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Microencapsulation of Polyunsaturated Fatty Acid-Rich Vegetable Oils with Buttermilk Using Ultra-High-Pressure Homogenization (UHPH) Technology: Application in Yogurt

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VICTORIA FERRAGUT PÉREZ, full professor of Food science and Technology at the Universitat Autònoma de Barcelona (UAB).

HEREBY NOTIFY: that Miss. FATEMEH AGHABABAEI has carried out her PhD under my supervision entitled "Microencapsulation of Polyunsaturated Fatty Acid-Rich Vegetable Oils with Buttermilk Using Ultra-High-Pressure Homogenization (UHPH) Technology: Application in Yogurt".

The present thesis entitled "**Microencapsulation of Polyunsaturated Fatty Acid-Rich Vegetable Oils with Buttermilk Using Ultra-High-Pressure Homogenization (UHPH) Technology: Application in Yogurt**" concludes my PhD project carried out at the Centre d'Innovació, recerca i transferència de tecnologia dels aliments (CIRTTA), Department of Animal and Food Science, Faculty of Veterinary, Autonomous University of Barcelona (UAB).

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My PhD was under supervision of Dr. Victoria Ferragut Pérez, Senior Professor of Food Technology at UAB.

Fatemeh Aghababaei

DEDICATION

To my parents

Hassan and **Azar**

The reason of what I become today

Thanks for financial support and love.

Without their warm help, this achievement would not be possible.

To my brother

Aria

How lucky I am to have you in my life.

Thanks for your support, and you have been my soulmate forever.

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Abstract

Ultra-high-pressure homogenization (UHPH) is a versatile technology that has the ability to inactivate microorganisms and enzymes, to give rise to submicron emulsions of great physical and chemical stability, and to produce changes in colloidal structures. As a consequence of this last aspect, UHPH have great potential to lead to restructuring of the protective layer of the droplets according to the composition of the emulsifying agents used, with repercussions on the techno-functional properties, and protection against oxidation.

This project aims to investigate the emulsions obtained using UHPH technology, incorporating Buttermilk (BM) with a variable composition of buttermilk together with vegetable oils rich in polyunsaturated fatty acids, especially α-linolenic and linoleic acid. Dehydration of emulsions by spray-drying will serve to extend the shelf life of these emulsions and facilitate their use at the industrial level. The final utility of these solid dry emulsions (SDE) will be their incorporation into a frequently dairy-based product such as yogurt.

This research has been divided into three parts. The first study aimed to examine the potential improvement of UHPH technology in producing buttermilk-stabilized polyunsaturated rich emulsions (BME) for further drying, compared with conventional homogenization. Oil-in-water emulsions formulated with 10% chia: sunflower oil (50:50); 30% maltodextrin (MD) and 4 to 7% BM were obtained by using conventional homogenization at 30 MPa and UHPH at 100 and 200 MPa, 30 ºC inlet temperature. Particle size analysis, rheological evaluation, colloidal stability, zeta-potential measurement, and microstructure observations were performed in the BME. Particularly, the application of UHPH technology improved the global quality characteristics of BME in terms of stability by generating smaller particle size. These formulations, especially the UHPH-processed emulsions, induced the aggregation of oil droplets when combining MD and BM. The presence of aggregates was relevant in different characteristics of emulsions such as rheological behavior and physical stability.

The next study consisted of spray drying of BM emulsions at a selected drying condition previously determined. Study performed was conducted by assessing physical and oxidative stability of the dried emulsions (SDE) which were characterized in terms of water content, water activity (Aw), flowing properties, water solubility, encapsulation efficiency (EE), color, and microstructure. Oxidation stability of SDE was analyzed in accelerated oxidation conditions at 50 °C for one month for primary and secondary oxidation analysis evolution on days 1, 7, 14, and 31 of storage. 7% BM UHPH-treated SDE showed the best primary oxidation stability during storage, while the 4% BM-UHPH-treated SDE exhibited better secondary oxidative stability. Results showed better ability of BM as encapsulating agent in UHPH-processed emulsions with 7% of BM. Hence, this formulation obtained to production of the yogurt.

In the last study, yogurt recombined milks were formulated using two concentrations of 7% BM-SDE (4% and 6%), 3% skimmed milk powder and 2% starter, all dispersed in UHT skim milk. Coagulation ability and yogurt characteristics were evaluated during 30-day cold storage, by assessing quality parameters, including texture and rheology, microstructure, physicochemical characteristics (colour, pH, total acidity, and water holding capacity), oxidative stability, main fatty acid profile, microbial assessment, and sensory evaluation. The CH yogurts exhibited higher textural parameters (firmness and consistency) and viscoelastic parameters (G' and G") compared to the UHPH yogurts at the same SDE concentration. However, UHPH yogurts generally showed better WHC values. UHPH yogurts also demonstrated higher stability to oxidation and higher PUFA content.

Resumen

La homogeneización a ultra alta presión (UHPH) es una tecnología versátil que tiene la capacidad de inactivar microorganismos y enzimas, dar lugar a emulsiones submicrónicas de gran estabilidad física y química, y producir cambios en las estructuras coloidales. Como consecuencia de este último aspecto, las UHPH tienen un gran potencial para dar lugar a reestructuraciones de la capa protectora de las gotas en función de la composición de los agentes emulsionantes utilizados, con repercusiones sobre las propiedades tecnofuncionales, y la protección frente a la oxidación.

Este proyecto tiene como objetivo investigar las emulsiones obtenidas mediante la tecnología UHPH, incorporando suero de mazada (BM) con una composición variable de suero de mazada junto con aceites vegetales ricos en ácidos grasos poliinsaturados, especialmente ácido α-linolénico y linoleico. La deshidratación de las emulsiones mediante secado por atomización servirá para prolongar la vida útil de estas emulsiones y facilitar su uso a nivel industrial. La utilidad final de estas emulsiones sólidas secas (SDE) será su incorporación a un producto frecuentemente lácteo como el yogur.

Esta investigación se ha dividido en tres partes. El primer estudio tenía como objetivo examinar la mejora potencial de la tecnología UHPH en la producción de emulsiones ricas en poliinsaturados (BME) estabilizadas con suero de mazada para su posterior secado, en comparación con la homogeneización convencional. Se obtuvieron emulsiones de aceite en agua formuladas con 10% de chía: aceite de girasol (50:50); 30% de maltodextrina (MD) y 4 a 7% de BM mediante homogeneización convencional a 30 MPa y UHPH a 100 y 200 MPa, 30 ºC de temperatura de entrada. En el BME se realizaron análisis del tamaño de las partículas, evaluación reológica, estabilidad coloidal, medición del potencial zeta y observaciones de la microestructura. En particular, la aplicación de la tecnología UHPH mejoró las características globales de calidad de la BME en términos de estabilidad al generar un tamaño de partícula menor. Estas formulaciones, especialmente las emulsiones procesadas con UHPH, indujeron la agregación de gotas de aceite al combinar MD y BM. La presencia de agregados fue relevante en diferentes características de las emulsiones, como el comportamiento reológico y la estabilidad física.

El siguiente estudio consistió en el secado por pulverización de emulsiones BM en una condición de secado seleccionada previamente determinada. El estudio realizado se llevó a cabo evaluando la estabilidad física y oxidativa de las emulsiones secas (SDE), que se caracterizaron en términos de contenido de agua, actividad del agua (Aw), propiedades de fluidez, solubilidad en agua, eficiencia de encapsulación (EE), color y microestructura. La estabilidad a la oxidación de los SDE se analizó en condiciones de oxidación acelerada a 50 °C durante un mes para la evolución de los análisis de oxidación primaria y secundaria en los días 1, 7, 14 y 31 de almacenamiento. El SDE tratado con BM-UHPH al 7% mostró la mejor estabilidad de oxidación primaria durante el almacenamiento, mientras que el SDE tratado con BM-UHPH al 4% mostró una mejor estabilidad de oxidación secundaria. Los resultados mostraron una mejor capacidad del BM como agente encapsulante en las emulsiones procesadas con UHPH al 7% de BM. Por lo tanto, esta formulación obtenida para la producción del yogur.

En el último estudio, se formularon leches recombinadas para yogur utilizando dos concentraciones de 7% de BM-SDE (4% y 6%), 3% de leche desnatada en polvo y 2% de iniciador, todo ello dispersado en leche desnatada UHT. Se evaluaron la capacidad de coagulación y las características del yogur durante 30 días de almacenamiento en frío, mediante la evaluación de parámetros de calidad, como la textura y la reología, la microestructura, las características fisicoquímicas (color, pH, acidez total y capacidad de retención de agua), la estabilidad oxidativa, el perfil de ácidos grasos principales, la evaluación microbiana y la evaluación sensorial. Los yogures CH mostraron parámetros texturales (firmeza y consistencia) y viscoelásticos (G' y G") más elevados que los yogures UHPH a la misma concentración

de SDE. Sin embargo, los yogures UHPH mostraron en general mejores valores de WHC. Los yogures UHPH también demostraron una mayor estabilidad a la oxidación y un mayor contenido de PUFA.

Resum

L'homogeneïtzació a ultra alta pressió (UHPH) és una tecnologia versàtil que té la capacitat d'inactivar microorganismes i enzims, de donar lloc a emulsions submicroniques de gran estabilitat física i química i de produir canvis en les estructures col·loïdals. Com a conseqüència d'aquest últim aspecte, les UHPH tenen un gran potencial per a la reestructuració de la capa protectora de les gotes segons la composició dels agents emulsionants utilitzats, amb repercussions en les propietats tecnofuncionals, i la protecció contra l'oxidació.

Aquest projecte pretén investigar les emulsions obtingudes mitjançant la tecnologia UHPH, incorporant sèrum de mantega (BM) amb una composició variable de sèrum de mantega juntament amb olis vegetals rics en àcids grassos poliinsaturats, especialment àcid α-linolènic i linoleic. La deshidratació de les emulsions per assecat per aerosol servirà per allargar la vida útil d'aquestes emulsions i facilitar-ne l'ús a nivell industrial. La utilitat final d'aquestes emulsions sòlides seques (SDE) serà la seva incorporació a un producte freqüentment derivat de lactis com el iogurt.

Aquesta investigació s'ha dividit en tres parts. El primer estudi tenia com a objectiu examinar la millora potencial de la tecnologia UHPH en la producció d'emulsions riques poliinsaturades (BME) estabilitzades amb llet de mantega per a un assecat addicional, en comparació amb l'homogeneïtzació convencional. Emulsions d'oli en aigua formulades amb un 10% de chía: oli de gira-sol (50:50); Es van obtenir un 30% de maltodextrina (MD) i un 4 a 7% de BM mitjançant homogeneïtzació convencional a 30 MPa i UHPH a 100 i 200 MPa, temperatura d'entrada de 30 ºC. L'anàlisi de la mida de les partícules, l'avaluació reològica, l'estabilitat col·loïdal, la mesura del potencial zeta i les observacions de la microestructura es van realitzar al BME. En particular, l'aplicació de la tecnologia UHPH va millorar les característiques de qualitat global de BME en termes d'estabilitat generant una mida de partícula més petita. Aquestes formulacions, especialment les emulsions processades amb UHPH, van induir l'agregació de gotes d'oli quan es combinaven MD i BM. La presència d'àrids va ser rellevant en diferents característiques de les emulsions com el comportament reològic i l'estabilitat física.

El següent estudi va consistir en l'assecat per aspersió d'emulsions de BM en una condició d'assecat seleccionada prèviament determinada. L'estudi realitzat es va dur a terme avaluant l'estabilitat física i oxidativa de les emulsions seques (SDE) que es van caracteritzar pel que fa al contingut d'aigua, activitat de l'aigua (Aw), propietats de flux, solubilitat en aigua, eficiència d'encapsulació (EE), color i microestructura. L'estabilitat d'oxidació de SDE es va analitzar en condicions d'oxidació accelerada a 50 ° C durant un mes per a l'evolució de l'anàlisi d'oxidació primària i secundària els dies 1, 7, 14 i 31 d'emmagatzematge. El SDE tractat amb BM UHPH al 7% va mostrar la millor estabilitat a l'oxidació primària durant l'emmagatzematge, mentre que el SDE tractat amb BM-UHPH al 4% va mostrar una millor estabilitat oxidativa secundària. Els resultats van mostrar una millor capacitat de BM com a agent encapsulant en emulsions processades amb UHPH amb un 7% de BM. Per tant, aquesta formulació s'obté a la producció del iogurt.

En l'últim estudi, les llets recombinades de iogurt es van formular utilitzant dues concentracions de 7% BM-SDE (4% i 6%), 3% de llet desnatada en pols i 2% d'inici, totes disperses en llet desnatada UHT. La capacitat de coagulació i les característiques del iogurt es van avaluar durant 30 dies d'emmagatzematge en fred, mitjançant l'avaluació de paràmetres de qualitat, incloent textura i reologia, microestructura, característiques fisicoquímiques (color, pH, acidesa total i capacitat de retenció d'aigua), estabilitat oxidativa, perfil d'àcids grassos principal, avaluació microbiana i avaluació sensorial. Els iogurts CH van mostrar paràmetres de textura (fermesa i consistència) i paràmetres viscoelàstics (G' i G") més alts en comparació amb els iogurts UHPH a la mateixa concentració de SDE.

Tanmateix, els iogurts UHPH generalment van mostrar millors valors de WHC. Els iogurts UHPH també van demostrar una major estabilitat a l'oxidació i un major contingut de PUFA.

Published papers

Paper I

Aghababaei, F., Cano-Sarabia, M., Trujillo, A. J., Quevedo, J. M., & Ferragut, V. (2021). Buttermilk as Encapsulating Agent: Effect of Ultra-High-Pressure Homogenization on Chia Oil-in-Water Liquid Emulsion Formulations for Spray Drying. *Foods*, *10*(5), 1059. https://doi.org/10.3390/foods10051059

Paper II

Varela, C., Aghababaei, F., Cano-sarabia, M., Turitich, L., Trujillo, A. J., & Ferragut, V. (2022). Characterization and oxidation stability of spray-dried emulsions with omega-3 oil and buttermilk processed by ultra-high-pressure homogenization (UHPH). *LWT*, *162*(January), 113493. https://doi.org/10.1016/j.lwt.2022.113493

Communications to Conferences

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36th EFFoST International Conference 2022 "Shaping the Production of Sustainable, Healthy Foods for the Future"
[Dublin, Ireland, 07/11/2022 – 09/11/2022]
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Authors: Fatemeh Aghababaei, Victoria Ferragut

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Title: Influence of Ultra-High-Pressure Homogenization (UHPH) on the oxidative stability of dried emulsions formulated with Buttermilk and omega-3 **- Poster presentation**
Authors: Carolina Varela, Fatemeh Aghababaei, Antonio Jose-Trujillo, Victoria Ferragut

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Abbreviation key

a*	Red-green color parameter
ALA	α-linolenic acid
b*	Yellow-blue color parameter
BM	Buttermilk
BME	Buttermilk emulsion
CCP	Calcium phosphate
CFU	Colony forming unit
CH	Conventional homogenization
CL	Creaming layer
CLSM	Confocal laser scanning microscopy
DE	Dextrose equivalents
DHA	Docosahexaenoic acid
EE	Encapsulation efficiency
EPA	Eicosapentaenoic acid
FA	Fatty acid
FFAA	Fatty acids
GA	Gum arabic
HC	High-cap
HP	High pressure
HPH	High pressure homogenization
L*	Luminosity
MD	Maltodextrin
MDA	Malondialdehyde
MFGM	Milk fat globule membrane
PUFAs	Polyunsaturated fatty acids
OSA	Octenyl succinic anhydride modified starch
O/W	Oil-in-water
PEG	Polyethylene glycol
PP	Pea protein
PUFA	Polyunsaturated fatty acid

PVA	Polyvinyl acetate
PVP	Polyvinyl pyrrolidone
RM	Recombined milk
SC	Sodium caseinate
SDE	Solid dry emulsion
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscope
SMP	Skimmed milk powder
SPI	Soy protein isolate
T_{in}	Inlet temperature
TSI	Total stability index
UHPH	Ultra high-pressure homogenization
WI	Whiteness index
WP	Whey protein
WPC	Whey protein concentrate
WPI	Whey protein isolate
YI	Yellowness index

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Chapter 1

Introduction

1.1. Food emulsions

1.1.1. Emulsions definition and types

Oil-in-water (o/w) food emulsions generally are complex systems containing a variety of dispersed particles, mainly oil droplets stabilized by surface active molecules, and other macromolecules dispersed in the continuous phase. They are well-known for imparting good mouthfeel aspects to food, but they also play an important role in the creation of structures in some processed foods. Emulsions are found in a wide range of meals, from the more natural, such as milk, cream, and whipped cream to the more complex, such as sausages and mayonnaises. Many meat products, as well as bread dough, rely on the existence of emulsions for their qualities, even though the emulsion structures in both cases might be quite complex.

Emulsions are colloidal dispersions consisting in two or more immiscible liquid phases, continuous and dispersed, of varying composition. The dispersed phase is also known as the internal phase, while the continuous phase is known as the external phase. The droplet dispersion in the continuous phase requires the intervention of an emulsifier adsorbed in the interphase.

According to distribution of oil and aqueous phases, emulsions are classified (McClements, 2004). The dispersed phase is formed by the substance that makes the emulsion droplets while the continuous phase contains the substance that makes up the surrounding liquid. Three main type of emulsion which is important in food (Fig. 1):

- Oil in water emulsion (O/W): where oil droplets are dispersed in an aqueous phase (e.g., mayonnaise, milk, cream, soups, and sauces).
- Water in oil emulsion (W/O): where water droplets are dispersed in an oil phase (e.g., margarine, butter, and spreads).
- Multiple emulsion: oil-in-water-in oil (O/W/O) and water-in-oil-in-water (W/O/W). The O/W/O emulsion, for example, contains oil droplets dispersed in aqueous droplets, which are then dispersed in a continuous oil phase. Various purposes can be served by them, such as reducing the total fat content or isolating one ingredient from another (Clegg et al., 2016; B. Kim et al., 2016).

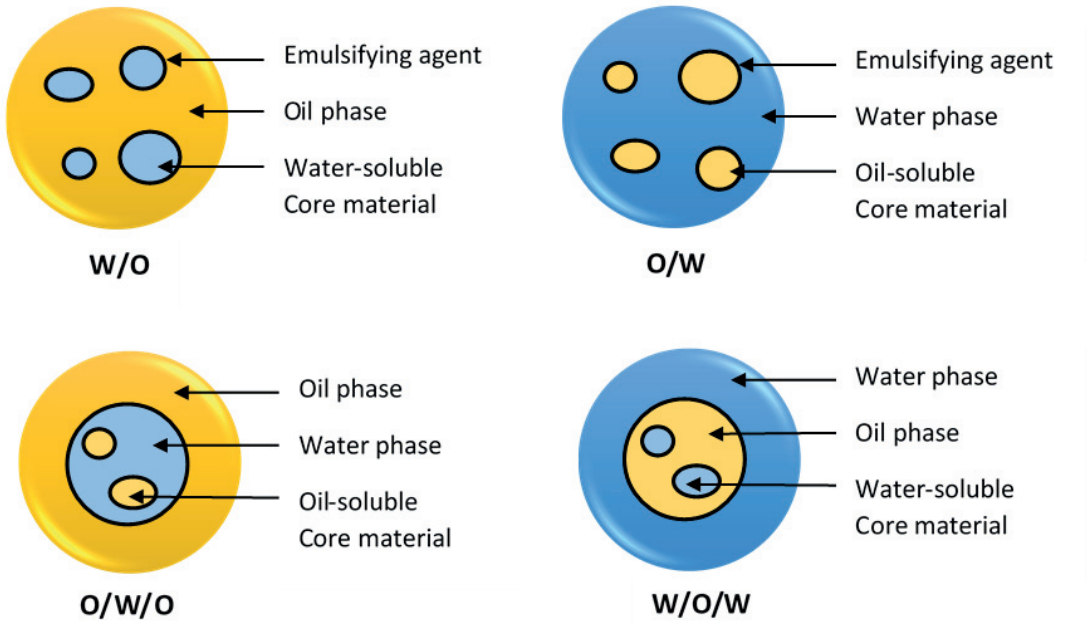


Fig. 1. Schematic illustration of the different emulsion systems (W/O, O/W, W/O/W, and O/W/O).

From the droplet size point of view, the IUPAC (2011) proposed a classification to clarify emulsion terminology. Depending on the droplet size, emulsions are divided into three categories: mini, micro, and macro emulsions (Fig. 2). Microemulsions are spontaneously generated emulsions with droplet widths ranging from a few to 100 nm. The sizes of mini emulsion droplets range from 50 nm to 1 µm and are generally stable over several days. Ultimately, macroemulsions are stable over several hours after preparation and typically have droplets diameters ranging from 1 to 100 µm (Slomkowski et al., 2011). Nano emulsions are not mentioned in this description because the nanometric dimension is already included in micro and mini emulsions.

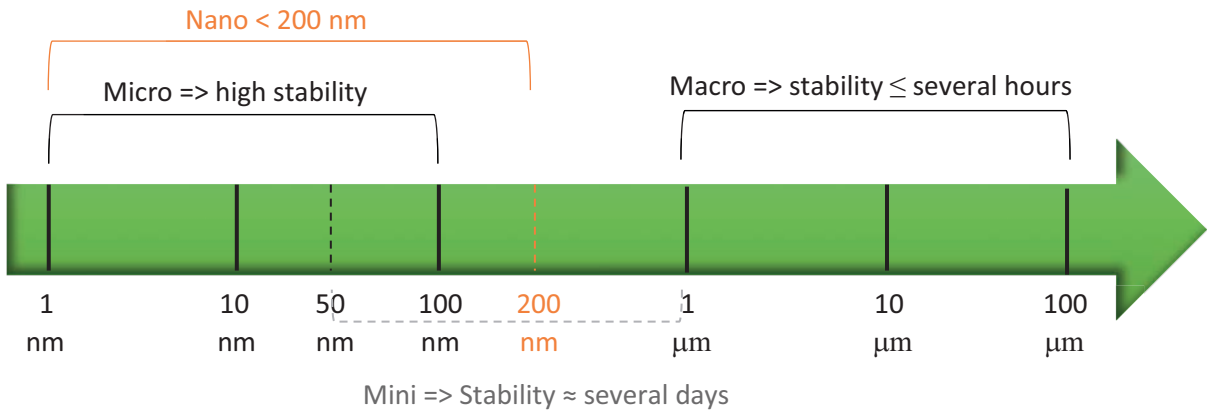


Fig. 2. Emulsion terminology

1.1.2. Emulsion stability

Emulsions are thermodynamically unstable because they represent a higher free energy state compared to the separate phases of the liquids that comprise it. This means that if left undisturbed, emulsions will eventually separate into their component phases over time. The stability of an emulsion depends on several factors, including Van der Waals forces, droplet charges, and the presence of macromolecules. Since the forces of attraction are inversely proportional to droplet size, control of homogenization is an essential variable for obtaining a stable emulsion (McClements, 2004). The electrical double layer around the droplets, due to electrolyte adsorption, also affects the stability of the emulsion. The formation of broad double layers, facilitated by moderate concentrations of monovalent ions, such as NaCl, is required to improve it. The effect on stability has to do with the creation of a cloud of charges that prevents the approach between droplets, thus minimizing aggregation and coalescence phenomena. However, if the charge on the droplets is neutralized, for example, due to changes in pH or the addition of high salt concentration, the repulsive force is reduced, and the emulsion becomes unstable. The presence of macromolecules also affects the stability of an emulsion (Schramm, 2005). Adsorbable macromolecules (mainly proteins) onto the oil-water interface create a protective layer that prevents coalescence. Non-absorbable macromolecules (polysaccharides), on the other hand, can act as steric stabilizers, physically hindering droplet coalescence. However, in both cases, whether they are adsorbable or non-adsorbable molecules, their concentration is a determining factor in the stability of the system. To promote good droplet protection, enough adsorbable molecules is required. However, to promote stability due to kinetic phenomena produced by thickeners, the quantities required are very small. Otherwise, destabilization due to molecular exclusion could occur. In summary, the stability of an emulsion is a delicate balance between several factors, including Van der Waals forces, droplet charges, and the presence of macromolecules. Understanding these factors is essential in the formulation of stable emulsions for various industrial and culinary applications (Smith, 2012).

There are several mechanisms by which emulsions can be destabilized, leading to their breakdown and separation into their component phases. The main mechanisms of destabilization are cremation, aggregation, coalescence, and phase separation (Robins, 2000).

- Creaming: is the formation of a surface layer of droplets from the continuous phase. It is a phenomenon due to the difference in density between the continuous phase and the aqueous phase. It is also inversely proportional to the droplet size and viscosity of the continuous phase. Creaming is a reversible process; the system being recovered by

resuspending by mixing. A common practice to avoid creaming is the use of hydrocolloids to increase the viscosity of the continuous phase. Another procedure used, especially in milk stabilization, is homogenization.

- Aggregation: This mechanism occurs when droplets in the emulsion begin to cluster together. The aggregation can be caused by factors such as changes in temperature or pH, or the presence of incompatible ingredients. As droplets aggregate together, the stability of the emulsion is reduced, and it can eventually produce phase separation though coalescence, if particle-particle forces are high enough.
- Coalescence: This mechanism occurs when two or more droplets in the emulsion come into contact and merge. Coalescence is a major contributor to emulsion breakdown, and it can be caused by factors such as changes in temperature or the presence of incompatible ingredients. Once coalescence occurs, the emulsion becomes unstable, and it will eventually separate into its component phases.
- Phase Separation: This mechanism occurs when the emulsion separates into its component phases due to a lack of stability. Phase separation can be caused by any of the other destabilization mechanisms, or it can occur due to factors such as changes in temperature, pressure, or the addition of incompatible ingredients.

To minimize these destabilization mechanisms and maintain emulsion stability, several strategies can be employed (Robins, 2000). These include:

- Using the right emulsifying agent: Choosing an appropriate emulsifying agent that is stable under the intended conditions of use is crucial for maintaining emulsion stability.
- Controlling temperature and pH: Emulsions can be destabilized by changes in temperature or pH. Controlling these factors can help to maintain stability.
- Avoiding incompatible ingredients: Certain ingredients, such as those with opposite charges or hydrophobic/hydrophilic properties, can cause emulsions to break down. Avoiding these ingredients can help to maintain stability.
- Optimizing emulsion and /or homogenization process: The emulsion process has a significant impact on emulsion stability. The optimization of this step is mainly aimed at creating a good protective layer and a sufficiently small particle size.

Stability of food emulsions is typically achieved by using surface active molecules (emulsifiers).

1.1.2.1. Emulsifiers

Emulsifiers are surface-active compounds that reduce the interfacial tension between the dispersed and continuous phases during emulsion formation and they can play a major role in relation to food texture. They are commonly used in food products for emulsification by adsorbing at the interface between oil and water, creating a protective layer around the emulsion droplets to prevent destabilization (Chen, 2015). Emulsifiers are amphiphilic molecules, which means that they have both, hydrophobic and hydrophilic domains. The former dissolves in or interacts with the fat or oil's hydrophobic surface, whereas the later dissolves in the aqueous phase.

The three major roles of emulsifiers in food processing and storage are (Chen, 2015):

- Controlling the condition of dispersion and aggregation of oil droplets or fat globules to increase emulsion/foam formation and stability.
- Interaction with carbohydrate and protein components to modify shelf life, texture, and rheological characteristics.
- Changing the size distribution of oil droplets or the structure of fat crystals to control the morphology and texture of fat-based products.

