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Electrosprayed particles derived from nano-emulsions as carriers of fish oil

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ABSTRACT

Fish oil encapsulated submicron particles were produced by electrospraying emulsions. Emulsions were homogenized by various pressures (1000 and 2000 bar) and passes (1, 2, 4, and 8). The physical properties of the emulsions were evaluated, namely droplet size, stability, microstructure, and rheology. Various physicochemical characterizations of the prepared particles were carried out, including the morphology and size of the electrosprayed particles, and the encapsulation efficiency of the fish oil. In optimised conditions, nano-emulsions were produced (d_{50} < 100 nm). It was found that the homogenization parameters of the emulsions affect the structure of the particles. Low emulsion viscosity combined with low oil droplet size and high stability yielded particles with the smallest diameters. The proposed emulsion electrospraying technology could be promising for the production of powdered ingredients enriched with omega-3.

Keywords: encapsulation, emulsion, fish oil, electrospraying

1 INTRODUCTION

High intake of omega-3 polyunsaturated fatty acids (PUFA) has been proven to potentially decrease health risks of various diseases such as cardiovascular, stroke, inflammation, and allergies [1]. Modern diets lead to insufficient daily intake of products rich in omega-3, such as the oily fish [2]. Thus, the development of novel foods enriched with PUFA's is in high demand by the food industry. Their major pitfall, however, is that they are highly susceptible to oxidation, driven by prooxidants such as oxygen, heat and light. Oxidation leads to the deterioration of the final food product not only in terms of their nutritional value, but also their quality characteristics [3].

A possible approach to overcome these limitations is the encapsulation of omega-3 oil, in which the oil is being protected from oxidation by the formation of a biopolymer barrier [4]. A wide variety of encapsulation techniques has been proposed to encapsulate sensitive compounds, but spray drying is the most commonly used [5-7]. However, these techniques require heating (170 - 220^{o} C), that damages the encapsulated compounds.

To this regard, electrohydrodynamic (EHD) processing, and specifically electrospraying, is beneficial for producing particles entrapping the active ingredients [8]. During electrospraying, a high-voltage electro-static field is used to charge the surface of a polymer solution or emulsion droplet which is formed at the end of a capillary tube [9].

Electrospraying presents advantages over conventional spraying systems, as it does not require heat and produces particles with small diameter (0.1 - 5 um). Smaller particles can be more easily incorporated to food matrices compared to the particles produced by spray drying which are relatively larger (1-100 um) [10].

Food grade ingredients are difficult to process by the electrohydrodynamic technique probably because of their complex structure [11, 12]. Several studies have used proteins as a wall material such as zein [13], whey protein [9], or amaranth protein [14], while others have used polysaccharides, such as starch [15], pullulan [9], or chitosan [16]. To the best of our knowledge, the possibility to use high concetrations of protein as wall material has not being studied yet.

Hence, the aim of the present study is to look at the possibility of producing submicron and nano particles loaded with fish oil and using whey protein isolate (WPI) as wall material. The study is evaluating the physical properties of the emulsions (e.g. viscosity, microstructure, size) as well as the properties of the electrosprayed particles (size, structure, encapasulation efficiency).

2 MATERIALS AND METHODS

2.1 Materials

Whey protein isolate (WPI), Lacprodan DI-9224, was kindly supplied by Arla Foods Ingredients (Viby J, Denmark), while maltodextrin, Fibersol-2AG was supplied by Prince International (Emmeloord, Netherlands). Tween 20 was purchased from Sigma- Aldrich (St Louis, USA). Fish oil was provided by Ourons (Gainsborough, UK) and used without further processing.

2.2 Preparation of the emulsions

The aqueous phase of the emulsions was prepared by mixing the precise amounts of the WPI, Tween 20 and maltodextrin with water through gentle stirring.

