JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Stability of Omega-3 LC-PUFA-rich Photoautotrophic Microalgal Oils Compared to Commercially Available Omega-3 LC-PUFA Oils

Eline Ryckebosch,[†,‡] Charlotte Bruneel,[†,‡] Romina Termote-Verhalle,[†,‡] Charlotte Lemahieu,[†,‡] Koenraad Muylaert,[§] Jim Van Durme,[#] Koen Goiris,[⊥] and Imogen Foubert*[,†,‡]

[†]KU Leuven Kulak, Research Unit Food and Lipids, Department of Molecular and Microbial Systems Kulak, , Etienne Sabbelaan 53, 8500 Kortrijk, Belgium

[‡]Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Kasteelpark Arenberg 20, 3001 Heverlee, Belgium

[§]KU Leuven Kulak, Laboratory of Aquatic Biology, Biology Department Kulak, Etienne Sabbelaan 53, 8500 Kortrijk, Belgium

[#]KaHo St.-Lieven, Association KU Leuven, Department of Microbial and Molecular Systems, Research cluster Food and Biotechnology, Research Group for Molecular Odour Chemistry, Technology Campus, Gebroeders De Smetstraat 1, 9000 Gent, Belgium

[⊥]Laboratory of Enzyme, Fermentation and Brewing Technology (EFBT), KU Leuven Faculty of Engineering Technology, Department of Microbial and Molecular Systems, Cluster for Bioengineering Technology (CBeT), Gebroeders De Smetstraat 1, 9000 Gent, Belgium

ABSTRACT: Microalgae are the primary producers of omega-3 LC-PUFA, which are known for their health benefits. Their oil may thus be a potential alternative for fish oil. However, oxidative and hydrolytic stability of omega-3 LC-PUFA oils are important parameters. The purpose of this work was therefore to evaluate these parameters in oils from photoautotrophic microalgae (*Isochrysis*, *Phaeodactylum*, *Nannochloropsis gaditana*, and *Nannochloropsis* sp.) obtained with hexane/isopropanol (HI) and hexane (H) and compare them with commercial omega-3 LC-PUFA oils. When the results of both the primary and secondary oxidation parameters were put together, it was clear that fish, tuna, and heterotrophic microalgae oil are the least oxidatively stable oils, whereas krill oil and the microalgae oils performed better. The microalgal HI oils were shown to be more oxidatively stable than the microalgal H oils. The hydrolytic stability was shown not to be a problem during the storage of any of the oils.

KEYWORDS: *eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), peroxide value, volatile oxidation products, free fatty acids, carotenoids, oxidation, hydrolysis*

INTRODUCTION

The omega-3 long-chain polyunsaturated fatty acids (omega-3 LC-PUFA), especially eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), were shown to provide significant health benefits when consumed regularly.[1] However, the recommended daily intake is in most countries of the world not reached,[2] mainly due to the low consumption of the main commercial source, fish, of which it is also known that stocks are decreasing.[3] Photoautotrophic microalgae are the primary producers of EPA and DHA and are thus suggested as the most promising alternative sources. Omega-3 LC-PUFA intake from this new source may be achieved by extracting the oil from the microalgal biomass and by subsequently adding it to different foodstuffs or using it as a supplement. Ryckebosch et al.[4] showed that food grade lipid extracts of different microalgae, such as *Isochrysis* (for DHA), *Nannochloropsis*, and *Phaeodactylum* (for EPA), contain a significant amount of omega-3 LC-PUFA and indeed have potential as an alternative to fish oil on the basis of their chemical composition.

However, not only is the composition of the oil important, but the obtained microalgal oils should also be stable during storage. After all, it is known that omega-3 LC-PUFA are easily oxidized, leading to off-flavors, a decrease of the nutritional value, and even formation of potentially toxic compounds.[5] Different studies showed that oxidation of fish oil occurs reasonably quickly, but decreases when the oil is stored at lower temperature.[6−8] Krill oil is, because of its high antioxidant capacity,[9] always referred to as an oxidatively stable omega-3 LC-PUFA oil, showing no oxidation during 30 days of storage at room temperature.[10] In photoautotrophically cultivated microalgae, a wide range of compounds, including carotenoids, phenolic compounds, tocopherols, and polysaccharides, that potentially work as antioxidants have been identified.[11−14] Co-extraction of (some of) these compounds may be why microalgal total lipid extracts showed a higher antioxidant capacity than fish oil.[15] However, to the best of our knowledge, the oxidative stability of photoautotrophic microalgae oils has until now never been investigated.

Previous research also showed that storage of microalgal biomass leads to lipid hydrolysis: especially when the microalgae are stored as a wet paste at temperatures above the freezing point,[16] but even spray- and freeze-dried microalgal biomass will hydrolyze during storage.[17] It is, however, not

Received: May 28, 2013
Revised: September 24, 2013
Accepted: September 25, 2013
Published: September 25, 2013

 © 2013 American Chemical Society

Table 1. Lipid Extraction Yield, Percentage Lipid Recovery, Lipid Class Content, Omega-3 Fatty Acid Content, and Carotenoid Content of the Microalgal Oils, Obtained with Hexane/Isopropanol (HI) and Hexane (H), and the Commercial Omega-3 LC-PUFA Oils

	Isochrysis galbana		*Nannochloropsis gaditana*		*Nannochloropsis* sp.		*Phaeodactylum tricornutum*		commercial oils			
	HI	H	HI	H	HI	H	HI	H	fish oil	tuna oil	DHA-S oil	krill oil
lipid extraction yield (g/100 g DW; mean ± SD, n = 3)	21.1 ± 0.9	14.7 ± 0.2	16.0 ± 1.0	3.9 ± 0.1	16.0 ± 0.9	5.9 ± 0.2	12.0 ± 0.4	4.5 ± 0.2				
lipid recovery (% of ref method; mean ± SD, n = 3)	76 ± 4	53 ± 1	57 ± 4	13.9 ± 0.9	53 ± 3	19.6 ± 0.7	67 ± 3	25 ± 1				
lipid class content (% of oil; mean ± SD, n = 3)												
neutral lipids (NL)	64.5 ± 0.5	87.1 ± 0.4	31.0 ± 0.2	45 ± 2	47.7 ± 0.3	75.5 ± 0.7	42 ± 1	80 ± 1	95 ± 2	95 ± 2	99.3 ± 0.8	39 ± 1
glycolipids (GL)	21.6 ± 0.4	5.1 ± 0.8	37.1 ± 0.5	26 ± 2	23.6 ± 0.3	10.1 ± 0.6	28 ± 1	10 ± 1	2.7 ± 0.6	2 ± 2	0.8 ± 0.9	3.6 ± 0.9
phospholipids (PL)	13.9 ± 0.3	7.8 ± 0.5	32.0 ± 0.5	29 ± 1	28.7 ± 0.6	14.4 ± 0.7	30.1 ± 0.3	10.3 ± 0.4	2 ± 2	3 ± 3	0.8 ± 0.3	57 ± 2
omega-3 fatty acid content (in mg/g oil; mean ± SD, n = 3)												
ALA (C18:3n-3)	27 ± 1	30 ± 2	8.9 ± 0.2	9.3 ± 0.3	0.8 ± 0.2	1.1 ± 0.1	1.7 ± 0.1	2.3 ± 0.1	5.02 ± 0.06	5.0 ± 0.3	0.58 ± 0.09	7.0 ± 0.2
SDA (C18:4n-3)	57 ± 3	61 ± 4	0.51 ± 0.06	0.54 ± 0.03	0.32 ± 0.01	0.31 ± 0.06	0.50 ± 0.03	0.58 ± 0.07	0.20 ± 0.04			
EPA (C20:5n-3)	2.3 ± 0.2	2.2 ± 0.4	128 ± 4	116 ± 4	120 ± 3	102 ± 8	99 ± 6	105 ± 7	147 ± 2	51 ± 2	4.7 ± 0.2	107 ± 6
DPA (C22:5n-3)									17.2 ± 0.2	9 ± 1	2.1 ± 0.2	2.28 ± 0.06
DHA (C22:6n-3)	56 ± 3	70 ± 5					5.6 ± 0.8	6.9 ± 0.2	98.6 ± 0.05	205 ± 14	321 ± 8	59 ± 3
carotenoid content (mg/g oil; mean ± SD, n = 3)												
astaxanthin												0.1–0.7[a]
carotene	3.8 ± 0.2	4.5 ± 0.2	4.9 ± 0.5	13.6 ± 0.7	5.2 ± 0.3	9.9 ± 0.6	4.2 ± 0.2	10.3 ± 0.8				
diadinochrome	0.87 ± 0.03	0.7 ± 0.1										
diadinoxanthin			3 ± 1	6.9 ± 0.2	4.4 ± 0.2	3.78 ± 0.01	6.9 ± 0.6	10 ± 2				
diatoxanthin	3.2 ± 0.2	1.6 ± 0.1					3.1 ± 0.2	4.4 ± 0.8				
fucoxanthin	41 ± 2	10.1 ± 0.3					38 ± 2	43 ± 8				
violaxanthin			2.8 ± 0.2	6.2 ± 0.3	14 ± 1	5.6 ± 0.1						
zeaxanthin			3.8 ± 0.5	2.6 ± 0.1	0.89 ± 0.04	0.589 ± 0.003						
total carotenoid content	49 ± 2	16.9 ± 0.7	15 ± 2	29 ± 1	25 ± 2	19.9 ± 0.7	52 ± 3	68 ± 11				

