ENCAPSULATION OF OMEGA-3 FATTY ACIDS BY PREMIX MEMBRANE EMULSIFICATION

Sarathiraja Ramakrishnan

Dipòsit Legal: T. 163-2014

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ENCAPSULATION OF OMEGA-3 FATTY ACIDS BY PREMIX MEMBRANE EMULSIFICATION

DOCTORAL THESIS

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UNIVERSITAT ROVIRA I VIRGILI

Tarragona

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CERTIFIQUEN:

Que el present treball, titulat "**Encapsulation of omega-3 fatty acids by premix membrane emulsification**", que presenta Sarathiraja Ramakrishnan per a l'obtenció del títol de Doctor, ha estat realitzat sota la nostra direcció al Departament d'Enginyeria Química d'aquesta universitat.

Tarragona, 25 de setembre de 2013

Dra. Carme Güell **Dra. Montserrat Ferrando**

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PREFACE

The Ph.D investigation reported in this thesis was carried out at the group of *Food Innovation and Engineering (FoodIE)* in the Departament d'Enginyeria Química of the Universitat Rovira i Virgili, Tarragona, Spain and was supervised by Dr. Carme Güell Saperas and co-supervised by Dr. Montserrat Ferrando Cogollos.

As part of the thesis, we actively collaborated with the group of Química Analítica, Enològica i dels Aliments from the Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Tarragona, Spain to perform oxidation stability tests of the microcapsules.

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TO MY PARENTS.....

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ABSTRACT

Microcapsules or microspheres are widely used in many different applications in chemical, food, cosmetic and pharmaceutical industries to encapsulate, protect and deliver bioactive compounds. The food industry is more and more focused in developing healthier foods by re-designing the production processes and enriching the foods with bio compounds. Omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) compounds, have proved positive effects on the prevention and treatment of many diseases. The most important natural source of omega-3 fatty acid is fish oil, with physiological and health benefits, can be employed by the food industry to enrich foods in poly-unsaturated fatty acids (PUFA). However, the use of fish oil has some limitations, mainly associated to its oxidative susceptibility and the fishy odour and flavour. The usual approaches to minimize oxidation and odour are done using microencapsulation technology, which offers the possibility to protect against oxidation and favors ease incorporation into food products.

This thesis is divided into seven chapters. **In Chapter 1** gives an overview on the preparation of fish oil microcapsules by different microencapsulation techniques, describing the processes, the effect of operation parameters on the oil encapsulation efficiency (OEE) and the effect of the emulsifiers and wall materials on the final properties and stability of the microcapsules. Since the production of fish oil microcapsules often consist of producing a fish oil/water emulsions followed by drying, the chapter reviews the emulsification technologies, emphasizing on membrane emulsification. Regarding the drying step, the chapter is mainly focused on spray-drying. As the last a part of the thesis project is devoted to the modification of the mechanical structure of the wall material, a part of the introduction is also dedicated to review some of the methodologies used to determine the strength of the microcapsules wall. **Chapters 2 and 3** present the hypothesis of the work and the objectives, respectively.

In Chapter 4, the thesis presents the preparation of fish oil microcapsules combining a low energy emulsification method (premix membrane emulsification) with spray drying. This kind of approach has not been previously studied and opens the possibilities to use more sustainable production technologies in the production

of food microcapsules. Oil-in-water (O/W) emulsions are prepared by a two-step emulsification method that uses a rotor-stator homogenizer followed by membrane emulsification. Membrane emulsification conditions and emulsion formulation have been selected as operation parameters. The results show that the emulsification method, the type and amount of emulsifier and wall material affect the final amount of encapsulated oil. Microcapsules produced by membrane emulsification and stabilized with 2% Tween 20 or 10% whey protein present the highest values (higher than 50%) of oil encapsulation efficiency. It has been found that the OEE increases when decreasing the droplet size of the emulsions as well as with the increase of the amount of wall material employed during drying. Morphology analysis show that microcapsules obtained from O/W emulsions produced by premix ME are more round in shape, without visible cracks on the surface and no vacuoles on the inside. Oxidation stability tests performed on some selected samples indicate that the microcapsules with higher stability are the ones produced with higher amount of wall material and have less surface oil.

Chapter 5, presents the research carried out to study the effect of the emulsification methods and the type and amount of wall material on the most significative physic-chemical properties of fish oil microcapsules. The emulsification technologies studies are a conventional one, rotor-stator homogenization, and a novel technology based on the use of membranes, premix membrane emulsification (ME). Premix ME consists of the production of a coarse emulsion by mechanical means followed by droplet break-up when the coarse emulsion is forced through a membrane. The emulsions produced had an oil load of 10% and 20% and were stabilized using whey protein (isolate and hydrolyzate at 1 or 10%) and sodium caseinate with concentrations of 2% and 10%. Regarding the material used to build the microcapsule wall, whey protein, maltodextrin or combinations of them were used at three different oil:wall ratios (1:1; 1:2; 1:3). The results presented in this chapter clearly show that premix ME is a suitable technology for producing O/W emulsions stabilized with proteins, which have a smaller droplet size and are more monodisperse than those produced by rotor stator emulsification. However, protein concentrations of 10% are required to reduce the droplet size down to 2-3 μm. Small and monodisperse emulsions have been found to produce microcapsules with lower surface oil content, which increases oil encapsulation efficiency and

presents lower levels of oxidation during storage at 30 ºC. Of all the possible combinations studied, the one with the highest oil encapsulation efficiency is the production of a 20% O/W emulsion stabilized with 10% sodium caseinate followed by the addition of 50% maltodextrin and drying.

Chapter 6, this chapter presents to the study on the effects of the addition of whey protein fibrils (WPF) to the wall material on the microcapsule morphology, mechanical properties, and the oil encapsulation efficiency. A Nylon membrane was used to prepare oil-in-water emulsions with a 20% oil load and microcapsules were produced by spray drying after the addition of maltodextrin and whey protein fibrils. Three different ratio oil/wall material ratios were used, 1:1, 1:2 and 1:3. This study demonstrates that the OEE is directly related to the amount of wall material used. When the ratio of oil/wall material is increased, the oil encapsulation efficiency also increased. Regarding inner and outer morphology of the microcapsules, a higher ratio of oil/wall microcapsules produces rounder, month and more rigid capsules. The presence of protein fibrils did not influence the inner and outer morphology of the microcapsules. To study the mechanical properties of the microcapsules, a methodology based on the use of an atomic force microscope was developed. This methodology allowed to calculate the Young modulus from the force curves. The results show that the Young modulus of the capsules increases as more wall material was present. This study has been the first step in demonstrating the use of AFM to obtain mechanical properties of fish oil microcapsules produced by spray-drying and opens the door to obtain reliable mechanical strength values when different wall materials are employed to build the microcapsules. **Chapter 7,** presents the general conclusions of the work and states possible research topics that can follow the findings of this thesis.

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RESUMEN

Las microcápsulas o micro-esferas se utilizan ampliamente in diversas aplicaciones de la industria química, cosmética, alimentaria y farmacéutica para encapsular, proteger y liberar controladamente compuestos bioactivos. La industria alimentaria está cada vez más enfocada al desarrollo de alimentos más sanos mediante el rediseño de los procesos de producción y la incorporación de productos bioactivos. Los ácidos grasos omega-3, especialmente el ácido eicosapentaenioco y el decosahexaenoico, han demostrado ser beneficiosos en la prevención y tratamiento de numerosas patologías. Una de las fuentes naturales más importantes de ácidos grasos omega-3 es el aceite de pescado, que puede ser empleado por la industria alimentaria para enriquecer alimentos en ácidos grasos poli-insaturados. No obstante, el uso de aceite de pescado presenta algunas limitaciones, como su susceptibilidad a la oxidación y su característico aroma y sabor. De forma habitual, la manera de evitar tanto la oxidación del aceite de pescado y de enmascarar sus propiedades sensoriales es mediante su encapsulación, que permite proteger el aceite frente a la oxidación y favorece su incorporación en las matrices alimentarias.

La presente tesis se ha dividido en siete capítulos. En el **Capítulo 1** se ofrece una visión general del estado del arte en la producción de microcápsulas de aceite de pescado. En primer lugar se hace un repaso a las distintas técnicas de encapsulación, que a menudo combinan una etapa de emulsificación del aceite en agua para pasar a un secado posterior. En el capítulo se exponen las distintas técnicas utilizadas, así como el efecto de las variables de proceso y los materiales utilizados en la eficiencia de encapsulación de aceite (OEE, del inglés). Entre las técnicas de emulsificación, se hace un repaso a las más convencionales, para centrarse en la emulsificación por membranas, que es la técnica empleada durante este trabajo. Por lo que se refiere a la etapa de secado, en el Capítulo 1 se presta especial atención al secado por atomización, ya que es la técnica más extendida en la industria alimentaria para la microencapsulación. Dado que la parte final de la tesis se dedica a la modificación de la pared de las microcápsulas y su efecto en la resistencia mecánica de las mismas, la última parte del capítulo hace un breve repaso a las metodologías empleadas en la determinación de las propiedades

mecánicas de las microcápsulas. En los **Capítulos 2 y 3** se presentan las hipótesis del trabajo y los objetivos del mismo, respectivamente.

En el **Capítulo 4** se estudia la posibilidad de obtener microcápsulas de aceite de pescado combinando una tecnología de emulsificación que requiere un aporte energético reducido (emulsificación por membrana premix) con el secado por atomización. Se trata del primer estudio que se realiza con este enfoque, combinando emulsificación por membranas y secado por atomización, abriendo así las puertas para la industria alimentaria en el empleo de tecnologías de producció más sostenibles. En este capítulo se han escogido las condiciones de la emulsificación y la formulación de las emulsiones de aceite de pescado como variables de estudio. Las emulsiones aceite-en-agua (O/W, del inglés) se han preparado mediante una proceso de emulsificación en dos etapas: la primera etapa consiste en la preparación de una pre-emulsión mediante un agitador mecánico que posteriormente se hace pasar a través de una membrana para reducir el tamaño de las gotas y su distribución de tamaño. Los resultados muestran que la emulsificación por membranas produce emulsiones con tamaño de gota menor y más monodispersas que las que se pueden obtener mediante agitación mecánica. Las microcápsulas producidas a partir de emulsiones O/W obtenidas mediante emulsificación con membranas y estabilizadas con 2% de Tween 20 o 10% de proteínas de suero de leche son que presentan unos valores de eficiencia de encapsulación más altos, por encima del 50%. Los resultados muestran que la OEE aumenta cuando disminuye el tamaño de gota y cuando se aumenta la cantidad de material empleado. El análisis morfológico de las microcápsulas indica que aquellas obtenidas a partir del secado de emulsiones producidas mediante emulsificación por membrana premix presentan una forma esférica más homogénea, sin defectos visibles en la superficie y sin vacuolas en el interior. Los tests de estabilidad oxidativa realizados señalan que las microcápsulas que presentan una estabilidad más elevada son aquellas con mayor cantidad de material de pared y menos cantidad de aceite superficial (no encapsulado).

El **Capítulo 5** presenta el trabajo experimental dedicado al efecto del método de emulsificación y la clase y cantidad de material empleado en las propiedades físico-químicas más relevantes de las microcápsulas. La tecnologías de emulsificación que se comparan son la homogenización mediante un agitador mecánico y el

proceso de emulsificación mediante membranas. En este caso las emulsiones estudiadas contenían un 10 y un 20% de contenido en aceite y fueron estabilizadas con proteína de suero de leche y con caseinato sódico. En cuanto al material empleado para formar la pared de las microcápsulas, se empleó maltodextrina, proteína de suero de leche, caseinato sódico o mezclas del polisacárido con alguna de las dos proteínas, manteniendo siempre la relación entre la cantidad de aceite y la de material de pared en 1:, 1:2 o 1:3. Los resultados presentados en este capítulo muestran claramente la tecnología de emulsificación por membranas es indicada para la producción de emulsiones O/W estabilizadas con proteínas, presentado unos valores de tamaño de gota siempre inferiores a los obtenidos en las mejores condiciones de homogenización mecánica. Además, estas emulsiones presentaban un menor grado de dispersión en el tamaño de gota. No obstante, es preciso señalar que las concentraciones de proteína necesarias para la obtención de tamaños de gota entre 2-3 μm tiene que fijarse alrededor del 10%. La relación directa entre el pequeño tamaño de gota y monodispersidad de las emulsiones con una mayor eficiencia de encapsulación es evidente. Además estas microcápsulas presentan cantidades de aceite superficial no encapsulado menores lo que también influye en una mayor estabilidad oxidativa durante el almacenado a 30ºC. De todas las combinaciones estudiadas, se puede concluir que la mayor eficiencia de encapsulación se obtiene a partir de emulsiones con un 20% de aceite, estabilizadas con un 10% de caseinato sódico y con una cantidad de material de pared tres veces superior a la de aceite.

El **Capítulo 6** presenta un estudio sobre la adición de proteínas de suero de leche desnaturalizadas en forma de fibras al material de la pared de las microcápsulas de aceite de pescado. Mediante una membrana de Nylon se obtuvieron emulsiones O/W con un 20% de carga de aceite, a estas emulsiones se les añadió maltodextrina y fibras de proteína antes del secado por atomización. Se estudiaron tres relaciones diferentes de aceite/material de pared: 1:1, 1:2 y 1:3. Una vez obtenidas las cápsulas, éstas se caracterizaron para obtener la OEE, la morfología interna y externa y su resistencia mecánica. Como era de esperar, el aumento en la relación aceite/material de pared incrementó la OEE. Por otra parte, el análisis morfológico no presentó modificaciones en la cápsulas producidas con adición de fibras respecto a las obtenidas solamente con maltodextrina. En el presente trabajo

se adaptó una metodología para determinar mediante un microscopio de fuerza atómica (AFM, del inglés) los valores del módulo de Young, obteniendo así datos sobre la resistencia mecánica de las microcápsulas. Los valores obtenidos demuestran que el aumento del valor del módulo de Young está relacionado con un aumento de cantidad de material de pared. Este estudio presenta por primera vez la aplicación de AFM para la determinación de propiedades mecánicas de microcápsulas de aceite de pescado obtenidas por atomización. El método desarrollado abre la puerta a la posibilidad de obtener datos objetivos referentes a la resistencia mecánica de este tipo de microcápsulas preparadas con distintos materiales. El **Capítulo 7** presenta las conclusiones generales del trabajo y lista las posibles líneas de investigación que podrían continuar con los trabajos realizados en esta tesis.

CHAPTER 1

INTRODUCTION

UNIVERSITAT ROVIRA I VIRGILI
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1.1 MICROENCAPSULATION

Microencapsulation is the process of enclosing small solid particles, liquid droplets or gas bubbles with a thin film of coating or shell material that can be triggered to release their content under specific environmental conditions (Augustin *et al.,* 2000; Hogan *et al.,* 2001; Kolanowski *et al.,* 2006). In microencapsulation, the material inside the microcapsules is referred to as the core material, whereas the coating is sometimes called a shell or wall material. The term microcapsule describes particles with diameters between 1 and 1000 μm. Depending on the method of microencapsulation employed, microcapsules can be classified into two basic categories according to their morphology as mono-core type and multiple-nuclear (matrix) type as shown in Figure 1.1.

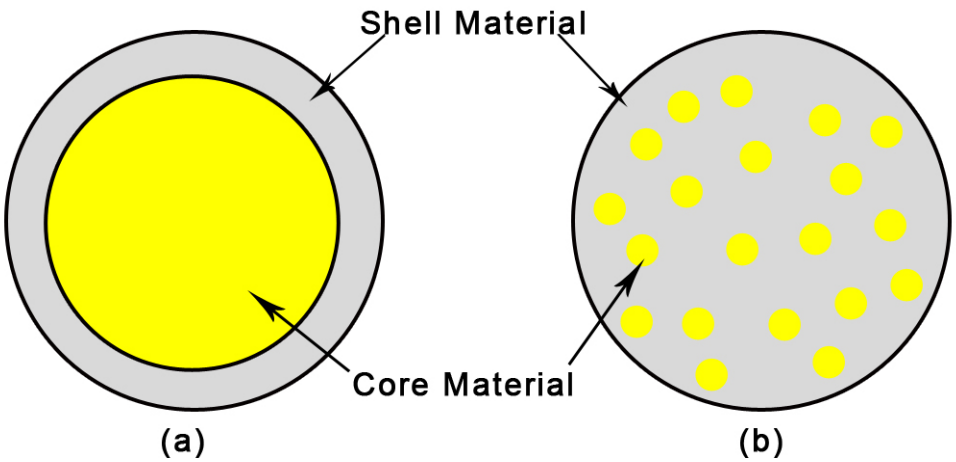

Figure 1.1. Two basic structures characteristic of many commercial microcapsules (a) mono-core type (b) multi nuclear (matrix) type.

Microencapsulation technologies have been used extensively in the pharmaceutical and food industries for many reasons, being the main ones (1) controlled and/or site-specific release of encapsulating drugs (Yoshizawa, 2004; Krishnamachari *et al.,* 2007) (2) protection of the encapsulated materials against oxidation and external environmental conditions like light or moisture (Lin *et al.,* 1995a; Kagami *et*

al., 2003), (3) masking of odour, taste and colour of encapsulated materials (Bruschi *et al.,* 2003), (4) Improving shelf-life of encapsulated materials, (5) isolation of encapsulated materials from undesirable phenomena, and (6) ease of use handling as powder-like encapsulating materials.

1.2 MICROENCAPSULATION OF FISH OIL

Omega-3 fatty acids, also called ω-3 fatty acids, n-3 fatty acids or polyunsaturated fatty acids (PUFA), are considered as essential fatty acids. It is also known that PUFA play an important role in human nutrition and disease prevention. The main sources of omega-3 fatty acids are fish oils and marine oils, but they can be found in other type of products, for example flaxseeds and nuts. Fish oil is a good source of the long chain omega-3 polyunsaturated acids that includes α – linolenic acid (ALA, 18 carbons and 3 double bonds – Figure 1.2 a), eicosapentaenoic acid (EPA, 20 carbons and 5 double bonds – Figure 1.2 b) and docosahexaenoic acid (DHA, 22 carbons and 6 double bonds – Figure 1.2 c). EPA and DHA are essential components for a healthy nutrition and have many beneficial effects on disease conditions like neurological benefits (Dyall and Titus 2008), anti-depressive effects and cardiovascular benefits (Bays, 2008 and Wang *et al.,* 2006).

The most notable benefit of omega-3 fatty acids is in the area of cardio vascular diseases, coronary artery disease, inflammations and allergies. It also improves proper development and function of central nervous system (Salem *et al.,* 1992; Stone, 1997; Simopoulos, 1999). Dietary recommendations suggest that the consumptions of omega-3 PUFA should be 0.65 g of DHA plus EPA per day (0.22 per day of each as minimum). However, the intake of long-chain omega-3 fatty acids in many developed countries is found to be in average 0.15 g/day, which is clearly below the recommend level (Kolanowski *et al.,* 2006). Fish oils, an important source of omega-3 fatty acids, are suitable for direct incorporation into foods, but due to their high degree of unsaturation it is difficult to protect them from oxidation. The usual approaches to minimize oxidation are done using microencapsulation technology, which offers the possibility to protect them against oxidation and favors ease of incorporating into food products like enriched bread, infant formula and diet powder (Kolanowski and Laufenberg, 2006). Typically, the fish oil is homogenized

with an emulsifier in water and the resultant mixture is dried rapidly to convert into powder. Commercially fish oils are formulated as soft gel capsules, emulsions or powder. In generally, though, the emulsion form is bulky and less easily administrated than a solid dosage form. Typically oil encapsulation is carried out by two steps. (i) Preparation of oil-in-water emulsion (O/W), (ii) drying.

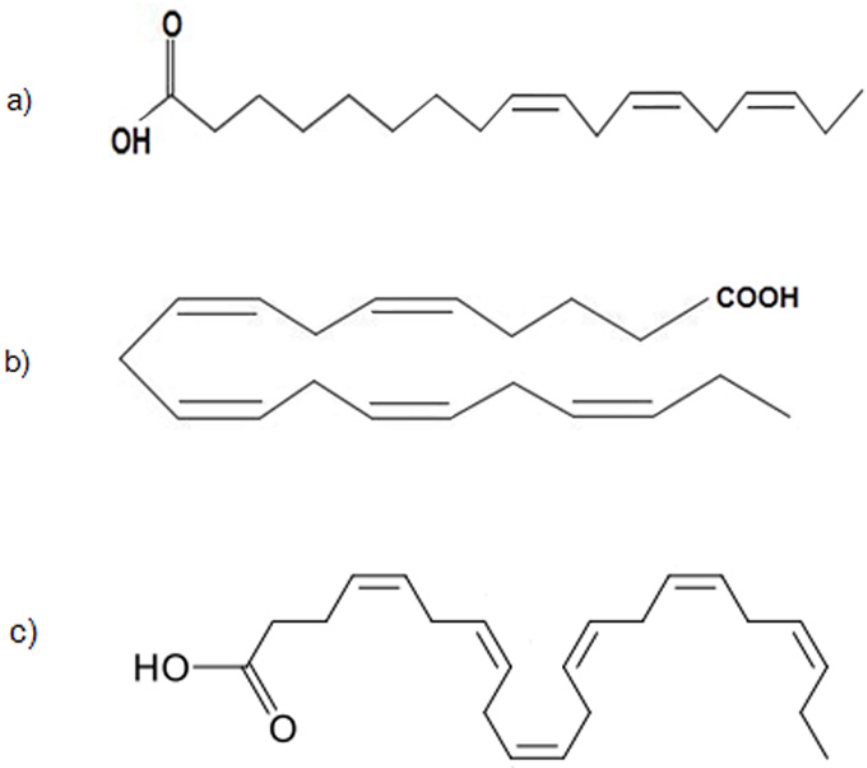

Figure 1.2. Structures of (a) α – linoleic acid (b) eicosapentaenoic acid and (c) docosahexaenoic acid.

1.3 EMULSIONS AND EMULSIFICATION TECHNOLOGY

Emulsions are liquid systems composed of two immiscible phases referred to as continuous and disperse phase, respectively. In O/W emulsions, the oil is the dispersed phase and water is the continuous phase. Alternatively, in water-in-oil emulsions (W/O), the water is dispersed phase and the continuous phase is oil. Multiple emulsions can also be produced, like water-in-oil-in-water (W/O/W) or oil-

in-water-in-oil (O/W/O) emulsions. However, one characteristic that all emulsions have in common is that they are thermodynamically unstable. To enhance the stability of the emulsions a specific type of ingredients, generally referred as stabilizers, can be used. The stabilizers can be divided into two categories, named emulsifiers or texture modifiers, depending on its stabilization mode. Emulsifiers are surface-active molecules that absorb to the surface of freshly formed droplets, while texture modifiers enhance emulsion stability by increasing the viscosity of the continuous phase. Nevertheless, there are some physicochemical mechanisms that are responsible for emulsion instability, being the most important sedimentation, flocculation, coalescence and phase inversion.

The emulsifier plays two main roles in an emulsion; one is lowering interfacial tension between the oil and water phases. Another is that the emulsifier stabilizes the droplets against coalescence and/or aggregation (Joscelyne and Trägardh, 2000). Emulsifiers consist of one or more hydrophilic head groups and one or more hydrophobic (or lipophilic) tails. For this reason they sometimes are called amphiphilic (both lipophilic and hydrophilic nature). Depending on the neutral, negative or positive charge of their head group, emulsifiers are classified as non-ionic, anionic and cationic, respectively. Their hydrophilic-lipophilic balance (HLB) describes the balance between the hydrophilic and hydrophobic parts of the emulsifier. The HLB is measured in a scale of 0 to 20 based on the molecular weight of the hydrophilic portion versus the molecular weight of the compound, and it is an indication of the solubility of the emulsifier. A low HLB value means that the emulsifier is mainly soluble in oil and suitable to stabilize W/O emulsions, while a surfactant with a high HLB value is mainly soluble in water and suitable for the stabilization of O/W emulsions.

1.3.1 Conventional emulsification techniques

Emulsions play an important role in the formulation of foods. Traditionally, there are different methods for preparation of oil-in-water emulsions, such as high pressure-homogenizers, ultrasound homogenizers or rotor/stator systems. Depending on the emulsification process, the diameter of the droplets lies between 0.1 μm and 0.1 mm. Generally, the homogenization systems require a large amount of energy to break up the droplets into smaller ones. However, over the last twenty years there

has been increasing interest in a technique for making emulsion known as membrane emulsification. Contrary to traditional methods, membrane emulsification needs low energy input to produce emulsions and results in narrow droplet size distributions (Joscelyne and Trägardh, 1999).

The **rotor-stator** (Figure 1.3 a) assembly includes a rotor which rotates at high speed inside the stationary stator, which contains numerous holes or slots. Initially, emulsions are made from a premix emulsion (i.e. mixing of both oil and water phases). This premix emulsion is passed through the gap between a numerous holes with high speed rotor. Due to high shear exerted by the rotor, the droplets are broken into small ones. In **high pressure systems** (Figure 1.3 b), the droplets are broken in the nozzle by turbulence and cavitation. The premix emulsion is pushed through a small orifice at high pressure. Due to the high shear force and high energy input of the system, the emulsion droplets are broken down into small droplets. In **ultra sound systems**, the premix emulsion is pushed through high intense ultra sound waves, being the droplets broken down to smaller droplets (Figure 1.3 c).

These methods require a high energy input (10^6-10^8 J m^{-3}) to produce emulsions with a fine droplet-size distribution, and this often results in polydisperse emulsions and causes difficulties when trying to control droplet size distribution (Charcosset *et al.,* 2004). Emulsions produced by high energy input subject the particles in the dispersed phase to high shear and thermal stress which may have undesirable effects on sensitive ingredients contained in the emulsion such as proteins and starches (Vladisvljević and Williams, 2005).

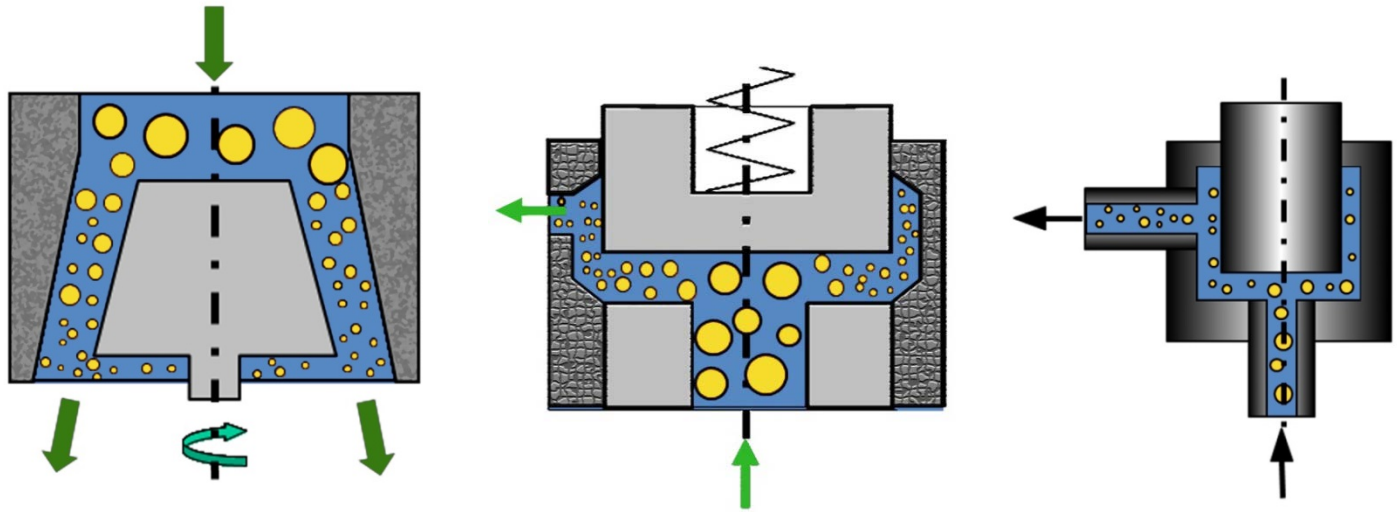

Figure 1.3. Schematic representations of three traditonal homogenization methods : a) rotor stator, b) high pressure, c) ultra sound systems. (adapted from webpage http://lvt.blt.kit.edu/english/1087.php).

1.3.2 Emulsification with microstructured systems

To overcome these problems, new methods of emulsification, which use microstructured systems, have received much attention in literature. Examples of these systems are cross-flow emulsification/direct membrane emulsification (ME), microchannel emulsification and premix membrane emulsification. These techniques are attractive for the better control of the emulsion droplet size and distribution, the mildness of the process conditions, the low energy consumption and easy for industrial-scale application (Nakashima *et al.,* 2000, Joscelyne and Trägardh 2000, Van der Graff *et al.,* 2004). Application of membrane emulsification includes to production of oil-in-water, water-in-oil and multiple emulsions. Other materials prepared by membrane emulsification include solid-in-oil-in-water dispersions, coherent solids (silica particles, solid lipid microspheres, solid metal powder), and structured solids: solid lipid microcarriers, gel microbeads, polymeric microspheres, core-shell microcapsules *and* hollow polymeric micro particles (Vladisavljević and Williams, 2005).

In direct membrane emulsification (Figure 1.4), as introduced by Nakashima *et al.,* (1991), the emulsion is formed when the dispersed phase is pressed through a microporous membrane while the continuous phase flows along the membrane surface. Droplet size can be controlled primarily by the choice of the membrane, the cross flow velocity, and the transmembrane pressure (Nazir *et al.,* 2010).

The most commonly used membranes are hydrophilic Shirasu porous glass (SPG), ceramic aluminum oxide (α-Al_2O_3), zirconium oxide, microporous silica glass, stainless steel membranes and polymeric membranes, such as polytetrafluoroethylene (PTFE) and polycarbonate membranes. The main advantages of direct emulsification are low shear stress, low energy requirement and uniform droplet size, which allow the use of less surfactant, and ease of design and scale out (Van der Graff *et al.,* 2004). The limitation of this method is the low dispersed phase flux obtained through the conventional membranes (like SPG or ceramic) which have a relatively high resistance, and therefore recirculation of the continuous phase is often required to increase the amount of dispersed phase. In addition, the required membrane area is rather large and this makes this technology too expensive for large-scale application.

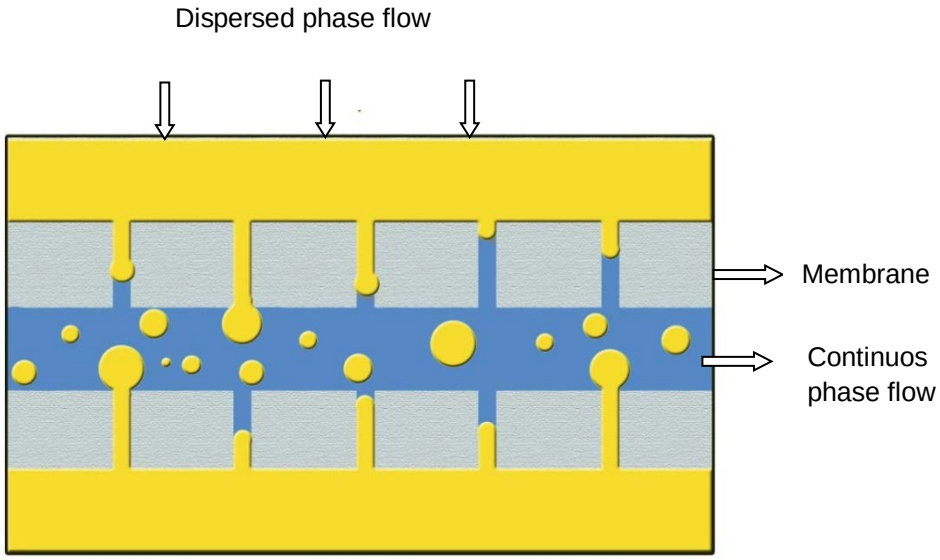

Figure 1.4. Schematic representation of direct membrane emulsification.

