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Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil

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Abstract

The purpose of this work was to evaluate the nutritional value of the total lipid extract of different omega-3 long chain polyunsaturated fatty acids producing photoautotrophic microalgae in one study. It was shown that microalgae oils from *Isochrysis*, *Nannochloropsis*, *Phaeodactylum*, *Pavlova* and *Thalassiosira* contain sufficient omega-3 LC-PUFA to serve as an alternative for fish oil, which was used as the 'golden standard'. In the microalgae oils an important part of the omega-3 long chain polyunsaturated fatty acids are present in the polar lipid fraction, which may be favourable from a bioavailability and stability viewpoint. Consumption of microalgae oil ensures intake of sterols and carotenoids. The intake of sterols, including cholesterol and phytosterols, is probably not relevant. The intake of carotenoids is however definitely significant and could give the microalgae oils a nutritional added value compared to fish oil.

Keywords

Lipids

Eicosapentaenoic acid (EPA)

Docosahexaenoic acid (DHA)

Carotenoids

Phytosterols

1. Introduction

Omega-3 polyunsaturated fatty acids (omega-3 PUFA) are a specific group of polyunsaturated fatty acids where the first double bond is located between the third and fourth carbon atom counting from the methyl end of the fatty acid. There are short chain (SC, ≤ C18) and long chain (LC, ≥ C20) omega-3 PUFA. The important health benefits are associated with omega-3 LC-PUFA and particularly with eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (Gogus and Smith, 2010). In scientific literature, intake of 250 mg EPA+DHA/day has shown to give primary prevention against cardiovascular disease (Kris-Etherton, Grieger & Etherton, 2009) and this is also the intake recommended by WHO and EU. It has, however, been shown that current global omega-3 LC-PUFA intake is insufficient (Sioen, De Henauw, Van Camp, Volatier & Leblanc, 2009).

The main commercial source of omega-3 LC-PUFA is fish. Concerns about the potential danger of contaminants such as mercury, however, often discourage people from eating fish. Another more recently recognized and serious issue is the global decline in wild-harvest fish stocks. If omega-3 LC-PUFA are to be used in new applications (e.g. feed additives in aquaculture, incorporation into margarines), marine fish are at risk of becoming scarce due to intensive fishing. Thus, new sources of omega-3 LC-PUFA must be found to answer the growing demand for the omega-3 LC-PUFA market.

Alternative sources of omega-3 LC-PUFA are microalgae, krill, calamari or genetically modified crops. Microalgae might be the most promising alternative, since they are the primary producers of EPA and DHA. They can be cultured either photoautotrophically or heterotrophically, with each system having its advantages and disadvantages (Ryckebosch, Bruneel, Muylaert & Foubert, 2012). Photoautotrophic omega-3 LC-PUFA producing microalgae are mainly marine planktonic species, belonging to different phyla. Literature shows that the most promising species belong to the *Bacillariophyta* (e.g. *Chaetoceros*, *Phaeodactylum*, *Skeletonema*, *Thalassiosira*), *Chlorophyta* (e.g. *Tetraselmis*), *Cryptophyta* (e.g. *Cryptomonas*, *Rhodomonas*), *Haptophyta* (e.g. *Isochrysis*, *Pavlova*), *Heterokontophyta* (e.g. *Nannochloropsis*) and *Rhodophyta* (e.g. *Porphyridium*) (e.g. Ryckebosch et al., 2012a). The data currently available in literature are, however, fragmentary and comparison of different species is difficult due to the use of different extraction methods in different articles. Furthermore, only a few articles can be used to calculate the amount of omega-3 LC-PUFA to mg/g oil since the amounts of lipid, fatty acid methyl esters (FAMEs) and omega-3 LC-PUFA have to be provided.

The purpose of this work was, therefore, to evaluate the nutritional value of the total lipid extract of different omega-3 LC-PUFA producing photoautotrophic microalgae in one study. It was determined whether microalgae oils contain sufficient omega-3 LC-PUFA to serve as an alternative for fish oil, which was used as the 'golden standard'. Additionally, we investigated if microalgae oils are a source of other nutritionally interesting components, such as carotenoids, phytosterols and antioxidants, which could give the microalgae oils a nutritional added value compared to fish oil.

