JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Influence of Drying and Storage on Lipid and Carotenoid Stability of the Microalga *Phaeodactylum tricornutum*

Eline Ryckebosch,[*,†] Koenraad Muylaert,[‡] Mia Eeckhout,[§] Tony Ruyssen,[§] and Imogen Foubert[†]

[†]Research Unit Food & Lipids, Department of Molecular and Microbial Systems Kulak, Leuven Food and Nutrition Research Centre (LFoRCe), K.U.Leuven Kulak, Etienne Sabbelaan 53, 8500 Kortrijk, Belgium

[‡]Laboratory of Aquatic Biology, Biology Department Kulak, K.U.Leuven Kulak, Etienne Sabbelaan 53, 8500 Kortrijk, Belgium

[§]Faculty of Biosciences, Department of Food Science and Technology, University College Ghent, Valentin Vaerwyckweg 1, 9000 Gent, Belgium

ABSTRACT: The influence of short-term storage and spray- and freeze-drying of fresh microalgal paste on the stability of lipids and carotenoids of *Phaeodactylum tricornutum* was investigated. Furthermore, the effects of storage time (14 and 35 days) and condition (vacuum packed vs non vacuum packed, −20 °C vs 4 °C vs 20 °C) after spray- and freeze-drying were studied. Total lipid content, free fatty acid content, carotenoid content and degree of lipid oxidation were measured. No effects of spray- and freeze-drying and subsequent storage were found on total lipid content, except for short-term storage of the fresh microalgal paste, which led to pronounced lipolysis and therefore a lower total lipid content. Freeze-dried microalgae were found to be more susceptible to lipolysis upon storage than spray-dried microalgae. On the other hand, spray-dried microalgae were more susceptible to oxidation than freeze-dried microalgae, possibly due to breakdown of protecting carotenoids upon spray-drying. Hardly any effect of storage condition was observed for any of the parameters tested.

KEYWORDS: algae, lyophilization, freeze-drying, spray-drying, lipolysis, carotenoids, lipid oxidation

INTRODUCTION

Microalgae are unicellular organisms that contain chlorophyll and produce oxygen (O_2) and immobilize carbon dioxide (CO_2) from the atmosphere through photosynthesis. There are about 100,000 different types of microalgae living not only in the oceans but also in fresh water (lakes, ponds, and rivers).[1]

Microalgae have a great potential as a source of nutritionally interesting and bioactive ingredients that can enhance the nutritional value of food or feed. Microalgae contain potentially interesting proteins (10−40% of DW), lipids (10−30% of DW) and carbohydrates (5−30% of DW). The composition however varies according to species.[2] Moreover, microalgae are cultivated as a source of highly valuable functional ingredients such as carotenoids, fatty acids, phycobiliproteins, polysaccharides, tocopherols, and phenolic compounds.[3]

When the microalgal biomass is to be used in the food, feed, or pharmaceutical industry, it should be possible to preserve and store it, e.g. to permit transport from production to consumer. The most obvious solution hereto is to dry the microalgal biomass. This leads not only to the removal of water to minimize microbial growth and deterioration by chemical reactions but also to a weight and volume reduction making the product more convenient and cheaper to transport, store, and use.[4] A main concern is however the fact that the biochemical composition and thus the nutritional value might be altered by the drying and storage technique.[5] Drying is often also the most expensive step of the whole processing method.

The choice of drying method depends on the production scale and the properties and application of the product that needs to be dried. Freeze-drying (lyophilization) and spray-drying are the most commonly used techniques for high value products.[6] Freeze-drying is the most gentle drying method. Due to the expensive equipment and the high energy consumption, it is however only used for applications that require the conservation of the biochemical composition. Spray-drying has its application in fast and uninterrupted drying of solutions and emulsions.[7] Because of the high cost, the applications are limited to products with a high added value. When the spray-dried product is vacuum packed, it can be stored for several months.[8] However, spray-drying can lead to damage of different thermolabile components. For certain applications, it is also necessary to add antioxidants.[7]

Despite the importance of drying and storage for future commercial application of microalgae, it is a research area with a very limited number of publications. The research of Esquivel et al.[5] showed that air and freeze-drying of the microalgae *Phaeodactylum tricornutum* and *Chaetoceros* sp. caused a loss of 70% of the lipids immediately after drying, while this was not the case when freezing. Morist et al.[8] studied the influence of spray-drying of *Spirulina platensis* on, among others, the fatty acid profile. No significant differences were detected in the content of the most abundant fatty acids (palmitic acid, γ-linolenic acid, linoleic acid, oleic acid, and palmitoleic acid in order of abundance). Babarro et al.[9] investigated the influence of preservation by freezing and freeze-drying and subsequent storage (7 days and 3 months) on the biochemical composition of a fresh culture of *Isochrysis galbana*. Preservation method did not show an influence on the lipid concentration, but after 3 months storage of the freeze-dried material, the percentage of polyunsaturated fatty acids was decreased from 37 to 26%. Zepka et al.[10]

Received: June 27, 2011
Accepted: August 25, 2011
Revised: August 25, 2011
Published: August 25, 2011

ACS Publications © 2011 American Chemical Society

observed a clear effect of the tray drying conditions (temperature and tray thickness) on the total lipid concentration but not on the ratio of saturated to unsaturated fatty acids of *Aphanothece microscopic Nägeli*. When processing Spirulina, Oliveira et al.[4] noted an effect of air drying conditions (temperature and sample thickness) on the degree of oxidation as measured by the TBA value.

