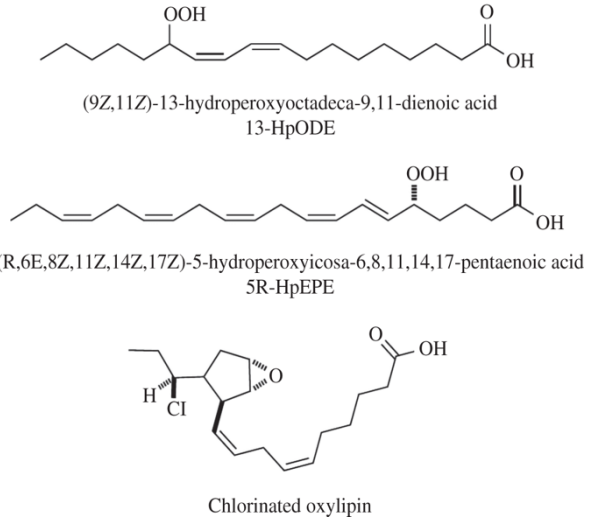

Figure 4: Oxylipins in algae. 13-HpODE is identified in *Chondrus crispus* (Rhodophyta, *Gigartinales*) (Fontana et al., 2007); 5R-HpEPE in *Skeletonema marinoi* (Bacillariophyta, *Thalassiosirales*) (D'Ippolito et al., 2005) and the chlorinated oxylipin in *Egregia menziesii* (Ochrophyta, *Laminariales*) (Dembitsky & Srebnik, 2002).

Several species of freshwater and marine microalgae produce high quantities of lipids, generally about 10-25% of dry weight and up to about 50-60% in some diatoms or *Chlorella* species (Chlorophyta) for example (**Table 1**; Mata, Martins, & Caetano, 2010; Mimouni et al., 2015, 2012). The C16 and C18 SFA and MUFA are present in all microalgal groups but in variable amounts ranging from 5 to 40% and 1.8 to 40% of total fatty acids, respectively (Hu et al., 2008; Mimouni, Couzinet-Mossion, Ulmann, & Wielgosz-Collin, 2018). However, the n-3 and n-6 long-chain polyunsaturated fatty acids (LC-PUFA) arachidonic acid (C20:4n-6, ARA), α-linolenic acid (C18:3n-3, ALA), γ-linolenic acid (C18:3n-6, GLA), docosahexaenoic acid (C22:6n-3, DHA) and eicosapentaenoic acid (C20:5n-3, EPA), which are of interest for human health applications, are mainly present in marine species (De Jesus Raposo, de Morais, & de Morais, 2013; Mimouni et al., 2012) while also available from freshwater species such as *Dunaliella* sp. (Chlorophyta, *Chlamydomonadales*) (Bhosale, Rajabhoj, & Chaugule, 2010; Chen et al., 2011; Sheffer, Fried, Gottlieb, Tietz, & Avron, 1986) or *Chlorella* sp. (Chlorophyta, *Chlorellales*) (Buono, Langellotti, Martello, Rinna, & Fogliano, 2014; Petkov & Garcia, 2007) under specific conditions of culture. Species rich in EPA include Diatoms (Bacillariophyceae) such as *Phaeodactylum tricornutum* (Bacillariophyta), *Odontella aurita* (Bacillariophyta, *Eupodiscales*), *Nitzschia sp.* (Ochrophyta, *Bacillariales*), *Thalassiosira pseudonana* (Bacillariophyta, *Thalassiosirales*) and *Skeletonema costatum* (Bacillariophyta, *Thalassiosirales*); (Mata et al., 2010; Mimouni et al., 2012) and also the Rhodophyte *Porphyridium cruentum* (Rhodophyta, *Porphyridiales*), the Dinophyte *Crypthecodinium cohnii* (*Miozoa*, *Peridiniales*), and the Prymnesiophyte *Isochrysis sp.* (Haptophyta, *Isochrysidales*) (including the species *Tisochrysis lutea*) (**Table 1**; Mata et al., 2010; Mimouni et al., 2012). Interestingly, the Prymnesiophyte *Diacronema lutheri* (previously *Pavlova lutheri*, Haptophyta, *Pavlovales*) displays high contents of both DHA and EPA (De Jesus Raposo et al., 2013) (**Table 1**). The LC-PUFA of marine microalgae are incorporated into TAG (neutral lipids) as storage and into polar lipids such as phospholipids and galactolipids as membrane constituents (Ulmann et al., 2017).

Table 1: Approximate amount of total lipids, DHA and EPA in some marine and freshwater microalgae

Classes	Species	Total Lipid content (% of dry weight)[a,b]	DHA (molar %)[b]	EPA (molar %)[b]
Bacillariophytes (Diatoms)	*Nitzschia sp.*	16-47[a]	<1	25-30
	Odontella aurita	7-13[b]	1-2	>25
	Phaeodactylum tricornutum	18-57[a]	2	26
	Skeletonema costatum	13-51[a]	1-5	10-20
	Thalassiosira pseudonana	20-24[a,b]	1	15
Chlorophytes	*Chlorella vulgaris*	5-58[a]	<1	1-5
	Dunaliella salina	6-25[a]		
	Dunaliella primolecta	23[a,b]	<1	<1
	Tetraselmis suecica	8-23[a]	<1	1-5
Dinophytes	*Crypthecodinium cohnii*	20-51[a]	<1	45
Prymnesiophytes	*Diacronema lutheri*	20-35[a,b]	10-20	>20
	Isochrysis sp.	7-40[a]	10-20	<1
Rhodophytes	*Porphyridium cruentum*	9-19[a]	<1	21

[a] Data from Mata et al. (2010); [b] Data from Mimouni et al. (2012).

The lipid content of microalgae can be modulated by culture conditions and several stresses (Sayanova et al., 2017). For example, the addition of sodium salts of organic acids, *i.e.*, sodium acetate, pyruvate, citrate and malate, to the culture medium of the marine microalgae *Thraustochytrium* sp. T01 (Labyrinthulomycetes, *Thraustochytriaceae*) increases DHA production by 40-46% (Chandrasekaran, Dhanraj, & Chadha, 2018). As another example, fermentation strategies using heterotrophic microalgae such as *Schizochytrium mangrovei* (*Thraustochytriaceae*) can influence the production of lipids and especially of DHA (Hoang et al., 2018). However, most of the studies aim to increase lipid production, which could be achieved by modifying the light spectrum exposure of *Dunaliella salina* and *Nannochloropsis oculata* (Ochrophyta, *Eustigmatophyceae*) cultures (Gilbert et al., 2013), under osmotic stress in *Chlamydomonas reinhardtii* (Chlorophyta, *Chlamydomonadales*) (Yang, Suh, Kang, Lee, & Chang, 2018), by nutrient stress such as nitrogen-limitation in *Chlorella vulgaris* and *Chaetoceros muelleri* (Ochrophyta) (Adamakis et al., 2018; Lin et al., 2018) or phosphate-limitation in *Thalassiosira weissflogii* (Lin et al., 2018). However, the increase in the overall amount of FA is not sufficient and particular attention must also be paid to their nature; nutrient limitation, for example, leads to a lower proportion of PUFA (Lin et al., 2018), which could be interesting for biodiesel production but not for human or animal health/feeding purpose. In addition, the effect of stress or culture conditions are also species specific (Pavón-Suriano et al., 2018). Environmental contaminants such as ethinylestradiol (among several other chemicals) could also have an impact

on lipid metabolism; in this case, ethinylestradiol impairs the production of LC-PUFA, affects the galactolipid versus phospholipid balance and triggers the recycling of FA from membrane lipids to TAG in *Phaeodactylum tricornutum* (Conte et al., 2018).

Unlike microalgae, macroalgae produce a low quantity of lipids generally about 1 to 6% dw (Rodrigues et al., 2015). Chain length and degree of unsaturation are higher than those of terrestrial plants (Kumari, Kumar, et al., 2013). Palmitic acid is the most common SFA (Gressler et al., 2010). A high content in PUFA is typical for seaweeds, especially ALA, EPA, ARA and stearidonic acid (C18:4n-3, STA) (Dawczynski, Schubert, & Jahreis, 2007; Murata & Nakazoe, 2001). FA distribution can be linked to the phylum and some FA can serve as taxonomic markers (**Table 2**; Galloway, Britton-Simmons, Duggins, Gabrielson, & Brett, 2012; Kumari, Bijo, Mantri, Reddy, & Jha, 2013). LC-PUFA are more abundant in red and brown seaweeds than in green (Schmid et al., 2014). Phaeophyta demonstrate a higher total lipid content than other phyla and SFA and PUFA are the main classes of FA representing up to 80% of FA (**Table 2**; Wielgosz-Collin, Kendel, & Couzinet-Mossion, 2016). C18 and C20 are the major PUFA present in similar quantities and DHA is rarely isolated from brown seaweeds except in Fucales and Scytosiphonales (Wielgosz-Collin et al., 2016). High proportions of ARA (13% of total FA), EPA (13% of total FA) and linoleic acid (C18:2n-6, LA) and lower content of palmitic and stearic acids than Rhodophyta are typical for Phaeophyta (**Table 2**; Galloway et al., 2012; Schmid et al., 2014).

Table 2: Fatty acid distribution characteristics of macroalgal phyla

Phylum	Specificity	References
Phaeophyta	• High content of C18 and C20 PUFA (13% of ARA and 13% EPA) • High content of LA • Lower content of palmitic and stearic acids than Rhodophyta • Very low content of DHA except for Fucales and Scytosiphonales	(Galloway et al., 2012; Schmid et al., 2014; Wielgosz-Collin et al., 2016)
Rhodophyta	• High content in C20 PUFA (11% of ARA and 17% of EPA) • High content of palmitic and oleic acids • Absence or very low content of DHA • Halogenated FA specific for *Asparagopsis*	(Dawczynski et al., 2007; Dembitsky & Srebnik, 2002; Galloway et al., 2012; Schmid et al., 2014; Wielgosz-Collin et al., 2016)
Chlorophyta	• High content of C18 PUFA • Lower content of C20 PUFA • 5% of LA • 7% of STA • Presence of ALA • C16:3n-3 specific for *Codium*	(Galloway et al., 2012; Goecke et al., 2010; Khotimchenko, 1993; Khotimchenko et al., 2002; Li et al., 2002; Schmid et al., 2014)

Rhodophyta are characterized by a high quantity of C20 PUFA with 4 and 5 double bonds (around 11% of ARA and 17% EPA) (Dawczynski et al., 2007; Galloway et al., 2012; Schmid et al., 2014; Wielgosz-Collin et al., 2016). Abundant quantities of palmitic and oleic acids are determined in red seaweeds whereas DHA is absent or present at low concentrations (**Table 2**; Dawczynski et al., 2007; Schmid et al., 2014). Halogenated FA such as short chain chlorinated, brominated or iodinated fatty acids have been identified specifically in *Asparagopsis* (Rhodophyta, *Bonnemaisoniales*) red seaweeds (Dembitsky & Srebnik, 2002). Chlorophyta phylum is characterized by a high content in C18 PUFA (LA and ALA) (Khotimchenko, Vaskovsky, & Titlyanova, 2002; Li, Fan, Han, & Lou, 2002) but the C20 content (ARA and EPA) is significantly lower than in Phaeophyta and Rhodophyta (**Table 2**; Schmid et al., 2014). Contents around 5% of LA, 7% of STA and presence of ALA are markers for Chlorophyta seaweeds (Galloway et al., 2012). C16 PUFA have chemotaxonomic value for green seaweeds (Khotimchenko, 1993). Indeed, hexadecatrienoic acid C16:3n-3 is specific to the *Codium* green seaweed genus(Chlorophyta, *Bryopsidales*) (Goecke et al., 2010).