[a]Not measured; estimated range communicated by the krill oil producer.

known whether the hydrolytic enzymes are coextracted and whether they still work in the oily environment.

The purpose of this work was therefore to evaluate the oxidative and hydrolytic stability of omega-3 LC-PUFA-rich microalgae oils obtained with the food grade solvents hexane/isopropanol (3:2) or hexane. The following species were used: *Isochrysis galbana*, *Phaeodactylum tricornutum*, and *Nannochloropsis gaditana* as well as *Nannochloropsis* sp. The oils extracted therefrom were compared with four fresh commercially available omega-3 LC-PUFA oils: fish oil, tuna oil, heterotrophic DHA-rich algal oil (DHA-S oil), and krill oil. The oxidative stability was not evaluated by measuring the antioxidant capacity, but by following specific markers for lipid and omega-3 LC-PUFA oxidation during 8 weeks of storage at 37 °C.

MATERIAL AND METHODS

Chemicals. Solvents included *n*-hexane p.a. 99% (Chem-Lab, Zedelgem, Belgium), 2-propanol HPLC 99.5% (Acros, Thermo Fisher Scientific, Geel, Belgium), and chloroform HPLC and methanol super gradient HPLC (Labscan, Boom B.V., Meppel, The Netherlands).

Reagents included xylenol orange disodium salt p.a., iron(II)sulfate, and barium chloride (Sigma-Aldrich, Bornem, Belgium) as well as anhydrous iron(III)chloride (Merck, VWR, Leuven, Belgium).

The internal standard was lauric acid (Nu-check Prep, Elysian, MN, USA).

Microalgae and Commercial Omega-3 LC-PUFA Oils. Biomass of omega-3 LC-PUFA producing photoautotrophic microalgae was obtained from European companies: in-house *N. gaditana* and Pt1 8.6 (CCMP2561) *P. tricornutum* from LGem (Voorhout, The Netherland); in-house *Nannochloropsis* sp. and *I. galbana* from Proviron (Hemiksem, Belgium). The biomass composition of these microalgae was given earlier.[4] The four microalgae were extracted with two food grade solvent systems, hexane/isopropanol (HI, 3:2) and hexane (H). These solvents were chosen because the former showed to be the best nonhalogenated solvent mixture, whereas the latter is currently the most commonly used to extract vegetable oils.[18] For the extraction, 6 mL of the solvent (mixture) was added to 100 mg of freeze-dried microalgae, followed by cell disruption with a bead beater, twice for 60 s at 30 Hz. Subsequently, the biomass was extracted four times with the extraction (solvent) mixture. The extraction on a 100 mg scale was repeated in 28-fold or more, and all of the extracts were combined to obtain enough microalgal oil for the whole setup. No further refining was conducted on these oils. The extraction yield and percentage lipid recovery for each combination of microalgae and solvent are reported in Table 1 as is the composition of the oils. The methodologies used to determine the lipid class content, the omega-3 LC-PUFA content, and the carotenoid content are mentioned below.

Fresh 18/12 refined fish oil made from anchovy, mackerel, and sardines, tuna oil, DHA-rich oil from *Schizochytrium* sp. (DHA-S oil), and krill oil were obtained from Bioriginal (Den Bommel, The Netherlands). These oils were fully processed according to state of the art methods. For fish, tuna, and DHA-S oils, this means extraction from their biomass and refining and deodorization, including removal of phospholipids, free fatty acids (FFAs), heavy metals, PCBs, dioxins, etc. For krill oil this means extraction from the biomass, normally without further refining. The oils were stored at −20 °C until the storage test started. The composition of the oils is provided in Table 1. The methodologies used to determine the lipid class content, the omega-3 LC-PUFA content, and the carotenoid content are mentioned below.

Storage test. The eight obtained microalgal oils and the four fresh commercial oils (10−15 mg) were stored at 37 °C in 20 mL amber screw-cap vials for headspace analysis for 8 weeks without taking extra precautions to prevent oxidation. This way a slightly accelerated oxidation was induced, allowing differences in oxidative stability to be measured in a reasonable time frame. Three parameters were monitored during storage. To study the oxidative stability, the primary oxidation products were determined by measuring the peroxide value (PV), whereas the secondary oxidation products were studied by measuring volatile components using HS-SPME-GC-MS, both at specific time points (t = 0, 1, 2, 4, 6, and 8 weeks) during storage. Furthermore, the hydrolytic stability was studied by measuring the free fatty acid content at the beginning and end of the storage test. The methods used to determine the different parameters are described below. At each time point two vials for each analysis type were transferred to −80 °C until analysis. The mean and standard deviation of the two repetitions are each time reported.

Analysis of Lipid Class Content. The lipid class content (neutral lipid, glycolipid, and phospholipid contents) of the extracts was determined using silica solid phase extraction (SPE) as previously described in Ryckebosch et al.[19] Briefly, the SPE column is conditioned with 10 mL of chloroform. Then, approximately 10 mg of lipids in 100 μL of chloroform is applied to the column. Elution with 10 mL of chloroform yields the nonpolar lipids, 10 mL of acetone gives the glycolipid fraction, and 10 mL of methanol yields the phospholipids. The lipid class content was determined in triplicate.

Analysis of Fatty Acid Content and Composition. To determine the fatty acid content, the lipid extracts were methylated with 1% sulfuric acid in methanol, followed by extraction of the required methyl esters with hexane, according to the method of Christie[20] with slight adjustments previously described by Ryckebosch et al.[19] The obtained fatty acid methyl esters (FAMEs) 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, i.d. 0.32 mm, film 0.25 μm (GRACE, Lokeren, Belgium) is used with the following time−temperature program: 70−180 °C (5 °C/min), 180−235 °C (2 °C/min), 235 °C (9.5 min). Peak areas are quantified with Chromcard for Windows software (Interscience). FAME standards (Nu-check) containing a total of 35 different FAMEs are analyzed for provisional peak identification, which are then confirmed by use of GC-MS (Trace GC Ultra, ISQ single-quadrupole MS, Thermo Scientific, Interscience) using an Rxi-5 Sil MS column of length 20 m, i.d. 0.18 mm, film 0.18 μm (Restek, Interscience). Quantitative and qualitative determination of the fatty acids was performed in triplicate.

Analysis of Carotenoid Composition and Content. For the determination of the carotenoid content and composition, 2 mg of the extract was dissolved in 10 mL of methanol. 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 the method of Wright et al.[21] For quantification, calibration curves were composed 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 to quantify. The analysis was performed in triplicate.

