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Effect of temperature towards lipid oxidation and non-enzymatic browning reactions in krill oil upon storage

F.S.H. Lu [a], I. Bruheim [b], B.O. Haugsgjerd [c], C. Jacobsen [a,*]

[a] Division of Industrial Food Research, Technical University of Denmark, Søltofts Plads, Building 221, 2800 Kgs. Lyngby, Denmark
[b] Olympic Seafood AS, P.O. Box 234, N-6099 Fosnavaag, Norway
[c] Nofima, Division Aquaculture, BioLab, Kjerreidviken 16, 5141 Fyllingsdalen, Norway

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ABSTRACT

The main objective of this study was to investigate the effect of temperature towards lipid oxidation and non-enzymatic browning reactions in krill oil upon storage. Krill oil was incubated at two different temperatures (20 and 40 °C) for 28 or 42 days. The oxidative stability of krill oil was assessed by peroxide value and anisidine value, measurement of lipid derived volatiles, lipid classes and antioxidants. The non-enzymatic browning reactions were assessed through the measurement of pyrroles, free amino acids content and Strecker-derived volatiles. The increase of incubation temperature firstly increased the lipid oxidation in krill oil and subsequently the non-enzymatic browning reactions. The occurrence of these reactions was most likely due to the reaction between α-dicarbonyl or carbonyl compounds with amino acids or ammonia. In addition to tocopherol and astaxanthin esters, the formation of pyrroles might help to protect the krill oil against lipid oxidation.

1. Introduction

Krill oil has emerged as a new source of long chain *n*–3 polyunsaturated fatty acids (LC PUFA) recently. Just like fish oil, krill oil is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Le Grandois et al., 2009). However, EPA and DHA in krill oil are predominantly bound to phospholipids (PL), mostly to phosphatidylcholine. This structural difference provides krill oil with a better bioavailability and oxidative stability (Burri, 2011; Schuchardt et al., 2011; Thomsen et al., 2013). In addition, krill oil contains astaxanthin, which is a powerful antioxidant and this may prevent the oxidation of EPA and DHA (Tou, Jaczynski, & Chen, 2007). Encapsulated krill oil has been used as a dietary supplement, due to its numerous health benefits (Bunea, El Farrah, & Deutsch, 2004; Ierna, Kerr, Scales, Berge, & Griinari, 2010; Sampalis et al., 2003). For instance, krill oil is more effective than fish oil in improving the physical and emotional symptoms of premenstrual syndrome (Sampalis et al., 2003) and in treating hyperlipidaemia (Bunea et al., 2004). Other health benefits of krill oil are their therapeutic value for metabolic syndrome, non-alcoholic fatty liver disease, attention deficit/hyperactivity deficit disorder and inflammation (Deutsch, 2007; Ierna et al., 2010; Sampalis et al., 2003).

To date, only a few studies have been carried out to investigate the oxidative stability of krill oil or foods enriched with krill oil. For instance, studies on oxidative stability and sensory properties of surimi seafood or novel nutraceutical egg products fortified with krill oil or a blend of krill oil with other *n*–3 fatty acids rich oils were recently performed (Kassis, Gigliotti, Beamer, Tou, & Jaczynski, 2011; Pietrowski, Tahergorabi, Matak, Tou, & Jaczynski, 2011; Sedoski, Beamer, Jaczynski, Partington, & Matak, 2012). The most recent study is on fermented milk products fortified with a neat or pre-emulsified mixture of fish oil and krill phospholipid or other marine phospholipids (Lu, Thomsen, et al., 2013). These studies reported that krill oil fortified products had acceptable sensory properties and this might be due to the high oxidative stability of krill oil. Furthermore, our recent studies showed that oxidative stability of marine PL might be influenced by the presence of pyrroles, which are antioxidative compounds formed as a result of non-enzymatic browning reactions between the primary amine group of phosphatidylethanolamine (PE) or amino acids with the lipid oxidation products in marine PL (Lu, Nielsen, Baron, & Jacobsen, 2012; Lu, Nielsen, Baron, Diehl, & Jacobsen, 2013; Thomsen et al., 2013). Therefore, it is hypothesised that the oxidative stability of krill oil might also be influenced by non-enzymatic browning reactions, as krill oil contains a significant amount of amino phospholipids (PE) and residual amino acids (Thomsen et al., 2013).