As well as in microalgae, lipid content and FA distribution is modulated by external parameters (Bhaskar, Miyashita, et al., 2004; Colombo et al., 2006; Denis et al., 2010; Floreto et al., 1994a, 1994b, 1996; Khotimchenko & Levchenko, 1997; M. K. Kim, Dubacq, Thomas, & Giraud, 1996; McCauley et al., 2016; Mishra et al., 1993; Tasende, 2000). Cold conditions of growth such as cold or deep water increase the total lipid content of red and brown seaweeds (Bhaskar, Miyashita, et al., 2004; Colombo et al., 2006; Denis et al., 2010; M. K. Kim et al., 1996; Mishra et al., 1993). FA are longer and more unsaturated for cold species than for tropical or temperate water species (Bhaskar, Miyashita, et al., 2004; Colombo et al., 2006). For example, the PUFA content of seaweeds from the Canadian coast is twice as large as the one from the south China coast (52.1 vs 22.3% respectively) and the

mean of chain length is 17.8 carbons for the cold water species whereas it is 16.3 carbons for the temperate water species (Colombo et al., 2006). In culture conditions, nitrogen starvation leads to a lower total lipid content, a higher SFA and low unsaturated (one or two double bonds) content and a lower PUFA content (McCauley et al., 2016; Mishra et al., 1993). Phosphorus deprivation has no significant impact on the total lipid content. However, the starvation of phosphorus decreased the storage FA content such as palmitic acid content and increased the PUFA content such as hexadecatetraenoic acid C16:4n-3 content (Floreto et al., 1996). Light intensity does not affect the total lipid content. However, light is necessary for an efficient lipid production (Floreto et al., 1994b). Seaweed FA are not affected by anchoring on the seabed whereas total lipid content is reduced if seaweeds are attached on the seabed (Khotimchenko & Levchenko, 1997). The stage of development of the seaweeds influences also the FA content and distribution. Gametophytes of the Rhodophyta *Chondrus crispus* (Rhodophyta, *Gigartinales*) exhibit more MUFA such as palmitoleic C16:1n-7 and oleic C18:1n-9 acids than sporophytes, whereas sporophytes are richer in SFA such as palmitic and stearic acids and in ALA (Tasende, 2000). For the red seaweed, *Gracilaria verrucosa* (Rhodophyta, *Gracilariales*), a difference in ARA content is also noticed between cystocarps and branches (Khotimchenko & Levchenko, 1997).

3. Isolation and purification of fatty acids from algae

3.1 Isolation of fatty acids from algae

Isolation of FA can be done just after the extraction of lipids from algae (see chapter 10 for lipid extraction). Chemical saponification is the most used method to release FA from glycerol or sugar moieties. For chemical saponification, an alkali hydro alcoholic solution is refluxed over a short period time (generally 1-2 hours) or stirred at room temperature over a longer time (17-24 hours) for the hydrolysis of the ester linkages. These parameters do not significantly influence the FA profile (Cartens, Molina Grima, Robles Medina, Giménez Giménez, & Ibáñez González, 1996). Then, after acidification, FA can be recovered directly (Zhang, Wang, Gao, Huang, & Zhang, 2018) or by liquid-liquid extraction (Kendel et al., 2015). The saponification can also take place during the extraction of FA. Alkali is directly added to the solvent extraction system and FA are recovered after acidification and liquid-liquid partition (Cartens et al., 1996; Guil-Guerrero, Belarbi, & Rebelloso-Fuentes, 2001; Paul, Neveux, Magnusson, & de Nys, 2014). This direct saponification on biomass increases the yield of FA extracted, reduces operating time and possible degradation (Cartens et al., 1996; Molina Grima et al., 1994). It should be noted that as a lot of studies are based on the identification and quantification of FA, the saponification is often replaced by a transesterification to Fatty Acid Methyl Esters (FAME) that can be injected directly in a gas chromatography system for analysis or used for further purification (Mansour, 2005; Nagappan & Kumar Verma, 2018).

Supercritical fluid extraction is an eco-friendly alternative method to isolate lipids from seaweeds. Until now, supercritical carbon dioxide ($ScCO_2$) is the most commonly supercritical fluid used, due to its low critical pressure and temperature (73.9 bar and 31.1 °C). $ScCO_2$ is currently used for the extraction of microalgae but it is little used for seaweed lipid extraction (Grosso, Valentão, Ferreres, & Andrade, 2015; Terme et al., 2017; Terme, Boulho, Kucma, Bourgougnon, & Bedoux, 2018).

3.2 Purification of fatty acids from algae

Purification of FA can be done from the free fatty acids (FFA) or esters (glycerides or FAME) and is generally conducted to obtain FA with a high added value, *i.e.*, PUFA, in a multistep process for high purity (Namal Senanayake, 2013; Robles Medina, Molina Grima, Giménez Giménez, & Ibáñez González, 1998; Shahidi & Wanasundara, 1998). The separation techniques reviewed here include physicochemical methods such as crystallization by urea inclusion compounds or use of low temperatures and distillation, chromatographic methods such as reverse phase chromatography, silver nitrate adsorption, centrifugal partition chromatography (CPC) and supercritical fluid chromatography and enzymatic methods.

3.2.1 Urea crystallization

Urea fractionation is widely used both for analytical and preparative purposes for FA concentration of marine oils, but not often used for algal oils. It is based on the formation of hexagonal crystals enclosing straight chain molecules during crystallization (Smith, 1952). The affinity of FA and esters with urea decreases when unsaturated bonds are present in the chain. Thus, SFA and MUFA are first precipitated from the solution while PUFA remain in solution (Iverson & Weik, 1967; Strocchi & Bonaga, 1975). The parameters governing the crystallization have been extensively studied as the urea adductability values (binding properties between urea and FA) (Iverson & Weik, 1967), the stability constants and the heats of formation of crystals (Schlenk, 1954; Swern, 1964). To form complexes, the urea concentration should be near saturation; the fractionation is greatly influenced by urea:FA ratio and temperature can be adjusted according to the desired concentration of FA.

An algal oil from the Dinoflagellate *Crypthecodinium cohnii* was enriched in DHA from 47.7 to 97.1% of the total FA fraction by using an urea:FA ratio of 3:1 at 4°C in methanol (Namal Senanayake & Shahidi, 2000). The soluble fraction comprised 98.2% of PUFA whereas the urea complexing fraction comprised 66.9% of SFA and MUFA. When the urea:FA ratio increases, more DHA is complexed and a lower recovery yield of DHA is obtained (Namal Senanayake & Shahidi, 2000). For the Rhodophyta *Porphyridium cruentum*, an EPA and ARA enriched fraction is targeted (Guil-Guerrero et al., 2001). The urea:FA ratio of 4:1 was tested in methanol at 4 and 28°C (Guil-Guerrero et al., 2001). Both temperatures resulted in a similar lipid concentrate composition with 57% ARA and 34% EPA but 28°C led to a better recovery yield because it is the optimal crystallization temperature for EPA. This enriched fraction also contained interesting FA such as LA (8.2%) and homo-GLA (1.3%). All the SFA were eliminated with 0.2% palmitoleic acid remaining (Guil-Guerrero et al., 2001). Extraction of the diatom *Phaeodactylum tricornutum* produced an enriched oil in EPA by urea fractionation with an urea:FA ratio of 4:1 at 28°C (Cartens et al., 1996). Methanol and ethanol were both tested for dissolving urea. SFA and MUFA are effectively removed with both solvents (Cartens et al., 1996). Methanol leads to a more concentrated fraction in EPA, DHA and ARA (55.2, 1.5 and 5.9% respectively) but ethanol recovers more EPA than methanol; 84% of the total EPA amount was recovered by an ethanolic solution of urea instead of 79% for the methanolic urea solution. However, overall yields (45% for methanol and 53% for ethanol) are acceptable in comparison with other purification processes (Cartens et al., 1996). When a second urea crystallization was used to purify EPA from the diatom *Phaeodactylum tricornutum*, the EPA content reached 80.7% of total FA but with a very low yield (23%; Zhang et al., 2018). This fraction also comprised 12.2% ARA, 1.1% LA and approximately 2% MUFA and SFA (Zhang et al., 2018). For *Isochrysis galbana*, purification with a 4:1 urea:FA ratio at 4°C in methanol was efficient to produce a STA, EPA, DHA enriched fraction representing 85.4% of the urea concentrate FA. It was noticed that the recovery yield of DHA in urea concentrate is 100% (Robles Medina, Giménez Giménez, et al., 1995; Robles Medina, Molina Grima, & García Sánchez, 1995). The purification can also be conducted on FAME obtained by transmethylation of lipids from *Porphyridium cruentum* (Cohen & Cohen, 1991). The EPA methyl ester proportion increased from 47.5 to 81.9% of the fraction, whereas the SFA methyl ester content decreased from 35.6 to 3.1% of the fraction (Cohen & Cohen, 1991). The fraction was also enriched in ARA (5.4 to 6.6%). The previous FA profile seems to be an important factor for enrichment. By using a strain of *Porphyridium cruentum* identified as a rich source of ARA (41.7%), the urea purification in the same conditions led to a 80% ARA enriched fraction (Cohen & Cohen, 1991).

3.2.2 Low temperature crystallization

Low temperature crystallization is based on the melting points of FA. Using different temperatures and successive filtrations, it is possible to purify PUFA from algae as SFA and MUFA have higher melting points than PUFA. Fatty acid esters or FA can be purified by low temperature crystallization depending on the solvent (Privett, 1971; Wang, Wang, Wang, Jin, & Wang, 2018; Zhang et al., 2018). Acetone is used for methyl esters while hydrocarbons are used for acids (Privett, 1971). A docosapentaenoic acid (C22:5, DPA) rich fraction was obtained from *Schizochytrium* sp. oil (Wang et al., 2018). A crystallization at -80°C over 8h in acetone enriched the fraction in DPA from 35.8 to 51.2% and decreased the content of SFA and MUFA from 41.7 to 15.1% (Wang et al., 2018). Low temperature crystallization of *Phaeodactylum tricornutum* lipids led to an EPA rich fraction (Zhang et al., 2018). After 24h at -80°C in hexane, EPA represented 40.7% of the total FA of the fraction whereas SFA and MUFA are reduced from 61.9 to 17.9% (Zhang et al., 2018).

3.2.3 Distillation

Distillation is based on the boiling points of FAME. The longer the FAME carbon chain is, the higher is the boiling point, with straight chains having higher boiling points than their branched chain analogues (Cason, Allinger, Sumrell, & Williams, 1951). Industrial application of fractional distillation is possible as apparatus and techniques have been developed to accommodate up to 20kg of esters with a high performance column (Privett, 1971). The main drawback of this technique is the use of heating that can lead to degradation of FA by loss of unsaturation, formation of cyclic compounds or isomerization (Privett, 1971). To avoid degradation, the distillation temperature must be below 200°C. However, temperatures greater than 200°C are necessary for LC-PUFA distillation. However, a decrease in pressure can lead to a decreased boiling point allowing lower distillation temperature use (Namal Senanayake, 2013). On the other hand, decreased pressure results in a loss of distillation column efficiency. This method, called molecular distillation, is applied to fish and aquatic organisms oil enrichment in some n-3 PUFA but is poorly applied to algae (Namal Senanayake, 2013).

