Available online at www.sciencedirect.com
ScienceDirect
Aquaculture 266 (2007) 200–210
Aquaculture
www.elsevier.com/locate/aqua-online

Oxidative stability and changes in the particle size of liposomes used in the *Artemia* enrichment

Óscar Monroig [a], Juan Carlos Navarro [a], Francisco Amat [a], Pedro González [b], Francisco Hontoria [a,*]

[a] *Instituto de Acuicultura de Torre de la Sal (CSIC), 12595 Torre de la Sal, Castellón, Spain*
[b] *Transtechnics, S.L. Jules Verne, 19, 08006 Barcelona, Spain*

Received 7 September 2006; received in revised form 12 December 2006; accepted 12 December 2006

Abstract

The oxidative stability and the particle size of several types of liposomes were assessed in order to characterize their behaviour when submitted to the aggressive conditions of the *Artemia* enrichments. Results show that all liposomes tested in this study were much more oxidatively stable than a commercial product based on fish oil emulsion. Whereas the initial thiobarbituric acid reactive substances (TBARS) concentration in the emulsion was only slightly higher than in liposomes, the concentration of TBARS in the emulsion increased up to values three orders of magnitude above those registered by liposomes after 21 h of incubation. Among the different liposome formulations, results indicate that vesicles composed of phospholipids containing long-chain highly unsaturated fatty acids (krill phospholipid extract) were generally less stable than those composed of shorter length-chain and more saturated acyl chains.

In regards to the particle size changes during enrichment, all liposomes maintained their original size during the experimental period when incubated without nauplii. In the presence of nauplii, liposomes did not exhibit notable changes in their size, except for unilamellar vesicles prepared by the extrusion methodology and formulated with soybean phosphatidylcholine. The implications of the results on the capability of liposomes to be used in *Artemia* nauplii enrichments are discussed.

Keywords: *Artemia* enrichment; Liposomes; Oxidative stability; Particle size; Larviculture

1. Introduction

Deficiencies in the nutritional value of live prey used in the rearing of marine fish larvae has been solved through enrichment procedures with products containing high levels of essential nutrients. During this boosting process, live prey such as rotifers and *Artemia* nauplii are incubated in aqueous media where the enrichment diet is dispersed, thereby facilitating its incorporation through the passive filtering behaviour of these organisms (Léger et al., 1986). A broad variety of enrichment products are available and can be classified as those based on microalgae (Wickins, 1972; Watanabe et al., 1978, 1982), spray-dried cells of *Schizochytrium* sp. (Barclay and Zeller, 1996), microcapsules (Southgate and Lou, 1995) and oil emulsion products (Léger et al., 1986; Han et al., 2000; Copeman et al., 2002). All of them contain significant levels of highly polyunsaturated fatty acids (HUFA) as eicosapentaenoic (EPA, 20:5$n-3$), docosahexaenoic (DHA,

* Correspoding author. Tel.: +34 964 319500; fax: +34 964 319509.
E-mail address: hontoria@iats.csic.es (F. Hontoria).

0044-8486/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2006.12.016

22:6$n-6$) and arachidonic acids (ARA, 20:4$n-6$) which are essential for marine fish larvae (Sargent et al., 1997; Izquierdo et al., 2000). In addition to these compounds, several studies have focused their attention on the importance of other nutrients such as vitamins (Merchie et al., 1997; Halver, 2002) and free amino acids (Rønnestad et al., 1999, 2003; Tonheim et al., 2000) on the early development of marine fish larvae. Although some effort has been made on the formulation of novel products which provide simultaneously all essential nutrients (Dhert et al., 2005), the different water or lipid solubility of such essential compounds has limited the success.

Since liposomes can include both hydrophilic and lipophilic substances, some authors have proposed their use as an alternative tool to deliver essential nutrients to marine fish larvae through their bioencapsulation in live prey (Ozkizilcik and Chu, 1994; Hontoria et al., 1994; McEvoy et al., 1996; Tonheim et al., 2000; Monroig et al., 2003, 2006a,b). Simply stated, liposomes can be defined as phospholipid vesicles that enclose an aqueous internal compartment and with variable sizes ranging from some dozens of nanometres to several microns, (New, 1990). The previously described characteristics (i.e., the existence of an internal space, their phospholipid based composition and the small particle size) differentiate liposomes from other commercially available enrichment products, and hence determine their behaviour as enrichment diets.

As a preliminary attempt to study how liposomes behave under the *Artemia* enrichment conditions, Monroig et al. (2003) assessed the capacity of several types of vesicles to retain water soluble compounds dissolved in the internal aqueous space. It was concluded that lamellarity, the incubation time and the membrane formulation determine the leakage rate of water soluble compounds from the aqueous compartment to the external space out of the membrane. However, other parameters are necessary to be studied for the proper assessment of the liposome capability for the live prey enrichment.

Lipid peroxidation, as an estimator of the oxidative stability, has been described to be an important degradation process of polyunsaturated fatty acids (Gurr and Harwood, 1991; Frankel, 1998). As aforementioned, most of the enrichment diets are basically composed of polyunsaturated fatty acids and hence are susceptible to peroxidize when exposed to the aggressive conditions of live prey enrichments i.e. illumination, high temperature and dissolved oxygen (McEvoy et al., 1995). Therefore, the oxidation process produces terminal toxic compounds such as aldehydes and ketones which can be accumulated in the live prey through their passive filtering behaviour. Finally, consumption of such harmful diets can lead to detrimental effects (Hipkiss et al., 1997; Tesoriere et al., 2002; Del Rio et al., 2005) on larvae. Alternatively, the phospholipid composition of liposomes can preserve the oxidative stability during enrichment since several studies have demonstrated the antioxidant properties of these compounds in comparison to other lipid classes such as triacylglycerols and fatty acid ethyl esters (King et al., 1992a,b; Song et al., 1997).

Particle size is an important property for the enrichment products as it can determine the bioencapsulation efficiency in the live prey (Gelabert-Fernández, 2002; Han et al., 2005). Generally speaking, commercial enrichment diets produce several micron-sized particles when dispersed in the enrichment medium (Léger et al., 1987; Southgate and Lou, 1995; Han et al., 2005). On the other hand, the unilamellar liposomes used in the present study are smaller with diameters varying around 150 nm. Likewise, incubation of liposomes in the enrichment medium itself can favour the occurrence of different phenomena such as fusion and aggregation, which can modify the original size distribution (New, 1990), and therefore the bioencapsulation efficiency in the nauplii.

In order to better characterize the behaviour of liposomes during the *Artemia* enrichment, we studied the oxidative stability of several liposome formulations when submitted to standard enrichment conditions, together with the changes in the vesicle size throughout the process. For this reason, the concentration of TBARS formed during the incubation of liposomes was used as an estimator of oxidative stability. In a second experiment, changes in the particle size of liposomes were monitored throughout the incubation period by means of a light scattering laser technique.

2. Materials and methods

2.1. Preparation of liposomes

The oxidative stability and the changes in the vesicle size were assessed in five liposome formulations submitted to standard *Artemia* enrichment conditions. Three different phospholipid sources (see Table 1 for fatty acid and lipid class composition) were used in the preparation of liposomes: krill phospholipid extract (KPE), dipalmitoyl phosphatidylcholine (DPPC), and soybean phosphatidylcholine (SPC), all from Avanti Polar Lipids Inc. (Alabaster, AL, USA).

All five liposomes were unilamellar vesicles and were prepared using two different methodologies. Firstly, multilamellar liposome suspensions obtained by simple hydration of a phospholipid film with seawater (Monroig et al., 2003) were extruded using a LiposoFast engine