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Optimization and evaluation of fish oil microcapsules

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ABSTRACT

Fish oil microcapsules were prepared using two natural polysaccharides, alginate and chitosan, as the wall materials. A response surface methodology (RSM) was used to optimize the conditions for fish oil encapsulation efficiency (FOEE). The FOEE was investigated with respect to three key-variables in the RSM: ratio of inner oil phase to aqueous phase (X_1, w/w); concentration of the aqueous phase (X_2, wt%); and ratio of the aqueous phase to outer oil phase (X_3, v/v). The optimal formulation obtained from the RSM model, *i.e.*, 2.7:1 (X_1), 1.6 wt% (X_2), and 11.5:1 (X_3), gave a FOEE of 28%. The model was validated and the fish oil microcapsules prepared under the optimized conditions were characterized in terms of particle size, polydispersity index (PDI), zeta potential, surface morphology, and *in vitro* release. The average droplet size, PDI, and zeta potential were 915 nm, 0.038, and +5.2 mV, respectively. The fish oil microcapsules were highly uniform microspheres, and had an accumulative release rate of 77.7% in 270 min in a gastrointestinal model, indicating their potential as an alternative carrier for the controlled release of fish oil. In conclusion, formulating optimal microencapsulation conditions by the RSM can be applied to the microencapsulation of various oil-soluble nutrients for food applications.

Introduction

Fish oils have been studied for decades because they are rich in polyunsaturated fatty acids (PUFAs), which are essential nutrients for human health. Both docosahexaenoic acid (DHA, C22:6 n-3) and arachidonic acid (ARA, C20:4 n-6) are very important PUFAs that come from the n-3 and n-6 families, respectively. These PUFAs are found in high concentrations in neural tissues such as retina photoreceptor cells and brain gray matter (Anderson & Sperling, 1971). DHA and ARA are mainly involved in the growth and function of neural tissues. Specifically, DHA plays an important role in neural and visual development, and ARA is required for the normal growth and function of the vascular system in neural tissues (Crawford & Sinclair, 1972). ARA and eicosapentaenoic acid (EPA, C20:5 n-3) are precursors of prostaglandin, a metabolite of PUFA that is necessary for brain development. Prostaglandin and its receptors are highly expressed in the brains of newborns where they regulate blood supply and nitric oxide synthase production (Crawford et al., 2003; Wright et al., 2001). Prostaglandin receptors exist in astrocytes and oligodendrocytes, and are thought to be involved in the regulation of myelin production and oligodendrocyte differentiation (Althaus & Siepl, 1996). Accumulated data from experimental and epidemiological studies have demonstrated the important role of PUFAs in intelligence and recognition memory in early life (Birch et al., 2010; Drover et al., 2012; Jensen et al., 2010). Unfortunately, fish oil has an unpleasant odor that limits its use in food industry applications. The highly unsaturated fatty acids in fish oil can easily be oxidized to degrade their nutritional value (Augustin, Sanguansri, & Bode, 2006) and even produce harmful compounds (Song & Miyazawa, 1997).

Microencapsulation is a commonly used processing technique to protect food ingredients (Shahidi & Han, 1993). This method is employed to entrap particles or droplets in coating materials and has been widely applied in the food industry to mask off-taste and color, prevent oxidation, and protect functional compounds (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Preparation of a stable emulsion is the first and key step in the microencapsulation process. Membrane emulsification (ME) differs from conventional emulsification methods (Berendsen, Güell, & Ferrando, 2015), such as the use of ultrasonic homogenizers (Nakashima, Shimizu, & Kukizaki, 2000), because ME is a more efficient technique that produces highly uniform droplets in a single

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step (Charcosset, Limayem, & Fessi, 2004; De Luca, Di Renzo, Di Maio, & Drioli, 2006; Vladisavljević & Williams, 2006). This emulsion formation approach requires less energy input than other methods and does not appreciably raise the system's temperature nor subject the emulsion to high shear (Schadler & Windhab, 2006; Vladisavljević & Williams, 2006). Both shear and thermal stresses during processing may have adverse effects on system components such as sensitive bioactive nutrients (Charcosset et al., 2004; Schröder & Schubert, 1999). Premix ME (PME) was first introduced in the 1990s (Suzuki, Fujiki, & Hagura, 1998) and has become a promising technique for emulsification because of its simplicity and feasibility for large-scale production (Vladisavljević & Williams, 2005).