3.2.4 Chromatography

FA can be separated according to the carbon chain length and degree of unsaturation using reverse phase, silver nitrate adsorption, CPC or supercritical fluid chromatography at analytical or preparative scales. The separation can be conducted whether on FFA or on fatty esters.

For chromatography, the choice of detector is a key point. The mass spectrometer is the most suitable and most used detector for identification. As it is a destructive method, it cannot be used for purification of fatty acids. However, the analytical and separation methodology can be optimized using this detector, and subsequently used for purification without the detector. The differential refractometer (refractive index detector) needs a sufficient concentration of FA in the sample and an elution gradient is not feasible with this detector (Robles Medina et al., 1998). Most FA do not absorb ultraviolet radiation or interfere with the solvent absorption. Therefore, their conversion to UV-visible sensitive derivatives is necessary for detection (Robles Medina et al., 1998). A multiple wavelength detection can help with the identification of PUFA. The wavelength of 192 nm is quite PUFA selective with sensitivity increasing with the number of double bonds. The wavelength of 217 nm can be used for EPA and DHA detection (Robles Medina, Giménez Giménez, et al., 1995) and 220 nm for triacylglycerol detection because of the specific absorbance due to the carbonyl (C=O) group (Shukla, 1988).

Reverse phase chromatography of FA is based on the adsorption of hydrophobic soluble molecules from a polar solvent to a non-polar sorbent under pressure such as in high-pressure liquid chromatography. Octadecylsilyl (C18) and Octasilyl (C8) stationary phases are used (Aveldano, VanRollins, & Horrocks, 1983; Cartens et al., 1996; Cohen & Cohen, 1991; Mansour, 2005; Molina Grima et al., 1994, 1995; Robles Medina, Giménez Giménez, et al., 1995; Robles Medina et al., 1998). C18 columns lead to a better separation of FA whereas C8 allows a faster separation of very long FA (Aveldano et al., 1983). The separation depends on chain length, degree and configuration of unsaturation. Elution time increases with chain length and decreases with the number of unsaturation for the same chain length (Robles Medina et al., 1998). FFA separate faster than the corresponding esters because they are more polar. The most commonly used mobile phases are 60 to 100% methanol or acetonitrile in water (Aveldano et al., 1983). Acetonitrile seems to be more selective probably due to its lower viscosity, but this solvent cannot dissolve all the FA and separations are longer than with methanol. For biocompatible (*i.e.* non-toxic and/or food-grade) separations, methanol can be replaced by ethanol but the flow rate must be decreased and the resolution is lower (Robles Medina, Giménez Giménez, et al., 1995). Reverse phase chromatography was used to obtain three fractions rich in STA, EPA and DHA respectively from an *Isochrysis galbana* urea concentrate at the semi-preparative scale with a good purity (Molina Grima et al., 1994, 1995). *Phaeodactylum tricornutum* led to a 93.6% EPA fraction after high performance liquid chromatography (HPLC) (Cartens et al., 1996). At the semi-preparative scale, 98% of the EPA contained in the diatom was recovered with a 94% purity. An EPA concentrate was also obtained from *Porphyridium cruentum* (Cohen & Cohen, 1991). The reverse phase chromatography enriched the EPA fraction from 81.9 to 97.3% and decreased the ARA content from 6.6 to 1.4%. LC-PUFA methyl esters from *Scrippsiella* sp. CS-295/c (Haptophyta, *Coccolithophyceae*) were isolated with repeated injections using preparative HPLC with a high purity (Mansour, 2005).

Silver nitrate silicic acid HPLC is a quick semi-preparative fractionation methodology for highly unsaturated fatty esters with 3 to 6 double bonds (Robles Medina et al., 1998). The fractionation is based on the formation of a complex between the column silver ions and double bonds by charge transfer (Guil-Guerrero et al., 2001). Elution time increases with the number of double bonds because sample-column interactions are stronger. Therefore, SFA are eluted first followed by unsaturated FA. The order of elution for unsaturated esters is determined by the number, the position and the geometric configuration of double bonds (Scholfield, 1979). For a good purification, the ratio of the fatty esters and stationary phase should be 4% (w/w) (Belarbi, Molina Grima, & Chisti, 2000). Various concentrations of acetone in hexane as the mobile phase allowed the separation of fatty esters from *Porphyridium cruentum* urea concentrate (Belarbi et al., 2000). 5% acetone in hexane enriched the fraction in ARA while 10% enriched the fraction in EPA (Belarbi et al., 2000). This method was also applied to *Monodus subterraneus* (Ochrophyta, *Xanthophyceae*) oil and led to an EPA rich fraction with more than 92% of purity. The scale-up of this process was conducted with *Porphyridium cruentum* by increasing the column diameter and demonstrated the possibility of EPA and ARA large scale production by this method (Belarbi et al., 2000).

The use of supercritical fluids for non-toxic and food-safe production of high value natural bioactives has been reviewed recently (Catchpole et al., 2012). Supercritical carbon dioxide ($ScCO_2$) is generally used for extraction of non-polar lipophilic compounds and a wide range of polar co-solvents can be used to improve the extraction of polar compounds (Catchpole et al., 2012). Combining $ScCO_2$ and chromatography principle led to a separation of FA on the basis of the chain length ($ScCO_2$ properties) and the degree of unsaturation (chromatography solid phase properties) (Montañés, Catchpole, Tallon, Mitchell, & Lagutin, 2013). Supercritical-fluid chromatography has been used for the isolation of EPA ethyl esters from an algal oil (Montañés et al., 2013).

The particle size, the stationary phase, sample loading, temperature/pressure, are the most important parameters for achieving separation of PUFA (Montañés et al., 2013). Under optimized conditions ($ScCO_2$, 7.5 $mL.min^{-1}$, 170 bar, 333K, GreenSep Nitro column), EPA with greater than 95% purity is obtained in a single pass from an algal oil (Montañés et al., 2013).

Centrifugal partition chromatography (CPC) is a liquid–liquid chromatography without sorbent using two immiscible solvent systems (Becerra et al., 2015; Boulho et al., 2017; Namal Senanayake, 2013; Wanasundara & Fedec, 2002; Yoon, Chin, & Kim, 2010). CPC is derived from counter-current chromatography and relies on the partition of compounds between the two solvent systems (Becerra et al., 2015). In a CPC system, the liquid stationary phase remains in the partition cells thanks to the centrifugal force created by the spinning rotor (800-1000 rpm) while the liquid mobile phase passes from cells to cells by ducts (Wanasundara & Fedec, 2002). This technique is particularly suitable for the isolation of molecules from complex natural matrices on a large scale (Becerra et al., 2015; Yoon et al., 2010). CPC has been used for the purification of fucosterol from the brown alga *Lessonia vadosa* (Ochrophyta, *Laminariales*) and recovered between 70 and 93% of fucosterol present in the extracted fraction with a high purity (more than 97%) (Becerra et al., 2015). CPC has also been used for the isolation and purification of PUFA from microalgae. The purification of an oil extract from *Skeletonema costatum* led to a fraction rich in essential FA (45% of C20:5, 43% of C16:4, 7.5% of C18:4 and 4.5% of C22:6) with a 42% yield (Bousquet & Le Goffic, 1995). After purification by CPC of a microalgal oil containing 39.7% DHA and 15.2% DPA n-6, samples at 84.6% DHA and 84.9% DPA n-6 were prepared using a solvent system composed of heptane/methanol/water (Wanasundara & Fedec, 2002).

3.2.5 Enzymatic purification of fatty acids

Highly PUFA-enriched triglycerides can be obtained by lipase-catalyzed reactions (Ishihara et al., 2000; Namal Senanayake & Shahidi, 2002; Robles Medina et al., 1998). Lipases are enzymes that can catalyze the synthesis or hydrolysis of an ester linkage in acylglycerols in a reversible way depending on the water content in the reaction media (Robles Medina et al., 1998). Therefore, lipases can catalyze exchanges of FA in acylglycerols (Marangoni & Rousseau, 1995). The type of enzymes, concentration and ratios of surfactants, initial composition of FA mixtures, solvents, water content and elimination method (*i.e.* evaporation or freeze-drying), temperature, pH, agitation method and speed, and type of reactor are all parameters that affect the enzymatic ratio rate and the type of product (Robles Medina et al., 1998). However, mild temperature and pH conditions are typically used for lipases (Gandhi, 1997), allowing their use for oils enriched in PUFA. As the acylglycerol form of FA is preferred for improved nutrition applications, this method can be taken in account for obtaining PUFA for medical or dietetic purposes (Namal Senanayake, 2013).

Lipase-catalyzed esterification of glycerol and PUFA from microalgae leads to a triglyceride-rich fraction (Robles Medina et al., 1999). The lipase Novozyme 435 isolated from *Candida antarctica*, and immobilized on acrylic resin, is used in hexane without water, at 50°C, with 1g of molecular sieves added at the beginning of the reaction, with a glycerol:PUFA molar ratio of 1.2:3, an agitation rate of 200 rpm and a concentration of 100 mg of lipase for 9 mL of hexane (Robles Medina et al., 1999). With these conditions, a triglyceride yield of 96.5% was obtained from *Phaeodactylum tricornutum* after 72h (Robles Medina et al., 1999). The triglyceride fraction comprised 42.5% of EPA (Robles Medina et al., 1999). With another microalga, *Porphyridium cruentum*, the triglyceride yield was lower, *i.e.*, 89.3% after 96h of reaction and the fraction comprised 45.6% of EPA and 43.4% of ARA (Robles Medina et al., 1999). The same enzyme was used in a one-pot process of extraction and purification of triglycerides from *Chlorella sp.* KR-1 (O. K. Lee, Kim, Na, Oh, & Lee, 2013). Almost all the total lipids were extracted and the FAME production was 293 mg per biomass gram in 6h at 60°C. The triglyceride fraction comprised 25% palmitic acid, 17% oleic acid and 15% LA (O. K. Lee et al., 2013). The total PUFA content represented 21% of the total FA. Twelve lipases derived from *Penicillium camemberti* (lipase G), *Candida rugosa* (lipase AY), *Penicillium roqueforti* (lipase R), *Rhizopus oryzae* (lipase F-AP), *Aspergillus niger* (lipase A), *Mucor javanicus* (lipase M), *Rhizopus* sp. (lipase Ta), *Alcaligenes* sp. (lipases PL and QL), *Candida cylindracea* (lipase QF and MY) and *Rhizomucor miehei* lipozyme-IM were tested for STA and hexadecatetraenoic acid (C16:4n-3) purifications from *Undaria pinnatifida* (Phaeophyta, *Laminariales*) and *Ulva pertusa* (Chlorophyta, *Ulvales*) (Ishihara et al., 2000). For *Undaria pinnatifida*, all the enzymes except R, PL and QL enriched the fraction in STA. The best results were obtained with lipase M with 61.6% of free STA in the fraction with 75.7% of recovery. For *Ulva pertusa*, all the enzymes except QL enriched the fraction in C16:4n-3 up to 30.6% of total FA content. For STA, the best results were obtained with the lipozyme-IM. Thus, STA represented 42.6% of the total FA content. The lipase M led to a fraction with 35.5% of STA and 30.6% of C16:4n-3. The recovery was 79.5% for C16:4n-3 and 69% for STA from *Ulva pertusa* with this lipase (Ishihara et al., 2000). Lipase AK from *Pseudomonas fluorescens* led to an enriched fraction in EPA up to 75.9% from *Phaeodactylum tricornutum* cells (Ramírez Fajardo et al., 2006). This study demonstrated that the initial concentration of EPA did not influence the final concentration or the

recovery yield of EPA. Lipase-catalyzed reactions were used for DHA incorporation in borage oil (*Borago officinalis* L.; Namal Senanayake & Shahidi, 2002). Here, the source of DHA was an algal oil. From an algal oil with 47.5% of DHA, a borage oil with 35.6% of DHA was obtained with 165 *Candida antarctica* lipase units, at 50°C during 25h (Namal Senanayake & Shahidi, 2002).