2. Material and methods

2.1. Microalgae biomass and fish oil

Biomass of omega-3 LC-PUFA producing photoautotrophic microalgae was obtained from European companies: *Isochrysis T-iso*, *Phaeodactylum tricornutum*, *Rhodomonas salina, Tetraselmis suecica* and *Thalassiosira pseudonana* from SBAE (Sleidinge, Belgium), *Nannochloropsis gaditana* from LGem (Voorhout, The Netherlands), *Nannochloropsis oculata* from Proviron (Hemiksem, Belgium) and *Porphyridium cruentum* from Necton (Olhão, Portugal). Biomass of *Pavlova lutheri* was produced in-house. The inoculum of this species was obtained from Proviron (Hemiksem, Belgium). The algae were produced under controlled conditions in 130 L pilot-scale plexiglass tubular airlift photobioreactors. They were cultured in the '*Pavlova* – medium' optimized by the company which provided the inoculum. The medium was filter sterilized before addition to the photobioreactors (0.2 µm PTFE filters). The reactors were illuminated continuously (125 µmol photons m^{-2} s^{-1}, Philips Cool White fluorescent tubes) and the medium was mixed with filter sterilized air (flow rate: 25 L min^{-1}). The culture was maintained at pH 7.6 by automated addition of CO_2 to the stream of air. The biomass was harvested at the end of the logarithmic phase by centrifugation and the wet biomass was immediately freeze-dried. All biomass were stored at -80°C until extraction.

Fresh 18/12 (i.e. containing a ratio of EPA/DHA of 18/12) refined fish oil made from anchovy, mackerel and sardines was obtained from Bioriginal (Den Bommel, The Netherlands). The oil was stored at -20°C until characterization.

2.2. Total lipid extraction

Total lipids were extracted from the microalgae according to the method previously optimized by Ryckebosch, Muylaert & Foubert (2012). The advantage of this method is the complete oil extract is obtained from the microalgae. In summary, each biomass was extracted four times with chloroform/methanol (1:1): twice with and twice without the addition of water. The oil from all the four extractions was combined. Each sample was extracted six times.

2.3. Analysis of lipid class content

The lipid class content (neutral lipid (NL), glycolipid (GL) and phospholipid (PhL) content) of the nine microalgal total lipid extracts and the fish oil sample was obtained by fractionation using silica solid phase extraction (SPE) followed by gravimetric quantification according to the method previously described (Ryckebosch et al., 2012b). Each sample was analyzed three times.

2.4. Analysis of fatty acid content and composition

To determine fatty acid (FA) composition, the total lipid extracts, different lipid class samples and fish oil sample were methylated according to Ryckebosch et al. (2012b). The FAMEs obtained were separated by gas chromatography with cold on-column injection and flame ionization detection (FID) (Trace GC Ultra, Thermo Scientific, Interscience, Louvain-la-Neuve, Belgium). An EC Wax column of length 30 m, ID 0.32 mm, film 0.25

µm (GRACE, Lokeren, Belgium) was used with the following time-temperature program: 70°C – 180°C (5°C/min), 180°C – 235°C (2°C/min), 235°C (9.5 min). Peak areas were quantified with Chromcard for Windows software (Interscience, Louvain-la-Neuve, Belgium). FAME standards (Nu-check, Elysian, USA) containing a total of 35 different FAMEs were analyzed for provisional peak identification, which was then confirmed by GC-MS (Trace GC Ultra, ISQ Single Quadrupole MS, Thermo Scientific, Interscience, Louvain-la-Neuve, Belgium) using an Rxi-5 Sil MS column of length 20 m, ID 0.18 mm, film 0.18 µm (Restek, Interscience, Louvain-la-Neuve, Belgium). For quantification in mg FA/g oil, an internal standard of fatty acid (C20:0, C19:0 or C20:1, depending on the species) was added to the oil before methylation. A conversion factor based on the difference in molecular weight between the FA and the FAME (0.952 for α-linolenic acid (C18:3ω-3) (ALA); 0.951 for stearidonic acid (C18:4ω-3) (SDA); 0.956 for EPA; 0.960 for docosapentaenoic acid (C22:5ω-3) (DPA); 0.959 for DHA) was used to calculate the amount of FA from the amount of FAME. Each sample was analyzed three times.

2.5. Analysis of carotenoid content and composition

For the determination of the carotenoid content and composition, each total lipid extract and fish oil (2 mg) was dissolved in methanol (10 ml). This solution and a 1/10 dilution were analyzed by high performance liquid chromatography (HPLC) coupled to a photodiode array detector (PAD) (Alliance, Waters, Zellik, Belgium) according to Wright et al. (1991). To express the carotenoids as mg/g oil, calibration curves were created for each carotenoid. Alloxanthin, diadinoxanthin, diatoxanthin, lutein, neoxanthin, violaxanthin and zeaxanthin were purchased from DHI (Hørsholm, Denmark). β-carotene was purchased from Sigma-Aldrich (Bornem, Belgium). When the area of a carotenoid exceeded the calibration curve, the 1/10 dilution was used. Each sample was analyzed three times.

