



## Impact of processing on n-3 LC-PUFA in model systems enriched with microalgae



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We dedicate this paper to our colleague, Dr. Koen Goiris, who passed away much too early on June 24th, 2017.

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### ABSTRACT

Microalgae have already shown their potential as an alternative source of n-3 LC-PUFA. In this study, 5 different microalgal species (*Isochrysis*, *Nannochloropsis*, *Phaeodactylum*, *Porphyridium* and *Schizochytrium*) were added to an acidic model system and screened on their potential use in acidic food matrices. The impact of mechanical and thermal processing on the model systems was studied by analyzing the amount of n-3 LC-PUFA, free fatty acids, carotenoids, lipid polymers and the oxidative stability. A (limited) reduction of n-3 LC-PUFA was observed. Thermal alterations combined with the presence of free fatty acids seemed to be the causing factor for this decrease. Furthermore, the oxidative stability of model systems enriched with photoautotrophic microalgae was significantly higher than of those enriched with heterotrophic microalgae. It can therefore be concluded that photoautotrophic microalgae low in initial free fatty acid content are a promising source of n-3 LC-PUFA in thermally processed acidic food systems.

### 1. Introduction

The health benefits of omega-3 poly-unsaturated fatty acids are generally accepted and are mainly associated with the consumption of omega-3 long chain poly-unsaturated fatty acids (n-3 LC-PUFA) containing a minimum of 20 carbon atoms, such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). Their role in the prevention of cardiovascular diseases and in brain development has been proven in different epidemiological and clinical studies (Calder, 2014), although a recent study has reported conflicting results of n-3 LC-PUFA supplementation (Aung et al., 2018). A daily intake of 250 mg n-3 LC-PUFA has been recommended (FAO, 2010). Food naturally rich in n-3 LC-PUFA, n-3 LC-PUFA enriched food and n-3 LC-PUFA supplements can attribute to consuming a higher level of n-3 LC-PUFA. However, supplements only reach a small part of the population (Sioen, Devroe, Inghels, Terwecoren, & De Henauw, 2010). The consumption of foods naturally rich in n-3 LC-PUFA, fatty fish and fish oil, is low in most countries and a change in diet would be needed to meet the recommended intake (Molendi-Coste, Legry, & Leclercq, 2011; Sioen et al., 2010). In contrast, no change in eating habits is needed to

enhance the intake of n-3 LC-PUFA via widely consumed enriched food products which reach a larger proportion of the population (Anbudhasan, Surendraraj, Karkuzhali, & Ramasamy, 2014). In this respect, there is an increased interest to enrich food products with n-3 LC-PUFA.

Microalgae, rich in n-3 LC-PUFA, could provide a sustainable alternative source to fish and fish oil, given reducing global fish stocks (Ryckebosch, Bruneel, Muylaert, & Foubert, 2012). Microalgae are unicellular organisms, including photoautotrophic and heterotrophic species and are the primary producers and a vegetarian source of n-3 LC-PUFA. They are characterized by a high growth rate and have been shown to have a limited competition with agriculture since they do not require arable land (Morales-Sánchez, Martínez-Rodríguez, Kyndt, & Martínez, 2014; Rawat, Ranjith Kumar, Mutanda, & Bux, 2013). Microalgae can be cultivated in seawater or fresh water. Seawater microalgae do not depend on limited fresh water supplies and for microalgae that grow in freshwater, water demand is lower than for terrestrial crops because microalgae do not actively transpire water (Posten & Schaub, 2009). Photoautotrophic microalgae obtain their energy from photosynthesis. For industrial production, they are

**Abbreviations:** DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n-3 LC-PUFA, omega-3 long chain poly-unsaturated fatty acids; TAG, triacylglycerol