Analysis of Primary Oxidation Products: Peroxide Value. The PV was determined by measuring iron oxidation (FOX) according to the method of Wrolstad et al.[22] with some adjustments. Microalgal or commercial oils (10−15 mg) were dissolved in chloroform/methanol 7:3 (9.9 mL), and the absorbance of this mixture at 560 nm forms the blank of the sample. Subsequently, a xylenol orange solution (10 mM, 50 μL) was added to the sample solution and homogenized, and then an iron(II) chloride solution (50 μL) was added and homogenized again. After exactly 5 min, the absorbance of this solution was determined at 560 nm. PV (expressed as mequiv active oxygen/kg sample) was calculated using the equation

$$PV = [(A_S - A_B) \times m_i]/(W \times 55.84 \times 2)$$

where A_S is the absorbance of the sample, A_B is the absorbance of the blank, m_i is the inverse of the slope (i.e., 1/slope) obtained from a standard curve of iron(III), W is the weight of the sample (g), and 55.84 is the atomic weight of iron.

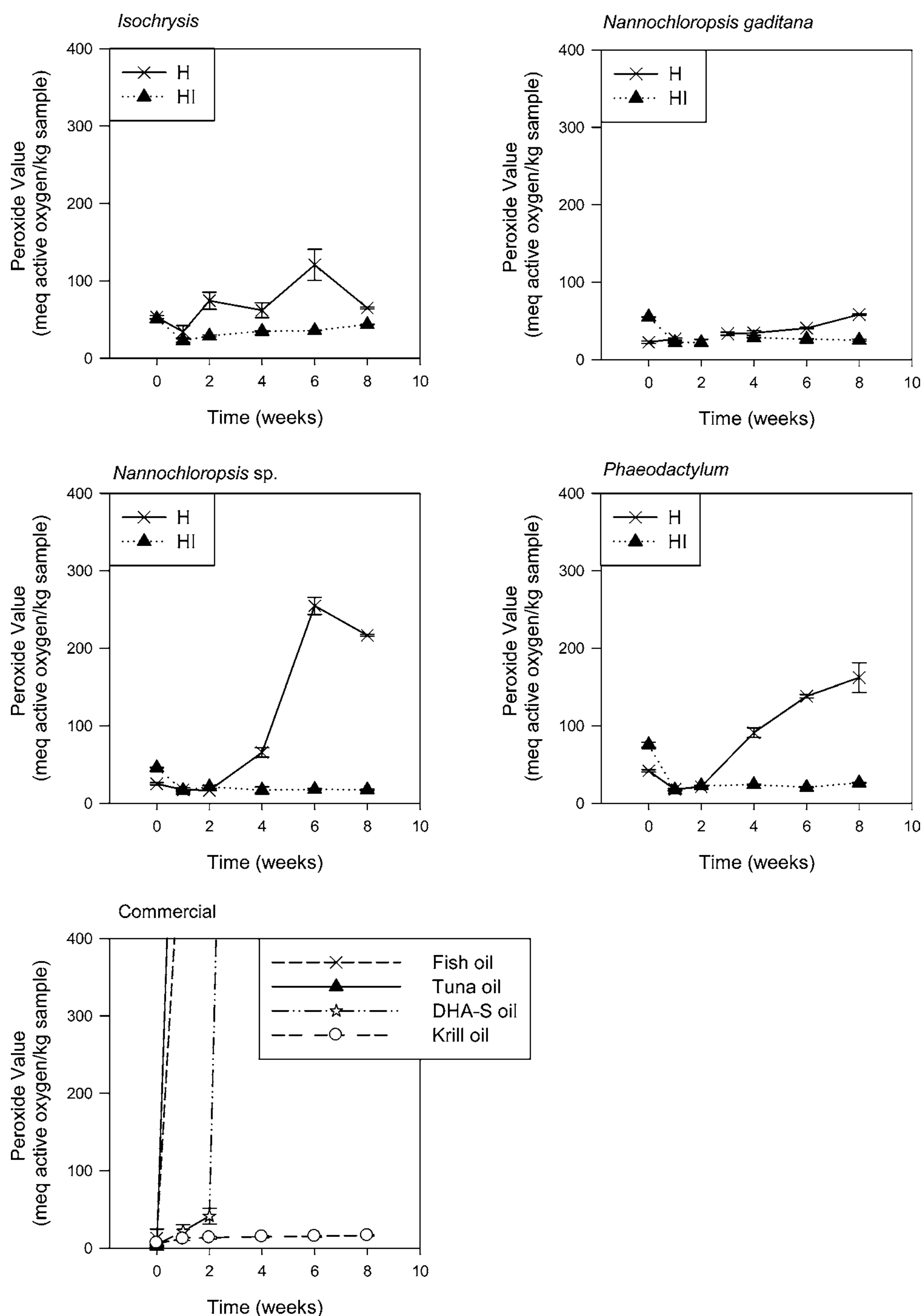

Figure 1. Peroxide value (in mequiv active oxygen/kg oil; mean ± SD; n = 2) as a function of storage time at 37 °C of the different microalgal oils, obtained with hexane/isopropanol (HI) and hexane (H), and the commercial omega-3 LC-PUFA oils.

When polymerization had occurred in the oil, it was no longer soluble in chloroform/methanol, and thus the measurement could no longer be performed. This was the case with fish and tuna oil from t = 2 weeks and with DHA-S oil from t = 6 weeks.

Analysis of Secondary Oxidation Products: Volatiles with Headspace–Solid Phase Microextraction–Gas Chromatography–Mass Spectrometry (HS-SPME-GC-MS). The isolation of the volatiles originating from lipid oxidation was performed with an autosampler (multi-PurposeSampler or MPS, Gerstel, Mülheim an der Rur, Germany), equipped with a headspace–solid phase microextraction unit. The conditions were as follows: the microalgal or commercial oil in the hermetically sealed 20 mL vials was incubated for 30 min at 60 °C in a thermostated agitator. Afterward, the headspace was extracted on a well-conditioned CAR/DVB/PDMS SPME fiber (Supelco, Sigma-Aldrich N.V., Bornem, Belgium) for 35 min at 60 °C. The volatiles were then analyzed with GC-MS, using splitless injection, helium as a carrier gas (1 mL/min), and a cross-linked methyl silicone column (HP-PONA), of length 50 m, i.d. 0.20 mm, film thickness 0.5 μm (Agilent Technologies, Diegem, Belgium). The following temperature program was applied: 40 °C (5 min), from 40 to 250 °C (5 °C/min), 250 °C (5 min). Injector and transfer lines were maintained at 250 and 280 °C, respectively. The total ion current (70 eV) was recorded in the mass range from 40 to 250 amu (scan mode) using no solvent delay and a threshold of 50.

Identification of volatile organic compounds in the headspace was performed using the Wiley 275 library. Because of their link with oxidation of omega-3 LC-PUFA,[6,23−25] the following volatile indicator components were chosen to follow in time: (E,E)-2,4-heptadienal,