Though several food emulsifiers naturally occur (proteins, lecithin, mono and diglycerides), some of them are considered food additives. Nowadays, most approved food emulsifiers are esters (or partial esters) derived from animal or vegetable fatty acids. Some of the most widely used are as follows:

- **Small-molecule surfactant**

In general, molecular weight of surfactants range from a few hundred to several thousand of Daltons. They typically have a hydrophobic tail and a hydrophilic head (Fig. 3), allowing them to adsorb at the interface and to form a protective layer around the dispersed droplets. This creates a stable emulsion by preventing the droplets from coalescing and separating. Additionally, the surfactant molecules can also provide a steric hindrance, preventing the droplets from coming into close contact and reducing the risk of Ostwald ripening, which can lead to instability of the emulsion over time. Overall, the use of small-molecule surfactants as emulsifiers in emulsions is a widely employed strategy due to their versatility, effectiveness, and compatibility with a wide range of applications.

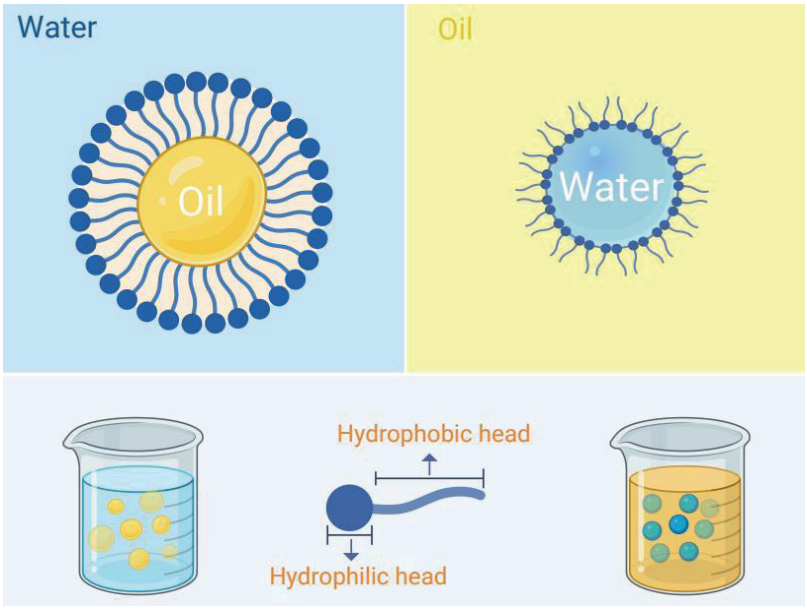


Fig. 3. Oil-in-water and water-in-oil emulsions. They are immiscible liquids consisting of oil and water forming a single phase by an emulsifier such as the surfactants.

– **Proteins**

Protein-stabilized emulsions are usually characterized by thin interfacial membranes which is frequently electrically charged. Thus, steric and electrostatic repulsion are mechanism involved in preventing droplet flocculation (McClements, 2004). As consequence of protein charges, protein-stabilized emulsions are highly sensitive to pH and ionic strength effects and are likely to flocculate at pH values near the isoelectric point of the proteins and at high ionic strengths (Dickinson, 2010). Milk, meat, fish, eggs, and some plant proteins are the most well-known emulsifiers used in the food industry.

Milk protein ingredients are used as emulsifiers in a wide range of emulsion-based food products such as beverages, frozen desserts, ice creams, sport supplements, infant formula, and salad dressings. Cow milk contains approximately 3.5% protein. Some inherent characteristics of milk proteins are provided in Table 1. Casein (80% wt/wt) and whey proteins (20% wt/wt) are the two major categories of milk proteins. Casein contains four major protein fractions: α_{S1} (44%), α_{S2} (11%), β (32%), and κ (11%). They exist in their natural state as micelles, which are typically between 50 and 250 nm in diameter and are held together in part by mineral ions (such as calcium phosphate). The properties of casein micelles change significantly when pH is reduced, owing primarily to colloidal phosphate solution; at even lower pH, increased salt bridge formation predominates. Temperature has a significant effect as well: when it is lowered,

the micelles become more voluminous, presumably due to protruding hairs of (primarily) κ-casein.

Casein coagulation can be accomplished by reducing the pH to their isoelectric point (pH 4.6) or by using some enzymes, such as rennet, that hydrolyzes the hydrophilic component of the micelle, κ-casein, that is responsible of its stabilization. After milk coagulation, casein (solid fraction) and whey protein (liquid fraction) can be separated (Chen, 2015). Caseins may be present in a variety of other molecular clusters in commercial ingredients, depending on how the proteins were isolated, such as sodium caseinate, calcium caseinate, acid casein, and rennet casein.

Curd formation is an important step in the production of cheese since large amounts of whey left over from this process can be used to manufacture functional whey protein ingredients. They are usually sold as powder, with a light cream to white appearance and a bland flavor. Whey protein concentrates (25-80% protein) or isolates (>90% protein) are the most common forms of these powders.

Table 1. Properties of milk proteins (Masum et al., 2023).

Protein	**Isoelectric point**	**Molecular weight (Da)**	**Amino acid residues**	**Hydrophobicity (kJ/residue)**
Casein				
α_{S1}-Casein-B	5.0	23.614	199	4.9
α_{S2}-Casein-A	5.2-5.4	25.230	207	4.7
β-Casein-A^2	5.2	23.983	209	5.6
κ-Casein-B	5.6	19.023	169	5.1
Whey proteins				
β-Lactoglobulin-B	5.3	14.176	123	4.7
α-Lactalbumin-B	4.8	18.363	162	5.1
Serum albumin	4.7	66.267	582	4.3

– **Buttermilk**

Buttermilk is the liquid fraction obtained from butter production, and it is largely considered as a by-product in dairy industries. Fig. 4 shows a schematic representation of different processing steps used in manufacturing of butter. Churning causes cream (oil-in-water emulsion) to separate into two distinct phases: an aqueous phase known as buttermilk and the oil phase which constitute the butterfat. The mechanical destabilization of refrigerated cream (to maintain fat globules in solid state) causes this separation. The contact with air and repeated physical collisions disrupt the thin membrane that surrounds and stabilizes fat globules, resulting in a partial coalescence and, eventually, the formation of a solid phase (i.e., butter) from which buttermilk is easily separated by draining.

In many ways, buttermilk and skim milk are very similar. More than 80% of their proteins are milk proteins, such as caseins and whey proteins. However, due to the presence of milk fat globule membrane (MFGM) components, its composition differs significantly from that of skim milk, both in quantity and composition. MFGM make up nearly 20% of buttermilk proteins (Conway et al., 2014).

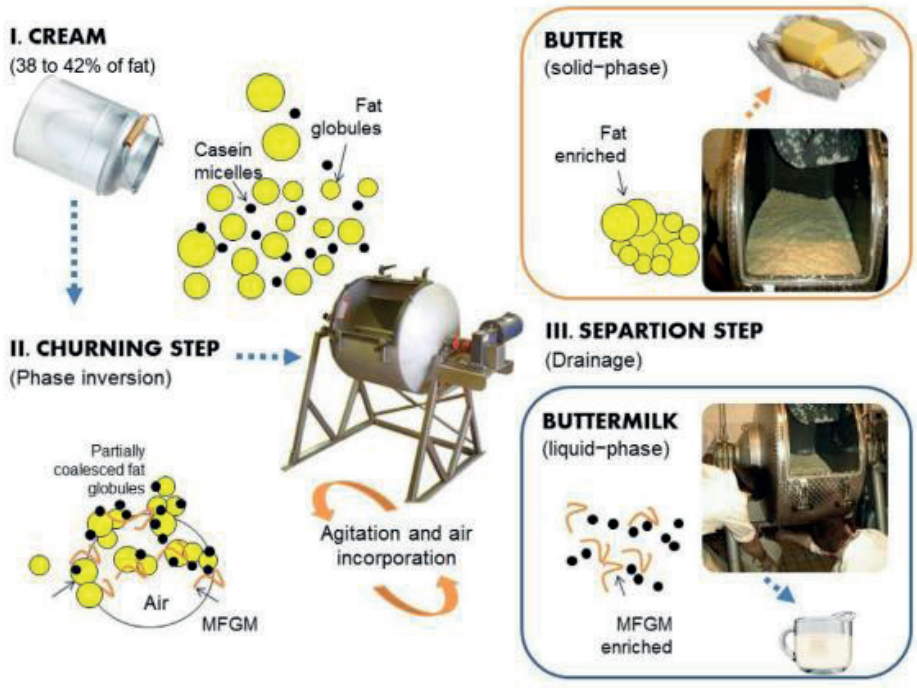


Fig. 4. The different processing steps used in the manufacture of butter are depicted schematically, along with their impact on the milk matrix structure. (I) Pasteurized cream is churned to induce phase inversion; (II) the milk fat globule membrane (MFGM) is ruptured; and (III) the churned liquid (sweet buttermilk) is drained and butter grains are further processed (Conway et al., 2014).

In the fat fraction of milk, lipids from the MFGM represents 2 to 6% of total mass globule (Lopez, 2011), forming a natural encapsulating system of milk fat which is structured as a three layer of polar lipids (phospholipids, sphingolipids, Fig. 5). The other components of BM are proteins (casein and glycoproteins), lactose and mineral salts. Because of their surface-active properties, the proteins and phospholipids in BM play a techno-functional role as stabilizing agents in food emulsions, forming a viscoelastic layer at the oil-water interface and preventing coalescence (Phan et al., 2016).

Polar lipids, and proteins in the MFGM have been described as health-promoting components because they prevent infections, improve immunity, protect and support adequate growth in healthy infants, and improve brain and cognitive system development (Hernell et al., 2016; Lopez et al., 2017; Singh & Gallier, 2017). Thus, this system has got a lot of attention in recent years (Le et al., 2014).

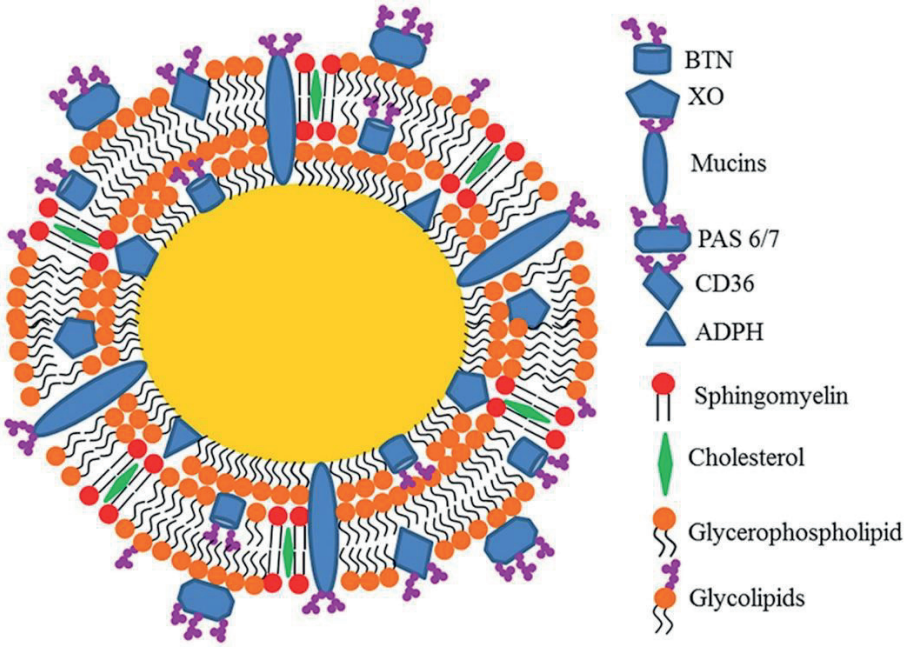


Fig. 5. The MFGM structure (Singh & Gallier, 2017).

As a result of their high content of phospholipids and other surface-active material, the MFGM fractions isolated from buttermilk have good emulsifying properties in the dairy, processed foods, bakery, and confectionary industries.

Several authors studied the potential application of buttermilk as an encapsulation agent. For example, Kumar et al. (2020) established that buttermilk can serve as a matrix for the encapsulation of omega-3-rich oil, which resulted in a stable emulsion for a period of 15 days. Similarly, buttermilk exhibited superior encapsulating properties compared to skim milk for omega-3 from fish oil (Augustin et al., 2015). In addition, 33.3% oil and 33.3% buttermilk powder mixed with 33.3% maltodextrin microcapsule achieved good encapsulation efficiency and the best stability during storage (Zhang et al., 2020).

1.1.2.2. Oil phase

It is well known that lipids (fats and oils) influence the nutritional, organoleptic, and physicochemical properties of food emulsions in many ways. Edible oils are vital sources of energy provider in human diets. In addition, oils are also a natural source of bioactive compounds. The most well-known oils in food industry with bioactive compounds are those containing unsaturated fatty acid rich oils such as olive, sunflower, rapeseed, and fish oils. They are well documented for their nutritional and health benefits and often associated with minor lipids that promote human health in significant ways. However, there has been a growing trend in recent years towards using oils that are rich in omega-3 fatty acids (FFAA). Omega-3 FFAA are considered essential because our bodies cannot produce them, and they must be obtained from the diet. Several studies have shown that omega-3 FFAA have numerous health benefits, including reducing the risk of cardiovascular disease, improving brain function, reducing inflammation, and supporting healthy immune function. In particular, the long-chain omega-3 FFAA, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are known for their potent health benefits (Swanson et al., 2012). Using oils rich in omega-3 FFAA can be a convenient way to increase the intake of these essential nutrients. However, it is important to choose high-quality oils that are fresh, pure, and properly stored to ensure that they provide the full range of health benefits.

- **Chia Oil**

Chia plant (*Salvia hispanica L.*), also known as Mexican chia or Spanish sage, is a flowering green plant endemic to Latin America; Mexico, Peru, Guatemala, and Argentina (Fig. 6a) (Sosa et al., 2016). Chia is a member of the Lamiaceae family, adapted to semiarid to arid territory with sandy loam soil, and can thus withstand long-term drought. (Ayerza, R., Coates, 2004; Ullah et al., 2016). The seed is the most important portion of the plant due to its high oil content. This seed also has a high concentration of proteins and essential amino acids, making it a

prospective source of bioactive peptides (Grancieri et al., 2019). In recent years, the crop has been extensively cultivated in Latin America and Australia as a main food and for its oil. Chia oil is rich in omega-3 FFAA (Fig. 6b), particularly α-linolenic acid (ALA), and also other polyunsaturated fatty acids (PUFAs), which has been associated with various health benefits, including reduced inflammation and improved cardiovascular health (Omolaja et al., 2020). When incorporated into emulsions, chia oil can enhance the nutritional profile of the product, providing consumers with a healthier alternative. Additionally, chia oil contains antioxidants such as tocopherols and phenolic compounds, which contribute to its oxidative stability and potential shelf-life extension in emulsions (Ixtaina et al., 2011). Other foods in which chia oil has been studied was the development of gelled emulsion for meat products, mainly emulsified meat products, like Frankfurt-type sausages and burgers, with interesting nutritional improvement (fat reduction and healthier lipid profile) and sensorial acceptance. (Heck et al., 2019; Lucas-González et al., 2020).

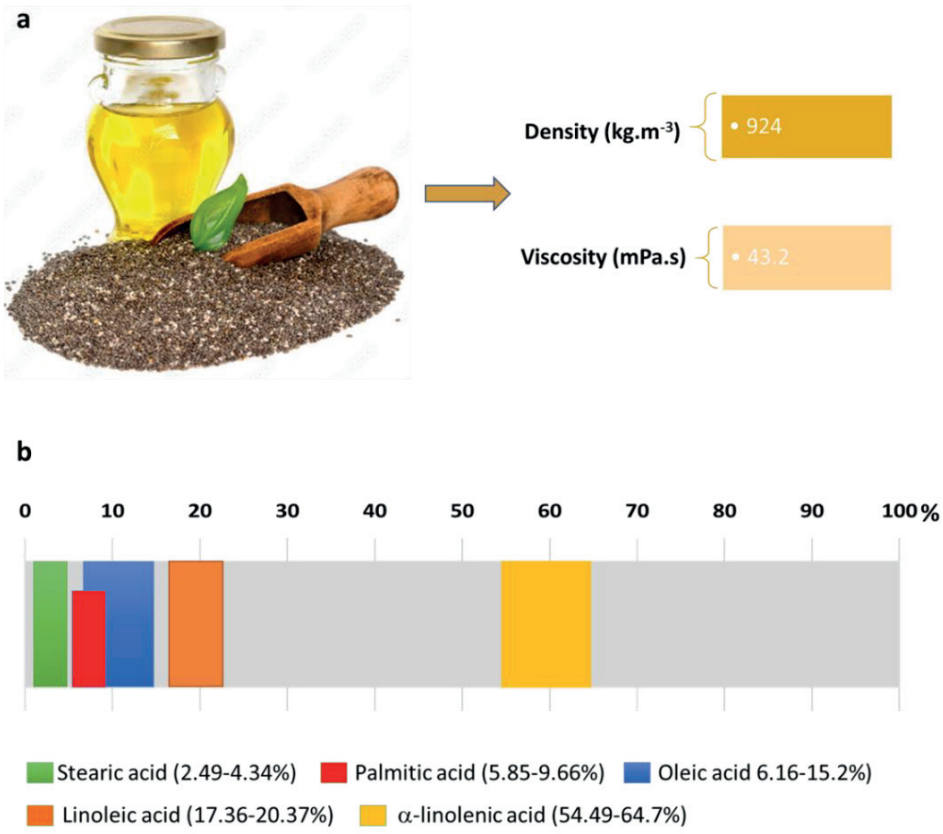


Fig. 6. **(a)** Bulk physical properties and **(b)** fatty acid composition of chia oil.

– **Sunflower oil**

Sunflower (*Helianthus annuus*) seeds is well-known as source of vegetable edible oil (Fig. 7a) in all around the word, which is partially premiere to other vegetable oils due to a higher concentration of unsaturated fatty acids and the presence of bioactive substances such as tocopherols and phytosterols (Debaeke & Izquierdo, 2021). It is the world's fourth-most-cultivated oil crop and contains the highest amount of vitamin E. On the other hand, sunflower oil contains the greatest amount of linoleic acid (Fig. 7b) and many other components, which makes it ideal for use in food in addition to other applications such as bio-lubricant, biofuel, and pharmaceutical industry. The presence of linoleic acid in sunflower oil can contribute to the sensory attributes of emulsions, providing a smooth and creamy mouthfeel, which is desirable in many food and cosmetic products (Agyei-Amponsah et al., 2019). Moreover, sunflower oil exhibits a good oxidative stability compared with other vegetable oils due to the high content of tocopherol and phenolic compounds, which make it an ideal choice for emulsions that require a long shelf life (Sainsbury, 2019).

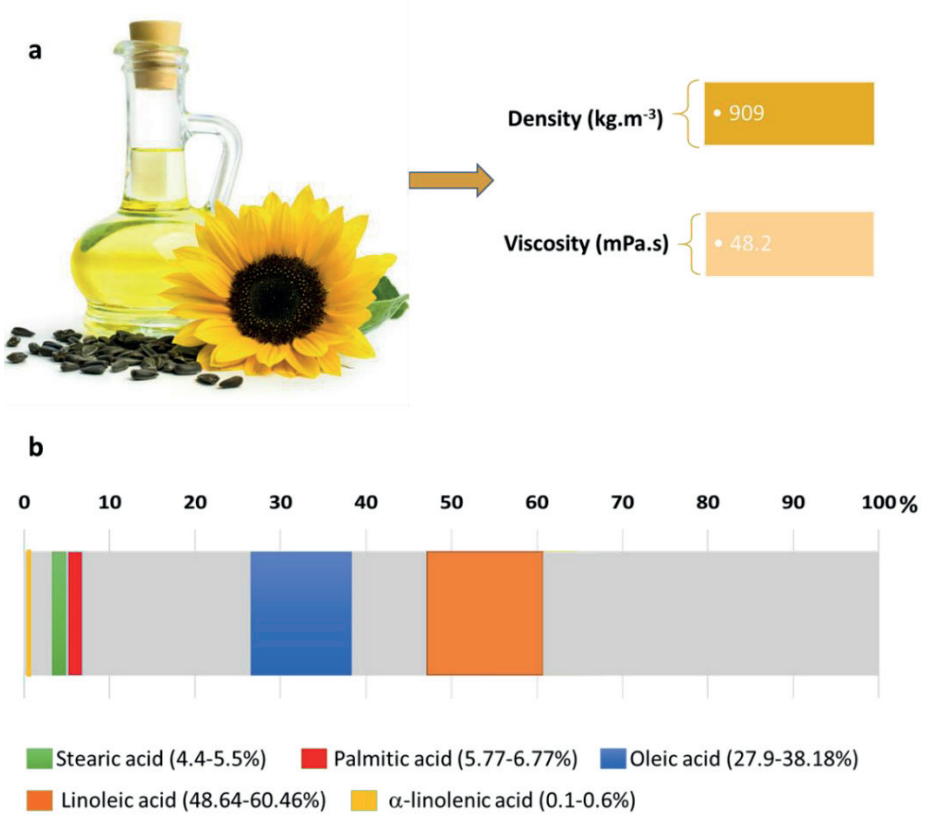


Fig. 7. **(a)** Bulk physical properties and **(b)** fatty acid composition of sunflower oil.

1.1.2.2.1 Lipid oxidation

As previously mentioned, PUFAs are prone to oxidation despite, in o/w emulsions they are protected by a emulsifier layer around oil droplets in the dispersed phase (García-moreno et al., 2016; Magdalena et al., 2015). Thus, their stability to oxidation is considered to be highly dependent on the properties of the interfacial layer (Genot et al., 2013).

Lipid oxidation is the primary process of vegetable oil degradation, which causes nutritional and sensory losses related to the generation of lipid oxidation products, and it is a major source of worry for the food industry due to the loss of nutritional value and functioning, as well as the buildup of potentially hazardous chemicals, are all negative consequences on human health. The cosmetics, pharmaceutical, and medical sectors, in addition to the food industry, use o/w emulsions to encapsulate, preserve, and release of bioactive lipids in their products (Echegaray et al., 2022).

Lipid autooxidation is a radical reaction mediated by catalysts such as light, oxygen in various forms, and transition metals. This reaction occurs in three stages: initiation, propagation, and termination. The production of hydroperoxides as primary products is odorless, but their degradation produces the final products responsible of the off-flavors, which are predominantly low-mass molecules such as aldehydes, ketones, alcohols, acids, esters, epoxides, and others (Rodriguez-Amaya & Shahidi, 2021). Apart from catalytic converters, many other parameters can affect lipid oxidation, including the nature and composition of the food matrix, physical state of the food and its physicochemical properties, availability of oxygen and type, heating rate, water activity, other food components and additives, processing, and storage conditions.

Several elements participate in the progression of lipid oxidation in emulsions due to their multi-phase and multi-component structure, molecule-molecule, colloidal interactions, and the division and dynamics of the implicated reactive entities (Fig. 8). Metal ions and free radicals existing in the aqueous phase cause lipid oxidation at the oil-water interface in o/w emulsions. As a result, reacting species must reach and cross the interface to access the oil phase.

Vacuum packing or controlled environment storage, low-temperature storage, encapsulation of sensitive added compounds, and the use of antioxidants are all strategies for minimizing lipid oxidation (Rodriguez-Amaya & Shahidi, 2021).

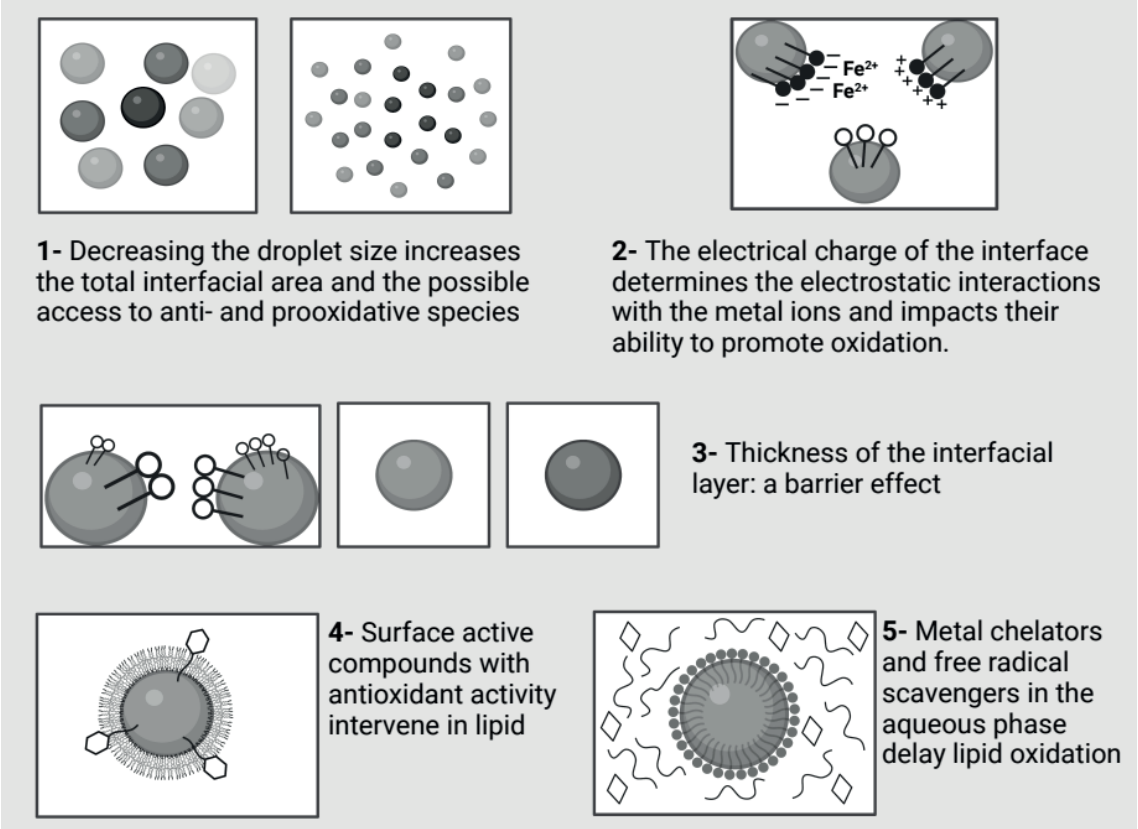


Fig. 8. Main interfacial parameters involved in lipid oxidation of oil-in-water emulsions.

1.2. Emulsion formation

1.2.1. Emulsification process and techniques

The process of dispersing two or more immiscible liquids together to form a semi-stable mixture is known as emulsification (Leong, 2016). During emulsification process, dispersed phase is broken up into small droplets. As mentioned before, to prepare an emulsion, oil, water, an emulsifier agent, and mechanical energy are needed. Normally, by rapid mixing of ingredients a coarse premix (pre-emulsion) is created. In this way, the dispersed phase is sufficiently broken up into large droplets to permit adsorption of emulsifiers and to be further homogenized (Wilde, 2009).

Emulsification is produced either by high-energy or low-energy mechanical process (Fig. 9). Most common emulsification at the food industry is performed by high energy homogenization.