From the above discussion, applications of lipases are well established and proven. Industrial lipase reaction systems are also available. Further development of lipase and lipase-catalyzed reactions can lead to the production of novel oils enriched in essential FA from natural sources and to the production of non-natural esters for use as bioactive compounds.

3.2.6 Highly pure polyunsaturated fatty acids

For purification of FA, two or more procedures are required to obtain highly purified molecules from algae (Ishihara et al., 2000; A. Mendes, Da Silva, & Reis, 2007; Robles Medina, Giménez Giménez, et al., 1995; Shinde, Ventre, Lloyd-Randolfi, & Lamont, 2018). A sequential procedure has been developed for concentration of DHA from the Dinoflagellate *Crypthecodinium cohnii* biomass (A. Mendes et al., 2007) using a low temperature crystallization followed by urea crystallization, purified DHA up to 99% of the FA fraction. The combination of lipase M from *Mucor javanicus* and medium pressure liquid chromatography was used to purify STA and hexadecatetraenoic acid (C16:4n-3) from the brown seaweed *Undaria pinnatifida* and the green seaweed *Ulva pertusa* (Ishihara et al., 2000). Highly pure fractions (more than 95% of purity) were obtained and the overall yields are over 60% for *Ulva pertusa* and 52.9% for *Undaria pinnatifida*. The key step for improving purity and yield is the optimization of the lipase reactions (Ishihara et al., 2000). A two-step process combining urea crystallization and HPLC led to three fractions enriched in STA, EPA and DHA from the Prymnesiophyte *Isochrysis galbana* with high yields and high purity (Robles Medina, Giménez Giménez, et al., 1995). A multistep process combining urea crystallization, distillation and silver ion chromatography used to purify n-7 FA like palmitoleic acid (C16:1n-7) from algal oils (Shinde et al., 2018). In this process, fractions enriched in n-3, n-6 and n-9 FA were also obtained from microalgae from genera such as *Nannochloropsis*, *Nitzschia*, *Thalassiosira* and *Phaeodactylum* (Shinde et al., 2018).

4. Health properties of fatty acids

Dietary lipids have a vast array of activities which can impact human health. Here, we describe the activities of lipids and FA from microalgae and macroalgae related to cosmetic, antivirals, cardiovascular diseases (CVD), metabolic syndrome (MetS) and cancer prevention and/or therapy.

4.1 Lipids, fatty acids from seaweeds and cosmetic or cosmeceutical uses

Lipids and FA from macroalgae may demonstrate four biological activities linked with cosmetics or cosmeceutical applications. They demonstrate skin hydration and skin elasticity enhancement efficacy and antimicrobial, anti-inflammatory and antioxidant activities (Al-Fadhli, Wahidulla, & D'Souza, 2006; Banskota, Stefanova, Sperker, Lall, Craigie, & Hafting, 2014; Banskota, Stefanova, Sperker, Lall, Craigie, Hafting, et al., 2014; Choi et al., 2012; Fabrowska, Kapuścińska, Łęska, Feliksik-Skrobich, & Nowak, 2017; Ishihara et al., 1998; M. Mendes, Pereira, Sousa Pinto, Carvalho, & Gomes, 2013; N.-H. Park et al., 2013; Plaza et al., 2010; Santos et al., 2017; Terme et al., 2018).

4.1.1 Skin care

Lipids and FA in cosmetics are used for their ability to protect the skin barrier and maintain the hydration level of the skin (Coderch, De Pera, Fonollosa, De La Maza, & Parra, 2002). In our skin, they may incorporate themselves into cell membranes and a lack of cutaneous lipids may exacerbate scratches and discomfort (Bonnet, 2018). Lipids and their derivatives are commonly used in cosmetic emulsions as emollients or surfactants. Bio-based lipids are of primary interest in this field. The lipid structure (FA chain length, saturation level and branching) defines the functionality of the ingredient. For example, short chain FA (less than 8 carbons) exhibits solubilizing and detergent properties, for intermediate chains (between 8 and 12 carbons), foaming properties and for long chains (more than 12 carbons), emulsifying properties. Emulsion fluidity is expected from short chains and stability provided by long chains fatty alcohols (Duprat-de-Paule, Guilbot, Roso, Cambos, & Pierre, 2018). Lipids in cosmetics can be used directly as active ingredients to improve skin appearance (Rabasco Alvarez, González Rodríguez, & Rodríguez, 2000). Emulsions in cosmetic products contain a large variety of oils (Duprat-de-Paule et al., 2018). From seaweed sources, few studies show their direct use in cosmetics (Fabrowska et al., 2017).

$ScCO_2$ extracts from the green seaweed *Cladophora glomerata* (Chlorophyta, *Chladophorales*), enriched in non-polar compounds such as FA improved *in vivo* skin hydration and *stratum corneum* water retention (Fabrowska et al., 2017). The skin hydration measured by corneometer increased up to +42-45% after four weeks

of application by a group of women of 20+ and 40+ years of age (Fabrowska et al., 2017). FA were observed to act as complementary occlusive compounds forming a barrier or seal which contributed to skin hydration (Fabrowska et al., 2017). An improvement in skin elasticity was also observed from an emulsion containing the *Cladophora glomerata* algae extract (Fabrowska et al., 2017).

4.1.2 Antimicrobial activity

Glycoglycerolipids from the red seaweed *Chondria armata* (Rhodophyta, *Ceramiales*) were isolated from the chloroform soluble fraction of a crude methanolic extract (Al-Fadhli et al., 2006). Glycoglycerolipids are compounds with one or more sugar residues glycosidically linked to a lipid containing a glycerol residue. Fractions PF_2 and PF_3, (PF refers to the Polarity of the Fraction isolated from the chloroform soluble fraction) containing the two major glycoglycerolipids ((2R)-2-*O*-(5,8,11,14-eicosatetranoyl)-3-*O*-*α*-D-galactopyranosyl-*sn*-glycerol and 2R-1-*O*-(palmitoyl)-2-*O*-(5,8,11,14,17-eicosapentanoyl)-3-*O*-*β*-D-galactopyranosyl-*sn*-glycerol) were assessed for their antimicrobial properties (Al-Fadhli et al., 2006). Antibacterial and antifungal activities were measured using the paper disk assay method and using as positive control Streptomycin for antibacterial assay and Nystatin for antifungal assay (Al-Fadhli et al., 2006). The diameter of the growth inhibition halos induced by the sample was evaluated. PF_2 and PF_3 showed mild antibacterial activity against *Shigella flexneri* and *Vibrio cholerae*, respectively (Al-Fadhli et al., 2006). PF_2 (130 µg/disk) exhibited antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and antifungal activity against *Aspergillus fumigatus* and *niger, Cryptococcus neoformans* and *Rhodotorula* sp.. PF_3 was effective against the bacteria *Klebsiella sp.* and yeasts *Candida albicans, Aspergillus fumigatus* and *Cryptococcus neoformans* (Al-Fadhli et al., 2006). Ethyl acetate extracts from two red seaweeds *Chondrus crispus* and *Gracilaria vermiculophylla* from an integrated multi-trophic aquaculture (IMTA) system exhibited higher antimicrobial activity when compared to other extraction solvents (methanol and diethyl ether) and cultivation regimes (IMTA or wild) (M. Mendes et al., 2013). All ethyl acetate extracts exhibited greater amounts of SFA (mean of 69% of total FA) than MUFA and PUFA (means of 8 and 22% respectively). The antimicrobial properties by the agar diffusion method assessed against *Staphylococcus aureus* and *Enterococcus faecalis* were higher than those of the positive control (lactic acid 30%). The anti-microbial activity appeared to be more effective against Gram-positive bacteria (M. Mendes et al., 2013). Thus, ethyl acetate was determined in this study to be the best solvent for isolation of antimicrobial compounds. Further studies should be performed in order to identify, isolate and characterize the specific compounds with antimicrobial activities (M. Mendes et al., 2013). STA and GLA from *Ulva linza* (previously the Chlorophyta *Enteromorpha linza*) have been identified as active compounds against Gram-negative oral pathogenic bacteria *Porphyromonas gingivalis* with a greater activity than the reference drug, Triclosan (Choi et al., 2012; N.-H. Park et al., 2013). The minimal inhibitory concentration (MIC) of STA and GLA were established at 9.76 $\mu g.mL^{-1}$ against 78.12 $\mu g.mL^{-1}$ for Triclosan. This seaweed could provide 500 mg of STA and 140 mg of GLA from 100 g of powder (Choi et al., 2012; N.-H. Park et al., 2013). Plaza and coworkers (2010) reported that pressurized liquid ethanol extracts of the brown seaweed *Himanthalia elongata* (Ochrophyta, *Fucales*) were more effective for antimicrobial activity (Minimal Bactericidal Concentration (MBC) and Minimal Fungicidal Concentration (MFC) between 6 and 12 $mg.mL^{-1}$) than water (MBC and MFC between 12.25 and 14 $mg.mL^{-1}$) or hexane (MBC and MFC between 7 and 14 $mg.mL^{-1}$) extracts against *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus niger*. These extracts contained greater amounts of FA compared to water and hexane extracts. In addition, ethanol extracts contained a large amount of palmitoleic and oleic acid (2.5 and 17.9% respectively) that could be related to the antimicrobial activity (Plaza et al., 2010). According to Zheng et al. (2005), LC-PUFA were selective inhibitors of FabI, an enoyl-acyl carrier protein reductase involved in the synthesis of FA in bacteria and so inhibited the FA biosynthesis of the tested microorganisms (Zheng et al., 2005). The brown seaweed *Bifurcaria bifurcata* (Ochrophyta, *Fucales*) is well known for its composition of diterpenes (Santos et al., 2017). Lipophilic extracts of *Bifurcaria bifurcata,* enriched in FA exhibited antioxidant activity, anti-inflammatory potential with inhibition of nitric oxide production and antibacterial activity *in vitro* (Santos et al., 2017). The dichloromethane extract was mainly composed of diterpenes (1892.8 ± 134.0 $mg.kg^{-1}$ dw), free SFA (550.4 ± 15.7 $mg.kg^{-1}$ dw), free unsaturated FA (397.1 ± 18.4 $mg.kg^{-1}$ dw) which represented 42% of the total FA, long-chain aliphatic alcohols (C14-C28) and sterols (406.5 ± 26.2 $mg.kg^{-1}$ dw). FA were the second most abundant compounds detected in the lipophilic extract (947.9 ± 21.9 $mg.kg^{-1}$ dw) with palmitic acid predominant for the saturated FA and octadec-9-enoic acid (C18:1n-9) for the unsaturated FA (Santos et al., 2017). Antibacterial activity of the lipophilic extract was assessed against *Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis* and *Pseudomonas aeruginosa* with Minimal Inhibitory Concentration (MIC) determination. Inhibition against both Gram-negative and Gram-positive bacteria was observed (against *Staphylococcus aureus* and *Escherichia coli* with MIC of 1024 and 2048 $\mu g.mL^{-1}$ respectively) but no growth inhibition against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* in the range of the tested concentrations (MIC > 2048 $\mu g.mL^{-1}$) (Santos et al., 2017).