2.6. Analysis of cholesterol and phytosterol content

For the determination of the sterol content, 5β-cholestan-3α-ol (200 µg; Sigma-Aldrich, Bornem, Belgium) was first added to the total lipid extracts or the fish oil sample, and then saponification was performed according to Abidi (2004), with some modifications. Briefly, 10-20 mg of the oil was stirred overnight with potassium hydroxide (1 mol / l) in ethanol (4 mL). Water (4 mL) was added and the reaction mixture extracted with diethylether (8 mL * 3). The ether extracts were combined and the solvent was removed using a rotary evaporator, giving the non-saponifiable fraction. Finally, the sterol components were silylated according to Toivo, Lampi, Aalto & Piironen (2000). For this, anhydrous pyridine (200 µL) and derivatization reagent (200 µL) containing 99% N,O-bis(trimethylsilyl)trifluoro acetamide (BSTFA) and 1% trimethylchlorosilane (TMCS) were added to the non-saponifiable fraction. To complete the silylation, solutions were incubated at 60°C for 1 hour. Before GC-analysis, the solution was diluted with 600 µL hexane. The silylated sterols were separated by GC-FID with cold on-column injection. An Rtx-5 column (length 30 m, ID 0.25 mm, film 0.25 µm) (Restek, Interscience, Louvain-la-Neuve, Belgium) was used with the following time-temperature program: 200-340°C at 15°C/min, 340°C (10 min). Peak

areas were quantified with Chromcard for Windows software (Interscience, Louvain-la-Neuve, Belgium). Peak identification was confirmed by GC-MS (Trace GC Ultra, ISQ Single Quadrupole MS, Thermo Scientific, Interscience, Louvain-la-Neuve, Belgium) using an Rxi-5 Sil MS column of length 20 m, ID 0.18 mm, film 0.18 µm (Restek, Interscience, Louvain-la-Neuve, Belgium). Cholesterol was identified separately. Values for the other peaks showing a sterol backbone were added together to give the total phytosterol content. Each sample was analyzed three times.

2.7. Analysis of antioxidant capacity

To analyze the antioxidant capacity, the Trolox equivalent antioxidant capacity (TEAC) assay was performed according to Li, Cheng, Wong, Fan, Chen & Jiang (2007), with slight modifications. This assay is based on the reaction of the antioxidant with the colored 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) radical with decoloration as a result. Briefly, the $ABTS^{\bullet+}$ stock solution was made by dissolving ABTS (7 mmol / l) and $K_2S_2O_8$ (2.45 mmol / l) in 5 mL MeOH. The $ABTS^{\bullet+}$ working solution was prepared by diluting the stock solution (3-4 times) with methanol to obtain an absorbance (734 nm) of 0.70 ± 0.05. The total lipid extracts and the fish oil sample were dissolved at 5-10 mg/mL in chloroform/methanol 1:1. 50 µL of this solution was mixed with 1.9 mL $ABTS^{\bullet+}$ working solution, incubated at room temperature for 10 minutes and the absorbance was measured at 734 nm. For quantification, a calibration curve was made with Trolox (1, 5, 10 and 15 µmol / l). Each sample was analyzed three times.

3. Results and discussion

3.1. Total lipid content of microalgae

The total lipid content of the microalgae (Figure 1) was species dependent and ranged from 8 to 30 % of dry weight. High lipid containing microalgae species were *Nannochloropsis gaditana*, *Nannochloropsis oculata* and *Pavlova*. Low lipid containing microalgae species were *Porphyridium* and *Rhodomonas*. These results are within the range found in literature and summarized by Ryckebosch et al. (2012a). It should be stressed these results were from one batch of each species. Total lipid content of microalgae is, however, depending on culture conditions and growth phase at the time of harvest (Ryckebosch et al., 2012a). Nevertheless, we have confirmed that microalgae previously shown to have high lipid contents, also had a high lipid content in this study.

3.2. Omega-3 fatty acid content

In the total lipid extracts, the omega-3 PUFA ALA, SDA, EPA, DPA and DHA were present at 0.3 – 92 mg/g oil; 0 – 43 mg/g oil; 3 – 193 mg/g oil; 0 – 2 mg/g oil and 0 –46 mg/g oil, respectively (Table 1). Total lipid extracts with high EPA content were obtained from *Nannochloropsis gaditana*, *Nannochloropsis oculata* and *Phaeodactylum*. Total lipids with high DHA content were extracted from *Isochryris* and *Pavlova*. Total lipids with high SDA content were found in *Isochrysis* and *Rhodomonas*. *Pavlova* is the only microalga that gave a total lipid extract rich in DHA that also contained a substantial amount of EPA. To our knowledge, there are no articles describing the amount of n-3 PUFA in mg/g oil, so recalculation was necessary for comparison. For most microalgae, this study gave results in the same range as those recalculated from the literature summarized in Ryckebosch et al., 2012a). The EPA content of the 'golden standard' fish oil was comparable with high EPA-containing total lipid extracts from the microalgae, but the fish oil DHA content was more than twice that measured in high DHA-containing total lipid extracts from microalgae. Fish oil contained less SDA than the high-SDA microalgae extracts, but more than all other microalgal total lipid extracts. It must be noted that crude microalgal total lipid extracts were compared with highly refined fish oil. It should, thus, be taken into account that 'food-grade' commercial extraction, potentially followed by concentration and refining, could alter the final EPA and DHA content in these extracts.

