Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 2, p. 318-326, 2014
http://www.innspub.net

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RESEARCH PAPER

OPEN ACCESS

Influence of casein and inulin on the properties of nano-particle encapsulation of fish oil

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Article published on February 15, 2014

Key words: Casein, inulin, fish oil, nano-mulsion, encapsulation.

Abstract

The purpose of this study was to investigate the influence of two components Casein and Inulin with known functional properties as wall material on properties of fish oil encapsulated powders. Two different level of concentration of fish oil and five different levels of combination Casein and Inulin were studied. Ultrasonic have been used for nano-emulsion preparation. The size and the pH of nano-emulsion were characterized. Results showed that, while both wall materials were capable of producing nano-emulsion of the size range of 97.4-148.433 nm, the Casein was the best wall material. The feed mixtures were dried in a mini spray dryer. Particle structure of fish oil encapsulated powder were evaluated by scanning electron microscope. The encapsulation efficiency, the moisture and the surface content of fish oil encapsulated powder were determined. The results revealed that sample containing 20%wt Casein and 8%wt fish oil were the best sample to achieve minimum amount of surface oil(31.67mg/100 g powder), maximum encapsulation efficiency (86.566%) and minimum moisture content(0.711).

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Introduction

Fish oil as functional foods that provide major health benefits. It is rich in long chain omega-3 fatty acids. There is an interest in increasing the amount of omega-3 oil in the diet due to reducing coronary heart disease, colon corner, diabetes, lowered blood pressure (Rubio-Rodrigueze, 2010; Yue *et al.,* 2007) However, fish oil has a strong taste and smell and very susceptible to oxidation. Encapsulation is a strategy that prevent unpleasant taste and protected fish oil against degradation and oxidation. It is a coating technology, small solid particle, liquid or gaseous as core materials are packaged within wall materials to form microcapsules (Pinto Ries *et al.,* 2006; Sukhorukova *et al.,* 2005; Quintanilla-Carvajal, 2010).

One of the most commonly used industrial technologies for encapsulation is spray drying. A critical point in the encapsulation by spray drier is the adequate selection of the wall material .Wall materials for encapsulation by spray-drying must have high water solubility, low viscosity, and drying properties (Rosenberg, 1997). Some of the most important factors to consider to selection of the appropriate wall material for the encapsulation are type of core, encapsulation process, type of consumption productions and economy. The wall may be single or multi layered, several shell 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 protein isolate(cho *et al.,* 2003), maltodextrin combined with modified starch and whey protein were used as wall material by Jafari *et al.,* (2008), whey protein isolate, chitosan and maltodextrin (Klaypradit and Huang.,2008). The wall material applied by Jafari *et al.,* (2008) was maltodextrin, modified starch and whey protein concentration.

In this study, Inulin & Casein with known functional properties have been used as wall material for fish oil encapsulation. An interesting possible encapsulation agent maybe Casein due to its technical and nutritive properties. Casein can form strong network structure within the continuous phase to hold the droplets in place by hydrophobic interactions (Semo *et al.,* 2007; summert *et al.,* 2003). Inulin is a polysaccharide composed of β(2-1) linked D-Fructose that is difficult to hydrolyze. When concentrations exceed 15%, inulin has the ability to form particle gels (De Vos *et al.,* 2010; Roberfroid, 2005; Dahl *et al.,* 2005).

The organic construct consumed as food comes packaged in units that carry the active components and protect the entrapped active materials until delivered to targeted human organs. It is very important study of Casein and Inulin on the properties of Nano-particle Encapsulation of Fish Oil for the Human health.

The aim of this study was to study the properties of fish oil encapsulated powder in different types and various wall material concentration.

Materials and methods

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^{o}C, RI=1.483). The wall material was Casein and Inulin. Casein (Casein souluble in alkali with bulk density 450 kg/m^3 and solubility 20.1 g/L (25^{o}C)) was provided by Merck Company and Inulin was obtained from Sigma Chemical Co (St Louise, Mo, USA). The emulsifier used was Polysorbate80 (Tween80) 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.

