Immobilization of fish oil in hydrogel alginate/oligochitosan microcapsules

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Introduction

Microcapsules have been used in food industry for a number of years, either in a natural form (for example as agglomerates of minerals) or formed during the specific process to provide a barrier against flavor, odor or oxygen. Microcapsules allow fragile materials to survive food processing and packaging conditions and can increase the variety of ingredients that can be applied as functional additives to food products (Brazel, 1999). Several liquid lipids are important nutrients. Polyunsaturated fatty acid such as linoleic, linolenic and arachidonic acids are essential nutrients for mammals. In Japan 'concentrated fish oil containing eicosapentaenoic acid and docosahexaenoic acid' has be approved as 'food for specified health use' by the Ministry of Health and Welfare in response to a proposal by several companies cooperating through the Health Food and Nutrition Food Association. Unfortunately, lipids are generally difficult to disperse in food products, and polyunsaturated fatty acids are susceptible to autooxidation, which results in off-flavor end potential toxicity (Matsuno, Adachi, 1993).
One of the major advantages of marine lipids microencapsulation is that the coating material creates barrier to oxidation, thereby masking off-flavor. The shelf life of active integredients, such as EPA and DHA, is also increased within the food matrix as the coating decreases the susceptibility of these oils to oxidative degradation. Although both synthetic and natural polymers can be use as shell materials, only those that are food-grade food additives can be applied in marine lipid microencapsulation technologies (Yulai et al., 2007).
Recently, we have proposed an effective immobilization method of cod liver oil in hydrogel alginate/Ca beads (Tarnowiecka et al., 2006). The most robust microcapsules with high mechanical resistance and sufficient stability during storage in water suspension has been obtained in case of system based on 1,5% alginate (Keltone HV) containing 20-30% of oil after the 15 min of gelling in 0,155 M $CaCl_2$ solution. We believe that after appropriate selection of capsule formation method and storage conditions the alginate/Ca system can be successfully used for immobilization of fish oils as food additives, especially in case of enrichment of aqueous based products such as milk, dairy products, juices and soft drinks.
The aim of this study was to evaluate the small alginate/Ca microcapsules of size below 800 μm with and without additional surface coating with oligochitosan as mechanical and oxidative stabilizer of immobilized fish oil stored in aqueous solution of varied pH.

Materials and methods

Biopolymers and other chemicals
Sodium Alginate – Keltone HV (ISP, USA) food grade, a molar mass of M_η= 440,000 g/mol,
Oligochitosan (M_n in range 400-2,000 g/mol) has been kindly provided by Kunpoong Bio Co., Ltd (Seoul, South Korea),
Cod liver oil (Lysi – Iceland) has been ordered via local representative (Island - Gdynia, Poland),
Calcium Chloride hexahydrate pure p.a. (Chempur, Poland),
All other chemicals in ultra pure form were provided by various chemical companies.

Capsule formation

All microcapsules were prepared at room temperature using classical two step emulsion based procedure. A specific amount of cod liver oil was mixed with an aqueous solution of sodium alginate (1,5% wt. %) and stirred using a mechanical homogenizer (Silent Crusher, Heidolph) at 15,000 rpm for 5 min in order to form an oil-in-water emulsion. In this method in 1st step 15 cm^3 specific alginate/oil emulsion was extruded as droplets with highest air-flow 350 dm^3/h (coaxial encapsulator Var-J1 Nisco, Switzerland) directly into 300 cm^3 aqueous 0,155 M solution of $CaCl_2$. In 2nd step gellified alginate/Ca^{2+}/oil beads were coated for 15 min in 1% oligochitosan of pH 5,5. Finally, after reaction both modified and unmodified capsules of 0,3-0,4 mm in diameter were collected, three times washed and stored similar to these obtained in one-step method, were stored at different temperatures, 4º and 37º C respectively, in dark place in different storage conditions (pH 4,4 and 8,0, in 0,9% NaCl, demineralized water).

Capsule characterization

The mechanical characterization of microcapsules have been described elsewhere (Tarnowiecka, et al., 2006).

Immobilization effectiveness and quality of immobilized oil

A known volume of microcapsules were grounded in a homogenizer in the presence of chloroform/methanol mixture in order to extract the oil from hydrogel matrix (modified Bligh&Dyer extraction procedure (Kołakowska et al., 2002). The oil was extracted from selected microcapsules directly after capsule preparation and respectively after 1, 2 and 4 weeks to observe the changes during storage. For all extracted oil samples peroxide value (PV) was determined using standard procedure described by Kolakowska at al (Tarnowiecka et al., 2006). Additionally there were analysed in 2:1 chloroform:methanol solution using the UV-VIS spectrometer (Helios γ, Spectro-Lab, USA) recording spectra in range of 200-400 nm.