* Corresponding author. Tel.: +45 45252559; fax: +45 45884774.
E-mail address: chja@food.dtu.dk (C. Jacobsen).

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In fact, the non-enzymatic browning reaction resulting from oxidised lipids has gained considerable attention recently and has been reviewed extensively by Hidalgo and Zamora (2005). However, studies on non-enzymatic browning reactions in marine PL are scarcely available in the literature except some studies on marine PL from our laboratory (Lu et al., 2012; Lu, Nielsen, et al., 2013) and from Thanonkaew, Benjakul, Visessanguan, and Decker (2006). Thanonkaew et al. (2006) investigated browning development in the marine PL liposome system from the aspect of pyrrolisation. However, they did not provide information about Strecker degradation in their marine PL system. Our recent studies proposed that non-enzymatic browning development in marine PL could be divided into two pathways: (a) pyrrolisation to form pyrroles and (b) Strecker degradation of amino acids if amino acids residues or peptides are present in marine PL. On the basis of studies performed by Hidalgo and Zamora (2005), we hypothesised that lipid oxidation produces α-dicarbonyl derivatives analogous to those of carbohydrates and therefore lipid oxidation products are engaged in non-enzymatic browning reactions. Examples of α-dicarbonyl derivatives from lipid oxidation are unsaturated epoxy keto fatty esters, epoxyalkenals and hydroxyalkenals (tertiary lipid oxidation products with two oxygenated functional groups).

In addition, our findings showed that non-enzymatic browning reactions in marine PL were influenced by the chemical composition of marine PL (the level of PE and residual amino acids), and the marine PL manufacturing processes (temperature and condition of marine PL extraction). Previously, a pilot study was carried out in our laboratory to investigate the lipid oxidation and non-enzymatic browning reactions in krill oil incubated at 40 °C, in comparison to fish oil (Thomsen et al., 2013). The present study is a continuation of this pilot study with the main objective to further investigate the non-enzymatic browning reactions in krill oil, especially from the aspect of pyrazines and pyridines formation, which has not previously been discussed. The secondary objective is to investigate the effect of temperature towards lipid oxidation and non-enzymatic browning reactions in krill oil upon storage.

2. Materials

Krill oil (Rimfrost Sublime) was obtained from Olympic Seafood (Fosnavaag, Norway). This krill oil was obtained from fresh Antarctic krill (*Euphausia superba*) through gentle enzymatic hydrolysis (with proteolytic enzymes, pH 7, at 50 °C, 30–45 min), followed by removal of krill exoskeleton and the water soluble proteins. The lipid-rich sediment was then dried under vacuum and the krill oil was obtained by solvent extraction. It contained approximately 12/100 g of EPA and 7/100 g of DHA. In addition, a low level of transition metals was found in this krill oil: 0.124 ppm of iron and 1.000 ppm of copper.