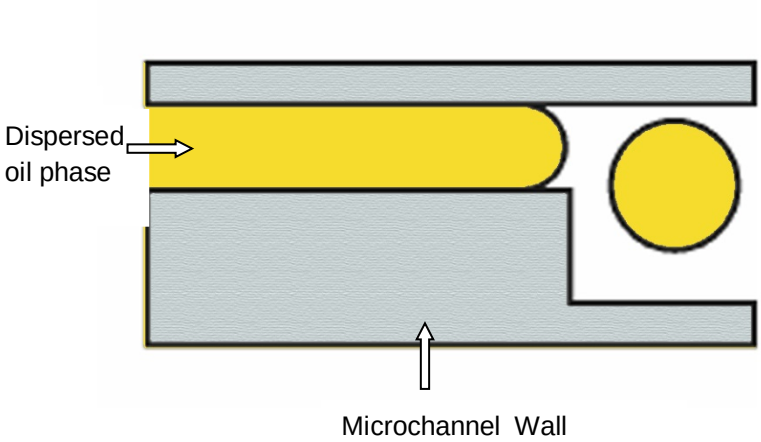

Figure 1.5. Schematic representation of microchannel emulsification (adapted from Van der Zwan, Ph D Thesis, 2008).

Microchannel emulsification is a relatively new technique (Figure 1.5), as introduced by Sugiura and co-workers (2001), in which the emulsion droplets are produced by forcing the dispersed phase through microchannels. The interfacial tension is the driving force in the droplet formation process that is divided into inflation and detachment processes. The dispersed phase is forced into a disk-like shape on the terrace in the membrane.

One of the main advantages of this process is that the emulsion droplet size is only determined by the geometry of the microchannel and the fact that no flow of the continuous phase is needed during the droplet formation. These make the microchannel emulsification technique attractive for producing monodisperse emulsions. The main disadvantage of this technique for practical application is its inherent low productivity since the dispersed phase flux is less than 0.01 $m^3/(m^2\ h)$ for a microchannel plate of 1 cm^2 (Liu *et al.,* 2010).

1.3.3 Premix membrane emulsification

In the early 1990s, Suzuki *et al.,* (1998) introduced premix membrane emulsification, which is carried out in two steps. To obtain O/W emulsions, initially, both oil (i.e., disperse phase) and water (i.e., continuous phase) are homogenized using a rotor–stator system to form a coarse premix emulsion. After this, the coarse premix emulsion is forced through a porous membrane. In the pores, the oil droplets are broken into smaller ones, although the coarse premix emulsion usually needs to be passed through the membrane several times, referred to as cycles, to obtain a narrow droplet size distribution. Figure 1.6 shows a schematic representation of a premix membrane emulsification system.

The energy costs for premix membrane emulsification are relatively low, since no cross-flow is required. Compared to other classical methods such as high pressure homogenization, rotor stator or ultra-sonic systems, less energy is required (i.e. 10^4-10^6 J m^{-3}) to produce small droplets.

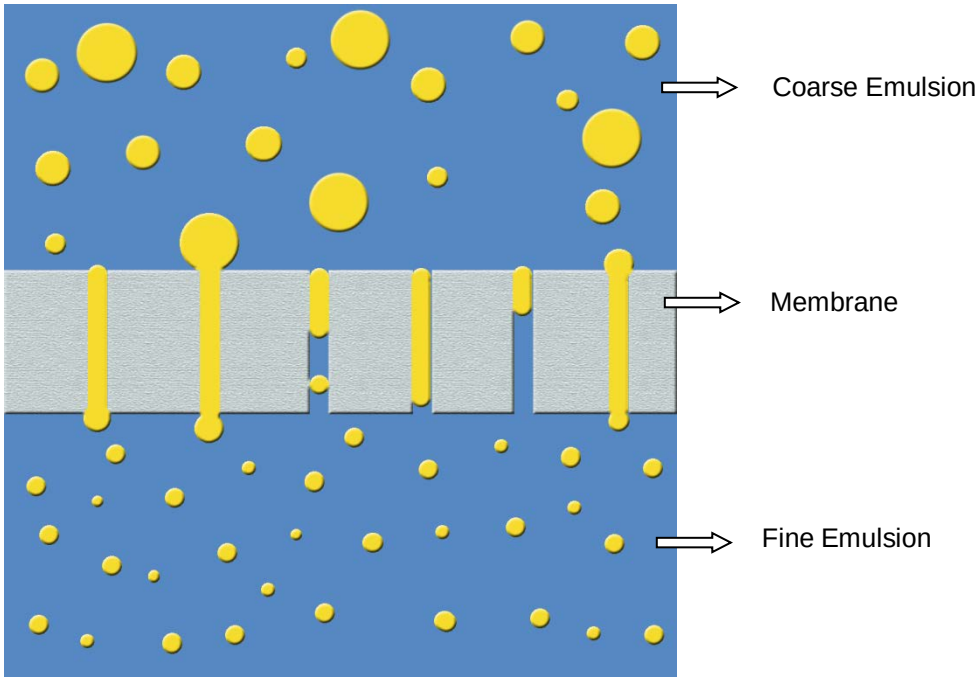

Figure 1.6. Schematic representations of premix membrane emulsification systems.

Premix ME provides several advantages over direct ME. In premix ME, the experimental setup is simpler than direct ME, e.g., no moving parts such as cross-flow pump, except the pre-emulsification step. In premix ME, the optimal transmembrane fluxes are typically above 1 $m^3m^{-2}h^{-1}$, which is one to two orders of magnitude higher than in direct ME (Vladisavljević and Williams 2005). One of the disadvantages of premix ME is the higher emulsion polydispersity compared to direct ME, however a higher degree of monodispersity can be achieved by increasing the number of homogenization cycles. Another possible drawback of premix ME is related to membrane fouling if proteins are used as stabilizers.

1.4 TECHNOLOGIES FOR PRODUCTION OF MICROCAPSULES

Microencapsulation technology is widely used in the pharmaceutical and food industries (Gibbs *et al.,* 1999; Madene *et al.,* 2006) and provides a means of converting fish oil into a dry powder. It was firstly discovered by Bungen burg de Jong and Kan in 1931, which dealt with the preparation of gelatin spheres through a coacervation process (Robinson and Lee, 1987). In the food industry, the most

widely employed technologies for encapsulating lipophilic compounds are based on the production of an oil-in-water emulsion which is then followed by either spray drying (Hogan *et al.,* 2001a; Kagami *et al.,* 2003; Gharsallaoui *et al.,* 2012; Aghbashlo *et al.,* 2012), freeze drying (Heinzelmann *et al.,* 1999), molecular inclusion (Yoshii *et al.,* 1996), enzymatic gelation (Cho *et al.,* 2006), or coacervation (Lamprecht *et al.,* 2001). Generally, microencapsulation methods are classified as chemical and physical methods.

1.4.1 Chemical methods

Chemical methods include coacervation, solvent evaporation, interfacial and in-situ polymerization. Coacervation is a colloid phenomenon that is carried out under continuous agitation to encapsulate liquids and solids (Versic, 1988). A large number of coating materials have been evaluated to carry out microencapsulation by coacervation, being the most studied and reported gelatin/acacia gum. However, other coating systems such as chitosan, soy protein, polyvinyl alcohol, gelatin/carboxymethylcellouse and guar gum/dextran have also been studied (Desai and Park, 2005; Gouin, 2004). Complex coacervation has been used for microencapsulation of oil carriers for hydrophobic drugs (Jizomoto *et al.,* 1993) and omega -3 PUFA (Lamprecht *et al.,* 2001; Jouzel *et al.,* 2003).

By the solvent evaporation process it is possible to produce microcapsules for a wide variety of liquid and solid core materials. Initially, the coating material is dissolved in a volatile solvent, and then the core material is added under continuous agitation to obtain the desired size of the microcapsule. When the desired emulsion droplet size is formed, the stirring rate is reduced and the evaporation of the solvent occurs continuously under atmospheric or reduced pressure at appropriate temperature to achieve solid microcapsules (Soppimath *et al.,* 2001; Chiao and Price 1994).

Interfacial polymerization is a process whereby a monomer is made to polymerize (Torres *et al.,* 1990) at the interface of two immiscible substances. For example, a solution of the pesticide and a di-acide chloride is emulsified in water and an aqueous solution containing an amine and a polyfunctional isocyanate is added. Condensed polymer walls form instantaneously at the interface of the emulsion

droplets. In few microencapsulation processes, the direct polymerization of a single monomer is carried out on the particle surface, for example, cellulose fibers are encapsulated in polyethylene while immersed in dry toluene (Jadupati *et al.,* 2012). All these methods are used in small scale in industrial or commercial applications.

1.4.2 Physical methods

Physical encapsulation methods include extrusion, freeze drying, spray drying and spray-chilling. Among these techniques, the most common way of producing encapsulated fish oil is by spray drying the O/W emulsion, since it is a very efficient and flexible process that quickly removes water by vaporization and that can be carried out with readily available equipment. There is also the possibility to produce microcapsules by assembly of oppositely charged biopolymers to build the shell of a desired thickness (Rosier-Miranda *et al.,* 2012), referred as layer-by-layer (LbL) electrostatic deposition.

Spray Drying

Spray drying is a single continuous drying step process of transformation of a feed from a fluid state into a dried particulate form by spraying the feed into a hot drying medium (Masters, 1991), yielding fine particles. The feed can be a solution, a suspension, a dispersion or an emulsion. To produce oil microcapsules, the feed is usually an O/W emulsion, which contains (dissolved or dispersed) the wall material. The dried product can be in the form of powders, granules or agglomerates with low moisture content. The characteristics of the final dried products depend upon the physical and chemical properties of the feed, process parameters of dryer. Encapsulation by spray drying has been used in the food industry from the late 1950s to provide flavour oils with some protection against degradation/oxidation (Gouin, 2004). For food applications there are several publications reporting spray drying for oil encapsulation using protein based wall materials with or without blending with maltodextrin (Hogan *et al.,* 2003; Kagami *et al.,* 2003; Bae and Lee 2008). Modified cellulose (Kolanowski *et al.,* 2004; Kolanowski *et al.,* 2006), chitosan (Klinkesorn *et al.,* 2005) and starch (Drusch and Berg, 2008) have also been employed as wall materials for fish oil encapsulation by spray drying. Figure 1.7 shows a schematic representation of the spray drying equipment. The feed is

pumped to the atomizing device, when the droplets are formed in the nozzle they contact with the hot air in the drying chamber. The solid particles formed are collected by a separation device.

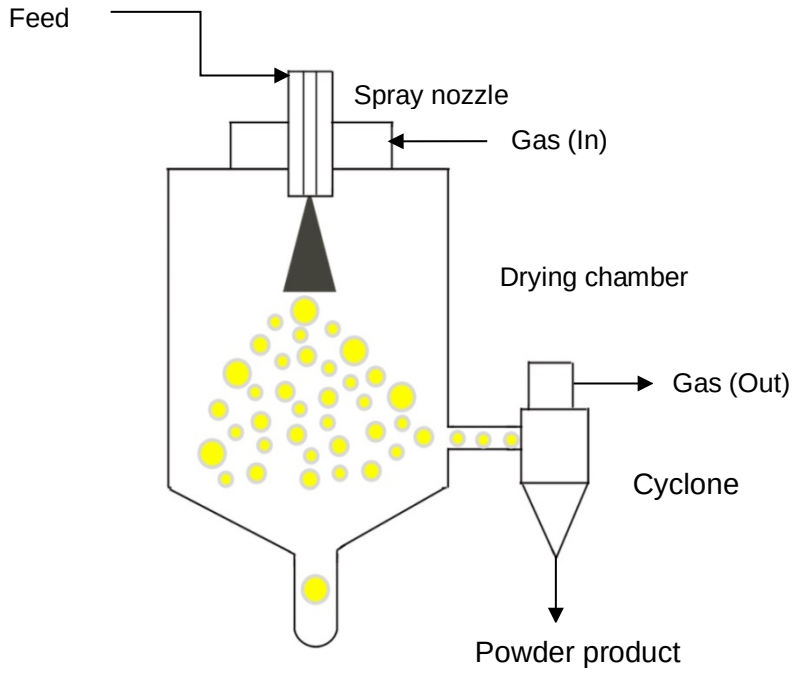

Figure 1.7. Schematic representation of a typical spray drying set up.

The spray drying process can be dividing into five steps.

1) Concentration: the feed stock is normally concentrated prior to introduction into the spray dryer.

2) Atomization process: in this step, the feed solution is sprayed into the drying chamber with the help of an atomizer or nozzle. There are different types of nozzle available, like rotary nozzles, single fluid pressure nozzles, ultrasonic nozzles and pneumatic nozzles. The selection of the atomizer is based on the characteristics of the feed and the qualities desired for the final dried product.

3) Spray-air contact: in this step, the atomized liquid is brought into contact with the hot gas medium. Generally, the hot drying gases are passed as either in co-current or counter current direction to the atomizer in the drying chamber. Co-current involves the droplets and drying air flowing in the same direction i.e., entering the dryer from the same end. In case of counter current direction, the droplets and the drying air enter the dryer from opposite ends of the dryer. Some spray dryers incorporate both layouts and are termed as mixed-flow dryers.

4) Drying step: in this step, rapid moisture evaporation takes place. The hot air converts the suspension into solid and the solvent into vapour.

5) Separation: this is the final step of spray drying where the dried particles are collected. Cyclones, bag filters and electrostatic precipitators may be used for the final separation stage.

The advantages of spray drying are its ability to handle heat-sensitive materials, readily availability of machinery and its variety, good keeping qualities of microcapsules, variety of particle sizes that can be produced and excellent dispensability of particles in aqueous media (Ashady, 1993; Re, 1998; Kolanowski *et al.,* 2006).

1.5 FACTORS AFFECTING ENCAPSULATION BY SPRAY DRYING

1.5.1 Initial emulsion

As mentioned in an earlier section, oil encapsulation can be carried out by preparing an O/W emulsion followed by spray drying. The emulsion features are one of key roles in determining the retention of volatiles and the surface oil content of the final encapsulated powder. The most significant parameters of the emulsion to consider before drying are emulsion size and distribution, viscosity and emulsion stability.

Most research studies have shown that the emulsion size has a considerable effect on the encapsulation efficiency of oils during the drying process. Some reports clearly show that reducing emulsion size can result in encapsulated powders with higher retention of volatiles and lower content of non-encapsulated oil at the surface of the powder particles (Risch and Reineccuis, 1988; Sootitantawat *et al.,* 2003;

Sootitantawat *et al.,* 2005; Jafari *et al.,* 2008). Risch and Reineccuis (1988) studied the effect of emulsion size of orange peel oil (0.9 to 4.0 µm) and their results suggested that small emulsion size yields a larger percentage of retention of orange oil with small amount of surface oil.

Similarly, Sootitantawat *et al.,* (2003) produced emulsions in the range of 0.5 to 5.9 µm and observed that the emulsion with the smallest droplet size had the higher retention of orange oil. In 2008, Jafari *et al.,* studied the effect of fish oil/water emulsion droplet size in the range of 0.21 to 4.6 µm produced by different emulsification systems. The results of the work suggested that the smallest emulsion size yields the highest oil encapsulation efficiency.

Minemoto *et al.,* (2002), Liu *et al.,* (1999) and Sootitantawat *et al.,* (2003) results suggest that microcapsules produced from emulsions with small droplet size oxidize slower than powders produced from emulsions with bigger emulsion droplet size, possible because of lower amounts of non-encapsulated oil on the surface of dried particles. The droplet size distribution is also an important parameter if considering that the packing efficiency of the oil droplets during the atomization process increases with a more narrow size distribution. The viscosity of the emulsion is also important since it affects flowing during emulsification and the formation of the droplets during atomization. Finally, higher emulsion stability is critical during the drying process since it ensures a homogeneous production of the microcapsules.

1.5.2 Wall materials type and amount

After the emulsion has been produced, it is a common practice to add the wall forming material before the drying step. Depending on how this material is added and how the drying step is performed, different microcapsule structures can be obtained. The wall is designed to protect the core from deterioration and release it under desired conditions (Young *et al.,* 1993). The selection of wall material is therefore of paramount importance for the success of the encapsulation, since the stability of the microcapsules and their controlled release depends mainly on the composition and structure of the wall. Typically, wall materials for microencapsulation of oil by spray drying must have emulsifying properties, high water solubility, low viscosity and drying properties (Shahidi and Han, 1993; Bae

and Lee, 2008). Moreover, the cost should be also considered in case of products for the food industry (Gharsallaoui *et al.,* 2007). In the spray drying process, a number of studies on the microencapsulation properties of different wall materials for food ingredients are available, including starches (ex. maltodextrin) and sugars (lactose, sucrose) (Onwulata *et al.,* 1995; Strange *et al.,* 1997; Shiga *et al.,* 2001; Bae and Lee, 2008), Arabic gum (Kim and Morr, 1996; McNamee *et al.,*1998), mesquite gum (Beristain *et al.,* 2001) and milk proteins (whey protein and sodium caseinate) (Sheu and Rosenberg, 1995; Hogan *et al.,* 2001).

Carbohydrates

Carbohydrates (ex. maltodextrin and starches) are considered good encapsulating agents because they exhibit low viscosities at high solid contents, good solubility and excellent protection for encapsulated core materials such as fish oil, milk fat, soya oil and orange oil (Kenyon, 1995; Varavinit *et al.,* 2001). The degree of protection is directly related to dextrose equivalent value (DE) of the carbohydrates. Higher-DE systems are less permeable to oxygen and result in powder with high encapsulation efficiency (Jafari *et al.,* 2008).

Starches that have been oxidized or incorporated with lipophilic groups where generally found to have good emulsifying and oil retention properties with low viscosities and high solids concentration (Shahidi and Han, 1993). The degree of protection is directly related to the dextrose equivalent (DE) of the hydrolysed starch, higher-DE systems are less permeable to oxygen and result in powder with higher encapsulation efficiencies (Sheu *et al.,* 1998; Re *et al.,* 1988; Hogan *et al.,* 2001; Jafari *et al.,* 2008). However, the lack of emulsifying properties of these ingredients results in poor retention of flavor during spray drying (Bangs *et al.,* 1990; Westing *et al.,* 1998). In order to improve the emulsifying capabilities of starch molecules, side chains of lipophilic succinic acid are inserted into starch to produce modified starches. Example of modified starch such as Capsul® FP, N-lok®, Hi-Cap® IFM and Encapsul® 855 (from Ingredion) are used in flavor and oil encapsulation (Trubiano *et al.,* 1988; Tesch *et al.,* 2002). Sugars such as maltose, lactose or sucrose have also been reported as encapsulating agents due to their high solubility and low cost (Konstance *et al.,* 1995; Onwulata *et al.,* 1995).

Maltodextrins are hydrolysed starches that are of low cost and provide good oxidative stability to encapsulated oil but exhibit poor emulsifying capacity, emulsion stability and low oil retention (Kenyo, 1995). Maltodextrin alone cannot be used as encapsulating material if an O/W emulsion has to be produced. In order to improve the properties, it has been suggested to blend it with other materials, like milk proteins or Arabic gum, which gives good encapsulation properties with high retention or encapsulation efficiency.

Kagami *et al.,* (2003) produced fish oil microcapsules using a combination of sodium caseinate and maltodextrin of DE 18 as wall material. Their results show that fish oil microcapsules prepared with blends of maltodextrin and sodium caseinate have good oxidation stability and good encapsulating properties. Calvo *et al.,* (2011) produced walnut oil microcapsules with wall blends of maltodextrin and sodium caseinate with 1:1 oil/wall ratio, maltodextrin and carboxymethylcellouse with 1:1.5 oil/wall ratio. They found the highest encapsulation efficiency when maltodextrin and carboxymehtylcellulose were used as encapsulation agents and the ratio of oil/wall materials was 1:1.5.

The combination of maltodextrin with whey protein or Arabic gum was reported to be effective for the encapsulation of cardamom oil (Sankarikutty *et al.,* 1998), avocado oil (Bae and Lee 2008), citrus oil (Thevenet *et al.,* 1995), soy oil (McNamee *et al.,* 2001) rice flavor (Apintanapong *et al.,* 2003), pine flavor (Lee *et al.,* 2005) and bixin (Barbosa *et al.,* 2005).

From all the revised literature it seems desirable to use carbohydrates in combination with other wall materials such as modified starches, Arabic gum and milk proteins.

Proteins

Proteins such as milk proteins (whey proteins, sodium caseinate) and gelatin are reported as good encapsulation materials for microencapsulation by spray drying. Proteins exhibit many desirable characteristics for a wall material such as solubility, film formation, the ability to interact with water, emulsification, stabilisation of emulsion droplets and high binding properties (Jafari *et al.,* 2008; Gharsallaoui *et al.,* 2007; Hogan *et al.,* 2001a; Rosenberg and Sheu, 1996). They have been used

alone or in combination with other materials to encapsulate a variety of flavour and oil compounds.

Sodium caseinate produced from skim milk is widely used as an ingredient in the food industry. Its functional properties include emulsification, water-binding, fat-binding, thickening, gelation and whipping. Sodium caseinate is especially important as a stabilizer for emulsions, because of its ability for rapidly conferring a low interfacial tension during emulsification and because of its strong amphiphilic characteristics (Berli *et al.,* 2002).

Sodium caseinate is well-used wall material because of its good solubility and emulsifying properties and its stability during drying process (Dickinson, 1999). Investigations have proven sodium caseinate to function well for encapsulating anhydrous milk fat, orange oil, soy bean oil, caraway essential oil, fish oil and fatty acids (Rosenberg *et al.,* 1998; Hogan *et al.,* 2003; Jimenez *et al.,* 2004; Kim *et al.,* 1996).

Whey protein and sodium caseinate have been proved to function well for encapsulation anhydrous milk fat (Young *et al.,* 1993; Rosenberg *et al.,* 1993), fish oil (Hogan *et al.,* 2001; Kagami *et al.,* 2003; McClements, 2004), orange oil (Kim *et al.,* 1996), soy bean oil (Hogan *et al.,* 2001) and fatty acids (Kagami *et al.,* 2003; Hogan *et al.,* 2001). Jimenez *et al.,* (2004) produced linoleic acid powder through spray drying with whey protein alone and found an oil encapsulation efficiency about 89.6% with a surface oil content of 1.77 g/100 g powder.

Hogan *et al.,* (2003) encapsulated soya oil through spray drying with whey protein concentrate alone and found that encapsulation efficiency correlated with oil/protein ratio. For a powder with an oil/protein ratio of 0.25, the encapsulation efficiency value found by these authors was around 55%. Whey protein has also been successfully used to encapsulate anhydrous milk fat, resulting in an oil encapsulation efficiency greater than 90% (Young *et al.,* 1993).

Recently, Leagako and Dunford (2010) produced fish oil microcapsules with whey protein (1:2 oil/wall ratio) by spray drying testing three different types of nozzles: a pressure nozzle with 1 liquid channel, a pressure nozzle with 2 liquid channels and a sonic atomizer with 2 liquid channels, and by freeze drying. The microcapsules

prepared with spray drying with 2-fluid pressure nozzle resulted in an encapsulation efficiency value around 91.6% with a surface oil content of 2.6±0.03 (% w/w). The microcapsules produced with freeze drying showed an encapsulation efficiency value around 83.3% with a surface oil content of 5.3±0.03 (% w/w). Microcapsules produced using the ultrasonic nozzle had lower oil encapsulation efficiency compared to the ones obtained using pressure nozzles and freeze dried microcapsules at the same oil to wall ratio. The authors attribute this finding to nozzle clogging during the microencapsulation process. This study demonstrated that new nozzle designs that eliminate emulsion preparation for microencapsulation applications could be viable options to conventional nozzles.

Moreover, protein based fibrils are interesting because of their functional properties, particularly whey protein fibrils have been reported to be effective stabilizers at air-water and oil-water interfaces and to able to form films at the interfaces with higher interfacial modulus. Their charge can be adjusted by changing the pH of the solvent, which makes them excellent materials for encapsulation (Sagis *et al.,* 2008; Bolder *et al.,* 2010; Rosier-Miranda *et al.,* 2012). In the literature, microcapsules produced with layer by layer deposition of whey protein isolate fibrils and polysaccharides yields microcapsules with high mechanical stability and give the shell a structure of fiber-reinforced microcapsules (Sagis *et al.,* 2008).

Other proteins such as soy protein isolate have been also described as effective wall materials for retaining orange oil during spray drying, reporting oil encapsulation efficiency around 85.7% (Kim *et al.,* 1996).

1.5.3 Operating conditions

In order to obtain good microencapsulation efficiency of food oils during spray drying, the operating parameters such as feed temperature and air inlet and outlet temperature must be optimized.

Table 1.1 Experimental conditions optimized for the encapsulation of food ingredients by spray drying.

Core material	***Wall material***	***Air inlet temperature (ºC)***	***Air outlet temperature (ºC)***	***References***
Fish oil	whey protein isolate (Bipro)	180 ºC	90±2 ºC	Legako *et al.,* (2010)
Refined cold-pressed fish oil	n-octenylsuccinate-derivatized starch, glucose syrup	170 ºC	70 ºC	Drusch *et al.,* (2006)
Fish oil	whey protein concentrate, modified starch (Hi-Cap 100)	180 ºC	65 ºC	Jafari *et al.,* (2008)
Fish oil	methylcellulose, maltodextrin	160 ºC	65 ºC	Kolanowski *et al.,* (2006)
Avocado oil	whey protein isolate, maltodextrin	180 ºC	80 ºC	Bae and Lee (2008)
Fish oil	whey protein concentrate, whey protein isolate, milk protein concentrate, sodium caseinate, skim milk powder	140- 180 ºC	NR	Aghbashlo *et al.,* (2012)
Fish oil	sodium caseinate, whey protein isolate, soy protein isolate, skim milk powder	180 ºC	80 ºC	Augustin *et al.,* (2006)
Fish oil	whey protein isolate, whey protein hydrolysate, maltodextrin	190 ºC	90 ºC	Ramakrishnan *et al.,* (2012)
Soya oil	sodium caseinate, carbohydrates	180 ºC	95 ºC	Hogan *et al.,* (2001)
Short chain fatty acid	maltodextrin, gum Arabic	180 ºC	90 ºC	Teixeira *et al.,* (2004)
Bixin	gum Arabic, maltodextrin, sucrose	180 ºC	130 ºC	Barbosa *et al.,* (2005)

NR – Not reported

Feed temperature modifies the viscosity of the emulsion, its fluidity and it will affect the quality of the final spray dried powder. When feed temperature increases, viscosity and droplet size should be decreased but high temperature can cause volatilization or degradation of some heat sensitive agents. So the flow rate of feed and its temperature must be optimized before it passes through the spray dryer. The influence of spray dryer inlet temperature is directly proportional to the microcapsule drying rate and the final water content.

Numerous studies reported (Hogan *et al.,* 2001; Augustin *et al.,* 2006; Drusch *et al.,* 2006; Bae and Lee 2008; Legako *et al.,* 2010) that, optimum inlet temperature for food oils encapsulation is between 160-220ºC, leading to a rapid drying and giving optimum flavour retention. When the air inlet temperature is low, the low evaporation rate occurs and it leads to high water content, poor fluidity and easiness of agglomeration (Gharsallaoui *et al.,* 2007). In case of high inlet temperature, the high evaporate rate occurs and leads to degradation of microcapsule ingredients or also a loss of volatiles (Zakarian and King, 1982).

The outlet air temperature is also an important factor to determine the encapsulation efficiency of food oils. It is quite difficult to predict the outlet temperature in advance, because it depends on the drying characteristics of the material. In spray drying, the outlet temperature cannot be directly controlled since it depends on the air inlet temperature. Numerous reports suggest the ideal air outlet temperature for food oils and flavours to be in the range of 50 to 90ºC. Table 1.1 shows a summary of experimental conditions optimized for the encapsulation of some different food ingredients by spray drying.

The other process parameter that controls oil encapsulation efficiency is the oil-to-wall material ratio, which usually ranges from 0.1:1 to 1:1 (Tan *et al.,* 2005). Recent studies show that when the oil-to-wall material ratio increases, so, too, does the oil encapsulation efficiency (Bae and Lee, 2008).

1.6 MECHANICAL PROPERTIES OF MICROCAPSULES

The need to investigate the mechanical properties of microspheres/microcapsules arises from requirements in quality control, design of new materials or tailor the mechanical properties (Zhang *et al.,* 2012). It is important to consider the specific nature of the microspheres, since they can be mono core type, multinuclear matrix type or even microbial cells that can be used to encapsulate target compounds. The methodology to determine the mechanical properties is adjusted depending on the type of microspheres/microcapsules. In the following paragraphs a general description of the most employed methods to characterize single microcapsules will be presented, focusing on the ones employed for multinuclear matrix type, since this is the type produced by spray drying to encapsulate fish oil in this project.

The techniques that have been described to characterize the mechanical strength of single microcapsules are atomic force microscopy (AFM), texture analyser, micromanipulation, nanomanipulation and micropipette aspiration.

Atomic force microscopy

Atomic force microscopy (AFM) is one type of scanning probe microscope (SPM) invented in the 1980s. In AFM the sample is scanned by a tip, which is mounted to a cantilever spring. While scanning, the force between the tip and the sample is measured by monitoring the deflection of the cantilever (Figure 1.8). A topographic image of the sample is obtained by plotting the deflection of the cantilever versus its position on the sample, besides the measurement of the force curves will provide valuable information regarding local material properties such as elasticity (Butt *et al.,* 2005).

Atomic force microscopy has been applied to study the mechanical properties of microcapsules (Lulevich *et al.,* 2004), interactions of polyelectrolyte multilayers (Jiang *et al.,* 2002), differentiation of cancer cells upon adhesion to particles (Gaikwad *et al.,* 2011), adhesion of melamine formaldehyde microcapsules on fabrics (Liu, 2010) and even lipid bubbles (Santos *et al.,* 2012).

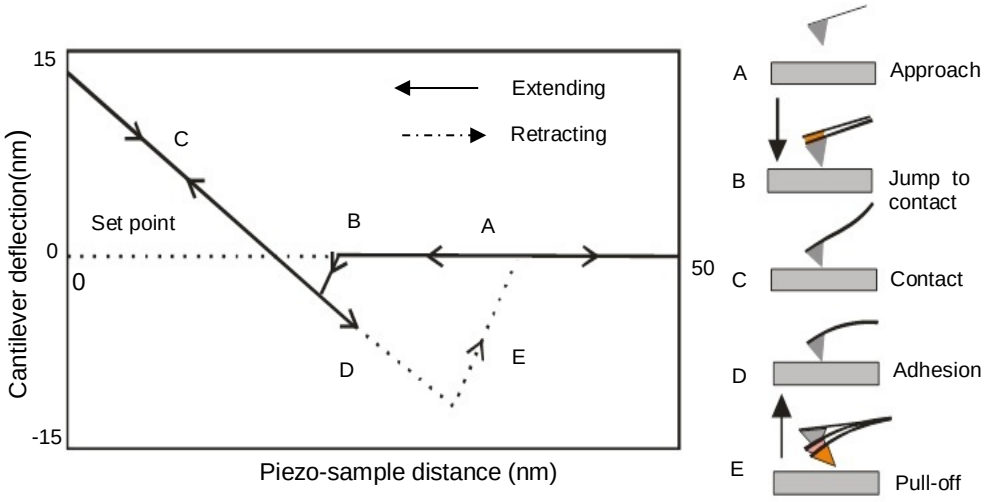

Figure 1.8. Typical force curves obtained by AFM indicating the tip-sample interaction points (adapted from Liu, 2010).

Texture Analyser

Another commonly applied method to evaluate the mechanical strength of single microcapsules is texture analyzer. With this technique a specific force is placed on the microcapsules. A texture analyzer consisting of a penetrometer with a stress gauge (Edwards-Levy and Levy 1999) provided a measure of a particle´s resistance to compressive force. The texture analyser is applicable to particles ranging from a few hundred micrometers to a few millimeters in diameter (Rosinski *et al.,* 2002), but the limitation of this method is its difficulty to measure when the particle size goes down to few micrometers. The limitation may be overcome by using a novel micro-manipulation technique that offers the capability to obtain the force versus deformation data when compressing a single particle to rupture.

Micromanipulation

The micromanipulation method has been used to study the mechanical strength of encapsulates of different size, shell thickness and shell composition. This technique was developed by Zhang *et al.,* (1999) and is based on squeezing a microparticle between two parallel surfaces. Force measurements were obtained at specific points and the compression speed could be varied. Visual observation of the process was provided by an optical microscope coupled with the micromanipulator.