Taken together, the above evidence suggests that macroalgal FA with antimicrobial activity could be potentially used as preservative in cosmetic products or to maintain the stability of the skin microbiome.

4.1.3 Anti-inflammatory activity

PUFA isolated from *Undaria pinnatifida* and *Ulva australis* inhibited the production of metabolites involved in the lipoxygenase pathway (Ishihara et al., 1998). Indeed, C16:4n-3 and STA significantly suppressed the production of B_4 and C_4 leukotrienes and 5-hydroxyeicosatetraenoic acid in MC/9 mouse mast cells (Ishihara et al., 1998). The anti-inflammatory activity of these seaweeds is of interest against skin inflammation, common in cutaneous diseases such as atopic dermatitis (Banskota, Stefanova, Sperker, Lall, Craigie, Hafting, et al., 2014). A lipophilic extract of *Bifurcaria bifurcata* inhibited lipopolysaccharide (LPS) induced nitric oxide production to 6 and 40% at 50 and 25 $\mu g.mL^{-1}$ in murine RAW264.7 cells (Santos et al., 2017). EPA and ARA isolated from *Chondrus crispus* also inhibited nitric oxide production in murine LPS-induced macrophages RAW264.7 to 50 and 62% at 100 µM respectively (Banskota, Stefanova, Sperker, Lall, Craigie, & Hafting, 2014). These results are limited to mouse macrophages and more research must be done with cell lines from human skin for anti-inflammatory assessment.

4.1.4 Antioxidant activity

Free radicals and reactive oxygen species (ROS) may induce skin damage associated with aging and inflammatory disorders (Oresajo, Pillai, Manco, Yatskayer, & McDaniel, 2012; Pillai, Oresajo, & Hayward, 2005). Lipid-based antioxidant compounds which may help to prevent and control oxidative damage are therefore of huge interest in cosmetics and cosmeceuticals. Ethanol extracts of the brown seaweed *Himanthalia elongata* exhibited antioxidant activity according to TEAC assay (radical cation inhibition) (Plaza et al., 2010). These activities could be linked to lipid content but deeper study should be done (Plaza et al., 2010). A lipophilic fraction of *Bifurcaria bifurcata* exhibited strong antioxidant activity evaluated by DPPH and ABTS *in vitro* assays. For DPPH, the antioxidant activity of the lipophilic fraction was expressed by an IC_{50} of 365.6 ± 10.0 $\mu g.mL^{-1}$ compared to ascorbic acid (IC_{50} of 4.08 ± 0.05 $\mu g.mL^{-1}$) and BHT (IC_{50} of 14.32 ± 0.69 $\mu g.mL^{-1}$). For ABTS assay, the lipophilic fraction exhibited an IC_{50} of 116.3 ± 2.5 $\mu g.mL^{-1}$ compared to Trolox (IC_{50} of 365.6 ± 10.0 $\mu g.mL^{-1}$) (Santos et al., 2017). Neutral lipids, glycolipids and phospholipids extracted by solvent extraction (chloroform/methanol) and supercritical carbon dioxide ($ScCO_2$, $ScCO_2$ + Ethanol 2%, $ScCO_2$ + Ethanol 8%) from two seaweeds, the red macroalgae *Solieria chordalis* (Rhodophyta, *Gigartinales*) and the brown *Sargassum muticum* (Ochrophyta, *Fucales*) demonstrated free radical scavenging activity with DPPH from 16 to 86.6% after a 30 min incubation at 37°C (Terme et al., 2018). All lipids classes from *Solieria chordalis* and *Sargassum muticum* demonstrated DPPH free radical scavenging activities at 1 $mg.mL^{-1}$. The antioxidant activity of the phospholipid class of *Solieria chordalis* $ScCO_2$ + Ethanol 8% and *Sargassum muticum* $ScCO_2$ was greater than the chloroform/methanol solvent, respectively (EC_{50} of 1.1 ± 0.1 and 1.0 ± 0.1 $mg.mL^{-1}$ against 4.9 ± 0.5 and 4.8 ± 0.1 $mg.mL^{-1}$) (Terme et al., 2018). Phospholipids with PUFA were likely extracted selectively and exhibited antioxidant properties *in vitro* (Terme et al., 2018). The glycolipid and phospholipid fractions represented up to 90% of the lipid fraction from *Sargassum muticum*. $ScCO_2$ appears to be a good eco-responsible technique for the extraction of antioxidant lipids since antioxidant activity of glycolipids and phospholipids from *Sargassum muticum* $ScCO_2$ are twice that of the chloroform/methanol solvent system (Terme et al., 2018). More research needs to be done on macroalgal lipids to assess their *in vitro* activity on skin cells (keratinocytes and fibroblasts) for anti-ageing and skin hydration assessment and potential relationships to antioxidant activity for example.

4.2 Preventive effects of n-3 PUFAs on cardiovascular disease and metabolic syndrome

Numerous investigations have been carried out in an attempt to examine the effect of n-3 PUFA intake for the management of CVD and CVD risk factors, and there is a large body of evidence supporting their cardioprotective effects (AbuMweis, Jew, Tayyem, & Agraib, 2018; Bird, Calder, & Eggersdorfer, 2018; Innes & Calder, 2018; Manuelli, Della Guardia, & Cena, 2017; O'Mahoney et al., 2018). CVD are the leading cause of mortality among developed countries, even in apparently healthy individuals. In addition to genetic predisposition and a few non-modifiable factors including age, gender and ethnic origin, the development of CVD is strongly associated with the presence of metabolic syndrome (MetS; also referred to as Syndrome X, insulin resistance syndrome or dysmetabolic syndrome) and the deregulation of clinical parameters such as increased very low density lipoprotein (VLDL) and triglyceride levels, decreased high density lipoprotein (HDL) levels, obesity, diabetes and hypertension (Gonzalez-Chávez et al., 2018). Lifestyle and diet alterations, especially an n-3 LC-PUFA enriched diet, could help to modulate these factors and reduce MetS and CVD risk.

4.2.1 Cardioprotective effects of n-3 polyunsaturated fatty acids

Cardioprotective effects of n-3 LC-PUFA were initially reported in a landmark study comparing the diets and CVD rates of Greenland Inuit to the Danish population (Bang, Dyerberg, & Sinclair, 1980). These benefits of n-3 LC-PUFA were further supported by epidemiological studies associating fish consumption with a reduced CVD mortality in different populations (Daviglus et al., 1997; Kagawa et al., 1982; Kromhout, Bosschieter, & Coulander, 1985; Newman, Middaugh, Propst, & Rogers, 1993). Various mechanisms have been proposed to explain the reduction in cardiovascular risk by n-3 PUFA, including prevention of cardiac arrhythmias, decreased plasma triglycerides, reduction of blood pressure, decreased platelet aggregation and reduction of inflammation (Calder, 2004). Interestingly, the effects of DHA and EPA are not identical as suggested by a recent systematic review (Innes & Calder, 2018), which discussed that DHA has a greater triglyceride-lowering effect, a greater effect on vascular function improvement and lowering of heart rate and blood pressure, and finally that only DHA increased HDL- and low density lipoprotein (LDL-) cholesterol concentration and LDL particle size (Innes & Calder, 2018). Consumption of n-3 PUFA has also been related to the regulation of eicosanoid concentrations, influencing several physiological functions in relation to the increase of plasma LDL-cholesterol levels, protection against cardiovascular, coronary heart diseases, atherosclerosis, diabetes, hypertension and metabolic diseases (Jung, Torrejon, Tighe, & Deckelbaum, 2008; Kaur, Chugh, & Gupta, 2012; Zárate, el Jaber-Vazdekis, Tejera, Pérez, & Rodríguez, 2017).

Nutrition feeding studies in healthy animal models showed that ALA, the metabolic precursor of EPA and DHA, could reduce myocardial damage during cardiomyopathic disease in hamsters fed a high-fat diet with ALA representing 52% of total lipids (Fiaccavento et al., 2006). Reduced atherosclerosis, antithrombotic and anti-inflammatory effects have also been reported in C57/B16 mice either wild-type or deficient for LDL-receptor, or deficient for APOE gene and fed diets rich in ALA (Bassett et al., 2011; Holy et al., 2011; Winnik et al., 2011). In addition, a diet enriched in n-3 LC-PUFA, when fed after the onset of the ischemia/reperfusion period, reduced apoptosis in the limbic system of the brain, reduced circulating pro-inflammatory cytokines and attenuated post-myocardial infarction depression in a rat model of ischemia/reperfusion (Gilbert et al., 2013). However, a protective effect of n-3 PUFA against CVD was not always detected in normal animals, although present in diabetic animals, as shown in a myocardial ischemia/reperfusion injury rat model (Xie et al., 2011). At the cellular level, recent studies reported the involvement of the peroxisome proliferator activated receptor (PPAR)-δ signaling pathway and enhanced ceramide levels in the cytotoxicity of DHA toward rat cardiomyocytes (Samokhvalov et al., 2015). In addition to an impact on PPAR-α and -γ signaling, n-3 PUFA may act through inhibition of cAMP-dependent Protein Kinase C (PKC) activation and calcium release at the intracellular level, and through inhibition of both Na^+-H^+ and Na^+-Ca^{2+} ions exchange as well as by reducing ligand availability for angiotensin-II, endothelin-1 and thromboxane receptors (Poudyal, Panchal, Diwan, & Brown, 2011).

Early randomized controlled trials showed a reduction in risk of cardiovascular mortality after increasing consumption of fatty fish or n-3 LC-PUFA dietary supplements (Burr et al., 1989; Gissi-Prevenzione Investigators, 1999; Singh et al., 1997). However, more recent studies do not show significant effects of EPA and/or DHA supplementation (Aung et al., 2018; Kromhout, Yasuda, Geleijnse, & Shimokawa, 2012; Maki, Palacios, Bell, & Toth, 2017; Manson et al., 2019). This lack of effect of n-3 PUFA in recent prevention studies may have several explanations, among which is the frequent use of statin therapy in study patients (Maki et al., 2017; Sethi, Bajaj, Khosla, & Arora, 2016). Indeed, the mechanisms of action of n-3 PUFA overlap with the pleiotropic effects of statins, such as anti-thrombotic and antioxidant effects, and improving endothelial function (Bird et al., 2018; Sethi et al., 2016). In addition, statins may interfere with cytochrome P450 enzymes and PPAR to affect PUFA concentration and eicosanoid production (Bird et al., 2018). Interestingly, DHA from algal oil reduced blood TAG and increased HDL- but also LDL-cholesterol in patients without coronary disease, demonstrating a positive effect in the risk of initiation of factors involved in CVD (Bernstein, Ding, Willett, & Rimm, 2012).

