Int. J. Bio-Inorg. Hybd. Nanomat., Vol. 2, No. 4 (2013), 485-490 ISSN: 2251-8533

International Journal of Bio-Inorganic Hybrid Nanomaterials

Evaluation of Casein and Inulin Effects on Droplet Size and pH of Nano-emulsion, Morphology and Structure of Microcapsules of Fish Oil

Mahnaz Hashemiravan[1*], Maryam Saberi[2], Nazanin Farhadyar[3]

[1] Assistant Professor, Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

[2] M.Sc., Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

[3] Assistant Professor, Department of Chemistry, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

Received: 4 September 2013; Accepted: 9 November 2013

ABSTRACT

Various compounds have been used for microencapsulating of fish oilso far, but in thiswork for the first time, inulin and casein which are both known for their functional properties, were used as the coating materialof fish oil. Microencapsulation of fish oil was done in 3 steps. First,the wall with 5 different concentrations of inulin and casein were mixed with distilled water. In the next step, an ultrasound generator was usedwith an intensity of 24 kHz for 120 seconds to prepare nano-emulsions and finally the emulsions were converted into powder in a lab-scale spray dryer. The emulsion pH was measured and emulsion droplet size was examined by a particle size analyzer. The microstructures of the powders were analyzed by scanning electron microscopy (SEM). The results showed that the type and concentration of the compounds used as the wallare effective on the properties of nano-emulsion. Comparing the two compounds and their concentrations demonstrated that caseinhas more desirable properties, as if the lowest size of 95.733 nm corresponds to the treatment with 20% casein. Moreover, a significant negative correlation was observed between the size and pH of the nano-size emulsion of fish oil. The comparison between 4% (w/w) and 8% (w/w) fish oil, showed that byincreasingthe ratio of core to wall from 5:1 to 5:2, the size of nano-emulsion significantly reduced ($p<0.01$). Microencapsulatedparticles containing higher concentrations of casein showed much less wrinkle and depression compared to the samples containing higher concentrations of inulin, because of the smaller size of the nano-emulsions containing higher concentrations of casein. In this study the emulsion droplet size wasat the nano-scale and the images showed the significance of wall material properties and their concentrationsaffecting droplet size and morphology ofmicrocapsules.

Keyword: Casein; Inulin; Fish oil; Nano-emulsion; Scanning electron microscope.

1. INTRODUCTION

Fish oil is inherently functional and possesses many components that are good for human health. There is an interest in increasing the amount of fish oil in the diet due to the many associated health of omega-3 fatty acids [1]. Encapsulation has been used to mask unpleasant taste in food sciences as well as to protect against light and airborne oxidation [2]. It is a coating technology, small solid particle, liquid or gaseous as

(*) Corresponding Author - e-mail: m_hashemiravan@yahoo.com.

core materials are packaged within wall materials to form microcapsules [3].

Severalshell or matrix materials have been tested for fish oil encapsulation. (Kolanowski et al., 2004) used modified cellulose as wall material,other wall materials studied were Pectin, Sodium alginate and chitosan (Diaz-Rojas et al., 2004), soybean proteinisolate (Cho et al., 2003), maltodextrincombined with modified starch and whey protein were used as wall material by (Jafari et al., 2008) [2, 4-7].

In this study fish oil was used as the core material and Casein combined with Inulin were used as the wall material. A casein micelle is, in effect, a natural nano-delivery system [8, 9]. Inulin is a Non-starch-polysaccharides consisting of a chain of fructose molecules. It is a polymer of β(2→1)-linked D-fructose units, constituting chains of different lengths each of them with a terminal glucose unit [10].

The aim of this study was to assess the influence of two different type of wall material (Casein and Inulin) on microcapsule morphology and properties of nano-emulsion.

2. MATERIALS AND METHODS

2.1. Materials

In this study, fish oil (HIDHA 25N, Nu-mega ingredients, Brisbane, Australia) was used as the core material (ρ=850 kg/m^3, η=86 mpa s at 25°C, RI=1.483). The wall material was Casein and Inulin. Casein (Casein soluble in alkali with bulk density 450 kg/m^3 and solubility 20.1 g/L (25°C)) was provided by Merck Company and Inulin was obtained from Sigma Chemical Co (St Louise, Mo, USA). The emulsifier used was Polysorbate 80 (Tween 80) that was supplied by Merck Company. Analytical grade hexane and 2-propanal were purchased from Merck Company. Distilled water used for the preparation of all solution.