Micromanipulation was proven to be a powerful technique, it was employed to study elastic or plastic behaviour of microcapsules (Sun and Zhang, 2001), compare mechanical strength of different wall materials (Sun and Zhang, 2002) and study the mechanical properties of microcapsules produced in tissue engineering and cell encapsulation purposes (Schuldt and Hunkeler, 2000; Rehor *et al.,* 2001; Zhao and Zhang, 2004). In addition to the above studies, micromanipulation was frequently employed to characterize the mechanical properties of other particles: alginate-chitosan microcapsule (Wang *et al.,* 2005) and hydroxyethyl methacrylated dextran particles (Chung *et al., 2005;* Yap *et al.,* 2008). The mechanical properties of single melamine formaldehyde encapsulates with diameters of 1-12 µm were determined using this method. The limitation of this technique is its inability to perform measurements in particles in the sub-micron range.

Nano manipulation

For single particles at the sub-micron scale, a novel environmental scanning electron microscopy (ESEM)-based nano-manipulation technique has been developed to characterize at nano-scale. ESEM is primarily used to visualize materials on nano-scales under wet mode. To enable the mechanical properties of single particles on such small scale to be measured, a nano-manipulation device with a force transducer was placed in the chamber of an environmental scanning electron microscope and was used to compress a single particle and then measure the force imposed on single particle (Liu *et al.,* 2005). Ren *et al.,* (2007) applied nano-manipulation technique to investigate the mechanical properties of melamine formaldehyde microcapsules.

Micropipette aspiration

Micropipette aspiration was employed by Jay and Edwards (1968) to measure the deformability of single nylon microcapsules containing erythrocyte hemolystate when their deformation was small. A micropipette with an inner diameter of several microns is used to suck the microcapsule with a defined, hydrostatically controlled pressure. The deformation of the object is monitored with optical microscope as a function of the applied suction pressure. This method could also be applied to study the mechanical properties of simple cells like red blood cells (Hochmuth *et al.,* 2000), neutrophils cells (Hochmuth *et al.,* 2000) and microgel capsules (Fiddes *et al.,* 2009). The main disadvantages of this method are the stress concentration at the pipette edge and the friction existing between the micropipette surface and the cell membrane, which may complicate the force calibration process and interfere with the mechanical response of the cell during aspiration (Lim *et al.,* 2006). Another disadvantages of this technique, is its difficulty to measure the rupture strength of microcapsules, which often occurs at relatively large deformations.

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CHAPTER 2

HYPOTHESIS

UNIVERSITAT ROVIRA I VIRGILI
ENCAPSULATION OF OMEGA-3 FATTY ACIDS BY PREMIX MEMBRANE EMULSIFICATION
Sarathiraja Ramakrishnan
Dipòsit Legal: T. 163-2014

2. HYPOTHESIS

Fish oils are highly demanded in the food industry due to the presence of omega-3 poly unsaturated fatty acids which have numerous health benefits. However, fish oils are highly susceptible to oxidation and have a characteristic fishy odour. To prevent the oxidation, mask the unfavorable sensory properties and extent shelf life of the product microencapsulation techniques are used**.** Traditionally, oil encapsulation is carried out by a two-step process: (i) emulsion preparation (ii) drying. The emulsification step plays a key role in optimizing the oil encapsulation efficiency (OEE), since the emulsion droplet size and distribution correlates with this parameter. Three factors that optimize the OEE are (1) a smaller emulsion droplet size, (2) a narrower emulsion droplet size distribution, and (3) a higher oil-to-wall material ratio.

Traditionally, O/W emulsions are produced by rotor–stator or high-pressure homogenization that require a high energy input to obtain emulsions with a small and narrow droplet size distribution. Besides the fact that traditional emulsification technologies often result in polydisperse emulsions, they have difficulties when trying to control the droplet size distribution. In recent years, premix membrane emulsification (ME) has been proved to produce stable O/W emulsions with a fine droplet size distribution at mild operation conditions and with a low energy input.

Typical wall materials such as milk proteins, and particularly whey protein and sodium caseinate, have been reported to be excellent encapsulating agents for oil encapsulation due to their amphiphilic properties. Some studies show that blending a protein (sodium caseinate and whey protein) with a polysaccharide, such as maltodextrin, provides good encapsulation properties. In the spray drying step, the oil to wall ratio is one of the key parameters in improving microencapsulation efficiency. A high ratio of wall systems generally yields better encapsulation.

After preparation, fish oil microcapsules have to be incorporated into a food matrix and they should be able to withstand the manufacturing process, therefore their mechanical strength is an important parameter. It has been described that denatured proteins in form of fibrils enhance the mechanical resistance of the shell in layer-by-layer microcapsules.

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This work combines for the first time the production of fish oil-in-water emulsions by premix membrane emulsification followed by spray drying to obtain food grade fish oil microcapsules. It also combined, for the first time, whey protein fibrils with a polysaccharide to build the microcapsule wall.

CHAPTER 3

OBJECTIVES OF THE WORK

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ENCAPSULATION OF OMEGA-3 FATTY ACIDS BY PREMIX MEMBRANE EMULSIFICATION
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3. OBJECTIVES OF THE WORK

The main objective of the present study was to prepare and evaluate the properties of fish oil microcapsules produced combining premix membrane emulsification followed by spray drying. In order to fulfill the main objective, the following particular objectives were defined to test the above mentioned hypotheses:

1) To prepare fish oil microcapsules from suitable O/W emulsions by combining premix membrane emulsification with spray drying.

2) To investigate the effect of the emulsification technique (rotor-stator or premix ME) and effect of the oil/wall material ratio on the oil encapsulation efficiency, the microcapsule morphology, and the oil stability.

3) To establish how the emulsion formulation, membrane type and microcapsule material and amount affect relevant physicochemical parameters such as microcapsule size, surface oil, oil encapsulation efficiency, humidity and oil oxidation stability.

4) To study the effect of the addition of whey protein fibrils (WPF) to the wall material, on the microcapsule inner and outer morphology and on the mechanical properties.

CHAPTER 4

FISH OIL MICROCAPSULES FROM O/W EMULSIONS PRODUCED BY PREMIX MEMBRANE EMULSIFICATION

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4.1 INTRODUCTION

Fish oils are in high demand by the food industry because they contain omega-3 poly unsaturated fatty acids which have numerous health benefits (Uauy and Valenzuela 2000; Larsson *et al.,* 2004). Long chain omega-3 fatty acids play an important role in the prevention and treatment of coronary artery disease, hypertension, arthritis and immune response disorders (Stone, 1997; Salem *et al.,* 1998; Simopoulos *et al.,* 1999). Fish oil contains significant amounts of omega-3 fatty acids that include α-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), all of them with beneficial health effects to humans. However, omega-3 fatty acids have a very strong odor and their constituent highly unsaturated long chain fatty acids can easily become oxidized (Augustin and Sanguansri 2006). To minimize or control these negative attributes, fish oil can be formulated as solid microcapsules. Microencapsulation can provide controlled release of encapsulated compounds, helps in masking odour and/or taste of encapsulated materials, and simplifies handling, storage and delivery of the powder-like materials produced.

Microencapsulation technology is widely used in the pharmaceutical and food industry (Gibbs *et al.,* 1999; Madene *et al.,* 2006) and provides a means of converting fish oil into a dry powder. In the food industry, the most widely employed technologies for encapsulating lipophilic compounds are based on the production of an oil-in-water (O/W) emulsion which is then followed by either spray drying (Hogan *et al.,* 2001a; Kagami *et al.,* 2003; Gharsallaoui *et al.,* 2011; Aghbashlo *et al.,* 2012), freeze-drying (Heinzelmann *et al*., 2000), molecular inclusion (Yoshii *et al.,* 1996) enzymatic gelation (Cho *et al.,* 2006) or coacervation (Lamprecht *et al.,* 2001). Of these techniques, the most common way of producing encapsulated fish oil is by spray drying the emulsion because it is a very efficient and flexible process that quickly removes water by vaporization and that can be carried out with readily available equipment.

A microcapsule consists of a core surrounded by a single or a multi-layered wall. The most common wall materials are low molecular weight carbohydrates such as sugars or maltodextrin, proteins like gelatin (Young *et al.,* 1993a; Sheu and Rosenberg 1998) and hydrocolloids such as gum arabic or mesquite gum (Buffo

and Reineccius 2000). The problem with some of these wall materials such as polysaccharides is that they lack emulsifying properties; therefore there is the need of using an emulsifier during the emulsification process. Generally, however, proteins and whey protein in particular can be used to stabilize emulsions and can act as effective encapsulation agents, thus providing an effective barrier against the oxidation of microencapsulated oil (Sheu and Rosenberg 1995; Kagami et al., 2003). Maltodextrin (MD) is a filler matrix, which is cheap, highly soluble in water and provides an effective barrier in encapsulation (Rosenberg *et al.,* 1993). Of all the encapsulating materials used, mixtures which use whey protein as the emulsifying and film-forming agent and/or maltodextrin as the matrix-forming material have shown very good results when applied to the microencapsulation of oil/fats and volatiles (Young *et al.,* 1993b; Sheu and Rosenberg 1998; Kagami *et al.,* 2003).

As mentioned previously, oil encapsulation typically involves a two-step process of (i) emulsion preparation, and (ii) drying. The emulsion plays a key role in optimizing the oil encapsulation efficiency because the emulsion droplet-size distribution correlates with this parameter. Some studies have shown that emulsion droplet size has a pronounced effect on the oil encapsulation efficiency of microcapsules obtained by spray drying. Jafari *et al.,* (2008) studied the effect of the emulsion size of fish oil droplets between 0.21 to 4.6 µm produced by different emulsification systems. Their results suggested that a smaller droplet size yields higher encapsulation efficiency. Risch and Reineccius (1998) and Soottiantwat *et al.,* (2003) produced emulsions with different droplet-size distributions covering the range of 0.5 to 5.9 µm and observed that the emulsions with the smallest droplet-size distribution had the highest retention of orange oil. These reports clearly indicate that emulsions with small droplet-size distribution showed higher oil encapsulation efficiency than those with larger droplets. Figure 4.1 presents a pictorial description of the influence of the droplet size and its distribution on the oil encapsulation efficiency:

Case A: the starting O/W emulsion has a small droplet size and it is monodisperse, with span values lower than 1, each droplet formed during spray drying contains more similar number of oil droplets which are entrapped by the wall material when the water is removed during spray drying. The fact that the oil droplets have similar

volume has a positive effect on their packing inside the microcapsules, enhancing the amount of encapsulated oil.

Case B: the starting O/W emulsion has big droplets and a wide size distribution, each droplet formed during spray drying might contain less and bigger oil droplets than case A, therefore the capsules formed after drying will contain less volume of oil. We can also assume that the polydispersity of the oil droplets prevents them to be efficiently packed, leaving more free volume in the microcapsule, to be occupied by the wall material and resulting in lesser amount of encapsulated oil and higher amount of surface oil.

The other process parameter that controls oil encapsulation efficiency is the oil to wall material ratio, which usually ranges from 0.1:1 to 1:1 (Tan *et al.,* 2005). Recent studies show that when the oil to wall weight ratio increases, so too does the oil encapsulation efficiency.

Different methods of producing O/W emulsions include mechanical stirring, high-pressure homogenization and membrane emulsification (Charcosset *et al.,* 2004) Mechanical stirring by using rotor stator homogenization and high pressure homogenization require a high energy input to produce emulsions with a fine droplet-size distribution, and this often results in polydisperse emulsions and causes difficulties when trying to control droplet size distribution. Membrane emulsification (ME) has been used in the last decade to produce emulsions with narrow droplet size distribution. In the early 1990s, Suzuki *et al.,* (1998) introduced premix membrane emulsification, which is carried out in two steps. To obtain O/W emulsions, initially both oil (i.e. disperse phase) and water (i.e. continuous phase) are homogenized using a rotor-stator system to form a coarse premix emulsion. After this, the coarse premix emulsion is forced through a porous membrane. In the pores, the oil droplets are broken into smaller ones, although the coarse premix emulsion usually needs to be passed through the membrane several times, referred as cycles, to obtain a narrow droplet size distribution. In premix ME, the experimental setup is simpler than direct ME e.g no moving parts such as cross flow-pump, except the pre-emulsification step. In premix ME, the optimal transmembrane fluxes are typically above 1 m^3/m^2 h, which is one to two orders of magnitude higher than in direct ME (Vladisavljevic and Williams 2005). When

comparing emulsion produced by mechanical methods, the energy cost for premix ME are relative low since no high-energy input, shear and extensional stresses are needed in except during the pre-emulsification step (Nazir *et al.,* 2010).

The aim of this study was to produce fish oil microcapsules by combining premix membrane emulsification with spray drying. The O/W emulsions were prepared using premix ME, a low-energy emulsification technique, using Nylon or nitrocellulose mixed esters membranes. The study investigated the effect of the emulsification technique (rotor stator or premix ME) and the effect of the oil:wall material ratio on the oil encapsulation efficiency, the microcapsule morphology and the oil stability. Two oil:wall material ratio were studied: 1:1 and 1:3, using maltodextrin and/or whey protein. As far as the authors know this has been the first attempt to produce fish oil microcapsules by combining membrane emulsification with spray drying.

4.2. MATERIALS AND METHODS

4.2.1 Materials

The O/W emulsions in this study were prepared using Menhaden fish oil (Sigma – Aldrich, CAS-No.8002-50-4, Spain) as the disperse phase and different concentrations of emulsifiers dissolved in distilled water as the continuous phase. The emulsifiers were: Tween-20 (polyoxyethylene sorbitan monolaurate, from Sigma – Aldrich, CAS-No. 9005-64-5, Spain), whey protein isolate (WPI, Bipro from Davis Co Food Inc – Switzerland, Ref No. LE 003-0-919) and whey protein hydrolyzate (WPH, Biozate from Davis Co Food Inc – Switzerland, Ref. No. JE 031-1-420). The wall material for the production of microcapsules was maltodextrin (Sigma Aldrich– Spain, Dextrose equivalent 16.5-19.5, CAS No. 9050-36-6), and/or whey protein isolate and hydrolyzate. Analytical grade hexane and petroleum ether were purchased from Sigma-Aldrich, Spain. Distilled water was used for the preparation of all solutions. All general chemicals used in this study were of analytical grade. The composition of all the emulsions used in this study is presented in Table 4.1.

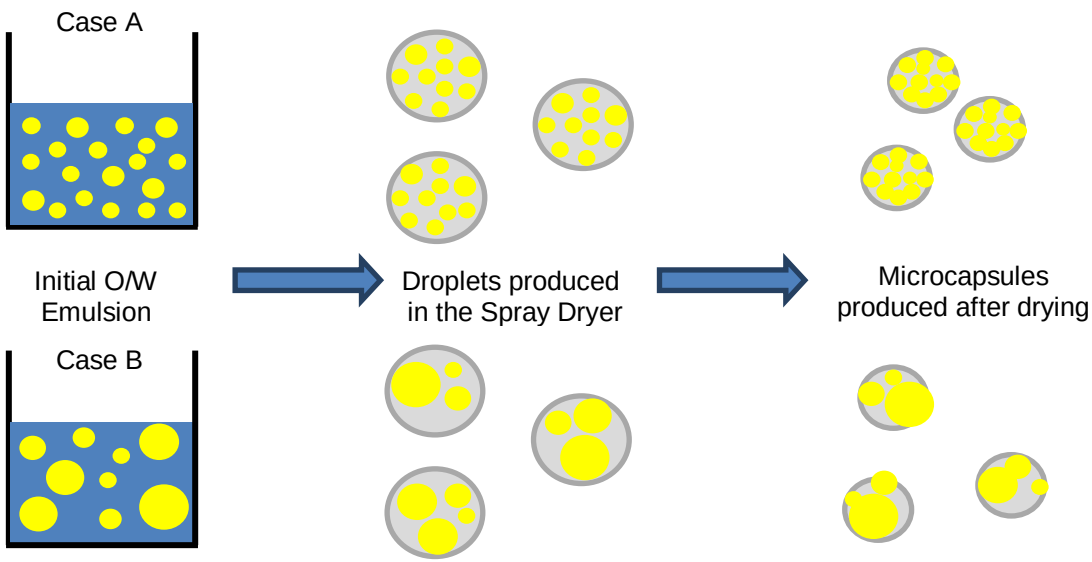

Figure 4.1. Pictorial description of the influence of the droplet size and its distribution on the oil encapsulation efficiency.

4.2.2 Preparation of the O/W emulsions

Fish oil/water emulsions were prepared by membrane emulsification. The required amount of each emulsifier was dissolved in water by the following procedures: 2% Tween 20 was dissolved in water stirring at 300 rpm for 15 min, whey protein 1% and 10% was dissolved in water stirring at 300 rpm for 1.5 h and 4-6 h, respectively. After the continuous phase was prepared the appropriate amount of fish oil was added to produce the emulsion. Figure 4.2 shows the schematic diagram of the premix membrane emulsification process, which consists of a two-step emulsification system. The first step was to prepare a coarse emulsion by homogenizing the water and oil phases in a rotor stator (Ultra-Turrax®, model T18, IKA). This coarse O/W emulsion was placed in the premix reservoir and forced through the membrane using nitrogen at 700 kPa. The outlet fine emulsion was collected in an Erlenmeyer flask and weighed using a precision balance. The balance was connected to a PC computer to record the time and mass data every 1 second. To reduce the droplet size of the emulsions, the premix emulsion was passed through the membrane three times (cycles) without any intermediate cleaning of the membrane. The experiments were carried out using two different 47 mm diameter polymeric membrane discs. One was made of nylon (0.8 µm mean pore size, Whatman ref. 7408-004) and the other was made of nitrocellulose mixed esters (MCE) (0.8 µm mean pore size, Sterlitech Corporation ref. MCEB0847100SG). These two membranes have previously tested by our group providing good results to produce O/W emulsions (Trentin *et al.,* 2010; Trentin *et al.,* 2011). The effective membrane diameter was 41 mm, giving an effective filtration area of 1.32×10^{-3} m^2 for the membrane module used in the present work. At the beginning of each experiment the membrane was conditioned by wetting with water.

Average permeate flux of the emulsion through the membrane during premix ME was calculated using mass and time data according to equation 4.1.

$$J = \frac{m}{t * A} \qquad [4.1]$$

where J (kg m^{-2} s^{-1}) is the permeate flux, m (kg) is the total mass of the emulsion registered in the balance, t (s) is the time required for the total mass to pass through the membrane and A (m^2) is the effective membrane area.

Fish oil/water emulsions were also prepared using mechanical stirring alone. As Table 4.1 shows, the emulsions were prepared using 10% oil fraction and stabilized with 2% Tween 20 or 10% WPH. The emulsification conditions such as rotor speed and time were adjusted to obtain the lowest possible value for the droplet size distribution, and were maintained at 15500 rpm or 20000 rpm for 75 minutes, depending on the emulsifier.

4.2.3 Production of the fish oil microcapsules

Oil:wall material ratio namely 1:1 and 1:3 were used to produce the fish oil microcapsules as shown in Table 4.1. For producing 1:1 fish oil:wall ratio, 10% WPH or 10% WPI were employed. For microcapsules obtained with a 1:3 fish oil:wall material ratio, 1% WPI or 1% WPH plus 30% maltodextrin or 10% whey protein plus 20% maltodextrin was employed (see Table 4.1). When maltodextrin was used as a wall material, it was added at the end of the emulsification step and mixed until dissolved.

To obtain the microcapsules, the O/W emulsions prepared by membrane emulsification or by mechanical stirring were spray-dried using a laboratory scale spray dryer (Buchi mini spray dryer b290, Switzerland). The operation conditions in the spray dryer were: air inlet temperature and outlet temperature of 190 °C and 90 °C, respectively and an emulsion flow rate of 400 mL/h. The dried powder was collected and stored in opaque, air tight containers at 4°C for further analysis.

Table 4.1. Formulation of O/W emulsions and emulsifying conditions employed.

Continuous phase	***Disperse Phase***	***Emulsifier (concentration)***	***Wall material***	***Emulsifying method***	
				Rotor stator	***Membrane***
Distilled water – 90% (v/v)	Menhaden Fish oil 10% (v/v)	Tween - 20 2%	30% MD	15,500 rpm for 2 min	Nylon
		Tween - 20 2%		15,500 rpm for 2 min	MCE
		Tween - 20 2%		15,500 rpm for 75 min	-----
		WPI 1%	30% MD	15,500 rpm for 2 min	Nylon
		WPI 1%			MCE
		WPI 10%	-----	20,000 rpm for 4 min	-----
		WPI 10%			Nylon
		WPH 1%	30% MD	15,500 rpm for 2 min	Nylon
		WPH 1%			MCE
		WPH 10%	----	20,000 rpm for 4 min	Nylon
		WPH 10%			MCE
		WPH 10%			-----
		WPH 10%	20% MD	20,000 rpm for 4 min	Nylon
		WPH 10%			MCE
		WPH 10%		20,000 rpm for 75 min	-----

4.2.4 Emulsion characterization

The droplet-size distributions of the O/W emulsions from both the rotor-stator and the premix ME (initial coarse emulsion and emulsions from cycles 1-3) were obtained by laser diffraction measurements using a Malvern Mastersizer 2000 equipped with a Hydro 2000 SM dispersion unit (Malvern Instruments Ltd., Worcestershire, England). The droplet size of the emulsions was expressed as the surface area mean diameter $d_{3,2}$. Droplet-size measurements were taken in triplicate for each sample. The span value, calculated according to equation [4.2] was taken as an indication of the dispersity of the droplet size. The lower the span value, the more monodisperse the emulsion.

$$span = (d_{90} - d_{10})/d_{50} \qquad [4.2]$$

where d_{90}, d_{50} and d_{10} are the droplet/particle diameters corresponding to 90, 50 and 10 vol.%, respectively, on the relative cumulative size distribution curve.

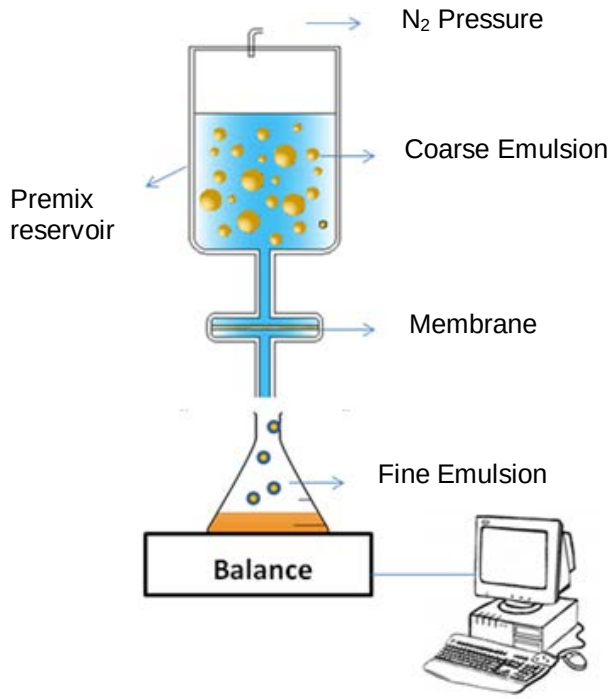

Figure 4.2. Schematic diagram of the premix emulsification process.

4.2.5 Powder particle size and moisture

The size distribution of the spray-dried powders was analyzed by laser diffraction using the Malvern Mastersizer 2000 equipped with Scirocco 2000 sample dispersion unit. Particle size was expressed as $d_{3,2}$, each sample was analyzed in triplicate and the average data were reported.

The moisture content of the powders was measured gravimetrically by drying 2 g of the powder samples in an air oven at 105° C for 12h, and it was expressed as the percentage per 100 g of powder.

4.2.6 Total oil, surface oil and oil microencapsulation efficiency

The total oil content in the microcapsules was analyzed using a procedure based on the Rose-Gottlieb method (Richardson 1985), which is widely accepted for quantitative determination of fat in milk and milk powders. Two grams of dried powder were dispersed in 20 mL of water heated at 65°C. After stirring gently, 4 mL of 25% NH_4OH was added and the solution was heated at 65°C for 15 min in a shaking water bath. Then, the solution was cooled at room temperature and the oil was extracted by applying three liquid-liquid extraction as follows: first, 50 mL of diethyl ether and 50 mL of light petroleum ether; second, 10 mL of ethanol, 50 mL of diethyl ether, and 50 mL of light petroleum ether; and third, 50 mL of diethyl ether and 50 mL light petroleum. After the extraction steps, the final solution was filtered through a filter paper containing anhydrous Na_2SO_4. The solvent was evaporated in a rotary evaporator (Heidolph Laborota 4000) and the extracted oil was dried to constant weight using a stream of nitrogen.

Surface oil or non-encapsulated oil was determined using a modified method described by Varavinit *et al.,* (2001). Hexane (20 mL) was added to an accurately weighed amount (2 g) of powder followed by stirring for 10 min. The suspension was then filtered and the residue rinsed three times with 20 mL of hexane. The residual powder was then dried to vaporize all residual solvent at 60 ºC to a constant weight. The difference in the powder's weight before and after extraction and washing with hexane was then used to calculate the free oil content as a percentage. Finally, the oil encapsulation efficiency (OEE) was calculated using equation [4.3]:

OEE = [(Total oil – Surface oil) / Total oil] x 100 [4.3]

4.2.7 Microcapsules surface and inner morphology analysis

To study the outer morphology of the microcapsules, the particles were mounted on cylindrical stubs coated with conductive carbon tape. An environmental scanning electron microscope (FEI, Quanta 600) was used to analyze the surface morphology of the dried powders. Digital images where taken at an accelerating voltage of 20kV and magnification of 2900x.

To study the inner structure of the microcapsules, the particles were mounted on a SEM stubs using double-sided sticky tape following the procedure by Bae and Lee (2008). The specimens were coated with gold (EMITECH K575XD Turbo Sputter Coater) and subsequently examined and photographed using a Scanning Electron Microscope at 15 kV (JOEL JSM-6400).

4.2.8 Determination of propanal content by static headspace gas chromatography

The propanal content of the microcapsules was determined by using a static headspace sampler (G1888 from Agilent Tachnologies, Waldbronn, Germany) coupled to a gas chromatograph (HP 6890 from Hewlett–Packard, Waldbronn, Germany) equipped with a quadrupole mass spectrometer with a diffusion pump (HP 5973, from Hewlett–Packard, Waldbronn, Germany). The chromatographic separation was carried out with an HP5-MS chromatographic column (30 m x 0.25 mm i.d., 0.25 µm film thickness) with an oven temperature program of 40ºC (5 min), 70ºC/min to 300ºC (10 min). The carrier gas was helium with a flow rate of 1.8 mL/min.

For each analysis, 3 mL of sample headspace were introduced into the injection port of the gas chromatography. The loop and transfer line temperatures were 75º and 80ºC, respectively, and the pressurization and injection times were 0.3 and 0.6 min, respectively. Chromatographic injection was made in splitless mode at 200ºC.

Mass spectra were recorded by electronic impact (EI) ionization at 70 eV with a temperature of 230ºC in the ion source and 150ºC in the mass quadrupole.

To prepare the samples, 1.00 g of the microcapsules was placed into a 10 mL vial together with 10 mg/L of 2 methyl propyl acetate (internal standard, ISTD) and dissolved in 2 mL of EDTA (0.5%). The vial was hermetically sealed with PTFE/silicone septum and the sample was thermostatted for 30 min at 70ºC under constant stirring. All the samples were prepared and analyzed in triplicate.

The propanal distribution constant between the sample (dissolved microcapsules) and the headspace strongly depends on the material used to elaborate the microcapsules; therefore, to ensure that the propanal content of each sample was properly quantified, we constructed three different calibration graphs using three matrices: 1% WPH + 30% MD, 10% WPH and 10% WPH + 20% MD. In each case, the calibration graph was obtained from each specific matrix spiked with 10 ppm of internal standard (ISTD) and four different concentrations of propanal in the range 10-60 ppm. The peak area ratios [propanal/ISTD] were plotted against the [propanal/ISTD] concentration ratios. Then, the amount of propanal in each sample was determined considering the calibration line constructed with the matrix more similar to the sample analyzed.

4.3 RESULTS AND DISCUSSION

As mentioned in the previous section, fish oil microcapsules can be produced from a fish oil/water emulsion that is subsequently dried. The novelty of the approach adopted in the present study is that it uses membrane emulsification which is a low energy technology to produce fish oil/water emulsions stabilized with whey proteins which are then dried by spray drying. Therefore, the first part of this section presents the results of producing fish oil/water emulsions from premix ME and then presents the physical and chemical characterization of the fish oil microcapsules.

4.3.1 Production of fish oil/water emulsions by Premix ME

It has been mentioned in the literature (Hu *et al.,* 2003a) that proteins used to stabilize oil/water emulsions can also play a role preventing oil oxidation. To further study the effect of proteins on the oxidation of fish oil microcapsules, control

samples were prepared using the widely employed food emulsifier Tween 20. A coarse emulsion with 10% (v/v) menhaden fish oil and 2% (v/v) Tween 20 was prepared by rotor stator at 15500 rpm for 2 minutes. The coarse emulsion was then passed three times through a Nylon or MCE membrane (0.8 μm pore size diameter) using 700 kPa of pressure; the flux and droplet-size diameter were measured for each cycle. After the third emulsification cycle and before drying, the appropriate amount of maltodextrin was added to the emulsions to obtain a final concentration of 30% (v/v). Figure 4.3 presents the fluxes and droplet size of the emulsions stabilized with Tween 20 after each cycle. The plots in Fig. 4.3A show that the coarse emulsion prepared with the rotor stator has the biggest droplet size (cycle 0). After this, the coarse emulsion passed through the Nylon or MCE membrane and the droplet size diameter was significantly reduced (Fig 4.3A, cycle 1). At the end of the 3rd emulsification cycle, the values of the droplet diameter (given as $d_{3,2}$) were close to 1.1 – 1.3 μm, regardless of the membrane. It is clear that droplet break-up occurs predominately during premix membrane emulsification and that the final values of the droplet size diameter are limited by the membrane pore size which in the present study is 0.8 μm (Joscelyne and Tragardh 2000; Van der Graaf *et al.,* 2004; Lambrich and Schubert 2005; Charcosset 2009).

Fig. 4.3B shows the flux evolution versus the number of cycles for premix ME with Nylon and MCE membranes during the production of O/W emulsions stabilized with Tween 20. The flux increases as the number of cycles increases because the average diameter of droplets is reduced to reach a constant value, and then the pressure applied is mostly used to push the emulsion through the membrane, as mentioned by Vladisavljevic *et al.,* (2004). Figure 4.3B also shows that the flux values were slightly higher for the Nylon than for the MCE membrane.

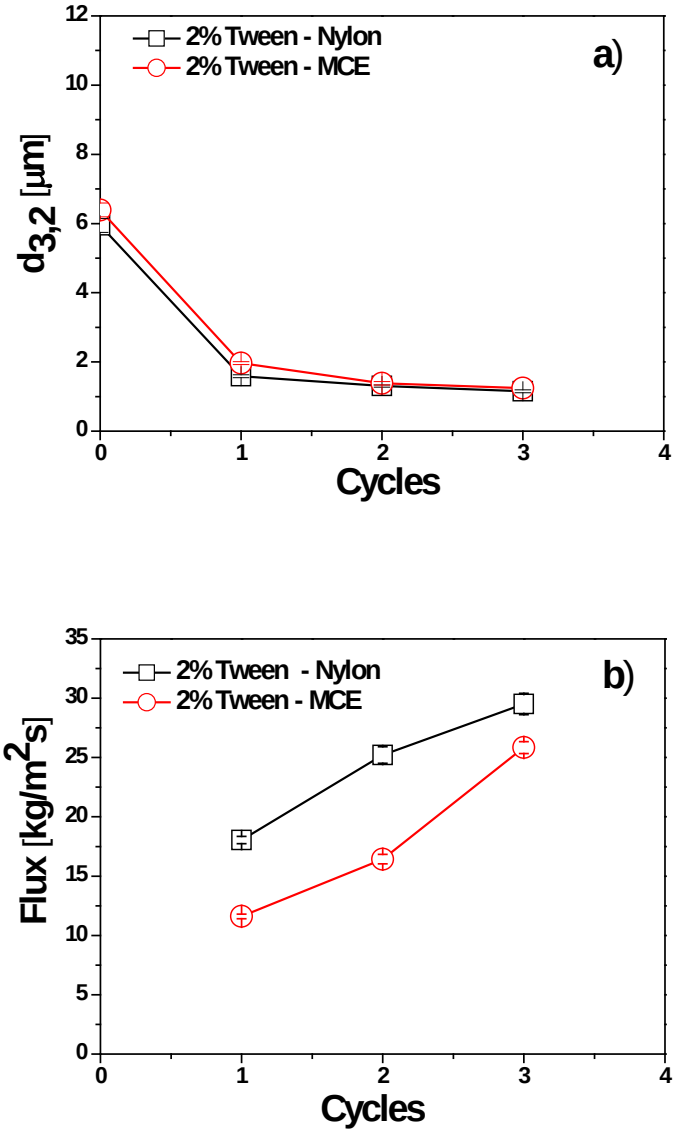

Figure 4.3. Oil droplet size as $d_{3,2}$ (a) and flux (b) evolution with the number of cycles during the production of O/W emulsions stabilized with Tween-20 by premix ME with nylon and MCE membranes. (a) $d_{3,2}$ minimum error: 0.03μm; maximum error: 0.2 μm. (b) Flux minimum error: 0.5 kg m^{-2} s^{-1}; maximum error: 1.5 kg m^{-2} s^{-1}.