Results and discussion

Most of the formed microcapsules show high deformations > 80% and good mechanical stability during storage at different solutions. Microcapsules modified 1% chitosan possess larger endurance mechanical than non-coated (Fig. 1). All parameters such as low temperature, low pH and additional modification by chitosan have slowed down the process of destabilization of fish oil/polyelectrolyte microcapsules (Tab. 1, Fig. 1).

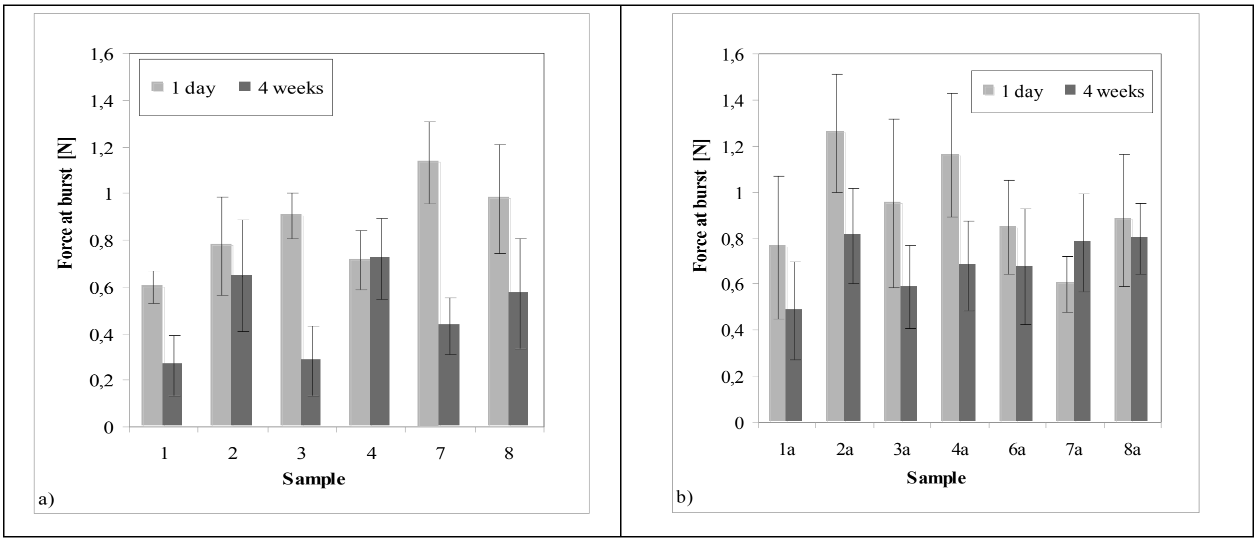

Figure 1: Change of mechanical properties (bursting force) of different alginate microcapsules containing fish oil during storage at: a) 37°C and b) 4°C

Uncoated microcapsules have been completely dissolved already after one day storage at pH 8,0, both at 37°C or 4°C (Fig. 1). Modification of microcapsules by coating in 1% chitosan solution has induced their storage time in solution of pH 8,0 to 1 week at 37°C and four weeks at 4°C (Fig. 1).
All performed experiments shown that microencapsulation in hydrogel alginate/Ca systems have significantly slowed down fish oil oxidation process (Tab 1 and 2) in comparison to free uncoated oil stored in the same temperature. Uncoated microcapsules and coated with 1% oligochitosan H (stored at 37° C) had the highest oxidative stability, in comparison to free oil. Specifically, it is confirmed by the peroxide values and analysis of lipids spectra during storage at 37°C (Tab. 1-2).

Table 1: Oxidation change of immobilized and free fish oil after different storage time (0-4 weeks) – (1,5 % Keltone HV containing 30% of oil, coated with oligochitosan H)