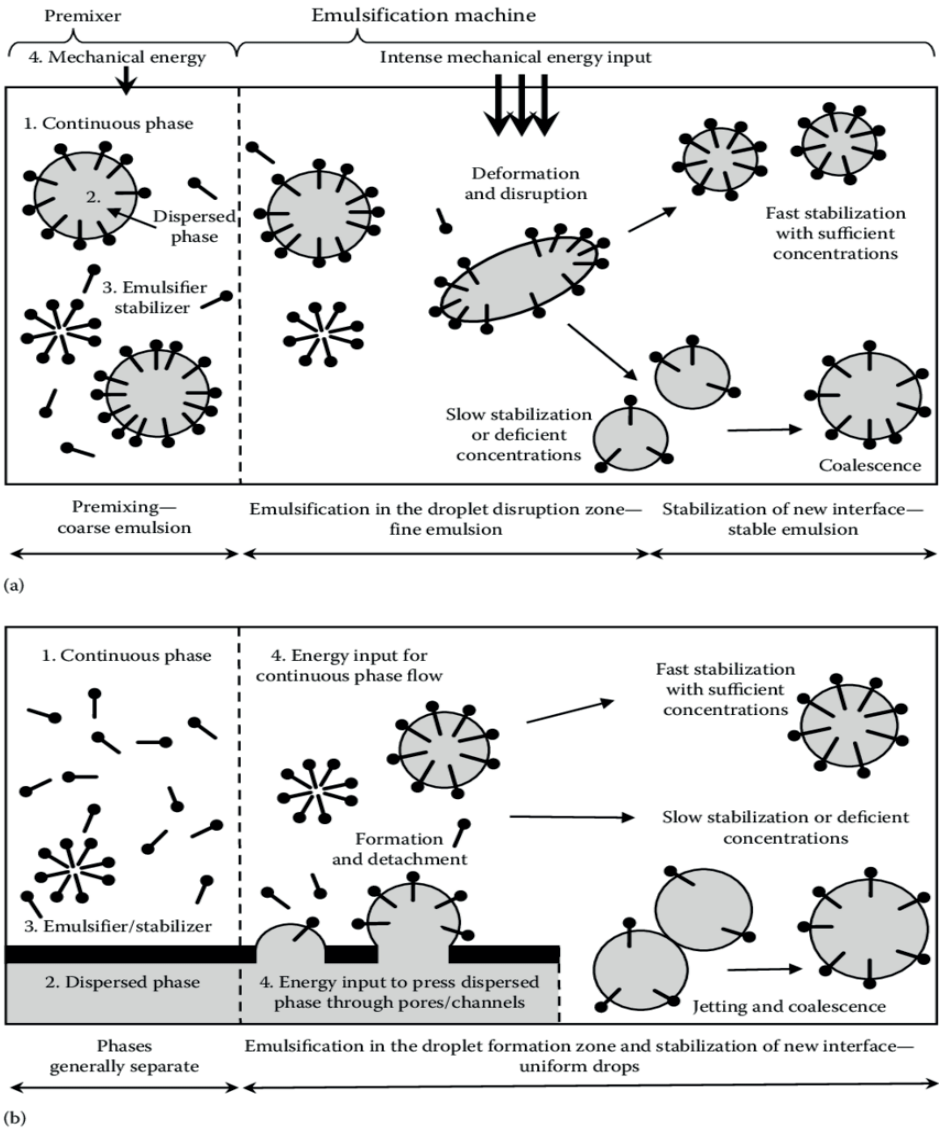


Fig. 9. (a) High-and **(b)** low-energy emulsification processes (Werner, 1995).

High-energy emulsification uses significant mechanical input to break up a coarse premix of emulsion droplets. Energy can be generated by stirring, shearing turbulent, and ultrasound procedures. In many high-energy methods, a variety of technical designs and apparatus can be used to achieve droplet size reduction to obtain a stable emulsion; however, the duration and strength of the mechanical energy applied determine how well the droplets are disrupted (Rayner, 2018).

Emulsification devices most used at industry include: (1) low-speed stirrer; (2) high-speed shear stirrer; (3) high-pressure homogenizer (HPH); and (4) ultrasonic devices. Furthermore, there

are various types of HPH equipment, ranging from prototypes to industrial scale: MICROFLUIDIZER® (Microfluidics International Corporation, USA), NANOJET (Haskel, USA), EMULSIFLEX (Avestin, Canada), STANSTED (Stansted Fluid Power Ltd., UK) and YPSICON which are equipment that achieve pressures greater than 100 MPa (Poliseli Scopel, 2012), thus they can be considered ultra-high-pressure (UHPH) generators, also called dynamic high pressure. Homogenization technology has improved greatly in the last twenty years and HPH equipment's are now accessible at lab, pilot, and production scales. The benefit of HPH over other technologies is that they produce more homogeneous droplet size distributions because the product is subjected to intense shear and cavitation forces that efficiently reduce the diameter of the original droplets (Emam Hebishy, 2013).

1.2.2. Ultra-high-pressure homogenization

In recent years, there has been a growing interest in new minimally processed food products with high nutritional and sensory quality, but also, with guaranteed safety. That has forced the food industry to seek alternative technologies to thermal treatments with as little effect as possible on the nutritional and sensory properties of food.

UHPH is a versatile technology that has the most of these requirements, due to the high pressure applied range from 100 to 400 MPa (Patrignani & Lanciotti, 2016). Different types of UHPH equipment have been developed over the past decades to seek this effect for the physicochemical and microbiological stabilization of foods, pharmaceutical, and cosmetic industries at moderate pressures (up to 100 MPa) to disperse nonmicellar phases, stabilize emulsions, and/or prepare products with desirable rheological properties (Hayes et al., 2005). Pressures above 200 MPa have been proposed to produce microbial and enzymatic inactivation, in addition to modifying the rheological and/or coagulation properties of milk and milk products (Thiebaud et al., 2003). Sophisticated homogenization valves with ceramic seats and needles covered with artificial diamond and capable of withstanding pressure levels of up to 350–400 MPa have been specifically designed for UHPH equipment (Dumay et al., 2013).

This technology allows for the stabilization and sanitization of liquid products simultaneously depending on the inlet temperature of the product and pressure applied. To control the desired effects the system is divided into three basic steps: (a) preheating the product up to the inlet temperature (T_{in}), (b) product passing through the ultra-high pressure valve, and (c) instant cooling (Fernández Avila, 2016). Fig. 10 shows the UHPH system that has been used in this work.

Fig. 10. The UHPH system that used in the present PhD thesis.

The food product undergoes a rapid increase in pressure using a pressure intensifier, which takes only a few seconds. It is then forced through an extremely narrow valve gap, typically a few micrometers wide (Fig. 11), accompanied of a drop-in pressure at the exit of the valve. All this results in different important effects: (i) the generation of high mechanical forces and elongational stress in the laminar flow at the valve entry and within the valve gap, and (ii) the occurrence of turbulence, cavitation, and collisions with solid surfaces as the fluid exits the gap.

Initially, as the pressure builds up in the pressure intensifier (P_1; Fig. 11a), the fluid temperature rises by 2-3 °C per 100 MPa due to the heat of compression generated during the process (T_1). As the fluid passes through the HP-valve, its velocity increases due to the reduction in pipe size and the pressure drop (ΔP). The shear effects and partial conversion of mechanical energy into

heat cause the fluid temperature increase, measured immediately at the output of the HP-valve (T_2; Fig. 11a), to rise linearly by 14-18 °C per 100 MPa (Dumay et al., 2013). When processing whole milk or o/w emulsions at T_{in} between 4 to 24 °C, temperature increases from 17 to 21 °C per 100 MPa (Cortés-Muñoz et al., 2009; Thiebaud et al., 2003). To prevent excessive heat exposure that could affect heat-sensitive biomolecules, the liquid is subjected to a rapid cooling at the exit of HP valve.

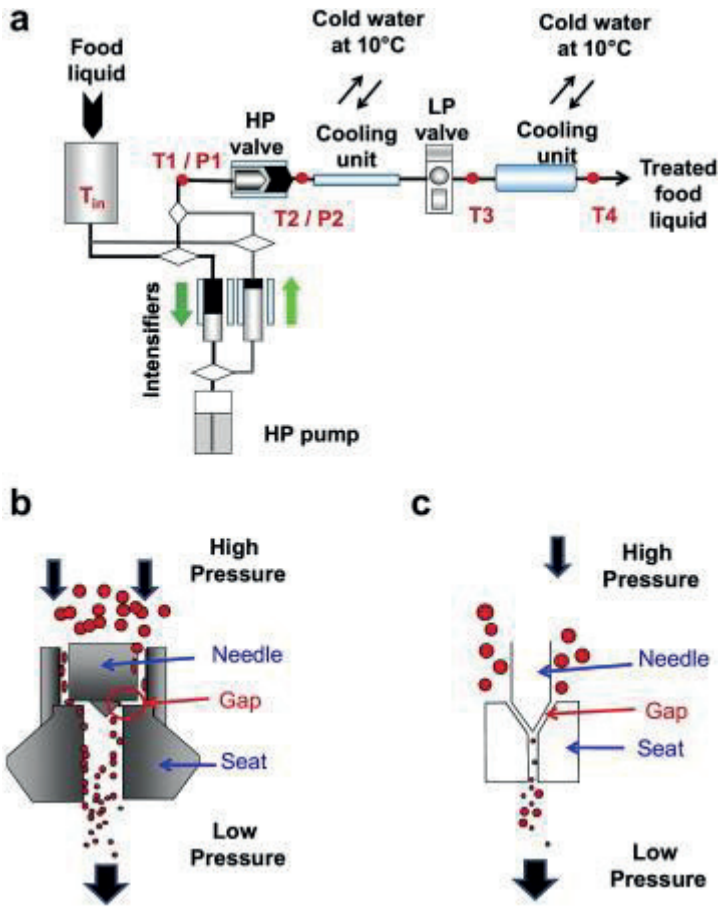


Fig. 11. (a) Schematic representation of high-pressure homogenizer with twin-intensifiers. T_{in}, initial fluid temperature in the feeding tank; T_1/P_1, temperature and pressure probes located at the HP-valve inlet; T_2/P_2, temperature and pressure probes located at the HP-valve outlet; T_3 and T_4, temperature probes after the first and the second cooling devices. **(b)** Schematic representation of a sharp-angle HP-valve from Stansted™. **(c)** Schematic representation of a Y-shape HP-valve (Dumay et al., 2013).

By recording temperature and pressure values throughout the UHPH-processing, it became feasible to assess the percentage of energy input utilized as mechanical energy for particle disruption or dissipated as thermal energy through temperature elevation. Cortés-Muñoz et al. (2009) determined that, based on dispersion and emulsion formulation, mechanical energy

accounted for 37-59% of the total energy input (with the remaining 63-41% being lost as dissipated heat). In the case of a convergent HP-valve with sharp angles (Stansted^{TM}) (Fig. 11b), a rapid increase in fluid velocity up to 200-250 m/s and intense velocity gradients up to 10^7-10^9 s^{-1} occur in the valve-gap as calculated by Floury et al. (2004) according to Poiseuille's law (laminar flow).

As mentioned before, several physical phenomena successively and/or simultaneously are involved in the UHPH process which are responsible of droplet and cell disruption. Each mechanism is manifested to varying degrees as a function of several elements such as product viscosity and operational parameters (pressure, temperature, flow rate):

- Cavitation: is a process that occurs when liquid moves from a low-pressure zone where cavities/air bubbles form to a high-pressure region where they collapse each other. Cavitation intensities increase dramatically with homogenizing pressure (Floury et al., 2004), and decrease with increasing fluid viscosity (Diels et al., 2005).
- Shear stress: is defined as the inertial forces inside particles passing through a material cross-section. In a laminar flow, it is the only mechanism that provides to droplet disruption (Floury et al., 2004); however, in this type of homogenizer, both laminar and turbulent regimes coexist.
- Turbulence: is a mechanism triggered by the inertial forces created in the very turbulent area located at the exit valve (Donsì et al., 2009).
- Extensional stress: droplets/cells that have been disturbed by elongational forces are stretched (Floury et al., 2004).
- Impingement: is the influence of the droplets or cells upon the UHPH valve walls.

All these mechanical forces result in modification of particles in the processed fluid conferring them new structural and physicochemical properties by a top-down (particle size reduction) or bottom-up (particle reassembly) process. Particularly, when the processed fluid is forced through the very narrow HP-valve gap, particles (emulsion oil droplets, fat globules, microorganisms) or polysaccharide macromolecules can be broken by the mechanical associated forces. Thereby, emulsions processed by UHPH exhibited an excellent stability *vs* time due to the narrow size distribution of the nano-/submicron droplets (Cortés-Muñoz et al., 2009; Juliane Floury et al., 2003). During UHPH treatment, shear forces cause agglomerates to break, hydrophobic substances to disperse, and the sample to homogenize. This leads to a decrease in particle size, which improves the bioavailability and functionality of encapsulated

substances, as well as facilitates their incorporation into various formulations (Comuzzo & Calligaris, 2019).

In addition to the improvement of colloidal stability, the UHPH technology is of great interest for its preservative effect, due to the destruction of microorganisms and enzymes as proven in different buffers, milk, and fruit juices. Microorganisms can be considered as microparticles that can be broken by shearing actions combined with temperature changes. Protein unfolding and aggregation can cause enzyme inactivation (Dumay et al., 2013).

UHPH treatment at 200-300 MPa has demonstrated to be equally successful as thermal pasteurization (Trujillo et al., 2021), while mantaining better sensory and nutritional properties. It has been also describerd the capacity of UHPH for sterilizing some food products such us vegetable beberages (Poliseli-Scopel et al., 2014) when 300 MPa, 80 °C T_{in} (144 °C/0.7 s at the homogenization valve) were used. In addition to reducing microbial burden, UHPH-induced inactivation of indigenous milk enzymes such as alkaline phosphatase, plasmin-plaminogen, lactoperoxidase system, or lipases has been at treatments of 200-300 MPa (Pereda et al., 2008). A decrease in pectin methylesterase residual activity by 50–80% was found in orange juice after multi-pass UHPH at 250 MPa (T_{in} = 22 °C) (Welti-Chanes et al., 2009) or by 96% after single-pass UPHP at 300 MPa (T_{in} = 10 or 20 °C) (Velázquez-Estrada et al., 2012) while multi-pass HPH at 170 MPa was insufficient to inactivate PME isoforms (Lacroix et al., 2005).

1.2.3. Physical effects of UHPH on emulsions

1.2.3.1. Particle size and physical stability

The physical stability of emulsions is directly affected by particle size. The smaller the dispersed particles, the more stable the system. In contrast, the more rapidly particles grow in size, the more unstable the system becomes (de Morais et al., 2006). By increasing pressure of the UHPH treatment has been shown in several studies to reduce particle size (Gaillard et al., 2022; C. Wang et al., 2020), which increase stability by reducing sedimentation, creaming, and phase separation, thus improving the overall appearance and quality of the product. The stability enhancement observed in UHPH-treated products can be attributed to several underlying mechanisms. In milk UHPH-treated, for example, the lowest particle size distribution was achieved at pressures up to 200 MPa (Pereda et al., 2007). However, at 300 MPa, an increase in particle size was observed, due to the droplets aggregation most likely due to inadequate protein concentration for the greater surface area generated, (Floury et al., 2002). The fat

globule size is also affected by the inlet temperature during UHPH treatment of milk. According to some authors (Datta et al., 2005; Hayes et al., 2005), the lipid state (liquid, solid, or partially solid and liquid) has a significant influence in the extent of globule size reduction.

Under pressures ≥ 300 MPa, proteins undergo denaturation, in which their native structure is lost to a greater or lesser degree, modifying their functional properties. In general, the level of denaturation and aggregation of proteins depends on the intensity of mechanical forces and/or temperature. In addition, HP can induce the formation of large aggregates by favoring the association of proteins and other macromolecules. Aggregate formation has also been described in UHPH-treated emulsions prepared with sodium caseinate containing sunflower oil (Hebishy, Buffa, et al., 2017) and several vegetable beverages, such as tiger nut, almond and soybean (Codina-Torrella et al., 2017; Cruz et al., 2007; Valencia-Flores et al., 2013). In the aforementioned studies, the presence of these structures was higher and more abundant as the pressure increased from 100 to 300 MPa, and by increasing the inlet temperature of the samples. However, in general, particle size of those aggregates were lower than oil droplets produced by CH.

1.2.3.2. Rheological properties

Understanding the rheological properties of food products is critical for product development, quality control, sensory assessment, equipment design, and equipment evaluation. Fluid flow behavior can range from Newtonian to non-Newtonian, time-dependent and viscoelastic, and a specific food can display all of these characteristics depending on its origin and components concentration (Rao, 2010).

The role of protein structure in the rheological study of emulsions is complex and fundamental rheological tests provide critical information its behavior, depending on time and on molecular mechanisms involved in its structure (Oakenfull et al., 2017). Colloidal food systems, and especially dairy products, are complex in their composition and structure, and can show wide variation of rheological properties in different conditions. The rheological properties of these products are strongly influenced by temperature, concentration and physical state of the dispersion (Van Vliet & Walstra, 1980).

UHPH treatments can reduce the viscosity of emulsions due to reduced droplet size and enhanced protein dispersion favoring migration to the o/w interface. It has been reported that the apparent viscosity of soymilk was reduced after UHPH treatment (Zamora & Guamis, 2015).

Moreover, in a study conducted by Cruz et al. (2007), in soymilk that were treated at 200 and 300 MPa presented lower viscosity values compare to untreated soymilk, which showed higher particle size. On the contrary, when pressures of 200 and 300 MPa were applied to emulsions with 10 and 20% oil concentrations, resulted in emulsions with similar viscosities to the CH emulsions, nevertheless viscosity increased dramatically when the same pressure was applied to emulsions with 30% oil, with a complete change in shear thinning behavior (Hebishy et al., 2019). A similar trend was observed in another work performed by these authors, which used whey protein isolate to obtain emulsions with oil concentration from 10 to 50% (v/v) under homogenization pressures of 100 and 200 MPa. When oil content raised from 10% to 50% in emulsions treated at 200 MPa, viscosity increased, and flow behavior changed from Newtonian to shear-thinning. This increase in viscosity was more accused in 50% than in 30% oil emulsions (Hebishy, Zamora, et al., 2017a).

1.2.3.3. Color

Food color is influenced by various factors, including pigments, chemical reactions, and physical structure. The reflection and transmission of light may be altered due to the new state of particle distribution caused by the UHPH treatment on the food fluid, thus affecting color parameters, especially luminosity (L*), which is highly dependent on the matrix and the particle size (Moisés et al., 2022). The L* parameter increased significantly in UHPH-treated almond beverages as compared to the base product and pasteurized products on the same day of production and during storage (Ferragut et al., 2015). As mentioned previously, this can be explained by the reduction of particles size by UHPH. Similarly, Pereda et al. (2007) demonstrated that UHPH positively affects the lightness of milk. Milk lightness increased compared to untreated milk due to the increase in light reflected by the fat globules in homogenized milk. However, Serra et al. (2007) found a negative effect on the lightness of skimmed milk treated in the range of 200–300 MPa. These authors related this effect to the aggregation of casein micelles, which reduces the surface of reflection. In another studies conducted by (Cruz et al., 2007; Poliseli-Scopel et al., 2013), authors also attributed the reduction of lightness in soy milk which was related to the lipid-protein and protein aggregates causing a decrease of light reflection. As a result, aggregation state, particle distribution, and type of chromophores in food matrix, as well as the strength of pressure applied, determine the effect of quality color.

1.2.3.4. Oxidative stability

Lipid oxidation in food emulsion is a matter of concern; this is why some new technologies can be a strategy and must be developed to reduce the rate of oxidation (Waraho et al., 2011).

Homogenization systems enhance the interfacial area between oil and water, allowing easier access of oxygen and reactive species to the lipid phase, which would promote oxidation. However, in contrast to CH, in milk, UHPH brings about significant changes to the lipid bilayer structure of the fat globule membrane, leading to the release of encapsulated antioxidants. This release increases the availability and effectiveness of antioxidants in scavenging free radicals and inhibiting oxidation reactions.

Additionally, the shear forces generated during UHPH contribute to the formation of new colloidal structures and the rearrangement of surfactant molecules. These changes result in improved emulsion stability by reducing the exposure of lipids to oxidizing agents. The shear forces effectively reduce the likelihood of lipid oxidation by minimizing the contact between lipids and oxidizing agents.

There have been little studies about the effects of UHPH on o/w emulsions in terms of their oxidative stability. Protein material, which is commonly used as an emulsifier in food emulsions, may undergo structural changes in droplets layers in some extent. Depending on the type and concentration of protein used (Fernandez-Avila & Trujillo, 2016b; Hebishy, Buffa, et al., 2017; Hebishy, Zamora, et al., 2017b) a thick interfacial layer is formed, preventing lipid oxidation (Fernandez-Avila & Trujillo, 2016b).

Some studies (Hebishy, Buffa, et al., 2017; Hebishy, Zamora, et al., 2017b) also demonstrated that o/w emulsions treated with UHPH and prepared with WPI (at 200 MPa), and SC (200-300 MPa) had improved physical and oxidative stability compared to emulsions treated by CH. (Fernandez-Avila & Trujillo, 2016a) treated emulsions enriched in conjugated linoleic acid (CLA, 6%, v/v) and stabilized by soy protein isolates (4%, w/v) by UHPH (200 MPa) to obtain submicron to be incorporated into UHT milk. These authors reported that UHPH produced emulsions with low droplet size, and high oxidative stability. In another work, (Hebishy et al., 2015) was studied the effect of UHPH (100 and 200 MPa) on the antioxidant capacity of whey protein-stabilized emulsions containing 20% sunflower oil. They found that UHPH led to emulsions exhibiting remarkable stability against oxidation in comparison to those treated with CH.

Other studies corroborate the protective effect of UHPH against oxidation (Pereda et al., 2008), demonstrating that UHPH can behave as protective technology against oxidation on emulsion beverages, particularly at 200MPa. As reported in another study (Poliseli-Scopel et al., 2014), where UHPH soymilk was treated at 300 MPa, revealed that in comparison with the counterpart soymilk treated by UHT, the UHPH soymilk had a lower hydroperoxide index and hexanal levels. Hence, the effect of stability to oxidation of UHPH depends to a great extent on the food matrix and their composition.

1.3. Microencapsulation

Encapsulation is a technique to selectively trap desirable compounds (active/core/internal) inside protective materials (wall material/ shell/ external/ encapsulant). This can be achieved at the nanometer (nanoencapsulation), micrometer (microencapsulation), or millimeter scale. In the food industry, microencapsulation plays a crucial role in preserving various food ingredients within microscopic-sized shells or coatings, offering protection and controlled release (Masum et al., 2023). Microencapsulation offers several advantages in the food industry, including (Bamidele & Emmambux, 2021; Boostani & Jafari, 2021; Raza et al., 2020):

- Protection against environmental stressors: It helps prevent the degradation of sensitive food additives and bioactive compounds when exposed to factors like high temperature, light, moisture, extreme pH conditions, or oxygen.
- Isolation of food additives and bioactive compounds: Microencapsulation aids in isolating food additives and/or bioactive compounds present in manufactured foods, reducing unwanted interactions with other food components during production and storage. For instance, it minimizes interactions between metals and unsaturated oils, thereby mitigating oxidation.
- Controlled release of core material: Encapsulation helps reduce or delay the rate of vaporization or transfer of the core material to the external environment. This is particularly beneficial for retaining flavor compounds more effectively.
- Masking unpleasant flavors: Functional ingredients in foods, such as polyphenols and peptides, often have unpleasant flavors. Encapsulation can mask these flavors, making them more palatable while still providing their intended health benefits.
- Encapsulation can improve the bioavailability of bioactive compounds, enhancing their absorption and utilization in the body.

Table 2 provides examples of food ingredients that can benefit from encapsulation. In this thesis, chia and sunflower oils were encapsulated to serve as sources of omega-3 and omega-6 FFAA.

Table 2. Encapsulated functional ingredients and some examples of components (Masum et al., 2023).

Ingredient	**Examples**
Flavoring agent	Sweeteners, seasonings, spices
Acids, bases, and buffers	Citric acid, lactic acid, sodium bicarbonate
Lipids	Omega-3 oils (chia seed, fish, algal oils), conjugated linolenic acid, milk fat
Enzymes and microorganisms	Proteases, probiotic bacteria
Artificial sweeteners	Aspartame, saccharin
Antioxidants	Flavanols, polyphenols
Preservatives	Sorbic acid, calcium propionate
Essential oils	Orange oil, rosemary oil
Minerals	Calcium, iron, zinc
Amino acids and peptides	Milk bioactive peptides
Vitamins and provitamins	Vitamins A, K, C, D
Phytochemicals	Lutein, coenzymes Q10, curcumin

Throughout the 20th century, encapsulation techniques and methods to generate capsules of varied sizes, morphology, active substances, and materials have been developed. The process chosen is highly dependent on the capsule design and function. The terminology used to designate the parts that make up a microcapsule is varied. The encapsulated substance is known as an active, core, payload, internal phase, encapsulate, or fill. The shell, wall, coating, external phase, support phase, or membrane are substances that surrounds the active compound.

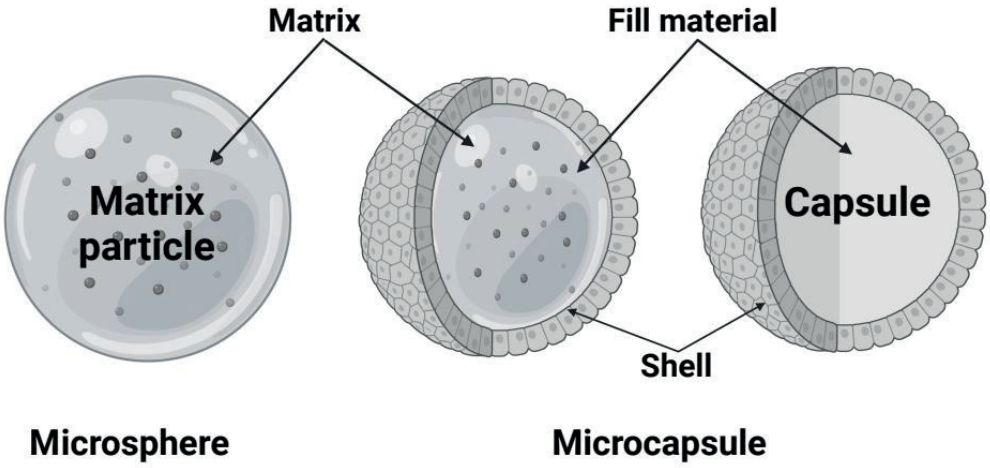


Fig. 12. Encapsulation morphology

The terminology used to designate the parts that make up a microcapsule is varied. The encapsulated substance is known as an active, core, payload, internal phase, encapsulate, or fill. The shell, wall, coating, external phase, support phase, or membrane are substances that surrounds the active compound. Generally, the shell material is insoluble and nonreactive with the core and represents for 1% to 80% of the weight of the microcapsule. Sugars, gums, proteins, natural and modified polysaccharides, lipids, waxes, or synthetic polymers make up the microencapsulation shells (Sobel et al., 2014).

Encapsulation materials are mostly limited in the food industry. To encapsulate hydrophobic actives, a hydrophilic substance must be employed. Edible oils and fats are examples of hydrophobic actives. Encapsulation has been accomplished using a wide range of polysaccharides, proteins, and polymers (Table 3).

Table 3. Materials used for microencapsulation of hydrophobic actives (Sobel et al., 2014).