3. Methods

3.1. Storage of krill oil at elevated temperature

The above mentioned krill oil was incubated at two different temperatures (20 and 40 °C) for 42 and 28 days, respectively. The samplings were made on Day 0, 1, 3, 7, 14, 21, 28 or 42. Eight or seven different jars of krill oil (covered with aluminium foil to avoid contamination) were prepared in two incubators (Refritherm 200, Struers AS, Denmark) and each jar represented one sampling point. Hence, eight jars were used for incubation at 20 °C and seven for incubation at 40 °C. Stirring was made at the sampling time to minimise the need to open the incubator and hence reduce the temperature fluctuation. The major drawback of this experimental design is that some of the volatiles generated in the krill oil were released to the air and could not be measured, especially in the krill oil incubated at 40 °C. This is due to the fact that the release of volatiles in krill oil increased when the incubation temperature increased from 20 to 40 °C (Steenson, Lee, & Min, 2002). In other words, more volatiles were retained in krill oil incubated at 20 °C than 40 °C. The samples were collected in amber bottles and stored in darkness at −40 °C prior to the analyses. The oxidative stability of krill oil was assessed by classic techniques, such as peroxide value (PV) and anisidine value (AV), and also advanced techniques such as a) measurement of both lipid and Strecker-derived volatiles by thermal desorption unit combined with a dynamic headspace unit (TDU/DHS–GC/MS), (b) measurement of pyrroles, (c) measurement of lipid classes, (d) measurement of antioxidants level such as tocopherols and astaxanthin esters and (e) the measurement of free amino acids content in krill oil before and after incubation.

3.2. Measurement of lipid oxidation

3.2.1. Determination of lipid-derived volatiles

The determination of lipid-derived volatiles was done mainly by thermal desorption unit combined with a dynamic headspace unit (TDU/DHS–GC/MS). However, classic DHS-GC/MS (using pear-shaped purge bottle and water bath) was also used to collect volatiles from the selected krill oil samples. For TDU/DHS–GC/MS, approximately 1 g of krill oil in a 10-mL vial was incubated at 60 °C for 4 min with agitation using the TDU/DHS autosampler system (Multipurpose Sampler MPS; Gerstel, Mülheim an der Ruhr, Germany). The volatiles were trapped into Tenax GR tubes (GERSTEL GmbH & Co. nKG, Mülheim an der Ruhr, Germany) by purging the headspace of the vial with nitrogen (trapping flow rate of 50 mL/min) for 20 min. The volatiles were then desorbed (at 280 °C) from the trap in a TDU into the injection port of a gas chromatograph (HP 6890 Series; Hewlett Packard, Palo Alto, CA) with a DB-1701 column, 30 m × 0.25 mm × 1.0 μm (J&W Scientific, Folsom, CA). The oven had an initial temperature at 35 °C for 3 min, then an increment of 3.0 °C/min to 140 °C. Subsequently an increment of 5.0 °C/min to 170 °C and lastly an increment of 10.0 °C/min to 240 °C and held for 8 min. The individual volatile compounds were analysed by HP 5973 MS inert mass-selective detector (Agilent Technologies, Santa Clara, CA). MS settings: electron ionisation mode, 70 eV; mass-to-charge ratio scan between 30 and 250. Some of the volatile compounds were tentatively identified by the MS-library Wiley 138 K. The volatiles were quantified through the abundance values obtained from MS analysis. Therefore, further identification and quantification of these compounds by using authentic standards is required for future work.

For classic DHS–GC/MS, volatiles from 4 g of the selected krill oil samples were collected by purging the oil with nitrogen (150 mL/min) for 30 min at 60 °C, and the volatiles were trapped on Tenax GR tubes (Perkin-Elmer, CN, USA) packed with 225 mg Tenax GR (60–80 mesh, Varian, Middelburg, Netherlands). The volatiles were then desorbed (200 °C) from the trap in an automatic thermal desorber (ATD-400, Perkin-Elmer, Norwalk, CT) and cryofocused on a Tenax GR cold trap. Volatiles were separated by gas chromatography (G1530 A series, Hewlett–Packard, Palo Alto, CA) and analysed by the same method as described above. The measurement was performed in triplicate.

3.2.2. Determination of tocopherol and astaxanthin ester content

The tocopherol content was determined according to AOCS Method Ce 8-89 (AOCS Official method Ce 8-89, 1998). Approximately 0.05 g of marine PL were dissolved in heptane (10 mL) and the extract was analyzed by HPLC–FLD (Agilent 1100 series, Agilent Technologies, Santa Clara, CA) on a Water Spherisorb (R)