4.2.2 Prevention of cardiovascular disease risk associated with metabolic syndrome and diabetes

Numerous nutrition studies have been conducted in animals with an enhanced risk of CVD, namely obesity, dyslipidemia and/or diabetes models, which highlighted the preventive effects of n-3 PUFA (Mimouni et al., 2015; Ulmann et al., 2017). The efficacy of n-3 PUFA to reduce the risk of CVD is linked to a reduction of factors involved in MetS development, mainly through modification of the serum lipid profile (Manuelli et al., 2017). The main effects of n-3 LC-PUFA, mainly EPA and DHA, on plasma lipids were a reduction of adiposity, a diminution of TAG synthesis and a decrease of the concentration of plasma TAG in obese patients (Micallef, Munro, Phang, & Garg, 2009; Ulmann et al., 2017). In hypercholesterolemic rats, DHA supplementation reduced total weight gain, adiposity index, plasma HDL-cholesterol and glucose concentrations regardless of the dose and form (*i.e.* DHA ethyl esters and DHA re-esterified TAG) of supplementation (Tabernero et al., 2013). Reductions in serum

cholesterol and TAG levels, and a decrease in platelet aggregation were also observed in hypercholesterolemic rats fed EPA and DHA (Adan, Shibata, Sato, Ikeda, & Imaizumi, 1999). Consumption of n-3 PUFA had beneficial effects on obesity with increased β-oxidation or adiponectin levels in obese rats (Shirouchi et al., 2007). Moreover, the body mass index and waist circumference of obese patients were inversely correlated with EPA and DHA intakes (Micallef et al., 2009) due to increased lipolysis and decreased lipogenesis in the liver (Harris & Bulchandani, 2006). In the same way, a significant decrease in blood TAG and increase in HDL-cholesterol were observed in patients with dyslipidemia after consumption of 3 g PUFA per day in a proportion of 0.9:1.5 for EPA and DHA respectively during 3 and 6 months (Derosa et al., 2009). A decrease of TAG and increase of HDL-cholesterol in blood were also reported in type 2 diabetes patients receiving 460 mg of EPA and 380 mg of DHA ethyl esters per day for eight weeks (Valdivielso, Rioja, García-Arias, Sánchez-Chaparro, & González-Santos, 2009) and similar conclusions have been drawn from a meta-analysis of type-2 diabetes randomized controlled trials (O'Mahoney et al., 2018).

Concerning the use of microalgal n-3 PUFA as dietary source of PUFA in human or animal nutrition protocols, several studies highlighted the benefits of microalgae (Mimouni et al., 2015; Ulmann et al., 2017, 2014). In hypertriglyceridemic men, the concentration of fasting TAG and the number of small dense LDL particles were decreased after 45 days of a diet containing 7.5 g per day of DHA oil from *Crypthecodinium cohnii*, corresponding to about 3 g of DHA per day (Kelley, Hubbard, & Erickson, 2007). The use of the EPA-rich diatom *Odontella aurita* as a diet supplement reduced the risk factors for high-fat-diet induced MetS including hyperlipidemia, platelet aggregation and oxidative stress in high-fat fed rats (Haimeur et al., 2012). Moreover, the ability of *Odontella aurita* to reduce CVD risk factors in high-fat fed rats was similar or even greater than that of fish oil, potentially due to a synergistic interaction of n-3 PUFA with other bioactive components of the microalga such as pigments or phytosterols (Haimeur et al., 2016). In alloxan-induced diabetic rats, diets supplemented with either *Nannochloropsis oculata* or *Isochrysis galbana* at 50 mg/day decreased blood levels of glucose, TAG and cholesterol (Nuño et al., 2013). Additionally, *Chlorella*, a freshwater microalgal genus rich in GLA, was demonstrated to prevent dyslipidemia in rat and hamster models fed with high-fat diets and subsequently prevented hyperlipidemia as well as atherosclerosis (J. Y. Cherng & Shih, 2005; Yamagishi, Nakamura, & Inoue, 2005). Consumption of *Chlorella pyrenoidosa* in the diet enhanced the hypoglycemic effects of exogenous insulin in streptozotocin-induced diabetic mice (J.-Y. Cherng & Shih, 2006; Jong-Yuh & Mei-Fen, 2005). Our current studies are investigating the potential of the two Diatoms *Phaeodactylum tricornutum*, *Tisochrysis lutea* and the Haptophyta *Diacronema lutheri*, to reduce MetS-related risk factors in high-fat fed rats (Mayer, Côme, Guéno, et al., 2018; Mayer, Côme, Ulmann, et al., 2018). *Phaeodactylum tricornutum* supplementation has a real impact on MetS (Mayer et al., 2019). Nevertheless, caution in the interpretation of the above results is mandatory since they were obtained using whole microalgal cells that provide a variety of bioactive molecules with potential synergistic effects.

4.3 Contribution of n-3 polyunsaturated fatty acids in cancer risk factor prevention and/or therapy

4.3.1 Epidemiological studies highlight the benefit of n-3 polyunsaturated fatty acid rich diet

Increasing evidence suggests that n-3 PUFA have anticancer activity and improve the effect of conventional cancer therapy (Chénais & Blanckaert, 2012; de Lorgeril & Salen, 2012; Ulmann et al., 2017, 2014; Vaughan, Hassing, & Lewandowski, 2013).

High fat consumption is commonly associated with increased risk for several types of diet-related cancers such as breast, colonic or pancreatic tumors (Chénais & Blanckaert, 2012; de Lorgeril & Salen, 2012; Ulmann et al., 2017, 2014; Vaughan et al., 2013). Furthermore, epidemiological studies highlight a 4 to 5 fold greater rate of breast cancer in Western countries than in Japan, associated at least in part, with a diet low in fatty fish rich in PUFA (Holmes & Willett, 2004; Hursting, Thornquist, & Henderson, 1990; Kaizer, Boyd, Kriukov, & Tritchler, 1989; Kinlen, 1991; Sasaki, Horacsek, & Kesteloot, 1993). These observations suggested that diets rich in n-3 and n-6 LC-PUFA may help to reduce the risk of cancers (de Lorgeril & Salen, 2012; Vaughan et al., 2013). However, while dietary n-3 PUFA appear to possess a preventive effect on cancer (Hursting et al., 1990; Kaizer et al., 1989; Sasaki et al., 1993), n-6 PUFA have been related to a higher risk of inducing tumor formation and inflammatory processes (Chénais & Blanckaert, 2012; Weisburger, 1997). On the other hand, SFA and MUFA have been shown to have either no effect on tumor formation or a moderate tumor-promoting effect (de Lorgeril & Salen, 2012; Weisburger, 1997). Recent population studies have shown that n-3 PUFA consumption reduced the risk of hepatocellular carcinoma (Gao et al., 2015) and enhanced survival after breast cancer (Khankari et al., 2015). Population studies show that intake of n-3 PUFA is not associated with an overall increased incidence of colorectal cancer (M. Song et al., 2015) but may have a protective role against colorectal cancer, by reducing the risk of tumor initiation (Hodge et al., 2015; M. Song et al., 2015). Nevertheless, different n-3 PUFA exposures may exhibit

different associations with cancer risk, and observational studies on colorectal, prostate and breast cancers provide only limited evidence of a possible role of n-3 PUFA in cancer prevention because of insufficient homogeneity of the observations (Gerber, 2012; Hodge et al., 2015; Holmes et al., 2004; Khankari et al., 2015; MacLean et al., 2006; Shen, Zhou, Dong, Ding, & Wu, 2012; M. Song et al., 2015).

4.3.2 Antiproliferative and pro-apoptotic effects of n-3 polyunsaturated fatty acids

Understanding the cellular and molecular mechanisms of the anticancer effect of n-3 PUFA remains an important area of current research. An increasing number of investigations have been performed in diverse human cancer cell lines using purified n-3 PUFA, mainly DHA, and highlighting their antiproliferative and pro-apoptotic effects (D'Eliseo & Velotti, 2016; Moloudizargari et al., 2018; E. Song & Kim, 2016; Ulmann et al., 2017; VanderSluis, Mazurak, Damaraju, & Field, 2017). It is noteworthy that n-3 PUFA had no pro-apoptotic effects on non tumoral cells including the human MCF-10A and H184-A1N4 mammary epithelial cell lines (Rovito et al., 2015; Tsai et al., 2017), embryonic kidney HEK-293 cells and human hepatocyte-like WRL-68 cells, as well as SD rat primary cortical neuronal cells and murine peritoneal macrophages (So, Liu, & Leung, 2015). Cell proliferation was reduced by DHA whether it originated from fish oil or microalgae (Judé et al., 2006; van Beelen et al., 2009). DHA acted as an anti-proliferative agent by lengthening the cell cycle at the G_2/M transition (Newell, Baker, Postovit, & Field, 2017) such as in the MDA-MB-231 breast cancer cell line (Barascu, Besson, Le Floch, Bougnoux, & Jourdan, 2006), or mainly by G_0/G_1 cell cycle arrest (Newell et al., 2017) such as in LA-N-1 neuroblastoma cells (So et al., 2015). The antiproliferative effects of DHA also involved increased cell sensitivity to ROS (Siddiqui, Harvey, & Stillwell, 2008; E. Song & Kim, 2016) through the downregulation of superoxide dismutase 1 (SOD1) (Ding, Vaught, Yamauchi, & Lind, 2004) and glutathione peroxidase 1 (GPX1) (Vibet et al., 2008). In addition, DHA was recently reported to induce the expression and nuclear translocation of the oxidative stress sensitive transcription factor NFE2L2/Nrf2 (Pettersen et al., 2016; Tsai et al., 2017) and to increase the expression of oxidative stress-induced growth inhibitor 1 (OSGIN1) in MCF-7 and Hs578T breast cancer cells (Tsai et al., 2017). However, DHA also increased the activity of antioxidant enzymes (SOD, CAT and GPX) in breast cancer tissues (Geng, Zhou, Liu, Wang, & Chen, 2018), suggesting that the anti-/pro-oxidant effects of DHA may be dependent of the cell type and concentration used.

Apoptosis was induced by DHA and, to a lesser extent by EPA (Sam, Ahangar, Nejati, & Habibian, 2016; Sun, Jia, Hou, Liu, & Gao, 2017; Wan, Fu, & Ababaikeli, 2016), but not by ALA (Mason, Klaire, Kharotia, Wiggins, & Thompson, 2015). Apoptosis was enhanced in DHA-treated cells through impairment of the activity of protein kinase Akt/PKB, p53 activation and increased caspase-3, caspase-9 and Bax pro-apoptotic enzyme levels or activity and decreased survival and Bcl-X_L in various cancer cell lines and with DHA concentration ranging from 10 to 200 µM (Ahangar, Sam, Nejati, & Habibian, 2016; Chamras, Ardashian, Heber, & Glaspy, 2002; Han et al., 2016; J. Liu et al., 2016; Mason et al., 2015; M. Park, Lim, & Kim, 2018; Sam, Esmaeillou, & Shokrgozar, 2017; Sam, Tavakoli-Mehr, & Safaralizadeh, 2018; So et al., 2015; Sun et al., 2017; Tasaki et al., 2017; Wan et al., 2016; M. Xue et al., 2017; Y. Yin, Sui, Meng, Ma, & Jiang, 2017; Table 3).