Polysaccharides (unmodified)	Polysaccharides (modified)	Polysaccharides (gums)	Proteins (vegetable)	Proteins (animal)	Polymers
Sugar	Dextrin	Gum arabic	Soy	Gelatin	PEG
Strach	Cyclodextrin	Alginate	Wheat	Casein	PVA
Glucose syrup	OSA strach	Carrageenan	Corn	WPC	PVP
Maltodextrin	Cellulose	Pectin		WPI	Cellulose derivatives
				Caseinate	Chitosan

OSA, Octenyl succinic anhydride modified starch; PEG, polyethylene glycol; PVA, polyvinyl acetate; PVP, polyvinyl pyrrolidone; WPC, whey protein concentrate; WPI, whey protein isolate.

For creating an appropriate formulation to microencapsulate an active ingredient various consideration such as formulation design, material selection, and process selection need to be considered. Despite the difficulties related to process selection, many technologies offer a wide range of alternatives for developing and manufacturing microcapsules.

1.3.1. Microencapsulation techniques

To mix the shell and core components into microcapsules, several encapsulation techniques can be used. Atomization, spray coating, coextrusion, and emulsion-based procedures are the four broad types utilized to generate microspheres (Sobel et al., 2014). Each generic methodology offers a separate manner of constructing an encapsulated product, with varying benefits and drawbacks. Table 4 summarizes other various techniques for microencapsulation. Phase separation, solvent evaporation, interfacial polymerization, coextrusion, coacervation, nanoencapsulations, and liposomes are all chemical processes involved in the creation of microcapsules.

Table 4. Methods used in microencapsulation (Sobel et al., 2014).

Physical Methods	**Chemical methods**
Spray drying	Phase separation
Spray cooling/chilling	Solvent evaporation
Spinning/rotating disk	Coacervation
Fluidized bed (drying, granulation, and coating)	Interfacial polymerization
Extrusion	Liposome
Coextrusion	Coextrusion
Molecular encapsulation	Nanoencapsulation
Emulsion-based processes	

Apart from different encapsulation methods, some techniques are commonly used for emulsion microencapsulation. Each method has its advantages and limitations, as follows:

- Spray Drying: is a widely used method for encapsulating emulsions. In this process, the emulsion is atomized into fine droplets and sprayed into a drying chamber where hot air is circulated. The rapid evaporation of the solvent results in the formation of solid particles or powder (fully explained in section 3.1.1).
- Freeze Drying (Lyophilization): the process of freeze-drying has been utilized to produce powders with specific properties. This method is particularly appropriate for sensitive bioactive compounds as it utilizes low temperatures to freeze the feed emulsion. The frozen solution is then subjected to very low pressures, causing the formed ice crystals to sublimate. However, it is considered an expensive drying technology due to the use of a pump to create a vacuum condition. Consequently, the complete drying process, which can take between 24 to 48 hours, is associated with high energy consumption (Rezvankhah et al., 2019).
- Coacervation: Coacervation: is a process based on liquid-liquid phase separation, has proven to be an efficacious technique for the generation of microspheres and microcapsules. The core material is dispersed in the polymer solution that forms the

wall, and the encapsulation is achieved by separating the phases through modifications in environmental factors such as pH, ionic strength, and temperature. The technique can be bifurcated into two categories, namely simple coacervation and complex coacervation (Sun et al., 2021).

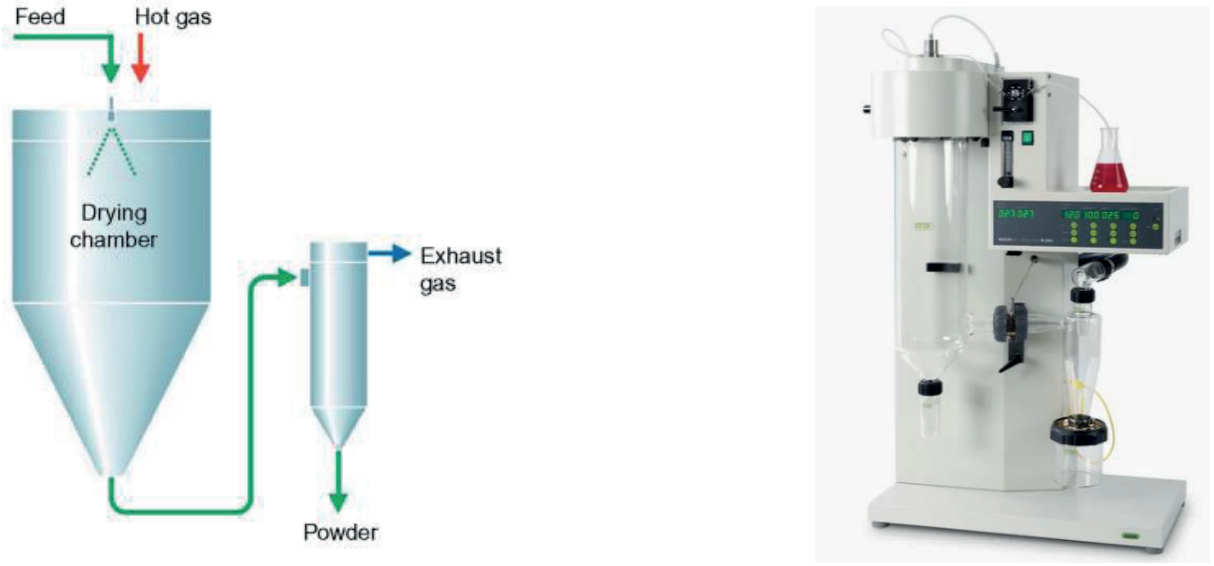
- Spray Chilling: is a microencapsulation methodology that bears resemblance to spray drying; it employs cooled air for the solidification of particles as opposed to heated air for solvent evaporation (Desai & Park, 2005). The carriers utilized in this technique commonly comprise lipids, such as fatty acids, oils, triacylglycerols, waxes, or combinations of these materials. Typically, the active material is dispersed throughout the molten carrier, resulting in emulsions or suspensions. This mixture is then atomized into a cooled chamber, where the droplets solidify. Furthermore, this technique is relatively low-cost in comparison with other methodologies and is considered convenient to scale up (Okuro et al., 2013).

These are some of the methods employed for encapsulating emulsions to obtain powder or solid particles. The choice of method depends on various factors such as the nature of the emulsion, desired particle characteristics, stability requirements, and the properties of the encapsulated material. However, in food industries, the most common technique to obtain powders is spray drying.

1.3.1.1. Spray drying

Spray drying, one of the oldest and commonly utilized techniques used to encapsulate bioactive compounds, has undergone a rapid revolution to address the challenge of transporting large quantities of materials by reducing volume and weight. This process involves transforming a fluid feed into a dry particulate or powder form by spraying it into a hot drying medium (Cal & Sollohub, 2010). Fig. 13a illustrates a common spray drying system, and fig. 13b is a benchtop Buchi B-290 spray dryer that has been used in this study. The encapsulation process consists of four essential steps: preparation of the feeding emulsion, atomization into the drying chamber, and dehydration of the atomized particles. The first step is the preparation of the emulsion, which involves dispersing the ingredients (oil and emulsifying agents) into a solution containing the wall material or carrier agent. Commonly used wall materials for encapsulating bioactive compounds include maltodextrin, gum Arabic, and mixtures thereof (Abdul Mudalip et al., 2021). The premix is then homogenized. Homogenization is important for achieving a stable emulsion with low viscosity, which results in smaller liquid droplets without air inclusion

in the particles. High viscosity solutions can lead to the formation of elongated and large droplets, which negatively affect the drying rate and atomization process.



a **b**

Fig. 13. (a) General spray dryer system. **(b)** Benchtop Buchi B-290 spray dryer.

The third step is atomization, where the liquid feedstock is converted into small liquid droplets by using a nozzle or atomizer. These droplets are sprayed into a hot air stream supplied to the drying chamber. There are three common types of atomization used in spray drying: pressure nozzle atomization, two-fluid nozzle atomization, and centrifugal atomization (Szczap & Jacobs, 2023). Pressure nozzle atomization, as shown in Fig. 14a, requires a positive displacement and high-pressure feed pump to operate. The average particle size produced depends on the feed flow per nozzle and spraying pressure. Two-fluid nozzle atomization, as shown in Fig. 14b, is achieved by contacting the feed with a compressed gas, such as air, which provides the atomization energy (Abdul Mudalip et al., 2021). The products produce from this nozzle may contains an average particle size, depending on the feed flow per nozzle, compressed gas rate and pressure. This method can produce extremely fine particles (10-30 μm) due to its relatively high wear resistance (Kemp et al., 2016).

Centrifugal atomization, as shown in Fig. 14c, works by passing the fluid across or through a rotating wheel or disk. The atomizer motor supplies the energy required for atomization, and the average particle size produced depends on the diameter and rotational speed of the motor (Tian et al., 2017).

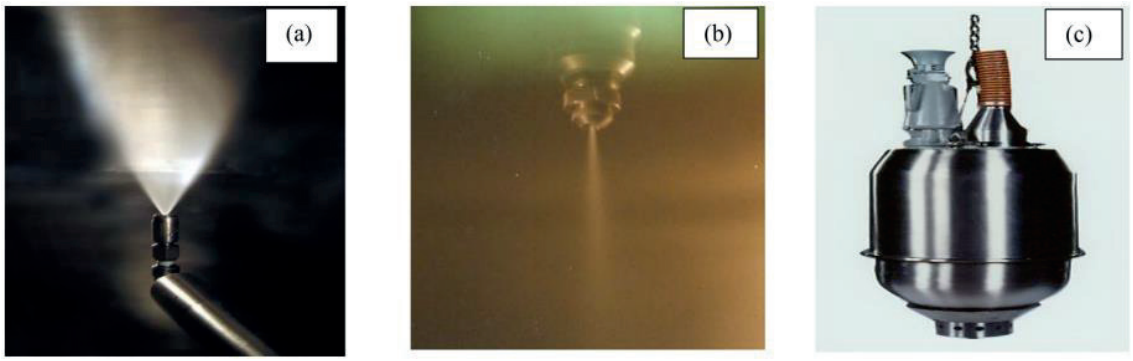


Fig. 14. (a) The pressure nozzle atomization; (b) The two-fluid nozzle atomization; and (c) The centrifugal atomization (Gharsallaoui et al., 2007).

During the atomization stage, a surface for heat and mass transfer is created between the hot air and the liquid sample, promoting the evaporation of solvents, such as water. The evaporation process occurs instantaneously in the co-current atomization operation mode, where the hot air inlet temperature ranges from 150 to 220°C, and the dry powder temperature is moderate (50 to 80°C). This mode of operation provides advantages as it minimizes the effect of thermal degradation. However, the counter current spray drying mode exposes the dry powder to high temperatures, sometimes exceeding 80°C, which is not suitable for bioactive compounds sensitive to high temperatures (Gharsallaoui et al., 2007). The evaporation process dehydrates the liquid sample, causing shrinkage and resulting in dried particles known as encapsulated powder. Finally, the encapsulated powder is collected using a cyclone, electrostatic precipitator, or filter bag. Spray dryers' range in size from less than one liter per hour to hundreds of liters per hour. Table 5 summarizes the optimization of encapsulation conditions of several oil sources. The optimized spray-drying conditions for various ingredients within different wall materials were shown to be different.

Table 5. Optimization of spray drying process conditions for various types of oils.

Core materials	Wall materials		Core: wall	Spray dyer temperature Inlet/outlet (°C)	EE%	Ref.
	Protein	Carbohydrate				
Algal oil	BM	MD	1:1, 1:2	180/ 80	89-94	(Zhang et al., 2020)
Chia oil	-	MD (10 and 20DE) + WPI+ GA		160/ 60	42-96	(Alcântara et al., 2019)
Chia oil	WP	GA	1:5	150/ 82	46-65	(Wangkulangkool et al., 2023)
Chia oil	SPI	GA	1:2	130, 160, 190/52	89-99	(M. Gabriela Bordón et al., 2021)
Chia oil	PP	MD and HC		150/82	87.71-91.97	(Vélez-Erazo et al., 2021)
Chia oil	SPI	GA	1:2	160/90	96.97–98.57	(María Gabriela Bordón et al., 2021)
Echium oil	-	GA	1:2- 1:3	150/na	-	(Comunian et al., 2017)
Chia and carp oil	WP or WP permeate	GA	1:2.8	125/105	43-90	(Lehn et al., 2018)

ARA-rich fungal oil	WPC	MD and Inulin	1:3	180/80	89	(Korma et al., 2019)
Concentrate of fish oil	-	HC	1:4	155-100	97	(Benito-Román et al., 2018)
Pumpkin seed oil	WPC	MD+ goma guar	1-1:1.6	180, 130/ 90	69-74	(Ogrodowska et al., 2017)
Sacha inchi	-	MD and HC	1:10-1:3	170/ 94-100	81-96	(Sanchez-Reinoso & Gutiérrez, 2017)
Fish oil	BM	SMP	1:1.6- 1:2-1:3	180/80	98	(Augustin et al., 2015)
Fish oil	WPI	-		160/na	50-67	(Y. Wang et al., 2016)
Chia oil	SC	Lactose	1:4	170/68-70	68.7	(Castejón et al., 2021)

BM: Buttermilk; GA: Gum arabic; HC: Hi- Cap®; MD: Maltodextrin; PP: Pea protein; SC: Sodium caseinate; SMP: Skimmed milk powder; SPI: Soy protein isolate; WP: Whey protein; WPI: Whey protein isolate.

1.3.2. Wall materials

In the context of encapsulation, a wall material refers to the substance used to create a protective barrier around an object or material. Wall materials play a crucial role in encapsulation processes, preventing the degradation and interaction of encapsulated compounds with external factors such as oxygen, moisture, light, and heat (Xu et al., 2022). These materials provide a versatile platform for encapsulation, offering control over the release kinetics, protection against environmental stresses, and enhancement of the bioavailability of active compounds. Moreover, the choice of wall material also affects the physicochemical characteristics of encapsulated particles, such as size, morphology, and surface properties, which further influence their stability and performance in food systems. A variety of factors influence the selection of wall materials, including expected product objectives and requirements, the nature of the core material, the encapsulation process, economics, and whether the coating material is approved by the Food and Drug Administration (US) or the European Food Safety Authority (Europe) (Madene et al., 2006).

The following attributes should be present in an ideal wall material (Desai & Park, 2005).

- Simple workability throughout encapsulation and high rheological characteristics at high concentrations
- The potential to emulsify or spread the active ingredient and stabilize the resulting emulsion.
- Non-reactivity with the substance to be encapsulated throughout manufacturing and long-term storage.
- The capacity to seal and keeps the active substance inside its structure throughout production and storage.
- The capacity to entirely release the solvent or other ingredients employed during the encapsulation process under drying or other desolventization conditions.
- The capacity to protect the active substance from environmental conditions to the greatest extent possible (e.g., humidity, light, heat, oxygen,).
- Acceptable solvent solubility in the food industry (e.g., ethanol, water).
- Chemical nonreactivity with the active core materials.
- Food-grade status, an affordable price.

In section 3, the number of materials used for microencapsulation is mentioned (Table 3). Modified starch, maltodextrin, gum, and other materials are hydrated for use as the carrier or

wall material in encapsulation. Maltodextrin is a hydrolyzed starch that is useful for encapsulating active substances due to its low viscosity at high concentrations, superior oxidation resistance, and inexpensive cost.

1.3.3. Maltodextrin

Maltodextrins (MD) are nutritional saccharide mixes composed of oligomers and polymers of d-glucose units. These units are joined together in varying-length chains primarily through α-1-4 bonds. Commercial maltodextrins typically contain three to roughly 20 glucose units and have a dextrose equivalent (DE) value of fewer than 20 (Table 6). The production process of maltodextrins involves heat treatment and gelatinization of food-grade starch, commonly corn starch in the United States or wheat starch in Europe. This is followed by partial hydrolysis using safe acids and enzymes (Tiefenbacher, 2017).

MD derived from acid-catalyzed hydrolysis tend to have a high proportion of linear chains that are prone to retrogradation. To produce MD with low hygroscopicity and high-water solubility, a combination of acid catalysis and subsequent amylase-catalyzed hydrolysis is employed. The resulting MD are usually white, free-flowing powders with a bland taste and residual moisture of around 4% to 6% (Lloyd & Nelson, 1984).

MD dissolve rapidly in water, forming sticky solutions. Those with low DE values have a non-sweet flavor and contribute to desirable mouthfeel. There are various applications such as sugar replacement to reduce sweetness in fillings or beverages, as stabilizers, thickeners, and bulking agents in meals. Maltodextrins are also commonly used as co-components in spray-drying processes and as carriers for noncaloric sweeteners, vitamins, spices, and minerals, typically in powder form. Moreover, maltodextrin acts as an oxidation inhibitor, enhancing the stability of encapsulated food components. However, MD exhibit weak emulsifying capability, oil retention, and emulsion stability.

Table 6. Maltodextrins composition (Tiefenbacher, 2017).

DE (Dextrose equivalent)	Glucose	Maltose	Higher saccharides	Sweetness (Sucrose = 10)
10	0.7	2.8	96.5	2
15	1.3	4.7	94	3.3
18	1.6	5.7	92.7	4

MD play an important role as a wall material in the spray-drying process of emulsions. Their functionality in this process includes the following aspects (Xiao et al., 2022):

- Encapsulation: MD act as a protective coating around the emulsion droplets during spray drying. The emulsion, which typically contains oil or fat dispersed in water, is converted into small droplets. MD form a film around these droplets, effectively encapsulating the lipophilic components and protecting them from degradation or interaction with the surrounding environment.
- Powder formation: Spray drying involves the conversion of liquid emulsion into a dry powder form. MDs, with their low moisture content and free-flowing properties, contribute to the formation of fine, free-flowing powders during the drying process. They help stabilize the emulsion droplets and facilitate the formation of dried particles.
- Improved stability: MDs contribute to the enhanced stability of the encapsulated food components within the spray-dried emulsion. They act as oxidation inhibitors, protecting sensitive compounds from degradation caused by exposure to oxygen. By forming a barrier around the encapsulated components, maltodextrins help maintain their integrity and preserve their sensory and nutritional properties.
- Texture and reconstitution properties: The choice of MD as a wall material in spray-dried emulsions can influence the texture and reconstitution properties of the resulting powder. MDs provide desirable mouthfeel characteristics to the reconstituted product and can contribute to its solubility in water. They also play a role in controlling the particle size and density of the spray-dried powder, which can impact its dispersibility and rehydration properties.

Some authors discussed the benefits of using MD in their research (Balasubramani et al., 2015; Tengse et al., 2017). The result of a study indicated that the bulk density and flowability of powders were improved with the addition of MD. Moreover, water sorption isotherms demonstrated that the addition of MD improved powder water absorption (Shi et al., 2020). According to Siccama et al. (2021), increasing the concentration of MD resulted in spray-dried powders with reduced moisture content, less unwanted agglomeration, and an increase in the retention of 1-octen-3-ol, as well as other alcohols and an aldehydes. Furthermore, the proportion of maltodextrin in formulations protected the preservation of a critical flavor component, 1-octen-3-ol. Carneiro et al. (2013) demonstrated that by mixing MD with other protectors might be recommended as a suitable wall material candidate for microencapsulation of flaxseed oil, resulting in high oxidative stability and encapsulation efficiency.

1.4. Fermented dairy products

Fermented foods are very important in human history; the fermentation process was used to extend the shelf-life of different food products. The food undergoes fermentation by edible microorganism, which hydrolyze the macromolecules in the food as polysaccharides, proteins, and lipids, to products with flavors, aromas and textures attractive and pleasant to consumers (Steinkraus, 1997). Moreover, fermentation is one of the oldest techniques of prolonging milk's shelf life. With an estimated value of more than 80 billion euros, fermented dairy products are the most important food industry from an economic point of view (Patrignani et al., 2020), and have a long history and a strong reputation as foods with high nutritional content and various health-promoting properties, such as improved lactose metabolism, lower blood cholesterol, reinforcing immune systems and lowering cancer risk (S. H. Kim & Oh, 2013; Vasiljevic & Shah, 2007). There are around 400 products derived from fermentation of milk. Fermented milks, such as yogurt, have long been an essential component of nutrition and diet among the around 3500 traditional fermented foods that exist globally. More crucially, current findings firmly support an inverse relationship between dairy food intake, particularly low fat varieties and fermented goods, notably yogurt, and the prevalence of type 2 diabetes (Nestel, 2017). On the other hand, they contribute to bone homeostasis by containing calcium, phosphorus, proteins, and micronutrients (Rizzoli & Biver, 2017).

1.4.1. Yogurt

Yogurt is one of the most widely consumed fermented dairy products in the world. Its nutritional benefits and potential health advantages make it highly appealing to consumers. It has a high protein content, calcium, potassium, magnesium, vitamin B2, B6, and B12 (Ayar & Gurlin, 2014). Moreover, yogurt is recognized as a functional food, capable of delivering considerable number of probiotic bacteria providing certain health advantages (Castro et al., 2015). Consumer demand for yogurt and yogurt-related products has increased as a result of these well-known potential health advantages, making it the fastest growing dairy category in the global fermented milk products market (Weerathilake et al., 2014). European legislation (WHO/FAO, 2003) states that the lactic acid bacteria in yogurt (*L. bulgaricus* and *S. thermophilus*) must be viable and present in the final product in a minimum amount of 1×10^7 CFU/g or mL. These thermophilic bacteria growth at temperatures between 42 and 43°C. In the initial phase of fermentation, *S. thermophilus* plays significant role by consuming lactose (which comprises approximately 20-30% of the milk), which is transformed into lactic acid.

Lactic acid reduces the acidity level of yogurt to a pH ranging of 4.5 to 3.7. In this environment, S. *thermophilus* reduces its activity, while *L. bulgaricus* begins to exhibit more activity. This bacterium generates acetaldehyde, which imparts the characteristic taste and sour aroma of yogurt (Alakali et al., 2009). Apart from lactic acid, acetaldehyde, and diacetyl, acetoin, acetone, and 2-butanone are the most important components of yogurt aroma and flavor. Off-flavor development in yogurt is caused by prolonged storage, which is mostly due to the generation of undesired aldehydes and fatty acids during lipid oxidation (Cheng, 2010).

The acidification of milk causes a disturbance in the structural properties of casein micelles, resulting in the approach of caseins to their isoelectric point (pH 4.6). This leads to protein attraction by means of electrostatic and hydrophobic interactions in the system, ultimately forming a solid mass known as curd or coagulum (Lee & Lucey, 2010; Lobato-Calleros et al., 2014).

During acidification of milk, casein micelle has many changes in its integrity. When the pH level decreases to approximately 5.5 to 5.0, the caseins undergo dissociation. Furthermore, as the pH value continues to decrease and approaches the range of 4.6-4.3, the colloidal calcium phosphate (CCP) solubilizes and diffuses into the aqueous phase of the milk. The alterations lead to a reduction of calcium within the casein micelles, ultimately resulting in the coagulation. Furthermore, the charge of caseins undergoes modification, leading to the destabilization at its isoelectric point. This change reduces the negative charge of the casein micelles, which lows the electrostatic repulsion between casein-charged groups and generates aggregates forming a three-dimensional gel matrix (Fig. 15) (Béal & Helinck, 2014).

The resulting curd possesses a gel solid-like texture in which the casein micelles form a network that includes serum and milk fat globules in its final structure (Lobato-Calleros et al., 2014; Sodini et al., 2010; Yildiz, 2016). Related to this topic, Hassan et al. (1995) describe yogurt as a gel with a "continuous three-dimensional network of interconnected molecules or particles that encapsulate a significant volume of a continuous liquid phase".

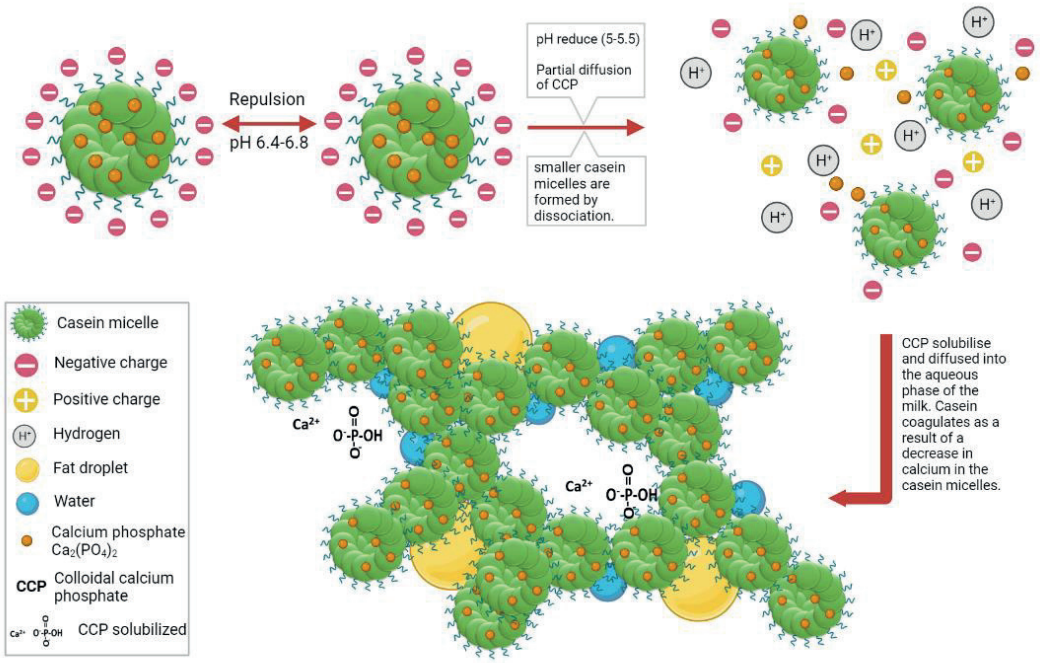


Fig. 15. Yoghurt network formation, interactions with the casein micelles.

One of the most crucial nutrients in dietary patterns are the omega-3 polyunsaturated fatty acids (O–3PUFAs), as their regular consumption has vast potential to improve the health of vital organs within the body. Dairy products, including yogurts, appear to be prospective candidates for fortification with O-3FAs within legal limits, primarily derived from plant-based sources or in encapsulated form, in order to increase the consumption and reach the recommended intake levels for the consumers. In a study conducted by Eker & Karakaya. (2020), the influence of the addition of chia seeds, germinated seeds, and sprouts on the nutritional and beneficial properties of yogurt was investigated. These authors demonstrated that, the addition of chia seed to the yogurt improved the nutritional value of yogurt in terms of fatty acid profile and nutritional content. Chia seed caused an increase in the amount of a-linolenic acid (18:3) and linoleic acid (18:2) in the final product compared to the other types of yogurt. Moreover, another study by Kowaleski et al. (2020) showed that the addition of chia increased the levels of crude protein, total lipids, dietary fiber, and PUFAs, especially omega-3 and the minerals (calcium, copper, potassium, magnesium, zinc, iron, and manganese), adding value to the final product.