2.2. Methods

2.2.1. Preparation of emulsions

All emulsion produced in two stages. In pre-emulsion, Casein with various concentrations (5,10,15, 20 g/100g) and Inulin (5, 10, 15, 20 g/ 100g) concentration were solved in distilled water with heating and string in a boiling water bath for 1h at 60°C. Then left it for 24 h. Afterward, we mixed fish oil (4, 8 g/ 100 g) concentration and tween 80 (1g/ 100g) then added to the pre-emulsion. The solution was placed in asonicator (model S 4000-010, misonix) for 2 min.

2.2.2. Particle size determination

The mean particle size and the size distribution of the nano-emulsions were measured by dynamic light scattering (DLS) using PMX 200C ParticleMatrix (German). The nano-emulsion was diluted 40-fold in deionized water before measurement.

2.2.3. pH determination

The pH Measurement bypHmeter (Swiss, Metrohm 827) was carried outimmediately afterthe ultrasound.

2.2.4. Spray-drying

The emulsions prepared were spray dried with a laboratory scale Buchi spray drier (Mini Spray drier B-290, Switzerland) equipped with 0.7 mm diameter nozzle. Spray drying conditions were similar for all samples. The air flow, rate of feeding and aspiration were 600(l/h), 2(mL/min) and 100%, respectively. The inlet and outlet air temperatures were maintained at 120°C and 65 ± 5°C. The fish oil encapsulated powders were collected from the collecting chamber and stored in opaque, air tight containers at 4°C until analysis.

2.2.5. Scanning electron microscopy of encapsulated powders

Themorphology were evaluated with a scanning electron microscope (Model TESCAN//VEGA, England). The samples were placed on the SEM stubs using a two-sided adhesive tape. The specimens were subsequently coated with a thin layer of gold using a magnetron sputter coater (Model Emitech, England).

2.2.6. Statistical design

The independent variables were the ratio of core material to coating material (1:5; 2:5) and the concentration of wall material [Casein, Inulin]. Statistical designs are presented in Table 1.The dependent variables were the emulsion size, the pH of emulsion and the size of nano-emulsion.

Table 1: *The formulations of encapsulated fish oil powders.*

Formulation	Casein (%)	Inulin (%)	Fish oil (%)	Tween 80 (%)	Distilled water
F_1	0	20	4	1	75
F_2	20	0	4	1	75
F_3	10	10	4	1	75
F_4	15	5	4	1	75
F_5	5	15	4	1	75
F_6	0	20	8	1	71
F_7	20	0	8	1	71
F_8	10	10	8	1	71
F_9	15	5	8	1	71
F_{10}	5	15	8	1	71

Table 2: *pH nano-emulsion of casein at various concentration (5, 10, 15 and 20%wt) mixed with inulin 5, 10, 15 and 20%wt, containing 4 and 8%wt fish oil. Values in the same column shown with similar letters are not significantly different.*

Nano emulsion	pH (mean ± SD)
F_1	4.520±0.091[b]
F_2	5.756 ± 0.60[a]
F_3	5.773±0.011[a]
F_4	5.803±0.015[a]
F_5	5.799±0.013[a]
F_6	5.826±0.025[a]
F_7	4.520±0.070[b]
F_8	5.750±0.070[a]
F_9	5.820±0.020[a]
F_{10}	5.843±0.020[a]