Preparation of emulsions

All emulsion produced in two stages. In pre-emulsion, Casein with various concentration(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 60ºC. Then left it for 24h. Afterward, we mixed fish oil (4, 8g/100 g) concentration and tween 80(1g/100g) then added to the pre-emulsion. The solution were placed in an sonicator (model S 4000-010,misonix) for 2 min.

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 120ºC and 65 ± 5ºC. The fish oil encapsulated powders were collected from the collecting chamber and stored in opaque, air tight containers at 4ºC until analysis.

Scanning electron microscopy of encapsulated powders

The morphology 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).

Analysis of moisture content

Moisture content was determined according to AOAC (1996) methods. 2g powder samples were dried in a vacuum oven at 105ºC until constant weight was reached percent loss in weight was reported as water content.

Surface oil content measurements

The surface oil content was determined using the method described by Klaypradit & Huang (2008) for fat extraction.10g powders samples were accurately weighed and added to 100ml of solvent in a volumetric flask and suspension was shaken frequently by hand. Then, it was placed on a shaker and extraction was extended for 15 mins . Then clear phase was purified with purifying paper 4. The Filtrate liquid was placed on Water bath German made in 70ºC to obtaining a stable weight. Finally Container containing surface oil were weighed using the scale and it was deducted from the empty Container to the amount of the oil.

Total oil content measurements

Total oil content was determined by the method of Klaypradit & Huang (2008). Firstly the Container were numbered based on the treatment number and then they were weighed and 10 ml pure water 50ºC was added to the Container inside and mixed for 2 min using shaker. Then the mixture was added as much as one third of the mixture isopropanal nad hexzan and mixed for 5 min and centrifuged applying model H-11N Japan made centrifugator circling 8000 Rpm .then clear phase was purified with purifying paper 4. The Filtrate liquid was placed on Water bath German made in 70ºC to obtaining a stable weight. Finally Container containing total oil were weighed using the scale and it was deducted from the empty Container to the amount of the oil.

Encapsulation efficiency (EE)

EE was defined as the measurement of the remaining content of the core material which is encapsulated in the wall material compare to the starting core material content:

$$EE = \frac{[\text{Total oil content-surface oil content}]}{\text{Starting oil content}}$$

Statistical design

The independent variables considered 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, the moisture content and encapsulation efficiency of encapsulated powder.

Table 1. The formulations of encapsulated fish oil powders.

Formulation	casein (%)	inulin(%)	fish oil(%)	tween80(%)	distilled water(%)
F1	0	20	4	1	75
F2	20	0	4	1	75
F3	10	10	4	1	75
F4	15	5	4	1	75
F5	5	15	4	1	75
F6	0	20	8	1	71
F7	20	0	8	1	71
F8	10	10	8	1	71
F9	15	5	8	1	71
F10	5	15	8	1	71

Result and discussion

Emulsion size

Average emulsion size of the oil/water emulsion ranged from 95.733 to 148.433(nm) and is in the Table 2.

Table 2. The pH values and emulsion size of samples.

Formulation	pH	Emulsion Size(nm)
F_1	$4.520{\pm}0.091^b$	148.433 ± 2.578^a
F_2	5.756 ± 0.60^a	101.266 ± 6.690^{cd}
F_3	$5.773{\pm}0.011^a$	$120.200{\pm}8.728^{bc}$
F_4	$5.803{\pm}0.015^a$	$128.466{\pm}6.612^{ab}$
F_5	$5.799{\pm}0.013^a$	$111.100{\pm}5.707^{bcd}$
F_6	$5.826{\pm}0.025^a$	$115.100{\pm}4.371^{bcd}$
F_7	$4.520{\pm}0.070^b$	$95.733{\pm}12.433^d$
F_8	$5.750{\pm}0.070^a$	$99.433{\pm}2.179^{cd}$
F_9	$5.820{\pm}0.020^a$	$97.400{\pm}5.911^{cd}$
F_{10}	$5.843{\pm}0.020^a$	$115.800{\pm}3.321^{bcd}$

These results confirmed that the emulsion size depended significantly on the type and concentration of wall material and percent oil load. Factors with affect the emulsion size include concentration and type of emulsion, pH value, type of phase and the method of producing of emulsification. All emulsion were produced were of oil-in-water type at same conditions. The only agent changed was its pH, because of different concentrations and kinds of wall materials so this factor in investigating the emulsion size was used. The emulsion size and the pH of samples, correlation coefficient was found to be -0.294 where the correlation is significant at the 0.05 level (Fig 1). Negative correlation value means with the increasing emulsion size, pH value decreases.