The aim of this research was to evaluate different techniques of preservation and subsequent storage for *Phaeodactylum tricornutum*, a saltwater microalgae species rich in the polyunsaturated fatty acid eicosapentaenoic acid (EPA). The influence of short-term storage of fresh microalgal paste and spray- and freeze-drying and subsequent storage under different conditions on the stability of the lipids and carotenoids was investigated. Apart from the lipid and carotenoid content, also the lipid degradation products such as free fatty acids and oxidation products were measured, as they provide a more sensitive means to monitor lipid stability.

MATERIALS AND METHODS

Chemicals. *Solvents:* chloroform HPLC, methanol super gradient HPLC, and dichloromethane HPLC (Labscan, Boom B.V., Meppel, The Netherlands); *n*-hexane GC-grade (Fluka), 2,2,4-trimethylpentane (isooctane) (Sigma-Aldrich, Sigma-Aldrich N.V., Bornem, Belgium).

Reagents: anhydrous sodium sulfate (Sigma-Aldrich), sulfuric acid (Sigma-Aldrich), sodium chloride (Sigma-Aldrich), diisopropylethylamine (Sigma-Aldrich), dimethylamine (Sigma-Aldrich, Sigma-Aldrich N.V., Bornem, Belgium), bis(2-methoxyethyl)aminosulfur trifluoride (TCI Europe N.V., Zwijndrecht, Belgium).

Internal standard: arachidic acid (Nu-check Prep Inc., Elysian, Minnesota).

Microalgae Cultivation and Harvest. *Phaeodactylum tricornutum* CCAP 1055/1 was obtained from Culture Collection of Algae and Protozoa, United Kingdom. The cells were grown in batch culture in 30 L Plexiglas columns, containing 25 L of WC-medium[11] to which 30 g/L artificial sea salt (Homarsel, Zoutman Industries, Roeselare, Belgium) was added. The culture was continuously illuminated with two fluorescent lights (Sylvania GRO-LUX, 36 W), aerated with 0.3 μm filtered air and kept at 15−17 °C.

Growth was monitored by measuring the optical density at 550 nm. The microalgae were harvested in the stationary phase (day 12) by centrifugation.

Experimental Setup. The microalgae were harvested and divided into three batches: (1) The fresh microalgal paste was stored at 4 °C for 48 h. (2) The fresh microalgal paste was diluted with supernatant to 2 L, stored overnight at 4 °C and subsequently spray-dried using a mobile minor Niro-Atomizer. The inlet air temperature was set to 180 °C, while the outlet air temperature was set to 70 °C. The atomizer wheel rotated at a maximum speed of ca. 25,000 rpm. (3) The fresh microalgal paste was stored overnight at −80 °C and subsequently freeze-dried in a Heto Drywinner model DW3. The total lipid content, FFA content, carotenoid content, and lipid oxidation were determined 2 days after harvest for each of these groups. Subsequently, the spray- and freeze-dried microalgae were vacuum packed and stored in a dark environment at different temperatures: −20 °C, 4 °C, and 20 °C. Non vacuum packed spray- and freeze-dried microalgae were stored in a dark environment at 4 °C. The total lipid content, FFA content, carotenoid content, and lipid oxidation were determined after 14 days and 35 days.

Chemical Analyses. *Determination of Total Lipid Content.* Microalgae were extracted according to the chloroform/methanol/water extraction described earlier.[12] Briefly, 400 mg of fresh algae paste or 100 mg of lyophilized or spray-dried algae was mixed with 4 mL of methanol. 2 mL of chloroform and 0.4 mL of water were added. The mixture was vortexed for 30 s. 2 mL chloroform and 2 mL of water were added, and the mixture was vortexed again. The tubes were centrifuged at 2000 rpm for 10 min. The upper layer was removed, and the lower layer was transferred into a clear tube. The solids were reextracted with 4 mL of chloroform/methanol 1:1. The combined solvent layers were passed through a layer of anhydrous sodium sulfate using Whatman no. 1 filter paper in a funnel. The solvent was removed using a rotary evaporator. The lipid content was determined gravimetrically. The extraction was performed in triplicate.