1.4.1.1. Yogurt types

Yogurt products can be divided into approximately five categories:

- Set type yogurt is incubated and cooling in the final packaging and has a firm jelly-like structure.
- Stirred yogurt is incubated in a container, and the last coagulum is broken by stirring before cooling and packaging. The texture of stirred yogurt will be less firm compared to set yogurt, like that of very thick cream.
- Drinking yogurt is like stirred yogurt in that the coagulum is broken before cooling. To break the coagulum in drinking yogurt, vigorous agitation is required. After packing, little if any reformation of the coagulum will happen.
- Frozen-type yogurt is inoculated and incubated similarly to stirred yogurt. Nevertheless, cooling is accomplished by pumping through a freezer/chiller/whipper in the same fashion as ice cream. The whipper/freezer, as well as the size and distribution of the ice crystals created, have the most impact on the end product's texture.
- Concentrated yogurt is inoculated and fermented in the same way as stirred yogurt. Following the breakdown of the coagulum, the yogurt is concentrated by boiling out part of the water; this is commonly done under vacuum to reduce the temperature demanded. Heating low-pH yogurt frequently results in completely denatured proteins and unpleasant, gritty textures. This is commonly referred to as “strained” yogurt since the liquid generated from the coagulum after heating used to be strained out in a way similar to preparing soft cheese (S. H. Kim & Oh, 2013).

Stirred yogurt, drinking yogurt, and set yogurt are the three most common types of yogurts. The production steps in the manufacture of stirred, drinking, and set yogurt are illustrated in Fig. 16. The former is made by fermenting yogurt in a tank and packing the yogurt mixture into individual containers. The latter is made by packaging the yogurt mixture into separate containers prior to fermentation (Horiuchi et al., 2009). The first step in making all kinds of yogurt is to make a yogurt mix and heat up. The yogurt mix is designed to contain the required quantity of milk fat, milk protein, and other nonfat milk solids, as well as additional sweeteners, stabilizers, flavors, and colors. The protein level and the solid non-fat content affect the texture and firmness of set and stirred yogurt. Moreover, stabilizers, fruits, aromas, colors, sweetening agents, and preservatives are all used in several types of yogurts.

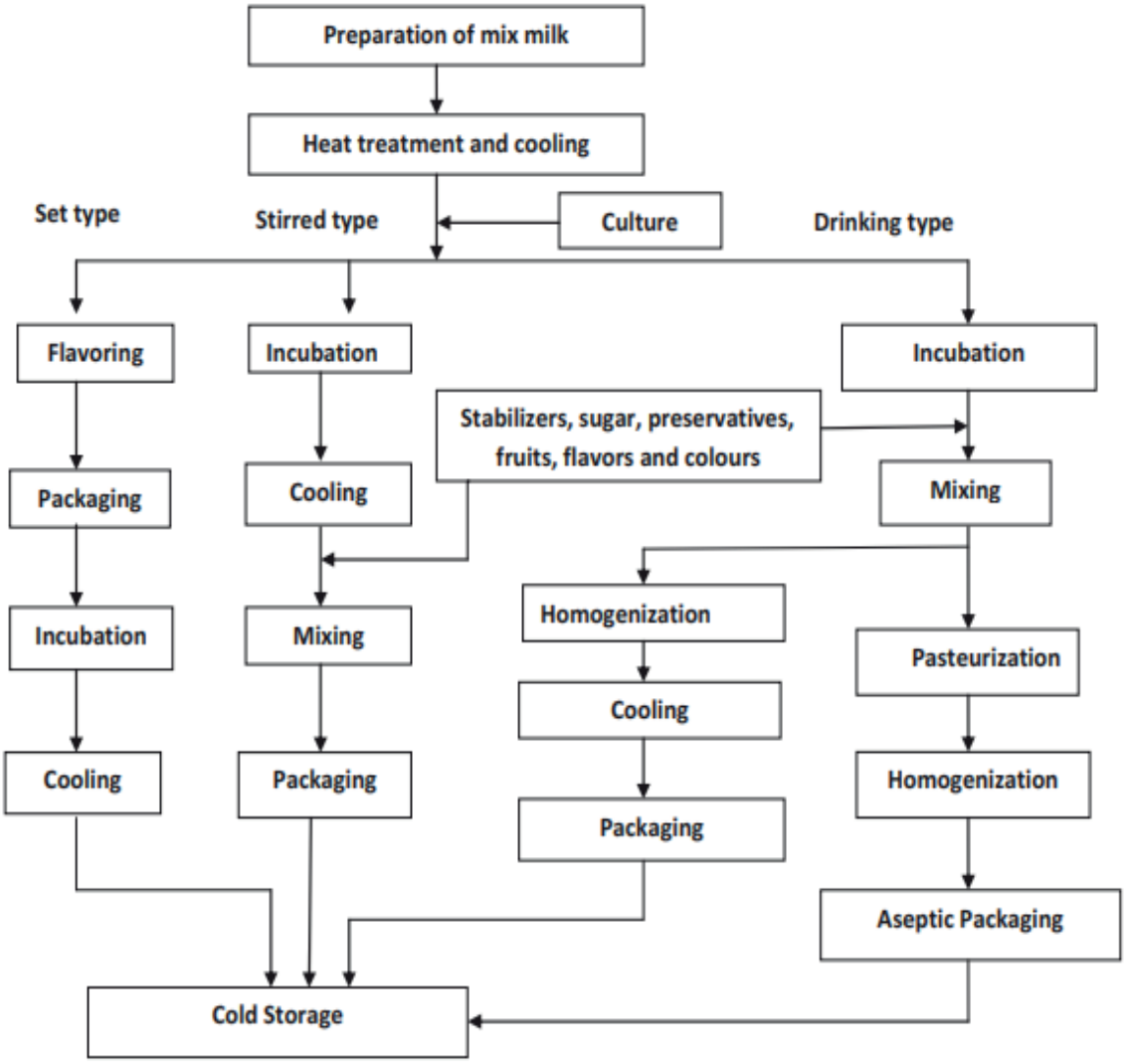


Fig. 16. Flow chart of different yogurt preparation.

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Chapter 2

Objectives and working plans

2.1. Objectives

2.1.1. General objective

The primary objective of this PhD research is to comprehensively investigate the distinctive properties and attributes of functional emulsions produced through the utilization of Ultra-High-Pressure Homogenization (UHPH) technology, which will subsequently undergo spray drying. These emulsions were formulated by combining varying concentrations of buttermilk with polyunsaturated fatty acid-rich vegetable oils, such as chia oil and sunflower oil. The ultimate purpose of these emulsions is to serve as a substitute for milk fat in stirred yogurt, and to assess their overall quality.

2.1.2. Specific objectives

To achieve the objectives abovementioned, the following specific objectives have been conducted:

1. Formulation optimization and emulsion production: Investigate and compare the formulation and emulsion production capabilities of commercial buttermilk for the purpose of generating emulsions suitable for subsequent spray drying. Specifically, compare emulsions obtained through Ultra-High-Pressure Homogenization (UHPH) with those produced using conventional homogenization (CH) methods. Identify the most effective formulations.

2. Spray-dried emulsion (SDE) characterization and oxidative stability assessment: Perform a comprehensive characterization and oxidation evaluation (primary and secondary) in accelerated conditions of SDEs containing PUFAs oil at varying concentrations (4% and 7%) of buttermilk, processed through both UHPH and CH techniques.

3. Evaluation of stirred yogurt: Investigate the coagulation process and the key quality attributes of stirred yogurt incorporating the SDE as a substitute for milk fat.

2.2. working plans

According to the objectives, Figures 1–4 schematically represent the experimental design and analysis of all experiments conducted in the present thesis.

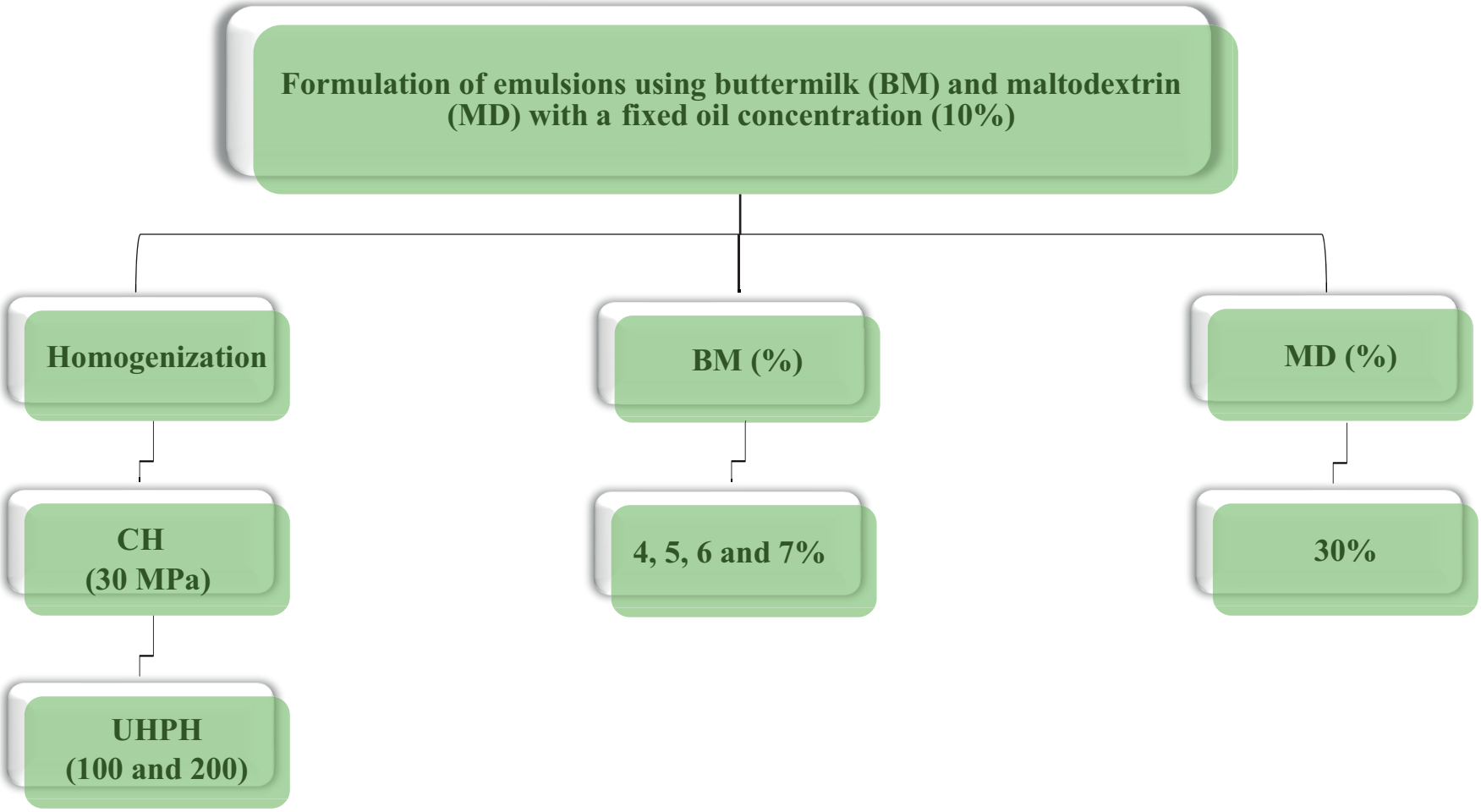


Fig. 1. Protocol for formulating of emulsions and homogenization treatments applied.

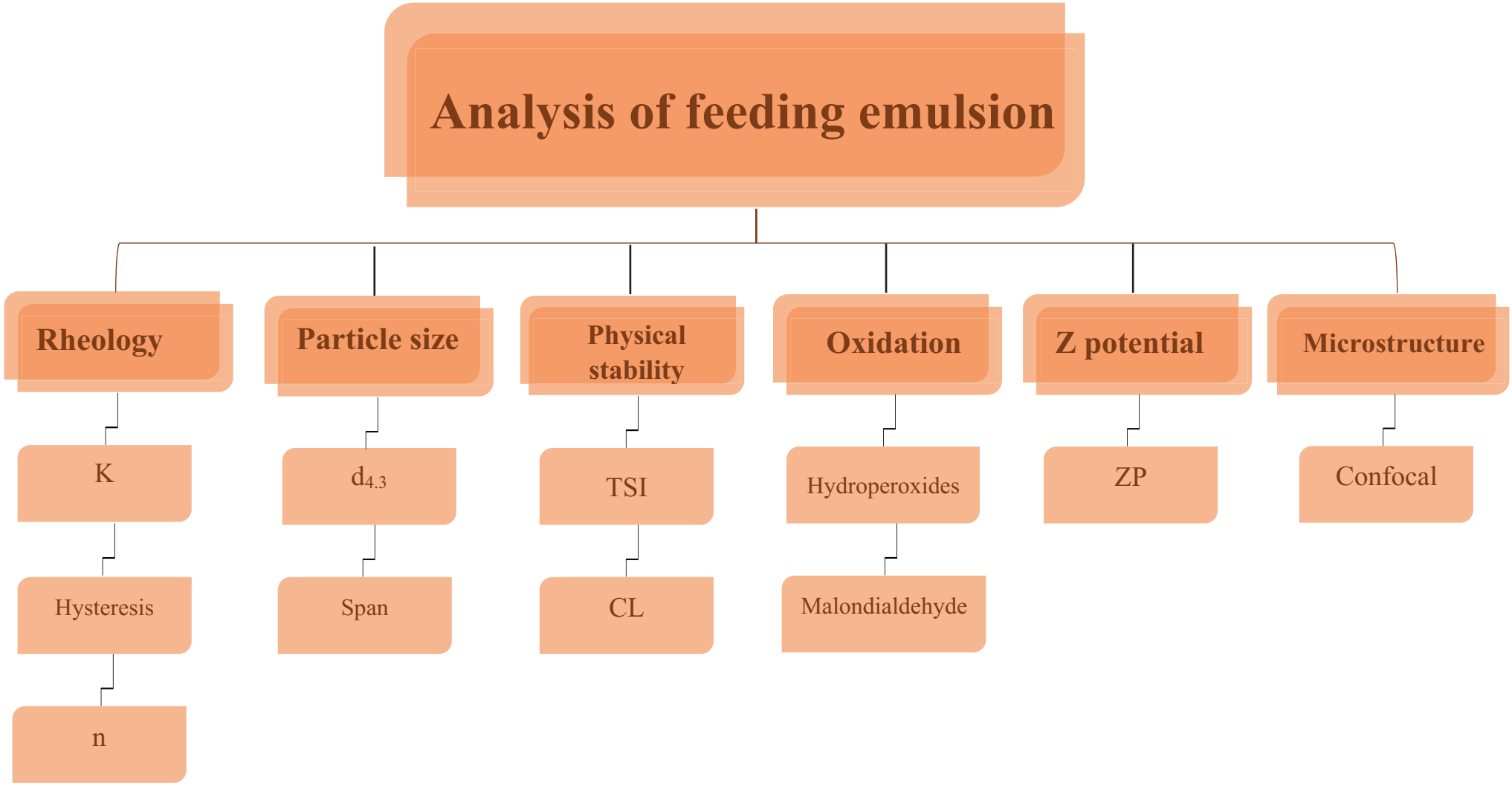


Fig. 2. Working plan corresponding to evaluate the physical and oxidative stability of feeding emulsions.

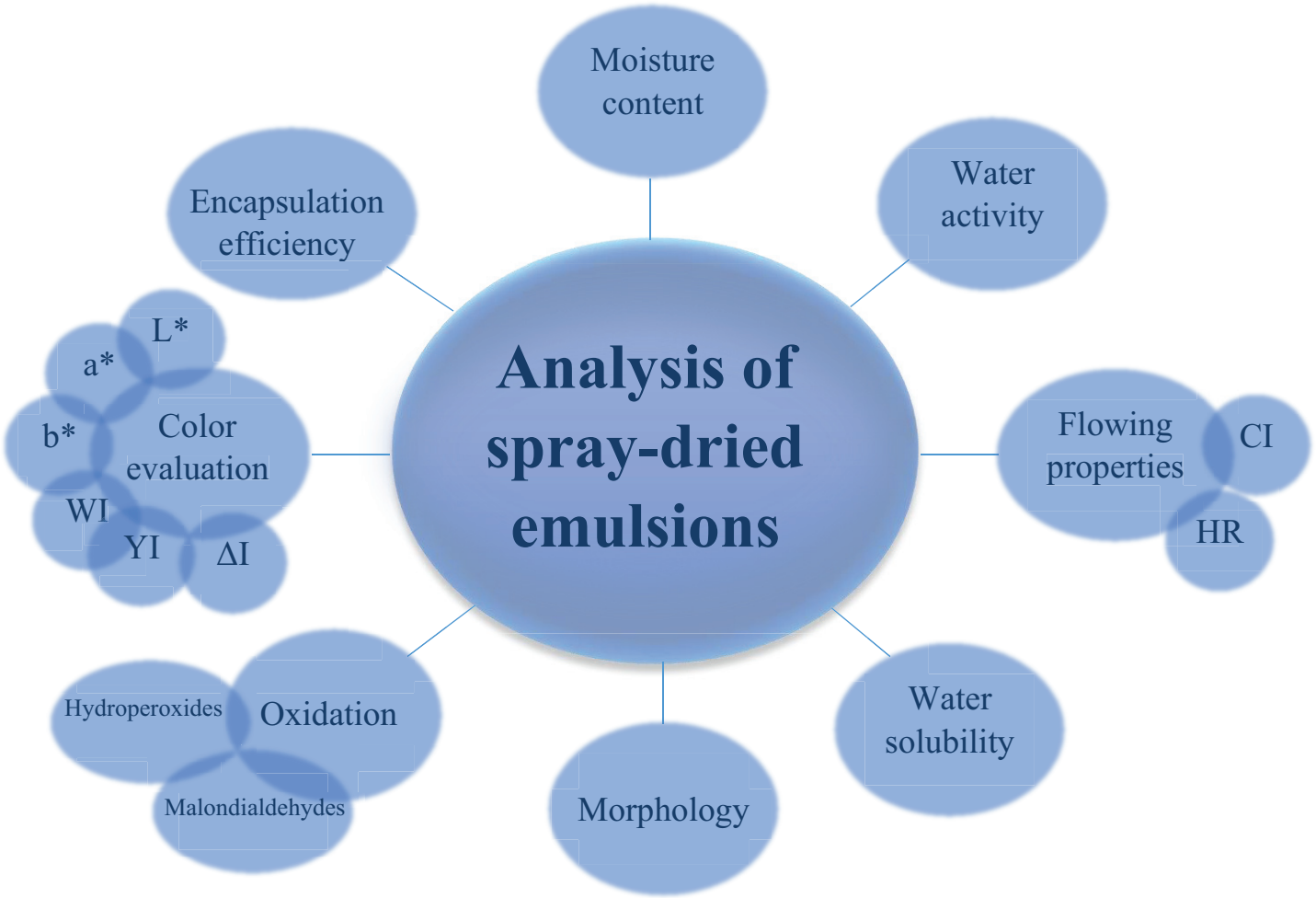


Fig. 3. Working plan corresponding to the study of the characterization and evaluation the oxidative stability of SDE.

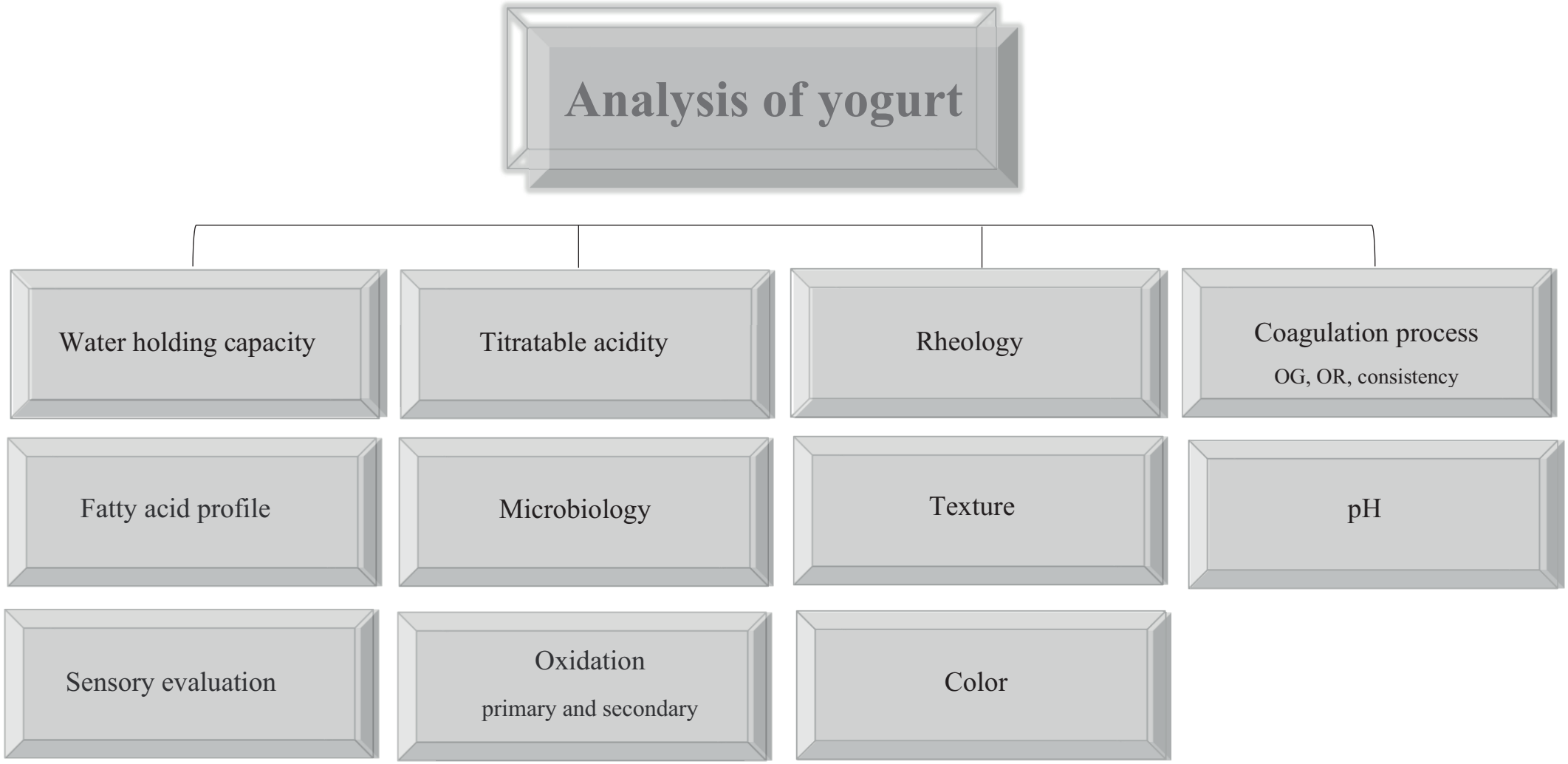
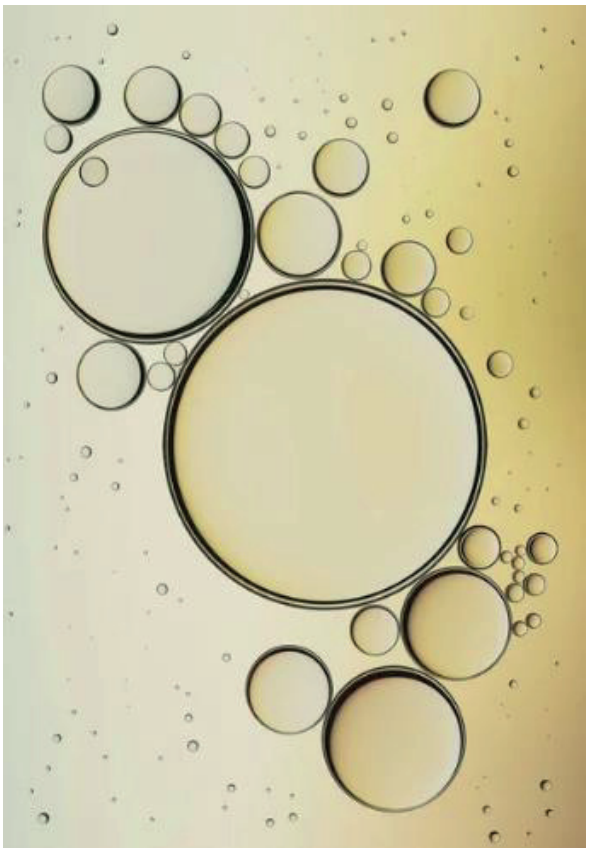


Fig. 4. Working plan corresponding to the study of physico-chemical characteristics of yogurt.



Chapter 3

***Experiment 1*:**

Buttermilk as Encapsulating Agent: Effect of Ultra-High-Pressure Homogenization on Chia Oil-in-Water Liquid Emulsion Formulations for Spray Drying

3.1. Introduction

Consumer tendency to make food choice is greatly based on their healthy characteristics. Those tailored functional foods contain added bioactive ingredients, with a physiological function in the human organism (Kaur & Das, 2011). Within the functional foods offered in the market, there are protective foods for preventing chronic diseases and/or improving some natural protective systems' development. The functional ingredients of interest include a wide range of bioactive components. Among those, omega-3 and some phospholipids have health-promoting activities.

Buttermilk (BM) is the liquid fraction obtained from butter production. It is largely considered as a by-product in the dairy industry, usually incorporated into feed. However, when processed by concentration and spray drying, it is used as an ingredient in food formulations as a partial substitute of milk solids. BM is rich in milk fat globule membrane (MFGM) residues. The MFGM is the natural encapsulating system of milk fat, consisting in a triple layer of phospholipids and proteins, so that it has a great potential as a functional ingredient. In food emulsions, the proteins and phospholipids content of BM has a techno-functional role as stabilizing agents due to their sur-face-active properties, which adsorbed at the interface oil-water create a viscoelastic layer preventing from coalescence (Phan, Le, Van de Walle, Van der Meeren, & Dewettinck, 2016). From a bio-functional point of view, gangliosides, polar lipids and proteins of the MFGM have been described as health-promoting components by preventing infections, improving immunity, protecting and supporting adequate growth in healthy infants, and improving brain and cognitive system development (Hernell, Timby, Domellöf, & Lönnerdal, 2016; Lopez et al., 2017; Singh & Gallier, 2017). At the same time, the use of BM as encapsulating agent, gives a great opportunity for revaluating this by-product of the dairy industry and also providing benefits from an environmental point of view by reducing its waste.

It has been confirmed the potential of BM as a new ingredient for encapsulation in atomized emulsions compared to milk proteins, and as a delivery system for bioactive compounds, such as rich fatty acids (FA) omega-3 oils (Augustin et al., 2015; Zhang et al., 2020). The use of whole BM stream is an opportunity for higher value applications in functional food by capitalizing the dual functionality of BM, i.e., techno-functional and physiological functionality (Augustin et al., 2015). In this sense, the commercial BM could be a valuable ingredient for multiple ap-plications.

Chia (*Salvia hispanica L.*) seed oil is a vegetable source with a high content of ome-ga-3 FA, with up to 67.8% of the total lipid fraction (Gundstone & Padley, 1997). Consumption of omega-3 FA must be incorporated to the human diet since they are essential, and are involved in multiple physiological regulations and protection against heart disease, among others (Kris-Etherton, Harris, & Appel, 2002). In the last revision of FAO/WHO report (FAO, 2008) for consuming recommendation of fats and fatty acids, omega-3 FA dietary intake recommendations were established between 0.5 and 2% of the total fat intake to prevent coronary heart diseases. The use of oils high in linolenic acid such as chia oil is an interesting tool to increase the contribution of omega-3 FA to the diet.

The most common way to incorporate a lipophilic compound to food products is by emulsions encapsulation (McClements, Decker, & Weiss, 2007). Therefore, emulsions consisting in omega-3 FA rich oils encapsulated by buttermilk surface active compounds is a potential way to incorporate those bioactive substances to produce functional foods.