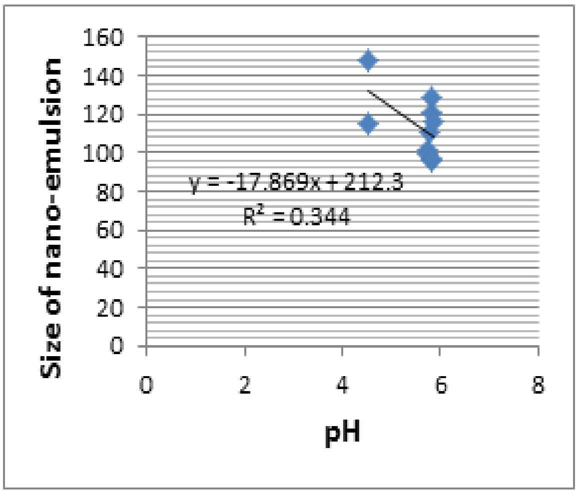

Fig. 1. Relationship between emulsion size and pH of the emulsion.

These are in agreement with the results of Dichinson *et al.*, 1997 who found that reducing pH from natural to acidic leads to increasing the emulsion size. The finding of our study showed that treatment containing 20%wt Inulin had the least pH of emulsion (pH= 4.52±0.07) Incorporating casein into emulsion composition had a profound influence on the pH, the pH of emulsion had increased significantly (p<0/05) when 15% wt Inulin mixed with 5% wt Casein (pH=5.843±0.02). This can be explained by properties of Casein. It has high electric charge, due to phosphate groups linked serine.

Another important result was a higher the pH of emulsion for Casein samples with reducing emulsion size compared with their Inulin counterparts, suggesting that emulsifying capabilities of Inulin are limited and lower than Casein. In Casein , there are many hydrophobic sites having emulsifying capabilities. In addition interaction between Casein and Inulin has smaller emulsion size compared with emulsion containing only Inulin.

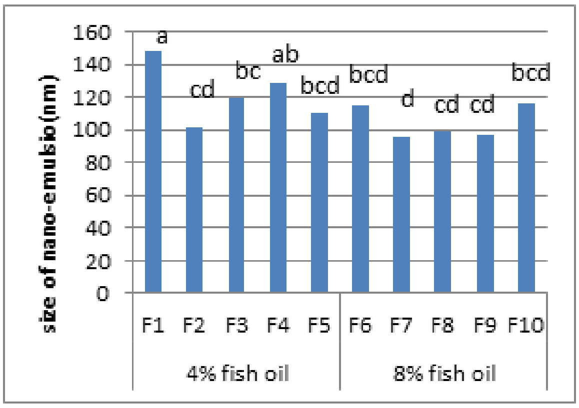

Fig. 2. Effects of fish oil and kind of wall material and its concentration on the size of nano-emulsion.

Moisture content

Moisture of samples is shown in the table 3 which ranged from 0.711 to 1.617 %. Results showed that type of wall material had very significant effects ($p \leq 0.01$) on the moisture powder. Casein compared to Inulin, the casein has a lower water binding capacity. Because its base in the number of linking groups with water in Inulin and Casein molecules are different. As hydrocolloids are capable of increasing water absorption and retention of moisture content. Also, the investigation results showed that concentration of wall material and fish oil had significant influence ($p \leq 0.05$) on the moisture content of the fish oil encapsulated powders. The moisture of spry dried powders was increased with increasing concentration of Inulin. The moisture of samples were decreased when the concentration of fish oil was increased from 4% to 8%, because the rate of dry matter increased in treatments of encapsulated powder with 8% fish oil. Obviously, the moisture of samples was decreased when the rate of dry matter was increased.