Determination of Free Fatty Acid (FFA) Content. Prior to extraction, 0.45 mg of arachidic acid (C20:0) in chloroform (150 μL) was added as an internal standard. The FFA content was determined by selective formation of dimethyl amide derivatives according to Kangani et al.[13] The dimethyl amide derivatives were analyzed on a GC8000 (Carlo Erba Instruments, Interscience, Louvain-la-Neuve, Belgium) containing a cold on-column injection port and a flame ionization detector (FID). An EC Wax column of length 30 m, i.d. 0.32 mm, film thickness 0.25 μm (GRACE, Lokeren, Belgium) was used. The following temperature program was applied: 100 °C to 160 °C (10 °C/min), 160 °C to 240 °C (2 °C/min), 240 °C (7 min). Peak areas were quantified with Chrom-card for Windows software (Interscience, Louvain-la-Neuve, Belgium). The areas of the peaks that were not present in the blank were summed and compared to the area of the internal standard (C20:0). The determination of FFA content was performed in duplicate.

Determination of Carotenoid Content. The carotenoids were extracted from the microalgae with methanol, followed by the measurement of the absorbance at 470, 665, and 652 nm (Ultrospec 1000 UV/visible spectrophotometer) according to Wellburn.[14] From these absorbance measurements, he developed an equation to determine the total amount of carotenoids taking into account the spectrophotometric interference of chlorophyll a and b. Since *P. tricornutum* does not contain chlorophyll b,[15] this term was removed from the equation, yielding the following equations to calculate the amount of carotenoids:

$$C_a = (15.65A_{665}) - (7.34A_{652})$$

$$X_{carotenoids} = \frac{(1000A_{470}) - (2.86C_a)}{221} \times DF \times V$$

where C_a is the concentration of chlorophyll a, A_{665} is the absorbance at 665 nm, A_{652} is the absorbance at 652 nm, A_{470} is the absorbance at 470 nm, $X_{carotenoids}$ is the amount of carotenoids extracted (μg), DF is the dilution factor, and V is the volume (= 5 mL).

The extraction was repeated five times, since additional extractions only yielded 1% surplus of the total carotenoids. The total amount of carotenoids (g/100 g DW) was calculated as the sum of the amount of the five consecutive extractions, divided by dry weight. The determination of carotenoid content was performed in duplicate.

Determination of Lipid Oxidation. The degree of lipid oxidation was determined by measuring the primary lipid oxidation products using a spectrophotometric method. The UV absorbance at 233 and 268 nm (Ultrospec 1000 UV/visible spectrophotometer) monitors the formation of conjugated dienes (CDs) and trienes (CTs) of polyunsaturated fatty acids respectively. The sum of the extinction values of CD and CT was used as a measure of the degree of oxidation.

Carotenoid containing oils may interfere in this assay by giving higher than expected absorbance values at 233 nm, due to the presence of double bonds in the conjugated structures of carotenoids. Nevertheless, the relative increase of the extinction values is a good measure for the progress of the oxidation reaction.[16] The determination of lipid oxidation was performed in duplicate.

Statistics. Results were statistically evaluated using one-way or multiple-way analysis of variance (ANOVA) and a post hoc Tukey's test with $\alpha = 0.05$ (Sigmaplot 11, Systat Software Inc., Chicago).