The inactivation of the PI3K/Akt pathway is the main apoptosis pathway mentioned in the literature (see Table 3 for references). However, some other apoptotic pathways such as the Bax-independent pathway could be also involved in HL-60 promyelocytic leukemia (Miura et al., 2004). In the breast cancer cell line MCF-7, DHA-induced apoptosis was linked to both increased death receptors (DR-4, TRAIL and Fas) expression and mitochondrial release of the caspase activator SMAC/Diablo (M. Xue et al., 2017). The inhibition of the STAT3 survival pathway has been reported for several cell types, including pancreatic and renal cancer cells and multiple myeloma (D'Eliseo, Di Renzo, Santoni, & Velotti, 2017; D'Eliseo, Manzi, Merendino, & Velotti, 2012; M. Park et al., 2018; Tasaki et al., 2017). The activation of the MAP Kinase pathway, especially that of p38 and JNK, was also reported a few times to activate apoptosis and/or autophagy in the human prostate cancer cell line DU145 and KF-28 ovarian cancer cells (Sun et al., 2017; Tanaka et al., 2017). In addition, DHA and EPA are natural ligands of PPAR-α and PPAR-γ, thereby. DHA-induced apoptosis may be linked to PPAR-γ overexpression in an epithelial ovarian cancer cell line (Wan et al., 2016), and to PPAR-α overexpression in breast cancer tissue (Table 3; Geng et al., 2018). Dopamine-conjugates of DHA and EPA induced breast cancer cell death through recruitment of PPAR-γ (Rovito et al., 2015). However, the role of PPAR-α and PPAR-γ in controlling cancer cell proliferation and apoptosis is controversial (Yousefnia, Momenzadeh, Seyed Forootan, Ghaedi, & Nasr Esfahani, 2018). Besides apoptosis, recent studies also highlighted that DHA may also induce pyroptosis, a form of programmed cell death mediated by inflammatory caspases (*i.e.* caspase-1, -4, -5 in humans) and leading to pore formation in cell membrane by the N-terminal region of GasderminD, in breast cancer cells (Pizato et al., 2018) and autophagy in breast, colon, and lung cancer cells as well as in glioblastoma cell lines (S. Kim et al., 2017; Pettersen et al., 2016; Rovito et al., 2015; Zajdel, Wilczok, & Tarkowski, 2015). Distinctive features of immunogenic apoptosis include the cell surface exposure of calreticulin and/or HSP90 in pre- or early-apoptotic stages and the release of non-histone chromatin protein high mobility group box 1 in late-apoptosis, which will stimulate antitumor immune responses through

antigen presenting cells (Serrano-del Valle, Anel, Naval, & Marzo, 2019). DHA has been reported to promote immunogenic apoptosis and activated autophagy in multiple myeloma cells and inhibited the STAT3 survival pathway (**Table 3**; D'Eliseo et al., 2017). This study also reported the activation of autophagy by DHA in peripheral blood macrophages and dendritic cells, suggesting DHA acted as an immune stimulator, enhancing the presentation of tumor antigens by phagocytic cells (D'Eliseo et al., 2017).

Table 3: Examples of cell death signaling pathways triggered by n-3 PUFA in human cancer cells

Type of cancer	Cell lines	n-3 PUFA	Antiproliferative effect	Signaling pathway elements	Reference
Acute lymphoblastic leukemia	Molt-4	DHA	Apoptosis	↑p53, ↓survivin	(Sam et al., 2017)
Adrenocortical carcinoma	SW13, H295R, (*)	DHA	Apoptosis, G_0/G_1 arrest	↓Akt, ↓mTor	(J. Liu et al., 2016)
Breast cancer	Human breast cancer tissue	DHA	Apoptosis	↑PPAR-α, TLR-4, ↑cAMP, GMPc, oxidative stress enzyme	(Geng et al., 2018)
	MCF-7	DHA	Apoptosis	↑p53 (PI3K), ↑NFE2L2, ↑ROS, ↑OSGIN1	(Tsai et al., 2017)
	MCF-7	DHA	Apoptosis	↑Fas, DR4, TRAIL	(M. Xue et al., 2017)
	BT-474	DHA	Apoptosis	↓Akt, ↓Erk-1/2	(Mason et al., 2015)
	MCF-7, SKBR3, MDA-MB-231	DHA, EPA**	Apoptosis, autophagy	↑PPAR-γ	(Rovito et al., 2015)
Colorectal cancer	HCT116	DHA	Apoptosis	↑p53, ↓survivin, miR-1G1	(Sam et al., 2018)
	HCT116, (*)	DHA	Apoptosis	↓survivin, β-catenin blockage	(Han et al., 2016)
	HCT116, HCT8, (*)	DHA	Apoptosis	↑TNF-α, ↓miR-21, ↓AMPK ↓RIP1 kinase	(Fluckiger et al., 2016)
	LS174T	DHA, EPA	Apoptosis	↓survivin	(Ahangar et al., 2016; Sam et al., 2016)
Glioblastoma	D54-MG, U-87-MG, U-251-MG	DHA	Apoptosis, autophagy, G_0/G_1 arrest	↓Akt, ↓mTor, ↑AMPK	(S. Kim et al., 2017)
Lung cancer (non-small cell)	A549	DHA	Apoptosis	↓Akt, PI3K, ↑ROS	(Y. Yin et al., 2017)
	A549	DHA, EPA	Apoptosis, autophagy	↑ROS	(Zajdel et al., 2015)
Multiple myeloma	RPMI-8226, OPM-2	DHA	Apoptosis, autophagy	↓STAT3	(D'Eliseo et al., 2017)
Ovarian cancer	KF-28, HAC-2	DHA, GLA	Apoptosis	↑MAPK (JNK, p38), ↑ROS	(Tanaka et al., 2017)
	TOV-21G	DHA, EPA	Apoptosis	↑p53, ↑PPAR-γ	(Wan et al., 2016)
Pancreatic cancer	PANC-1	DHA	Apoptosis	↓survivin, ↓STAT3, ↓EGFR, ↓NF-κB	(M. Park et al., 2018)
Prostate cancer	DU-145	DHA, EPA	Apoptosis	↑p53, PI3K/Akt, ↑MAPK (JNK, p38), ↑TNF-α, ↑NF-κB	(Sun et al., 2017)
Renal cancer	Caki-1, 786-O	DHA	Apoptosis, G_0/G_1 arrest, G_2/M arrest	↓Akt, ↓STAT3, ↑EGFR	(Tasaki et al., 2017)

(*) Publication including animal model. ** dopamine-conjugated DHA or EPA.

Studies have been conducted in animal models of chemically-induced tumors or tumor xenografts that reported antiproliferative and pro-apoptotic effects of DHA (Fluckiger et al., 2016; J. Liu et al., 2016), its metabolic precursor GLA (Das, Prasad, & Reddy, 1995) or dietary fats (Han et al., 2016; Ramesh & Das, 1995). Moreover, animal studies that demonstrated the potential preventive effects of n-3 PUFA against several types of cancer have been reported using the *fat*-1 transgenic mouse that express n-3 desaturase missing in mammalian cells. These studies focused on colorectal cancer and colitis-associated cancer (Algamas-Dimantov et al., 2014; Han et al., 2016; Jia et al., 2008; Lim, Han, Dai, Shen, & Wu, 2009; M. Liu et al., 2016; Nowak et al., 2007; Weylandt et al., 2011), but have also targeted breast (Zou et al., 2013), liver (Lim et al., 2009; Weylandt et al., 2011), lung (Xia,

Wang, & Kang, 2005), pancreatic (Mohammed et al., 2012) and prostate (Lu et al., 2008) cancers as well as melanoma (Xia et al., 2006) and adrenocortical carcinoma (J. Liu et al., 2016).

4.3.3 Anti-invasive and anti-metastatic effects of n-3 polyunsaturated fatty acids

A few studies have shown that the diet can affect the metastatic potential of cancer cells known to have a high metastatic phenotype such as breast cancer (Bougnoux et al., 2009; Bougnoux, Hajjaji, Maheo, Couet, & Chevalier, 2010). The anti-metastatic effect of DHA was highlighted in several cancer cell lines (Chénais & Blanckaert, 2012; Ulmann et al., 2017, 2014). DHA, at a concentration ranging from 10 to 100 µM, reduced the invasive potential of the MDA-MB-231 breast cancer cell line (Blanckaert et al., 2015, 2010; Yun et al., 2016) as well as 0.1% (v:v) fish oil with the tamoxifen-resistant MCF-7 (TamR) cells (Davison, Nicholson, Hiscox, & Heard, 2018). DHA also suppressed the invasiveness of A549 lung adenocarcinoma (Y. Yin et al., 2017) and renal cancer cell lines Caki-1 and 786-O (Tasaki et al., 2017). This anti-invasive effect of DHA was also demonstrated in several human colorectal cancer cell lines including and may be linked to inhibition of granzyme B, a serine protease most commonly found in the granules of cytotoxic lymphocytes (CTL), natural killer cells (NK cells) and cytotoxic T cells (D'Eliseo et al., 2016). The mechanisms and signaling pathways involved in the anti-metastatic effects of DHA remain to be understood and the current data are insufficient. However, this anti-metastatic effect is corroborated by *in vivo* animal studies using *fat*-1 transgenic mice capable of producing n-3 FA from the n-6 type, leading to abundant n-3 FA with reduced levels of n-6 FA in their organs and tissues, without the need of a dietary n-3 supply (Kang, 2007). The use of *fat*-1 transgenic mice has shown a decrease of tumor growth and diminution of lung metastasis of syngeneic breast cancer cells in this DHA-rich environment (Yun et al., 2016).

4.3.4 n-3 polyunsaturated fatty acids as adjuvant of chemotherapy

Several studies have highlighted the potential of n-3 PUFA, either fish oil or purified EPA/DHA, as an adjuvant in the treatment of cancer (Corsetto, Colombo, Kopecka, Rizzo, & Riganti, 2017; Murphy, Mourtzakis, & Mazurak, 2012; Siddiqui et al., 2011; Volpato & Hull, 2018). Indeed, DHA enhanced the cytotoxic effects of docetaxel in prostate (LNCaP, PC3 and DU145) and mammary (MDA-MB-231) cancer cell lines through increased apoptosis (Chauvin et al., 2016; Shaikh, Brown, Schofield, Wahle, & Heys, 2008). A similar enhancement of apoptosis was observed in vitro when combining DHA with diverse chemotherapeutic drugs, including lomustine with glioblastoma cell lines (Harvey et al., 2015), all-*trans* retinoic acid with the breast cancer cell line MCF-7 (Abdolahi, Shokri, Hosseini, Shadanian, & Saboor-Yaraghi, 2016), cisplatin with gastric cancer cells (Sheng, Chen, Liu, Li, & Cao, 2016); or with other traditional adjuvants such as dexamethasone in multiple myeloma cells (Dai, Li, & Geng, 2017) or curcumin in breast cancer cell lines or an *in vivo* mouse model of 7,12-dimethylbenz[a]anthracene (DMBA) induced mammary tumorigenesis (Altenburg et al., 2011; Siddiqui et al., 2013). Combining DHA-rich fish oil with either 5-fluorouracil, irinotecan or oxaliplatin also enhanced apoptosis in the colorectal cell line HT-29 by 120, 43 and 55% respectively (Granci et al., 2013). Even radiation therapy such as single X-ray radiotherapy (up to 4Gy) may be improved by n-3 PUFA exposure of colorectal HT-29 and LST174T cell lines (Cai et al., 2014). *In vivo* experiments using a model of N-methylnitrosurea-induced mammary tumor showed that the reduction of the tumor size induced by epirubicin was 40% greater when about 0.7 g DHA was provided daily in the diet (Colas et al., 2006). Furthermore, this effect of DHA was linked to a selective increase of oxidative damage as demonstrated by elevated lipid hydroperoxide level in tumors (Hajjaji, Besson, & Bougnoux, 2012). In addition, a clinical phase-II trial showed that the addition of DHA to anthracycline-based treatment targeting metastatic breast cancer improved the outcome of chemotherapy (Bougnoux et al., 2009). Moreover, DHA has the potential to specifically sensitize tumoral cells to chemotherapy or radiotherapy while protecting non-tumor cells (Bougnoux et al., 2010; Hajjaji & Bougnoux, 2013). Indeed, high incorporation of DHA during chemotherapy was devoid of adverse side effects and can improve the outcome of chemotherapy or radiotherapy (Bougnoux et al., 2010; Hajjaji & Bougnoux, 2013).

