Immobilization of fish oil in alginate microcapsules as potential food additives

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Introduction

Nowadays the food industry is expecting more and more sophisticated properties from novel food additives and these can be provided by various technologies including microencapsulation. Microencapsulation has been already industrially applied to mask the unpleasant taste of certain ingredients and also to simply convert liquids to solids. Over the last decade the growing interest by food technologists in the enormous potential of microencapsulation, including carefully fine-tuned controlled release properties, is demonstrated by the exponential increase in the number of publications related to this topic. Liposome entrapment and spinning disk, as well as coacervation, have experienced the most rapid growth in interest from researchers and technologists [1].

Fish oil is an excellent source of nutritional factors such as lipid soluble vitamins and essential fatty acids including n-3 polyunsaturated fatty acids (PUFA). There is a close relationship between the consumption of fish oil and the decrease of cholesterol and triglycerides level in blood, together with a decrease in blood pressure and formation of clots responsible for cardiovascular diseases. However, fish oil has unpleasant taste and odor, which constitutes a serious inconvenience for its use as a dietary supplement or therapeutic applications. Moreover, due to high content of polyunsaturated fatty acids fish oils during storage easily undergoes oxidation when exposed to air or light. The successful incorporation of immobilized fish oil into foods without changing important properties like stability, taste etc. may provide a means of increasing intakes of n-3 polyunsaturated fatty acids (n-3 PUFA) [2].

Over the last decade one can observe a growing interest in immobilization systems based on alginate, since it is one of the few abundantly available and naturally derived biocompatible anionic polysaccharides. One of these methods could be the immobilization of dispersed fish oil as microcapsules within the biopolymeric matrix based on alginate/Ca polyelectrolyte complex [3]. However, in case of the food industry, two major drawbacks limit the use of such system, which are difficulties in scale up of the process and too high porosity of hydrogel matrix. Recently a few new processes have been proposed to facilitate the large-scale production of alginate beads, which makes possible to envision a commercial encapsulated food ingredient based on the alginate bead technology [1]. So for no one has extensively investigated the potential application of commercial food grade alginate samples as matrix and change of such capsules and immobilized oil during storage.

Therefore, herein the application and characterization of standard alginate/Ca microcapsules containing dispersed cod liver oil will be presented. The influence of various reaction parameters on capsules mechanical resistance and porosity will be discussed. Furthermore, the effects of storage in water on mechanical resistance and immobilized oil oxidation stability with the potential application of additional protective coating have been evaluated.

Materials and methods

Sodium Alginate - Keltone HV and Manugel GHB (ISP, USA), both food grade, with intrinsic viscosity [η] of 880 and 612 mL/g in 0.1 M NaCl at 20°C, what corresponds to a molar mass (MM) of 440,000 and 306 000 respectively, were used for capsule preparations.

Oligochitosan sample (400-2,000 g/mol) in H form has been kindly provided by Kunpoong Bio Co., Ltd (Seoul, South Korea) – pH of 1% solution 7,8%.

Cod liver oil (Lysi – Iceland) has been ordered via local representative Island, Gdynia, Poland.

All other chemicals in ultra pure form were provided by various chemical companies.
Capsule Formation. A specific amount of cod liver oil was mixed with an 1-2% aqueous solution of sodium alginate (in total 50 ml of solution) and stirred using a mechanical homogenizer (Silent Crusher M, Heidolph) at 15,000 rpm for 5 min at room temperature in order to form an oil-in-water emulsion. All microcapsules were prepared at room temperature using classical two step procedure [4]. In this method in 1st step 5 ml of alginate/oil emulsion was extruded as droplets using the disposable syringe with a 0.7 mm flat-cut needle directly into 0.05-0.5 M solution of $CaCl_2$ (each time the alginate/$CaCl_2$ solution volume ratio was constant 1/20). Four different concentrations of alginate were applied (1%; 1.25%; 1.5% and 2.0%). Once gellified after 5-30 minutes of reaction and washed with water, alginate/Ca^{2+}/oil beads (2.5 cm^3) in 2nd step were coated for 20 min in 50 cm^3 0.1-1% solutions of oligochitosan (Table). Finally, after reaction alginate/oil/Ca^{2+}/oligochitosan capsules of 2.5-3.0 mm in diameter were collected, three times washed and stored similar to these obtained in one-step method.
To determine the effectiveness of oil immobilization after each encapsulation process all solutions from gelling and washing bath where mixed with 10 ml of hexane. The concentration of fish oil in hexane extract was determined using standard UV spectrometric method at 327 nm wavelength.
Capsule characterization. The methods of cut-off determination and mechanical characterization of microcapsules have been described elsewhere [4].
Content 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 [5]). 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 [5].

Results and discussion
Already preliminary experiments have indicated that one of the most important parameter during formation of mechanically stable fish oil/alginate microcapsules is type of applied alginate. In case of Keltone HV all microcapsules were from 2 to 5 times more resistant than for Manugel GHB based systems [6]. In this case the molar mass and G/M co-monomer block composition have significant influence on stability of alginate based microcapsules [4]. Therefore, in further experiments only Keltone HV has been used. Furthermore, based on our other unpublished results as the most optimal reaction time and calcium chloride concentration, which result in highest immobilization effectiveness and mechanical resistance of microcapsules, 15 min and 155 mMol $CaCl_2$ have been selected respectively [6].
There was no significant effect of alginate concentration on immobilization effectiveness (Tab. 1), however one could observe a typical increase of mechanical resistance for concentrations higher than 1.5%. Hereafter for further experiments systems based on 1,5% Keltone HV has been selected.