Primary oil-in-water emulsions with an oil percentage of 10% wt. were prepared and subsequently mixed in a propeller stirrer at 200 rpm for 2 min (Eurostar 60, IKA, Staufen, Germany). During the second homogenization process, the emulsions were further treated with an ultra high pressure homogenizer (Emulsiflex C-50, Avestin,

Ontario, Canada). Homogenization was carried out at varying pressure (1000 and 2000 bar), while the passes varied from 1 to 8. The resulting emulsions had a final concentration of 90% wt. aqueous phase containing 10% wt. WPI, 2% wt. Tween 20, and 2% wt. maltodextrin and 10% wt. fish oil.

2.3 Emulsions' physical properties

The droplet size distribution of the emulsions was determined using laser scattering technique (Malvern Mastersizer 3000, Malvern Instruments Ltd, UK). The refractive indices of fish oil and water were taken as 1.473 and 1.330 respectively, while the obscuration range was fixed between 8% and 20%. The stirrer function was applied to 1000 rpm to avoid the flocculation of the droplets during the measurement period. The Mie theory was used for the analysis.

The stability of emulsions upon storage at ambient temperature was performed with intervals of 24 hours and total time of 7 days. Emulsion samples (approximately 40 mL) were put into test tubes and stored at 4° C. The upper height of separation after storage was being recorded for all the tested emulsions. The stability is presented by the serum index (SI), which was calculated using following equation:

$$SI\ \% = \frac{H_s}{H_e} * 100 \quad (1)$$

where Hs is the height of the serum layer and He is the total height of the emulsion. Therefore, a lower SI represents a more stable emulsion.

Rheological measurements of the emulsions were performed on a stress-controlled rheometer (Physica MCR 301, Anton Paar, Graz, Austria) equipped with a double-gap geometry (DG-26.7). The temperature was kept constant (25.0 ± 0.1 °C) using a water bath. The apparent viscosity was determined versus the imposed shear rate from 0.1 to 100 s^{-1}. 10 points per decade were measured while the whole measuring time was 10 min.

2.4 Electrohydrodynamic process

The emulsions were processed using a Fluidnatek LE10 system from Bioinicia, S.L., Valencia, Spain equipped with a high voltage power supply (0–30 kV). Emulsions were introduced in a 5 mL plastic syringe and were electrosprayed under fixed flow-rate and voltage using a stainless-steel needle. The tip-to-collector distance was set at 13 cm, the flow rate at 0.02 mL/min, and the voltage at 18 kV.

2.5 Properties of the particles

The size distribution of the produced particles was determined using laser scattering technique (Malvern Mastersizer 3000, Malvern Instruments Ltd, UK) with an Aero S dry module accessory.

The microstructure of the electrosprayed particles was determined using a Quanta SE microscope at an accelerating voltage of 5 kV equipped with an image acquisition system (Quanta 650, Thermofisher Scientific, Massachusetts, USA). Samples were sputtered with gold (Q300T, Quorum, Laughton, UK) under vacuum before examining their structure. Several pictures were taken from various positions representing the overall structure of each sample.

The fish oil encapsulation efficiency was determined by dividing the fish oil concentration found in the particles by the initial fish oil concentration added to the emulsions. The particle concentration was evaluated after the ATR- FTIR analysis of the particles using an Nexus 470 equipment (Nicolet, Massachusetts, USA).

2.6 Statistical analysis

Statistical analysis of the results was performed with Statgraphics Centurion XV (Statgraphics, Rockville, MD, USA).

RESULTS AND DISCUSSION

Fish-oil loaded emulsions were homogenized with a high pressure homogenizer at two different pressures (1000 and 2000 bar) and 4 different passes (1, 2, 4, and 8). The physical properties of the emulsions are evaluated and presented in Table 1.

Table 1: Droplet size and serum index of the emulsions and particle size after the emulsion electrospraying.