Emulsions are unstable thermodynamic colloidal systems, which exhibit destabilization phenomena such as creaming, aggregation, and coalescence. These phenomena, despite an adequate formulation, take place to some extent during storage. In addition to the quality of emulsifiers, one of the most effective mechanisms to stabilize emulsions is the reduction of the droplet size in terms of both thermodynamic stabilities, by decreasing the attractive interaction of droplets, and kinetics, by delaying the creaming destabilizing process. To produce stable emulsions, high-energy mechanical devices, such as high-pressure homogenizers or sonication equipment, are used at industry. These homogenizers create intense breakdown forces to reduce the size of the pre-emulsion oil droplets. Ultra-high-pressure homogenization (UHPH) is a versatile technology that has the ability to inactivate microorganisms and enzymes, to give rise to submicron emulsions of great physical stability, and to produce some modifications in colloidal structures, due to the high pressures applied ranged from 100 to 350 MPa (Dumay et al., 2013; Roach & Harte, 2008). UHPH have a potential to produce restructuring of the protective layer of the droplets according to the composition of the emulsifying agents used, with repercussions on the techno-functional properties. Several studies (Desrumaux & Marcand, 2002; Dumay et al., 2013; Fernández-Ávila, Escriu, & Trujillo, 2015; Hebishy, Buffa, Juan, Blasco-Moreno, & Trujillo, 2017) performed in emulsions using this technology have demonstrated the improvement of colloidal stability when compared to conventional homogenization.

To make easier handling, transport, and preservation for a longer time of emulsions with encapsulated functional compounds, a common practice is dehydration. Spray drying is one of the most used techniques for the microencapsulation of oils due to the high availability of equipment and low production costs compared to the other methods. Obtaining a homogeneous and stable BME previously to spray drying is decisive (Soottitantawat et al., 2005), and it is directly related to a reduced droplet size distribution, ideally submicronic (Jafari, Assadpoor, Bhandari, & He, 2008).

This study aims to investigate the ability of commercial BM to produce BME suit-able for further spray drying. It is intended to compare the emulsion characteristics of conventional high-pressure homogenization with UHPH treatment for elucidating the ability of this technology for encapsulating omega-3 oil with BM. The drying of emulsions previously processed by UHPH has not been studied yet, thus it is hypothesized that those BME could lead to improved characteristics of powders obtained. For this purpose, this study was mainly focused in the BME characteristics and, in less extent, in those obtained by spray drying.

3.2. Materials and Methods

3.2.1. Materials

Maltodextrin (MD) Glucidex® 19-Maltodextrin was purchased from Roquette Freres (Lestrem, France) with 19 DE. Buttermilk powder (BM) had the following com-position provided by the company: 30% protein, 7% fat, 52% lactose, less than 4% moisture and 7% ash, and was purchased from Activa Food-Tech, S. A. (Girona, Spain). Crude Chia oil (20% C-18:2, >56% C-18:3 according to the specifications) was obtained from Interfat Natural Oils (Barcelona, Spain). Crude Sunflower oil (4–9% C-16:0, 1–7% C18:0, 15–85% C18:1, 50–72% C18:2) was purchased from Gustav Heess Oils (Barcelona, Spain). All other chemical used were of analytical or better grade.

3.2.2. Emulsion Preparation

Four formulations of oil-in-water emulsions were prepared with an oil mixture of chia and sunflower (50:50), MD as wall material and BM as emulsifier. Continuous phase of emulsions was prepared by dispersing individually MD (50% w/w) and BM (30% w/w) with a Thermomix (Vorwek, Wuppertal, Germany) at 2000 rpm for 5 min. The resulting aqueous dispersions were stored overnight at 4 °C for complete hydration. To prepare pre-emulsions, the corresponding weights of MD dispersion to final concentration of 30% (w/w), BM dispersions (to 4–7% w/w)

and water were mixed. Oil was slowly added to the pre-warmed (40 °C) aqueous phase and stirred by using a conventional rotor-stator mixer (Charles Ross & Son Company, Hauppauge, NY, USA) at 15,000 rpm for 5 min. The final solid content of the emulsions varied from 44 to 47% (w/w). Coarse emulsions were further homogenized using conventional or UHPH treatments at 40 °C inlet temperature. Conventional homogenization (CH) of pre-emulsions was performed in a benchtop Homolab (FBF Italia, Sala Baganza PR, Italy) at 30 MPa. Subsequently, heat treatment of emulsions was made at 65 °C, 30 min. UHPH treatments at 100 and 200 MPa were processed in an Ypsicon equipment Model A-60, which is a high-pressure continuous device (60 L/h) (Ypsicon Advance Technologies, S.L., Barcelona, Spain) that works up to 300 MPa. Working temperatures of samples were 40 °C inlet, 60 ± 2 and 80 ± 3 °C, respectively to 100 and 200 MPa at the high-pressure valve, and 25 °C outlet temperature, reached after a quick cooling by a heat exchanger connected to the UHPH equipment. Residence time in the high-pressure valve was less than one second. Emulsions were collected in Pyrex bottles for further sampling and analysis. In Table 1, the designation and composition of emulsions produced and analyzed are detailed.

Table 1. Name of samples and formulation composition of initial emulsions.

Sample Name	H(MPa)	Oil% (*w*/*w*)	MD% (*w*/*w*)	BM% (*w*/*w*)	TS%(*w*/*w*)
4CH	30	10	30	4	44
4UH100	100	10	30	4	44
4UH200	200	10	30	4	44
5CH	30	10	30	5	45
5UH100	100	10	30	5	45
5UH200	200	10	30	5	45
6CH	30	10	30	6	46
6UH100	100	10	30	6	46
6UH200	200	10	30	6	46
7CH	30	10	30	7	47
7UH100	100	10	30	7	47
7UH200	200	10	30	7	47

CH (emulsions processed with conventional homogenizer); UH (emulsions processed with ultra-high-pressure homogenizer); H (homogenization pressure); Oil (50:50, chia:sunflower); MD (maltodrextrin); BM (buttermilk); TS (total solids).

3.2.3. Emulsion Characterization

3.2.3.1. Particle Size Analysis

Particle size and distribution of emulsions were measured in the fresh samples after UHPH or CH treatments using a Mastersizer laser diffraction 2000 analyzer (Malvern Instruments Ltd., Worcestershire, UK). Emulsion were added directly to the recirculating measuring cell containing distilled water or 0.5% SDS solution until 5–9% obscuration was achieved. The optical model based on the Mie theory of light scattering by spherical particles was used. The optical model used were a refractive index of 1.460 for BM, a refractive index of 1.332 for water, and absorption of 0.01. Results were expressed as the volume-weighted mean diameter ($d_{4.3}$, μm) and Span index. Measurements were made separately for water and SDS as dispersing media. Span is a parameter which indicates the homogeneity of the particle size distribution and was calculated using Equation (1).

$$\text{Span} = (d_{90}-d_{10})/d_{50} \quad (1)$$

where d_x (μm) is the size point below which x% of the particles is contained.

3.2.3.2. Zeta Potential

The zeta-potential of emulsions was measured using a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK). Emulsions were diluted 1:100 with ultrapure water and allowed to equilibrate at 25 °C for 120 s in the cuvette prior to analysis. The measurement was performed on the same day of homogenization using an automatic voltage selection. Zeta-potential was calculated using the Smoluchowski model using the software provided by Malvern Instruments.

3.2.3.3. Rheological Evaluation

Flow curves were performed on fresh emulsions (24 h after treatment) at 20 °C with a controlled stress rheometer (Haake Rheo Stress 1, Thermo Electron Corporation, Karlsruhe, Germany). A concentric cylinder probe was used. Samples were loaded into the probe for 5 min before starting the test in order to reach equilibrium.

Flow curves were obtained in ascending and descending shear rates in the range of 0.1 and 100 s^{-1} for 60 s, respectively. Ostwald de Waele rheological model (Equation (2)) were fitted for descending curves, and the rheological parameters (K, n) were obtained.

From the difference between the area under the ascendant and descendant curves, the hysteresis was calculated as indicative of thixotropic behavior.

$$\sigma = K\gamma^n \quad (2)$$

where s is the shear stress (Pa), K is the consistency index (Pa.s^n), g is the shear rate s^{-1}, and n is the flow behavior index (n = 1 indicates Newtonian behavior $n \neq 0$ indicates non-Newtonian behavior).

3.2.3.4. Physical Stability

Colloidal stability of the emulsions was evaluated by using Turbiscan MA 2000 optical analyzer device (Formulaction, Toulouse, France). Emulsions were transferred into borosilicate glass tubes of 27.5 mm dimeter up to 40 mm height, and sodium azide (0.04%) was added to prevent microbial growth. Three tubes of each sample were prepared and stored at 20 °C for 8 days. The evolution of stability was analyzed at 0, 1, 4, 6, and 8 days. This equipment provides a powerful technique for characterization of dispersions, detecting variations in stability phenomena by measuring backscattering (BS) along the sample tubes. Stability index (TSI, Formula 3) and creaming layer thickness evolution was determined by using the software (Turbisoft 2.3.1.125 version) provided by the manufacturer.

$$\mathrm{TSI} = \frac{\sum \mathrm{h}[\mathrm{scan_i(h)} - \mathrm{scan_{i-1}(h)}]}{\mathrm{H}} \quad (3)$$

where $scan_i$ (h) is mean BS for each i of measurement, $scan_{i\text{-}1}$ (h) is mean BS for i-1 measurement, and H is the height of a sample. Higher TSI indicates stronger destabilization caused by particle aggregation and/or dynamic migration.

3.2.3.5. Confocal Observations

A confocal laser-scanning microscope (Leica TCS SP5, Leica Microsystems GmHB, Mannheim, Germany) was used to observe the structure of fresh emulsions (24 h after production). The protein and oil were fluorescently labelled together and separately from phospholipid components of the samples. Proteins were labelled by Fast Green FCF (Sigma-Aldrich, St. Louis, MO, USA), prepared at concentration of 1% in distilled water and subsequent addition of 10% to the emulsion, excited by a 633 nm laser, and detected at 650–750 nm. Oil was labelled by Nile Red (5H-Benzo-phenoxazine-5-one, 9-diethylamino; Sigma-Aldrich, St. Louis, MO, USA), by dissolving 1 mg/mL in acetone. About 100 µL of Nile Red

solution was added to 1 mL of emulsion. Excitation was made at a 488 nm laser and detected at 500–600 nm. Phospholipids were labelled by Liss Rhod PE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-lissamine rhodamine B sulfonyl; 1 mg/mL; Avanti Polar Lipids Inc., St. Louis, MO, USA), by adding 40 µL to 1 mL emulsion, excited by a 561 nm laser and detected at 575–630 nm. All fluorescently labelled samples were mounted on cavity plates and examined at room temperature with a 100× oil immersion objective.

3.2.4. Spray Drying

BME were dried in a Mini Spray-Dryer B-290 (Büchi Labortechnik AG, Flawil, Switzerland). The samples were tempered at 25 °C, and the drying working conditions were 150 °C inlet temperature, 80% aspiration, and 30% feed flow. To improve conservation and reduce possible oxidative damage, the solid samples were collected in aluminum bags that were heat-sealed and stored at –80 °C for further analysis.

3.2.5. Microstructure of SDE

The morphology of the solid emulsions was observed by SEM, using the Quanta ™ 650 FEG scanning electron microscope (FEI Company, Hillsboro, OR, USA), with an accelerating beam voltage (HV) of 5 kV. Samples were prepared by fixing a small amount of powder on metal discs with double-sided carbon tapes, which were then platinum-plated in a Leica EM ACE600 vacuum chamber (Leica Microsystems, Wetzlar, Germany).

3.2.6. Encapsulation Efficiency

Extraction of free oil from SDE was carried out as described by Gonzalez et al. 2016. Dried emulsions (2.00 ± 0.01 g) were weighed and transferred to a beaker containing 30 mL of petroleum ether, stirred for 1 min and filtered. The filter paper was washed with 10 mL of petroleum ether through a pre-weighed flask to evaporate organic solvent under vacuum. Finally, the flask was heated at 105 °C in an oven to constant weight for 1 h. Encapsulation efficiency (EE) was determined according to Equation (4).

$$\mathrm{EE} = \frac{[\mathrm{TO}-\mathrm{SO}]}{\mathrm{TO}} \times 100 \qquad (4)$$

where, TO is the total oil contained in the microcapsules, and SO is the free oil on surface.

3.2.7. Statistical Analysis

Results are presented as mean ± standard deviation. All data were subjected to a one-way analysis of variance (ANOVA) test using the Minitab Express™ version 1.5.3 (Minitab, State College, PA, USA). Significant differences between means were determined by Tukey test. A confidence level of 95% ($p < 0.05$) was used. At least two individual productions of each formulation and treatments were performed. All analysis were replicated three times.

3.3. Results and Discussion

3.3.1. BME Characterization

3.3.1.1. Particle Size and Distribution

The particle size distribution of BM stabilized emulsions is shown in Fig. 1. In Table 2, the volume weighted ($d_{4.3}$) mean values and Span index for emulsions dispersed in water or 0.5% SDS, respectively, are shown.

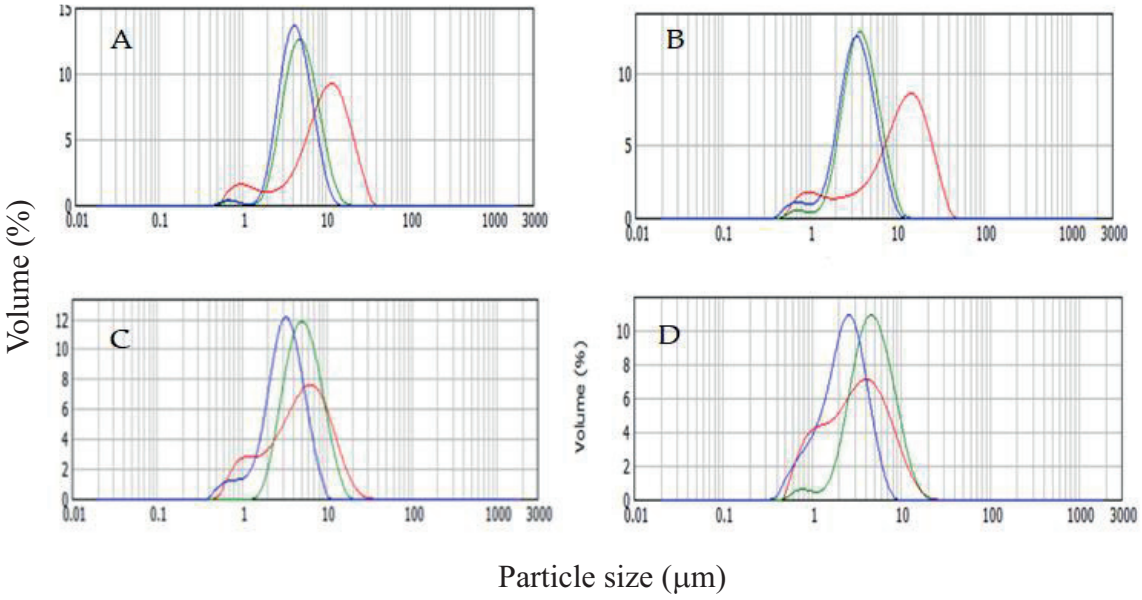


Fig. 1. Particle size distribution curves measured by laser diffraction of emulsions containing different BM concentration: (A) 4%, (B) 5%, (C) 6% and (D) 7%, processed by CH (red), UHPH at 100 MPa (green) and UHPH at 200 MPa (blue).

A bimodal distribution was observed in most of samples represented by a small population of particles in the range of 0.5–1 μm and a big population, which varied in function of BM concentration and homogenization conditions applied. The exception to these distribution curves were the UHPH processed emulsions with 7% BM at 200 MPa, which shifted to a unimodal distribution. The span values indicated narrower peaks of UHPH-treated emulsions compared to CH, indicating a higher homogeneity in the particle size of the former emulsions. In most of BM concentrations formulations, the CH processed emulsions showed higher particle size than UHPH emulsions. $D_{4.3}$ values of CH-processed emulsions decreased as BM concentration increased from 4 to 7%, although differences only were significant ($p < 0.05$) between 4–5 and 6–7% BM concentrations. In UHPH-treated emulsions, increasing pressure from 100 to 200 MPa caused a reduction in $d_{4.3}$ within the same BM concentration, although not always significant. The most accused reduction of $d_{4.3}$ in UHPH treatments was observed in UH200-treated samples as BM concentration increased from 4 to 7%.

Table 2. Droplet size parameters ($d_{4.3}$ and Span index) of fresh emulsions (24 h after production) dispersed in water (w) and SDS.

Emulsions	$d_{4.3}$ (w) μm)	Span (w)	$d_{4.3}$ (SDS) (μm)	Span (SDS)
4CH	13.1 ± 2.50 ^{a}	1.8 ± 0.100 ^{bcd}	2.60 ± 0.500 ^{b}	1.9 ± 0.300 ^{b}
4UH100	5.45 ± 0.02 ^{bc}	1.27 ± 0.01 ^{e}	0.69 ± 0.070 ^{d}	1.00 ± 0.04 ^{cd}
4UH200	4.64 ± 0.03 ^{bcd}	1.19 ± 0.02 ^{e}	0.495 ± 0.008 ^{d}	1.00 ± 0.04 ^{cd}
5CH	11.5 ± 0.90 ^{a}	2.04 ± 0.02 ^{bc}	2.60 ± 0.400 ^{b}	1.7 ± 0.100 ^{b}
5UH100	4.16 ± 0.05 ^{bc}	1.2 ± 0.100 ^{e}	0.56 ± 0.020 ^{d}	1.15 ± 0.01 ^{c}
5UH200	3.65 ± 0.04 ^{de}	1.35 ± 0.02 ^{de}	0.40 ± 0.010 ^{d}	0.89 ± 0.06 ^{cd}
6CH	5.93 ± 0.10 ^{b}	2.21 ± 0.01 ^{b}	1.92 ± 0.010 ^{c}	1.7 ± 0.100 ^{b}
6UH100	5.08 ± 0.10 ^{bcd}	1.39 ± 0.04 ^{de}	0.42 ± 0.040 ^{d}	0.9 ± 0.100 ^{cd}
6UH200	3.44 ± 0.03 ^{de}	2.0 ± 0.600 ^{bc}	0.32 ± 0.010 ^{d}	0.75 ± 0.03 ^{d}
7CH	4.5 ± 0.500 ^{bcd}	2.7 ± 0.300 ^{a}	3.9 ± 0.8000 ^{a}	2.5 ± 0.400 ^{a}
7UH100	4.4 ± 0.900 ^{bcd}	1.59 ± 0.09 ^{cde}	0.43 ± 0.010 ^{d}	0.83 ± 0.03 ^{cd}
7UH200	2.48 ± 0.08 ^{e}	1.61 ± 0.09 ^{cde}	0.30 ± 0.010 ^{d}	0.73 ± 0.03 ^{d}

Means with different letters in the same column are significantly different at $p < 0.05$.

When comparing $d_{4.3}$ values of emulsions dispersed in water and in 0.5% SDS, droplet aggregation phenomena was revealed. Values of $d_{4.3}$ emulsions dispersed in SDS were

considerably lower than those dispersed in water in all cases, which was caused by the breakdown of aggregates by the dispersing agent. In the case of UHPH-processed emulsions, particle size of individual droplets (SDS dispersed droplets) agrees with those reported by Fernandez-Avila et al. (2016), who studied UHPH-treated emulsions prepared with soy protein at 4% and 10% soy oil. In this study, particle size of oil droplets in emulsions processed at 100 and 200 MPa were of 0.3 and 0.4 μm, respectively, which correspond to values found in the present study for formulations with 6 and 7% BM processed at 100 and 200 MPa.

The aggregate formation in UHPH-treated emulsions (Hebishy, Buffa, Juan, Blasco-Moreno, & Trujillo, 2017) prepared with sodium caseinate and sunflower oil, and several vegetable beverages, such as tigernut, almond, and soy (Codina-Torrella, Guamis, Ferragut, & Trujillo, 2017; Cruz et al., 2007; Valencia-Flores, Hernández-Herrero, Guamis, & Ferragut, 2013), have also been described. In the mentioned studies, the presence of these structures were bigger and more abundant as pressure increased from 100 to 300 MPa and increasing inlet temperature of samples. Authors attributed the aggregation formation to partial protein denaturation caused by the combined effect of high pressure and temperature increase in the high-pressure valve during UHPH treatment. In-let temperature of emulsions in UHPH treatments in this study was of 40 ± 3 °C, which rise to 60 ± 4 and 80 ± 3 °C at 100 and 200 MPa, respectively, at the pressure valve, for less than one second. Samples were quickly cold down at 25 ± 2 °C in the heat exchanger connected to the UHPH equipment. Thus, these UHPH conditions were not especially conducive to the aggregate formation. Apart from forces acting in UHPH treatments, responsible for partial denaturation and disintegration of whey proteins and casein micelles, respectively, aggregate formation could be explained by a combined effect of factors. On the one hand, depletion flocculation could be the most possible mechanism of aggregation due to the effective reduction of individual droplets in the UHPH treatments (Berton-Carabin, Ropers, & Genot, 2014). Moreover, the presence of 30% of MD in the formulations could have contributed to droplet aggregation, probably due to the thermodynamic incompatibility between carbohydrates and proteins (Kruif & Tuinier, 2001).

3.3.1.2. Microstructure

BM composition provides techno-functional components involved in the formation and stability of emulsions. Milk proteins, especially caseins, and polar lipids from MFGM are the surface-active components of this ingredient. Polar lipids contain ionic groups, exerting a repulsive force between oil droplets. Proteins stabilize emulsions by forming a viscoelastic layer at the oil–water interphase.

The effect of this adsorbed layer on emulsion stability combines electrostatic and steric repulsion, pre-venting from coalescence. In foods containing both polar lipids and proteins, binding each other through electrostatic and hydrophobic interactions (Mcclements, D J, 2015) may take place. Thus, combined layers of polar lipids and proteins from BM are the most likely oil droplets protection of these emulsions.

The CLSM images (Fig. 2) of freshly prepared emulsions were obtained with selective staining. On the one hand preparations stained to visualize the lipid (red) and protein (green) structures together were made. On the other hand, preparations were stained to observe the polar lipids (cyan). Images showed the difference in micro-structure between CH and UHPH emulsions. A background of protein (green) was observed in CH emulsions, which had a lower surface area of droplets to be protected by surface-active components compared to UHPH-treated emulsions. The presence of aggregates in UHPH-processed emulsions was observed in all formulations independently of BM concentration (not shown). In those emulsions, proteins can be seen located as part of aggregates formed by small oil droplets and acting as bonding material between them.

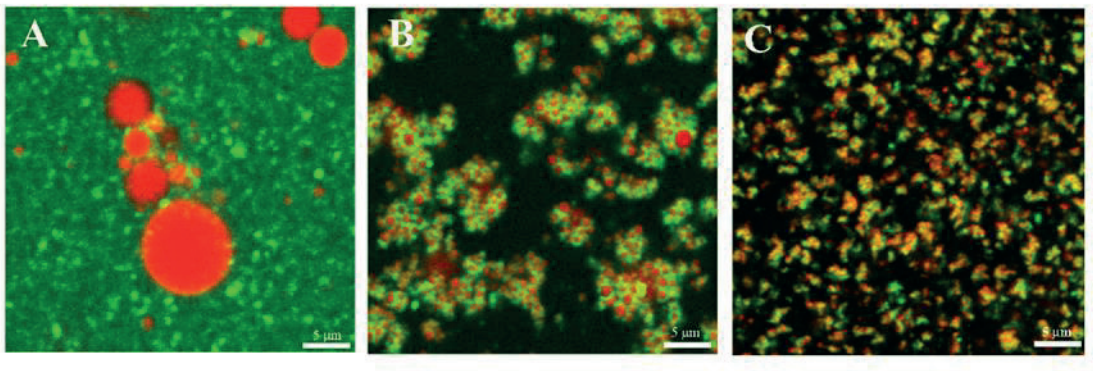


Fig. 2. CLSM images of emulsions containing 5% BM showing the oil droplets aggregates: (**A**) 5CH, (**B**) 5UH100 and (**C**) 5 UH200. Scale bar 5 μm. Oil (red) stained with Nile Red; protein (green) stained with Fast Green FCF.

The difference in aggregates size and arrangement between droplets produced in emulsions is noticeable (Fig. 3). While in CH emulsions the attachment within droplets appeared weak, in UHPH-treated emulsions, there were considerably lower sizes than the previous ones, and the attachment of droplets into the aggregates appeared tightly joined. At 100 MPa, those structures were bigger with irregular shape, while at 200 MPa, a smaller size and regular shape were observed, constituting a more homogeneous disperse phase than those emulsions treated at 100

MPa. This pattern was observed in all BM concentration emulsions. Images of column 3 in Fig. 3, polar lipids distribution around oil droplets can be observed. All images showed a good and homogeneous coverage of droplets. From images, it seems that at least a high percentage of oil droplets are well protected by polar lipids, which probably interacted with proteins to create a combined oil droplets protection.

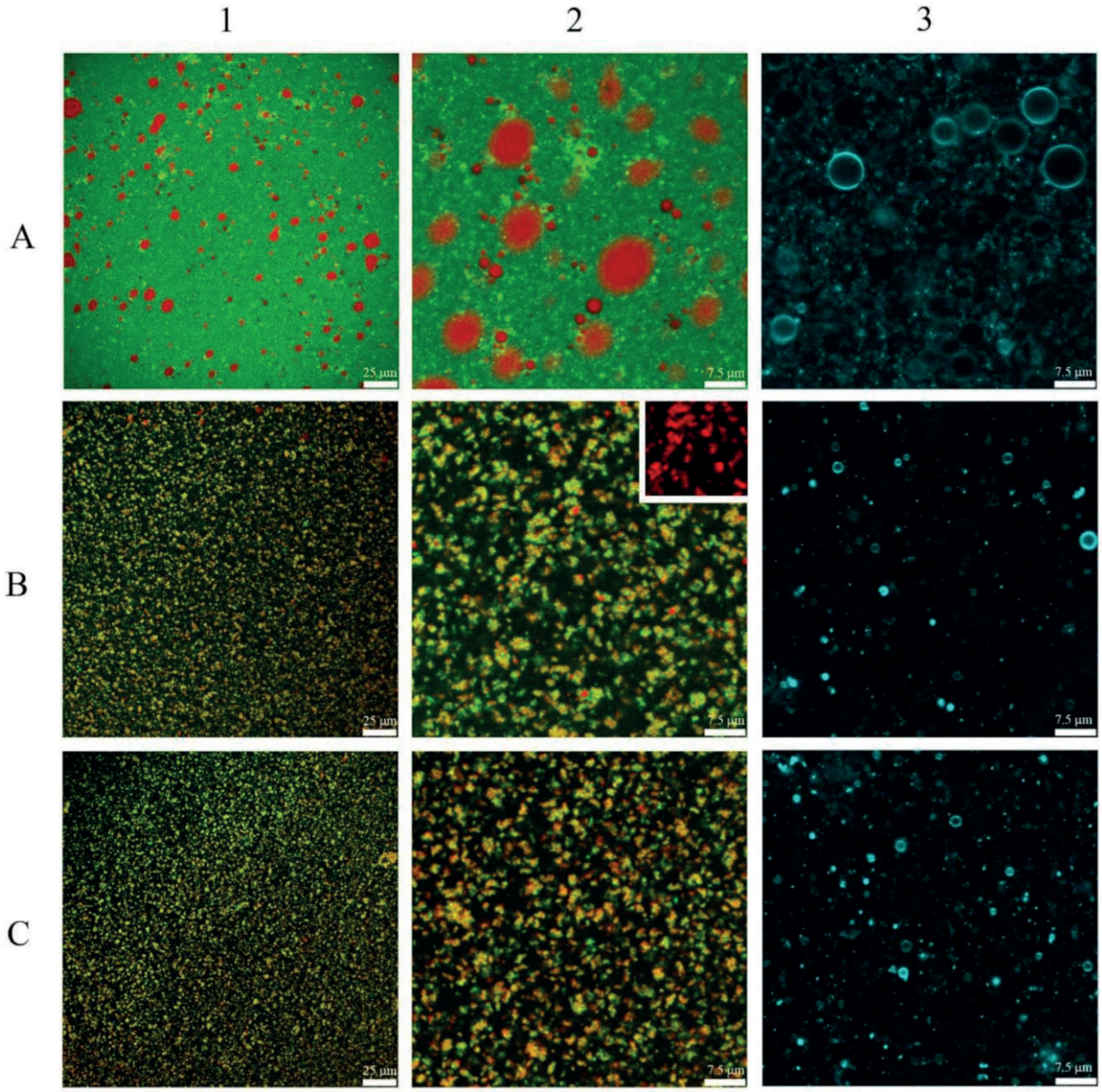


Fig. 3. CLSM images of emulsions containing 7% BM. Columns 1 (scale bar 25 µm) and 2 (scale bar 7.5 µm) are images of the overlay of green labelled protein (stained with Fast Green FCF) and red labelled fat (stained with Nile Red). Column 3 (scale bar 7.5 µm) corresponds to images of cyan labelled polar lipids labelled images (stained with Liss Rhod PE). **(A)** 7CH, **(B)** 7UH100, and **(C)** 7UH200. (B2) Detail of fat labelled aggregates.