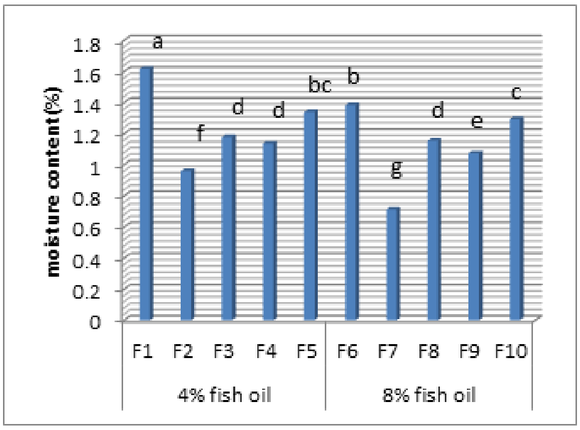

Fig. 3. Effects of fish oil and kind of wall material and its concentration on the moisture content.

Surface oil

The surface oil content of samples are given in Table 3. Differences in type of wall material had significant effect ($p<0.05$) on surface oil value Surface oil content of samples with Casein were found to be significant lower than the samples produced with Inulin. This could be attributed to the greater emulsifying ability of Casein which are mainly related to have many hydrophobic sites. Also, the investigation results showed that concentration of wall material had very significant influence ($p<0.01$) on the surface oil content of fish oil encapsulated powders. This trend could be well explained by the droplet size, similar to the results of Jafari *et al.,* (2008). These workers found that reduces the emulsion size significantly decreased the surface oil content (by using Hi-cap and wpc as the wall material) .The reason could be small oil droplets embedded more efficiently within the wall matrix of microcapsules. Emulsion droplet size plays an important role in the surface oil content of encapsulated powders. According to the theory Thijssen *et al.*, 1968, when water content reduces, diffusion coefficient of the oil phase components decreased several times more than water, so while drying the emulsion, water is continuously removed from the shell with a certain speed, but the oil phase components are spread out of the capsule with a negligible velocity because they are trapped inside the mass of material (wall material) being dried. This solid coating, as a semi-permeable membrane, allows water molecules to exit while reducing or even stopping the outflow of oil components through microcapsules. The results obtained in this work are consistent with the theory Thijssen.

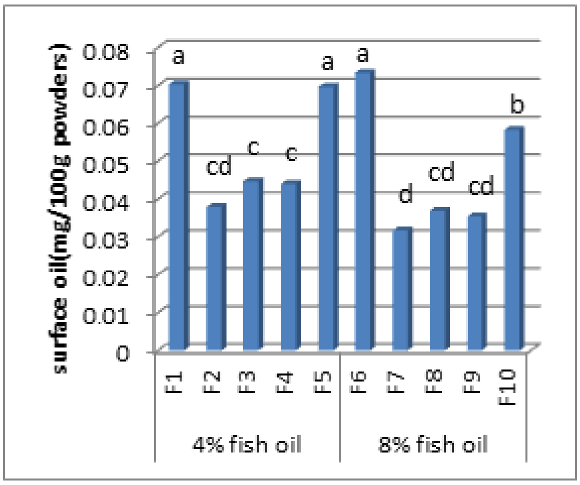

Fig. 4. Effects of fish oil and kind of wall material and its concentration on the surface oil.

Encapsulation efficiency

The EE results of the treatments evaluated are shown in table 3.

There were significant differences ($p<0.05$) between the treatment containing Casein and the ones containing Inulin which showed the smallest value (63.06%). This could be explained by properties of Casein. It has enhanced emulsifying properties and forms a better membrane at the oil-water interface, why it can cover fish oil with a better microencapsulation. It sounds because of high levels of Proline in the chemical structure of casein, it can have a proper orientation into the oil droplet sat the biphasic water-oil environments that this feature helps the oil droplets have an appropriate and effective coverage. Besides This increase may be due to the unique characteristics of the functional groups hydroxyl and carboxyl present in casein that can have a good reaction with the surfactant tween 80 and form a more compact layer with less permeability around the oil droplets.The overall results proved that concentration of wall material and concentration of fish oil had a very significant influence ($p<0.01$) on the encapsulation efficiency.When comparing fish oil encapsulated powders consisting of 8% with those made with 4%, it was found that samples containing 4% fish oil generally manifested lower percentage of EE with a bigger emulsion size.