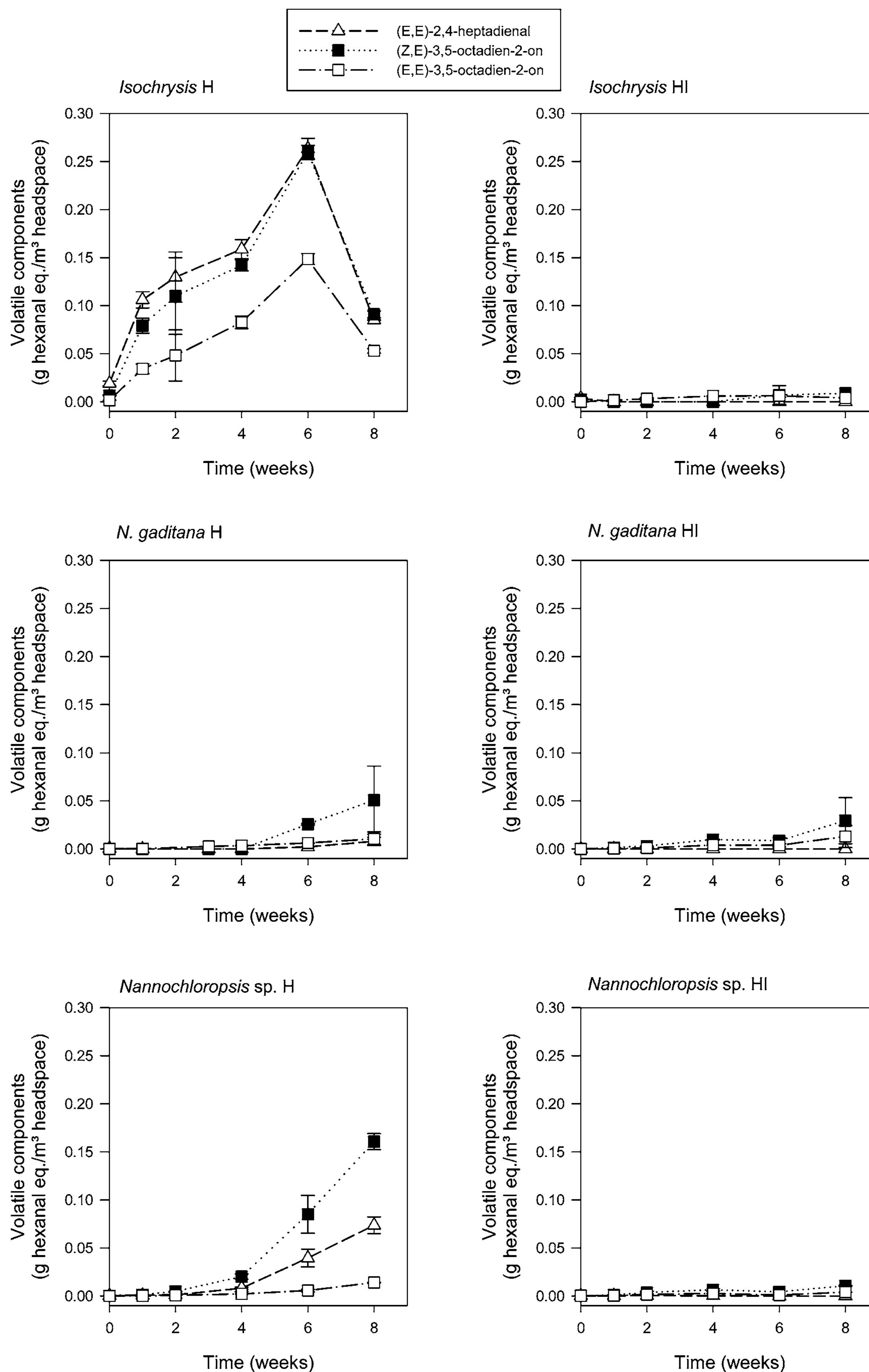

Figure 2. continued

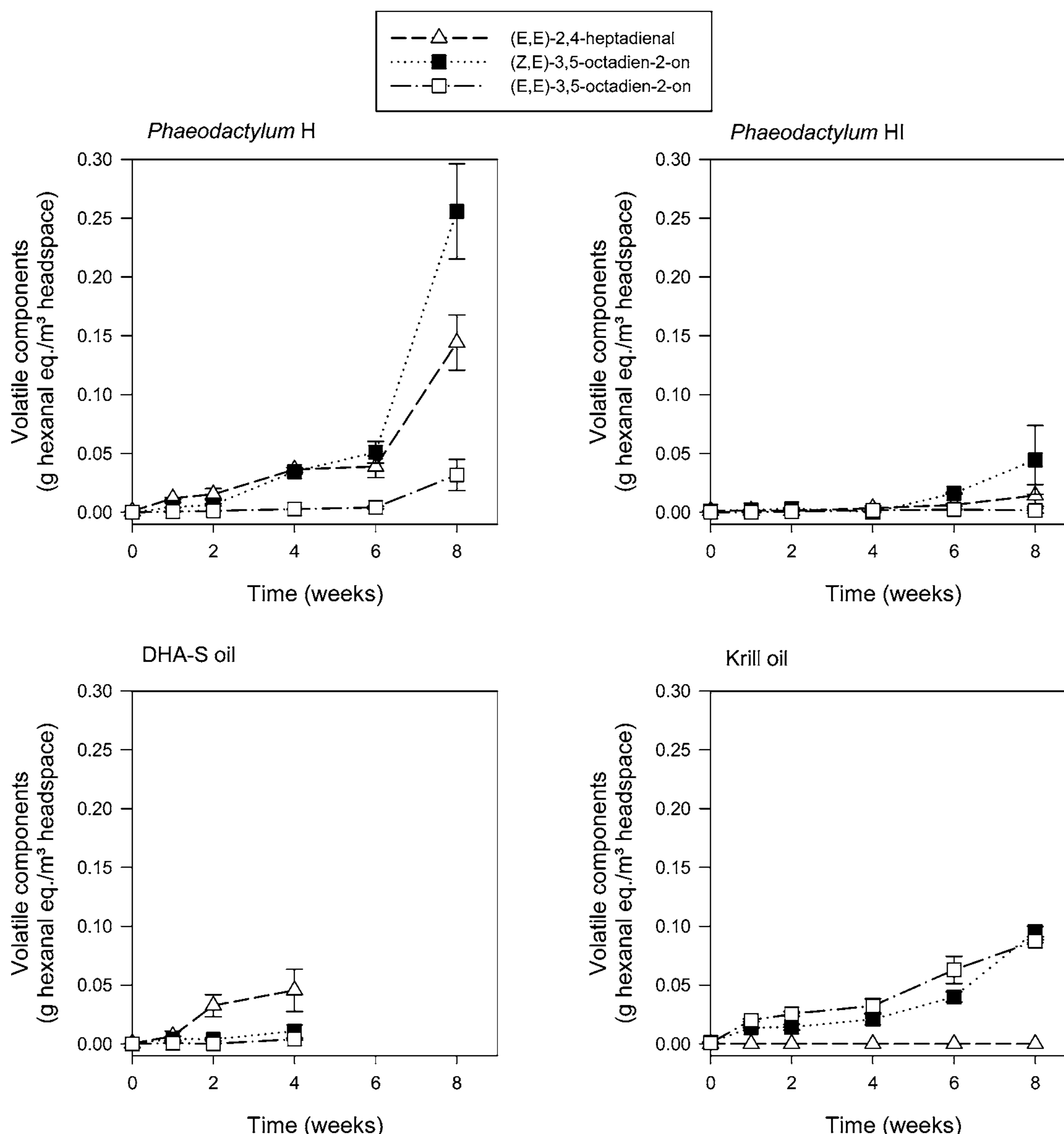

Figure 2. Volatile components (in g hexanal equiv/m^3; mean ± SD; $n = 2$) as function of storage time at 37 °C of the microalgal oils, obtained with hexane/isopropanol (HI) and hexane (H), and the commercial omega-3 LC-PUFA oils.

(*Z*,*E*)-3,5-octadien-2-one, and (*E*,*E*)-3,5-octadien-2-one. The semi-quantitative concentrations of the identified volatile compounds were expressed as grams of hexanal equivalents per cubic meter and calculated as the area of the volatile indicator component divided by the response factor of the external standard hexanal.

When polymerization had occurred in the oil, the HS-SPME-GC-MS measurement was no longer relevant. This was the case with fish and tuna oil from $t = 2$ weeks and with DHA-S oil from $t = 6$ weeks.

Analysis of Hydrolysis Products: Free Fatty Acids. The free fatty acid content was determined according to the procedure described by Ryckebosch et al.,[17] which is based on the method for selective formation of dimethyl amide derivatives of Kangani et al.[26] Lauric acid (C12:0) in chloroform was added to the oil as an internal standard. The dimethyl amide derivatives were analyzed on GC-FID following the method previously described.[4]

At $t = 8$ weeks, polymerization had occurred in fish, tuna, and DHA-S oils and thus their FFA content could not be determined.

Statistics. Results were statistically evaluated using a t test with $\alpha = 0.05$ (Sigmaplot 11, Systat Software Inc., Chicago, IL, USA).

RESULTS

Formation of Primary Oxidation Products during Storage of Omega-3 LC-PUFA Oils. The PV as a function of storage time for the different microalgal and commercial oils is shown in Figure 1. It is clear that the PVs obtained for all microalgae oils, even when freshly extracted and hence not oxidized, were high (mostly >20). This may be explained by the presence of carotenoids (Table 1) and chlorophyll, absorbing at the wavelength where measurements for PV are performed.[27] Therefore, not the absolute value of the PV was used to evaluate the oxidative stability of the oils, but the time point when the PV started to rise as well as the slope of the curve.