Taking the evidence discussed above, DHA is a safe, natural compound that can greatly improve the anticancer properties of anticancer drugs by additive or synergistic interactions (Corsetto et al., 2017; Siddiqui et al., 2011; Volpato & Hull, 2018). In addition, n-3 PUFA reduced the risk of obesity related breast cancer (Manni, El-Bayoumy, & Thompson, 2017) and had protective effects toward the cardiotoxicity of anthracyclines, the most extensively used chemotherapeutics (Serini, Ottes Vasconcelos, Nascimento Gomes, & Calviello, 2017; H. Xue, Ren, Denkinger, Schlotzer, & Wischmeyer, 2016). Thus, current results of cohort studies and investigations in cell lines or animal models demonstrated that DHA could reduce tumor cell number by acting as soon as the cell begins its neoplastic transformation through a decrease in proliferation and an increase in apoptosis; this might explain the low level of breast cancer in populations with a high intake of DHA such as Japanese, Scandinavians, people from Greenland and Nunavut (Bougnoux et al., 2010). According to Stark et al. (2016), regions with high EPA + DHA blood levels (> 6%) included the Sea of Japan, Scandinavia, Greenland, Nunavut, Mongolia and some other

areas with indigenous population or populations not fully adapted to Westernized food habits (Stark, Van Elswyk, Higgins, Weatherford, & Salem, 2016). More importantly, DHA may also reduce the invasive potential of certain cancers, which is one of the main complications with advanced cancers.

4.4 Antiviral activities

Sulfolipids like 1,2-di-O-acyl-3-O-(α-D-sulphoquinovosyl)-glyceride with two palmitic acids seemed to be responsible for the antiviral activities of extracts from the green seaweed, *Ulva fasciata*, the red seaweed *Laurencia papillosa* (Rhodophyta, *Ceramiales*) and the brown seaweed *Sargassum vulgare* against Herpes Simplex Virus 1 and 2 (HSV-1 and HSV-2) *in vitro* (El Baz, El Baroty, Ibrahim, & Abd El Baky, 2014; Plouguerné et al., 2013). Palmitic acid extracted from *Sargassum fusiforme* also demonstrated antiviral activity against Human Immunodeficiency Virus 1 (HIV-1) blocking virus entry and infection *in vitro* (D. Y. W. Lee et al., 2009).

5. Potential commercial applications

Algal use by local populations across the globe is an old tradition such as in China, Japan, and Korea. In recent decades, progress in biotechnology has increased the development of commercial and industrial scale of algal bioactives. Macroalgae are mainly used for the production of phycocolloids, particularly, the Rhodophyta and Phaeophyta. However, their richness in the essential n-3 and n-6 PUFA and their long-chain metabolites and derivatives EPA and DHA (Banskota, Stefanova, Sperker, Lall, Craigie, & Hafting, 2014; Khotimchenko, 2002) has strengthened their importance for human and animal diets.

Integrated multi-trophic aquaculture (IMTA) systems are based on the use of organisms from different trophic levels where the excretion of upper-level organisms becomes a resource utilized by the lower levels (Pliego-Cortés et al., 2019). The use of aquaculture wastewater as a nutrient feed for seaweeds is recognized as a way to obtain algal biomass (Chopin et al., 2001; Neori et al., 2004). Very recently, Peñuela et al. (2018) showed that the PUFA content was doubled in the biomass of the carragenophyte *Solieria filiformis* cultured in an IMTA system (Peñuela et al., 2018). Coupling ecological aquaculture or bioextraction to obtain algal biomass with the use of environmentally friendly food-safe techniques and the biorefinery concept could be a research strategy focus into the 'Blue Economy' that highlights the sustainable use of oceanic resources by providing economic and ecological benefits.

The successful use of microalgae in biotechnology relies on the choice of the algal strain in accordance with their specific culture conditions to obtain the desired products with desirable properties (Pulz & Gross, 2004). Currently, only a small number of microalgal species are produced on an industrial scale for their lipid content (Gouveia, Batista, Sousa, Raymundo, & Bandarra, 2008). They are mainly produced as human nutritional supplements and additives for animal feed (Kovač, Simeunović, Babić, Mišan, & Milovanović, 2013). The most important species in terms of annual world production are the Chlorophytes *Chlorella sp.* (2000 tonnes/year), *Dunaliella salina* (1200 tonnes/year) and *Haematococcus pluvialis* (Chlorophyta, *Chlamydomonadales*, 300 tonnes/year), the dinoflagellate *Crypthecodinium cohnii* (240 tonnes/year for DHA oil) and *Schizochytrium* sp. (10 tonnes/year for DHA oil production) (Van der Voort, Spruijt, Potters, De Wolf, & Elissen, 2017).

For human nutrition, microalgae are sold in different forms including tablets, capsules and liquids or incorporated into snack foods, pasta, candies or beverages (Liang, Liu, Chen, & Chen, 2004). In our diet, PUFA are generally supplied by fish and fish oils. However, fish oil as a food additive has drawbacks including an unpleasant taste and fishy smell. When one considers that PUFA fish are derived from their diet and especially from microalgae consumption, it makes sense to consider microalgae as a direct source of essential PUFA (Jiang, Chen, & Liang, 1999). The Rhodophyte *Porphyridium cruentum* has been cultivated in artificial seawater and used for ARA production (Pulz & Gross, 2004). Bacillariophytes including *Phaeodactylum tricornutum* can accumulate lipid up to 60% dw under nitrogen-starvation making them a good source of PUFA, especially EPA (Borowitzka, 1997).

The global value for n-3 oils was around € 320 million in 2014 (Van der Voort et al., 2017). Infant formula and dietary supplements are the most important applications for DHA-rich oils (Van der Voort et al., 2017). TAG obtained from *Isochrysis sp.* and *Isochrysis galbana* 2307 exhibited FA composition and positional distribution in TAG, crystallization and melting properties similar to that of human milk fats (He et al., 2019). Therefore, they are promising sources of TAG for infant formulas. Algal oils accounted for 3% of the EPA and DHA volume and 18% of the market value in recent years and the demand is growing related to the demand of nutrition and food labelling with terms such as "vegetarian" or "natural" (Van der Voort et al., 2017).

The economic value of algal cultivation is not the only parameter to consider in commercial applications of algae. The sustainable development of algae cultivation must also be taken into account to meet the needs of the present without compromising the ability of future generations to meet their own needs (World Commission on Environment and Development, 1987). DHA can be produced by green strategies related to waste consumption.

Indeed, *Schizochytrium* sp. grown on cane molasses as a carbon source and algal-residue (*Schizochytrium* residue after a first FA extraction) as a nitrogen source has been used to produce DHA (F. W. Yin et al., 2019). The technical feasibility and sustainability of *Nannochloropsis gaditana* cultivation was demonstrated in the circular economy framework at the semi-industrial scale (López et al., 2019). The use of flue gases from a coal fired power plant produced a biomass in accordance with regulations for animal feed (López et al., 2019).

6. Conclusion and future trends

Very few numbers of algal species are used for their FA content for commercial purposes, despite a wide range of biological activities potentially impacting human health and wellness. The cultivation conditions whether *in vitro* or at sea must be controlled for a high lipid quantity and purity. Molecular biology and genetic engineering could be promising approaches to increase the lipid content or FA profile of a specific interest by strain selection or gene overexpression. In the context of climate change, the biorefinery approach allows the global valorization of biomass by making successive extractions of compounds of interest. Improvement of processes for extraction and purification of FA will extend the development of new markets with high added-value without the drawback of the costs of cultivation.

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8. List of abbreviations

13-HpODE (9Z,11Z)-13-hydroperoxyoctadeca-9,11-dienoic acid
5R-HpEPE (R,6E,8Z,11Z,14Z,17Z)-5-hydroperoxyicosa-6,8,11,14,17-pentaenoic acid
ABTS 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid
ALA α-linolenic acid (C18:3n-3)
AMPK Adenosine monophosphate activated protein kinase
APOE Apo lipoprotein E
ARA Arachidonic acid (C20:4n-6)
Bcl-X_L B-cell lymphoma-extra large
BHT Butylated hydroxytoluene
cAMP Cyclic adenosine monophosphate
CAT Catalase
CPC Centrifugal partition chromatography
CVD Cardiovascular diseases
CTL Cytotoxic lymphocytes
DHA Docosahexaenoic acid (C22:6n-3)
DMBA 7,12-dimethylbenz[a]anthracene
DPA Docosapentaenoic acid (C22:5)
DPPH 2,2-diphenyl-1-picrylhydrazyl
DR-4 Death receptor 4
dw dry weight
EC_{50} Half maximal effective concentration
EGFR Epidermal growth factor
EPA Eicosapentaenoic acid (C20:5n-3)
Erk Extracellular signal-regulated kinases
FA Fatty acids
FAME Fatty acid methyl esters
FFA Free fatty acids
GLA γ-linolenic acid (C18:3n-6)
GMP Guanosine monophosphate
GPX Glutathione peroxidase
HDL High density lipoprotein
HIV Human immunodeficiency virus
HPLC High performance liquid chromatography
HSP Heat Shock Protein
HSV Herpes simplex virus
IC_{50} Half maximal inhibitory concentration
IMTA Integrated multi-trophic aquaculture
JNK c-Jun N-terminal kinase
LA Linoleic acid (C18:2n-6)
LC-PUFA Long-chain polyunsaturated fatty acids
LDL Low density lipoprotein
LPS Lipopolysaccharide
MAPK Mitogen-activated protein kinase
MBC Minimal Bactericidal Concentration
MFC Minimal Fungicidal Concentration
MIC Minimal Inhibitory Concentration
miR MicroRNA
MetS Metabolic syndrome
mTor Mammalian target of rapamycin
MUFA Monounsaturated fatty acids
NFE2L2 Nuclear factor erythroid 2 Like 2
NF-κB Nuclear factor κ B
NK Natural Killer
NMI Non-methylene-interrupted fatty acids
Nrf2 Nuclear factor (erythroid-derived 2)-like 2
OSGIN Oxidative stress-induced growth inhibitor
PF Polarity of the Fraction
PI3K Phosphoinositide 3-kinase
PKB Protein Kinase B
PKC Protein Kinase C
PPAR Peroxisome proliferator activated receptor
PUFA Polyunsaturated fatty acids
RIP Receptor-interacting protein
ROS Reactive oxygen species
$ScCO_2$ Supercritical carbon dioxide
SD rat Sprague Dawley rat
SFA Saturated fatty acids
SOD Superoxide dismutase

STA Stearidonic acid (C18:4n-3)
STAT Signal transducer and activator of transcription
TAG Triacylglycerols
TamR Tamoxifen-resistant
TEAC Trolox Equivalent Antioxidant Capacity
TLR Toll like receptor
TNF Tumor necrosis factor
TRAIL Tumor-necrosis-factor related apoptosis inducing ligand
UV Ultraviolet
VLDL Very low-density lipoprotein

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