OXIDATIVE STABILITY OF FISH OIL WITHIN A FILLED HYDROGEL FOR USE IN MEAT PRODUCTS

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Abstract – The effect on lipid oxidation of the encapsulation of fish oil within protein-rich filled hydrogel microspheres (an oil-in-water-in-water emulsion: $O/W_1/W_2$) and the storage time (2 ºC, 19 days) was determined by comparison with a conventional oil-in-water (O/W) emulsion stabilized by sodium caseinate. Both systems were prepared with 7.5% fish oil rich in n-3 fatty acids. Samples were characterized by particle size measurement and morphology through optical microscopy. Lipid oxidation was monitored by measurement of lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS). Results showed that the O/W emulsion had a monomodal distribution with $D[_{4,3}]$ between 0.72 and 0.79 µm and filled hydrogel microspheres presented a polydisperse distribution with most particles between 6 and 20 µm. These distributions agree with optical microscopy images. Storage time had little effect on particle size and distribution. According to the lipid hydroperoxides and TBARS measurements, filled hydrogel microspheres showed significantly lower oxidation rates than the O/W emulsion over the storage time. The encapsulation of fish oil in this delivery system is an interesting approach for controlling lipid oxidation in omega-3-enriched meat products.

Keywords – Emulsions, Encapsulation, Filled hydrogel microspheres, Lipid oxidation

I. INTRODUCTION

Long chain n-3 fatty acids (n-3 LCFAs) have been identified as critical nutrients for human health. Because fish oil is the main dietary source of n-3 LCFAs, it has been used in various forms and levels to enrich different food products, including meat products [1, 2]. Unfortunately, incorporation of n-3 LFCAs in food systems is potentially problematical due to their susceptibility to lipid oxidation, a reaction that leads to the generation of unacceptable flavours, thus limiting the possibilities of incorporation of these valuable fatty acids in foods [2]. Therefore, if beneficial n−3 fatty acids are to be used in foods, their oxidative stability must be increased. Emulsion-based delivery systems may also be used as an alternative to protecting these compounds with antioxidants. Researchers have shown that food proteins can be used as emulsifiers to produce physically stable oil-in-water emulsions while also inhibiting lipid oxidation [3]. Hence they are potentially useful multifunctional ingredients that can be used to aid the incorporation of oxidatively unstable lipids in foods [4].

Conventional oil-in-water (O/W) emulsions are the most common type of emulsion-based delivery systems. O/W emulsions are popular because of their low production costs and their ease of manufacture. Despite these advantages, conventional emulsion-based delivery systems have limited potential in terms of their ability to inhibit lipid oxidation. Thus, more sophisticated emulsion-based delivery systems are necessary in applications where oxidative stability is critical. Filled hydrogel microspheres are one example of this type of delivery system, which consists of oil droplets (O) trapped within a hydrogel matrix (W_1), which is dispersed within an aqueous medium (W_2) that may be described as an $O/W_1/W_2$ type of structured emulsion [5]

Filled hydrogel microspheres can inhibit oxidation because they contain concentrated antioxidant biopolymers in close proximity to emulsion droplets and can physically inhibit interactions between aqueous phase prooxidants and lipids [6].

As an approach to reducing fat content and improving the lipid profile of healthier meat products, fish oil can be incorporated using hydrogel filled microspheres. However, before this system can be used in meats, it is necessary to determine the level of oxidative stability that this delivery system can provide.

The objective of this study was therefore to compare the oxidative stability of fish oil in both filled hydrogel microspheres and conventional oil-in-water emulsions stabilized by sodium caseinate.

II. MATERIALS AND METHODS

Preparation of conventional O/W emulsion

An oil-in-water emulsion with 7.5% fish oil (Omevital 18/12 TG Gold, Cognis GmbH, Germany) containing 160 mg EPA/g and 115 mg DHA/g plus a combination of tocopherols as antioxidants was emulsified with sodium caseinate (DMV, The Netherlands) based on the procedure described by Matalanis *et al.* [7]

Preparation of filled hydrogel microspheres

Preparation of solutions. Solutions were prepared according to Matalanis *et al.* [5] using pectin (GRINDSTED® Pectin USP, Danisco, Denmark) and sodium caseinate (DMV, The Netherlands). Briefly, a pectin-rich solution containing 4.03% pectin and 0.22% sodium caseinate and a sodium caseinate-rich solution with 0.52% pectin and 14.2% sodium caseinate were prepared. Following dispersion, the pH of the solutions was adjusted to pH 7. An oil-in-water emulsion with 50% fish oil was prepared as previously described for conventional O/W emulsion.