When looking at the commercial oils, it was observed that the PV of krill oil remained low, whereas for fish and tuna oils increased PVs were already measured at week 1. Sullivan Ritter and Budge[6] also measured an increase of the PV for three fish oils stored at 40 °C as a function of storage time, which was in accordance with our results. Due to the large quantity of oil stored in their experiment, the oxidation, however, occurred

much more slowly than in our study, leading to lower PVs after 1 week. Sullivan Ritter and Budge[6] also did not notice the sudden increase as observed in our study. However, Sullivan et al.[7] did show a sudden increase of the PV of fish oil at 40 °C immediately at the start of the storage test, whereas at 4 °C the PV increased steeply only after some time.

The PV of DHA-S oil rose slowly until week 2 and then suddenly rose to a high value. To better compare the oxidative stability of krill oil, given the soft slope of the curve, the minimum and maximum PVs were used to estimate the slope. A value of 2.7 mequiv active oxygen/(kg sample·time) was obtained. The results on krill oil should be looked at with care, because it has been stated that the oxidation of krill oil occurs through a different mechanism, not measurable through classic methods.[28,29] However, up till now not enough research has been performed on this subject.

When the PVs of microalgal oils over time are evaluated, it is clear that both the HI and H curves remained reasonably low compared to fish, tuna, and DHA-S oils. However, more primary oxidation products were formed during storage of the hexane (H) oils compared to the hexane/isopropanol (HI) oils. The H curves of *Nannochloropsis* sp. and *Phaeodactylum* had increased steeply at week 4 with a higher slope for *Nannochloropsis* sp. when compared to *Phaeodactylum*. The curve of *N. gaditana*, on the other hand, did not rise until week 8. For *Isochrysis* the whole H curve rose to a PV of about 70 mequiv active oxygen/kg sample and fluctuated there. To evaluate the HI curves, the same method was used as for krill oil. The HI curve of *Nannochloropsis* sp. increased the most (1.9 mequiv active oxygen/(kg sample·time)), whereas that of *N. gaditana* increased the least (1.1 mequiv active oxygen/(kg sample·time)). The curves of *Phaeodactylum* and *Isochrysis* showed an intermediate increase (1.5 and 1.3 mequiv active oxygen/(kg sample·time), respectively). It is clear that the stability of the different HI oils varies less than that of the different H oils, although it seems that the order of stability between the microalgae is similar in both HI and H oils.

In conclusion, it seems that, on the basis of the PV, the oxidative stability of the oils increases in the following direction: fish oil = tuna oil < DHA-S oil < microalgae H oils < krill oil < microalgae HI oils. Furthermore, it looks like the *N. gaditana* H oil is the most stable of all H oils, although the *Isochrysis* H oil could not be ranked properly due to its fluctuating course. The differences between the PV curves of the HI oils are probably too small to rank these oils.

Formation of Secondary Oxidation Products during Storage of Omega-3 LC-PUFA Oils. The volatile components as markers of the omega-3 LC-PUFA oxidation, (*E,E*)-2,4-heptadienal, (*Z,E*)-3,5-octadien-2-one, and (*E,E*)-3,5-octadien-2-one, of the different microalgae and commercial oils as a function of storage time are given in Figure 2. (*E,E*)-2,4-Heptadienal was previously identified in DHA-enriched triacylglycerol (TAG) fish oil and fish oil-enriched mayonnaise and found to increase during storage.[23,30−32] (*E,Z*)- and (*E,E*)-3,5-octadien-2-one were also detected in different fish oils, fish oil-enriched mayonnaise, and fish oil-enriched milk and correlated well with storage time and sensory characteristics.[6,31−33]

As mentioned before, the samples were no longer measured when polymerization had occurred, which was the case for fish and tuna oil from $t = 2$ weeks and for DHA-S oil from $t = 6$ weeks. Polymerization is to be expected as oxidation of oil occurs, because it was already suggested as an excellent indication of oxidation at low temperature. Furthermore, it was shown that results on polymer content showed the same general trend as the *p*-anisidine value,[34] whereas a significant increase of polymers was shown to indicate the end of the induction period.[35] Full polymerization thus shows that the oxidation is already at an advanced stage, which can be seen as a third parameter to track oxidation. The polymerization of fish, tuna, and DHA-S oils thus shows that oxidation was already at an advanced stage at $t = 2$ weeks and $t = 6$ weeks, respectively.

To evaluate the volatile components formed during time, the same technique was applied as for the PV. The most interesting evaluation parameters are the induction period and the slope over time. Due to the specificity of the volatiles, the values at $t = 8$ weeks could also be taken into account.

In the evaluation of krill oil, the only commercial oil that could be measured during the whole 8 weeks of storage, it was observed that the volatile components of krill oil showed a slow increase starting from $t = 1$ week. The increase was somewhat higher than the increase in volatiles observed during storage of the microalgae HI oils, although somewhat lower than that of most of the microalgae H oils. This also means that more secondary oxidation products are formed during storage of the H oils than during storage of the HI oils. The H oil of *Isochrysis* showed the highest values for the volatiles, with values also already increasing at $t = 1$ week and subsequently decreasing after $t = 6$ weeks. This is a natural phenomenon of oxidation, because formed aldehydes and other secondary oxidation products can undergo further scission to produce carbonyls and alkanes of shorter chain length. The H oil of *N. gaditana* showed the lowest values for the volatiles of all H oils.

In conclusion, with the time at which the oils were polymerized, the induction time for occurrence of the volatiles, and the values of the volatiles at $t = 8$ weeks taken into account, it seems that the oxidative stability of the oils increases in the following direction: fish oil = tuna oil < DHA-S oil < microalgae H oils < krill oil < microalgae HI oils. The *N. gaditana* H oil appears to be the most stable of all H oils. The differences between the HI oils are probably too small to rank these oils.

Formation of Hydrolytic Products during Storage of Omega-3 LC-PUFA Oils. The FFA content of the different microalgal and commercial oils at the start and after 8 weeks of storage at 37 °C is shown in Table 2. The FFA content of fish, tuna, and DHA-S oils could not be determined after 8 weeks of storage, because the oils were then completely polymerized. The FFA content of the commercial oils was rather low (<5%), which was expected because refining of the oils removes most of them. The somewhat higher FFA content in krill oil can be explained by the fact that krill oil is not refined to the same extent as fish oil. The FFA content of the HI oils of both *Nannochloropsis* species and *Phaeodactylum* is in the same range (<5%) as the commercial oils. The FFA content of the H oils of *N. gaditana* and *P. tricornutum* was higher, but still below 10%. Both oils of *Isochrysis* contained a high amount of FFA (>20%). Furthermore, it seems that the FFA content as a function of storage time remains mostly constant, except for the H oil of *Isochrysis*, where a decrease in the FFA content was observed. This is probably due to the fact that the technique to determine the FFA content was validated in a lower concentration range[26] and therefore not accurate for measuring higher FFA contents (>20%).

In conclusion, it was shown that no hydrolysis occurs during storage of the oils. As no other studies have already investigated

Table 2. Free Fatty Acid Content as a Function of Storage Time at 37 °C of the Microalgal Oils, Obtained with Hexane/Isopropanol (HI) and Hexane (H), and the Commercial Omega-3 LC-PUFA Oils[a]

	fatty acid content (mg/100 mg oil; mean ± SD; n = 3)	
	t = 0 weeks	t = 8 weeks
Isochrysis galbana		
H	29 ± 3 b,x	20 ± 2 a,y
HI	21 ± 1 a,x	25.5 ± 0.9 a,x
Nannochloropsis gaditana		
H	8.6 ± 0.5 b,x	10 ± 2 b,x
HI	2.5 ± 0.4 a,x	3.6 ± 0.2 a,x
Nannochloropsis sp.		
H	5.0 ± 0.2 a,x	4.9 ± 0.6 a,x
HI	3.6 ± 0.3 a,x	3.5 ± 0.2 a,x
Phaeodactylum tricornutum		
H	8.6 ± 0.5 b,x	9.1 ± 0.1 b,x
HI	4 ± 1 a,x	4.3 ± 0.3 a,x
commercial oils		
fish oil	1.97 ± 0.04	*b*
tuna oil	2.1 ± 0.9	*b*
DHA-S oil	0.5 ± 0.2	*b*
krill oil	3.2 ± 0.5 x	3.4 ± 0.3 x

[a]Different letters (a, b) show the significance of differences between solvent systems within one microalga and storage time (α = 0.05). Different letters (x, y) show the significance of differences between storage times within one solvent system (α = 0.05). [b]FFA content could not be determined because the samples were completely polymerized.

the hydrolytic stability of oils from autotrophic microalgae, this conclusion could not be compared with the literature.