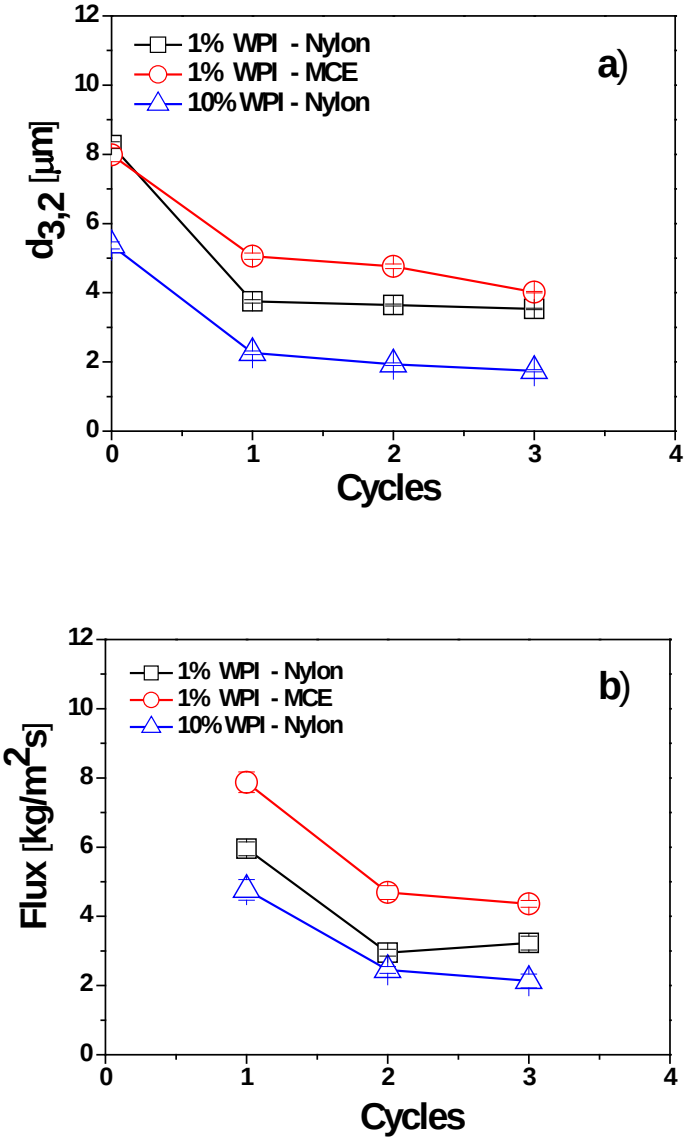

Figure 4.4. Oil droplet size as $d_{3,2}$ (a) and flux (b) evolution with the number of cycles during the production of O/W emulsions stabilized with whey protein concentrate (WPI) by premix ME with nylon and MCE membranes. a. $d_{3,2}$ minimum error, 0.02μm; maximum error, 0.1μm. b. Flux minimum error, 0.5 kg m^{-2} s^{-1}; maximum error, 1.2 kg m^{-2} s^{-1}.

To produce O/W emulsions stabilized with whey protein, 10% menhaden fish oil with two different concentrations of WPI or WPH (1% and 10%) was first homogenized using a rotor-stator (see conditions in Table 4.1). The resulting coarse emulsion was passed 3 times through the Nylon or MCE microfiltration membranes at 700 kPa of nitrogen pressure. Figures 4.4 and 4.5 present the flux evolution during the emulsification cycles and the values of the droplet-size distribution of the O/W emulsions after each cycle for WPI and WPH, respectively. As can be seen in Figure 4.4A, the highest reduction in the droplet size is obtained during the first membrane emulsification cycle, regardless of the protein concentration or the membrane employed. When 1% WPI was used, the final droplet size value was 3.5 μm for the Nylon membrane and 4.2 μm for the MCE membrane, whereas the emulsions prepared using 10% WPI presented a smaller droplet size (1.2 μm) than those prepared with 1% WPI. These results agree with the fact that using a higher amount of emulsifier will decrease the interfacial tension, favoring droplet break up and also enhance the stabilization of the droplets formed, resulting is smaller oil droplets.

Figure 4.4B shows the progress of flux versus the number of cycles during premix membrane emulsification at 700 kPa of pressure. The fluxes decreased from cycle 1 to 3, regardless of the membrane and the WPI concentration. The highest flux value was observed in cycle one for 1% WPI using the MCE membrane, while the lowest flux was obtained for 10% WPI at the third cycle with the Nylon membrane. A comparison of the plots in Figures 4.3B and 4.4B clearly shows the effect of protein fouling during membrane emulsification. When membrane fouling is mainly due to oil droplets (Figure 4.3B), a decrease in the droplet size increases the flux, as previously reported by Vladisavljevic *et al.,* (2004). However, when fouling is due to both the oil droplets and the presence of proteins used as emulsifiers, a decrease in droplet size during premix membrane emulsification is not translated into an increase in the flux, as was already reported by Trentin *et al.,* (2010) and Trentin *et al.,* (2011). From Figure 4.4 it is clear that 10% WPI produces O/W emulsions with smaller droplets, but the flux obtained during emulsification is lower than that obtained using 1% WPI, this being mainly attributed to protein fouling, since a higher protein concentration will also result in more severe membrane fouling.

Whey protein hydrolyzated was also employed to produce O/W emulsions by premix membrane emulsification. Coarse O/W emulsions were prepared with a rotor-stator homogenizer using 10% menhaden fish oil with 1% or 10% WPH (conditions in Table 4.1). The coarse emulsion was repeatedly passed three cycles through the Nylon and MCE microfiltration membranes at 700 kPa. Figures 4.5A and 4.5B show the evolution of the droplet size diameter and the flux with the number of cycles, respectively. The results display similar trends to those mentioned for WPI: the smallest droplet size is obtained when the highest protein concentration is used, whereas the flux has the opposite trend. Again more severe protein fouling is responsible for the low fluxes during the production of emulsions with 10% WPH concentration. Regarding the flux values, it can be seen that the MCE membrane gives the highest fluxes, regardless of the protein employed. It is also important to notice that a hydrolyzed whey protein gives higher fluxes than a whey protein isolate. It is well reported that whey protein hydrolyzation modifies solubility, viscosity and foaming and emulsifying properties (Guadix *et al.,* 2006)**,** therefore it can be assumed that the hydrolyzation process of the whey protein produces smaller peptides that have a lower impact in membrane fouling during membrane emulsification.

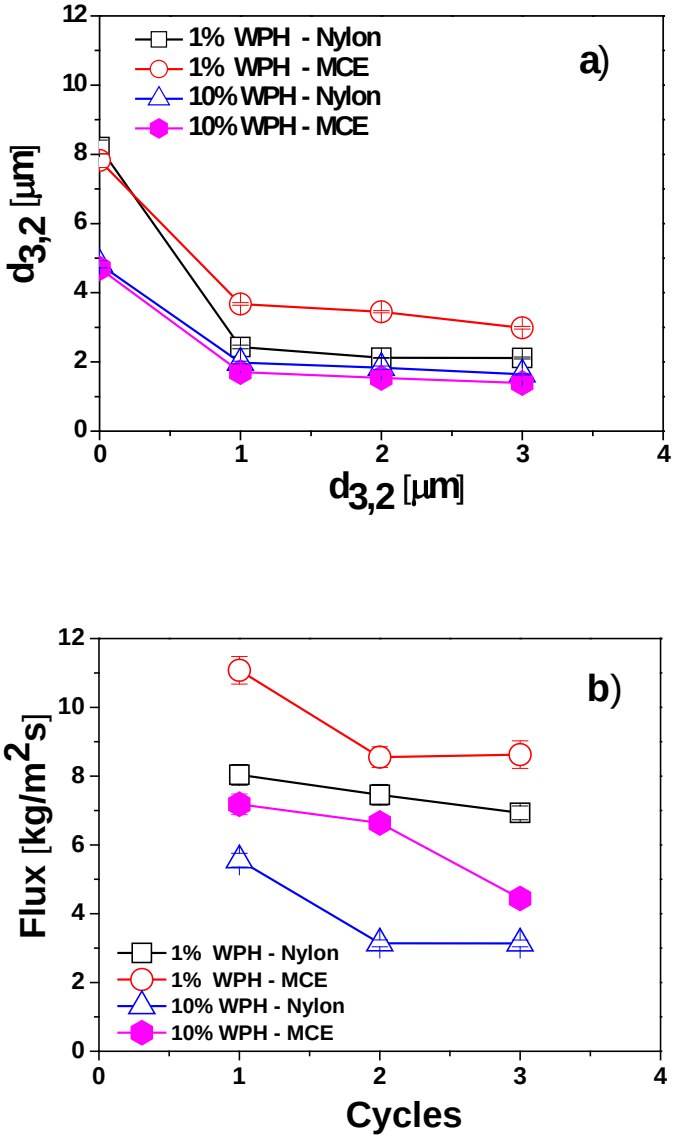

Figure 4.5. Oil droplet size as $d_{3,2}$ (a) and flux (b) evolution with the number of cycles during the production of O/W emulsions stabilized with whey protein hydrolysate (WPH) by premix ME with nylon and MCE membranes. a. $d_{3,2}$ minimum error, 0.01μm; maximum error, 0.2μm. b. Flux minimum error, 0.5 kg m^{-2} s^{-1}; maximum error, 1.3 kg m^{-2} s^{-1}.

4.3.2 Production of fish oil microcapsules by spray drying

In order to investigate the influence of the emulsification system on the properties of fish oil encapsulated powder, O/W emulsions were prepared as described above using either premix ME or a high energy input technique (rotor – stator homogenization), following the process conditions presented in Table 4.1. For the rotor-stator process the mixing time was adjusted to obtain the lowest possible values of droplet size, meaning that emulsification took place at 15500 or 20000 rpm for 75 minutes, depending on the emulsifier type and concentration. The energy density (E_v), defined as the ratio between the energy input and the volume of the emulsion, has been used by several authors to compare the efficiency of different emulsification techniques. According to Schuchmann and Danner (2004) E_v corresponding to rotor-stator processes is of two orders of magnitude higher than the one required for membrane emulsification. Regarding the droplet size of the emulsions, these authors show that rotor-stator homogenization produces bigger oil droplets than membrane emulsification even though higher energy inputs are required. Comparing the droplet size of the emulsions from rotor-stator or from premix ME (Table 4.2) it can be seen that, for the same emulsion formulation, the droplet size of the emulsions produced by rotor stator is about three times bigger than the one of emulsions produced by premix ME, being this in agreement with previous work (Schuchmann and Danner 2004).

All the O/W emulsions produced in this study by either premix ME or rotor-stator homogenization were processed under the same spray drying conditions: air inlet temperature and outlet temperature of 190 °C and 90 °C, respectively, and emulsion flow rate at 400 mL/h. The wall material was a combination of maltodextrin and/or whey protein (WPI or WPH). It should be noted that two different ratios of oil:wall material, 1:1 and 1:3, were studied. Once they had been produced, the microcapsules were stored at 4 ºC until characterization. Table 4.2 presents the main physical and chemical parameters that were used to characterize the fish oil microcapsules obtained after drying the O/W emulsions. External and internal morphology, powder size, moisture content, surface oil content and total oil content were the parameters selected for this study. The OEE, based on the values obtained for the total and surface oil and calculated using equation [4.3], is one of

the quality parameters used to determine the amount of oil successfully encapsulated by spray drying.

It can be seen that the microcapsules size measured by laser diffraction is highly dependent on the emulsion droplet size, the emulsification method and the amount of wall material employed (Table 4.2). For a 1:1 oil:wall material ratio microcapsules obtained from O/W emulsions produced by rotor-stator homogenization have a particle size of about 30 µm, while microcapsules from O/W emulsions produced by premix ME have a size between 4-7 µm and are much less polydisperse since they have lower span values. Increasing the oil:wall material ratio to 1:3, the particle size and polydispersity of microcapsules from O/W emulsions prepared by rotor-stator and premix ME is very similar (12 µm for rotor-stator and 9 – 14 µm for premix ME). The smaller microcapsules were obtained from O/W emulsions stabilized with Tween 20 or 10% WPH, which also corresponds to the emulsions with the smaller droplet size.

The other important parameter for oil microcapsule production is the OEE, which is strongly related to the amount of surface oil. The values in Table 4.2 clearly show that the OEE increases when the droplet size of the emulsion decreases and when the amount of wall material employed increases, as has already been indicated earlier. It should be mentioned that the droplet size of the emulsion depends on the emulsification method and the type and amount of emulsifier. Emulsions that used 10% WPI or WPH as emulsifier and as wall material exhibited very different OEE values depending on the emulsification method. When the rotor stator was used the values were 17% or 22% for WPI and WPH, respectively, while when ME was used the values increased to 29% and 34-36% for WPI and WPH, respectively. The emulsions produced with the rotor stator had a significantly bigger droplet size (4.4 or 4.8 µm) than those produced with membrane emulsification (1.75 or 1.64 µm). It seems reasonable to assume that when the oil droplets are smaller they will be more easily entrapped within the wall matrix of the microcapsule, and that furthermore the emulsions with the smallest droplet size are more stable during the spray drying process (Jafari *et al.,* 2008). Therefore, it is expected that, in general, emulsions produced by membrane emulsification will have less surface oil and yield better encapsulation efficiency as shown in Figure 4.1.

Table 4.2. Properties of microcapsules with 10% fish oil loading.

Emulsifier	***Wall***	***Emulsification method***	***Oil : Wall ratio***	***Droplet size of emulsion (µm)***	***Span value of emulsion***	***Moisture content (100g)***	***Powder particle size (µm)***	***Span value of powder***	***Surface oil content (g/1g powder)***	***Average wall thickness (µm)***	***(OEE) (%)***
10% WPI	___	Rotor stator	1:1	4.85±0.01	1.772±0.00	0.72± 0.1	30.82±0.42	2.78±0.12	0.2732±0.008	0.49 ±0.05	16.14± 1.11
		Premix ME –Nylon		1.77±0.00	0.821±0.01	0.65±0.07	6.90±0.31	2.10±0.00	0.2241±0.007	0.54 ±0.03	29.44± 2.07
10% WPH		Rotor stator		4.5±0.00	1.83 ±0.01	0.88 ±0.05	28.97±0.24	2.81±0.03	0.2491±0.004	0.43 ±0.02	22.21± 1.18
		Premix ME – Nylon		1.64±0.01	0.824±0.01	0.76±0.11	4.9±0.11	1.89±0.09	0.2189±0.003	0.56 ±0.07	34.1 ±3.31
		Premix ME – MCE		1.39±0.02	0.771±0.02	0.91 ±0.05	3.60±0.16	1.86±0.06	0.2432±0.001	0.60 ±0.09	36.77±1.58

Emulsifier	*Wall*	*Emulsification method*	*Oil : Wall ratio*	*Droplet size of emulsion (µm)*	*Span value of emulsion*	*Moisture content (100g)*	*Powder particle size (µm)*	*Span value of powder*	*Surface oil Content (g/1g powder)*	*Average wall thickness (µm)*	*(OEE) (%)*
1% WPI	30% MD	Premix ME – Nylon	1:3	4.93±0.01	2.43 ±0.03	3.24±0.36	10.78±0.20	2.19±0.08	0.1053±0.003	0.82±0.05	27.62±1.25
		Premix ME – MCE		4.82±0.02	2.289±0.06	2.14±0.47	10.5±0.30	2.29±0.14	0.1141±0.004	0.88±0.034	32.83±1.04
1% WPH		Premix ME – Nylon		4.32±0.04	2.67 ±0.04	1.30±0.19	14.5±0.15	2.18±0.04	0.1042±0.007	0.82 ±0.04	31.3±1.45
		Premix ME – MCE		4.18±0.01	2.469±0.02	3.08±0.37	13.16±0.11	2.16±0.10	0.1081±0.008	0.81 ±0.11	30.01±1.89
2% Tween - 20		Rotor stator		4.09±0.02	1.90 ±0.03	3.15±0.57	12.33±0.21	2.91±0.14	0.098±0.002	1.03±0.09	32.11±2.01
		Premix ME – Nylon		1.34±0.06	1.62 ±0.01	3.19 ±0.63	9.10±0.18	2.20±0.21	0.0698±0.001	0.94 ±0.12	40.56±1.59
		Premix ME – MCE		1.20±0.03	1.78 ±0.04	2.15 ±0.51	9.85±0.32	2.370±0.17	0.0715±0.005	0.91 ±0.03	39.46±3.51
10% WPH	20% MD	Rotor stator	1:3	4.12±0.08	1.72 ±0.00	1.86 ±0.67	11.21±0.41	2.63±0.02	0.089 ±0.004	0.81±0.06	42.53±2.35
		Premix ME-Nylon		1.44±0.01	0.82 ±0.01	1.44 ±0.44	8.73±0.21	1.97±0.03	0.0719±0.006	0.89±0.09	55.95±2.67
		Premix ME -MCE		1.242±0.04	0.61 ±0.03	1.21 ±0.62	9.29±0.30	2.12±0.01	0.0842±0.003	1.01±0.11	52.97±2.01

*size of emulsion measured after addition of MD. Values are average of triplicate analyses ± standard deviation.

The oil:wall material ratio was increased from 1:1 to 1:3 by producing microcapsules with 1% whey protein or 2% Tween 20 and 30% maltodextrin or with 10% whey protein and 20% MD. Since the emulsions produced using 2% Tween 20 had a smaller droplet-size distribution, the final value of the OEE was higher than for capsules produced from emulsions stabilized using 1% whey protein, regardless of its type. However, when 10% WPI or WPH was used to produce the emulsions, the OEE was higher than 50%, which is directly related to the small droplet size of the initial emulsion and the amount of wall material employed to produce the microcapsule.

Young *et al.,* (1993 b) reported a decrease in the surface oil content when combining whey protein and maltodextrin to encapsulate anhydrous milk fat powder. The results obtained in our study agree with their results because when the oil:wall material ratio was maintained at 1:3, the microcapsules produced by combining 10% whey protein and 20% MD had a lower surface oil content than that of the microcapsules produced using MD alone. The results obtained so far show that OEE is enhanced by increasing the quantity of wall material, as has been reported by other authors (Hogan *et al.,* 2001b; Bae and Lee 2008).

Regarding the moisture content of the microcapsules, which influences their stability, Table 4.2 shows that the values were lower than 1% (wet basis) for microcapsules produced using a 1:1 ratio for oil:wall material, whereas for microcapsules produced using a 1:3 oil:wall material ratio the moisture content increased to 3%. The moisture values of all the microcapsules produced in the present study are within the range of the specified moisture for dry products in the food industry (Masters 1991).

4.3.3 Morphological characterization of fish oil microcapsules

Fish oil:wall material ratio 1:1

The surface and inner morphology of the fish oil microcapsules was studied using ESEM and SEM images of the samples, respectively. Figure 4.6 shows the images obtained from microcapsules prepared using O/W emulsions stabilized with 10% WPH, produced by rotor stator or premix ME and dried without further addition of wall material. All the images from ESEM which show external morphology of

microcapsule (Fig. 4.6 A-D) were captured using 20.0 kV and 2900 magnification, whereas the images from SEM (inner morphology, Fig. 4.6 E-H) were obtained from broken microcapsules (see section 4.2.7) and captured using 15 kV. The wall thickness was obtained by directly measuring different positions of the wall on the images during analysis with the SEM microscope and the values for all the microcapsules are presented in Table 4.2.

Microcapsules obtained using a 1:1 oil:wall material ratio with 10% whey protein show a different external morphology depending on the emulsification method. Fig. 4.6 shows that microcapsules prepared from emulsions obtained by rotor stator (Fig.4.6A) were more irregular in shape, showed more shrinkage and lacked uniformity. The microcapsules prepared from emulsions produced by premix ME were rounder (Fig. 4.6 B-D), although most of them appear deflated, which can be indicative of a thin wall due to the low amount of wall material employed. The size of the microcapsules has a wide distribution and agglomerates can be clearly seen; however, they seem to have a smoother surface than the ones obtained from O/W emulsions produced by mechanical stirring.

Regarding the inner morphology of the microcapsules, some large vacuoles can be observed in the microcapsules made from emulsions prepared by rotor stator (Fig. 4.6E), and this may be because more air was trapped during emulsification and drying. All the microcapsules produced using an oil:wall material ratio of 1:1 seemed to have a thin wall (Fig.4.6 F-H) of between 0.4 to 0.6 μm measured from SEM images, regardless of the emulsification method employed to produce the O/W emulsion.

Fish oil:wall material ratio 1:3

Figure 4.7 presents the images of microcapsules obtained from O/W emulsions containing 10% oil stabilized with 1% whey protein and with a further addition of 30% MD, acting as wall material, which gave an oil:wall material ratio of 1:3. A comparison of the images in Fig. 4.6 A-D and in Fig. 4.7 A-D shows that the increase in the amount of wall material helps to produce more spherical capsules, which, in general, appear not to be deflated or have cracks on the surface (Fig. 4.7 A-D). However, there is still a wide size distribution. Regarding the inner

morphology of the microcapsules (Fig. 4.7 E-H), it seems that the use of 30% MD resulted in good wall thickness (0.81 – 0.88 μm), and no vacuoles caused by trapped air are visible inside the capsules. Fig. 4.7 E-H also show that this type of microcapsule has a very smooth outer surface.

Some capsules agglomerates could be observed when the microcapsules were produced by mechanical stirring and prepared from fish oil emulsions stabilized with 2% Tween 20 (Fig. 4.8A) and combined with MD before spray drying, in contrast to capsules from emulsions produced by premix ME where less agglomerates were visible (Fig 4.8 B-C).

Regardless of the emulsification method, all the microcapsules were round, presented a wide size distribution and appeared to have very smooth surfaces with no visible cracks. There were no deflated capsules, as a result of the thicker wall. The inner morphology of the microcapsules can be seen in Fig. 4.8 D-F, and again, as in the case of microcapsules produced from emulsions stabilized with 1% whey protein and maltodextrin, they show no vacuoles on the inside, a wall thickness of 0.91- 1.03 μm (Table 4.2) and a very smooth surface.

Microcapsules were also produced by rotor-stator and ME using the same oil:wall material ratio (1:3) but changing the proportions of the protein and polysaccharide to 10% whey protein and 20% MD added after emulsification. Fig. 4.9 shows the internal and external morphology of the microcapsules produced using this specific formulation. Microcapsules from emulsions produced by rotor stator have more agglomerates with visible cracks on the surface (Fig. 4.9A) and big vacuoles on the inside (Fig. 4.9D) indicating that air was entrapped during the process, while microcapsules from emulsions obtained by premix ME are more rounder, with a wide particle size distribution and no visible cracks on the surface (Fig. 4.9B-C). All the microcapsules produced by combining 10% whey protein and 20% maltodextrin have a wall thickness ranging from 0.81 to 1.01μm.

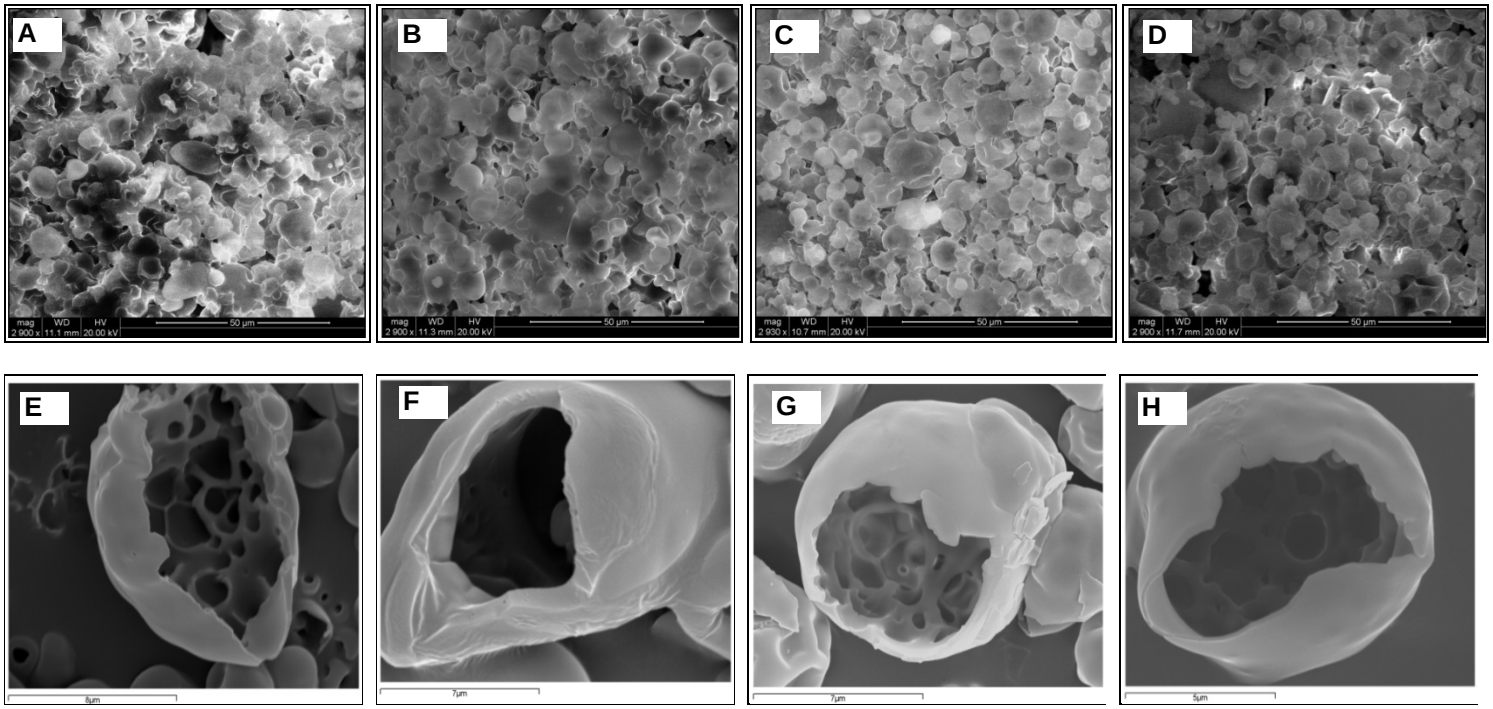

Figure 4.6. Images of the external (ESEM) and internal (SEM) morphology of the fish oil microcapsules produced by mechanical stirring or premix ME from 10% fish oil/water emulsions stabilized with 10% whey protein hydrolyzated and dried without further addition of wall material. A and E: 10% WPH – mechanical stirring; B and F: 10% WPI – Nylon; C and G: 10% WPH – Nylon; D and H: 10% WPH – MCE.

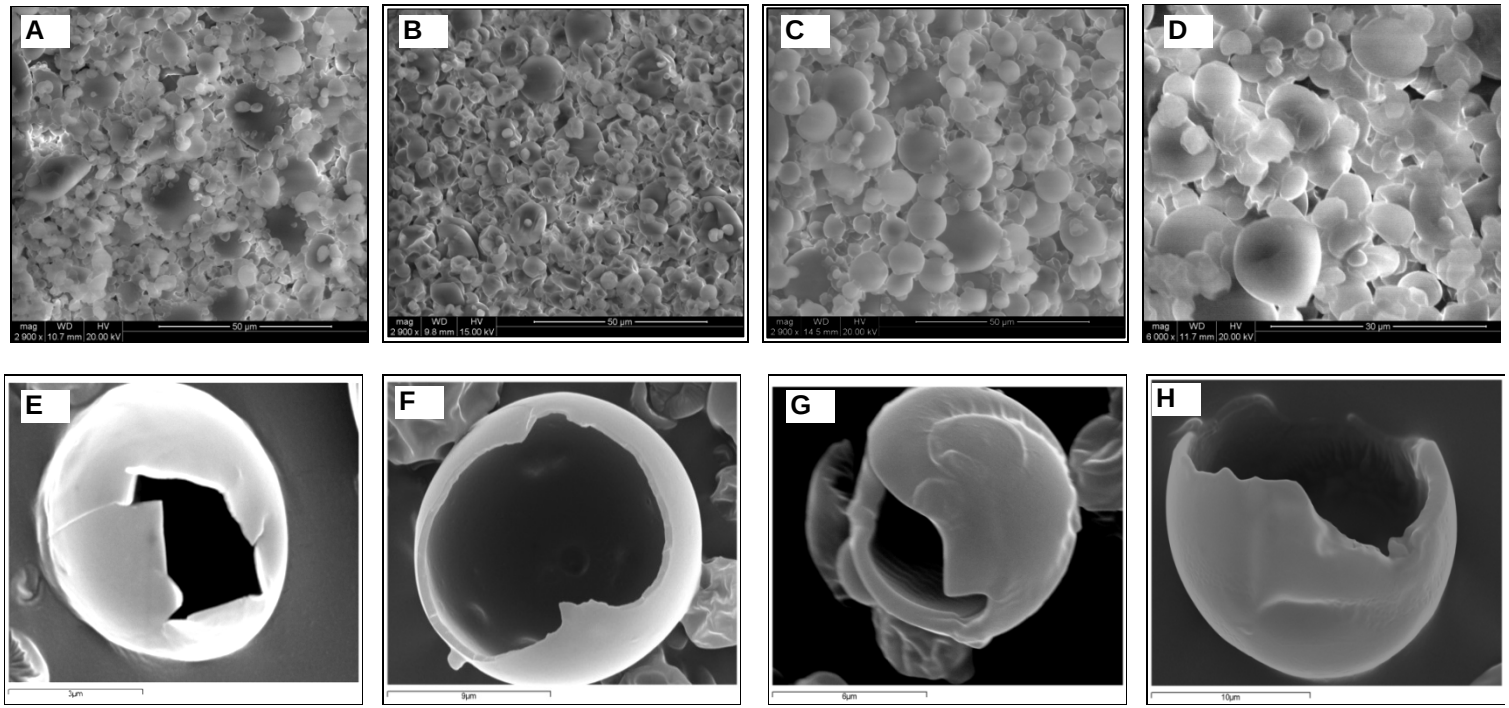

Figure 4.7. Images of the external (ESEM) and internal (SEM) morphology of the fish oil microcapsules produced by premix ME from 10% fish oil/water emulsions stabilized with 1% WPI or 1% WPH and dried after addition of maltodextrin. A and E: 1% WPI + 30% MD – Nylon; B and F: 1% WPI + 30% MD – MCE; C and G: 1% WPH + 30% MD – Nylon; D and H: 1% WPH + 30% MD – MCE.

