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Influence of Casein and Inulin on the Properties of Fish Oil Nano-emulsion

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ABSTRACT

The purpose of this study is to produce an oil-in-water nano-emulsion by ultrasonication. Casein combined with inulin used as continuous phase, while dispersed phase consisted of fish oil. The size of the nano-emulsion and the pH of nano-emulsion were characterized. Ultrasound has been used for preparing emulsion by 24 KHz intensity for 120 seconds. Prepared nano-emulsion was investigated by particle matrix. Result shows that ultrasonication is an efficient emulsification technique producing small emulsion droplets. Nano-emulsions were very small droplet size (<172.344 nm) .In the present work, our aim was to evaluate kind of continuous phase materials and its concentration on the pH of the Oil/water nano-emulsions and the size distribution of nano-emulsion droplet. Considering the difference in structure in our study shows the mean particle diameter of nano-emulsion droplets decreased with increasing fish oil concentration. This research proved that type and density of used components as continuous has effect on the properties of nano-emulsion. The ultrasonic technology used in this study could lead to application in the food industry.

Keyword: Oil in water nano-emulsions; Casein; Inulin; Fish oil; Ultrasonic.

1. INTRODUCTION

Nano-emulsions are heterogeneous systems consisting of two immiscible liquids in which one liquid is dispersed in another liquid as droplets with diameters of tens to a few hundred nanometers [1]. Nano-emulsions can be defined as oil-in-water (o/w) emulsions with mean droplet diameters ranging from 50 to 1000 nm. Usually, the average droplet size is between 100 and 500 nm.

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The terms sub-micron emulsion (SME) and mini-emulsion are used as synonyms [2]. The preparation of nano-emulsions requires high-pressure homogenization [3]. Accordingly, many emulsion such as stability; theology and color depend on the emulsion droplet size [4]. Development of oil-in-water nano-emulsion is emerging in the food industry as they can be used as delivery systems in transparent foods and beverages and to increase the bioavailability of lipophilic active components. This study was to produce an oil-in-water nano-emulsion by ultrasonication. Ultrasonic emulsification is very efficient in reducing droplet size [5]. And that the more hydrophobic the monomer is, the longer the sonication time required [6]. In our study casein and inulin used as continuous phase, while dispersed phase consisted of fish oil. Casein is a protein that is found in milk, which Ultrasound is defined as any sound of a frequency greater than the upper limit of human hearing, above 16 KHz up to 10 MHz [7]. The efficiency of the dispersion process is strongly dependent on the ultrasonication time at different amplitudes account for about 80% of cow milk protein and known functional properties. Casein is formed in micelles. Casein micelle is, in effect, a natural nano-delivery system [8]. Inulin is a Non-starch polysaccharides consisting of a chain of fructose molecules [9, 10]. The fructose molecules are connected by β (1-2) bonds and the last fructose is linked with a glucose by and α (1-2) bonds [9]. Inulin is the polysaccharides of indigestive dietary fiber that has the advantage of very low caloric value. Inulin could act as a substitute for sugar or fat [11]. The objective of this research was to study the effect of concentration of fish oil and kind of continuous phase material and its concentration on the size distribution of nano-emulsion droplets.

2. EXPERIMENTAL

2.1. Materials

Fish oil was purchased from Iran Quash Fish processing company. 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. Inulin was obtained from Sigma Chemical Co (St Louis, MO, USA). The emulsifier used was Polysorbate 80 (Tween-80) that was supplied by Merck Company. Distilled water was used for the preparation of all solutions. All general chemicals were of analytical grade and were used as received.

2.2. Preparation of nano-emulsions

All nano-emulsion were produced in two stages. In pre-emulsion, casein with various concentrations (5, 10, 15, 20 g/100 g) and inulin (5, 10, 15, 20 g/100 g) 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. Afterwards, we mixed fish oil (4, 8 g/100 g) concentration and Tween 80 (1 g/100 g) then added to the pre-emulsion. The solution was performed by 24 KHz sonicator (models 4000-010, misonix) for 120 seconds.

2.3. 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 Particle Matrix (German). The nano-emulsion was diluted 40-fold in deionizer water before measurement.

2.4. Experimental design and statistical analysis

A randomized complete design (RCD) was used to characterize the nano-emulsion of fish oil. The independent variable considered were the concentration of fish oil (4 and 8%wt) and the ratio of casein to inulin Table 1. The dependent variables were pH of Nano-emulsion and the size distribution of nano-emulsion droplet. All the experiments were performed based on a fully factorial design and the results represent the means of three replicates.

3. RESULTS AND DISCUSSION

3.1. The effect of kind of continuous phase material and its concentration on the pH of Nano-emulsion

According to the Table 2, the result shows that the

Table 1: *The formulation of nano-emulsion.*

Formulation	casein (%)	inulin (%)	fish oil (%)	tween80 (%)	distilled water (%)
A1B1	20	0	4	1	75
A1B2	0	20	4	1	75
A1B3	10	10	4	1	75
A1B4	15	5	4	1	75
A1B5	5	15	4	1	75
A2B1	20	0	8	1	71
A2B2	0	20	8	1	71
A2B3	10	10	8	1	71
A2B4	15	5	8	1	71
A2B5	5	15	8	1	71

kind and concentration of continuous phase has very significant influence on pH ($p < 0.01$). The pH of nano-emulsion of fish oil is changed by kind of continuous phase 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 casein. It happened Because of casein's properties that have relatively high electric charge and it is the result of the presence of phosphate groups bonded to serine.

3.2. The effect of increasing fish oil on the pH of nano-emulsion

According to the Table 2 increasing the concentration of fish oil from 4% to 8% shows that there was no signification change ($p < 0.05$) on the pH of nano-emulsions.