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cultivated under controlled circumstances in the presence of light and carbon dioxide. Besides n-3 LC-PUFA they contain other high value compounds like antioxidants (Ryckebosch, Bruneel, et al., 2012). Heterotrophic microalgae grow by fermentation on organic substrates without light (Morales-Sánchez et al., 2014). Although they contain a high amount of lipids (70%) they lack carotenoids (Perez-Garcia, Escalante, de-Bashan, & Bashan, 2011). Each cultivation system has advantages and disadvantages, but in general, microalgae offer a great opportunity to innovate and create healthy food products enriched in n-3 LC-PUFA. Previous studies show that in general, heterotrophic oil extracted from the biomass (Park, Kelleher, McClements, & Decker, 2004; Chee et al., 2005; Gallaher, Hollender, Peterson, Roberts, & Coupland, 2005; Lee et al., 2006; Serna-Saldivar, Zorrilla, De La Parra, Stagnitti, & Abril, 2006; Valencia, Ansorena, & Astiasarán, 2007; Lopez-Lopez, Cofrades, & Jiménez-Colmenero, 2009; García-Íñiguez de Ciriano et al., 2010; Kassis, Beamer, Matak, Tou, & Jaczynski, 2010; Tolasa, Lee, & Cakli, 2010; Pietrowski, Tahergerabi, Matak, Tou, & Jaczynski, 2011; Sedoski, Beamer, Jaczynski, Partington, & Matak, 2012; Berasategi et al., 2014; Lamas et al., 2015; Alejandro, Passarini, Astiasarán, & Ansorena, 2017), as well as the whole autotrophic microalgal biomass can serve as delivery system of n-3 LC-PUFA (Gouveia, Batista, Raymundo, & Bandarra, 2008; Gouveia et al., 2008; Fradique et al., 2013; Babuskin, Krishnan, Babu, Sivarajan, & Sukumar, 2014; Babuskin, et al., 2015; Batista et al., 2017; Garcia-Segovia, Pagan-Moreno, & Martinez-Monzo, 2017).

Due to their large number of double bonds, n-3 LC-PUFAs are, however, highly susceptible to lipid oxidation. Oxidation of n-3 LC-PUFA should be avoided, since it results in loss of nutritional value, undesired flavor and the presence of toxic compounds (Frankel, 2005). The presence of (natural) antioxidants in the food matrix can help to prevent the fatty acids from oxidation (Jacobsen, 2010). In this respect, fruit and vegetable based products (for example soups, sauces, smoothies and juices) might be an interesting matrix given their relatively high endogenous antioxidant capacity. Moreover, fruit and vegetable based products are essential in a healthy diet and also contain other high-value compounds, like antioxidants and micronutrients (Darmon, Darmon, Maillot, & Drewnowski, 2005).

Limited research has been performed on the use of microalgae in food products. A dozen studies have focused on the use of heterotrophic microalgal oil in egg products, dairy products, fish, meat and bread (Gheysen, Matton, & Foubert, 2018). Only one of them has taken the impact of typical food processing steps (mechanical and thermal treatments) as well as the oxidative stability of the end product into account. Lee et al. (2006) investigated the enrichment of cooked meat products with n-3 LC-PUFA from heterotrophic microalgal oil. They observed that the cooking step reduced the amount of n-3 LC-PUFA in all samples whether or not antioxidants were added. Only the samples without added antioxidants, however, showed an increase in the amount of oxidation during storage. These results indicate the importance of antioxidants to prevent oxidation in food products enriched with n-3 LC-PUFA. Given that the consumers are highly interested in natural antioxidants (Guedes, Amaro, & Malcata, 2011), it could be more beneficial to add photoautotrophic microalgae, containing endogenous antioxidants, as a source of n-3 LC-PUFA (Ryckebosch, Bruneel, et al., 2012). Studies focusing on the use of photoautotrophic microalgae in food products are, however, even more limited. Some studies about the enrichment of eggs, desserts, paste, bread and plant based oils with photoautotrophic microalgal biomass have been performed, but none of them have investigated the remaining amount of n-3 LC-PUFA after processing and the oxidative stability of the treated product upon storage.