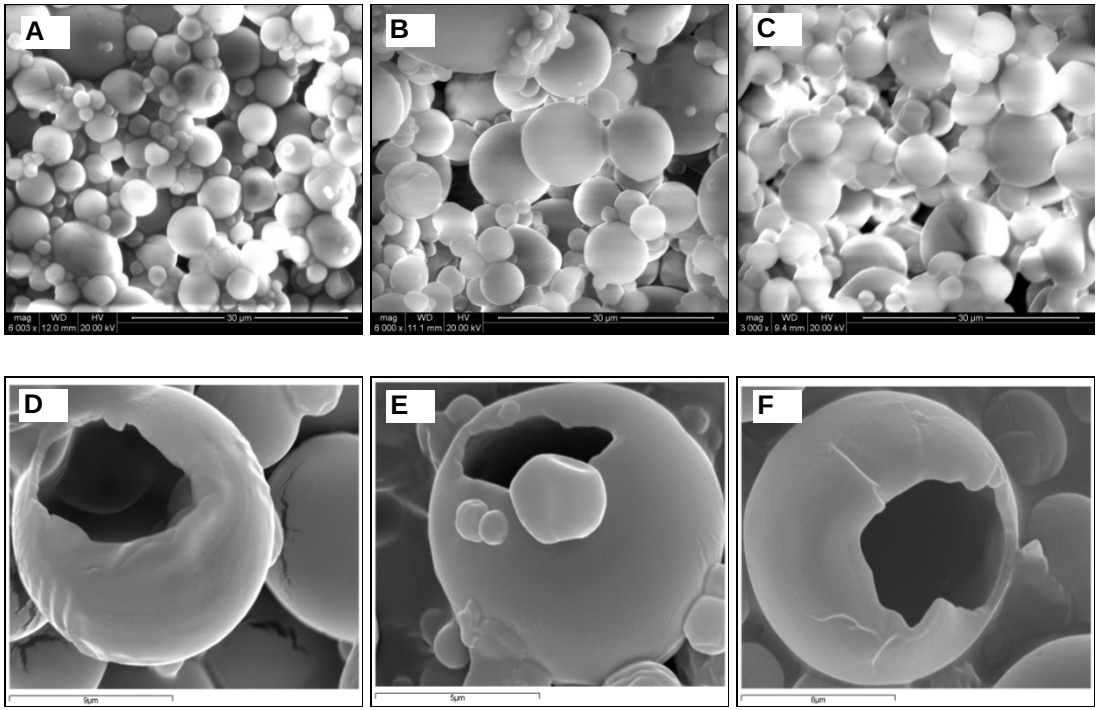

Figure 4.8. Images of the external (ESEM) and internal (SEM) morphology of the fish oil microcapsules produced by mechanical stirring or premix ME from 10% fish oil/water emulsions stabilized with 2% Tween 20 and dried after addition of maltodextrin. A and D: 2%Tween+ 30 % MD – mechanical stirring; B and E: 2% Tween + 30% MD - Nylon; C and F: 2% Tween + 30% MD – MCE.

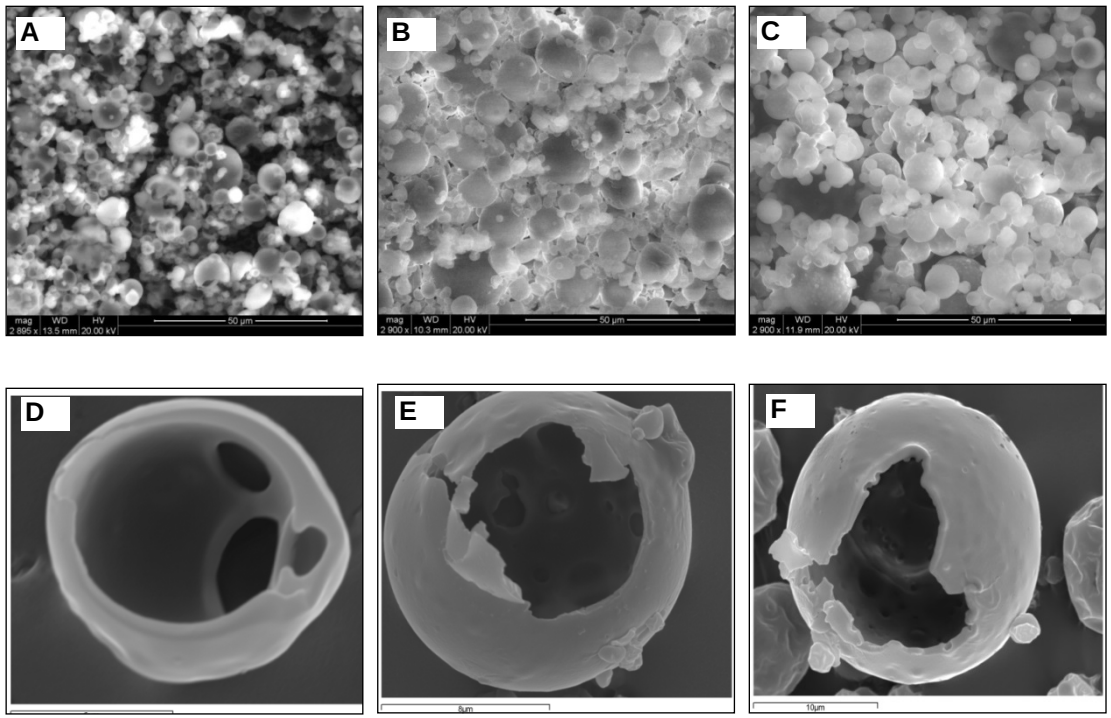

Figure 4.9. Images of the external (ESEM) and internal (SEM) morphology of the fish oil microcapsules produced by mechanical stirring or premix ME from 10% fish oil/water emulsions stabilized with 10% whey protein hydrolyzated and dried after addition of maltodextrin. A and D: 10%WPH + 20% MD – mechanical stirring; B and E: 10% WPH + 20% MD – Nylon; C and F: 10% WPH + 20% MD - MCE.

4.3.4 Oxidation stability of fish oil microcapsules

Fish oil encapsulation is carried out, among others, to protect its polyunsaturated fatty acids against oxidation. Selected samples of the microcapsules produced in this study were maintained at room temperature (20 ± 2 ºC) during 6 weeks and oxidation stability was monitored following propanal content according to the methodology described in section 4.2.8. Figure 4.10 presents the evolution of the propanal content of the microcapsules versus the storage time for a six weeks period, being the initial propanal content of the microcapsules 12 ± 2 ppm. The results point out the influence of the amount of wall material employed to build the microcapsules, since all the samples produced using an oil:wall material ratio of 1:3 show a lower propanal content at the end of the six weeks, less than 150 ppm for all the samples, compared to the sample produced using a ratio of 1:1, with a propanal content of about 400 ppm after 6 weeks. It is also important to point out that the microcapsules showing higher propanal content, which indicates higher oxidation, are the ones produced using 10% WPH, while the less oxidized microcapsules are the ones produced from O/W emulsions stabilized with Tween 20 or WPH dried after the addition of 30% MD.

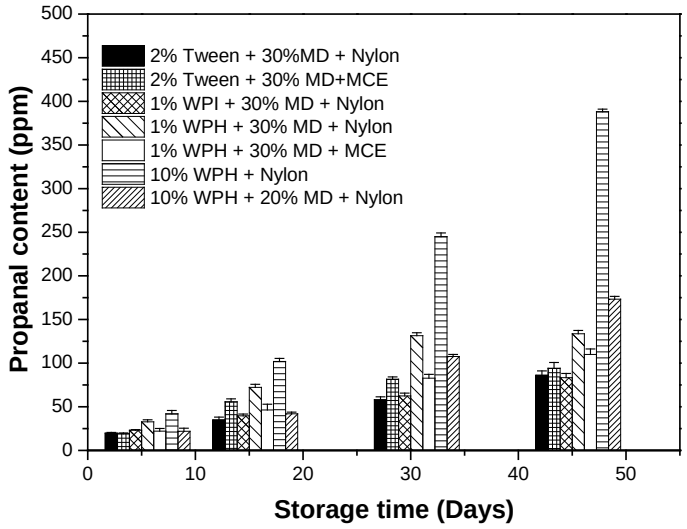

Figure 4.10. Propanal content (ppm) of the microcapsules versus storage time for six weeks at 20 ± 2 ºC.

The same trend was found by Bae and Lee (2008) during encapsulation of avocado oil by spray drying using mixtures of whey protein and maltodextrin to form the microcapsule wall. They found that the addition of MD to replace whey protein as a wall forming material increased the oxidation stability of the microcapsules. According to these authors, the hydrophilic nature of maltodextrin or the less oxygen permeability of a wall composed by a mixture of whey protein and maltodextrin could explain the results. These results can be a good start point in the selection of the most appropriate conditions for emulsion/microcapsule production to increase physical and chemical stability of the microcapsules.

4.4 CONCLUSIONS

This study demonstrates that premix membrane emulsification can be used as the first step in the production of fish oil microcapsules. The results obtained so far show that the combination of a low energy emulsification technique (premix membrane emulsification) with spray drying can produce fish oil microcapsules with an OEE of more than 50%. The increase in the amount of encapsulated fish oil is mostly dependent on the combination of two factors, namely the initial droplet size of the emulsion and the ratio between the oil and the wall material. Emulsions produced by premix membrane emulsification have smaller droplet size distribution and are more monodisperse than those produced by rotor-stator, which facilitates the encapsulation of the oil and the stability of the emulsion during the spray drying process. When the ratio of oil:wall material increases from 1:1 to 1:3 the OEE is increased by more favorable process conditions. The inner and outer morphology of the fish oil microcapsules varies depending on the emulsification technique; O/W emulsions obtained by premix ME will produce rounder microcapsules, with a smoother surface and without inner vacuoles. Studies on oxidation stability of the microcapsules show a correlation between the amount of wall material and the amount of surface oil with the stability of microcapsules.

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CHAPTER 5

INFLUENCE OF EMULSIFICATION TECHNIQUE AND WALL COMPOSITION ON PHYSICOCHEMICAL PROPERTIES AND OXIDATIVE STABILITY OF FISH OIL MICROCAPSULE PRODUCED BY SPRAY DRYING

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ENCAPSULATION OF OMEGA-3 FATTY ACIDS BY PREMIX MEMBRANE EMULSIFICATION
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5.1 INTRODUCTION

Microencapsulation is defined as a process in which small particles or droplets are surrounded by a coating, or embedded in a homogeneous or heterogeneous matrix, to produce small capsules (Gharsallaoui *et al.,* 2007; Calvo *et al.,* 2011). Microencapsulation of bioactive compounds for the food industry is mainly performed to prevent a premature interaction between the core material and the other ingredients present in the food matrix. The advantages for the food industry of having a target compound in encapsulated form are easy handling, storage and transportation and uniform mixing and dispersion in food formulations when small quantities need to be incorporated (Bae and Lee, 2008). Fish oil is a rich dietary source of omega-3 polyunsaturated fatty acids(PUFA) such as eicosopentaenoic acid (EPA) and docosohexaenoic acid (DHA) which have many health benefits to humans, such as the prevention and treatment of coronary heart diseases, arthritis and immune response disorders (Simopoulos *et al.,* 1999). However, fish oil has a strong odor and oxidizes rapidly due to its high content of unsaturated long chain fatty acids. These negative attributes can be controlled by microencapsulation techniques.

Solid oil microcapsules can be obtained by a two-step process, with the production of an oil-in-water (O/W) emulsion followed by a microencapsulation procedure such as spray-drying, freeze-drying, coacervation, rotational suspension separation, co-crystallization or polymerization (Desai and Park, 2005; Gibbs *et al.,* 1999; Gouin 2004; King, 1995; Shahidi and Han,1993). Of the many techniques for obtaining a solid microcapsule, spray drying is the most commonly used in the food industry due to its low cost, ease of operation and ready availability of equipment. Spray drying is a one-step continuous drying process in which a liquid product is atomized to a powder in a hot drying medium (Fuchs *et al.,* 2006; Partanen *et al.,* 2008). The oil encapsulation process using spray drying is usually carried out in two steps: (I) emulsification, and (II) spray drying. Both steps have to be carefully designed to enhance the oil encapsulation, to preserve the core material and to obtain faultless capsules. In emulsification, one of the most important parameters to be controlled is the droplet size distribution, since small and monodisperse emulsions correlate with higher oil encapsulation efficiency (Soottitantawat *et al.,* 2003; Soottitantawat *et al.,*

2005; Liu *et al.,* 2001; Jafari *et al.,* 2007; Risch *et al.*, 1988; Ramakrishnan *et al.,* 2012). Another important parameter is viscosity, since high viscosities interfere with the atomization process, leading to large elongated droplets that adversely affect the drying process (Gharsallaoui *et al.,* 2007). Emulsion stability and composition determine some of the key quality parameters of oil microcapsules such as surface free oil contents, morphology, encapsulation efficiency and oxidation stability.

After the emulsion has been produced, it is common practice to add the wall forming material before the drying step. Depending on how this material is added and how the drying step is performed, different microcapsule structures can be obtained. The wall is designed to protect the core from deterioration and release it under desired conditions (Young *et al.,* 1993).The selection of wall material is therefore of paramount importance for the success of the encapsulation, since the stability of the microcapsules and their controlled release depends mainly on the composition and structure of the wall. Typically, wall materials for microencapsulation of oil by spray drying must have emulsifying properties, high water solubility, low viscosity and drying properties (Shahidi and Han, 1993; Bae and Lee, 2008).In the spray drying process, a number of studies on the microencapsulation properties of different wall materials for food ingredients are available, including starches (ex. maltodextrin) and sugars (lactose, sucrose) (Onwulata *et al.,* 1996; Strange *et al.,* 1997; Shiga *et al.,* 2001; Bae and Lee, 2008; Davidov-Pardo *et al.,* 2013), Arabic gum (Kim and Morr, 1996; McNamee *et al.,* 1998), mesquite gum (Beristain *et al.,* 2001) and milk proteins (whey protein and sodium caseinate) (Sheu and Rosenberg, 1995; Hogan *et al.,* 2001) or milk-originated wall materials (Aghbashlo *et al.,* 2013).

Milk proteins, and particularly whey protein and sodium caseinate, have been reported to be an excellent encapsulating agent in oil encapsulation due to their amphiphilic properties (Hogan *et al.,* 2001). Maltodextrin has many of the desirable properties in encapsulating agents, such as low viscosity at high solid content and good solubility. However, maltodextrins alone cannot be used as wall material because they generally have no emulsification properties. An additional emulsifying agent for the encapsulation of oil is therefore required. Some studies shown that blending a protein (sodium caseinate and whey protein) with maltodextrin provides good encapsulation properties (Sankarikutti *et al.,* 1998; Bhandari *et al.,* 1992; Dian *et al.,* 1996; Kagami *et al.,* 2003). In the spray drying step, the oil to wall ratio is one

of the key parameters in improving microencapsulation efficiency. A high ratio of wall systems generally yields better encapsulation (Bae and Lee, 2008). It has also been widely reported that the temperature of the inlet and outlet air used in the spray drying process plays a role in encapsulation efficiency (Lee *et al.,* 2005). The temperature conditions during spray drying must ensure an end product without any cracks. Several studies have reported that for maltodextrin and protein walls, the inlet and outlet air temperature must be maintained between 160-220ºC and 70-125 ºC, respectively (Baranauskiene *et al.,* 2006; Watanabe *et al.,* 2004; Young *et al.,* 1993).

O/W emulsions are traditionally prepared using colloid mills, rotor-stator systems and high pressure homogenizers. These methods require a high energy input to produce emulsions with a fine droplet-size distribution, and this often results in polydisperse emulsions and causes difficulties when trying to control droplet size distribution (Charcosset *et al.,* 2004). Membrane emulsification (ME) is a relatively new emulsification technology employing low energy inputs of 10^4 – 10^6 J m^{-3}, that has been used to produce emulsions with a narrow droplet size distribution. In the early 1990s, Suzuki *et al.,* (1998) introduced premix membrane emulsification, which is carried out in two steps: (i) homogenization of both the oil (i.e. disperse phase) and water (i.e. continuous phase) using a rotor-stator system, forming a coarse premix emulsion; (ii) break-up of the oil droplets by forcing the coarse emulsion through the membrane pores several times until a small and narrow droplet size distribution is obtained.

In a previous study, we showed the feasibility of combining premix membrane emulsification and spray drying to produce fish oil microcapsules (Ramakrishnan *et al.,* 2012). To the best of our knowledge, this was the first study to combine membrane emulsification and spray drying for this purpose. This study extends the research and correlates emulsification process parameters (emulsification method, membrane type, oil fraction and emulsifier type and concentration) and microcapsule wall composition with the physical and chemical properties of fish oil microcapsules. Their key quality properties, such as surface oil content, oil encapsulation efficiency and oxidation stability, have been determined. Emulsions with a 10% or 20% oil fraction were prepared by rotor stator homogenization or by

membrane emulsification, using different amounts of Tween 20, whey protein and sodium caseinate as emulsifiers. For the wall composition, we studied whey protein, sodium caseinate, maltodextrin or different combinations of protein and polysaccharide. The results were used to select the process conditions that maximize the oil encapsulation efficiency and minimize oil oxidation during storage.

5.2 MATERIALS AND METHODS

5.2.1 Materials

In this study, antioxidant-free menhaden fish oil (Sigma – Aldrich, CAS-No.8002-50-4, Spain) was used as the core material for preparing O/W emulsions with a 10% or 20% oil load. The emulsions were stabilized with different concentrations of emulsifiers dissolved in distilled water as continuous phase. The emulsifiers used were Tween-20 (polyoxyethylene sorbitan monolaurate, from Sigma – Aldrich, CAS-No. 9005-64-5, Spain), whey protein isolate (WPI, Bipro from Davis Co Food Inc. – Switzerland, Ref No. LE 003-0-919), whey protein hydrolyzate (WPH, Biozate from Davis Co Food Inc. – Switzerland, Ref. No. JE 031-1-420) and sodium caseinate (Sigma – Aldrich, CAS-No.9005-46-3, Spain). Various combinations of maltodextrin (Sigma Aldrich– Spain, Dextrose equivalent 16.5-19.5, CAS-No. 9050-36-6), sodium caseinate and whey protein (WPI or WPH) were used to build the microcapsules. A detailed list with the composition of all the microcapsules prepared in this study is shown in Table 5.1.

Analytical grade hexane and petroleum ether were purchased from Sigma-Aldrich, Spain. Distilled water was used to prepare the solutions. The general chemicals used in this study were of analytical grade.

Table 5.1. Emulsification method, formulation of O/W emulsions, type and amount of wall material used for the production of the microcapsules, and oil/wall ratio of each microcapsule. Where no wall material is shown, the protein employed as emulsifier was also used as wall material. The concentration of emulsifier and wall material are based on the total volume of the system.

Emulsification method	***Continuous phase***	***Disperse Phase***	***Emulsifier (concentration)***	***Wall material***	***Oil:Wall Ratio***
Premix ME- nylon	Distilled water – 90% (v/v)	Menhaden Fish oil 10% (v/v)	Tween 20 (2%)	30% MD	1:3
Premix ME- MCE			Tween 20 (2%)	30% MD	1:3
Rotor stator			Tween 20 (2%)	30% MD	1:3
Premix ME- nylon			WPI (1%)	30% MD	1:3
Premix ME- MCE			WPI (1%)	30% MD	1:3
Premix ME- nylon			WPH (1%)	30% MD	1:3
Premix ME- MCE			WPH (1%)	30% MD	1:3
Premix ME- nylon			WPI (10%)	---	1:1
Rotor stator			WPI (10%)	---	1:1
Premix ME- nylon			WPH (10%)	---	1:1
Premix ME- MCE			WPH (10%)	---	1:1
Rotor stator			WPH (10%)	---	1:1
Premix ME- nylon			WPH (10%)	20% MD	1:2
Premix ME- MCE			WPH (10%)	20% MD	1:2
Premix ME – nylon			CAS (10%)	---	1:1
Premix ME – MCE			CAS (10%)	---	1:1
Rotor stator			CAS (10%)	---	1:1

Emulsification method	***Continuous phase***	***Disperse Phase***	***Emulsifier (concentration)***	***Wall material***	***Oil:Wall Ratio***
Premix ME- nylon	Distilled water – 80% (v/v)	Menhaden Fish oil 20% (v/v)	CAS (2%)	40% MD	1:2
Premix ME- MCE			CAS (2 %)	40% MD	1:2
Premix ME- nylon			CAS (10%)	10% MD	1:1
Premix ME- MCE			CAS (10%)	10% MD	1:1
Rotor stator			CAS (10%)	10% MD	1:1
Premix ME- nylon			CAS (10%)	30% MD	1:2
Premix ME- MCE			CAS (10%)	30% MD	1:2
Rotor stator			CAS (10%)	30% MD	1:2
Premix ME- nylon			CAS (10%)	50% MD	1:3
Premix ME- MCE			CAS (10%)	50% MD	1:3
Rotor stator			CAS (10%)	50% MD	1:3

5.2.2 Preparation of microcapsules

Fish oil microcapsules were obtained by first producing O/W emulsions by rotor stator or premix membrane emulsification using an organic microfiltration membrane, then adding the wall material, followed by a drying step in a spray drier. A flow diagram of the whole process including the process parameters studied is shown in Figure 5.1. The information of the organic microfiltration membranes employed in the study is presented in the section below.

5.2.2.1 Preparation of O/W emulsions

Premix Membrane Emulsification. The emulsions produced by premix membrane emulsification were prepared following the experimental procedure depicted in Figure 5.2. The method consists of producing a coarse emulsion by mixing the oil and the water containing the emulsifier using a rotor stator (Ultra-Turrax®, model T18, IKA). The coarse premix emulsion was then immediately passed through a membrane using nitrogen pressure (700 kPa). The resulting fine emulsion was collected in an Erlenmeyer flask and weighed using an electronic balance. The balance was interfaced to a computer to record the time and mass data at 1-second intervals. These data were used to calculate the flux during the emulsification process. The O/W emulsion was passed up to three times through the membrane to obtain a small droplet size.

Two types of polymeric membrane discs were used in this study. (i) Nylon (Whatman ref. 7408-004) (ii) nitrocellulose mixed esters (MCE) (Sterlitech Corporation ref. MCEB0847100SG). The mean pore size of the two membranes was 0.8 μm, and the effective membrane diameter was 41 mm, giving an effective filtration area of 1.32×10^{-3} m^2 for the membrane module used in the experiment. The membrane was conditioned by wetting with water at the beginning of each experiment.

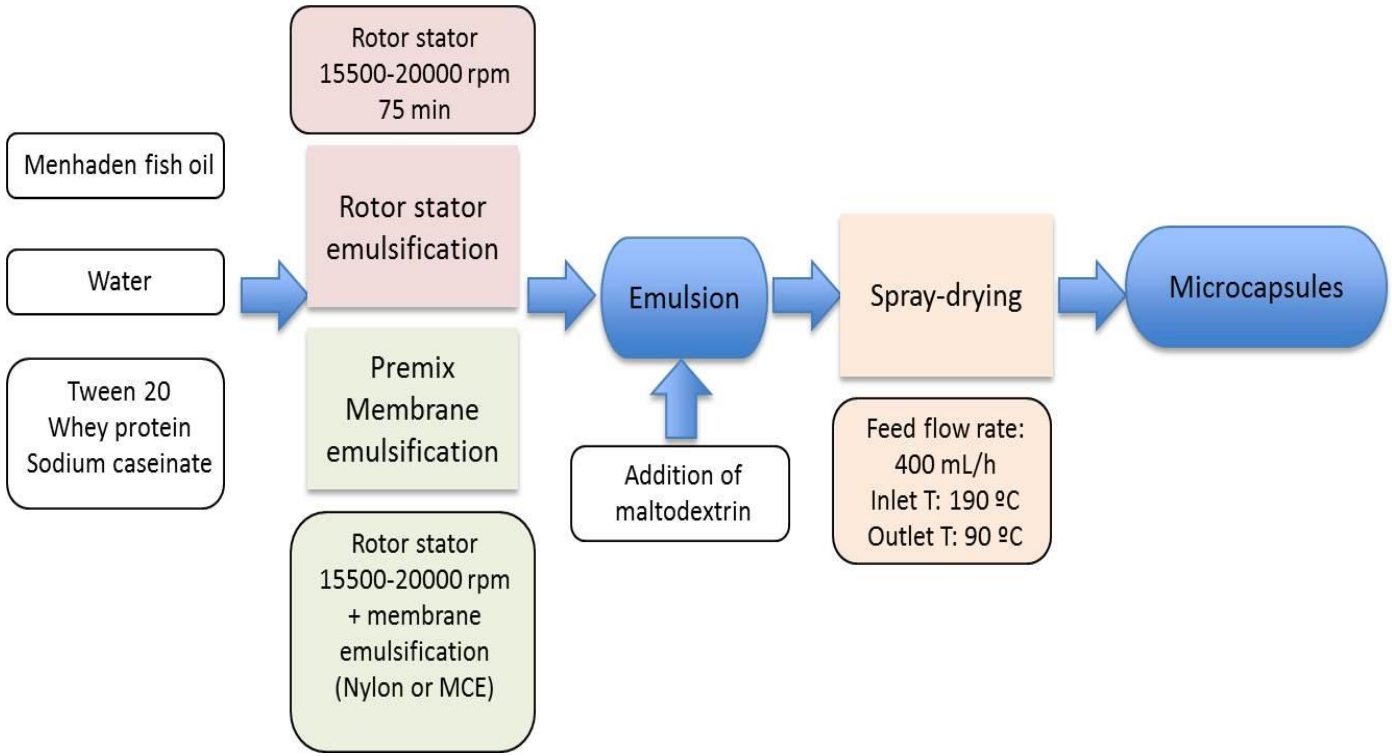

Figure 5.1. Flow diagram of the preparation process to produce fish oil microcapsules.

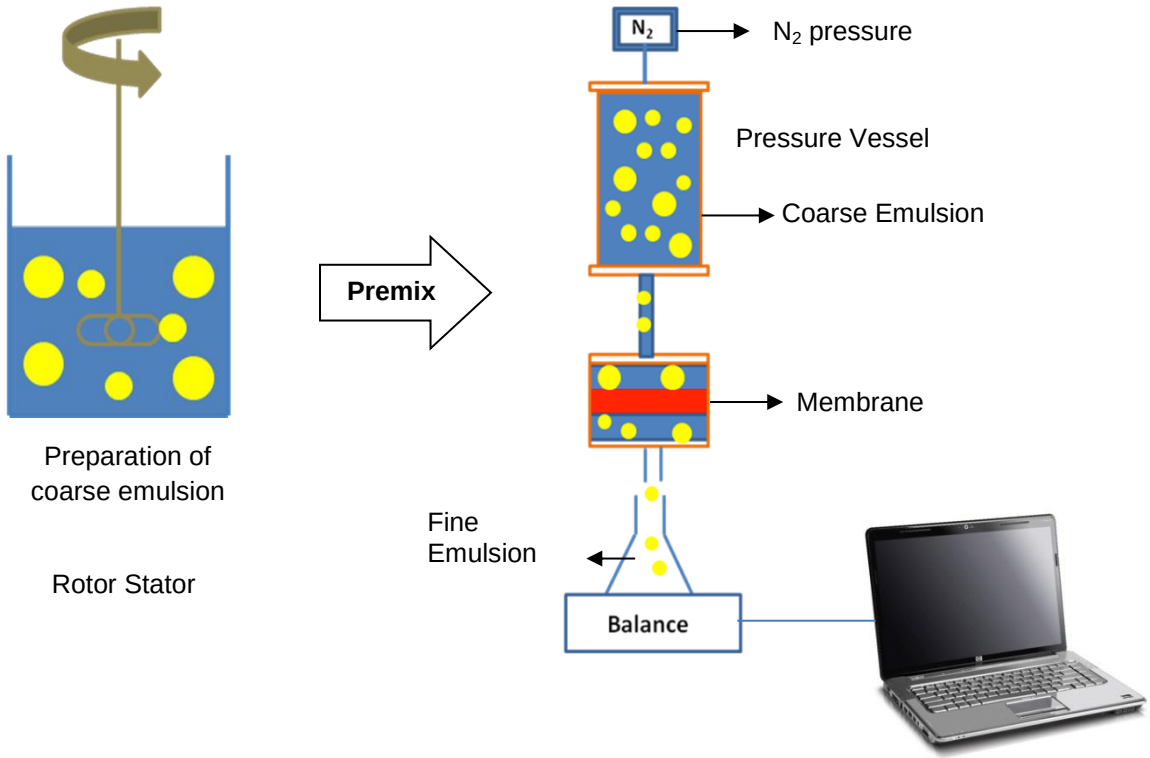

Figure 5.2. Diagram of the experimental set-up for premix membrane emulsification.

Rotor-stator emulsification. A rotor-stator homogenizer (Ultra-Turrax®, model T18, IKA) was employed to produce O/W emulsions by mechanical stirring. The speed and time employed were adjusted for each emulsion to obtain the smallest possible droplet size, and it was directly correlated with the type and amount of emulsifier used. Emulsions prepared with 2% Tween 20 were emulsified at 15500 rpm for 75 min, emulsions stabilized with 1 or 10% WPI/WPH 20000 rpm for 4 min were required, while emulsions stabilized with 10% CAS, 20000 rpm for 75 min were employed.

5.2.2.2 Spray-drying

The fish oil microcapsules were produced drying the O/W emulsions in a laboratory scale spray dryer (Buchi mini spray dryer b290, Switzerland). The O/W emulsions produced by membrane emulsification or rotor stator homogenization were mixed with the appropriate amount of maltodextrin (see Table 5.1) and fed (400 mL/h) into the spray-drier at room temperature. The inlet and outlet temperatures were maintained at 190 °C and 90 °C, respectively. The dried microencapsulated powder was removed from the dryer and immediately stored in 30 mL brown bottle closed with black rubber lid at 4° C until characterization was performed.

5.2.3 Emulsion and microcapsule characterization

5.2.3.1 Emulsion and microcapsule droplet size

The droplet size distribution of the emulsions produced by premix membrane emulsification (coarse emulsion and emulsions from cycles 1-3) and by rotor stator was measured using laser light diffraction in a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, England). The emulsion droplet size was expressed as $d_{3,2}$, (Sauter mean diameter) and the span value defined in equation [5.1] was used to measure the degree of monodispersity. Span values of 1 or lower are correlated with narrow droplet size distributions.

$$span = (d_{90} - d_{10})/d_{50} \qquad [5.1]$$

where d_{90}, d_{50}, d_{10} are the mean diameters at which 90, 50, and 10% (vol %) of the particles counted and calculated. All the emulsion samples were analyzed in triplicate and the average data are reported.

The particle size distribution ($d_{3,2}$) of the spray-dried powders was determined using the Malvern Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, England) equipped with a Hydro 2000 SM dispersion unit (Malvern Instruments Ltd., Worcestershire, England). Each sample was analyzed in triplicate and the data were reported as averages.

5.2.3.2 Oil Encapsulation Efficiency

The *total oil* content of the powders was analyzed gravimetrically by ether extraction based on the Rose-Gottlein method (Richardson, 1985), and expressed as mass per 100 g of powder (wet basis). The procedure consisted of dispersing two grams of microcapsules in 20 mL of water heated at 65°C. After stirring gently, 4 mL of 25% NH_4OH was added and the solution was heated at 65°C for 15 min in a shaking water bath. Then, the solution was cooled at room temperature and the oil was extracted by applying three liquid-liquid extraction: (1) 50 mL of diethyl ether and 50 mL of light petroleum ether; (2) 10 mL of ethanol, 50 mL of diethyl ether, and 50 mL of light petroleum ether; and (3), 50 mL of diethyl ether and 50 mL light petroleum. After the extraction steps, the final solution was filtered and the solvent was evaporated in a rotary evaporator (Heidolph Laborota 4000) and the extracted oil was dried to a constant weight using a stream of nitrogen.

The surface oil or non-encapsulated oil was determined according to the method described by Varavinit *et al.,* (2001). Hexane (20 mL) was added to an accurately weighed amount (2 g) of powder followed by stirring for 10 min. The suspension was then filtered and the residue rinsed three times with 20 mL of hexane. The residual powder was then dried to vaporize all residual solvent at 60 ºC to a constant weight. The non-encapsulated oil (surface oil) was determined by the mass difference between the initial powder amount and after extraction with hexane, and this was used to calculate the free oil and expressed as a percentage.

Finally, the oil encapsulation efficiency (OEE) was calculated using equation [5.2]:

$$OEE = [(total\ oil - surface\ oil)/total\ oil] * 100 \quad [5.2]$$

5.2.3.3 Moisture content

The moisture content of the powders was calculated by drying 2 g of powder in an air oven at 105°C, and the result was expressed the percentage per 100 g of powder (wet basis).

5.2.3.3 Microcapsules surface and inner morphology analysis

The inner and outer morphology of the microcapsules was characterized by electronic microscopy. The microcapsules were mounted on cylindrical stubs coated with conductive carbon tape to study their outer morphology. An environmental scanning electron microscope (FEI, Quanta 600) was used to analyze the surface morphology of the dried powders. Digital images were taken at an accelerating voltage of 20kV and magnification of 2900x.