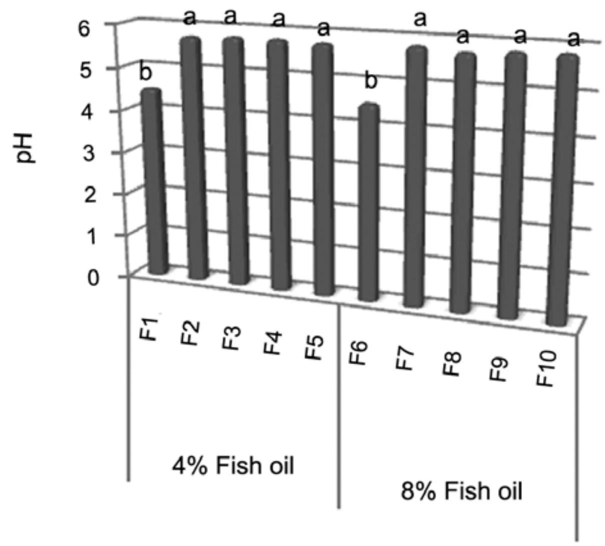

Figure 1: *Effects of fish oil and kind of wall material an dits concentration on the pH of nano-emulsion.*

3. RESULTS AND DISCUSSION

3.1. pHofNano-emulsion

According to the Table (2), the results show that thekindand concentration of combination of Casein and Inulin have very significant influence on pH (p<0.01) but increasing the concentration offish oilfrom 4% to 8% shows that there was no significant change (p<0.05) on the pH of nano-emulsions.

The pH of nano-emulsion of fishoilis changed by kind of wall material, when nano-emulsion was prepared at 20%wt inulin had the least pH of nano-emulsion on the other hand pH of Nano-emulsion had increased significantly when inulin mixed with case in (Figure 1). It happened because of case in's properties that have relatively high electric charged and it is the result of the presence of phosphate groups bonded to serine [11].

3.2. Size of nano-emulsion

The results show that thekind and concentration ofcombination of Case in and Inulin have very significant influence on size of nano-emulsion(p<0.01). Figure (2) shows that the sample which contains 20% casein,has the most pH (pH = 5.79) while it has the least mean size of emulsion droplets (95.733 nm). It is the result of casein's characteristics, because on the

***Table 3:** The mean size of nano-emulsion droplet of casein at various concentration (5, 10, 15 and 20%wt) mixed with inulin 5, 10, 15 and 20%wt, containing 4 and 8%wt fish oil. Values in the same column shown with similar letters are not significantly different.*

Nano emulsion	Size of nano emulsion (mean ± SD)
F_1	148.433±2.578[a]
F_2	101.266±6.690[cd]
F_3	120.200±8.728[bc]
F_4	128.466±6.612[ab]
F_5	111.100±5.707[bcd]
F_6	115.100±4.371[bcd]
F_7	95.733±12.433[d]
F_8	99.433±2.179[cd]
F_9	97.400±5.911[cd]
F_{10}	115.800±3.321[bcd]

higher pH from it is Isoelectric point change between 3.8 to 5.8. Clearly it depends on the kind of the case in ' s Fractions. So when case in presence in emulsion in the shared level of oil and water, it creates repulsion of electrostatic and also space prevent that avoids water closing each other [12].

The size of thenano-emulsion rang was from 95.733 to 148.433 nm and is listed in Table (3). It shows on