3.3.1.3. Rheological Behavior

Flow curves of emulsions were fitted to the Power law and rheological parameters K (consistency index) and n (flow behavior index) of emulsions for descendant curves are shown in Table 3. Flow curves and viscosity curves analysis revealed a non-Newtonian behavior with slight thixotropic character in all emulsions. All samples, to a greater or lesser extent, exhibited a low variation of apparent viscosity values as shear rate increased during shearing. However, time dependence of shearing varied from one to another samples, especially depending on homogenization treatment applied, and to a lesser extent, according to BM concentration. This behavior demonstrates a certain degree of structuration of emulsions due to interactions between colloidal particles, i.e., oil droplets and BM proteins. The no co- incidence of ascendant and descendant shearing curves observed were especially evident at low deformations values in CH and UH200 (from 0 to approximately 30 s^{-1}) independently of BM concentration of formulation. UH100 samples showed a more marked thixotropic character, with hysteresis area being detected nearly in all range of shear rate applied in the flow curves. Hebishy et al. (2019), found a Newtonian behavior in emulsions with 5% sodium caseinate and 10% oil when treated by UHPH or conventional homogenization. The presence of aggregates in the present formulations, and probably the contribution of 30% MD in the continuous phase are responsible of the thixotropic character of emulsions, contributing to a certain degree of structuration of the samples.

Table 3. Rheological parameters (consistency index, K, and flow behavior index, n) of descendant flow curves, and the hysteresis area from flow curves of fresh emulsions (24 h after production.

Emulsions	K (Pa.s ^{n})	n	Hysteresis (Pa/s)
4CH	0.07 ± 0.010 ^{abc}	0.96 ± 0.0100 ^{cd}	5.9 ± 1.20 ^{cd}
4UH100	0.07 ± 0.020 ^{abc}	0.96 ± 0.0100 ^{cd}	10.7 ± 1.1 ^{b}
4UH200	0.045 ± 0.007 ^{c}	0.980 ± 0.007 ^{bcd}	3.4 ± 0.10 ^{d}
5CH	0.042 ± 0.003 ^{e}	0.970 ± 0.009 ^{cd}	4.4 ± 0.60 ^{d}
5UH100	0.06 ± 0.0100 ^{abc}	0.98 ± 0.0200 ^{bcd}	7.8 ± 2.10 ^{bc}
5UH200	0.04 ± 0.0100 ^{de}	1.002 ± 0.001 ^{ab}	3.5 ± 0.20 ^{d}
6CH	0.045 ± 0.007 ^{de}	0.985 ± 0.004 ^{abc}	5.1 ± 0.60 ^{cd}
6UH100	0.083 ± 0.003 ^{ab}	0.95 ± 0.0100 ^{d}	10.1 ± 0.2 ^{b}
6UH200	0.05 ± 0.0100 ^{de}	0.97 ± 0.0100 ^{cd}	4.9 ± 0.10 ^{cd}
7CH	0.057 ± 0.006 ^{cde}	0.981 ± 0.002 ^{abcd}	6.2 ± 0.90 ^{cd}
7UH100	0.09 ± 0.0100 ^{a}	0.974 ± 0.003 ^{bcd}	14.7 ± 3.5 ^{a}
7UH200	0.043 ± 0.003 ^{e}	1.00 ± 0.0100 ^{a}	5.5 ± 0.10 ^{cd}

Means with different letters in the same column are significantly different at $p < 0.05$.

Consistency index and flow behavior index were calculated only in the descendant flow curve to assimilate the flow conditions during most of the operations at industry. K values of samples ranged from 0.02 to 0.09 Pa.s in all formulations. Although results did not reveal a clear pattern within samples in relation to homogenization treatment and BM concentration, some general behavior could be observed attributable to the presence of aggregates. Since viscosity manifestation depends to a great extent not only on particle size, but also on asymmetry of particles, aggregates could be the reason for the observed results. Aggregate formation and destruction do not follow a pattern since they are formed randomly. Thus, the particles shape and their asymmetry may vary to some extent in each individual emulsion production and during shearing. This could be the reason that caused a variability of frictional response during rheological measurements of emulsions with a partial disintegration during shearing. It could be hypothesized that aggregates formed in CH emulsions are easily disintegrated during shearing since those are weakly packed, as can be seen in Fig. 3. Thus, in the descendent curves, K value of CH and UH200 emulsions were closer in most of cases. It could be attributed to particle size. In UH200 emulsions, aggregate sizes were closer to the individual droplets of CH emulsions, as shown in $d_{4.3}$ (SDS) in Table 1.

We assumed that the shearing of UH200 emulsions was not sufficient to breakdown those aggregates, as hysteresis was observed in UH200 samples which showed the lowest values. Thus, viscosity manifestation in these emulsions could be attributed to the friction of small and tightly packed homogeneous aggregates. However, UH100 emulsions exhibited the highest K and hysteresis values. As mentioned previously, these samples had bigger aggregate sizes than UH200. Probably the pressure difference of UHPH from 100 to 200 MPa is responsible for different degrees in casein micelles disintegration and conformation (Roach & Harte, 2008) in both 100 and 200 UHPH treatments, producing the observed differences in aggregate size and interaction forces. In this sense, during shearing of UH100 emulsions, the manifestation of higher K values and hysteresis may be due to a partial destruction of aggregates, which provoked a higher structuration degree and internal friction during flowing.

In any case, the viscosity values in all emulsion formulations ranged in values far from the maximum suitable for spray dried, being the final objective of these emulsions. Di Battista et al. (2015) confirmed that to obtain a good atomization, viscosity of emulsion has to be lower than 0.3 Pa.s.

3.3.1.4. Physical Stability

In this study, the influence of homogenization treatments and BM concentration was evaluated by using Turbiscan® equipment during 8 days of storage at 20 °C. The backscattering (BS) at t = 0 was considered as reference to analyze the stability evolution of the system. The evolution curves during monitoring of samples by obtaining the difference of backscattering (ΔBS) gives information about the destabilization phenomena such as creaming, flocculation and coalesce, which are the common phenomena occurring in emulsions during storage. The reduction of BS in the bottom of the vial containing the sample and the increase of BS values in the top area were observed in all the ΔBS profiles of samples, indicating that creaming was the main destabilization mechanism in these emulsions.

The velocity of migration of the oil droplets and aggregates toward the surface is mainly determined by their particle size, the viscosity of the continuous phase, and by the density difference between the continuous and the disperse phases (Mcclements, D J, 2015). From the evolution of ΔBS profiles during eight days, a global stability index (TSI, Fig. 4) and creaming layer thickness (CL) at day 1 and day 8 (Table 4) were calculated.

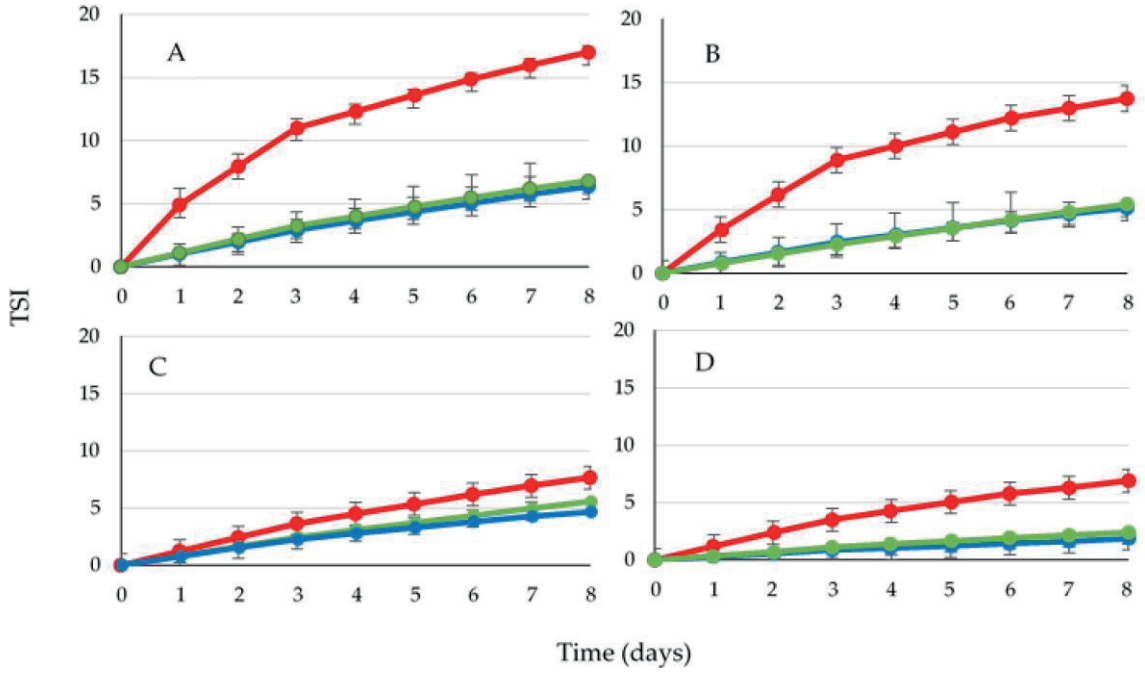


Fig. 4. Evolution during 8 days storage of stability index (TSI) of emulsions with different BM concentration and homogenization treatments: **(A)** 4% BM; **(B)** 5% BM; **(C)** 6% BM; **(D)** 7% BM. CH (red line); UH100 (green line); UH200 (blue line).

CH emulsions always presented higher values of TSI than UHPH samples, indicating that the former were less stable than the latter. UHPH samples barely showed differences in stability between 100 and 200 MPa. With the same tendency, the effect of BM concentration, was significant. At 4 and 5% of BM, the TSI difference was more marked between CH and UHPH samples than in 6 and 7% BM emulsions. Related to the CL values (Table 4), the results were concomitant with TSI values. No significant differences ($p < 0.05$) were found between UHPH treatments, while they were significant as compared to the CH emulsions.

Table 4. Stability parameters (creaming layer, CL) of fresh (1 day) and stored emulsions (8 days) from backscattering measurements curves, and zeta-potential of fresh emulsions (1 day).

Emulsions	CL-d1 (mm)	CL-d8 (mm)	Zeta-Potential (mV)
4CH	3.8 ± 0.4 ^{a}	9.7 ± 0.9 ^{a}	−26 ± 1 ^{bcd}
4UH100	0.8 ± 0.3 ^{c}	3.3 ± 0.6 ^{b}	−29 ± 2 ^{d}
4UH200	0.7 ± 0.3 ^{c}	2.8 ± 0.4 ^{b}	−22 ± 1 ^{a}
5CH	4.2 ± 1.0 ^{a}	12 ± 3.0 ^{a}	−28 ± 1 ^{cd}
5UH100	0.8 ± 0.4 ^{c}	4.4 ± 0.8 ^{b}	−23 ± 1 ^{ab}
5UH200	0.7 ± 0.2 ^{c}	4.2 ± 0.5 ^{b}	−25 ± 1 ^{abcd}
6CH	1.3 ± 0.8 ^{bc}	5.1 ± 2.0 ^{b}	−27 ± 2 ^{bcd}
6UH100	1.0 ± 0.1 ^{c}	4.4 ± 1.0 ^{b}	−23 ± 1 ^{abc}
6UH200	0.7 ± 0.2 ^{c}	3.1 ± 0.4 ^{b}	−28 ± 1 ^{abcd}
7CH	2.2 ± 0.400 ^{b}	4.8 ± 2.0 ^{b}	−27 ± 4 ^{cd}
7UH100	0.53 ± 0.05 ^{c}	2.3 ± 0.4 ^{b}	−25 ± 1 ^{abc}
7UH200	0.38 ± 0.08 ^{c}	2.1 ± 0.6 ^{b}	−25 ± 1 ^{abc}

Means with different letters in the same column are significantly different at $p < 0.05$.

Although the influence of BM concentration on CL in UHPH treatments was not significant statistically ($p < 0.05$), there was a correlation of creaming layer thickness with the type and degree of homogenization applied, which in turn was in line with the particle size values observed (Table 2). The presence of big aggregates in the CH samples and the flocculation phenomenon observed during the storage, which was observed in the ΔBS curves, explain the high tendency of these samples towards creaming compared to UHPH-treated emulsions. In the ΔBS curves, the flocculation phenomenon is observed if there is separation of the profiles in the middle of the vial and the different days of monitoring. In UHPH emulsions, the aggregation phenomena was found in all formulations from the beginning; however, aggregates behaved as small particles with limited mobility to the top. Flocculation was not observed during the storage in any of UHPH samples as the ΔBS profiles showed.

In addition to the type of homogenization applied, the BM concentration appeared as the main factor in TSI index and CL values. Increasing BM from 4–5 to 6–7% might increase the amount of adsorbed protein at the interface, with a closer packing layer formation resulting in higher stability (Guo & Mu, 2011).

Zeta-potential is the charge that develops at the interface between particles and the continuous, and it is used to evaluate the colloidal stability in dispersed systems. Zeta- potential is also related to the ionized groups from protein layers that surround oil droplets, when the pH was above the isoelectric point (Hu, McClements, & Decker, 2003). Values of zeta-potential were in the range from -22 to -29, which are near, but not in the optimum range values considered to have good stability due to electrostatically repulsion (i.e., higher/lesser than ±30 mV). Thus, the stability of these emulsions by electrostatic repulsion was not the most relevant contribution. There was no correlation of zeta-potential with homogenization procedure, nor with BM concentration. This variability in zeta-potential values might be related to the heterogeneous distribution of proteins into the aggregates observed, especially in UHPH emulsions, in which, zeta-potential values had less negative charge. Probably, the protein was occluded inside the aggregates, hindering the exposure to the surface of those colloidal structures.

3.3.2. Spray-Dried Emulsions

3.3.2.1. Encapsulation Efficiency

The degree to which wall material can prevent the internal oil extraction is given by the encapsulation efficiency (EE) (Hogan, McNamee, Dolores O'Riordan, & O'Sullivan, 2001). This is an important parameter taken into consideration for the quality of dried emulsions. In Fig. 5, comparison of the SDE with different formulations and homogenization treatments applied to BME can be seen.

The main formulation factors affecting encapsulation efficiency are total solid con- tent and the ratio of oil to wall material and oil content. In the present emulsions, total solid content varied from 44 to 47%, and oil content was from 21.3 to 22.7% (dry basis) corresponding to formulations with 4 to 7% BM. MD content remained constant (30%) in all emulsions. The corresponding ratio of oil to wall material varied from 1: 3.4 to 1:3.7 according to the increase of BM content. In agreement to previous studies reported by Botrel et al. (2014), the optimum for encapsulation efficiency in terms of mass ratio of oil to wall material could be 1:3 and less than 1:4 (w/w).

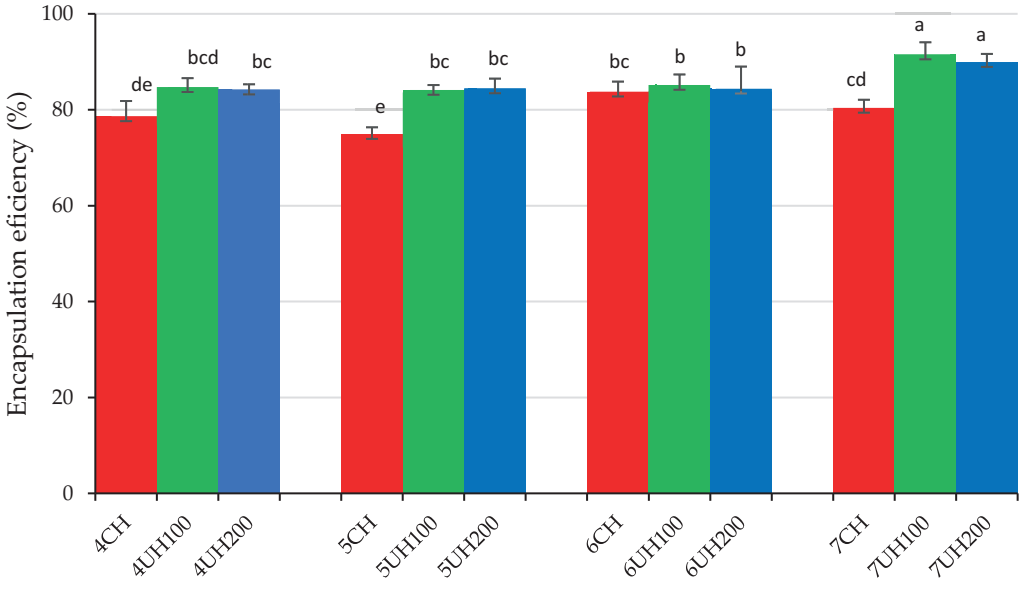


Fig. 5. Encapsulation efficiency of the dry emulsions from the different homogenization treatments (CH, UHPH at 100 MPa and UHPH at 200 MPa) applied to the feed emulsions and containing different percentages (w/w) of BM (4, 5, 6 and 7%).

Thus, the present formulations are in range. Values of free oil on the surface of powder particles decreased, in general, as total BM concentration increased for all samples. Within the same BM content, the homogenization technology applied significantly ($p < 0.05$) affected the free oil on the surface, which was always higher in CH emulsions, indicating the positive effect of UHPH technology on the encapsulation efficiency (Fig. 6). However, the pressure intensity applied in UHPH produced similar results. A significant improvement of encapsulation efficiency was observed in formulations with 7% BM, which had around 90% EE. Especially in those emulsions, the difference between CH and UHPH was more marked, indicating that probably the colloidal structures created in the UHPH processing could be responsible for maintaining a better oil encapsulation. The small oil droplets attached by proteins in the aggregates would create a protective system for oil migration to the surface. BM containing casein, whey protein, and phospholipids has good emulsifying properties contributing to oil protection. Zhang et al. (2020) reported encapsulation efficiency values between 89.6 and 94.3% in SDE formulated with BM, with or without MD as wall materials. Although the formulations differed substantially in BM content from that of the present study, those authors attributed to BM a great oil encapsulating capacity and found that increasing MD from 33 to 66.7% considerably decreased the encapsulation efficiency.

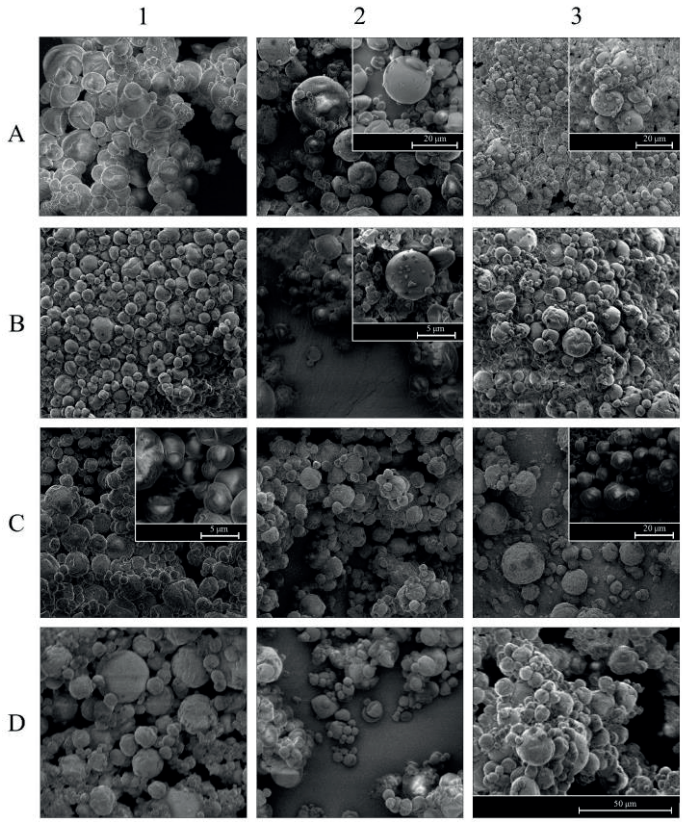


Fig. 6. SEM images (2691×) of emulsions obtained by the different homogenization treatments: (**1**) CH; (**2**) UHPH at 100 MPa; (**3**) UHPH at 200 MPa, with different BM concentration: (**A**) 4% BM; (**B**) 5% BM; (**C**) 6% BM; (**D**) 7% BM. Magnification of overlapped images are: (**A2**) 6177×; (**A3**) 4974×; (**B2**) 9767×; (**C1**) 20614×; (**C3**) 5267×.

3.3.2.2. Morphology

The morphological structure of the obtained microcapsules after the spray-drying of emulsions was examined by scanning electron microscopy. The SEM images of the dried emulsions are illustrated in Fig. 6. All the images showed microcapsules with similar aspect, always being spherical, without apparent fractures, and with more or less rough surfaces without noticeable differences between the powders with different percentages of BM or homogenization treatments.

In all the SDE, heterogeneity in particle sizes could be observed, ranging from 5 to 20 µm, clearly distinguishing at least two populations of different sizes. This size variety has also been observed in different studies (Benito-Román, de Paz, Melgosa, Beltrán, & Sanz, 2018; Carneiro, Tonon, Grosso, & Hubinger, 2013), and is a typical characteristic presented by particles that have been spray dried.

Depression and superficial folds were observed in some microcapsules; this may be due to the sudden contraction that occurs in the early stages of drying. The viscoelastic properties of emulsifiers influence the appearance of these folds (Botrel, Borges, Fernandes, & Lourenço Do Carmo, 2014), which may be due, in this study, to the presence of MD in the formulation of the emulsions. Korma et al. (2019) observed that emulsions formulated with whey proteins and maltodextrin had a higher number of dents than those formulated with whey proteins alone.

In dry emulsions obtained by conventional homogenization (Augustin et al., 2015), formulated with MD and BM at different concentrations, the integrity of the microcapsules observed in the SEM images of this study indicated that BM had good properties to form a film, which efficiently protected seaweed oil contained in capsules.

3.4. Conclusions

From this study, it can be concluded that commercial BM could be applied to stabilize emulsions with omega-3 oil for further spray drying. The application of UHPH technology improved the global quality characteristics of BME in terms of stability. These formula- tions, especially the UHPH-processed emulsions, induced the aggregation of oil droplets when combining MD and BM. However, the particle size of the colloidal structures in the UHPH-treated emulsions was similar or lower than the individual oil droplets produced by conventional homogenization. The presence of aggregates seems to be relevant in the rheological behavior of all emulsions studied, with a slight thixotropic character, although the equilibrium viscosity at relative low deformations showed adequate values for spray drying purposes. Results of SDE obtained to evaluate the influence of UHPH technology applied to the BME showed good behavior in terms of powder morphology and encapsulating efficiency. The formulation containing 7% BM presented the best general characteristics in both BME and SDE.

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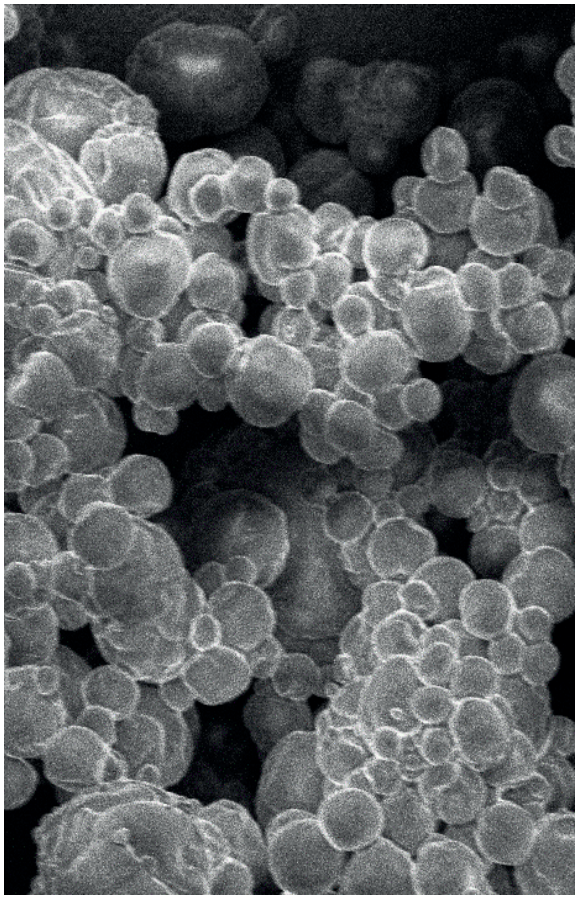

Chapter 4

***Experiment 2*:**

Characterization and oxidation stability of spray-dried emulsions with omega-3 oil and buttermilk processed by ultra-high-pressure homogenization (UHPH).

4.1. Introduction

Substituting or enriching the lipid fraction of foods with oils rich in polyunsaturated omega-3 offers the opportunity to consumers to in- crease the daily intake of these components, which in general is insufficient in the diet of most of the population (Kris-Etherton, Harris, & Appel, 2002). In addition, if buttermilk (BM) is also incorporated in the emulsion as an emulsifier, which is also recognized as a biofunctional compound (Ali, 2019; Hernell, Timby, Domello¨f, & Lo¨nnerdal, 2016; Singh & Gallier, 2017; Vanderghem et al., 2010), the potential advantages of the obtained emulsion are multiple. BM is a by-product of butter production, with a market maintaining an average annual growth rate of 1.16% from 2013 to 2016 (Ali, 2019). Its use in the food industry includes the production dry mixes, bakery products, and dairy products such as cheese or yoghurt (Ali, 2019). Thus, BM may increase the added value of food products to which it is supplemented, such as, infant formulae. BM is rich in milk fat globule membrane (MFGM), composed mainly by phospholipids, sphingolipids, and glycoproteins, all of them responsible for giving BM functional value (He et al., 2017; Lopez et al., 2017). These compounds have shown beneficial health effects, such as lowering of cholesterol levels and improved brain development and cognitive function in infants, within others (He et al., 2017; Lopez et al., 2017; Spitsberg, 2005). The use of oil-in-water emulsions is common in the pharmaceutical and food industries for encapsulating bioactive lipids as delivery systems of lipophilic functional components. Spray drying is the most widely used technology in the food sector for the preparation of solid emulsions due to its wide availability, versatility, and low cost.