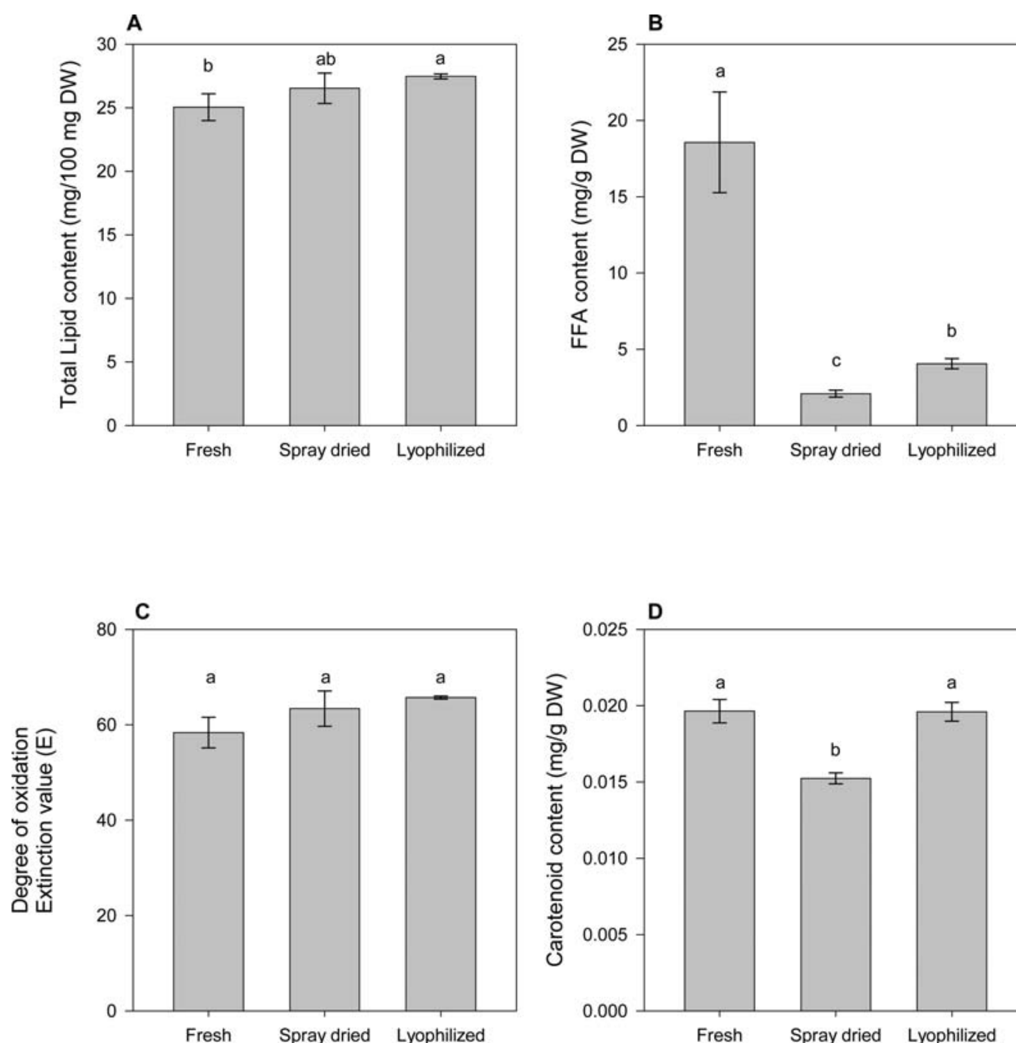

Figure 1. Influence of short-term storage, spray-drying and lyophilization of *P. tricornutum* on the total lipid content (A), FFA content (B), degree of oxidation (C), and carotenoid content (D).

RESULTS

Influence of Short-Term Storage of Microalgal Paste and Spray- and Freeze-Drying. Short-term storage of microalgal paste (48 h) or spray- or freeze-drying did not lead to significant differences in the degree of lipid oxidation (Figure 1C). However, the amount of FFA was significantly higher in the stored fresh microalgae than in the dried microalgae (Figure 1B). The total lipid content of the fresh microalgae was significantly lower than that of the freeze-dried microalgae (Figure 1A). Finally, the carotenoid content of the spray-dried microalgae was significantly lower than that of the fresh and freeze-dried algae (Figure 1D).

Influence of Storage Time and Conditions after Spray-Drying. The spray-dried microalgae were vacuum packed and stored at different temperatures: −20 °C, 4 °C, and 20 °C. Microalgae that were not vacuum packed were stored at 4 °C. The total lipid content, FFA content, carotenoid content, and degree of lipid oxidation of the spray-dried microalgae were determined after 14 and 35 days.

No significant interaction was found between storage time and storage condition for total lipid, FFA, and carotenoid content. The total lipid and carotenoid content were not significantly different between different time points and between different storage conditions (Figure 2A,D). On the other hand, the FFA content was significantly higher after 14 days of storage, but did not increase further upon additional storage. The FFA content was not significantly influenced by the storage condition.

There was a significant interaction between storage time and storage condition for the degree of oxidation. After 14 days some oxidation occurred in the vacuum packed algae at 4 °C and 20 °C and in the microalgae that were not vacuum packed. Vacuum packed storage at −20 °C resulted in a significantly lower degree of oxidation at this point of time. After 35 days, the oxidation was more pronounced and the extent was independent of the storage conditions (Figure 2C).

Influence of Storage Time and Conditions after Freeze-Drying. The freeze-dried microalgae were vacuum packed and stored at different temperatures: −20 °C, 4 °C, and 20 °C. Microalgae that were not vacuum packed were stored at 4 °C. The total lipid, FFA, and carotenoid content and the degree of lipid oxidation of the freeze-dried microalgae were determined after 14 and 35 days.

No significant interaction was found between storage time and storage condition for the total lipid and the carotenoid content.