3.3. The Effect of pH on the size distribution of emulsion droplet

It shows on the Table 3 that between the pH and the size of emulsion droplet 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 A_1B_2 contain 20% inulin had the least pH value (pH = 4.52) while it has the most size distribution of emulsion droplet (172.944 nm). According to researcher's study reducing of the pH from neutral to acidic caused increasing the size distribution of emulsion droplet [12].

Table 2: *The pH nana-emulsion of casein at various concentration (5, 10, 15 and 20%wt) mixed with inulin5, 10, 15 and 20%wt, containing 4and 8%wt fish oil.*

Nano-emulsion	pH
A_1B_2	4.520 ± 0.091^b
A_1B_1	5.756 ± 0.60^a
A B_3	5.773 ± 0.011^a
A_1B_4	5.803 ± 0.015^a
A_1B_5	5.799 ± 0.013^a
A_2B_1	5.826 ± 0.025^a
A_2B_2	4.520 ± 0.070^b
A_2B_3	5.750 ± 0.070^a
A_2B_4	5.820 ± 0.020^a
A_2B_5	5.843 ± 0.020^a

Table 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 (632.116 nm). It is the result of casein's characteristics, because on the higher pH from it is isoelectric point changed between 3.8 to 5.8. Clearly it depends on the kind of the casein's fraction.Therefore when casein presence in emulsion in the shared level of oil and water, it creates repulsion of electrostatic and also space prevent that water avoids closing each other [12].

3.4. The effect of kind of continuous phase material and its concentration on the size distribution of nano-emulsion

The size distribution of the oil/water emulsion rang was from 116.632 to 172.944 nm and is listed in Table 3. The size distribution of emulsion depends on several factors like the amount and type of emulsifiers, phase and the method of producing of emulsification and its pH. So we will investigate these items. Some agents 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 continuous phase 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 A_2B_1 sample that contain 20% casein and 8% fish oil. A_1B_2 sample had the least pH and also A_2B_1 sample had the most pH so these results shows that when pH is reduced the size of emulsification drops is increased.

3.5. The effect of concentration of fish oil on the size distribution of emulsion

As it shows in the Figure 1 when we increased the amount of fish oil from 4% to 8% the size distribution of emulsification drop of fish oil was decreased. According to Klinkesorna theory the ratio of materials, the speed of mixture, the rate of adding materials, and the order of adding has also an important role.

Table 3: *the mean size of nano-emulsion droplet of casein at various concentration (5, 10, 15 and 20%wt) mixed with inulin5, 10, 15 and 20%wt, containing 4and 8%wt fish oil.*

Nano-emulsion	The mean size of nano-emulsion droplet (nm)
A_1B_1	152.344 ± 16.690^{abc}
A_1B_2	172.944±22.578^{a}
A_1B_3	146.366±8.728^{abcd}
A_1B_4	168.066±26.612^{ab}
A_1B_5	151.255±35.707^{abc}
A_2B_1	116.632±12.433^{d}
A_2B_2	134.577±4.371^{bcd}
A_2B_3	125.577±2.179^{bcd}
A_2B_4	137.477±5.911^{bcd}
A_2B_5	123.877±23.321^{cd}

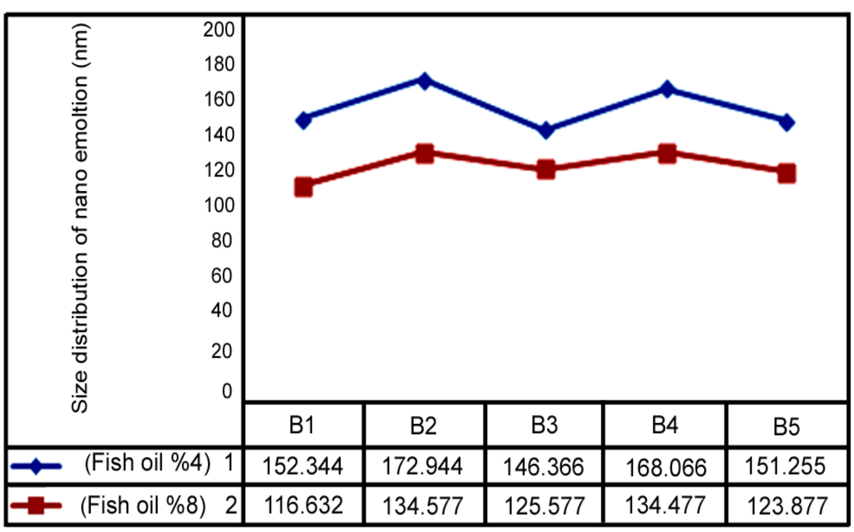

Figure 1: *the effect concentration of fish oil on the size distribution of emulsion.*

All samples that we investigated from these point of view are the same and the ratio of material that in the A_1B_1, A_1B2, A_1B_3, A_1B_4, A_1B_5 samples the ratio of the concentration of fish oil to the continuous phase material (the different ratios of inulin and casein) was 1 to 5. In the A_2B_1, A_2B_2, A_2B_3, A_2B_4, A_2B_5 treatments the ratio of concentration of fish oil to continuous phase material, the size distribution of emulsification drop was reduced.

4. CONCLUSIONS

The results of our study clearly indicated that inulin alone is unable to produce small mean size distribution of emulsion droplet since it was needed to corporate with casein in ratio 1:3, 1:1 and 3:1 respectively. Nano-emulsion is least size distribution of emulsion droplet when mixed with casein. Interaction between casein and inulin can co-adsorb on the oil/water interface, resulting in least size distribution of emulsion droplet caused by increased interfacial electrostatic stability.

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