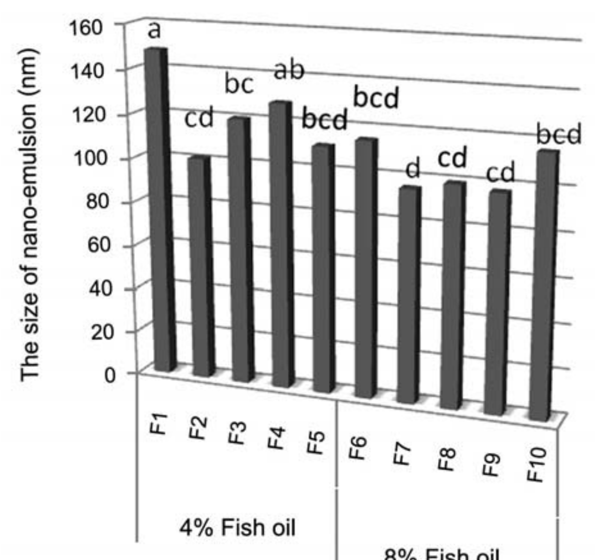

***Figure 2:** Effects of fish oil and kind of wall materialandits concentrationon the size of nano-emulsion.*

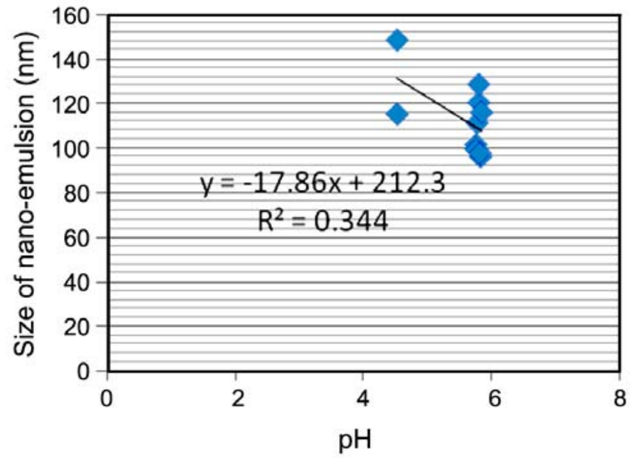

***Figure 3:** Relationship between emulsion size (d_{43}) and pH of the emulsion.*

the Figure (3) that between the pH and the size of emulsiondroplet has negative significant correlation ($p<0.01$). It means that the size of emulsion droplet was reduced by pH increasing. It mentioned on the Table (2) sample contain 20% inulinhad the least pH value (pH = 4.52) while it has themost size of emulsion droplet (148.433 nm). According to researcher's study reducing of the pH from neutral to acidic caused increasing the size distribution of emulsion droplet [12].

The size distribution of emulsion depends onseveralfactors like the amountand type of emulsifiers, phase and the method of producing of emulsification andits pH [13]. So we investigate these items. Some agents

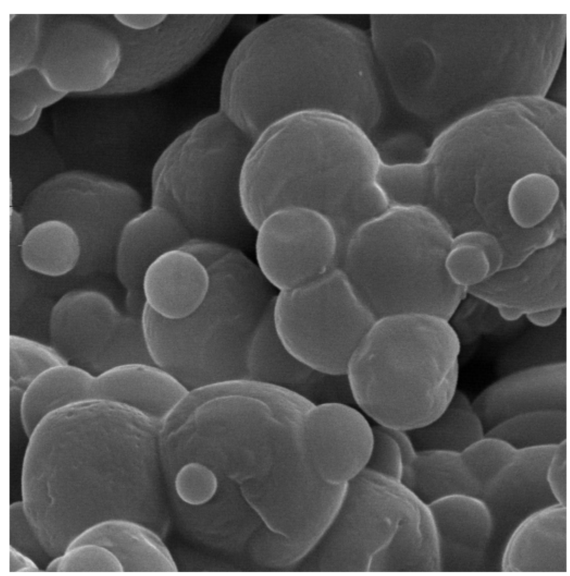

***Figure 4:** Scanning electron microscope (SEM) of encapsulated fish oil powders prepared with 20% casein.*

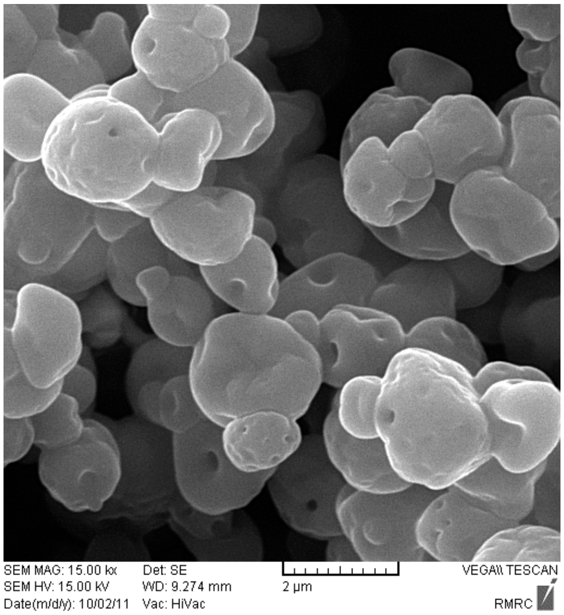

Figure 5: *Showed the presence of some defects, specifically the pores formed, which could explain the relatively high values of surface oil found in the fish oil encapsulated powder containing 20% Inulin.*

like the amount and kind of emulsifier (here we used Tween 80) and phase kind (oil in water) and method of emulsification production was the same for every single samples. The only agents that changed was pH, it is because of kind of wall materials and different concentration so we use this factor in analyzing the result. According to results that we mentioned on the Table (3), the least amount of size of emulsification drops belong to sample that contain 20% case in and 8% fish oil. F1 sample had the least pH and also F2 sample had the most pH so these results shows that when pH is reduced the size of emulsification drops is increased.