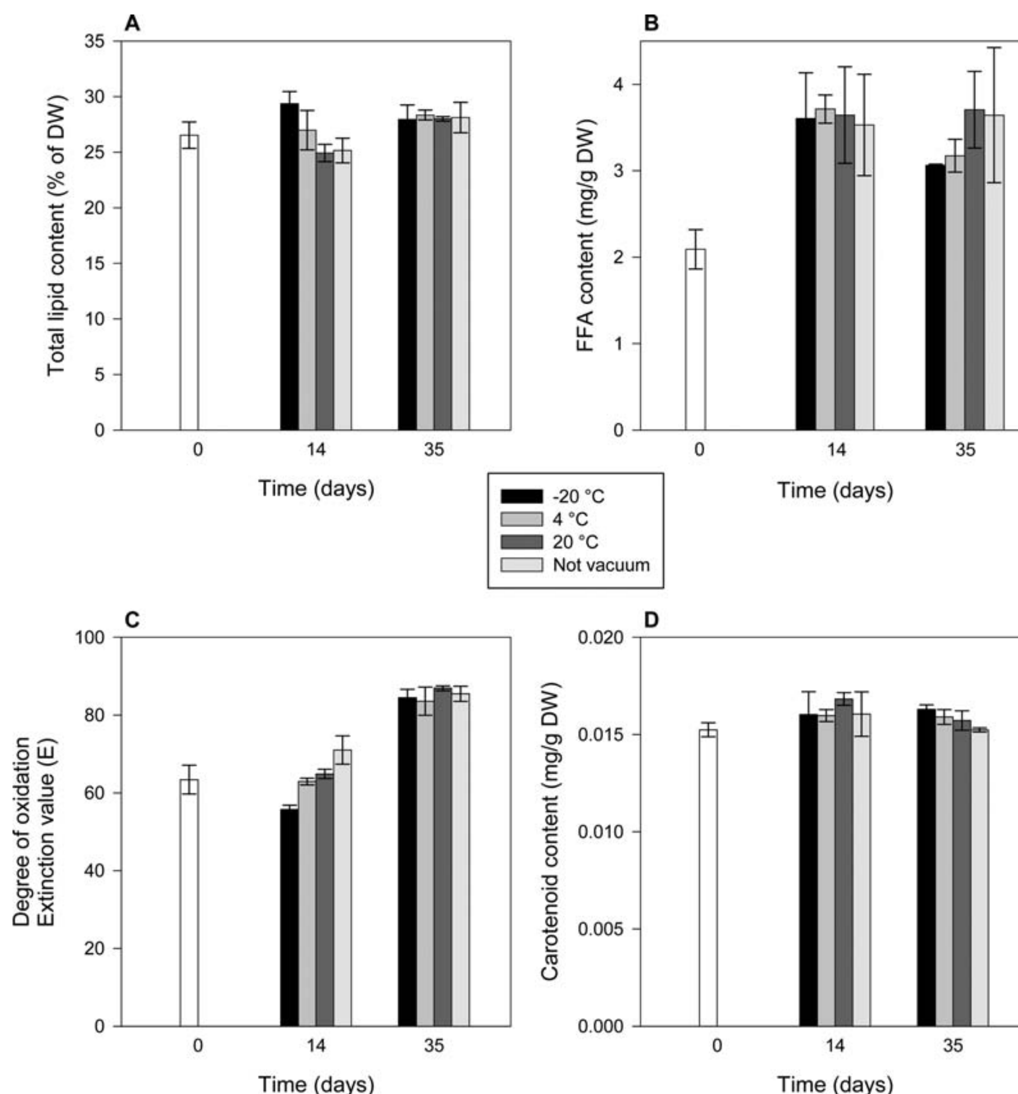

Figure 2. Influence of storage time and conditions on the total lipid content (A), FFA content (B), degree of oxidation (C), and carotenoid content (D) of spray-dried *P. tricornutum*.

Furthermore, the total lipid content was not significantly different between different points in time or between different storage conditions (Figure 3A). The carotenoid content was constant during the first 14 days, but decreased upon longer storage (35 days). The storage conditions did not have a significant influence on the carotenoid content (Figure 3C).

A significant interaction was found between storage time and storage condition for the FFA content and the degree of oxidation. For all storage conditions, the FFA content was significantly higher after 14 days of storage, and still increased upon additional storage (35 days). The increase was significantly less pronounced when the microalgae were stored at −20 °C (Figure 3B). The degree of oxidation was not significantly different for the different storage times. Storage at −20 °C and 4 °C resulted in a somewhat lower degree of oxidation than storage at 20 °C and non vacuum packed storage.

DISCUSSION

Influence of Short-Term Storage of Fresh Microalgal Biomass. When storing fresh microalgal biomass, lipolysis by naturally occurring lipases occurs. This degradation process releases free fatty acids from the glycerol backbone of the microalgal lipids, which can be seen by an increase in the amount of FFA. The lipolysis can also explain the decrease in the total lipid content. The free glycerol backbones, with or without sugar or phosphate group attached, remaining after total lipolysis, are, due to their high polarity, not extracted through the lipid extraction method used. From this experiment, it can thus be concluded that even short-term storage of fresh microalgal paste before further processing or use should be avoided, since it leads to the formation of FFA. These results coincide with Bergé et al.,[17] who also noted that any (short) delay between cell harvesting and addition of boiling water (to inactivate the lipases) resulted in FFA production in *Skeletonema costatum*, thus confirming the presence of high levels of lipases.