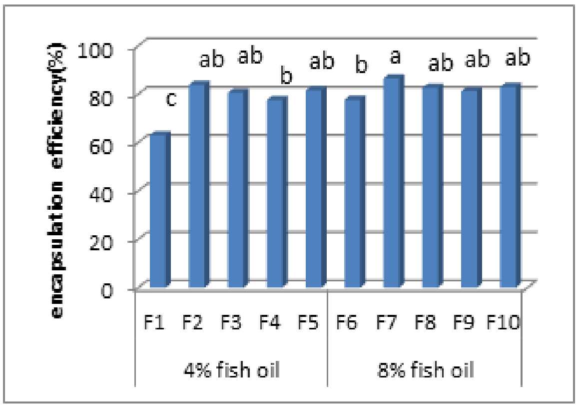

Fig. 5. Effects of fish oil and kind of wall material and its concentration on encapsulation efficiency.

The emulsion size influences the percentage of EE. Small oil droplets will be enclosed more efficiency within the wall matrix of the microcapsules. Interaction between Casein and Inulin can co-adsorb on the oil/water interface, resulting in increasing electrostatic stability.

In the photographs obtained by SEM (fig.6) observed that encapsulation powders containing Casein had smooth surface and less indentation than their Inulin counterparts. Figure 7 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. 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 sizes was 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 concentrations affecting the structure and morphology of microcapsules by the presence of cracks in the microcapsules containing greater amounts of inulin (Fig 8, 9, 10).

Table 3. The properties of fish oil encapsulated powders.

Formulation	moisture content (%)	Encapsulation Efficiency (%)	Surface Oil(mg/100g powders)
F_1	1.617 ± 0.015^{a}	63.060 ± 8.076^{c}	0.070 ± 0.002^{a}
F_2	0.959 ± 0.022^{f}	83.850 ± 5.021^{ab}	0.380 ± 0.001^{cd}
F_3	1.178 ± 0.068^{d}	80.573 ± 4.915^{ab}	0.044 ± 0.002^{c}
F_4	1.136 ± 0.003^{d}	77.530 ± 6.069^{b}	0.044 ± 0.013^{c}
F_5	1.339 ± 0.365^{bc}	81.5000 ± 5.060^{ab}	0.069 ± 0.001^{a}
F_6	1.383 ± 0.017^{b}	77.650 ± 3.258^{b}	0.073 ± 0.009^{a}
F_7	0.711 ± 0.013^{g}	86.556 ± 2.490^{a}	0.031 ± 0.002^{d}
F_8	1.154 ± 0.033^{d}	82.626 ± 0.501^{ab}	0.031 ± 0.003^{cd}
F_9	1.073 ± 0.007^{e}	81.150 ± 1.220^{ab}	0.035 ± 0.001^{cd}
F_{10}	1.292 ± 0.037^{c}	82.990 ± 0.010^{ab}	0.058 ± 0.026^{b}

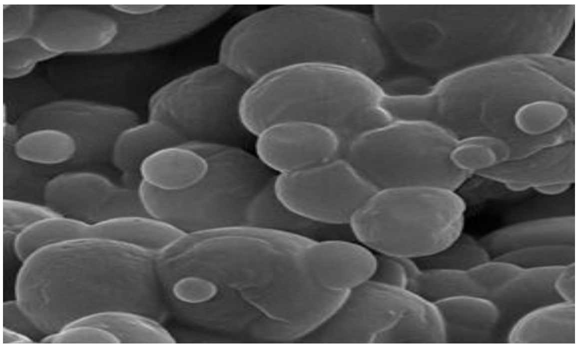

Fig. 6. Scanning electron microscope (SEM) of encapsulated fish oil powders prepared with 20% casein.

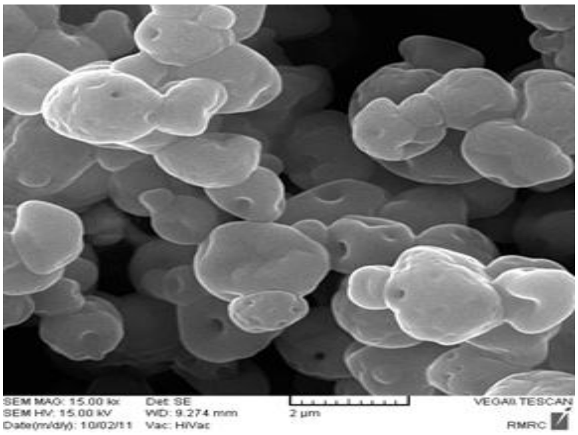

Fig. 7. Scanning electron microscope (SEM) of encapsulated fish oil powders prepared with 20% Inilin.