Formation of filled hydrogel microspheres. Filled hydrogel microspheres formation was a multistep process carried out according to Matalanis *et al* [5]. The general scheme for forming these microspheres involved creating an oil-in-water emulsion with 50% fish oil using a water-soluble surfactant (sodium caseinate) which is mixed with a biopolymer solution (pectin-rich and caseinate-rich solutions) that is capable of phase separation into an oil-in-water-in-water ($O/W_1/W_2$) dispersion. The environmental conditions were then altered by reducing the pH to 5 with 1 M citric acid, and transglutaminase (ACTIVA WM, Ajinomoto, Germany) was added to promote hydrogel particle formation. After that, the pH was adjusted to 7 with 1 M sodium hydroxide and then sample was stored overnight. Hydrogel microspheres were washed to remove the pectin-rich phase. A ratio of 1 part hydrogel microspheres to 4 parts 10 mM phosphate buffer (pH 7) was mixed and then centrifuged a using a Sorvall Evolution RC Centrifuge (Kendro Laboratory Products, USA) at 10000 g for 1 h. The washing solution (along with most of the continuous phase) was decanted, and the washed microspheres were collected.

Both the emulsion and the filled hydrogel microspheres were placed in plastic tubes and stored in a cold chamber (2 ºC). Analyses were carried out at 1, 5, 11, 19 days. Measurements were made in triplicate.

Filled hydrogel microspheres and emulsion characterization

Particle size distribution of oil globules in both emulsions was measured with a Malvern Mastersizer S laser diffraction particle size analyzer (Malvern Instrument Ltd, U.K). The measurement range was 0.05-900 μm. The mean droplet size ($D[_{4,3}]$), and the percentage of volume were determined based on the Mie Scattering theory.

A Nikon Optihot optical microscope (Nippon, Kogaki, K.K., Japan) was used to examine sample morphology.

Determination of lipid oxidation

Lipid hydroperoxide concentration was used as a measurement of the primary products of oxidation according to Matalanis *et al.* [7] and results were expressed as mmol hydroperoxide/kg oil. TBARS concentration was used as a determination of secondary oxidation products. TBARS was measured by a method adapted from Serrano [8] and results were expressed as mg malonaldehyde/kg oil.

III. RESULTS AND DISCUSSION

Filled hydrogel microspheres and emulsion characterization

Conventional O/W emulsion presented a monomodal distribution with ($D[_{4,3}]$) ranging from 0.72 to 0.79 μm (Fig. 1a, inset). However, a polydisperse distribution was recorded in the filled hydrogel microspheres, with most particles between 6 and 20 μm (Fig. 1b, inset). Matalanis *et*

al [7] reported similar results. The microscopic images confirm the monomodal distribution of the O/W emulsion (Fig. 1a) and the polydisperse distribution of the filled hydrogel microspheres (Fig. 1b). Variations in particle size distribution and mean droplet size ($D[_{4,3}]$) during storage time were small.