Microcapsule type		**Time** [week]			
		–	1	2	4
		Peroxide value [mqO/kg]			
I	Free oil/ 37°C	1,7±*0,30*	113,7±*6,87*	401,3±*5,53*	436,3±*11,1*
1	Oil+alginate/Ca capsules /water / 37°C	7,2±*0,75*	46,9±*1,67*	54,9±*1,87*	74,5±*5,93*
2	Oil+alginate/Ca/1% chitosan H capsules water /37°C	35,4±*0,91*	46,8±*3,39*	56,9±*1,51*	63,0±*1,62*
3	Oil+alginate/Ca capsules /pH 4,4/ 37°C	10,5±*1,71*	42,2±*6,21*	60,6±*5,65*	56,8±*0,42*
4	Oil+alginate/Ca/1% chitosan H capsules /pH 4,4/ 37°C	82,7±*1,72*	23,2±*1,20*	36,2±*4,66*	59,5±*0,51*
5	Oil+alginate/Ca capsules /pH 8,0/ 37°C	51,7±*0,68*	–	–	–
6	Oil+alginate/Ca/1% chitosan H capsules /pH 8,0/ 37°C	12,4±*3,44*	28,5±*2,53*	–	–
7	Oil+alginate/Ca capsules /0,9% NaCl/ 37°C	5,8±*0,51*	59,3±*4,23*	48,5±*0,38*	74,7±*5,88*
8	Oil+alginate/Ca/1% chitosan H capsules /0,9% NaCl / 37°C	49,1±*1,34*	19,0±*1,05*	81,4±*2,75*	34,5±*0,21*
II	Free oil /4°C	1,2±*0,06*	6,6±*0,63*	5,3±*0,40*	15,1±*0,98*
1a	Oil+alginate/Ca capsules /water / 4°C	2,3±*0,02*	10,6±*1,06*	8,1±*1,84*	32,0±*10,98*
2a	Oil+alginate/Ca/1% chitosan H capsules / water /4°C	3,7±*0,16*	26,7±*0,81*	49,8±*0,50*	170,2±*0,97*
3a	Oil+alginate/Ca capsules /pH 4,4/ 4°C	2,3±*0 06*	18,6±*2,20*	17,5±*1,42*	50,4±*0,000*
4a	Oil+alginate/Ca/1% chitosan H /pH 4,4/ 4°C	7,9±*0,41*	81,6±1,06	109,5±*1,84*	270,4±*0,92*
5a	Oil+alginate/Ca capsules /pH 8,0/ 4°C	2,7±*0,14*	–	–	–
6a	Oil+alginate/Ca/1% chitosan H capsules /pH 8,0/ 4°C	2,2±*0,01*	25,0±*2,18*	29,4±*0,50*	69,2±*1,12*
7a	Oil+alginate/Ca capsules /0,9% NaCl/ 4°C	1,8±*0,01*	8,4±*0,21*	12,0±*1,88*	32,8±*0,45*
8a	Oil+alginate/Ca/1% chitosan H capsules /0,9% NaCl / 4°C	4,2±*0,09*	45,9±*1,75*	62,9±*0,80*	142,2±*1,60*

Table 2. Conjugated di-, tri-enoic acids change of immobilized and free fish oil after different during storage time (0-4 weeks) – (1,5 % Keltone HV containing 30% of oil, coated with oligochitosan H)

Microcapsule type	Conjugated dienoic acid [233 nm] [1g/1%]				Conjugated trienoic acid [268 nm] [1g/1%]			
	Storage time [weeks]				Storage time [weeks]			
	–	1	2	4	–	1	2	4
I	**2,034**	**1,636**	**1,916**	**3,046**	**6,787**	**4,174**	**5,722**	**8,931**
1	1,385	1,522	1,791	2,746	4,484	4,172	5,255	6,960
2	1,362	1,453	1,657	2,275	4,318	4,943	5,664	6,954
3	0,264	2,307	0,092	1,006	4,406	4,509	5,183	6,799
4	1,360	0,100	1,147	1,894	4,392	4,734	5,041	7,130
5	1,780	–	–	–	4,237	–	–	–
6	1,960	1,617	–	–	4,323	4,885	–	–
7	1,377	1,768	1,833	2,018	4,210	3,986	4,902	6,523
8	1,260	1,681	1,291	2,048	4,251	4,663	6,188	6,367
II	**0,997**	**0,058**	**0,116**	**0,390**	**4,177**	**4,177**	**4,207**	**4,287**
1a	0,215	1,919	1,209	1,624	4,400	4,670	4,042	4,682
2a	0,833	1,910	0,890	1,517	4,230	4,362	4,089	5,008
3a	1,270	1,716	0,135	0,645	4,381	4,355	4,117	4,849
4a	1,202	0,614	0,911	2,238	4,600	4,303	4,330	5,715
5a	2,294	1,942	–	–	6,915	5,290	–	–
6a	1,786	1,494	0,986	1,501	5,397	4,071	4,083	4,700
7a	0,493	1,977	1,039	1,176	5,806	4,284	3,993	4,254
8a	1,275	2,027	1,143	1,483	4,112	4,339	4,098	5,111

Conclusions

The proposed within this paper method of immobilization of fish oil in alginate/Ca beads could be effectively applied for immobilization of fish oil. The additional coating with oligochitosan improves the mechanical and oxidative stability of oil stored in aqueous solutions of various pH.

Acknowledgments

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