The necessary intake of microalgal oil/day to reach the recommended intake of 250 mg EPA+DHA/day was calculated. However, since similar beneficial health effects were suggested for DPA as for EPA and DHA (Kaur, Cameron-Smith, Garg & Sinclair, 2011), the amount of DPA was also taken into account. Furthermore, the intake of SDA was also included, since it was shown that intake of SDA also causes a raise in the amount of EPA in red blood cells. The conversion of SDA to EPA ranges from 17 to 41 % (Krul et al. 2012). Therefore, a conversion factor of 1/6 (17 %) was taken into account. Thus, the required daily consumption of the studied microalgae total lipid extracts to reach an intake of 250 mg EPA + DPA + DHA + 1/6 * SDA (shortly, 250 mg omega-3 LC-PUFA) was calculated and is reported in Table 2. For fish oil 0.8 g must be consumed daily to achieve this intake. When

the microalgal total lipid extracts were to be used as such, the daily intake would range from 1.3 up to 12.5 g oil/day. Some microalgae, including both *Nannochloropsis* species, *Pavlova*, *Phaeodactylum* and *Thalassiosira* (intake < 2.5 g oil/day) thus definitely show potential as an alternative for fish oil since the amount to be consumed is feasible (about half a teaspoon/day). Other microalgae, including *Porphyridium*, *Rhodomonas* and *Tetraselmis,* probably deliver an oil that is too low in interesting omega-3 LC-PUFA to be potentially interesting for this purpose. Therefore, these three microalgal total lipid extracts were no longer taken into account in remainder of the nutritional evaluation. When *Isochrysis* was to be used, a relative high daily intake would also be required, but because of the interesting presence of DHA, this microalgae was not excluded.

3.3. Lipid class content

The lipid classes in the microalgae extracts are shown in Figure 2. NL varied the most while the percentage of GL and PhL (21 – 42 % of oil) varied less. In the literature, the same range of lipid classes was found (e.g. Alonso, 1998). The total lipid extract of *Isochrysis* was the only one to contain high levels of NL compared with polar lipids (GL + PhL), which is in contrast to the literature (Alonso, 1998). However, lipid class content also depends on growth phase at the time of harvest and culture conditions (e.g. Fidalgo, 1998). The commercially available fish oil underwent degumming, which removes GL and PhL (Rubio-Rodriguez, Beltran, Jaime, de Diego, Sanz & Carballido, 2010), leaving only the triacylglycerols (TAG) (NL fraction) in the final oil as observed.

3.4. Omega-3 LC-PUFA content in the lipid classes

The omega-3 LC-PUFA content in the different lipid classes (Table 3) is expressed as the ratio of the specific omega-3 LC-PUFA (EPA or DHA) against the sum of all FAMEs in this lipid class. EPA was highest in the GL class for *Isochrysis*, *Nannochloropsis*, *Pavlova* and *Thalassiosira*, and in the NL class for *Phaeodactylum*, *Rhodomonas*, *Porphyridium* and *Tetraselmis*. DHA was highest in the NL class for *Isochrysis* and *Rhodomonas*, and in the PhL class for *Phaeodactylum* and *Thalassiosira*. Thus, it seems EPA and DHA are not incorporated in a specific lipid class, although, preferential incorporation in polar lipid classes was expected, since EPA and DHA are structurally important fatty acids giving fluidity to cell membranes (Valentine & Valentine, 2004). As fish oil only contains NL, all of the omega-3 LC-PUFA were present in the NL class. Results found in literature were mostly in contrast to our results. Where we found the omega-3 LC-PUFA to be more abundant in the NL class, the literature showed these were more abundant in one of the polar classes (Cartens, Molina Grima, Robles Medina, Giménez Giménez & Ibañez González, 1996; Giménez Giménez, Robles Medina, Molina Grima, García Salas & Esteban Cerdán, 1998; Mendoza Guzmán, Jara Valido, Carmona Duarte & Freijanes Presmanes, 2010a & 2010b; Alonso, 1998; Molina Grima, Robles Medina, Giménez Giménez, Sánchez Pérez, Garcia Camacho & García Sánchez, 1994). Even in our previous results for *Phaeodactylum tricornutum,* it was shown that EPA content was highest in the GL class, followed by the PhL and NL class (Ryckebosch et al., 2012b). One possible

explanation is offered by Tonon, Harvey, Larson & Graham (2002) who showed EPA content in TAG (NL class) was higher following a longer incubation period. Another explanation can be found in the possible presence of free fatty acids, which are part of the NL class. Previously, it has been shown that microalgae contain substantial amounts of free fatty acids, formed during storage of the biomass (Ryckebosch, Muylaert, Eeckhout, Ruyssen & Foubert, 2011).