■ DISCUSSION

From the results of both the primary and secondary oxidation parameters, as well as the time at which full polymerization occurred, it is clear that the same conclusions could be drawn. Fish and tuna oil were shown to be the least oxidatively stable, whereas DHA-S oil performed somewhat better. Subsequently, the microalgae H oils were shown to be more stable than DHA-S oil, followed by krill oil, and the most stable oils were the microalgae HI oils. The H oil of *N. gaditana* appears to be the most stable of the microalgae H oils, whereas the microalgae HI oils could not be ranked because of the small differences between them.

Frankel et al.[36] published similar results on the oxidative stability of different fish oils and heterotrophic algae oils: the commercially available fish oils were oxidized more rapidly than the commercially available heterotrophic algae oil formulated with tocopherols and ascorbyl palmitate. They, however, also showed that the high oxidative stability of the heterotrophic algae oil was completely lost after chromatographic purification to remove tocopherols and other antioxidants. Krill oil showed no oxidation during 30 days of storage at room temperature.[10] Literature on the stability of photoautotrophic microalgae oils was, to the best of our knowledge, not published yet.

An explanation for the differences in stability between the oils can be found in their oil composition. During production, a mix of tocopherols was added to fish and tuna oil to protect the omega-3 LC-PUFA from oxidation.[37,38] However, even with these added antioxidants, they seemed to be quite unstable. This also explains the ongoing research on increasing fish oil stability, using microencapsulation[39,40] and addition of different antioxidants.[41,42] During production of DHA-S oil, tocopherols, ascorbyl palmitate, soy lecithin, and rosemary extract were added. Tocopherols act as antioxidant as already discussed above. Furthermore, a synergistic effect was observed when a mixture of tocopherols and lecithin was added to fish oil.[43,44] Rosemary extract contains natural phenolics, such as carnosic acid and rosmarinic acid, which have widely reported[42] stabilizing properties against oxidation. Ascorbyl palmitate is another well-known antioxidant. The synergistic effect and the extra antioxidants added can explain the higher stability of DHA-S oil compared to fish and tuna oil. Krill oil contains the natural fat soluble antioxidant astaxanthin, the singlet oxygen quenching activity of which is 10 times stronger than that of any other carotenoid such as zeaxanthin, lutein, canthaxanthin, and β-carotene and up to 500 times stronger than that of tocopherols.[45] Furthermore, krill oil contains more than 50% phospholipids (Table 1), which were shown to be more oxidatively stable than triacylglycerols,[46] whereas they can also inhibit oxidation of other oils.[47,48] This all can explain the clearly higher stability of krill oil when compared to the other commercial omega-3 LC-PUFA rich oils.

It was clear that all of the HI oils were more stable than the H oils of the same microalga. This phenomenon cannot be explained by the (total) carotenoid content of the microalgae oils (Table 1), because diverse results were found: the carotenoid content of *Isochrysis* and *Nannochloropsis* sp. was higher in the HI oil than in the H oil, whereas for *N. gaditana* and *Phaeodactylum* the carotenoid content in the H oil was higher than in the HI oil. Although some carotenoids have stronger antioxidant activity than others,[49] these differences are probably too small to explain the higher oxidative stability of all HI oils compared to the H oils. Two other possible explanations for the difference between the HI and H oils can be given. First, other antioxidants, such as phenolic compounds, can be present in the oils. Phenolic compounds, such as flavonols, benzoic acid derivatives, and cinnamic acid derivatives, have been shown to be present in microalgae biomass in amounts in the same order of magnitude as carotenoids.[12,14,50] Furthermore, they were shown to have a higher antioxidative capacity than tocopherols and carotenoids.[51] Moreover, carotenoids are particularly good at protecting against $^1O_2$ mediated photo-oxidation, a type of oxidation that will not take place in this setup due to the use of amber vials. Because the solubility of phenolic compounds was shown to be a lot smaller in hexane than in alcohols,[52] it is expected that they are more abundant in the HI than in the H oils, thus possibly explaining the higher oxidative stability of the HI compared to the H oils. A second possible explanation is the higher GL and PL content in the HI oil compared to H oil (Table 1). After all, it has been shown that omega-3 LC-PUFAs associated with phospholipids as well as glycolipids were more resistant to oxidation than when they are associated with TAGs.[46,53,54] Furthermore, it was also shown that addition of phospholipids inhibits the oxidation of other (TAG) oils.[47,48]

Comparison of the different microalgal H oils also clearly showed from both PV and HS-SPME-GC-MS results that the H oil of *Nannochloropsis* sp. was less stable than the H oil of *N. gaditana*. Because these two microalgae contain the same carotenoids, direct comparison of amounts was possible. The H oil of *N. gaditana* clearly contained a higher content of all carotenoids compared to the H oil of *Nannochloropsis* sp. This

can explain why the H oil of *N. gaditana* is more stable than that of *Nannochloropsis* sp. It was also clear that the *Phaeodactylum* H oil was less stable than the *N. gaditana* H oil. Yet the total carotenoid content of the *Phaeodactylum* H oil was more than double of the carotenoid content of the *N. gaditana* H oil. Two reasons to explain this observation are possible. First, the carotenoid composition of both oils is quite different: in the *Phaeodactylum* H oil the carotenoid content is mainly determined by fucoxanthin, whereas carotene is the main carotenoid in the *N. gaditana* H oil. The higher oxidative stability of the *N. gaditana* H oil might thus be explained by the higher antioxidant capacity of some carotenoids compared to others.[49] However, there is no clear ranking in the antioxidant capacity of all different carotenoids available to confirm this potential explanation. Second, carotenoids are also known to have pro-oxidant properties in certain conditions. Factors that determine the anti- or pro-oxidant activity of carotenoids include the concentration of the carotenoid and the partial oxygen pressure.[55] Because the carotenoid content in the *Phaeodactylum* H oil is particularly high and the oxygen pressure in the storage vials was initially 100%, this can also explain the lower oxidative stability of the *Phaeodactylum* H oil compared to the *N. gaditana* H oil. For the other H oils differences in stability were much less clear, and therefore no discussion is attempted. However, it seems that the carotenoid content and composition play important roles in the oxidative stability of the H oils.

As mentioned before, the differences between the HI oils of the different microalgae are too small to rank these oils; therefore, no explanations were pursued.

It should also be mentioned that this study compares crude, laboratory scale extracted microalgae oils with highly refined commercial omega-3 LC-PUFA oils. It has not yet been investigated whether microalgae oil will require refining and, if it does, how it will be performed. Furthermore, it is not clear if refined microalgae oil will show the same oxidative stability as the crude oils. These are definitely topics for further research.

Further research on oxidation of microalgae oils is definitely needed. Most importantly, it should be researched in more detail if the amount or types of polyphenols can explain the stability of the microalgae HI oils, or if other compounds might be responsible. Furthermore, further research should be performed on the antioxidant capacity of different carotenoids, so proper ranking could lead to an explanation of the stability of the microalgae H oils. Finally, a sensory panel should be used to check whether the parameters discussed before also correlate with sensory properties of microalgae oils. However, this might be difficult because all microalgae already have a specific odor ranging from grassy and fruity to fishy aromas.[56]

Hydrolytic degradation does not seem to be a problem during storage of any of the oils. In contrast, microalgal lipids hydrolyze considerably due to storage of the wet paste at temperatures above the freezing point, as was shown for *Scenedesmus* sp.:[16] the TAG content decreased while the FFA content increased from a negligible level to 70% in just 1 day. Even when spray- and freeze-dried microalgal biomass was stored, the FFA content almost doubled over 5 weeks.[17] This means that the high FFA content found in microalgal oils immediately after extraction can most probably be explained by the way the wet biomass was stored before drying, the technique used for drying, and even the way the dried biomass was stored. Nevertheless, once the oil was separated from the biomass, the FFA content remained relatively stable. This may indicate that hydrolytic enzymes are not coextracted with the oils or that they are not active in the lipid environment. Similarly, the FFAs in crude fish oil are also generated during enzymatic spoilage of the fish prior to processing, but not formed during the production process.[57] Refining will, however, remove most of them. In contrast, the microalgae oils tested in this study were not refined, explaining the slightly to much higher FFA content in these oils.