The characterization of the inner morphology of the microcapsules was performed using a Scanning Electron Microscope (SEM) (JOEL JMS-6400). The samples were mounted on SEM stubs using double-sided sticky tape following the procedure detailed by Bae and Lee (2008). The specimens were coated with gold (EMITECH K575XD Turbo Sputter Coater) and subsequently examined and imaged at 15 kV.

5.2.4 Stability of the microcapsules during storage

Representative samples of the microcapsules produced were kept in brown bottle closed containers at 30 ºC for two months. Samples were taken at 15-day intervals to determine the oil stability following the propanal formation. Propanal is one of the main volatile compounds formed during the oxidative decomposition of omega-3 fatty acids and it has been recommended to follow the oxidation stability of foods rich in this type of fatty acids (Shahidi and Spurvey 1996). The samples that underwent the stability tests and their main features are listed in Table 5.2. The propanal content of the microcapsules was determined using a static headspace

sampler (G1888 from Agilent Tachnologies, Waldbronn, Germany) coupled to a gas chromatograph (HP 6890 from Hewlett–Packard, Waldbronn, Germany) equipped with a quadrupole mass spectrometer with a diffusion pump (HP 5973, from Hewlett–Packard, Waldbronn, Germany). The chromatographic separation was carried out with an HP5-MS chromatographic column (30 m x 0.25 mm i.d., 0.25 µm film thickness) with an oven temperature program of 40ºC (5 min), 70ºC/min to 300ºC (10 min). The carrier gas was helium with a flow rate of 1.8 mL/min.

For each analysis, 3 mL of sample headspace were introduced into the injection port of the gas chromatography. The loop and transfer line temperatures were 75º and 80ºC, respectively, and the pressurization and injection times were 0.3 and 0.6 min, respectively. Chromatographic injection was performed in splitless mode at 200ºC.Mass spectra were recorded by electronic impact (EI) ionization at 70 eV, with a temperature of 230ºC in the ion source and 150ºC in the mass quadrupole.

To prepare the samples, 1.00 g of the microcapsules was placed into a 10 mL vial together with 2 mL of EDTA (0.5%) and the appropriate amount of 2 methyl propyl acetate (internal standard, ISTD) to obtain a final concentration of 10 mg/L of the ISTD. The vial was hermetically sealed with PTFE/silicone septum and the sample was thermostatted for 30 min at 70ºC under constant stirring. All the samples were prepared and analyzed in triplicate.

The propanal distribution constant between the sample (dissolved microcapsules) and the headspace depends heavily on the material used to produce the microcapsules (wall material);to ensure that the propanal content of each sample was properly quantified, we therefore constructed three different calibration graphs using three matrices: 1% WPH + 30% MD, 10% WPH and 10% WPH + 20% MD. In each case, the calibration graph was obtained from each specific matrix spiked with 10 ppm of internal standard (ISTD) and four different concentrations of propanal in the range 10-60 ppm. The peak area ratios [propanal/ISTD] were plotted against the [propanal/ISTD] concentration ratios. The amount of propanal in each sample was then determined considering the calibration line constructed with the matrix most similar to the sample analyzed.

5.2.5 Statistical analysis

All the data were expressed as the means of three replicates. Groups were compared using one-way ANOVA for repeated measurements using SPSS software (IBM SPSS Statistics 20). Duncan's test was used for post hoc analysis. A value of $P<0.05$ was considered significant.

5.3 RESULTS AND DISCUSSION

5.3.1 Effect of emulsification on the droplet size and dispersity of the emulsions

As mentioned above, the factors influencing the efficiency of the oil encapsulation obtained by spray drying include the droplet size of the initial O/W emulsion and its degree of dispersity. The emulsification method and the amount of emulsifier are directly correlated with these two features of the emulsion. We therefore tested two different techniques for producing 10% and 20% fish oil – water emulsions with different types and amounts of food grade emulsifiers: Tween 20, whey protein and sodium caseinate (see Table 5.1), and the techniques were premix membrane emulsification and rotor stator homogenization. For premix membrane emulsification, the coarse emulsion was always obtained by stirring at 15,500 rpm for 2 minutes when the emulsifier concentration was maintained at 1% or 2%, and 20,000 rpm for 4 minutes when the concentration used was 10%. The coarse emulsion was subsequently passed through a Nylon or MCE membrane three times. The emulsions prepared by rotor stator were obtained using the combinations of stirring and time mentioned in section 5.2.2.1.

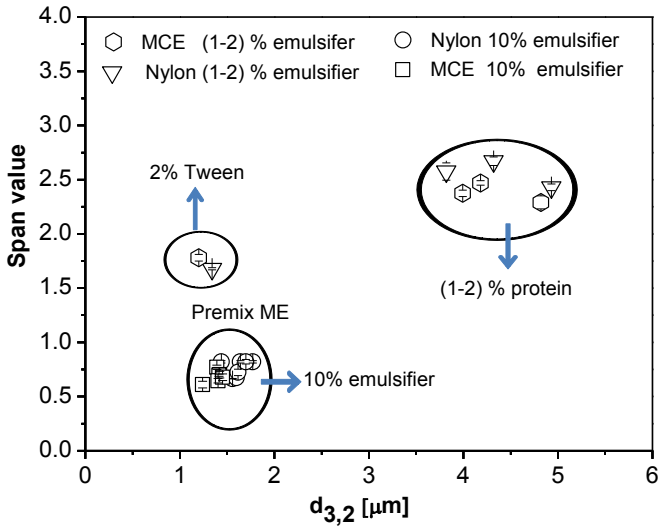

Figure 5.3. Final oil droplet size expressed as $d_{3,2}$ versus size distribution (span) for O/W emulsions with a 10% and 20% oil load prepared by membrane emulsification.

Figure 5.3 presents the final droplet-size of all the fish O/W emulsions produced by membrane emulsification versus the size distribution expressed as the span value, calculated according to equation [5.1]. When the concentration of whey protein isolate or hydrolyzate and sodium caseinate was 10%, it is clear in figure 5.3 that membrane emulsification produces emulsions with a droplet size between 1 and 2 μm and span values lower than 1, which means that they can be considered monodisperse. Since the droplet size for all the emulsions produced by premix ME are grouped together in figure 5.3, it is also clear that the type of membrane used has no significant effect on the final droplet size in our case. It has to be pointed out that both membranes used in this study have the same nominal pore size (0.8 μm). When the amount of emulsifier was reduced to 1% or 2%, membrane emulsification was observed to be no longer able to produce monodisperse O/W emulsions (Figure 5.3). The final droplet size of the O/W emulsions obtained is between 4 - 5 μm for emulsions stabilized with proteins, and between 1 and 2 μm for emulsions stabilized by Tween 20, while the span values are about 2.5 and 1.6, respectively. Tween 20 is a small non-ionic surfactant that can quickly stabilize the oil droplets, while proteins, being much bigger, will need more time to reach the oil-water interface during the emulsification process. Figure 5.3 shows that Tween 20 is more

effective when stabilizing the emulsions at low concentrations than proteins, since the droplet size is smaller and the span value is lower. However, proteins have an advantage over Tween 20 since they combine the emulsifying capacity with wall forming properties, which make them suitable for microencapsulation by spray drying. Our results show that when producing monodisperse O/W emulsions by premix ME stabilized by proteins, their concentration must be adjusted to values close to 10%. Regarding the influence of the oil load on the droplet size distribution of the O/W emulsions, our results show a similar tendency when the amount of oil is increased from 10% to 20 %. Figure 5.3 plots the droplet size and span values of emulsions with 10% and 20% of fish oil obtained by premix membrane emulsification. The differences in the droplet size clearly do not come from the oil load, but instead from the type and amount of emulsifier used. When the amount of emulsifier was 1% or 2%, the droplet size of the emulsions was bigger and had higher span values than those obtained with 10% emulsifier, regardless of the oil load.

It is interesting to compare the effect of the emulsification technique on the droplet size and its distribution. Figure 5.4 presents the droplet sizes of all the O/W emulsions stabilized with 10% protein (whey protein or sodium caseinate) obtained by premix ME and rotor stator homogenization. For the emulsions prepared by mechanical stirring, increasing the mixing time clearly reduces the droplet size of the emulsions. However, for O/W emulsions produced by rotor stator, no further reduction in the droplet size was observed in our case when the stirring time was increased to more than 75 minutes. Figure 5.4 shows that premix ME is much more effective for producing O/W emulsions with a small and more monodisperse droplet size than rotor stator homogenization.

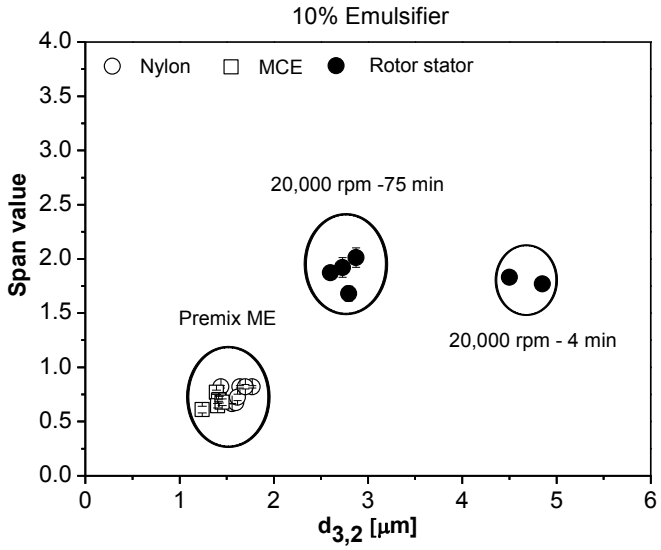

Figure 5.4. Final oil droplet size expressed as $d_{3,2}$ versus size distribution (span) of fish oil emulsions with a 10% emulsifier concentration prepared using different emulsification techniques.

The concept of energy density (energy input per unit of volume of emulsion produced, E_v), has been used to compare different emulsification processes by several authors (Lambrich *et al.,* 2005; Nazir *et al.,* 2010; Vladisavlevic *et al.,* 2004). In their studies, these authors agree that cross-flow membrane emulsification and premix membrane emulsification are able to produce emulsions with smaller droplet sizes than mechanical, high pressure or ultrasound systems with energy density values one or two orders of magnitude lower. We compared premix membrane emulsification with rotor stator while maintaining the transmembrane pressure and the power input constant, respectively, and varying the type and amount of emulsifier. The energy density for each type of emulsification process therefore remained constant. The E_v values for premix ME at 700 kPa are about $2x10^6$ J/m^3 (compared to 10^7-10^8 J/m^3 required for rotor stator to obtain emulsions smaller than 5 μm, (Schuchmann and Danner, 2004) which can result in a different droplet size depending on the type and amount of emulsifiers, but with no significant differences for a 10% or 20% oil load, as shown in Figures 5.3 and 5.4, since the droplet sizes

of the different emulsions are always grouped according to the emulsification method and amount of emulsifier.

5.3.2 Influence of the emulsion droplet size and amount of wall material on the microcapsule size

All the emulsions with a 10% or 20% oil load prepared in this study by premix ME or rotor-stator homogenization were spray dried under the same process conditions of emulsion flow rate and inlet and outlet temperatures (see section 5.2.2.2). After the spray drying process was completed, the dried powders were removed from the collection vessel of the drier and stored in airtight brown glass bottles at 4°C until characterization. Particle size distribution of the microcapsules using laser diffraction was performed in triplicate and the results are plotted versus the droplet size of the initial emulsion for 10% and 20% oil loads in Figure 5.5.

As can be seen in Figure 5.5a, the samples can be grouped in three areas: microcapsules produced from O/W emulsions obtained by premix ME and stabilized with 10% emulsifier which have a size between 3 and 12 μm; microcapsules produced from O/W emulsions obtained by mechanical stirring and stabilized with 10% emulsifier which have a size between 8 and 18 μm and microcapsules from O/W emulsions obtained under mild rotor stator homogenization conditions (20000 rpm for 4 minutes), which resulted in microcapsules of about 30 μm. This plot shows a clear relationship between the droplet size of the emulsion and the microcapsule size, with the smallest microcapsules always obtained from the emulsions with the smallest droplet size. There is of course another factor influencing the final powder size, which is the amount of wall material used, even though this will be discussed in the following section, it is important to mention at this point that the microcapsules obtained from premix O/W emulsions are divided in two groups. The group of five microcapsules with the smallest size (smaller than 8 μm, Figure 5.5a) correspond to microcapsules with 10% oil load and 10% whey protein (oil:wall ratio 1:1), therefore the smaller microcapsules are the ones with the lowest amount of wall building material. Figure 5.5b shows that if the initial emulsion was stabilized with less emulsifier, this has no significant effect on the size of the microcapsules. For O/W emulsions stabilized with 2% Tween 20, the droplet size was about 1.2 μm, while for O/W stabilized with 1% whey protein or sodium

caseinate it was between 4-5 μm, resulting in microcapsules of approximately 10 and 14 μm, respectively. According to our findings, an increase in the droplet size results in bigger microcapsules, but it is also true that a range of microcapsules between 4 and 12 μm can be obtained when starting from emulsions with similar droplet sizes, and we therefore have to analyze the amount of wall material which will also influence the viscosity of the emulsion and how the droplets are formed in the nozzle of the spray drier.

After the fish oil emulsions were prepared by either premix ME or rotor stator homogenization, the appropriate amount of maltodextrin to reach the formulations presented in Table 5.1 was added. Three different oil:wall ratios (1:1; 1:2 and 1:3) were studied, which were protein alone (only for the 1:1 ratio; 10% oil:10% whey protein), maltodextrin alone and mixtures of maltodextrin and protein.

Figure 5.6 shows the relationship between the microcapsule size and the amount of wall material (oil:wall ratio). The microcapsules produced from emulsions obtained by premix ME (Nylon or MCE) show a slight increase in the powder size when the amount of wall material used to build the microcapsule also increases. Emulsions produced by premix ME with a 20% oil load and stabilized with 10% sodium caseinate will result in microcapsules of about 8-9 μm when dried after the addition of 10% maltodextrin (oil: wall ratio of 1:1), while the same emulsion dried after the addition of 30% or 50% maltodextrin will result in capsules of about 12-13 μm. An opposite trend is observed for microcapsules produced from emulsions obtained from rotor-stator homogenization (Figure 5.6). An emulsion with a 20% oil load stabilized with 10% CAS was dried after the addition of 10% maltodextrin and the resulting microcapsules had a size of 16 μm, while after the addition of 30% and 50% maltodextrin, the size of the resulting microcapsules decreased to 14 and 12 μm respectively.

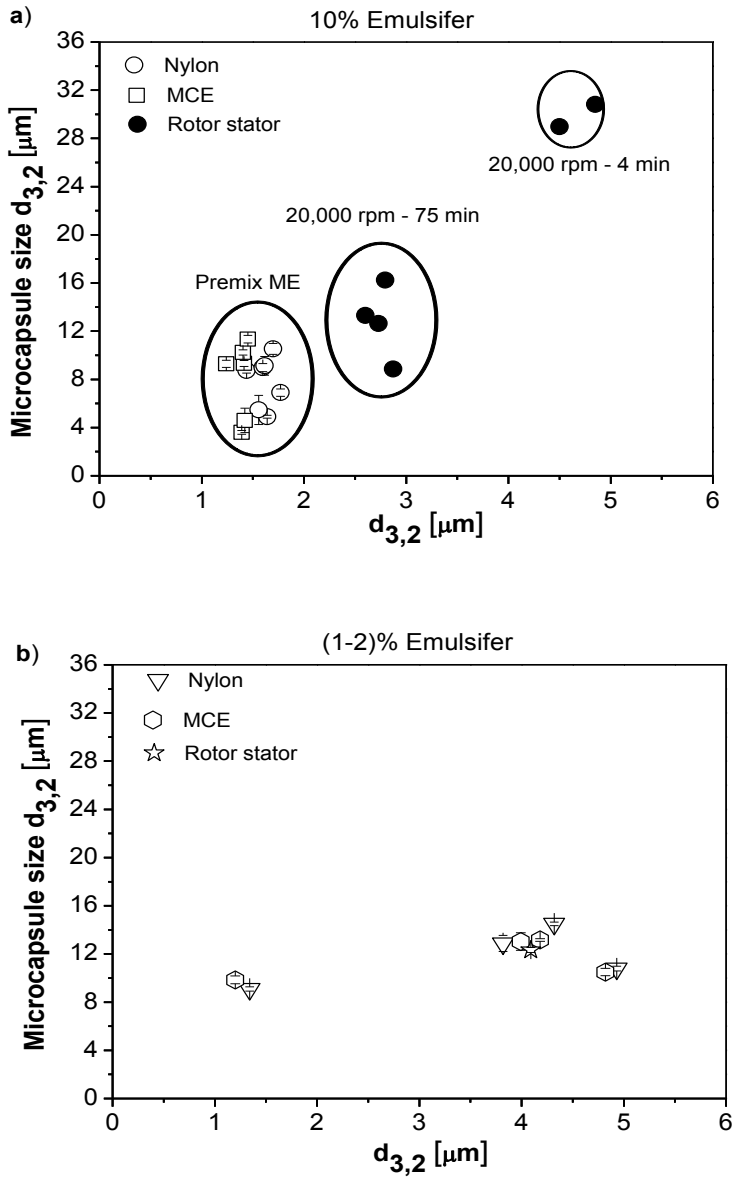

Figure 5.5. Final oil droplet size ($d_{3,2}$) versus microcapsule size distribution ($d_{3,2}$) obtained from fish oil emulsions prepared using different emulsification techniques with (a) 10% emulsifier concentration (b) 1 or 2% emulsifier concentration.

Figure 5.7 shows the relationship between the wall thickness of microcapsules versus the oil:wall ratio. The wall thickness of the microcapsules was analyzed by direct measurement from the SEM images of several microcapsules. The fish oil microcapsules produced in this study had wall thickness values of about 0.4 μm for a 1:1 oil:wall ratio to 1.2 μm for a 1:3 wall ratio. As expected, increasing the amount of wall building material used during the production of microcapsules increases the thickness of the microcapsules wall. For this property, the values plotted in Figure 5.7 show that emulsification methodology does not influence the thickness of the final capsule.

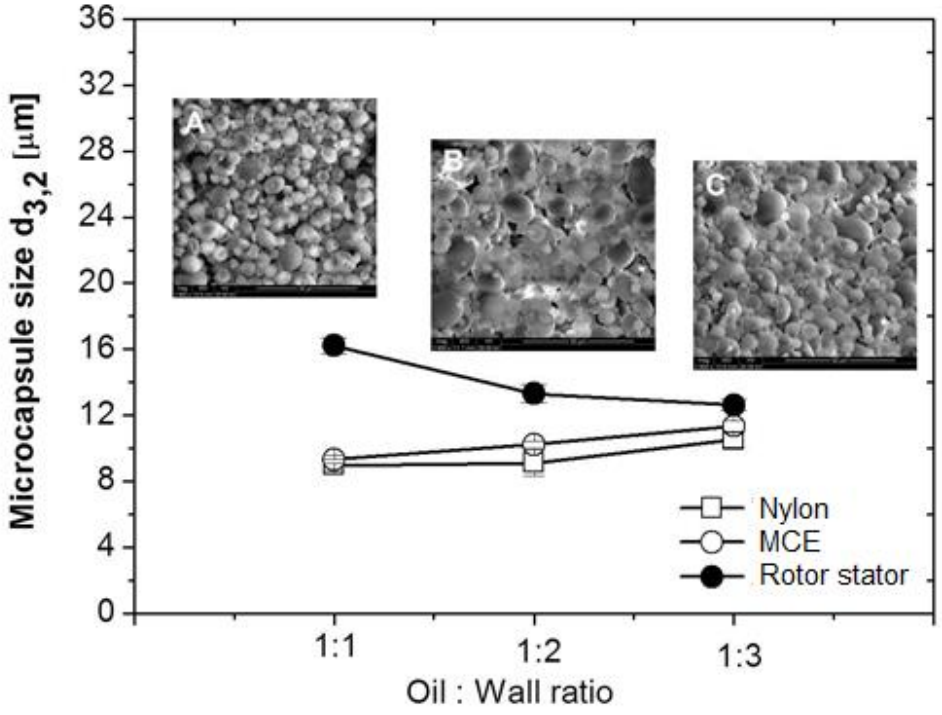

Figure 5.6. Dependence of the microcapsule size ($d_{3,2}$) on the oil:wall material ratio for microcapsules prepared from premix ME emulsions or rotor stator emulsions. The values plotted correspond to 20% fish oil + 10% CAS and + 10% MD for 1:1 ratio; 20% fish oil + 10% CAS + 30% MD for 1:2 ratio and 20% fish oil + 10% CAS + 50% MD for 1:3 ratio. SEM images (50 μm scale) correspond to: (a) 20% fish oil + 10% CAS + 10% MD-Nylon; (b) 20% fish oil + 10% CAS + 30% MD-Nylon and (c) 20% fish oil + 10% CAS + 50% MD-Nylon.

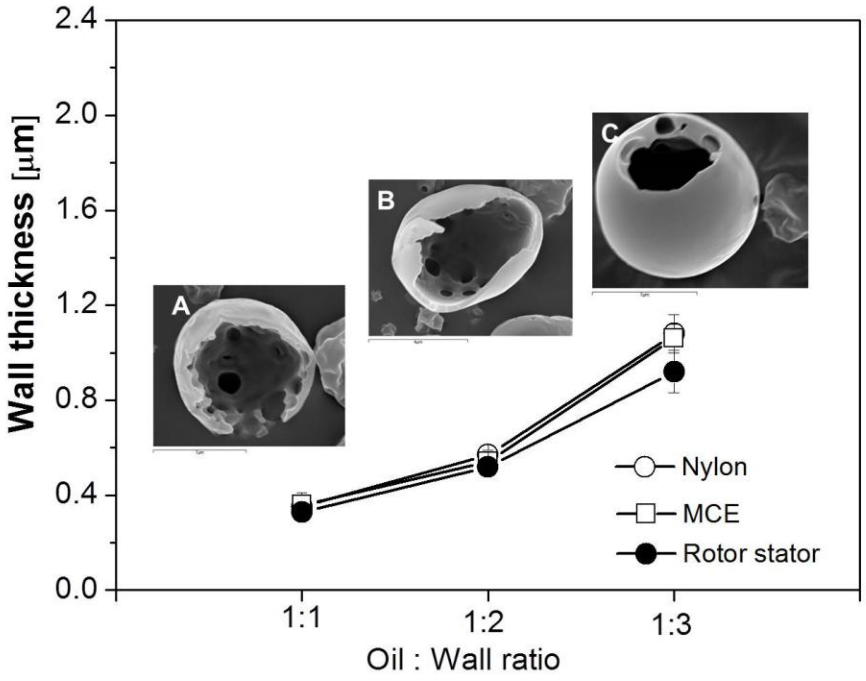

Figure 5.7. Dependence of average wall thickness on the oil:wall material ratio for microcapsules prepared from premix ME emulsions (open symbols) or rotor stator emulsions (solid symbols). The values plotted correspond to 20% fish oil + 10% CAS and + 10% MD for 1:1 ratio; 20% fish oil + 10% CAS + 30% MD for 1:2 ratio and 20% fish oil + 10% CAS + 50% MD for 1:3 ratio. SEM images (50 μm scale) correspond to: (a) 20% fish oil + 10% CAS + 10% MD-Nylon; (b) 20% fish oil + 10% CAS + 30% MD-Nylon and (c) 20% fish oil + 10% CAS + 50% MD-Nylon.

5.3.3 Influence of the droplet size and wall material amount on oil encapsulation efficiency

The results in the previous section show that a combination of premix ME with 10% protein emulsifiers favors the production of fish oil microcapsules of about 10 μm. Although a small powder size could be desirable for some processes or applications, the most widely used quality parameters are related to the surface oil and the amount of encapsulated oil. A surface oil increase is related to a decrease in the amount of encapsulated oil, besides it can increase the stickiness of the capsules and since the oil is not protected it can be quickly oxidized. The

combination of emulsification and drying to produce fish oil microcapsules must be optimized to reduce the amount of surface oil as much as possible. Figure 5.8 presents the surface oil content of the microcapsules produced in this study versus the oil:wall material ratio. The figure shows that an increase in the oil:wall ratio has a direct impact on the amount of surface oil, resulting in a clear reduction in this parameter, regardless of the composition of the wall material or the emulsification method used. However, the microcapsules obtained from O/W emulsions produced by rotor stator homogenization are those with the highest surface oil content. Since the efficiency of the oil encapsulation is also related to the droplet size of the emulsion, we expected that the microcapsules obtained from the emulsions with a larger and more polydisperse droplet size would have a higher surface oil content. In our case, the O/W emulsions with the biggest droplet size distribution were those produced by rotor stator homogenization, which are those with the largest amount of surface oil (Figure 5.8).

In this study, the amount of encapsulated oil is obtained by the difference between the total oil content of the microcapsules and the surface oil, and calculated using equation [5.2] as the oil encapsulation efficiency (OEE). The main feature of the emulsion influencing the OEE is the droplet size. As reported by other authors (Risch *et al.,* 1988; Minemoto *et al.,* 2002; Soottitantawat *et al.,* 2003; Soottitantawat *et al.,* 2005; Ramakrishnan *et al.,* 2012) a small droplet size distribution increases the OEE. Another important process parameter with a major influence on the OEE is the relationship between the amount of oil and wall material. As shown in Figure 5.8, an increase in the oil:wall ratio reduces the surface oil content, which is expected to increase the oil encapsulation efficiency. The results found in this study confirm previous findings (Calvo *et al.,* 2011; Brian *et al.,* 1998; Bae and Lee, 2008) regarding the increase of OEE when reducing the droplet size of the emulsions and when increasing the oil:wall ratio (Figure 5.9). Premix membrane emulsification produces emulsions with a droplet size distribution of about 1–2 μm when using 10% whey protein or sodium caseinate. When these emulsions are dried, the amount of wall material will have a major influence on the amount of encapsulated oil. The highest oil:wall material relation studied (1:3) is the one with the highest OEE, which was about 70% for 20% fish oil emulsions stabilized with 10% sodium caseinate and dried after adding 50% of maltodextrin.

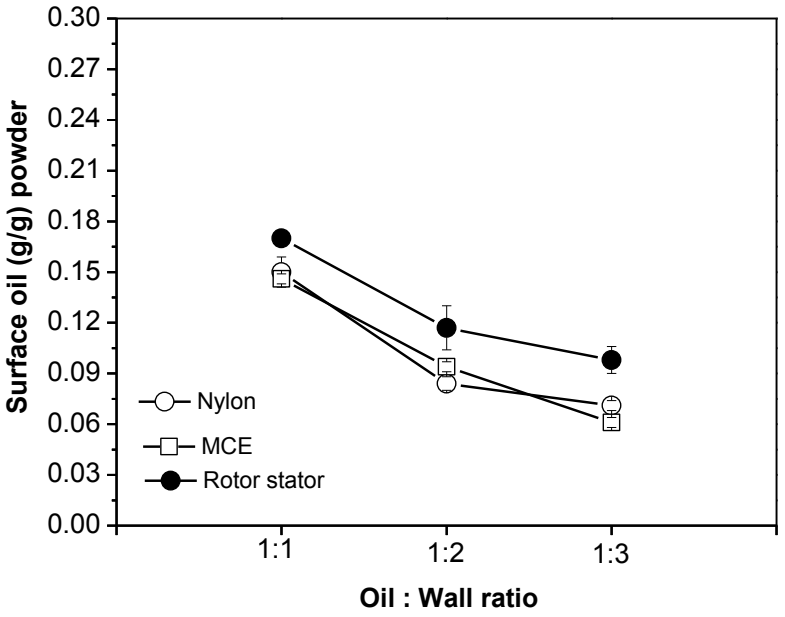

Figure 5.8. Influence of the oil:wall material ratio on the surface oil content of the microcapsules produced from emulsions prepared by premix ME (open symbols) or rotor stator homogenization (solid symbols). The values plotted correspond to 20% fish oil + 10% CAS and + 10% MD for 1:1 ratio; 20% fish oil + 10% CAS + 30% MD for 1:2 ratio and 20% fish oil + 10% CAS + 50% MD for 1:3 ratio.

The results in Figure 5.9 show a high correlation between the increase in the OEE and the increase in the oil:wall material, regardless of the emulsification method used to obtain the O/W emulsion. However, the emulsification method has an impact on the absolute value of the OEE. While the microcapsules obtained after drying mechanically-homogenized emulsions have values from 25% to 55% OEE for oil:wall ratios of 1:1 and 1:3 respectively, the microcapsules obtained from membrane-homogenized emulsions have values in the range of 30-40% for a 1:1 oil:wall ratio and more than 70% for a 1:3 oil:wall ratio. As discussed previously by other authors there is a clear relationship between the droplet size and dispersity of the emulsion and the final content of the surface oil in the microcapsules. The surface oil content affects the oil encapsulation efficiency, decreasing it, and also increases the oxidation stability of the microcapsules, this last item will be discussed below. It has been suggested that small and more monodisperse emulsions will be

more effectively packed during droplet formation in spray-drying (Ramakrishnan *et al.,* 2012) resulting in lower amount of surface oil, or that larger amounts of oil in the surface can be the result of large emulsion droplets breakdown during atomization (Soottitantawat *et al.,* 2003).

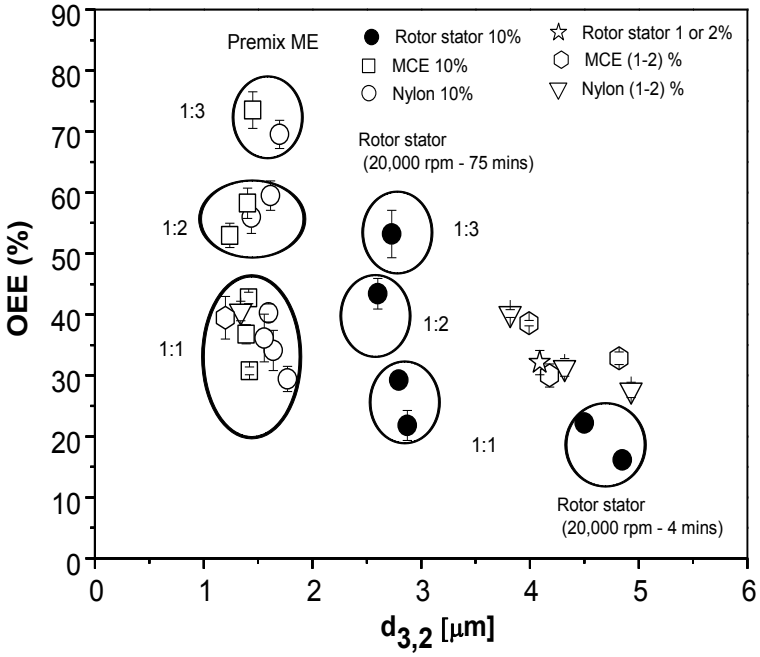

Figure 5.9. Influence of the droplet size of the emulsions ($d_{3,2}$) and the amount of wall material on the oil encapsulation efficiency of the fish oil microcapsules produced by spray drying from premix ME (open symbols) or rotor stator (solid symbols) O/W emulsions stabilized with 1, 2 or 10% emulsifier.

5.3.4 Oxidation stability of the fish oil microcapsules

One of the main reasons for encapsulating fish oil is to protect it against oxidation and mask the fishy odor. A successful encapsulation process should therefore provide capsules that can withstand storage, the processing needed to incorporate them into a food matrix as well as during the processing, preservation and storage of the food. As a first stage in ascertaining the stability of the microcapsules produced combining emulsification (membrane or mechanical) and spray-drying, selected samples of different microcapsules (Table 5.2) with a 1:2 oil:wall ratio (20% oil load) were stored in dark-brown bottles at 30 ±1 ºC for about two months. The selection of the samples allowed to compare the behavior of microcapsules with the same oil and wall material load from different formulations and production technologies. The oil load was selected at 20%, since in a previous study (Ramakrishnan *et al.,* 2012) the oxidation stability of fish oil microcapsules with a 10% oil load was already performed. Samples of the microcapsules were taken at various intervals to analyze its degree oxidation, expressed as the propanal content and analyzed according to section 5.2.4. It is important to consider, as mentioned previously, that the propanal distribution constant between the sample (dissolved microcapsules) and the headspace depends heavily on the material used to produce the microcapsules (wall material); therefore to ensure that the propanal content of each sample was properly quantified, different calibration graphs were constructed using three matrices. The results shown below were obtained using the proper calibration for each microcapsule.