Freeze- or spray-drying removes the water from the microalgal biomass, thus lowering the activity of the lipases.[18] This can probably explain the much lower FFA values after drying than after wet storage.

Influence of Storage of Microalgal Biomass after Freeze- or Spray-Drying. Spray- and freeze-drying of the microalgae

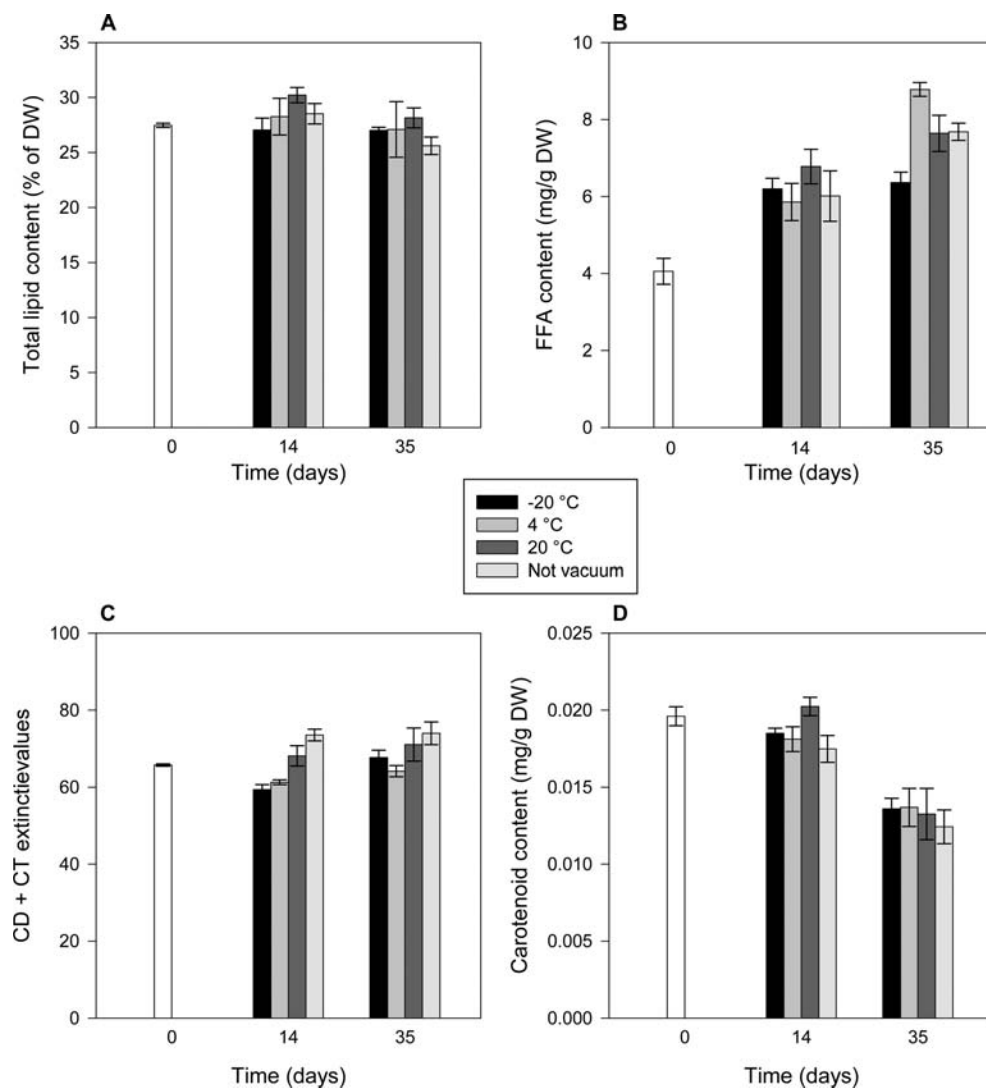

Figure 3. Influence of storage time and conditions on the total lipid content (A), FFA content (B), degree of oxidation (C), and carotenoid content (D) of freeze-dried *P. tricornutum*.

yielded different changes in chemical composition, and their subsequent storage again altered the chemical composition in a different way.

No differences were observed in the total lipid content on drying and storage of the microalgal biomass. This is in contrast to what Esquivel et al.[5] observed, but similar to the result of Babarro et al.[9] A possible explanation for these contradicting results is storage of the fresh microalgal paste prior to processing. As shown in this research, even very short term storage of fresh biomass can lead to pronounced lipolysis and lower amounts of measured lipid content.