From the above, it is clear that in the microalgae oils an important part of the omega-3 LC-PUFA were present in the GL and PhL while in fish oil, they were only present in the NL. It has been suggested that ingesting EPA and DHA from PhL instead of TAG allows the body to absorb them more efficiently and easily (Schuchardt, Schneider, Meyer, Neubronner, von Schacky & Hahn, 2011). This way the metabolic effects of microalgae could be similar to those of fish oil but at a lower dose of EPA and DHA, as was shown for krill oil (Ulven et al., 2011). Furthermore, omega-3 LC-PUFA incorporated into PhL may offer them more protection against oxidation, as was shown for DHA (Lyberg, Fasoli & Adlercreutz, 2005). No information could be found whether this increased bioavailability and oxidative stability is also valid when the omega-3 LC-PUFA are present in the form of GL.

3.5. Sterol content

All microalgae total lipid extracts contained phytosterols (Table 1). *Pavlova lutheri* clearly was the microalga with the highest amount of phytosterols. As expected, fish oil did not contain any phytosterols, but only cholesterol. Also the oil extracted from *Nannochloropsis* (*gaditana* and *oculata*) contained cholesterol, 3 to 5 times the amount measured in commercial fish oil. When compared to results available in literature, the same range of sterol contents was found. And, in accordance to our results, *Pavlova* was also the highest phytosterol containing microalga (e.g. Volkman, Jeffrey, Nichols, Rogers & Garland, 1989). The sterol content of microalgae can again be influenced by growth conditions and growth phase upon harvest, as was shown e.g. by Durmaz et al. (2008).

To make a nutritional evaluation, the intake of sterols when the required amount of oil is used to reach the daily consumption of 250 mg omega-3 LC-PUFA was calculated and is reported in Table 2. Intake of cholesterol due to consumption of fish oil is 6 to 7 times lower than due to *N. gaditana* and *N. oculata* respectively. Importantly, intake from both sources is much lower than the maximum intake of 300 mg cholesterol / day for healthy populations recommended by USDA (2005) and AHA (2006). Intake of phytosterols varies between 8 and 180 mg/day. Phytosterols are generally consumed in an amount of 200-400 mg/day in Western diets, while the recommendation of daily plant sterol intake published by various organizations over the last years ranges from 1 up to 25 g plant sterols and stanols/day (Ose, 2006). 1.6 to 2 gram of plant sterols or stanols has shown to be necessary to reduce serum cholesterol by 8-10 % (Marangoni & Poli, 2010). The maximum intake of phytosterols due to consumption of microalgae oil for omega-3 LC-PUFA is only 10 % of the necessary daily intake. Therefore, it is difficult to state that phytosterols are a nutritional added value of these microalgal oils. Nevertheless, it is clear that some microalgae, and especially *Pavlova*, are a good source of phytosterols, independently of the omega-3 LC-PUFA.

3.6. Carotenoid composition

All microalgae total lipid extracts contained carotenoids, while no carotenoids were found in the fish oil (Table 1). The amount of carotene gives the sum of all possible isomers of carotene (mainly alpha and beta), since they could not be chromatographically separated. It was the only carotenoid present in all microalgae total lipid extracts. All other carotenoids were only present in certain microalgae total lipid extracts. This was expected as the carotenoid composition is specific for an algae division/class. The major carotenoids found in this study were moreover in accordance to the taxonomically significant pigments in the algal divisions/classes (Jeffrey and Vesk, 2005). For quantitative comparison with the literature, the amount of carotenoids was recalculated from mg/g oil to mg/100 g DW. A wide range of results was found in the literature (Durmaz et al., 2008; Carvalho, Monteiro & Malcata, 2009; Kim et al., 2012; Rebolloso Fuentes, Acién Fernández, Sánchez Pérez & Guil Guerrero, 2000): some values are higher, some lower and some comparable to the results found in this study. As for other components, the amount of carotenoids is dependent on culture conditions and growth phase when harvested (e.g. Durmaz et al., 2008), which may explain the variation in results.