The propanal content evolution of the samples that underwent the oxidation stability test is plotted against storage time in Figure 5.10. As can be seen in this figure, during the first 15 days of storage there is a rapid increase in the oxidation of all samples, regardless of their nature and production methodology, from initial propanal values close to 12 mg propanal/kg (for all the microcapsules analyzed) to 30 to 80 mg propanal/kg, depending on the type and amount of emulsifier used and the emulsification method. For the same microcapsule composition (20% oil load, 10% CAS and 30% MD), the microcapsules produced from an O/W emulsion obtained by premix ME are less oxidized during storage at 30ºC (about 70 mg propanal/kg at the end of the test) than the microcapsules produced from an O/W emulsion obtained by rotor-stator homogenization (about 120 mg propanal/kg at the

end of the test). In addition to the difference in the amount of propanal produced after 55 days of storage, the oxidation curve is also different for these two types of microcapsules. Those from O/W emulsions obtained by premix ME oxidized quickly during the first 15 days of storage, reaching an almost stable plateau and showing almost no subsequent oxidation. However, the microcapsules from O/W emulsions obtained by rotor-stator continuously oxidized during the first four weeks of storage, reaching a plateau at that point, followed by a four-week period with a slight oxidation of the samples.

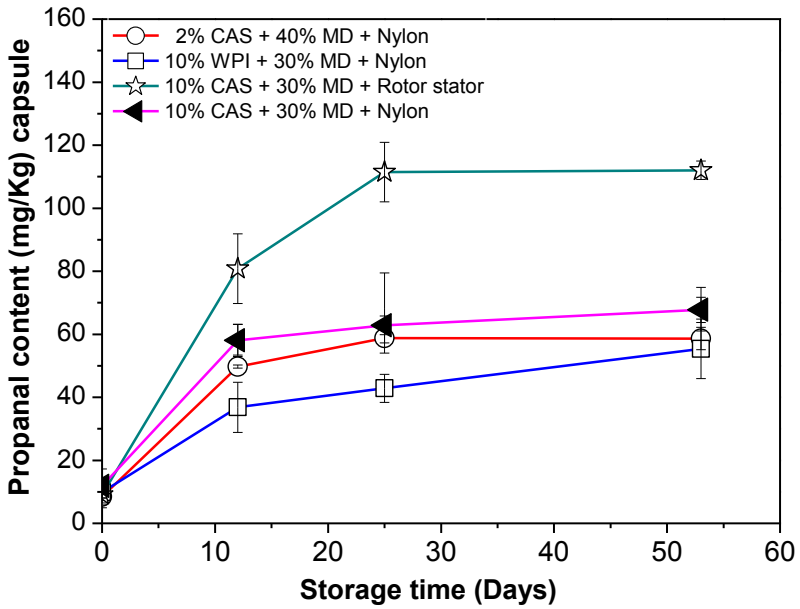

Figure 5.10. Propanal content evolution of fish oil microcapsules versus storage time at 30 ± 1 ºC.

The oxidation of the microcapsules can be correlated with their surface oil content and also to the permeability of the microcapsule wall to oxygen resulting in the oxidation of the encapsulated oil. From the microcapsule samples studied in the oxidation stability tests, those from rotor-stator emulsions have the highest surface oil content (Table 5.2) and they show the highest propanal formation during the test.

However, the three microcapsule samples from premix ME emulsions all have a very similar surface oil content, but exhibit a different oxidation pattern. The two samples build using 10% CAS and maltodextrin show higher oxidation during the first four weeks of storage than the one produced with 10% whey protein and maltodextrin. However, after eight weeks the amount of propanal of all microcapsules from ME was similar. The combination of whey protein and maltodextrin seems to give the highest protection to oxidation for the fish oil microcapsules during storage at 30 ºC. Several authors (Drusch *et al.,* 2007; Dzondo-Gadet *et al.,* 2005; Kagami *et al.,* 2003; Bae and Lee 2008, Ramakrishnan *et al.,* 2013) have found that combining polysaccharides, particularly maltodextrin, and whey protein to encapsulate different types of oils results in high oxidation stability. More recently, Carneiro *et al.,* (2013) have evaluated the performance of selected wall materials in the oxidation stability of encapsulated flaxseed oil, their results indicate that the combination of maltodextrin and whey protein results in the highest protection against oil oxidation, which has been attributed to the combination of the antioxidant activity of the whey protein and also to the molecular weight of the materials conforming the microcapsule wall, with an increase in the oxidation stability when the molecular weight profile of the compounds decreases. Our results agree with these authors, since the oxidation stability of fish oil microcapsules produced combining whey protein and maltodextrin show higher stability throughout the storage time. In our previous study (Ramakirshnan *et al.,* 2013) we found that an increase in the oil:wall ratio increased the oxidation stability of the fish oil microcapsules with an initial 10% oil load. The oxidation stability tests presented in this study correspond to microcapsules with an initial oil load of 20% and an oil:wall ratio of 1:2, therefore we can expect that microcaspules with the highest oil:wall ratio produced (1:3) would exhibit higher oxidation stability.

Table 5.2. Physic-chemical characterization after preparation of fish oil microcapsules used during the oxidation stability test.

Sample Name	***Powder particle size (µm)***	***Moisture content (100g)***	***Surface oil content (g/1g powder)***	***Total oil content (g/1g powder)***	***Average wall thickness (µm)***	***Oil encapsulation efficiency (OEE) (%)***
2% CAS + 40%MD + Nylon	12.89^{b}	$1.56^{a,b}$	0.087^{a}	0.145^{a}	0.54^{a}	40.15^{a}
10% CAS +30% MD+ Nylon	9.12^{a}	1.29^{a}	0.084^{a}	0.210^{b}	0.57^{a}	59.48^{c}
10% WPI + 30% MD+ Nylon	15.51^{c}	1.92^{c}	0.097^{a}	0.203^{b}	0.52^{a}	50.97^{b}
10% CAS+30% MD+Rotor stator	13.31^{b}	1.78^{c}	0.117^{b}	0.206^{b}	0.52^{a}	43.42^{a}

Means within the same column followed by different letters (a, b, c) are significantly ($P < 0.05$) different.

5.4 CONCLUSIONS

The results of the study demonstrated a clear relationship between the formulation and emulsification technology and the oil encapsulation efficiency and the oxidation stability of the fish oil microcapsules during storage. Premix ME can be used to obtain monodisperse O/W emulsions stabilized by proteins, however, the concentration of this type of emulsifier should be higher than the usual concentration of small surfactants, such as Tween 20, in order to ensure the production of small and monodisperse oil droplets. The increase in the amount of emulsifier does not have a negative influence on the process conditions; on the contrary, the protein added as emulsifier will also act as wall material during the drying step. Moreover, the combination of a whey protein and maltodextrin seems to have a protective effect against oxidation. In the two different membranes tested, our results show that they do not influence the droplet size of the emulsion, and therefore do not alter one of the most important quality parameters of the microcapsules - oil encapsulation efficiency. When comparing premix ME and rotor stator homogenization as a first step in the production of fish oil microcapsules, it is clear that the former is a more robust methodology, since it requires less energy input and results in smaller and monodisperse emulsions, which in turn yield OEE values up to 70%. Furthermore, since the higher degree of monodispersity of the O/W premix ME emulsions renders less surface oil in the microcapsules obtained after spray-drying, the oxidation stability of the samples during storage is also increased. It is also important to mention that for propanal analysis is extremely important to consider the effect of the wall material in the retention of this compound, and the proper calibration curves must be obtained for each system. Finally, the increase in the ratio between the oil load and the wall material always results in higher oil encapsulation efficiency, regardless of the emulsification methodology used. The results of this study indicate that under the proper operating conditions, membrane emulsification followed by spray drying is a suitable methodology for producing fish oil microcapsules with OEE higher than 70% and with a reasonable oxidation stability.

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CHAPTER 6

PRODUCTION OF REINFORCED FISH OIL MICROCAPSULES COMBINING PREMIX MEMBRANE EMULSIFICATION AND SPRAY DRYING

UNIVERSITAT ROVIRA I VIRGILI
ENCAPSULATION OF OMEGA-3 FATTY ACIDS BY PREMIX MEMBRANE EMULSIFICATION
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Dipòsit Legal: T. 163-2014

6.1 INTRODUCTION

Microencapsulation has become an important technique in the food industry, because its ability to protect sensitive ingredients and control their targeted release. One of the common ways to obtain oil microcapsules is to produce first an oil-in-water (O/W) emulsion followed by drying. From the different drying techniques, the most frequently used by the food industry is spray drying, which allows obtaining the so-called multinuclear microcapsules. It is possible to find in the literature a myriad of studies regarding the properties of different food-grade wall materials, including starches (ex. maltodextrin) (Carneiro *et al.,* 2013; Anwar *et al.,* 2011; Bae and Lee, 2008) and sugars (lactose, sucrose) (Polavarapu *et al.,* 2011; Drusch, 2007; Drusch *et al.,* 2007; Leroux *et al.,* 2003; Onwulata *et al.,* 1996; Strange *et al.,* 1997; Shiga *et al.,* 2001; Keogh *et al.,* 2001), Arabic gum (Kim and Morr, 1996; McNamee *et al.,*1998; Calvo *et al.,* 2010), mesquite gum (Beristain *et al.,* 2001) and whey protein and sodium caseinate (Chen *et al.,* 2013; Aghbashlo *et al.,* 2012; Bae and Lee 2008; Sheu and Rosenberg, 1995; Hogan *et al.,* 2001).

Regarding the emulsification technique, most of the reported studies use rotor-stator or high-pressure homogenization systems, which require high energy inputs and can damage shear sensitive substances. The combination of a low energy emulsification technique, such as premix membrane emulsification (ME) with spray drying to produce fish oil microcapsules has been recently proven (Ramakrishnan *et al.,* 2013a; Ramakrishnan *et al.,* 2013b).

Food grade microcapsules will be used by the industry in the formulation of traditional and novel foods for several purposes, most of them related to enrichment of the food in flavor or bioactive compounds. To produce these types of microcapsules it is mandatory to use food-grade ingredients and to ensure the properties of the target compounds will be maintained during food manufacture, storage and distribution. Therefore the compounds used to build the microcapsules as well as their physico-chemical properties are of paramount significance. Regarding the wall building materials, as mentioned previously, there is a large number of them that have been studied, being whey proteins often used, alone or combined with a polysaccaride. Whey proteins are a mixture of proteins, often used

as encapsulating materials because of their many desirable characteristics such as solubility, film formation properties, the ability to interact with water and binding properties (Jafari *et al.,* 2008; Gharsallaoui *et al.,* 2007; Hogan *et al.,* 2001a; Rosenberg and Sheu, 1996; Young *et al.,* 1993a).

Whey proteins can be denatured in the form of spherulites or fibrils, depending on the denaturation process conditions. Whey protein fibrils (WPF) are interesting because of its gelling properties, they are formed upon heating proteins at pH values far from the isoelectric point, at low ionic strength, where they are highly charged (Bolder *et al.,* 2010). It has been recently reported the production of polymer microcapsules using layer-by-layer (LbL) electrostatic adsorption of negatively charged high methoxyl pectin and whey protein fibrils (Sagis *et al.,* 2008; Rosier-Miranda, Ph D thesis). Those authors suggest that the presence of the whey protein fibrils in the microcapsule shell reinforces its structure and improves the mechanical properties. In this study, we will investigate the suitability of whey proteins fibrils as encapsulating materials for the production of fish oil microcapsules. However, it has to be considered that the WPF form gels at low concentrations, which will limit their amount when used in the production of microcapsules by spray drying, since the O/W emulsion has to be pumped during spray-drying.

Knowledge about mechanical properties of microcapsules is needed to ensure they possess enough robustness to withstand the manufacturing conditions (Fery *et al.,* 2007; Mercadé-Prieto and Zhang, 2012). The mechanical strength and permeability of the capsules can be controlled by selecting a suitable production technique or by the changing the characteristics of the encapsulating materials. Several methods for characterizing mechanical properties of single microcapsules have been reported in the literature, being micromanipulation and atomic force microscopy (AFM) the most commonly used (Mercadé-Prieto and Zhang, 2012).

The micromanipulation technique was developed by Zhang *et al.,* (1999) and consisted of squeezing a microparticle between two parallel surfaces. Force measurements were obtained at specific points and the compression speed could be varied. Visual observation of the process was provided by an optical microscope

coupled with the micromanipulator. It has been employed to study elastic or plastic behaviour of microcapsules (Sun and Zhang, 2001), to compare mechanical strength of different wall materials (Sun and Zhang, 2002) and to study the mechanical properties of microcapsules produced in tissue engineering and cell encapsulation purposes (Rehor *et al.,* 2001; Zhao and Zhang, 2004). In addition to the above studies, micromanipulation was frequently employed to characterize the mechanical properties of other particles: dextran-hydroy-ethyl-methacrylate, alginate-chitosan microcapsule, hydroxyethyl methacrylated dextran particles (Chung *et al.,* 2005; Wang *et al.,* 2005; Zhao and Zhang 2004; Yap *et al.,* 2008). The mechanical properties of single melamine formaldehyde encapsulates with diameters of 1-12 µm were determined using this method. The main limitation of this technique is related to its inability to perform measurements of particles in the sub-micron range.

Atomic force microscopy (AFM) has been applied in this study to measure the mechanical properties of fibrils based microcapsules. The microcapsules were placed inside the AFM equipped with a conic tip, which allows to obtain a topographic image of the sample from the scan obtained on tapping mode. Besides the topographic image of the sample, AFM allows to measure force-displacement data that can be used to calculate mechanical properties of the sample. More specifically, in this study the Young modulus, which is the ratio of stress and strain within the elastic region of the stress and strain curve, is obtained applying the Hertz's, one of the most often applied to study the behavior of elastic particles (Liu, Ph D Thesis, 2010).

The aim of this study was to use whey protein fibrils in the formulation of the microcapsule wall to produce reinforced fish oil microcapsules by combining premix membrane emulsification with spray drying. As far as the authors knowledge there are no previous applications of whey protein fibrils in encapsulation by spray drying. Since the main goal of combining whey protein fibrils with a polysaccharide is to modify the mechanical strength of the wall, we have developed and applied a methodology based on AFM to measure the Young moduli of the microcapsules and study the effect of the presence of protein fibrils in the mechanical properties. The effect of the WPF on the physico-chemical properties and morphology of the microcapsules has also been studied.

6.2 MATERIALS AND METHODS

6.2.1 Fish oil, emulsifiers and wall materials

In this study, menhaden fish oil (Sigma - Aldrich, CAS-No.8002-50-4, Spain) was used as the core material for preparing O/W emulsions with 20% oil load. The emulsions were stabilized with Tween 20 (polyoxyethylene sorbitan monolaurate, from Sigma - Aldrich, CAS-No. 9005-64-5, Spain) dissolved in acetate buffer (pH=3.1) as the continuous phase. Various combinations of maltodextrin (Sigma Aldrich- Spain, dextrose equivalent 16.5-19.5, CAS-No. 9050-36-6), and whey protein fibrils obtained from whey protein isolate, WPI, (ref no. LE 003-0-919, Bipro from Davis Co. Food, Inc., Switzerland) were used to build the microcapsules wall.

Analytical grade hexane and petroleum ether were purchased from Sigma-Aldrich. Acetate buffer (pH=3.1) was used for the preparation of all solutions. All general chemicals used in this study were of analytical grade. The formulation of the emulsions prepared in this study is shown in Table 6.1.

6.2.2. Preparation of the O\W emulsions by premix ME

The first step was to prepare an acetate buffer. It was necessary to work at an acid pH in order to avoid aggregation of the WPF. The buffer (pH = 3.1) contained 0.1 M of acetic acid and 0.1 M of sodium acetate. Tween-20 was then added to the buffer and stirred at 300 rpm for 15 minutes. After the continuous phase was prepared, fish oil was added. A coarse emulsion was prepared by using a rotor stator (15500 rpm, 2 minutes), which was subsequently passed through a microfiltration membrane to reduce the droplet size and its distribution. The membrane employed during ME was a 47 mm nylon disc membrane of 0.8 µm mean pore size (ref. 7408-004, Whatman). The experimental set up and O/W emulsion production method have been fully explained in previous chapters (See chapter 4 – section 4.2.2 and Chapter 5 – section 5.2.2.1)

Whey protein fibrils were prepared adding WPI to a 6M HCl solution adjusted to pH 2, and heated at 80 ºC under continuous stirring at 600 rpm for 10 hours. The stirring velocity was adjusted to obtain the proteins fibrils, since under lower shear stress the proteins denature in form of spherulites. The final solution was filtered

through a protein filter (FD 30/0.45 µm Ca-S from Schleicher and Schuell) to remove any traces of undissolved protein. The WPF solution was stored in closed containers at 4 ºC. A transmission electron microscope (TEM, JEOL model 1011) was used to obtain images of the whey protein fibrils and ensure they were properly produced.

6.2.3. Production of the Fish Oil Microcapsules

To produce the microcapsules, three different oil/wall material ratios were used, 1:1, 1:2 and 1:3. In each case, 100 mL of whey protein fibril solution containing 4 g of WPF was added to 100 mL of O/W emulsion. Then maltodextrin was added to reach the appropriate oil/wall ratio (Table 6.1). The solution was then stirred at 300 rpm for 4-6 h until maltodextrin was completely dissolved.

To obtain the microcapsules, the emulsions was spray-dried using a laboratory-scale spray dryer (Buchi mini spray dryer b290, Switzerland). The operation conditions in the spray dryer were air inlet and outlet temperatures of 190 and 90 ºC, respectively, and an emulsion flow rate of 400 mL/h. The dried powder was collected and stored in opaque, air-tight containers at 4 ºC for further analysis.

Table 6.1. Formulation of O/W emulsions and emulsifying conditions employed in the production of reinforce fish oil microcapsules.

No	*Continuous phase*	*Disperse phase*	*Emulsifier*	*Wall materials*		*Oil/wall ratio*
	Acetate Buffer (mL)	*Fish oil (mL)*	*Tween – 20 (g)*	*Maltodextrin (g)*	*Whey Protein Fibrils (g)*	
1	60	40	1.8	36	4	1:1
2				76	4	1:2
3				116	4	1:3

6.2.4. Emulsion and microcapsule size characterization

In previous chapters (see Chapter 4 – section 4.2.4 and Chapter 5 – section 5.2.3.1), it is fully explained how to measure the size of the emulsion, the span value and the microcapsule size by laser diffraction using the Malvern Mastersizer Instrument (Malvern Instruments, Ltd., Worcestershire, UK). The droplet size and distribution is expressed as $d_{3,2}$ (Sauter mean diameter) and span values calculated by equation 6.1.

$$span = (d_{90} - d_{10})/d_{50} \quad [6.1]$$

where d_{90}, d_{50} and d_{10} are the mean diameters at which 90, 50, and 10% (vol %) of the particles counted and calculated. The particle size ($d_{3,2}$) of spray dried powders was determined by laser diffraction using the Malvern Mastersizer 2000 equipped with a Hydro 2000 SM dispersion unit. All measurements were done in triplicate for each sample and the average value was reported.

6.2.5. Total Oil, Surface oil and Oil Encapsulation Efficiency

The total oil content in microcapsule was analysed using a procedure based on the Rose-Gottlieb method (Richardson, 1985) by ether extraction method. The procedure of total oil encapsulation efficiency method clearly explained in previous chapter (see Chapter 4 – section 4.2.6 and Chapter 5 – section 5.2.3.2). The surface oil content in microcapsules was determined using a modified method described by Varavinit *et al.,* (2001) by hexane extractions. The detailed procedure of surface oil content explained in previous chapter (see Chapter 4 and 5). The oil encapsulation efficiency (OEE) was calculated using Equation 6.2.

$$OEE = (\text{Total oil} - \text{Surface oil})/\text{Total oil} \times 100. \quad [6.2]$$

6.2.6. Microcapsule surface and inner morphology analysis

To study the outer morphology of the microcapsules, an environmental scanning electron microscope (FEI, Quanta 600) was used. The particles were mounted on cylindrical stubs coated with a conductive carbon tape. The digital images were taken at an accelerating voltage of 20 kV and magnification of x2900.

To study the inner structure of the microcapsules, a scanning electron microscope at 15 kV was used (JEOL JSM-6400). The particles were mounted on SEM stubs using double-sided sticky tape and then coated with gold (EMITECH K575XD Turbo Sputter Coater).

6.2.7. Methodology to calculate the Young modulus of fish oil microcapsules

To study the mechanical properties of the microcapsules, with and without WPF, the Young modulus was calculated. Young modulus indicates the stiffness off the wall material and is the most commonly used elastic modulus. To calculate it, an Atomic Force Microscope (AFM) (Agilent 5500 SPM/ AFM System) was used after developing the appropriate methodology.

The methodology developed to calculate the Young modulus is as follows:

1. Preparation of microcapsules for AFM analysis: surface oil was removed following the same experimental extraction procedure employed for the surface oil analysis. Two grams of microcapsules were treated with 20 mL of hexane, filtered and dried. Then, the dried microcapsules were fixed on to a cover glass using 10 mL of a 0.01% solution of polyethilenimine 750,000 (Sigma-Aldrich 18,197-8) (Figure 6.1a). After letting the sample dry, the cover glass was fixed to an AFM metallic disc (Pelco® - 16219, 20 mm) using double-sided tape (Figure 6.1b).

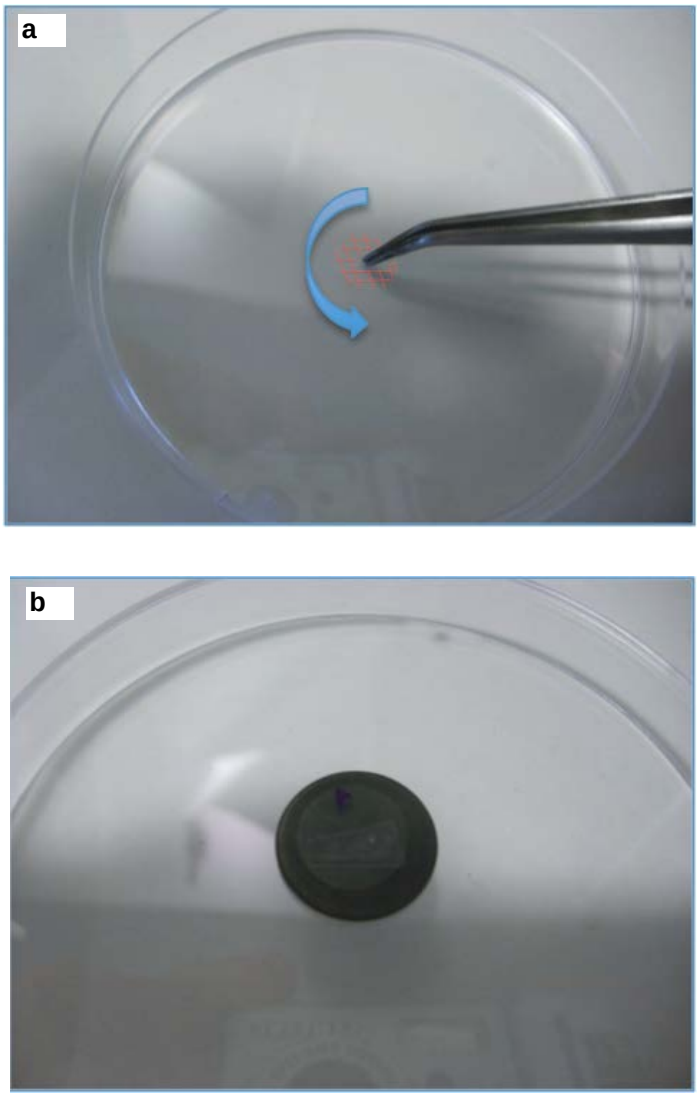

Figure 6.1. Image of the fixation of degreased microcapsules in the cover glass (a) and fixation of the cover glass in the AFM metallic disc (b).

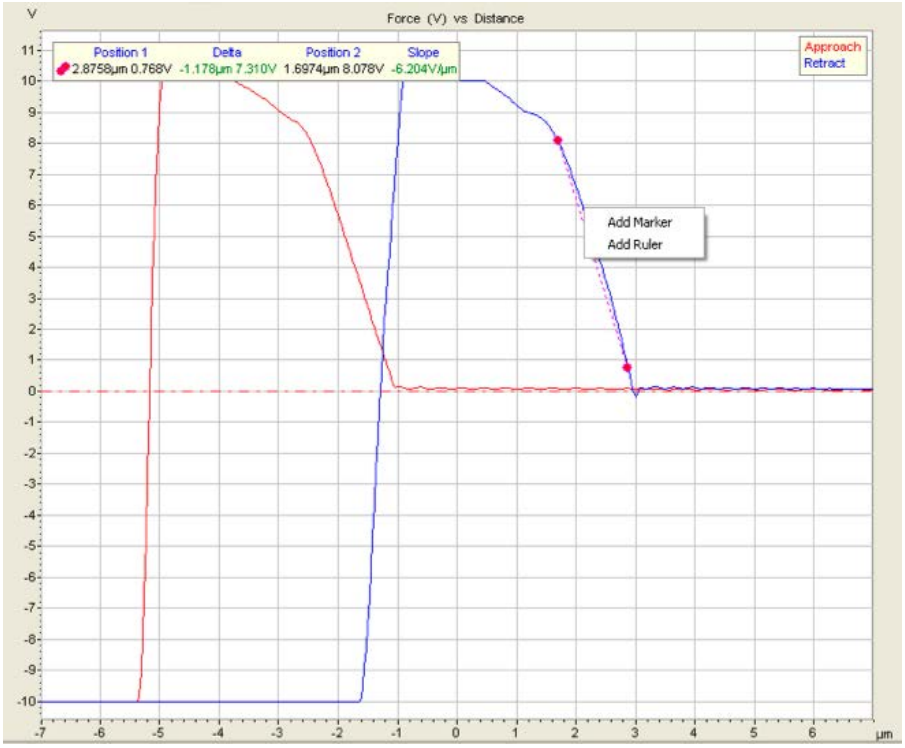

Figure 6.2. Typical attack and retract force curves obtained by AFM on the reinforced fish oil microcapsules.

2. AFM preparation and analysis: the AFM was equipped with the appropriate conic tip and the characteristic frequency of the tip was calculated, then the dry sample was placed inside the AFM and an area of (50x50) μm was selected and scanned using tapping mode. If the area contained microcapsules, it was possible to continue with the analysis, if not a new area had to be selected and scanned until the microcapsules were detected. Once the capsules are detected, a single microcapsule has to be selected to obtain the force curve. For each sample a total of fifty microcapsules were analysed, allowing to calculate a mean value of the force constant. In Figure 6.2 a typical representation of the attack and retract force curves obtained during AFM analysis is presented. The slope of the retract curve is used to calculate the force constant.

3. Calculation of the Young modulus: to calculate the Young modulus, Hertz's equation for conic tips was used, as shown on Eq. 6.3.

$$F = \frac{E \cdot R \cdot \delta}{1 - \nu^2} \qquad [6.3]$$

where *F* is the force constant (N/m) measured in the AFM, *E* is the Young modulus (Pa), *R* is the tip radius, ν is Poisson's ratio and δ is the tip penetration depth.

6.3 RESULTS AND DISCUSSION

6.3.1 Production of protein fibrils

As mentioned previously in this chapter, protein fibrils can be used as encapsulating material since they have been reported to be effective stabilizers at air-water and oil-water interfaces and to be able to form films at the interfaces with a high interfacial modulus (Isa *et al.,* 2010; Jung *et al.,* 2010; Jung *et al.,* 2011)

Whey protein fibrils were prepared from a 4% WPI solution heated at 80ºC under continuous stirring at 600 rpm for 10 hours. The stirring speed is extremely important since, as can be seen in Figure 6.3a, if the speed velocity is lower (300 rpm) the denaturation process leads to the production of whey protein spherulites, while when the stirring velocity increased to 600 rpm, whey protein was denatured in the form of fibrils (Figure 6.3b) that are of few hundred nanometers long.

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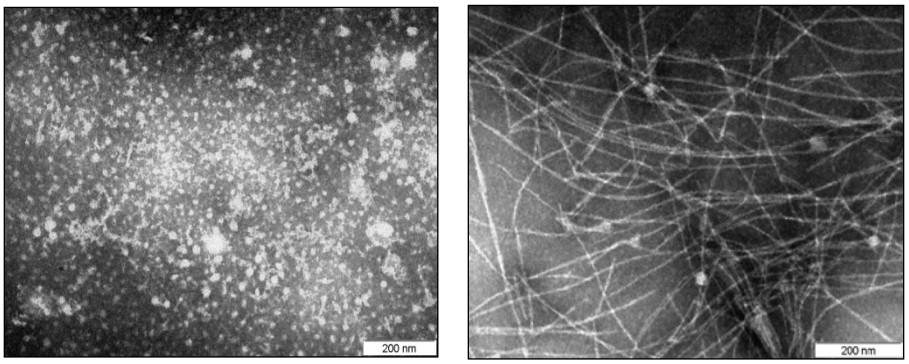

Figure 6.3. TEM images of denatured whey protein pH 2. (a) Samples heated for 10 hours at 80 ºC and 300 rpm continuous stirring. (b) Samples heated for 10 hours at 80 ºC and 600 rpm continuous stirring.

6.3.2. Production of emulsions and microcapsules

In this study, a nylon membrane with a pore size distribution of 0.8 µm was employed to prepare the fish O/W emulsions by premix membrane emulsification stabilized with 2% Tween 20. Initially, a coarse emulsion was prepared by rotor stator stirring at 15,500 rpm for 2 minutes. The droplet size of this emulsion was 8.5 µm with a span value close to 1.8. Then the coarse emulsion was passed three times through a nylon membrane using 700 kPa of nitrogen pressure. After the first emulsification cycle (cycle 1, Figure 6.4a) the droplet size of the emulsion has been reduced down close to 2 µm, and the emulsion was more monodisperse than the coarse one, since the span value was reduced to a value close to 1.4 (Figure 6.4b). The reduction in the droplet size of the emulsion continues during cycles 2 and 3, reaching a final value of 1.1 µm, with a span value close to 1 after the third cycle (Figure 6.4).

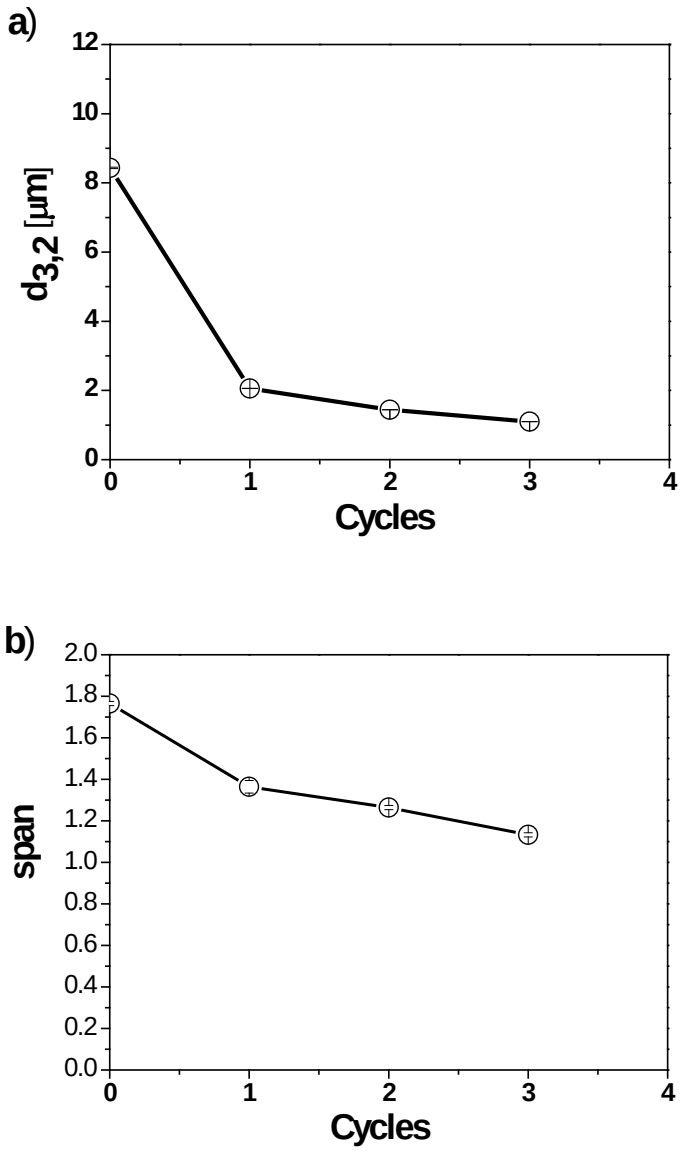

Figure 6.4 Oil droplet size as $d_{3,2}$ (a) and span value (b) evolution with the number of cycles during the production of O/W emulsions stabilized with Tween-20 by premix ME with nylon membrane. Cycle 0 corresponds to the value after the rotor-stator homogenisation. **a.** $d_{3,2}$ minimum error, 0.02; maximum error, 0.23. **b** Span value minimum error, 0.03; maximum error, 0.18.