From the above, it is clear that very little research has been performed on n-3 LC-PUFA enrichment of food products by microalgae addressing processing effects and oxidative stability during storage. Notwithstanding their high antioxidative capacity, there are, to the best of our knowledge, no studies focusing on the enrichment of fruit and

vegetable based products with microalgae. Before studying the interaction with real fruit and vegetable based products, in the present study aqueous acidic model systems (representative of acidic fruit and vegetable based products) were enriched with microalgal biomass. The effect of mechanical (high pressure homogenization) and thermal (pasteurization) processing on n-3 LC-PUFA and the endogenous antioxidants was investigated. All enriched model suspensions were characterized for their amount of n-3 LC-PUFA, free fatty acids, carotenoids and lipid polymers. Moreover, the oxidative stability of the enriched model systems was followed during a 12 week storage experiment by measuring the primary and secondary lipid oxidation products. It is hypothesized that the oxidative stability of the microalgal species may be affected by the presence of endogenous antioxidants. Therefore, suspensions enriched with photoautotrophic microalgae are expected to be more oxidatively stable than those enriched with heterotrophic microalgae. Furthermore, it is hypothesized that processing steps may influence the amount of endogenous antioxidants and by association, the oxidative stability.

## 2. Materials and methods

All solvents used for lipid extraction, determination of n-3 LC-PUFA, free fatty acids, lipid polymers, carotenoids and the measurement of primary oxidation (chloroform, methanol, toluene, hexane, dichloromethane, acetonitrile and ethyl acetate) were HPLC grade and purchased from Carl Roth (Karlsruhe, Germany) or Biosolve (Valkenswaard, the Netherlands).

### 2.1. Microalgal biomass

Five different n-3 LC-PUFA rich microalgae (4 photoautotrophic and 1 heterotrophic) were used in this study. The biomass was obtained from different companies: *Isochrysis* sp. and *Nannochloropsis* sp., currently named as *T-Isochrysis* sp. and *Microchloropsis* sp. respectively (Bendif, Probert, Schroeder, & de Vargas, 2013; Fawley, Jameson, & Fawley, 2015), (both photoautotrophic) from Proviron (Hemiksem, Belgium) and *Schizochytrium* sp. (heterotrophic) from Mara Renewables Corporation (Dartmouth, Canada) were delivered as freeze dried biomass. *Phaeodactylum* sp. and *Porphyridium* sp. (both photoautotrophic) were obtained from Necton (Olhão, Portugal) as a wet paste and were freeze dried at Proviron (Hemiksem, Belgium). All biomasses were stored at  $-80^{\circ}\text{C}$  until use. The n-3 LC-PUFA content, fatty acid composition and lipid class composition of the biomass is shown in Table 1. The biomass was characterized in terms of n-3 LC-PUFA and fatty acid composition according to the method described in Section 2.3.2 and lipid class composition according to the method described in Section 2.3.3.

### 2.2. Experimental set up

Fig. 1 shows a general overview of the experimental set up of this study. For each of the different microalgae a model suspension was made according to the steps shown in Fig. 1. At the beginning and after all the different processing steps, the suspensions were analyzed for the amount of n-3 LC-PUFA, free fatty acids, carotenoids and lipid polymers. Moreover, the processed model suspensions were stored for 12 weeks at  $37^{\circ}\text{C}$  and analyzed for the amount of primary and secondary lipid oxidation products at week 0, 2, 4, 8 and 12. For each of the five microalgal species used in this study, the suspension was prepared in twofold and independently treated from its duplicate. The different steps presented in Fig. 1 will be discussed more in detail in Sections 2.2.1–2.2.4.

#### 2.2.1. Preparation of model suspensions

An appropriate amount of freeze dried microalgal biomass (see Table 1) was suspended in demineralized water by mild stirring for

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