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Properties of *Calanus finmarchicus* biomass during frozen storage after heat inactivation of autolytic enzymes

Maria Bergvik [a,*], Ingrid Overrein [b,1], Michael Bantle [c], Jan Ove Evjemo [a], Turid Rustad [d]

[a] Norwegian University of Science and Technology (NTNU), Department of Biology, NO-7491 Trondheim, Norway
[b] SINTEF Fisheries and Aquaculture, Department of Marine Resources Technology, NO-7465 Trondheim, Norway
[c] NTNU, Department of Energy and Process Engineering, NO-7491 Trondheim, Norway
[d] NTNU, Department of Biotechnology, NO-7491 Trondheim, Norway

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ABSTRACT

Calanus finmarchicus is a marine zooplankton of interest for the aquaculture industry, as well as for nutraceuticals and the cosmetic industry. The chemical composition of *C. finmarchicus* rapidly changes *postmortem* due to autolytic processes; in particular phospholipids rapidly degrade to give free fatty acids. The aim of this study was to inactivate autolytic enzymes in *C. finmarchicus* by applying heat (72 °C, 5–30 min) through mixing with boiling, fresh water, and further to explore the effects of heat (70 °C, 15 min) combined with long time storage (−20 °C, 12 months) of treated and untreated material. Heat treatment (5 min) inactivated all tested enzymes and maintained the initial amount of phospholipids, total lipids and crude protein. Storage of untreated material led to complete degradation of all phospholipids, whereas heat treatment resulted in a stable product containing the initial amount of phospholipids and astaxanthin.

1. Introduction

Calanus finmarchicus is the dominant zooplankton species in the Norwegian and Barents Seas (Planque & Batten, 2000). The annual production in the Nordic Seas is roughly estimated to be 74 million tons by wet weight (Aksnes & Blindheim, 1996). This copepod has a generation time of 1–2 years in the Norwegian Sea, and has a dormancy period in deep waters during winter. Copepods hatch as nauplius larvae and progress through six naupliar stages and five copepodite stages before reaching sexual maturity. The females spawn at the time of the spring bloom, and the offspring feed to obtain large lipid deposits in summer and autumn (Marshall & Orr, 1972). The storage lipids of late copepodites and adults are in the form of wax esters, which are fatty acids esterified with fatty alcohols. Wax esters are the most common storage lipids in herbivorous zooplankton feeding on short blooms of phytoplankton (Sargent, Gatten, & Henderson, 1981). The profile of the fatty acids in wax esters will reflect the fatty acid composition of the dietary phytoplankton (Lee, Barnett, & Hirota, 1971), and may thereby contain large amounts of *n*-3 fatty acids. The phospholipids mainly consist of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Fraser, Sargent, & Gamble, 1989) and contains a high share of C22:6 *n*-3 and C20:5 *n*-3 (Farkas, Storebakken, & Bhosie, 1988). Due to its fatty acids, *C. finmarchicus* is a promising marine raw material and an interesting lipid source for commercial use.

World aquaculture has grown steadily in the last 50 years, with an annual growth rate of 8.7% (excluding China) since 1970. Meanwhile, for the last 10–15 years most of the world fisheries are fully or overexploited, and the maximum sustainable catch has been reached, of which 20–30% are used for animal feed (FAO, 2008). Further growth in the aquaculture sector requires alternative feed sources. Vegetable sources can replace part of the fishmeal and oil, but the need for *n*-3 highly unsaturated fatty acids (HUFA) like C22:6 *n*-3 and C20:5 *n*-3 will require new marine lipid sources (Turchini, Torstensen, & Ng, 2009). There has also been an increasing awareness of the importance of marine lipids in human nutrition, and beneficial health effects have been documented from consumption of *n*-3 HUFA which are mainly provided by marine lipids. C22:6 *n*-3 and C20:5 *n*-3 are HUFAs that play a vital role in membrane fluidity, cellular signalling, and gene expression. They have been shown to provide positive benefits in coronary heart disease, diabetes, immune response disorders, and mental health (Larsen, Eilertsen, & Elvevoll, 2011).

A rapid change in lipid- and protein composition *postmortem* is found in *C. finmarchicus* (Overrein, 2010), probably caused by digestive enzymes that are present in copepods (Bond, 1934). Phospholipids will be hydrolysed resulting in *lyso*-phospholipids

* Corresponding author. Tel.: +47 99621498; fax: +47 73596311.
E-mail address: maria.bergvik@bio.ntnu.no (M. Bergvik).
[1] Present address: Overreins LipoTek, NO-7048 Trondheim, Norway.

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and free fatty acids, and *lyso*-phospholipids are further hydrolysed by *lyso*-phospholipases and phospholipases. Proteolytic activity will degrade proteins to peptides and free amino acids. Solgaard, Standal, and Draget (2007), and Solgaard, Thorsen, and Draget (2009) have shown high proteolytic activity in *C. finmarchicus* and also identified a high degree of *postmortem* degradation and subsequent leaching of nutrients as challenges in processing raw material. Similar problems and characteristics are found for other zooplankton species (Grabner, Wieser, & Lackner, 1981), and are also well documented for krill (Ellingsen, 1982; Saether, Ellingsen, & Mohr, 1986, 1987).