Selecting appropriate and safe coating materials is important in food industries, especially with regard to nutritional supplements for children. Alginate is a natural polysaccharide that has attracted increasing attention because of its excellent biocompatibility, mucoadhesive biodegradability, and mild gelation characteristics (Steinbuchel & Byrom, 1991). Chitosan is produced by deacetylation of chitin, a naturally occurring polysaccharide, and has a number of functional properties that make it technically and physiologically useful in nutritional applications (Rinaudo, 2006). A chitosan–alginate complex can form by exploiting the strong electrostatic interaction between the amino groups of chitosan and the carboxyl groups of alginate by means of the coacervation method. This complex can lower the porosity of alginate beads and decrease leakage from the encapsulated cores (Sezer, 1999; Yao, Peng, Yin, Xu, & Goosen, 1995).

In this study we use response surface methodology (RSM) to optimize the preparation of chitosan–alginate microencapsulated fish oil with the aim of maximizing the encapsulation efficiency (EE). RSM is generally regarded as one of the most effective methods for evaluating the relationship between independent variables and the associated response of microcapsules (Baş & Boyacı, 2007). A microencapsulation process containing two-step PME and two-step solidification was employed. To the best of our knowledge, there is no two-step PME fish oil microencapsulation process report and only a two-step ME process has been used to prepare solid lipid microcapsules (Kukizaki & Goto, 2007). Particle size, polydispersity index (PDI), zeta potential, surface morphology, and *in vitro* release are evaluated to further confirm the RSM optimized conditions. Our ultimate goal was to determine if this promising method could be applied for microencapsulation of various oil components in the food industry in the near future.

Experimental

Materials

Sodium alginate was purchased from Acros Organics (New Jersey, USA). The manufacturer specifies that the alginate contained 65–75% guluronic acid subunits and 25–35% mannuronic acid subunits, and had a molecular weight range of 450–550 kDa, with a viscosity of 485 cP for a 1 wt% aqueous solution. Chitosan was obtained from Jinke Biochemical Co., Ltd. (Shandong, China); the degree of deacetylation was 90% and the viscosity-average molecular weight was 150 kDa. Fish oil was kindly provided by DSM Co., Ltd. (Columbia, USA). Hexaglycerin penta ester (PO-500) was supplied by Sakamoto Yakuhin Kogyo Co., Ltd. (Sakamoto, Japan). Sucrose esters of fatty acids HLB 15 (SE-15) were purchased from Jinhelai Food Additive Co., Ltd (Zhejiang, China). Sunflower oil was obtained commercially and used without further purification. Distilled water was used for the preparation of all solutions. All of other chemicals used in this study were of analytical grade.

Table 1
Coded and un-coded levels of independent variables.

Coded variable level	O_1-to-W ratio X_1 (w/w)	Aqueous phase concentration X_2 (wt%)	W-to-O_2 ratio X_3 (v/v)
−1	1	1	5
0	3	1.5	10
+1	5	2	15

Table 2
Box–Behnken experimental design and results.

Run	X_1	X_2	X_3	Y
1	3	1.5	10	26.997
2	5	1.5	5	15.603
3	3	1	5	15.380
4	3	1	15	17.772
5	3	2	5	14.505
6	1	1	10	19.927
7	3	1.5	10	27.088
8	3	1.5	10	27.734
9	1	1.5	5	16.047
10	3	2	15	23.256
11	3	1.5	10	28.211
12	1	1.5	15	22.335
13	5	1	10	12.641
14	5	1.5	15	17.272
15	5	2	10	18.735
16	1	2	10	17.457
17	3	1.5	10	27.598

Experimental design for response surface methodology

A 3-factor–3-level Box–Behnken design was used for the RSM to determine the optimum encapsulating conditions. The effects of the following three independent variables on one response variable, *i.e.*, fish oil encapsulation efficiency (FOEE (Y)), were evaluated: ratio of the inner oil phase to aqueous phase (X_1), concentration of the aqueous phase (X_2), and ratio of the aqueous phase to outer oil phase (X_3). The factors and their levels used in the design are shown in Table 1. The three coded levels (−1, 0, and +1) of the three variables were incorporated into the design, leading to 17 experiments (Table 2). All experiments were performed in triplicate. A quadratic polynomial regression model was used to predict the Y variable. For three independent variables and one response variable, the model Eq. (1) was expressed as:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3, \quad (1)$$

where Y represents the predicted response variable, β_0 represents the intercept, β_1, β_2, β_3 represent the linear coefficients, β_{11}, β_{22}, β_{33} represent squared coefficients, β_{12}, β_{13}, β_{23} represent interaction coefficients, and X_1, X_2, X_3 represent the above independent variables. This response surface model was also employed to predict the maximum EE from the three-dimensional (3D) surface, which is the projection of the response surface in a 3D plane (Box & Hunter, 1957).

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