Table 1. Effect of alginate concentration on properties of hydrogel microcapsules*

Alginate concentration [%] wt	Immobilization effectiveness [%]	Bursting force [N]
1.0	99.7	7.70 ±0.56
1.25	99.7	7.75 ±0.44
1.5	99.9	9.22 ±0.59
2	99.2	14.77 ±0.43

*1,5% Keltone HV/30% oil in distilled water, 15 min reaction time in 0,155 M $CaCl_2$ gelling bath

In the first stage the stability of emulsion based on fish oil/alginate solution using different concentration of hydrophobic phase (10-40% oil) has been compared (Fig. 1). These results clearly

demonstrate that all emulsions are stable during first day of storage, although after 2 days only emulsion containing 20% and 30% of oil have indicated no phase separation.

Fig. 1. Stability of emulsion (50 cm^3 of emulsion 1,5 % Keltone HV containg respectivelly: 10% (6), 20% (7), 30% (8), 40% (9) of fish oil) after different storage time (0 –directly after homogenisation, A – after 1 day, B – after 2 days) – emulsification 15,000 rpm for 5 min

Table 2. Effect of fish oil concentration on properties of alginate microcapsules*

Fish oil concentration [%] wt	Immobilization effectiveness [%]	Membrane cut-off [g/mol]	Bursting force [N]		
			1 day	7 days	30 days
distilled water					
10	98.3	119,000	10.06 ±0.09	10.12 ±0.07	12.00 ±0.75
20	99.1	94,000	9.93 ±0.33	9.74 ±0.12	8.28 ±0.71
30	99.3	116,000	8.64 ±0.79	9.37 ±0.52	7.63 ±0.70
40	99.2	82,000	6.00 ±0.58	6.61 ±0.57	4.36 ±0.43
0.9% NaCl					
10	99.4	100,00	6.36 ±0.68	4.65 ±1.85	4.05 ±0.35
20	96.3	79,000	3.38 ±0.46	3.36 ±0.45	3.23 ±0.29
30	99.4	112,000	2.71 ±0.17	2.89 ±0.41	2.55 ±0.27
40	98.9	83,000	1.9 ±0.10	1.7 ±0.29	1.98 ±0.12

*1,5% Keltone HV in distilled water and 0,9% NaCl, 15 min reaction time in 0,155 M $CaCl_2$ gelling bath

There is no significant effect of oil concentration within the alginate/Ca hydrogel matrix on its immobilization effectiveness and capsule membrane cut-off (Tab. 2). The immobilization effectiveness for all systems is above 98% and the membrane cut-off changes in relatively narrow range from 80,000 to 120,000 g/mol. Only mechanical strength of the capsules vary remarkably as a function of the oil concentration and ionic strength of aqueous solution applied during capsule preparation and storage, where typically capsule resistance growth with increase of oil concentration and lowering of ionic strength respectively. Based on all results of characterization (Fig. 1, Tab. 2) and considering the stability during storage the microcapsules containing between 20% and 30% of oil have been selected as the most promising systems.

Additional coating of alginate/oil/Ca beads with different concentration of oligochitosan H did not lead to any significant change of membrane cut-off or mechanical characteristic [6].

Evaluation of oxidative stability of fish oil samples either immobilized or in free form as aqueous suspensions showed that the alginate/Ca beads without oligochitosan outer layer gave a product of the highest stability after 4 weeks of storage (Fig. 2). Furthermore, extra oligochitosan H outer coating did not improve the shelf-life of the immobilized oil. One could even observe that after 2 weeks of storage there is a significant acceleration of oxidation process for microcapsules with increasing of oligochitosan concentration in comparison to free oil. This has been already observed for other systems immobilized in cellulose based matrixes using the spray drying process [7]. Such behavior could be explained by too high concentration of “antioxidant” which may accelerate the oxidation processes.

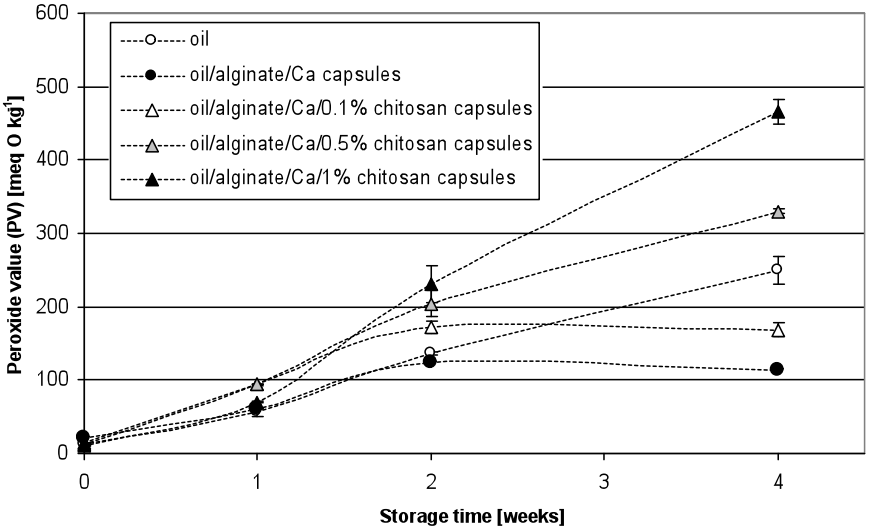

Fig. 2. Oxidation change of immobilized and free fish oil after different storage time (0-4 weeks) – (1,5 % Keltone HV containing 30% of oil, coated with oligochitosan H)

Conclusions

The method of effective immobilization of cod liver in alginate/Ca beads has been proposed. 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.

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