In conclusion, this study investigated the stability of microalgal omega-3 LC-PUFA-rich oils in comparison with commercial omega-3 LC-PUFA rich oils. From the results of both the primary and secondary oxidation parameters, it was clear that fish, tuna, and DHA-S oils were the least oxidatively stable oils, whereas krill oil and the microalgae oils performed better. The oxidative stability of the microalgae oils was also shown to be dependent on the solvent (mixture) used to extract the lipids: the oils obtained with hexane/isopropanol were more stable than oils obtained with hexane. This is probably due to the presence of more polar antioxidants such as polyphenols in these oils. All oils were shown to be hydrolytically stable during storage.

AUTHOR INFORMATION

Corresponding Author

*(I.F.) E-mail: Imogen.Foubert@kuleuven-kulak.be. Phone: +32 56 24 61 73. Fax: +32 56 24 69 97.

Funding

The research presented in this paper was financially supported by Flanders' Food − IWT (Omega-OIL project), a Research Grant of the Research Foundation − Flanders (FWO) (1.5.122.09N) and KU Leuven Kulak.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge the companies of the FF Omega-OIL project for fruitful discussions. We give special acknowledgment to Bioriginal for the samples of the commercial omega-3 LC-PUFA rich oils and Proviron and LGem for the samples of the microalgae. We also acknowledge Cedrick Veryser for his drive and accurate work during his holidays.

REFERENCES

(1) Gogus, U.; Smith, C. n-3 omega fatty acids: a review of current knowledge. *Int. J. Food Sci. Technol.* **2010**, *45* (3), 417−436.

(2) Meyer, B. J. Are we consuming enough long chain omega-3 polyunsaturated fatty acids for optimal health? *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2011**, *85* (5), 275−280.

(3) Myers, R.; Worm, B. Rapid worldwide depletion of predatory fish communities. *Nature* **2003**, *423*, 280−283.

(4) Ryckebosch, E.; Bruneel, C.; Termote-Verhalle, R.; Muylaert, K.; Foubert, I. Influence of extraction solvent system on extractability of lipid components from different microalgae species. *Algal Res.* **2013**, submitted for publication.

(5) Nawar, W. W. Lipids. In *Food Chemistry*, 3rd ed.; Fennema, O., Ed.; Dekker: New York, 1996; pp 225−319.

(6) Sullivan Ritter, J. C.; Budge, S. M. Key lipid oxidation products can be used to predict sensory quality of fish oils with different levels of EPA and DHA. *Lipids* **2012**, *47*, 1169−1179.

(7) Sullivan, J. C.; Budge, S. M.; St-Onge, M. Modeling the primary oxidation in commercial fish oil preparations. *Lipids* **2011**, *46*, 87−93.

(8) Frankel, E. N.; Satuega-Gracia, T.; Meyer, A. S.; German, J. B. Oxidative stability of fish and algae oils containing long-chain

polyunsaturated fatty acids in bulk and in oil-in-water emulsions. *J. Agric. Food Chem.* **2002**, *50*, 2094−2099.

(9) Gigliotti, J. C.; Davenport, M. P.; Beamer, S. K.; Tou, J. C.; Jaczynski, J. Extraction and characterisation of lipids from Antarctic krill (*Euphausia superba*). *Food Chem.* **2011**, *125* (3), 1028−1036.

(10) Bustos, R.; Romo, L.; Yanez, K.; Diaz, G.; Romo, C. Oxidative stability of carotenoid pigments and polyunsaturated fatty acids in microparticulate diets containing krill oil for nutrition of marine fish larvae. *J. Food Eng.* **2003**, *56* (2−3), 289−293.

(11) Chen, F.; Li, H. B.; Wong, R. N. S.; Ji, B.; Jiang, Y. Isolation and purification of the bioactive carotenoid zeaxanthin from the microalga *Microcystis aeruginosa* by high-speed counter-current chromatography. *J. Chromatogr., A* **2005**, *1064*, 183−186.

(12) Li, H.-B.; Cheng, K.-W.; Wong, C.-C.; Fan, K.-W.; Chen, F.; Jiang, Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chem.* **2007**, *102*, 771−776.

(13) Miranda, M. S.; Cintra, R. G.; Barros, S. B. M.; Mancini-Filho, J. Antioxidant activity of the microalga *Spirulina maxima*. *Braz. J. Med. Biol. Res.* **1998**, *31* (1), 1075−1079.

(14) Goiris, K.; Muylaert, K.; Fraeye, I.; Foubert, I.; Brabanter, J.; Cooman, L. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *J. Appl. Phycol.* **2012**, *24* (6), 1477−1486.

(15) Ryckebosch, E.; Bruneel, C.; Termote-Verhalle, R.; Muylaert, K.; Foubert, I. Nutritional evaluation of omega-3 LC-PUFA rich microalgae oils as an alternative for fish oil. *Food Chem.***2013**, submitted for publication.

(16) Chen, L.; Liu, T.; Zhang, W.; Chen, X.; Wang, J. Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. *Bioresour. Technol.* **2012**, *111*, 208−214.

(17) Ryckebosch, E.; Muylaert, K.; Eeckhout, M.; Ruyssen, T.; Foubert, I. Influence of drying and storage on lipid and carotenoid stability of the microalga *Phaeodactylum tricornutum*. *J. Agric. Food Chem.* **2011**, *59* (20), 11063−11069.

(18) Ryckebosch, E.; Cuéllar Bermúdez, S. P.; Termote-Verhalle, R.; Bruneel, C.; Muylaert, K.; Parra-Saldivar, R.; Foubert, I. Influence of extraction solvent system on the extractability of lipid components from the biomass of *Nannochloropsis gaditana*. *J. Appl. Phycol.* **2013**, submitted for publication.

(19) Ryckebosch, E.; Muylaert, K.; Foubert, I. Optimization of an analytical procedure for extraction of lipids from microalgae. *J. Am. Oil Chem. Soc.* **2012**, *89*, 189−198.

(20) Christie, W. W. *Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids*, 3rd ed.; The Oily Press: Bridgewater, UK, 2003.

(21) Wright, S. W.; Jeffrey, S. W.; Mantoura, R. F. C.; Llewellyn, C. A.; Bjornland, T.; Repeta, D.; Welschmeyer, N. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine-phytoplankton. *Mar. Ecol.: Prog. Ser.* **1991**, *77*, 183−196.

(22) Wrolstad, R. E.; Acree, T. E.; Decker, E. A.; Penner, M. H.; Reid, D. S.; Schwartz, S. J.; Shoemaker, C. F.; Smith, D. M.; Sporns, P. *Handbook of Food Analytical Chemistry*; Wiley: Hoboken, NJ, 2005.

(23) Lee, H.; Kizito, S. A.; Weese, S. J.; Craig-Schmidt, M. C.; Lee, Y.; Wei, C.-I.; An, H. Analysis of headspace volatile and oxidized volatile compounds in DHA-enriched fish oil on accelerated oxidative storage. *J. Food Sci.* **2003**, *68* (7), 2169−2177.

(24) Frankel, E. N. *Lipid Oxidation*, 2nd ed.; The Oily Press, PJ Barnes & Associates: Bridgwater, UK, 2005.

(25) Elmore, J. S.; Campo, M. M.; Enser, M.; Mottram, D. S. Effect of lipid composition on meat-like model systems containing cysteine, ribose, and polyunsaturated fatty acids. *J. Agric. Food Chem.* **2002**, *50*, 1126−1132.