To make a nutritional evaluation, the intake of the different carotenoids when the required amount of oil is used to reach the daily consumption of 250 mg omega-3 LC-PUFA was calculated and is reported in Table 2. Only the carotenoids which have proven additional beneficial properties, other than their antioxidant capacity are reported and the carotenoids behaving similar in terms of human metabolism and tissue storage (Johnson, 2002) are summed. From the table, it is clear that consumption of microalgae oils in general also ensures intake of carotenoids. As carotenoids are known to be antioxidants, they may guard the oil against lipid oxidation (Paiva & Russell, 1999) and addition of extra antioxidants might thus not be required. Furthermore, carotenoids have shown at least a trend to lower oxidative stress (Butalla, Crane, Patil, Wertheim, Thompson & Tomson, 2012; Martínez-Tomás et al., 2012), which makes that the induced oxidative stress due to the intake of PUFA can potentially be lowered. Moreover, intake of the 'potentially interesting microalgae oils' would provide an intake in the range of 4 to 11 mg carotene/day which is in the same range up to 3 times the recommended daily intake (ERNA, 2011) meaning the microalgae oils can give a substantial contribution to the presence of carotene in the human body. Consumption of oil of both species of *Nannochloropsis* could also raise intake levels of lutein and / or zeaxanthin to the value of 6 mg, recommended by ERNA (2011) for protection against eye diseases and skin conditions. Microalgae oils of specific species can also provide high amounts of fucoxanthin, alloxanthin and/or diatoxanthin. For fucoxanthin for example, the intake is ten- to twentyfold the amount that shows anti-obesity properties (Miyashita, Maeda, Tsukui, Okada & Hosokawa, 2009).

3.7. Antioxidant capacity

The antioxidant capacity of the microalgae total lipid extracts ranged from 37 to 93 µmol Trolox eq./g oil (Figure 3). The four oils with the highest antioxidant capacity were obtained from both *Nannochloropsis* species, *Rhodomonas* and *Tetraselmis*. Our results could be compared with the results of Goiris, Muylaert, Fraeye,

Foubert, Brabanter & De Cooman (2012), although the extraction protocol was different and a recalculation to µmol Trolox eq./g DW was needed. Some of the values, e.g. for both *Nannochloropsis* species, *Phaeodactylum* and *Porphyridium,* were in the same range as found by Goiris et al. (2012). For other microalgae, e.g. *Tetraselmis*, the results were completely different. A possible explanation is that the total antioxidant capacity of the microalgal biomass as measured by Goiris et al. (2012) is not only determined by lipid soluble molecules like carotenoids, but also by hydrophilic molecules like polyphenols, which may occur at lower concentrations in a lipid extract. The antioxidant capacity of the microalgae total lipid extracts was three to four times higher than the antioxidant capacity of fish oil. The origin of the antioxidant capacity of fish oil is the addition of antioxidants, such as vitamin E. The higher antioxidant capacity of the microalgae oils may mean that the omega-3 LC-PUFA in microalgal oils will be better protected against oxidation than in fish oil.

3.8 Global nutritional evaluation

Microalgal total lipid extracts contain a significant amount of omega-3 LC-PUFA. Therefore, some microalgae, including *Isochrysis* (for DHA), *Nannochloropsis gaditana*, *Nannochloropsis oculata* and *Phaeodactylum (for EPA), Pavlova* and *Thalassiosira* (for EPA and DHA) definitely show potential as an alternative for fish oil since the amount to be consumed is feasible. In the microalgae oils an important part of the omega-3 LC-PUFA are present in the GL and PhL. This can be interesting, since PhL may be absorbed more efficiently and omega-3 LC-PUFA associated with PhL may be better protected against oxidation. The same properties might be valid for GL, but more research is to be performed on this subject. Furthermore, consumption of microalgae oil ensures intake of sterols and carotenoids. The intake of sterols, including cholesterol and phytosterols, is probably not relevant, while the intake of carotenoids is definitely significant.

It should be mentioned that additional research must be performed on extraction with food grade solvents, since they can give more specific extraction of omega-3 LC-PUFA, sterols and/or carotenoids. But the aim of this study was to unravel the full potential of the microalgal oils, so that future extraction work can be directed in concordance with the results of this study.

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Figure Captions

Figure 1 – Total lipid content (in % of DW; mean ± SD; n = 6) of following microalgae species: 1 *Isochrysis T-iso*, 2 *Nannochloropsis gaditana*, 3 *Nannochloropsis oculata* , 4 *Pavlova lutheri*, 5 *Phaeodactylum tricornutum*, 6 *Porphyridium cruentum*, 7 *Rhodomonas salina*, 8 *Tetraselmis suecica*, 9 *Thalassiosira pseudonana*.

Figure 2 – Lipid class composition (in % of oil; mean ± SD; n = 3) of the total lipid extract of following microalgae species and fish oil: 1 *Isochrysis T-iso*, 2 *Nannochloropsis gaditana*, 3 *Nannochloropsis oculata* , 4 *Pavlova lutheri*, 5 *Phaeodactylum tricornutum*, 6 *Porphyridium cruentum*, 7 *Rhodomonas salina*, 8 *Tetraselmis suecica*, 9 *Thalassiosira pseudonana*, 10 Fish oil.