Emulsions/ particles	d_{50} (nm) (emulsions)	SI (%)	d_{50} (nm) (particles)
1000/1	1300^{h} (19)	20^{c} (1)	n/a
1000/2	632^{f} (3)	0^{a} (0)	974^{f} (6)
1000/4	245^{d} (7)	0^{a} (0)	501^{d} (10)
1000/8	128^{c} (10)	0^{a} (0)	n/a
2000/1	901^{g} (6)	9^{b}(0)	n/a
2000/2	441^{e}(12)	0^{a} (0)	635^{c}(8)
2000/4	103^{b}(5)	0^{a} (0)	198^{a}(3)
2000/8	95^{a} (3)	0^{a} (0)	n/a

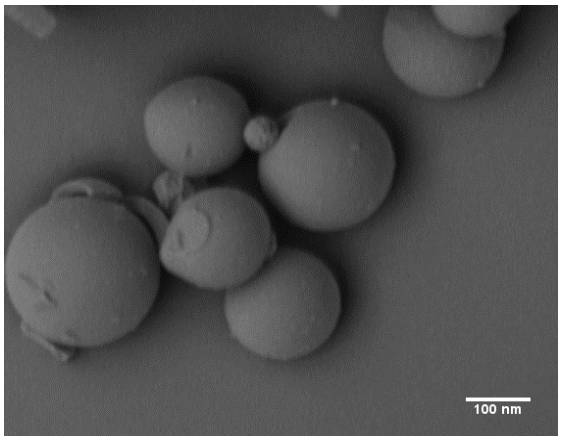
It can be clearly seen that all the emulsions homogenized at higher pressures (2000 bar), show significantly reduced droplet size and serum index (SI, Eq.1) compared to the emulsions homogenized by lower pressures (1000 bar). What is more, the passes are also affecting the physical properties of the emulsion, as by increasing the number of the passes, the size decreases significantly. At optimal conditions (2000 bar for 8 passes), the emulsions reached their minimum droplet size (95 nm).

This steady declining trend of the droplet size by increasing the intesity of the homogenization is well known [17-19].

Interestingly, all the emulsions, except the ones treated by 1 pass, do not show any instability, which is a promising result for the electrosprayability. Thus 1000/1 and 2000/1 emulsions were not selected to be electrosprayed due to their instability.

The rest of the emulsions have been processed with the electrohydrodynamic device. However, the emulsions homogenised for 8 passes (1000/8 and 2000/8) did not produce any powder, probably due to their high viscosity that affects the electrospraying process. All the other emulsions produced particles with different structures at the end of the process. Their average particle size can be seen in Table 1, while the structure of the 2000/4 electrosprayed emulsion can be seen in Figure 1. It is of interest to observe that by increasing the droplet size of the emulsions, the particle size of the produced particles is also increasing. This phenomenon is in accordance with the literature [8].

Figure 1: SEM image of electrosprayed fish oil-loaded particles for emulsion homogenized at 2000 bar for 4 passes.



The encapsulation efficiency (EE) of the fish oil has been evaluated. The EE shows differences between 1000 and 2000 particles, indicating that different emulsification conditions affect the amount of encapsulated fish oil. Specifically, lower EE values can be seen in 1000 particles (60- 80%) compared to 2000 particles (70– 98%). This could be directly related to the oil droplet size of the emulsions during the electrospraying process. In fact, 1000/2 and 1000/4 emulsions experience a more pronounced oil droplet size distribution than 2000/2 and 2000/4, leading to particles with larger diameters.

3 CONCLUSIONS

In this work, emulsions containing whey protein isolate (WPI) and fish oil were prepared by high pressure homogenizer and processed under various conditions of pressure and passes. The emulsions were further processed by the electrospraying technique. Parameters such as the size and distribution of emulsion and stability of emulsions during electrospraying, influence not only the emulsion storability but also the electrospraying process. 2000/4 emulsions gave rise to smooth, homogeneous particles with smaller size compared to the 1000/2. Emulsion electrospraying showed promising results for fish oil encapsulation, especially when using high pressures (2000 bar), as it produced more stable emulsions with smaller oil droplet sizes and lower viscosity. The EE showed differences between samples (60-98%), indicating that the emulsifying method could induce variability of the results in the amount of encapsulated oil. All in all, fish oil loaded sub-micron particles were successfully produced, leading to their potential use in order to enhance fish oil shelf life when incorporated within various food products.

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