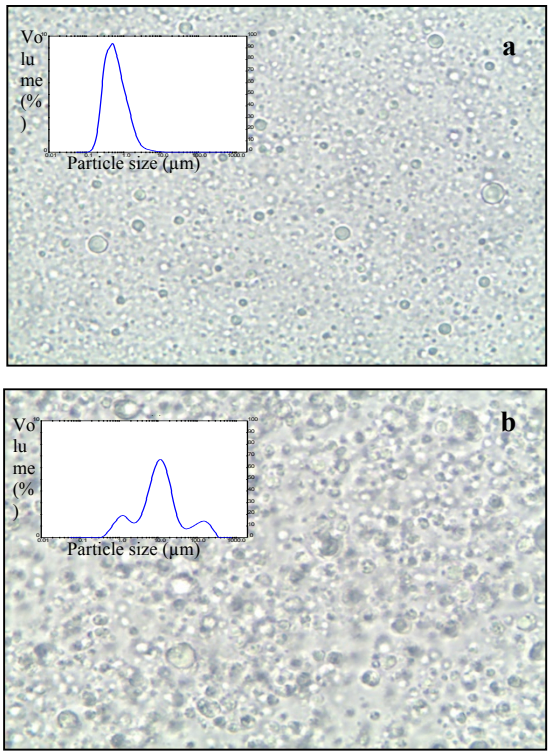

Figure 1. Optical microscopy images (a) oil-in-water emulsion and (b) filled hydrogel microspheres. Insets display particle size distribution.

Lipid Oxidation in Filled Hydrogel Microspheres and Emulsions

The concentration of hydroperoxides in O/W emulsions and filled hydrogel microspheres are presented in figure 2. At the outset of storage (day 1), both samples presented similar values. During the course of storage, hydroperoxide levels in the O/W emulsion increased substantially between 1 and 11 days and then stabilized until the end of storage. This behaviour may be due to conversion from primary to secondary oxidation products. The filled hydrogel microspheres showed modest increases in hydroperoxide levels over the storage time. Except day 1 of storage, the amount of peroxides produced was greater in the O/W emulsion than in the filled hydrogel microspheres at all times.

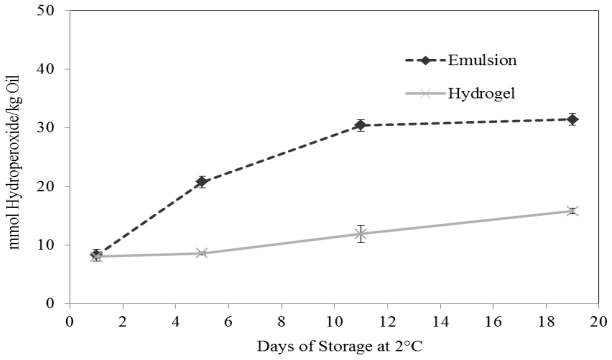

Figure 2. Hydroperoxide values during storage at 2 ºC in oil-in-water emulsion and filled hydrogel microspheres.

TBARS values in O/W emulsions increased sharply until day 11, while TBARS in filled hydrogel microspheres increased until day 5 and thereafter increased moderately until the end of storage (Fig 3). The TBARS values in O/W emulsions showed a normal oxidation pattern, consisting of a plateau or decline, presumably due to secondary products reacting with other components. In the case of the filled hydrogel microspheres, the oxidation process was not completed, presumably due to the protective effect of the system designed on fish oil lipids, which reduced their susceptibility to oxidation.

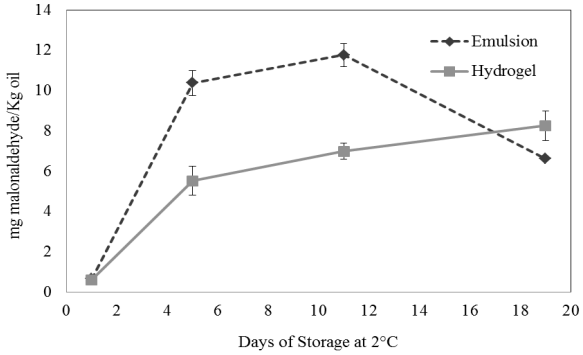

Figure 3. TBARS values during storage at 2 ºC in oil-in-water emulsion and filled hydrogel microspheres.

These results show that the O/W emulsion was oxidized faster than the filled hydrogel microspheres. The explanation for this behaviour may be that in the microspheres there was more protein close to the emulsion droplet; this would

make for more effective scavenging of free radicals, thereby delaying lipid oxidation. These results agree with studies conducted on oxidation of oil-in-water emulsions stabilized with caseinate, which have shown that the rate of lipid oxidation tends to decrease with increasing levels of casein [4, 9].

IV. CONCLUSION

The encapsulation of fish oil within hydrogel microspheres as a strategy to delay oxidative deterioration was successfully achieved in comparison with a conventional caseinate O/W emulsion. Filled hydrogel microspheres show potential as a means of incorporating fish oil in meat products to both reduce fat content and improve the lipid profile in this kind of products.

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