Figure 3 – Antioxidant capacity (in µmol Trolox eq./g oil; mean ± SD; n = 3) of the total lipid extract of following microalgae species and fish oil: 1 *Isochrysis T-iso*, 2 *Nannochloropsis gaditana*, 3 *Nannochloropsis oculata* , 4 *Pavlova lutheri*, 5 *Phaeodactylum tricornutum*, 6 *Porphyridium cruentum*, 7 *Rhodomonas salina*, 8 *Tetraselmis suecica*, 9 *Thalassiosira pseudonana*, 10 Fish oil.

Tables

Table 1 – Omega-3 fatty acid content (in mg/g oil; mean ± SD; n = 3), sterol content (in mg/g oil; mean ± SD; n=3) and carotenoid content (in mg/g oil; mean ± SD; n=3) of the total lipid extract from the microalgae species and of fish oil.

	Isochrysis T-iso	***Nannochloropsis gaditana***	***Nannochloropsis oculata***	***Pavlova lutheri***	***Phaeodactylum tricornutum***	***Porphyridium cruentum***	***Rhodomonas salina***	***Tetraselmis suecica***	***Thalassiosira pseudonana***	**Fish oil**
n-3 fatty acids										
ALA (C18:3n-3)	29 ± 4	0.3 ± 0.03	0.7 ± 0.1	10.0 ± 0.3	0.8 ± 0.1	1.42 ± 0.01	92 ± 5	68 ± 4	1.9 ± 0.1	7.7 ± 0.2
SDA (C18:4n-3)	43 ± 10	0.3 ± 0.1	-	17.0 ± 0.5	1.9 ± 0.1	-	43 ± 2	17.0 ± 0.5	20.4 ± 0.8	29 ± 1
EPA (C20:5n-3)	2.8 ± 0.7	175 ± 12	193 ± 24	92 ± 2	111 ± 5	35.6 ± 0.3	18 ± 1	16.3 ± 0.5	81 ± 2	184 ± 5
DPA (C22:5n-3)	-	-	-	-	1.08 ± 0.01	-	-	-	1.82 ± 0.01	16.8 ± 0.3
DHA (C22:6n-3)	46 ± 14	-	-	40.9 ± 0.9	8.3 ± 0.5	-	11.1 ± 0.8	0.8 ± 0.1	20.9 ± 0.8	105.2 ± 0.7
Saturated fatty acids	0.10 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.101 ± 0.004	0.08 ± 0.01	0.205 ± 0.001	0.059 ± 0.004	0.11 ± 0.01	0.127 ± 0.003	0.26 ± 0.01
n-6/n-3 ratio	0.273 ± 0.001	0.231 ± 0.001	0.208 ± 0.001	0.085 ± 0.003	0.063 ± 0.004	2.0 ± 0.1	0.0531 ± 0.0003	0.33 ± 0.01	0[$]	0.071 ± 0.001
Sterols										
Cholesterol	-	13.8 ± 0,6	20 ± 1	-	-	-	-	-	-	4.4 ± 0.1
Phytosterols	14.9 ± 0.1	17 ± 1	6.1 ± 0.3	97 ± 3	16.5 ± 0.6	26.5 ± 0.2	26 ± 3	10.9 ± 0.2	34 ± 3	-
Carotenoids										
Alloxanthin	-	-	-	-	-	-	6.8 ± 0.9	-	-	-
Carotene	2.4 ± 0.3	3.5 ± 0.03	3.6 ± 0.3	2.9 ± 0.1	2.0 ± 0.4	0.4 ± 0.1	5.7 ± 0.8	8.6 ± 1.9	4.3 ± 0.4	-
Diadinochrome[£]	2.1 ± 0.5	-	-	-	-	-	-	-	-	-
Diadinoxanthin	-	2.9 ± 0.1	1.6 ± 0.04	3.4 ± 0.2	3.7 ± 0.5	-	-	-	-	-
Diatoxanthin	1.5 ± 0.2	-	-	8.6 ± 0.6	1.1 ± 0.2	-	-	-	-	-
Fucoxanthin	19.2 ± 2.8	-	-	14.8 ± 0.7	38.3 ± 4.8	-	-	-	31.9 ± 3.4	-
Lutein	-	-	-	-	-	0.5 ± 0.2	8.7 ± 1.1	5.6 ± 0.6	-	-

Neoxanthin	-	-	-	-	-	-	-	5.6 ± 0.5	-	-
Violaxanthin	-	14.3 ± 0.3	11.5 ± 0.7	-	-	-	2.2 ± 0.3	7.8 ± 1.3	-	-
Zeaxanthin	-	3.4 ± 0.07	1.24 ± 0.1	-	-	3.2 ± 0.8	-	2.0 ± 0.2	-	-

[£] The diadinochrome content was estimated using the calibration curve of diadinoxanthin. [$] Thalassiosira contains no n-6 C_{18}-C_{20} fatty acids.