3.3. Morphology and structure of microcapsules of fish oil

The SEM images (Figure 4) show that microcapsules containing 20% case in have much less shrinkage and depression than those containing 20% inulin. External structure of the powder particles containing inulin was porous and has depression and some cracks shown in Figure 5. Adding Inulin to Casein had a profound impact on the structure and morphology of microencapsulated powders. Casein combined with high levels of inulin particles produces particles with rough surfaces, but combined with low doses of inulin had no tangible impact, though the uniformity of microcapsule sizeswas affected. It showed the slower rate of hardening of the walls of the sample matrix containing casein. The pictures showed the significance of wall material properties and their concentration saffecting the structure and morphology of microcapsules by the presence of cracks in the microcapsules containing greater amounts of inulin.

4. CONCLUSIONS

The study revealed that ultrasonic waves can be used to crush droplets and reduce their size to the extent of nano and also energy of the waves can be used to produce food nano-emulsions and products in which the particle size as a parameter has an important role in the product quality. Results showed that the smallest particle size was related to the sample containing 20% casein and 8% fish oil and the greatest particle size was related to the sample containing 20% inulin and 4% fish oil. By comparing the samples of 4% and 8% fish oil studies showed that by increasing the ratio of core (fish oil) to wall (combination of inulin and casein) from 1:5 to 2:5, the droplet size as the important characteristic of nano-emulsion decreased, thus the product quality improved. The lowest pH belonged to the treatment containing 20% inulin which was significantly ($p<0.05$) increased by adding of casein. A negative significant correlation was observed between the pH and the emulsion droplet size as by increasing pH the emulsion droplet size was reduced.

REFERENCES

1. Davidov-pardo G., Roccia P., Saldago D., Leon E., Pedroza R., A*m. J. Food Technol*., **6** (2008), 384.
2. JafariS.M.,Assadpoor E.,Bhandari B., & He Y., *Food Res. Int.*, **41** (2008), 172.
3. Loksuwan J., *Food Hydrocolloid*, **21** (2007), 928.
4. Kolanowski W., Ziolkowski M., WeiBbordt J., Kunz B., & Laufenberg G., *Eur. Food Res Technol.*, **222** (2006), 336.

5. Diaz-Rojas E.I., Pacheco-Aguilar R., Lizardi J., Arguelles-Morales W., Valdez M.A., RinaudoM., & Goycoolea F.M., *Food Hydrocolloid*, **18** (2004), 293.
6. Cho Y., Shin D.S., & Park J., *Korean J. Food Sci. Technol.*, **32** (2000), 132.
7. Klinkesorn U., Sophanodora P., Chinachoti P., Decher E.A., & McClements D.J., *Food Hydrocolloid*, **19** (2005), 1044.
8. Summer A.P., Formuggioni P., & Malacarne M., *Ann. Fac. Medic.*, **21** (2003), 169.
9. Semo E., Kesselman E., Danino D., & Livney Y.D., *Food Hydrocolloid*, **21** (2007), 936.
10. Barclay T., Ginic-Markovic M., Cooper P., & Petrovsky N., *J. Exc. Food Chem.*, **1** (2010), 27.
11. Y.H.Hui, 1992. *Dairy Science and Technology Handbook*, VCH Publisher; Eurika, California, USA.
12. Dichenson E., Radford S.J., Golding M., *Food Hydrocolloid*, **16** (2003), 153.
13. Izquierdo P., Esquena J., Tadros T.F., Dedern C., Garcia M.J., Azemar N., & Solans C., *Langmuir*, **1** (2002), 26.