A similar FFA content was found in the freeze- and spray-dried microalgae immediately after drying. On storage however, the FFA content increased more in the freeze-dried microalgae compared to the spray-dried microalgae. The lipases in the former sample thus appear to have a higher remaining activity. This can possibly be explained by temperature inactivation of the lipases during spray-drying. Unfortunately, very little is known about the properties (e.g., heat stability) of the lipases of marine diatoms[19] or even marine algae in general.[20] A possible difference in degree of dehydration between spray- and freeze-drying can be added to the explanation. Storage at −20 °C seems to decrease the amount of lipolysis, probably by decreasing the amount of free water and due to a lower enzymatic kinetic at low temperature.

After spray-drying, a 25% decrease in the amount of carotenoids was observed, while this was not the case when freeze-drying. Thermal breakdown and complexation are probably responsible for this decrease. Spray-drying has been shown before[21] to destroy carotenoids although almost all research deals with products containing carotenes, lutein and astaxanthin while the most important carotenoids in *Phaeodactylum tricornutum* are fucoxanthin and diadinoxanthin. In 2009, Prabhasankar et al.[22] published the first report regarding stability of fucoxanthin. They observed that it was not destroyed during pasta production (75 °C for 3 h) and cooking (8 min). Contradicting results and loss of only part of the carotenoids can probably be explained by the fact that the temperature sensitivity of carotenoids depends on the specific structure of the carotenoids, with components with a longer conjugated carbon−carbon double bond structure (such as β-carotene) being more susceptible to temperature.[23] The same authors also noted a dependency of the

temperature susceptibility on the formation of complexes of carotenoids with other components.

Further storage of the spray-dried microalgae did not lower the carotenoid content any further. On the contrary, while freeze-drying did not alter the initial carotenoid content, storage of freeze-dried microalgae caused degradation of the carotenoids to an even greater extent than spray-drying. On the other hand, spray-dried microalgae showed more oxidized lipids than freeze-dried microalgae during storage, although a significant difference could only be observed after 35 days of storage. Possibly there is a correlation between these results. After all, carotenoids are known for their inhibitive properties on lipid oxidation as they react more rapidly with radicals than do the unsaturated acyl chains.[24] So, it may be possible that during storage after freeze-drying the carotenoids protect the lipids from oxidizing but get destroyed themselves during the process. This clear correlation between the disappearance of carotenoids and the inhibition of lipid peroxidation was also clearly shown by Woodall et al.[24] But again the carotenoid structure has an important influence on their antioxidative capacity. Miller et al.[25] concluded from their research that carotenoids are more efficient antioxidants than xanthophylls and that carbonyl and hydroxyl groups on the ring structure of xanthophylls have a suppressive effect on their radical scavenging response. Although the most abundant carotenoids in *Phaeodactylum tricornutum* (fucoxanthin and diadinoxanthin) have not been tested, it can be estimated from the structure−function relationship that they will have a lower antioxidant potential than e.g. β-carotene. Thus after spray-drying, the most potent antioxidant carotenoids have probably already been degraded and are thus not available anymore to protect the lipids from oxidizing. Furthermore, the ultrastructure of microalgal biomass was possibly smaller when spray-dried than when freeze-dried, leading to a greater/larger contact surface with oxygen. This all leads to a more pronounced oxidation of spray-dried compared to freeze-dried microalgae. As the remaining carotenoids do not act as antioxidants (or do less so), they are not degraded in due course.

It could be concluded that neither freeze-drying nor spray-drying followed by storage has an effect on total lipid content. Two days of storage of fresh microalgal paste at 4 °C provokes extensive lipolysis, which leads to a lower total lipid content. Freeze-dried microalgae are more susceptible to lipolysis during storage than spray-dried microalgae. Spray-drying on the other hand leads to loss of carotenoids immediately after drying, and oxidation on storage. No pronounced differences were found between the different storage conditions for any of the parameters tested.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Eline.Ryckebosch@kuleuven-kortrijk.be. Tel: +32 56 24 64 01. Fax: +32 56 24 69 97.

Funding Sources

The research presented in this paper was financially supported by K.U.Leuven Kulak.

ACKNOWLEDGMENT

Lisa Moeyaert is acknowledged for performing most of the experiments in the framework of her MSc thesis.

REFERENCES

(1) Sumi, Y. Microalgae Pioneering the Future, Application and Utilization. *Sci. Technol. Trends, Q. Rev.* **2009**, *34*.

(2) Volkman, J. K.; Brown, M. R. Nutritional value of microalgae and applications. In *Algal cultures, analogues of blooms and applications*; Subba Rao, D. V., Ed.; Science Publishers: Enfield, NH, 2006; Vol. 1, pp 407−457.

(3) Plaza, M.; Herrero, M.; Cifuentes, A.; Ibanez, E. Innovative natural functional ingredients from microalgae. *J. Agric. Food Chem.* **2009**, *57*, 7159–7170.