Table 2 – Required consumption of microalgae oil to reach an intake of 250 mg omega-3 LC-PUFA per day. The sterol intake, saturated fatty acid intake and carotenoid intake (in mg/day; mean ± SD, n=3) due to this consumption. [$] Thalassiosira contains no n-6 C_{18}-C_{20} fatty acids.

	Isochrysis T-iso	***Nannochloropsis gaditana***	***Nannochloropsis oculata***	***Pavlova lutheri***	***Phaeodactylum tricornutum***	***Porphyridium cruentum***	***Rhodomonas salina***	***Tetraselmis suecica***	***Thalassiosira pseudonana***	**Fish oil**
Intake (g oil/day)	5 ± 1	1.4 ± 0.1	1.3 ± 0.2	1.84 ± 0.03	2.1 ± 0.1	7.0 ± 0.1	6.9 ± 0.5	12.5 ± 0.4	2.34 ± 0.04	0.80 ± 0.01
Saturated Fatty Acids	0.5 ± 0.1	0.17 ± 0.02	0.12 ± 0.02	0.19 ± 0.01	0.16 ± 0.01	1.44 ± 0.01	0.41 ± 0.04	1.3 ± 0.1	0.30 ± 0.01	0.21 ± 0.01
n-6/n-3 ratio	0.273 ± 0.001	0.231 ± 0.001	0.208 ± 0.001	0.085 ± 0.003	0.063 ± 0.004	2.0 ± 0.1	0.0531 ± 0.0003	0.33 ± 0.01	0[$]	0.071 ± 0.001
Sterols										
Cholesterol	-	20 ± 2	25 ± 4	-	-	-	-	-	-	3.5 ± 0.1
Phytosterols	67 ± 20	24 ± 3	8 ± 1	178 ± 6	34 ± 2	186 ± 2	178 ± 26	136 ± 5	80 ± 8	-
Carotenoids										
Carotene	11 ± 4	5.0 ± 0.3	4.7 ± 0.7	5.4 ± 0.2	4.1 ± 0.8	2.7 ± 0.7	39 ± 6	108 ± 24	10 ± 1	-
Lutein + Zeaxanthin	-	4.8 ± 0.4	1.6 ± 0.2	-	-	26 ± 6	60 ± 9	95 ± 9	-	-
Fucoxanthin + Neoxanthin	55 ± 9	-	-	17 ± 2	51 ± 8	-	-	70 ± 9	47 ± 6	-
Alloxanthin +	7 ± 1	-	-	16 ± 2	2.3 ± 0.4	-	47 ± 7	-	-	-

Diatoxanthin

Table 3 – Omega-3 LC-PUFA content in lipid classes: neutral lipids (NL), glycolipids (GL) and phospholipids (PhL) (in % of FAME of lipid class; mean ± SD; n = 3).

	Isochrysis T-iso	***Nannochloropsis gaditana***	***Nannochloropsis oculata***	***Pavlova lutheri***	***Phaeodactylum tricornutum***	***Porphyridium cruentum***	***Rhodomonas salina***	***Tetraselmis suecica***	***Thalassiosira pseudonana***
EPA (C20:5n-3)									
NL	0.3 ± 0.04	12.6 ± 0.2	19.6 ± 0.7	16.2 ± 0.2	31.7 ± 0.4	9.9 ± 0.3	8.1 ± 0.5	2.9 ± 0.2	14.3 ± 0.2
GL	0.9 ± 0.1	46.5 ± 2.0	68.3 ± 0.9	31.8 ± 1.8	27.2 ± 0.7	7.2 ± 0.4	1.6 ± 0.2	1.0 ± 0.1	16.0 ± 0.6
PhL	0.7 ± 0.00	21.5 ± 0.2	32.0 ± 0.6	10.0 ± 2.2	12.1 ± 1.3	1.4 ± 0.2	3.1 ± 0.2	2.5 ± 0.3	13.3 ± 2.2
DHA (C22:6n-3)									
NL	11.2 ± 1.5	-	-	6.7 ± 0.1-	1.7 ± 0.1	-	3.1 ± 0.3	-	3.1 ± 0.3
GL	5.5 ± 0.1	-	-	0.4 ± 0.1	0.4 ± 0.00	-	0.6 ± 0.1	-	0.5 ± 0.06
PhL	2.9 ± 0.4	-	-	13.5 ± 3.9	3.9 ± 0.2	-	1.9 ± 0.1	-	3.5 ± 1.2