Figure 6.5 shows the flux evolution versus the number of cycles during premix ME with the Nylon membrane during the production of an O/W emulsion stabilized with Tween 20. It can be clearly seen from Figure 6.5 that the flux value increases from cycle 1 to cycle 3 almost in a two-fold. If we compare the flux evolution obtained when proteins are used to stabilize the emulsions, it is clear that here we have the opposite trend, that is the flux increases with the number of cycles. This behavior has already been reported in Chapter 4, and it is attributed to the effect of membrane fouling. However, it should be noted that the emulsion prepared in Chapter 4 had a 10% oil load and was stabilized with 2% Tween 20, while in this study the emulsions have a 40% oil load and they wer stabilized with the same amount and type of emulsifier. The increase in the oil load resulted in a decrease of the flux during emulsification, from 29 kg m^{-2} s^{-1} to 5 kg m^{-2} s^{-1} for 10% and 40 % oil load, respectively. This reduction of the flux due to the increase in the oil load is a clear indication that oil contributes to membrane fouling in membrane emulsification processes, however as it has been found by other authors (Gelaw, Ph D Thesis, 2013) it seems that oil fouling is more restricted to the membrane surface, while protein fouling would probably be more severe in the internal structure of the membrane, where the proteins would reduce the size of the pores and/or block them.

At the end of the emulsification step, the appropriate amount of wall material, maltodextrin and whey protein fibrils (Table 6.1) was added and droplet size measured. As can be seen in Table 6.2 the droplet size of the emulsion after adding different amounts of maltodextrin and WPF increased. A similar increase in the droplet size of the emulsion after wall material addition has already been found during the production of fish oil microcapsules using maltodextrin, whey protein, sodium caseinate or combinations of the polysaccharide with one of the proteins. Since the only difference between the three systems presented in table 6.2 was the amount of maltodextrin, it seems that an increase in the amount of this polysaccharide results in a slight increase in the droplet size of the emulsion.

It should be mentioned that there is a difference in the formulation of the emulsions between the presented in this chapter and the ones presented in Chapters 4 and 5, that is the pH value. While all the emulsions presented in Chapters 4 and 5 were prepared close to neutral pH, in this case the emulsions were prepared at pH = 3, to

maintain the pH close to the one employed for whey protein fibrils production. As mentioned in the introduction of the chapter, WPF production requires moderate temperatures (about 80 ºC), proper shear and acidic conditions. If we want to increase the pH to neutral we pass through the isoelectric point (IP) of the whey proteins (4.5-5.5, depending on the composition). When that happens there is and aggregation of the proteins and it is not clear that a further increase in the pH will return the WPF to its original condition. Therefore we decided to adapt the pH of the emulsion to the one that ensured WPF would be kept in its original state.

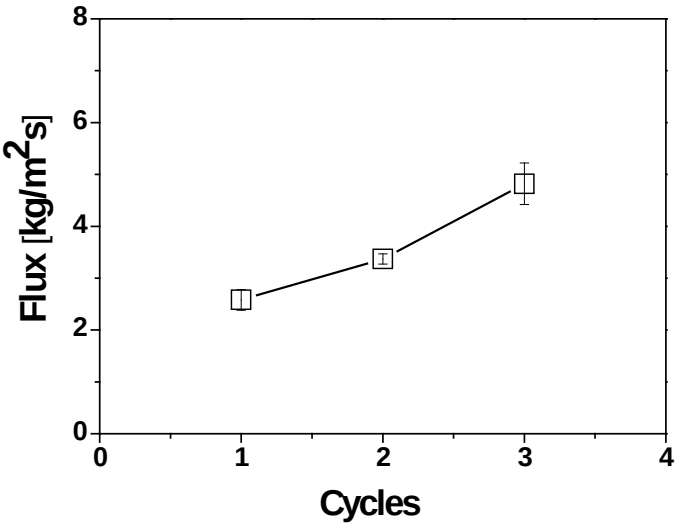

Figure 6.5. Flux evolution with the number of cycles during the production of O/W emulsions stabilized with Tween 20 by premix ME Nylon membrane. Flux minimum error: 0.2 kg m^{-2} s^{-1}; maximum error: 0.5 kg m^{-2} s^{-1}.

To further study the influence of the emulsification and the wall material addition on the droplet size distribution the plot of the particle size distribution obtained by laser diffraction can be useful. Figure 6.6 plots the distribution of the emulsion from cycle 1 (red) to cycle 3 (blue) and it is clear that there is a reduction on the average size of the droplets as well as in its distribution, since the curve presents only one peak which narrows with the emulsification cycle. After the addition of the wall material (Figure 6.6, black) it can be seen that the curve still has only one peak, but the average size has increased and the curve is broader. At this point it should be

mentioned that to add the wall material, some minor stirring has to be applied, this plus the presence of the polysaccharides can cause this shift in the droplet size distribution.

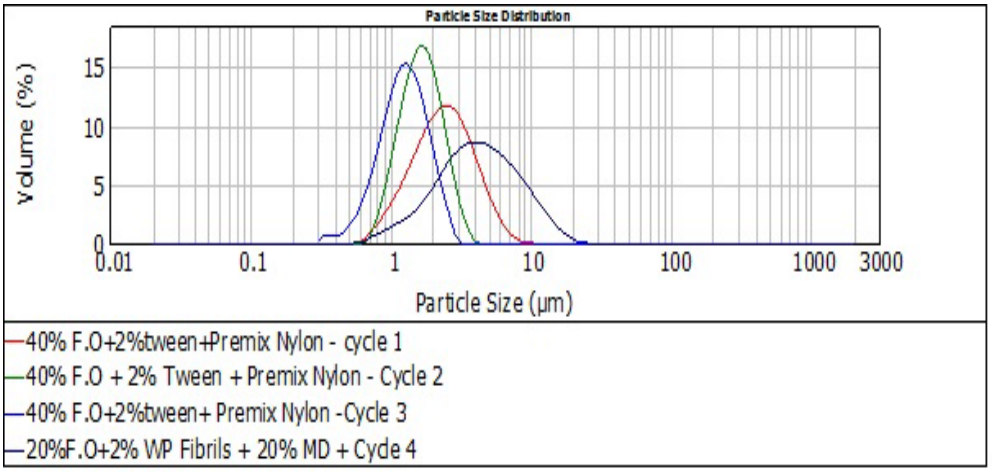

Figure 6.6. Particle size distribution of emulsion prepared by nylon membrane (cycle 1 to 3 – premix cycle, cycle 4 – addition of wall material into emulsion).

Figures 6.7 and 6.8 show the images of the emulsions prepared using 1:2 oil-to-wall material ratio before and after adding the wall material, respectively. The images were obtained using and optical microscope Leica DM2500-N.

From figure 6.7, it is possible to observe that before adding the wall material, the emulsion droplets were round in shape and appeared isolated. Figure 6.8, taken after addition of the wall materials (maltodextrin and protein fibrils), shows droplet aggregation that might be attributed to an enhancement of aggregation by the presence of the wall materials.

After the addition of the wall materials into the O/W emulsion, all samples were dried under the same spray drying conditions as mentioned in Chapters 4 and 5: air inlet and outlet temperature of 190 and 90 ºC, respectively, and an emulsion flow rate of 400 mL/h. All microcapsules samples were stored under nitrogen atmosphere in dark closed containers at 4ºC until characterization.

Table 6.2. Properties of the fish oil microcapsules prepared with whey protein fibrils.

Oil/wall ratio	*O/W Emulsion*			*Microcapsule Data*				
	Initial Droplet size (µm) Premix -3rd Cycle	*Span*	*Final droplet size (µm)– After addition of WPF and MD*	*Powder particle size (µm)*	*Span*	*Surface oil (g/1g powder)*	*(OEE) (%)*	*Young Modulus (GPa)*
1:1	1.08±0.02	1.08±0.05	2.86±0.2	22.52±1.32	2.97±0.12	0.245±0.014	29.90±7.61	0.599±0.129
1:2			3.01±0.11	17.36±1.72	3.13±0.08	0.144±0.077	38.98±12.4	0.665±0.073
1:3			3.49±0.2	13.44±0.98	3.319±0.14	0.064±0.004	49.80±5.27	0.968±0.014

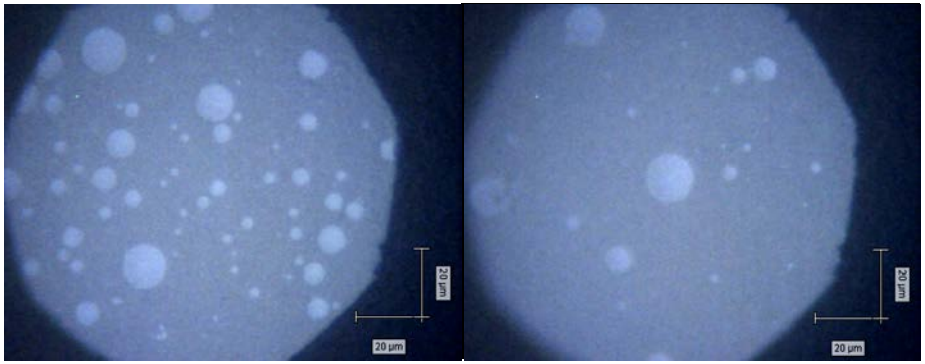

Figure 6.7. Images of O/W emulsions obtained with an optical microscope before adding a 1:2 oil-to-wall material ratio.

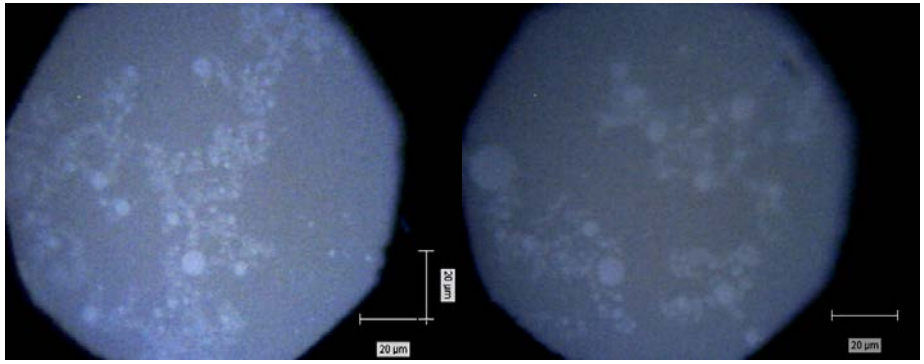

Figure 6.8. Images of O/W emulsions obtained with an optical microscope after adding a 1:2 oil-to-wall material ratio.

6.3.3. Microcapsule size and Oil encapsulation efficiency

Once the emulsion has been spray dried, there are two important parameters to study: (1) powder particle size and (2) OEE. Table 6.2 shows the values of the main physical and chemical parameters used to characterize the fish oil microcapsules obtained after drying the O/W emulsions. Concerning the morphology, it was studied using electronic micrographs and will be discussed later. Figure 6.9 shows the relationship between microcapsule size and oil/wall ratio. For a 1:1 oil/wall material ratio, the microcapsule have a particle size of about 22 μm, while increasing to 1:2 and 1:3 the oil/ratio the powder size decreases to 17 and 13 μm, respectively, and are more polydisperse since they have slightly higher span values (Table 6.2). There are several parameters that influence droplet formation during atomization, such as viscosity, density and surface tension. The particle size is influenced by the emulsion viscosity and the solids content, besides the operation conditions of the spray-drier. In our study all the operational parameters were maintained constant except for the amount of wall material and the percentage of protein fibrils in the wall material. Microcapsules produced using a 1:1 oil:wall ratio had the highest percentage of protein fibrils, while the ones produced using a 1:3 oil:wall ratio had the lowest WPF percentage. The amount of WPF can affect the viscosity of the feed solution pumped to the spray-drier, which might result in a slower droplet formation during atomization. The slower droplet formation obtained with higher viscosities is associated with bigger droplets which can even lead to irregularities in the final shape of the capsules. In our case, there were not significant shape irregularities in the microcapsules produced, but we can attribute the differences in the final size of the particles to the amount of WPF present in the feed solution.

Figure 6.10 shows the relationship between the oil encapsulation efficiency and the oil/wall ratio. The microcapsules produced with a 1:1 oil-wall ratio had the biggest powder size and lowest OEE, while the microcapsules produced with highest oil to wall ratio (1:3) were the ones with the highest value for the OEE, close to 50%. This trend agrees with previous findings that attribute a higher retention of flavors and oils to an increase of the wall material. Regarding the relationship between the particle size and the oil retention, this is still not clear with some authors finding a relationship between bigger sizes and higher retention of flavors and oils, while

others have found an optimum intermediate size for the retention (Jafari *et al.,* 2008). Even though the bigger microcapsules in our case are the ones showing the lower OEE, we must point out that they are the ones with the lower amount of wall material.

It is possible to compare the OEE values obtained with and without protein fibrils in the wall material. In Chapter 4, microcapsules with a 10% oil load were prepared from emulsions stabilized with 2% Tween 20 and obtained by premix ME with a Nylon membrane and using maltodextrin as a wall building material in a ratio of 1:3 (oil:wall material). The OEE for those microcapsules was close to 40.5% (see Table 4.2), while the values of the OEE for reinforced fish oil microcapsules, for the same oil:wall ratio but with a 20% oil load, are higher, about 50% (Figure 6.10). It is not possible to compare the OEE of microcapsules from 20% oil load emulsions stabilized with Tween 20, since this type of microcapsules have not been prepared during this study. However, the results presented in Chapter 5 for microcapsules produced from 20% oil load emulsions stabilized by whey protein or sodium caseinate present different values of OEE depending on the composition of the wall material. Table 5.2 shows that for a constant 1:2 oil:wall ratio the OEE varied from 40 to almost 60% depending on the type and amount of emulsifier (which affects the droplet size and distribution) and also the composition of the wall material. The preliminary values obtained when adding protein fibrils in the wall material seems to increase the OEE, however we must consider the limitation of WPF regarding the gel forming properties when the concentration is increased over 4%. The microcapsules prepared in this study had a 2% WPF content, therefore it could still be some improvement in the OEE with a slight increase in the whey protein fibrils concentration. However, it has also to be taken into account that he amount of WPF can affect the viscosity of the feed solution pumped to the spray-drier, which might result in a slower droplet formation during atomization. The slower droplet formation is associated with bigger droplets which can even lead to irregularities in the final shape of the capsules.

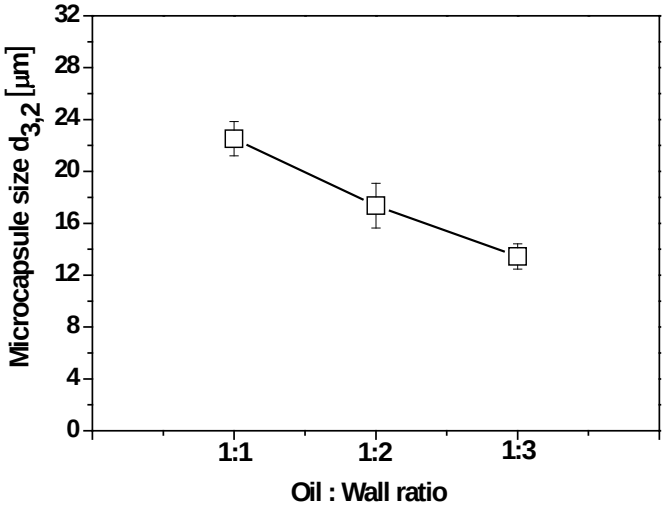

Figure 6.9. Microcapsules size ($d_{3,2}$) versus oil/wall material ratio for microcapsules prepared drying 20% fish oil emulsions. The wall material was composed of 4% whey protein fibrils and the required amount of maltodextrin to reach the appropriate oil-to-wall ratio. $d_{3,2}$ minimum error, 0.98; maximum error, 1.72.

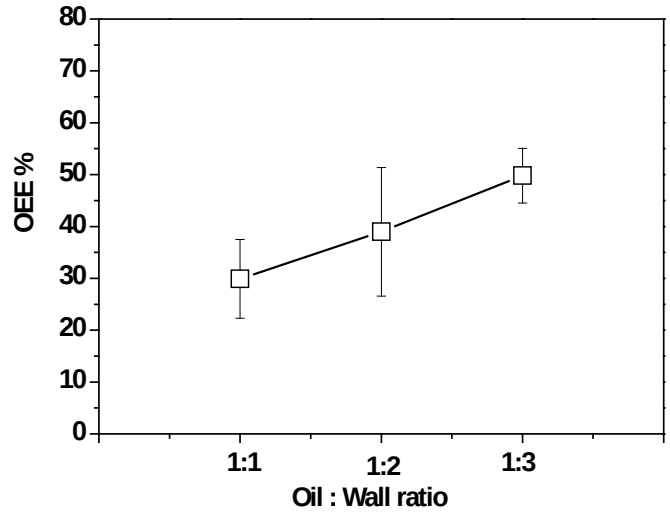

Figure 6.10. OEE versus oil/wall material ratio of microcapsules prepared drying 20% fish oil emulsions. The wall material was composed of 4% whey protein fibrils and the required amount of maltodextrin to reach the appropriate oil-to-wall ratio. OEE minimum error, 5.27; maximum error 12.49.

6.3.4. Morphological Characterization of Reinforced fish oil microcapsules

The surface morphology of the microcapsule was observed by ESEM. It can be seen that the microcapsules prepared by the three oil-wall ratio show a similar external morphology and more irregular in shape for the emulsions prepared with 1:1 and 1:2 ratios (Figure, 6.11 a - b), but look more spherical with the 1:3 ratio (Figure 6.11 c). In all cases, there is wide size distribution and agglomerates can be clearly seen.

Regarding inner morphology of microcapsules, the capsules produced with 1:1 oil/wall ratio presented a thin wall and some vacuoles can be observed (Figure, 6.11 d). Increasing oil/wall ratio from 1:1 to 1:2 and 1:3, the capsules presented a thicker wall and less vacuoles can be observed (Figure, 6.11 e - f). Our results show that, increasing oil/wall material resulted in thicker capsule and smoother surface and less vacuoles inside the microcapsules. From the morphological point of view, there are no differences between the capsules prepared with (Figure 6.11) and without (Figures 4.7-4.9) whey protein fibrils.

6.3.5 Analysis of the Young Modulus

The mechanical properties of the microcapsules produced in the present study will be determined by the wall material type, composition and amount. We have produced fish oil microcapsules using maltodextrin and WPF as wall building materials. The final amount of maltodextrin was increased to attain three different oil/wall ratios of 1:1, 1:2 and 1:3, while WPF amount added was maintained constant (Table 6.1), meaning that the concentrating of WPF decreased as the amount of maltodextrin increases. As mentioned earlier in this chapter, WPF will readily produced gels when the concentration increases above 4%, therefore it was considered to select half of this value as starting conditions for the experiments. The mechanical properties of the microcapsules were assessed using the elastic Young modulus, calculated following the methodology presented in section 6.2.7.

As a first result of this study we proved that it was possible to adapt the existing nano-indentation procedures based on AFM force measurements to obtain the Young modulus of fish oil microcapsules produced by spray drying. The values of the Young modulus for the fish oil microcapsules containing WPF in their wall,

calculated using equation (6.3) are presented in Figure 6.12. Each value has been calculated using the average force constant from fifty measurements and it shows values between 0.6 GPa for the capsules with less amount of wall material to approximately 1 GPa for the capsules with the highest amount of wall material. A similar trend has been reported for melamine-formaldehyde microcapsules with thicker shell presenting the highest Young modules values, even though in that case the values were between 1.5 and 2.5 GPa, depending on the operational production conditions (Su *et al.,* 2012).

In order to compare the influence of the WPF in the mechanical properties of the microcapsule wall, the Young modulus was also measured for fish oil microcapsules without WPF, but with other wall formulations. The values show the same trend (Figure 6.13), and increase in the Young modulus when the amount of wall material increases, but there is no significant difference of the values obtained with or without WPF. Contrary to what has been reported previously by other authors (Rosier-Miranda Ph D thesis, 2010), in this case the protein fibrils do not add an extra reinforcement to the wall structure. However, they have also found and increase in the Young modulus as the thickness of the shell increase, as in our case.

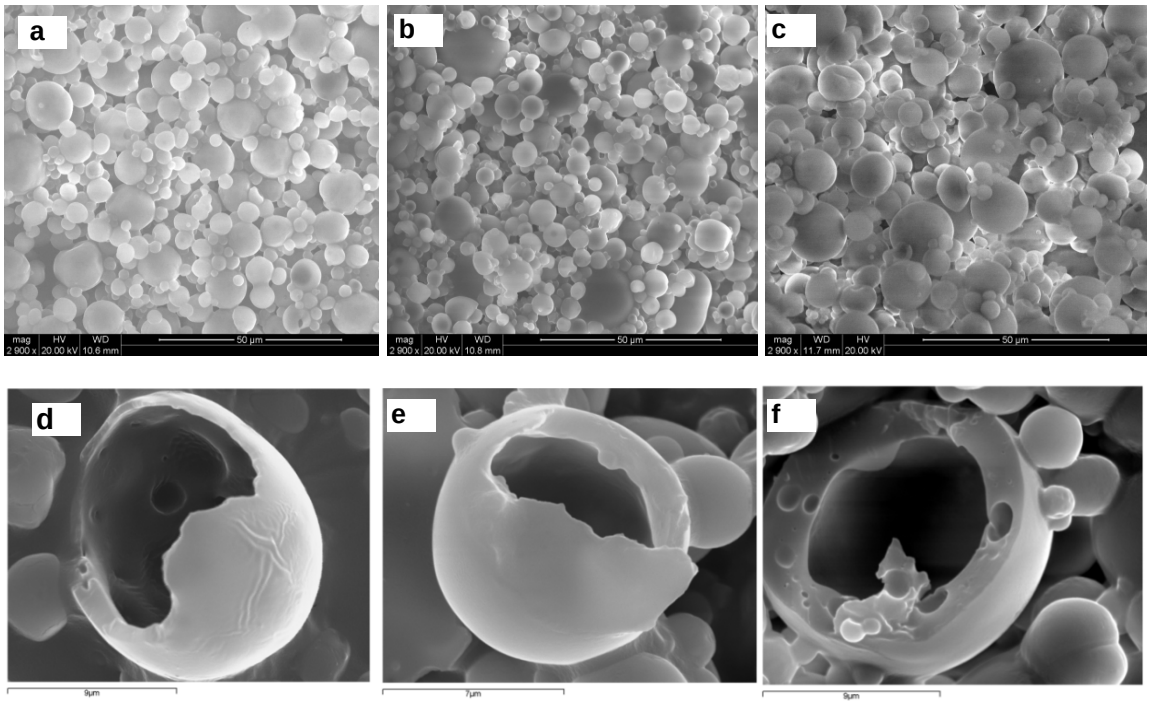

Figure 6.11. Images of the external and internal (SEM) morphologies of the fish oil microcapsules produced with a,d) 1:1 oil/wall b,e) 1:2 oil/wall c,f) 1:3 oil/wall ratio. Scale bars indicated in each image.

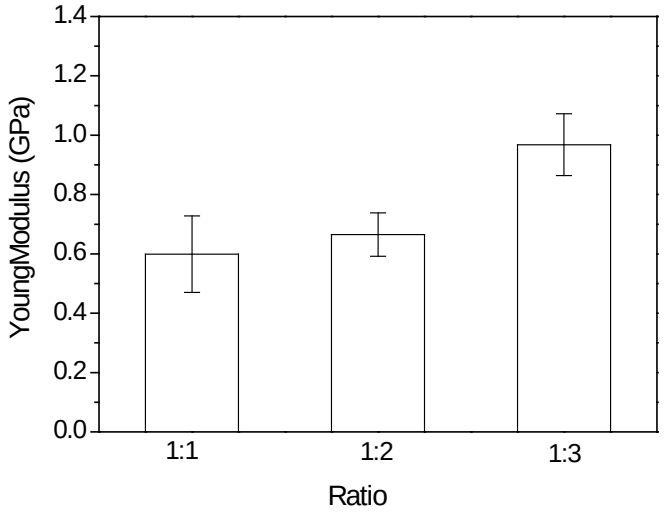

Figure 6.12. Young modulus of fish oil microcapsules with WPF as a part of the wall building material. The capsules were obtained by spray-drying using three oil-to-wall ratios 1:1, 1:2 and 1:3.

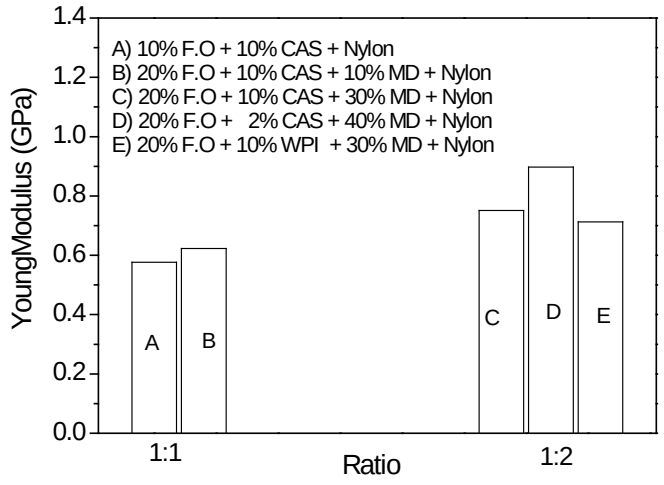

Figure 6.13. Young modulus of fish oil microcapsules without presence of whey protein fibrils as a part of the wall building material. The capsules were obtained by spray-drying with following formulations A) 10% Fish oil + 10% CAS +Nylon; B) 20% Fish oil + 10% CAS + 10% MD + Nylon ; C) 20% F.O + 10% CAS + 30% MD + Nylon ; D) 20% Fish oil + 10% WPI + 30% MD + Nylon.

6.4 CONCLUSIONS

This study investigates the effects of addition of whey protein fibrils (WPF) to the wall material on the microcapsule size, OEE and mechanical properties. The results indicate an slight increase of the emulsions droplet size distribution when adding the wall building material composed of maltodextrin and WPF. The values of oil encapsulation efficiency, though, are slightly higher than the one obtained the same process conditions, except for the oil load. With the addition of WPF it is still true that the oil encapsulation efficiency is directly related to amount of wall material employed reaching almost 50% when the highest amount of wall material was employed. The inner and outer morphologies of the microcapsules also vary depending on the oil-to-wall material ratio. A higher ratio produces rounder, smoother and more rigid capsules. A nano-indentation methodology based on the use of AFM has been successfully adapted and employed to measure mechanical properties of microcapsules. The methodology has allowed to learn that the Young modulus of the capsules increases as more wall material is added, even though it has not been possible to find significant differences between capsules prepared with or without WPF.

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CHAPTER 7

GENERAL CONCLUSIONS AND FUTURE WORK

UNIVERSITAT ROVIRA I VIRGILI
ENCAPSULATION OF OMEGA-3 FATTY ACIDS BY PREMIX MEMBRANE EMULSIFICATION
Sarathiraja Ramakrishnan
Dipòsit Legal: T. 163-2014

7. GENERAL CONCLUSIONS AND FUTURE WORK

In accordance with the general and specific objectives of the present study, the most significant results are that (1) premix membrane emulsification, a low energy input technique, combined with spray drying can be used to produce food grade fish oil microcapsules; (2) milk derived proteins (whey protein and sodium caseinate) can be used to stabilize fish oil emulsions produced by membrane emulsification; (3) milk derived proteins cause membrane fouling during premix membrane emulsification, reducing the permeate flux, however when combined with maltodextrin to build up the microcapsule wall, they increase the protection against oil oxidation; (4) oil encapsulation efficiency (OEE) depends on the initial droplet size of the emulsion and the amount of wall material and (5) protein fibrils can be used mixed with maltodextrin to build the microcapsule wall, with a slight increase in the oil encapsulation efficiency, but with no significant improvement of the mechanical properties at the concentration employed in this work.

The general conclusions of this thesis are:

- Emulsions produced by a low-energy emulsification technique (premix membrane emulsification) have a smaller droplet size distribution and are more mondisperse than those produce by high-energy emulsification technique (rotor stator), which facilitates the encapsulation of the oil and the stability of the emulsion during the spray-drying process.

- From the two organic microfiltration membranes studied (mixed cellulose esters and polyamide) no differences were observed regarding the droplet size of the resulting emulsion.

- The increase in the amount of encapsulated oil is directly related with the initial emulsion size and the oil-wall ratio. The emulsions produced by premix membrane emulsion had smaller droplet size than the ones obtained by mechanical stirring. For the same emulsion formulation and drying conditions, fish oil microcapsules produced from O/W obtained by membrane emulsification presented higher values of oil encapsulation efficiency (OEE).

- From all the conditions studied, membrane emulsification followed by spray drying allowed to obtain a 70% of OEE with a 20% fish oil load, using 10% sodium caseinate to stabilize the emulsions and 50% of maltodextrin to build the wall of the microcapsule.

- The inner and outer morphology of microcapsules depends on the emulsification technique and the oil-wall ratio. Microcapsules from premix membrane emulsification (either with cellulose mixed esters or polyamide membrane) and spray drying show that an increase of the oil-wall material ratio resulted in a more round shape, smoother external surface, a thicker walls without the presence of inner vacuoles.

- The average wall thickness of the microcapsules is directly correlated with the amount of the wall material employed. Thicker capsule walls are always observed in microcapsules produced with 1:3 oil – wall ratio.

- Oxidation stability of the microcapsules under storage conditions can be correlated with the surface oil content. When drying emulsions produced by premix membrane emulsification, the resulting microcapsules have less surface oil than the ones produced from emulsions obtained by mechanical stirring, which increases the oxidation stability.

- Whey protein fibrils can be introduced as a wall forming material for fish oil microcapsules, with a slight increase in the oil encapsulation efficiency.

- The elastic Young modulus of microcapsules with and without whey protein fibrils is in the same range, which indicates that the concentrations of fibrils used in this study do not change the mechanical properties of the microcapsule wall. However, there is a clear relationship between the amount of wall material and the value of the Young modulus.

Future work needs to focus in two main aspects:

a) **Facilitate the scale up/industrial application of emulsification with microstructured systems:** as seen in this thesis, a low energy emulsification technique can be combined with spray drying to produce food grade microcapsules, however, membrane fouling when using proteins as stabilizers is an important drawback. Cleaning and re-use of the membranes could be an option, but the relative high pressures required for premix membrane emulsification can damage the structure of commercial microfiltration membranes if used for several emulsification runs. As an alternative to premix membrane emulsification, packed bed emulsification has been described. In this kind of systems a packed bed of small glass beads acts as a dynamic membrane, which can be easily cleaned disintegrating the bed. The use of this kind of system would facilitate up-scaling of the process and would be an excellent continuation of the work developed in this thesis.

b) **Enhance the oil encapsulation efficiency:** to ensure the success of the encapsulation process, the amount of encapsulated oil has to be maximized. The results obtained in this thesis show the influence of several operational parameters in the oil encapsulation efficiency. Besides optimizing the conditions to obtain a small droplet size during emulsification, use an economically reasonable amount of wall material and combine a polysaccharide with a protein to build the microcapsule wall, it is possible to move forward and explore other options, like using protein-polysaccharides soluble complexes to stabilize the oil-water interface. The results of an ongoing project in our group show that these types of complexes can be used to stabilize oil-water interfaces and provide good protection to the encapsulated material. A possible continuation of the present work could be focused to test these types of soluble protein-polysaccharide complexes in the production of fish oil microcapsules to increase the efficiency of the encapsulation and, hopefully, the resistance against oxidation.