(4) Oliveira, E. G.; Duarte, J. H.; Moraes, K.; Crexi, V. T.; Pinto, L. A. A. Optimisation of Spirulina platensis convective drying: evaluation of phycocyanin loss and lipid oxidation. *Int. J. Food Sci. Technol.* **2010**, *45*, 1572–1578.

(5) Esquivel, B. C.; Lobina, D. V.; Sandoval, F. C. The biochemical composition of two diatoms after different preservation techniques. *Comp. Biochem. Phys. B* **1993**, *105*, 369–373.

(6) Ratti, C. Hot air and freeze-drying of high-value foods: a review. *J. Food Eng.* **2001**, *49*, 311–319.

(7) Grima, E. M.; Fernandez, F. G. A.; Medina, A. R. Downstream processing of cell-mass and products. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*; Richmond, A., Ed.; Blackwell Science Ltd: Oxford, U.K., 2004; pp 215−251.

(8) Morist, A.; Montesinos, J. L.; Cusido, J. A.; Godia, F. Recovery and treatment of Spirulina platensis cells cultured in a continuous photobioreactor to be used as food. *Process Biochem.* **2001**, *37*, 535–547.

(9) Babarro, J. M. F.; Fernandez Reiriz, M. J.; Labarta, U. Influence of preservation techniques and freezing storage time on biochemical composition and spectrum of fatty acids of Isochrysis galbana clone T-ISO. *Aquacult. Res.* **2001**, *32*, 566–570.

(10) Zepka, L. Q.; Jacob-Lopes, E.; Goldbeck, R.; Queiroz, M. I. Production and biochemical profile of the microalgae Aphanothece microscopica Nägeli submitted to different drying conditions. *Chem. Eng. Process.* **2008**, *47*, 1305–1310.

(11) Guillard, R. R. L.; Lorenzen, C. J. Yellow-green algae with chlorophyllide c. *J. Phycol.* **1972**, *8*, 10–14.

(12) Ryckebosch, E.; Muylaert, K.; Foubert, I. Optimization of an Analytical Procedure for Extraction of Lipids from Microalgae. *J. Am. Oil Chem. Soc.* **2011**, DOI: 10.1007/s11746-011-1903-z.

(13) 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*, 95–101.

(14) Wellburn, A. R. The spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *J. Plant Physiol.* **1994**, *144*, 309–311.

(15) Jeffrey, S. W.; Vesk, M. Introduction to marine phytoplankton and their pigment signatures. In *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*; Jeffrey, S. W., Mantoura, R. F. C., Wright, S. W., Eds.; UNESCO: Paris, France, 2005; Vol. 2, pp 37−84.

(16) Pegg, R. B. Measurement of Primary Lipid Oxidation Products. In *Handbook of food analytical chemistry*; 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., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ, 2005; Vol. 1, pp 515−529.

(17) Berge, J.-P.; Gouygou, J.-P.; Dubacq, J.-P.; Durand, P. Reassessment of lipid composition of the diatom, Skeletonema costatum. *Phytochemistry* **1995**, *39*, 1017–1021.

(18) Han, D.; Walde, P.; Luisi, P. L. Dependence of Lipase Activity on Water Content and Enzyme Concentration in Reverse Micelles. *Biocatal. Biotransform.* **1990**, *4*, 153–161.

(19) Cutignano, A.; d'Ippolito, G.; Romano, G.; Lamari, N.; Cimino, G.; Febbraio, F.; Nucci, R.; Fontana, A. Chloroplastic Glycolipids Fuel Aldehyde Biosynthesis in the Marine Diatom Thalassiosira rotula. *ChemBioChem* **2006**, *7*, 450–456.

(20) Illijas, M. I.; Terasaki, M.; Nakamura, R.; Iijima, N.; Hara, A. Purification and characterization of glycerolipid acyl-hydrolase from the red alga Gracilaria vermiculophylla. *Fish. Sci.* **2008**, *74*, 670–676.

(21) Tang, Y. C.; Chen, B. H. Pigment change of freeze-dried carotenoid powder during storage. *Food Chem.* **2000**, *69*, 11–17.

(22) Prabhasankar, P.; Ganesan, P.; Bhaskar, N.; Hirose, A.; Stephen, N.; Gowda, L. R.; Hosokawa, M.; Miyashita, K. Edible Japanese seaweed, wakame (Undaria pinnatifida) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chem.* **2009**, *115*, 501–508.

(23) Chen, B. H.; Tang, Y. C. Processing and Stability of Carotenoid Powder from Carrot Pulp Waste. *J. Agric. Food Chem.* **1998**, *46*, 2312–2318.

(24) Woodall, A. A.; Britton, G.; Jackson, M. J. Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: Relationship between carotenoid structure and protective ability. *Biochim. Biophys. Acta* **1997**, *1336*, 575–586.

(25) Miller, N. J.; Sampson, J.; Candeias, L. P.; Bramley, P. M.; Rice-Evans, C. A. Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.* **1996**, *384*, 240–242.