To avoid degradation of the raw material, enzymes in *C. finmarchicus* must be inactivated. Heat is the most commonly used denaturing agent in food processing and preservation. From a nutritional standpoint, denaturation of proteins often improves the digestibility and biological availability of essential amino acids (Damodaran, 2008). Enzyme inhibitors and pH alteration are other methods to inactivate enzymes, but for nutritional purposes, a neutral pH would be preferred and inhibitors often have antinutritional effects or are toxic. Drying is also a way to arrest enzymatic activity. However, some enzymes can still be active at low water activities (Parkin, 2008), and the enzymes will be reactivated once the product is rehydrated, as shown in *C. finmarchicus* (Bantle, Eikevik, & Rustad, 2009).

Products with a high content of HUFA are highly susceptible to oxidation during storage. Oxidation results in formation of a variety of volatile compounds giving an unpleasant rancid product; the products formed can also result in possible biological damage. High content of free fatty acids formed by autolytic activity is a parameter for poor quality because they cause off-flavour, reduce oxidative stability and cause foaming (McClements & Decker, 2008). A high content of HUFA gives an even higher oxidation potential. Several strategies can be used to avoid oxidation. Light, heat, oxygen, and pro-oxidants should be avoided to prevent oxidation, while antioxidants will reduce the reaction rate (McClements & Decker, 2008).

The main aim of the present study was to investigate the effect of heat on the activity of proteolytic and lipolytic enzymes in *C. finmarchicus*, and further to evaluate the stability of selected biochemical components during heating and low temperature storage.

2. Material and methods

2.1. Heat treatment experiment

Zooplankton biomass was obtained from two harvests in spring (14th May, 2008) using NTNU's research vessel R/V Gunnerus outside of Frøya off the middle part of the Norwegian coast (63°42′N, 9°00′E). A kite trawl developed at NTNU with plankton nets (mesh size 500 μm) was dragged through the water (1 knot speed) in the surface zone (0–10 m depth) for one hour. Immediately after landing the trawl on deck, the zooplankton was kept on ice and the experiment was started after 30 min. From the zooplankton batch a small sample was fixed in formalin (10%) for identification of species and stages. Ten grams of zooplankton were transferred to 50-mL centrifuge tubes, and boiling fresh water was added giving a total volume of 40 mL in all tubes. The immediate temperature in the mixture was 70 °C, and the tube was incubated in a water bath at 72 ± 2 °C for 5, 10, 15, 20, 25 and 30 min. After heat treatment the samples were immediately placed in ice water. The samples were filtered through a plankton net (mesh size 200 μm) and the two fractions (water and solids) were frozen in preweighed tubes on dry ice (−78.5 °C), which were reweighed on shore. All samples were divided for different biochemical analyses and flushed with

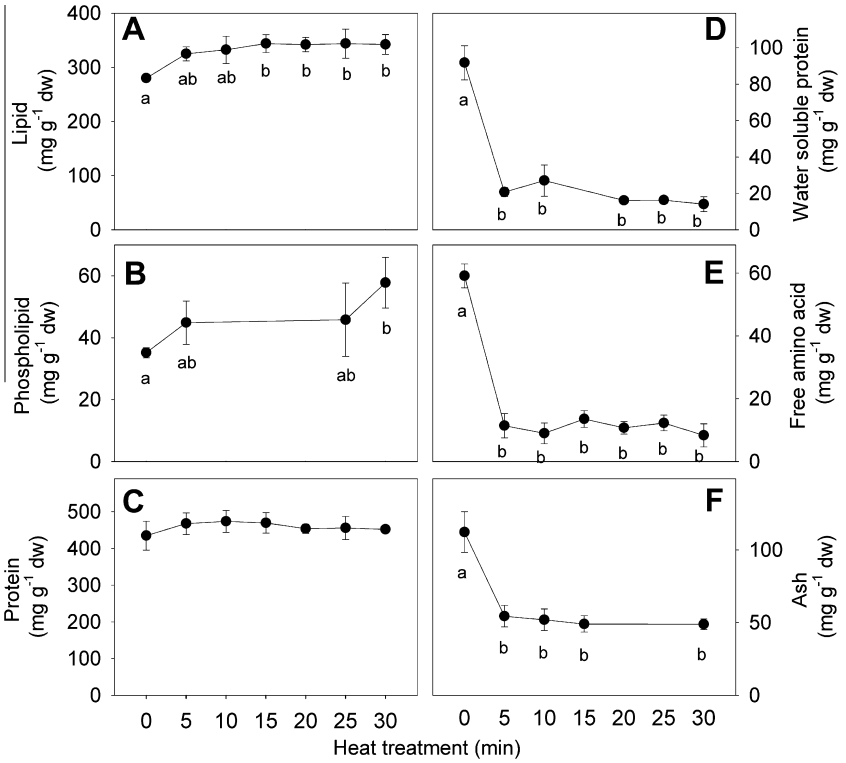

Fig. 1. Biochemical components of the biomass from heat treated *C. finmarchicus* showing total lipid (mg g^{-1} dw) (A), phospholipids (mg g^{-1} dw) (B), crude protein (mg g^{-1} dw) (C), water soluble proteins (mg g^{-1} dw) (D), free amino acids (mg g^{-1} dw) (E) and ash (mg g^{-1} dw) (F). Phospholipids are the sum of PC and PE. Lower case letters imply significant differences between samples.