(26) Kangani, C. O.; Kelley, D. E.; Delany, J. P. New method for GC/FID and GC-C-IRMS analysis of plasma free fatty acid concentration and isotopic enrichment. *J. Chromatogr., B* **2008**, *873* (1), 95−101.

(27) Hornero-Méndez, D.; Pérez-Gálvez, A.; Mínguez-Mosquera, M. I. A rapid spectrophotometric method for the determination of peroxide value in food lipids with high carotenoid content. *J. Am. Oil Chem. Soc.* **2001**, *78* (11), 1151−1155.

(28) Griinari, J. M. Novel omega-3 ingredients from krill (*Euphausia superba*). Presented at the Lipid Forum on Novel Sources for Omega-3 for Food and Feed, Copenhagen, Denmark, Nov 14−15, 2012.

(29) Bruheim, I.; Oterhals, A.; Ole Haugsgjerd, B.; Vogt, G.; Griinari, M.; Thomsen, B. R.; Lu Fung Sieng, H.; Jacobsen, C. Oxidative stability of krill (*Euphasia superba*) oil. Presented at the 104th AOCS Annual Meeting & Expo, Montréal, Québec, Canada, 28 April 28−May 1, 2013.

(30) Jacobsen, C.; Hartvigsen, K.; Lund, P.; Meyer, A. S.; Adler-Nissen, J.; Holstborg, J.; Hølmer, G. Oxidation in fish oil enriched mayonnaise. *Eur. Food Res. Technol.* **1999**, *210* (1), 13−30.

(31) Hartvigsen, K.; Lund, P.; Hansen, L. F.; Hølmer, G. Dynamic headspace gas chromatography/mass spectrometry characterization of volatiles produced in fish oil enriched mayonnaise during storage. *J. Agric. Food Chem.* **2000**, *48* (10), 4858−4867.

(32) Jacobsen, C.; Hartvigsen, K.; Thomsen, M. K.; Hansen, L. F.; Lund, P.; Skibsted, L. H.; Hølmer, G.; et al. Lipid oxidation in fish oil enriched mayonnaise: calcium disodium ethylenediaminetetraacetate, but not gallic acid, strongly inhibited oxidative deterioration. *J. Agric. Food Chem.* **2001**, *49* (2), 1009−1019.

(33) Venkateshwarlu, G.; Let, M. B.; Meyer, A. S.; Jacobsen, C. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil-enriched milk emulsion. *J. Agric. Food Chem.* **2004**, *52*, 311−317.

(34) Heinzelmann, K.; Franke, K.; Velasco, J.; Màrquez-Ruiz, G. Microencapsulation of fish oil by freeze-drying techniques and influence of process parameters on oxidative stability during storage. *Eur. Food Res. Technol.* **2000**, *211* (4), 234−239.

(35) Velasco, J.; Dobarganes, M.; Márquez-Ruiz, G. Oxidation of free and encapsulated oil fractions in dried microencapsulated fish oils. *Grasas Aceites* **2000**, *51* (6), 439−446.

(36) Frankel, E. N.; Satué-Gracia, T.; Meyer, A. S.; German, J. B. Oxidative stability of fish and algae oils containing long-chain polyunsaturated fatty acids in bulk and in oil-in-water emulsions. *J. Agric. Food Chem.* **2002**, *50* (7), 2094−2099.

(37) Hamre, K. Metabolism, interactions, requirements and functions of vitamin E in fish. *Aquacult. Nutr.* **2011**, *17*, 98−115.

(38) Seppanen, C.; Song, Q.; Csallany, A. S. The antioxidant functions of tocopherol and tocotrienol homologues in oils, fats, and food systems. *J. Am. Oil Chem. Soc.* **2010**, *87*, 469−481.

(39) Kolanowski, W.; Ziolkowski, M.; Weißbrodt, J.; Kunz, B.; Laufenberg, G. Microencapsulation of fish oil by spray drying - impact on oxidative stability. Part 1. *Eur. Food Res. Technol.* **2006**, No. 222, 336−342.

(40) Aghbashlo, M.; Mobli, H.; Rafiee, S.; Madadlou, A. Optimization of emulsification procedure for mutual maximizing the encapsulation and exergy efficiencies of fish oil microencapsulation. *Powder Technol.* **2012**, *225*, 107−117.

(41) Baik, M.-Y.; Suhendro, E. L.; Nawar, W. W.; McClements, D. J.; Decker, E. A.; Chinachoti, P. Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. *J. Am. Oil Chem. Soc.* **2004**, *81* (4), 355−360.

(42) Barret, A. H.; Porter, W. L.; Marando, G.; Chinachoti, P. Effect of various antioxidants, antioxidant levels, and encapsulation on the stability of fish and flaxseed oils: assessment by fluorometric analysis. *J. Food Process. Preserv.* **2011**, *35* (3), 349−358.

(43) Yi, O.-S.; Han, D.; Shin, H.-K. Synergistic antioxidative effects of tocopherol and ascorbic acid in fish oil/lecithin/water system. *J. Am. Oil Chem. Soc.* **1991**, *68* (11), 881−883.

(44) Yanishlieva, N. V.; Marinova, E. M. Stabilisation of edible oils with natural antioxidants. *Eur. J. Lipid Sci. Technol.* **2001**, *103* (11), 752−767.

(45) Shimidzu, N.; Goto, M.; Miki, W. Carotenoids as singlet oxygen quenchers in marine organisms. *Fish. Sci.* **1996**, *62* (1), 134−137.

(46) Song, J.-H.; Inoue, Y.; Miyazawat, T. Oxidative stability of docosahexaenoic acid-containing oils in the form of phospholipids,

triacylglycerols, and ethyl esters. *Biosci., Biotechnol., Biochem.* **1997**, *61* (12), 2085−2088.
(47) Khan, M. A.; Shahidi, F. Tocopherols and phospholipids enhance the oxidative stability of borage and evening primrose triacylglycerols. *J. Food Lipids* **2000**, *7* (3), 143−150.
(48) Nwosu, C. V.; Boyd, L. C.; Sheldon, B. Effect of fatty acid composition of phospholipids on their antioxidant properties and activity index. *J. Am. Oil Chem. Soc.* **1997**, *74* (3), 293−297.
(49) Müller, L.; Fröhlich, K.; Böhm, V. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. *Food Chem.* **2011**, *129* (1), 139−148.
(50) Kovacik, J.; Klejdus, B.; Backor, M. Physiological responses of *Scenedesmus quadricauda* (Chlorophyceae) to UV-A and UV-C light. *Photochem. Photobiol.* **2010**, *86* (3), 612−616.
(51) Podsedek, A. Natural antioxidants and antioxidant capacity of *Brassica* vegetables: a review. *LWT − Food Sci. Technol.* **2007**, *40* (1), 1−11.
(52) Panteli, E.; Saratsioti, P.; Stamatis, H.; Voutsas, E. Solubilities of cinnamic acid esters in organic solvents. *J. Chem. Eng. Data* **2010**, *55* (2), 745−749.
(53) Lyberg, A.-M.; Fasoli, E.; Adlercreutz, P. Monitoring the oxidation of docosahexaenoic acid in lipids. *Lipids* **2005**, *40* (9), 969−979.
(54) Yamaguchi, T.; Sugimura, R.; Shimajiri, J.; Suda, M.; Abe, M.; Hosokawa, M.; Miyashita, K. Oxidative stability of glyceroglycolipids containing polyunsaturated fatty acids. *J. Oleo Sci.* **2012**, *61* (9), 505−513.
(55) Young, A. J.; Lowe, G. M. Antioxidant and prooxidant properties of carotenoids. *Arch. Biochem. Biophys.* **2001**, *385* (1), 20−27.
(56) Van Durme, J.; Goiris, K.; De Winne, A.; De Cooman, L.; Muylaert, K. Chemical-analytical aroma analysis and sensory evaluation of five species of microalgae. *J. Agric. Food Chem.* **2013**, submitted for publication.
(57) de Koning, A. J. The free fatty acid content of fish oil: the effect of lime addition on the reduction of the free fatty acid content of fish oil during the fish meal and oil production process. *Int. J. Food Prop.* **2001**, *4* (1), 171−177.