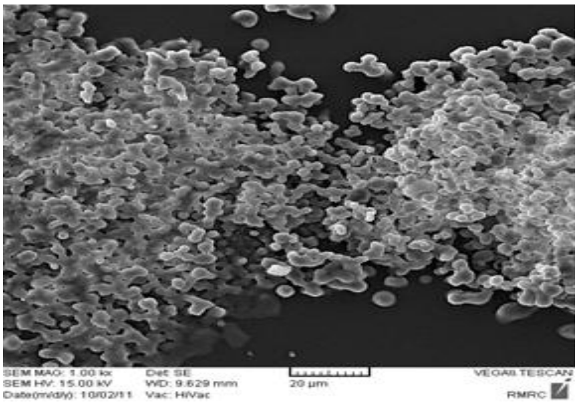

Fig. 8. Scanning electron microscope (SEM) of encapsulated fish oil powders prepared with 10% Inilin & 10% Casein.

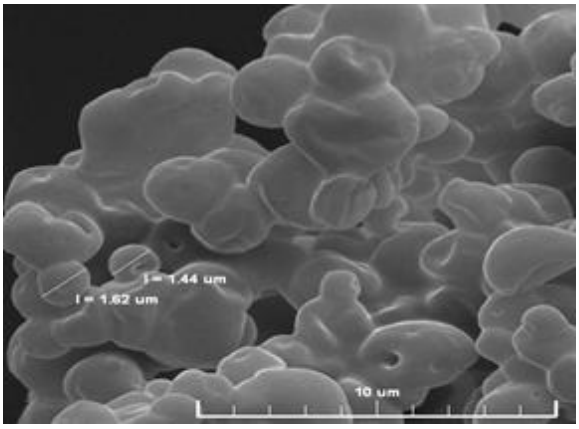

Fig. 9. Scanning electron microscope (SEM) of encapsulated fish oil powders prepared with 15% Inilin & 5% Casein.

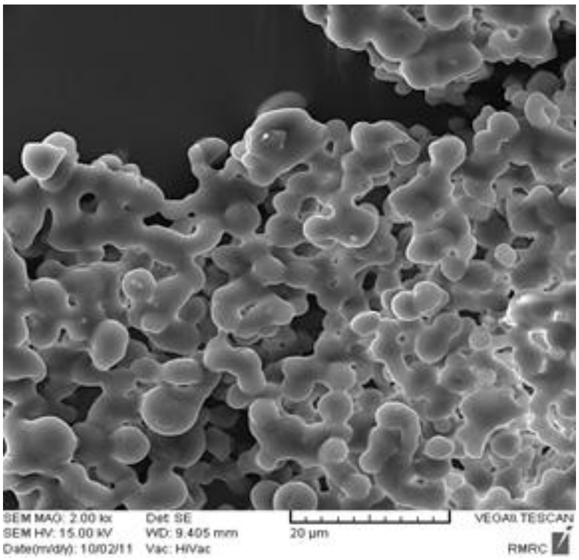

Fig. 10. Scanning electron microscope (SEM) of encapsulated fish oil powders prepared with 5% Inilin & 15% Casein.

Conclusion

These reports clearly showed that type and concentration of wall material influences the properties of the nano-emulsion and encapsulated powders. Casein plays a key role in properties of fish oil encapsulated powder. It has shown potential for encapsulation of fish oil due to their emulsifying properties. It was an efficient wall material for fish oil encapsulated powder with the lowest surface oil, moisture content and emulsion size. Inulin was unable to give an acceptable powder since it was needed to corporate with Casein. In fact, the properties of charged groups of casein causes the protein be introduced as a more suitable covering material than inulin for microencapsulation of fish oil by spray drying method. There was a strong correlation between emulsion droplet size and surface oil content. Also the smaller the emulsion size, the better the encapsulation efficiency.

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