Microencapsulation of oil in SDE requires droplets to be surrounded by a coating in a homogeneous or heterogeneous matrix to give small capsules providing a physical barrier between the core compound and other components of the product. Thus, the combination of an emulsifying agent to adsorb at the oil-water interface and a polysaccharide with high solid content and low viscosity is required to create the matrix in which droplets are embedded. Both types of compounds, emulsifiers, and polysaccharides, are commonly called wall materials. Maltodextrin (MD) is one of the most widely used polysaccharides in SDE combined with an emulsifying material, in this case BM.

Although drying of emulsions is a good system for the protection and release of bioactive compounds, unsaturated fatty acids are susceptible to oxidation. However, as reported by Augustin et al. (2015) whole BM powder was found to be better to high heat skim milk powder

as an encapsulant to produce recombined omega-3 oil powders, founding a lower oxidation in BM emulsions. Similarly, another study (Zhang et al., 2020) using algal oil encapsulated with 100% or 50% buttermilk mixed with 50% maltodextrin showed good EE and oxidation stability of spray dried emulsions.

Homogenization systems produce fine emulsions, which show different degrees of oil protection against oxidation. UHPH processing may work up to 400 MPa (Hebishy, Buffa, Juan, Blasco-Moreno, & Trujillo, 2017). Currently, the UHPH technology capable of reaching up to 400 MPa is under development. Therefore, it is considered an emerging technology with great potential for the food, pharmaceutical and other industries in which colloidal products require great physical stability, as well as a microbial reduction. Mechanisms acting in UHPH are great intensity physical forces on dispersed particles such as friction, compression, acceleration, and shear resulting in great size reduction of particles, including microorganisms. All this result in high physical stability of dispersions, and microorganisms' inactivation when compared with conventional high-pressure homogenization systems (Dumay et al., 2013; Fernandez-Avila, Arranz, Guri, Trujillo, & Corredig, 2015; Hebishy, Buffa, Guamis, Blasco-Moreno, & Trujillo, 2015). On the other hand, protein material usually used as emulsifiers in food emulsions, may also experience structural modifications to some extent, depending on the type of protein incorporated (Fernandez-Avila & Trujillo, 2016; Hebishy, Buffa, et al., 2017; Hebishy, Zamora, Buffa, Blasco-Moreno, & Trujillo, 2017), thus creating a thick interfacial layer in the oil-water interface which prevents against lipid oxidation (Fernandez-Avila & Trujillo, 2016). These studies showed that oil-in-water emulsions made with whey protein isolate and sodium caseinate and treated by UHPH exhibited a greater physical and oxidative stability than emulsions produced with conventional homogenization. With this premise and considering the good results in emulsion stability observed in a previous study (Aghababaei et al., 2020) with a similar formulation to the present study, it is expected that improved characteristics of SDE-UHPH-treated emulsions will be found compared to conventional homogenized ones. The effect of UHPH and its potential protection of spray-dried against oxidation, as well as the general characteristics of powders, could contribute to find new opportunities for this emerging technology, which has not yet been studied for this purpose. Hence, the main objective of this work was to characterize and evaluate the oxidative stability of spray-dried emulsions obtained from UHPH (100 and 200 MPa) and conventional (30 MPa) homogenized feeding emulsions formulated with 4 and 7% BM, 10% oil and 30% MD.

4.2. Material and methods

4.2.1. Materials

Glucidex® 19-Maltodextrin (MD) was purchased from Roquette Freres (Lestrem, France) with 19 DE. Buttermilk powder (BM) was obtained from Activa Food-Tech, S. A. (Girona, Spain) with the following composition: 30% protein, 7% fat, 52% lactose, less than 4% moisture and 7% ash. Crude chia oil (20% C-18:2, >56% C-18:3 according to the specifications) was obtained from Interfat, S. A. (Barcelona, Spain). Crude sunflower oil (4–9% C-16:0, 1–7% C18:0, 15–85% C18:1, 50–72% C18:2) was purchased from Gustav Heess (Barcelona, Spain). All other chemicals used were of analytical or better grade.

4.2.2. Emulsion preparation and analysis

Six different samples (Table 1) of emulsions were obtained. The preparation procedure is fully described elsewhere (Aghababaei, Cano-Sarabia, Trujillo, Quevedo, & Ferragut, 2021). After mixing of ingredients, coarse emulsions were further homogenized using conventional (CH) or UHPH treatments. CH was performed with a Homolab (FBF Italia, Sala Baganza PR, Italy) at 30 MPa. Subsequently, heat treatment of emulsions was made at 65 °C, 30 min. UHPH treatments at 100 and 200 MPa were processed in an Ypsicon equipment Model A-60, which is an ultra-high-pressure continuous device (60 L/h) (Ypsicon Advance Technologies, S.L., Barcelona, Spain) that works up to 300 MPa. Working temperatures of samples were the following: 40 °C inlet temperature; 60 ± 2 and 80 ± 3 °C, at the high-pressure valve, corresponding to 100 and 200 MPa respectively; and 25 °C outlet temperature, which was reached after a quick cooling by a heat exchanger connected to the UHPH equipment. Emulsions were collected in Pyrex bottles for further sampling and analysis. Emulsions were analyzed as fully described by Aghababaei et al. (2021) for particle size distribution, using a Mastersizer laser diffraction 2000 analyzer (Malvern Instruments Ltd., Worcestershire, UK) and confocal laser-scanning microscope (Leica TCS SP5, Leica Microsystems GmHB, Mannheim, Germany) was used to observe the structure of fresh emulsions (24 h after production).

Table 1. Emulsion formulations and treatments.

Sample name	H (MPa)	Oil % (w/w)	MD % (w/w)	BM % (w/w)	TS %(w/w)
4CH	30	10	30	4	44
4UH100	100	10	30	4	44
4UH200	200	10	30	4	44
7CH	30	10	30	7	47
7UH100	100	10	30	7	47
7UH200	200	10	30	7	47

H: homogenization conditions; CH: conventional homogenization treatment;
UH: ultra-high-pressure homogenization treatment; Oil: 50:50, chia:sunflower;
MD: maltodextrin; BM: buttermilk; TS: total solids.

4.2.3. Spray drying of emulsions

Emulsions were dried in a Mini Spray-Dryer B-290 (Büchi Labortechnik AG, Flawil, Switzerland). The samples were tempered at 25 °C, and the drying working conditions were 150 °C inlet temperature, 80% aspiration (32 m3/h), and 30% feed flow (9 mL/min). Spray-dried emulsions (SDE) were collected in aluminum bags for further analysis.

4.2.4. Spray-dried emulsion characteristics

4.2.4.1. Moisture content and Aw

Moisture content of SDE was determined gravimetrically by drying 2 g of powder until constant weight (AOAC standard method no. 990.20). Aw was determined using Aqualab equipment, Model Series 3 TE (Decagon Devices, Pullman, WA).

4.2.4.2. Flowing properties

The bulk (ρbulk) and tapped (ρtapped) densities were determined as described by Tatar, Tunç, Dervisoglu, Cekmecelioglu, and Kahyaoglu (2014), with minor modifications. About 5 mL of SDE was added into a 25 mL glass graduated cylinder, measured, and weighed. Then, the cylinder was gently tapped by hand fifty times and the volume was read directly from the cylinder. The weight of SDE divided by the volume was used to calculate respectively the bulk (non-compacted powder) and tapped (compacted) densities. The powder flowability was

evaluated using Carr's Index (CI) and the Hausner Ratio (HR) (Turchiuli et al., 2005), which were calculated following equations (2), (3), respectively:

$$CI = (\rho_{tapped} - \rho_{bulk})/\rho_{tapped} \times 100 \quad (2)$$

$$HR = \rho_{tapped}/\rho_{bulk} \quad (3)$$

2.4.3. Water solubility

Solubility of SDE was evaluated as described by Botrel, Borges, Fernandes, and Lourenço Do Carmo (2014) with minor modifications. The sample (1 ± 0.01 g) was weighed and added to a beaker with 20 mL of distilled water, while stirring at 200 rpm. When all the sample had been added, the stirring speed was increased to 1200 rpm for 3 min. Subsequent centrifugation at 1600 rpm for 15 min at 20 °C was performed. Then, 5 mL of the supernatant was shifted to a preweighed capsule and dried at 110 °C for 4 h until constant weight. The water solubility (WS) was calculated by equation (4):

$$WS\ (\%) = (s \times 4/m) \times 100 \quad (4)$$

where: s is the grams of solids in supernatant and m is the grams of sample.

4.2.4.4. Encapsulation efficiency

The EE, total oil content (TO), and surface oil content (SO) of SDE were determined as described by González, Martínez, Paredes, Leónn, and Ribotta (2016). Briefly, for TO determination, 4.0 ± 0.1 g of sample were extracted in a Soxhlet apparatus for 24 h, with 200 mL of n-hexane solvent. After complete evaporation of the solvent, the oil extracted was weighed and expressed as a percentage of oil (dry basis) of the SDE.

For SO determination, 2 ± 0.01 g of SDE were mixed with 30 mL of petroleum ether followed by shaking for 1 min at room temperature and filtered through Whatman no. 1 paper. Solids in the filter were washed with 10 mL hexane and organic phases were combined. The filtrated solution was then transferred to an oven at 105 °C for the complete evaporation of hexane. EE was determined by calculating the ratio of the total oil contained in the SDE (TO) and the free oil (SO) located on its surface, according to equation (5).

$$EE = (TO - SO) \times 100/TO \quad (5)$$

4.2.4.5. Color evaluation

Color parameters were assessed with a colorimeter Konica Minolta CR-410 (Konica Minolta, Osaka, Japan), using D_{65} light source and angle of 10° observer as references. ESD samples were placed in a 50 mL clear optical glass container filled up to 10 mm followed by a white stopper disk to standardize measurements. The bottom external surface was measured. Results were expressed in the CIE L*a*b* color space, where L* is the lightness, a* is the greenness-redness, and b* is the blueness-yellowness. The Whiteness Index (WI), Yellowness Index (YI), and total color difference (ΔE) were calculated using the following equations (6), (7), (8), respectively.

$$WI = 100 - [(100 - L^*) + a^{*2} + b^{*2}]^{0.5} \quad (6)$$

$$YI = 142.86 \times b^* \times L^{*-1} \quad (7)$$

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \quad (8)$$

4.2.4.6. Morphology and size

The morphology of SDE was observed by SEM, using the Quanta ™ 650 FEG scanning electron microscope (FEI Company, Hillsboro, OR, USA), with an accelerating beam voltage (HV) of 5 kV. Samples were prepared by fixing a small amount of powder on metal discs with double-sided carbon tapes, which were then platinum-plated in a Leica EM ACE600 vacuum chamber (Leica Microsystems, Wetzlar, Germany). Mean diameter of particle size of SDE samples were analyzed by measuring 100 particles per sample on the SEM photographs using the microscope software.

4.2.5. Oxidation stability

The oxidative stability of SDE was determined by the peroxide value and by quantification of malondialdehyde as secondary oxidation product. SDE were stored at 50 °C for 31 days to accelerate the oxidation process. Primary and secondary oxidation were determined in SDE on days 0, 7, 15, and 31. Feeding emulsions of the corresponding SDE were also analyzed on day 0 to evaluate the effect of the drying process on primary oxidation.

4.2.5.1. Primary oxidation

The hydroperoxide concentration was determined according to the previously described method by Hu, McClements, and Decker (2003). SDE (1 ± 0.01 g) were reconstituted in 10 mL distilled water. A sample of 300 μL emulsion was taken in triplicate and mixed with 1.5 mL of an isooctane: 2-propanol solution (3:1) in glass tubes, and vortexed for 30 s (10 s × 3). The organic phase of the mixtures was separated by centrifugation at 1000 rpm at 20–25 °C for 2 min. 200 μL of the organic phase was added to 2.8 mL of a methanol: 1-butanol solution (2: 1). Then, 1 μL of 3.97 M ammonium thiocyanate and 15 μL of iron solution prepared with 0.132 M BaCl2 and 0.144 M FeSO4 were added continuously. Finally, the test tubes were vortexed (10 s), and, after 20 min, the absorbance of the solution was measured at a wavelength of 510 nm in a spectrophotometer Dinko UV 2310 (Dinko Instruments, Barcelona, Spain). Hydroperoxide concentrations were determined by means of a standard curve, made from cumene hydroperoxide, in a concentration range of 0.002–2 mM.

4.2.5.2. Secondary oxidation

Quantification of malondialdehyde was made according to the method proposed by Papastergiadis, Mubiru, Van Langenhove, and De Meulenaer (2012) by HPLC. For sample preparation, 5 mL of emulsion were taken and mixed with 15 mL of 7.5 g/100 mL TCA containing 0.1 g/100 mL EDTA and 0.1 g/100 mL propyl gallate into 50 mL conical centrifuge tubes, and shaken horizontally for 15 min. Subsequently, they were centrifuged at 3500 rpm for 15 min and then, 1 mL of the supernatant was taken and added to glass tubes (with cap and screw) together with 3 mL of 40 mM TBA. The tubes were boiled for 40 min, then cooled to room temperature, and 1 mL of methanol was added to each one and vortexed for 10 s. Samples were placed in Eppendorf tubes and centrifuged at 3500 rpm for 15 min to avoid the presence of precipitates. The separation and quantification of malondialdehyde was carried out in an HPLC equipment composed of an automatic injector (Waters 717 Plus, Milford, Massachusetts, USA), a Perkin Elmer model 515 booster pump (Waltham, Massachusetts, USA), and a serial fluorescence detector 200 (Perkin Elmer). Aliquots (20 μL) of the sample were injected onto an Agilent Pursuit 3 C18 column (5 μm, 150 × 4.6 mm) at 40 °C analysis temperature. The mobile phase consisted of a 50 mM KH2PO4 buffer solution, methanol, and acetonitrile (72:17:11, pH 5.3) pumped isocratically with a flow of 0.8 mL/min. The fluorometric excitation and emission wavelengths were set at 525 and 560 nm, respectively. To quantify the amount of malondialdehyde, a standard curve was made from 1.1 to 3.3 tetraethoxypropane in a

concentration range of 0.25–25 μM. Peak quantification was performed using Turbochrom TC6 software (Perkin Elmer).

4.2.6. Statistical analysis

Results are presented as mean ± standard deviation. SDE characterization was subjected to a one-way analysis of variance (ANOVA) test using the Minitab Express™ version 1.5.3 (Minitab, State College, PA, USA). Significant differences between means were determined by the Tukey test. A confidence level of 95% ($p < 0.05$) was used. At least two individual productions of each formulation and treatment were performed. All analysis were replicated three times.

4.3. Results and discussion

4.3.1. Characteristics of emulsions

Processing of feeding emulsion determines the characteristics of microcapsules produced by spray drying (González et al., 2016). BM concentration as well as homogenization treatment influenced the particle size distribution and microstructure of feeding emulsions (Fig. 1). In CH emulsions, mean particle size ($d_{4.3}$) was about 13 and 4.5 μm corresponding to 4CH and 7CH, respectively. The particle size distribution curves were bimodal, which corresponded to individual and aggregated oil droplets. This fact was especially present in 4CH emulsions. UHPH-processed emulsions showed a particle size reduction in comparison to CH emulsion in both 4UH and 7UH. In Fig. 1 the CLSM micrographs are inserted, in which the structure of oil droplets can be observed. The most remarkable characteristic of UHPH-processed emulsions, independently of the BM concentration, was the formation of oil droplets aggregates which were smaller in particle size than the individual oil droplets of the CH emulsions, which was associated to a higher emulsion stability of UHPH-processed emulsions (Aghababaei et al., 2021). The strong physical forces acting in UHPH have been described to cause partial protein denaturation (Fernandez-Avila et al., 2015; Floury, Desrumaux, & Legrand, 2002; Serra, Trujillo, Guamis, & Ferragut, 2009) and casein micelle disintegration (Sharma, Jana, & Chavan, 2012), which facilitates the aggregation by means of protein-oil and protein-protein interactions. This phenomenon has also been described in other emulsions treated by UHPH such as those formulated with 1, 3 and 5% sodium caseinate (Hebishy, Buffa, et al., 2017), in which aggregates were only observed at 3 and 5% of that emulsifier. In our emulsions, which were formulated for further spray drying, the presence of 30% of MD could lead to depletion

flocculation, promoting the aggregation of individual oil droplets in presence of protein and carbohydrates, by their partial thermodynamic incompatibility (De Kruif & Tuinier, 2001).

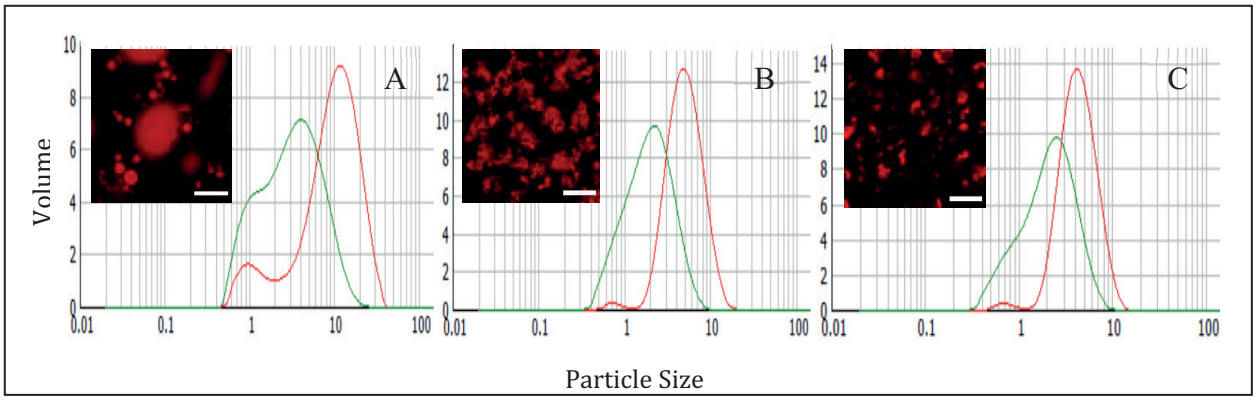

Fig. 1. Particle size distribution of 4% BM (red line) and 7% BM (green line) of emulsions processed by **(A)** CH, **(B)** UH100 and **(C)** UH200. CLSM images correspond to oil droplets and aggregates observed in 7% BM at the different homogenization treatments applied, labelled with Nile Red (scale bar 7.5 mm).

4.3.2. Characteristics of spray-dried emulsions

In this study, spray-drying conditions were kept constant according to preliminary trials to make sure that the SDE were not sticky and flowability was good enough for an acceptable yield. Total solid content of SDE was 44% for formulations containing 4% BM and 47% for formulations with 7% BM. In Table 2, characteristics of SDE are shown. Moisture content varied from 2.06 to 3.19% in all SDE with few differences ($p < 0.05$) being observed among them. 4CH sample was the SDE with the lowest water content and 7UH100 that which showed the highest water content of SDEs. It appeared that both homogenization treatment and BM concentration influenced water content. Probably, in CH treatments, water was easier to eliminate by spray drying because of the lower particle volume fraction observed in these sample (Fig. 1). This behavior of CH compared to UH samples was observed for both BM concentrations, 4 and 7%. In addition, when comparing the BM concentration SDE series, 7% SDE showed higher water content, which could explain certain difficulty for water evaporation in the drying process. On the other hand, the different colloidal structures, big droplets in CH or small aggregates in UH SDE, may indicate a different protein distribution between the continuous and oil-water interface of the feeding emulsions, modifying water elimination during the spray drying process. However, in terms of powder stability during storage, as reported by Klaypradit and Huang (2008), dry foods with moisture content between 3 and 10% have a good behavior.

Table 2. Characteristics of SDE from feeding emulsions containing 4 and 7 (%) BM and processed by different homogenization systems (CH and UHPH).

Sample	Moisture (%)	A_w	r_b (Kg/m^3)	r_t (Kg/m^3)	CI	HR	WS (%)	EE (%)	Size (mm)
4CH	2.06 ± 0.04^{c}	0.191 ± 0.01^{a}	455 ± 17^{a}	551 ± 11^{a}	17.3 ± 2.6^{a}	1.21 ± 0.03^{a}	94.1 ± 3.4^{a}	77.1 ± 2.6^{c}	10.3 ± 3.6
4UH100	2.64 ± 0.10^{b}	0.192 ± 0.01^{a}	438 ± 12^{a}	526 ± 17^{ab}	16.6 ± 1.1^{a}	1.20 ± 0.06^{a}	93.6 ± 2.9^{a}	83.0 ± 2.1^{b}	11.4 ± 5.5
4UH200	2.82 ± 0.30^{ab}	0.185 ± 0.01^{a}	454 ± 12^{a}	534 ± 10^{ab}	15.1 ± 0.7^{ab}	1.17 ± 0.01^{ab}	92.1 ± 3.3^{a}	84.1 ± 0.8^{b}	7.4 ± 6.3
7CH	2.76 ± 0.08^{ab}	0.130 ± 0.01^{a}	434 ± 09^{a}	470 ± 06^{c}	7.70 ± 0.7^{c}	1.08 ± 0.01^{cd}	96.8 ± 2.2^{a}	81.2 ± 1.3^{bc}	8.8 ± 5.2
7UH100	3.19 ± 0.06^{a}	0.151 ± 0.01^{a}	445 ± 13^{a}	506 ± 15^{b}	11.9 ± 1.1^{b}	1.13 ± 0.01^{bc}	91.7 ± 4.5^{a}	91.5 ± 2.5^{a}	6.3 ± 2.9
7UH200	3.07 ± 0.10^{ab}	0.131 ± 0.01^{a}	430 ± 07^{a}	465 ± 10^{c}	7.50 ± 1.0^{c}	1.08 ± 0.01^{d}	91.5 ± 1.3^{a}	89.4 ± 0.9^{a}	11.0 ± 4.5

Means in each column with different superscript letters were significantly different ($p < 0.05$). Values are mean and SD of three replications.

A_w: water activity; r_b: Bulk density; r_t: Tapped Density; CI: Carr Index; HR: Hausner Ratio; WS: Water Solubility; EE: Encapsulation Efficiency; Size: mean diameter ofparticle size analyzed.

Aw values ranged from 0.130 to 0.191 with no significant differences ($p < 0.05$) between them, although the highest values observed corresponded to those with lower BM content, as expected. These values are considered adequate for dry foods since they guarantee microbiological stability (Comunian et al., 2019).

One relevant characteristic of powders is bulk density, which varied in a narrow range, from 430 to 455 kg/m^3 for all samples with no significant differences ($p < 0.05$) observed between them. These values are in the range of most of the spray-dried emulsions reported in literature, which may vary broadly depending on composition and drying conditions used. Our SDE bulk density values were similar to those found by other authors (Sarkar, Arfsten Golay, Acquistapace, & Heinrich, 2016; Carneiro, Tonon, Grosso, & Hubinger, 2013) who also reported oil content similar to those used in this study. Some powders parameters related to bulk and tapped densities (eqs. (2), (3))), were calculated: Carr's Index (Carr, 1965) is a scale of flowability (values ≤ 10 are excellent and values > 38, are awful). The Hausner Ratio (Hausner, 1967) is a scale of cohesiveness from 1 (excellent) to > 1.6 (awful). Thus, both parameters, CI and HR, can be used to determine powder properties during processing and storage conditions (Quispe-Condori, Saldaña, & Temelli, 2011; Sanchez-Reinoso & Gutiérrez, 2017). Flowing properties of the present SDE showed a significantly different ($p < 0.05$) behavior, mainly depending on BM concentration regardless of the homogenization system used; i. e., all 4% BM SDE were similar but different from 7CH and 7UH200, which exhibited lower values of CI and HR. As shown in Table 2, SDE with 4% BM had values in the range corresponding to a fair flowability and cohesiveness (CI = 16–20 and HR = 1.19–1.25, are considered as fair on their respective reference scales). In these samples, although no statistical differences were observed, CI and HR values decreased as homogenization pressure increased, indicating a certain influence of this technological treatment. SDE with 7% BM had values mostly in the range of excellent flowability and cohesiveness, thus exhibiting very good handling properties, and showing that BM is a good encapsulating agent capable of producing powder emulsions with high quality characteristics of handling and transport.

Solubility in water is a relevant quality parameter of dry powders which mostly depends on its chemical composition and physical state (Dhanalakshmi, Ghosal, & Bhattacharya, 2011). All SDE produced in this study showed high solubility in water at room temperature. They did not exhibit significant variations in their solubility ($p > 0.05$), which ranged between 91.5 and 96.8%. This parameter is strongly influenced by the nature of the wall materials (carbohydrates and proteins), which in this work were constituted by 30% MD and 4 or 7% BM, both with

high solubility in water. Solubility values observed in this study were similar to those reported by other authors (Korma et al., 2019) in powder emulsions formulated with WPI and MD or inulin with 30% total solids.

The EE reflects the degree to which wall material can retain oil in microcapsules. Values of this parameter showed good encapsulation of oil, ranging between 77.1 and 91.5% in the different SDE. Both BM concentration and homogenization system influenced the results obtained. In formulations containing 4% BM, the UHPH treatment (100 or 200 MPa) improved significantly ($p < 0.05$) the capacity of oil retention of microcapsules compared to 4CH. On the other hand, SDE containing 4% BM and treated by UHPH showed similar values of this parameter than formulation 7CH, indicating the positive influence of BM concentration. The best EE was observed in 7UH100 and 7UH200 SDE, which showed similar values. Emulsifying properties of BM are attributed to its composition, rich in casein, whey proteins and phospholipids, which adsorbs at the oil-water interface exerting the desired covering of oil droplets. Thus, increasing BM content could improve the effective oil coverage, and in consequence, the EE as reported also by other authors (Wang, Che, Fu, Chen & Selomulya, 2016). On the other hand, as observed in the feeding emulsions, UHPH promoted the aggregation of oil droplets though protein-protein interactions. In consequence, oil could be hidden in those colloidal structures keeping better EE.

Color attributes are important characteristics for consumer acceptance. Table 3 summarizes the results of color measurements: CIE Lab parameters, color difference between homogenization treatments, and color difference between different BM concentrations for the same treatments of SDE. As well, two indexes used for near-white opaque materials (WI and YI) were calculated. L* did not show differences between treatments or BM concentration, ranging from 92.6 to 94.0. Red – green (a*) varied in a short range into the red tonality, while b* (yellow - blue), was the parameter that most contributed to the color differences and YI within samples, being formulations containing 7% BM those that showed the highest WI values ($p < 0.05$). Regarding color differences, by comparing the effect of homogenization treatment as well as the BM content, most of values were >1, which means that they can be classified as slightly noticeable (0.5–1.5) by the human eye (Sanchez-Reinoso & Gutiérrez, 2017).

Table 3. Color characteristics of SDE from feeding emulsions containing 4 and 7% BM processed by different homogenization systems (CH and UHPH).

Samples	L*	a*	b*	ΔE	WI	YI
4CH	92.9±0.5^{ab}	3.70±0.02^{a}	0.47±0.22^{c}	-	95.4±0.04^{e}	0.72±0.34^{c}
4UH100	92.6±0.1^{b}	3.60±0.02^{b}	0.60±0.07^{c}	1.60±0.07^{a}	95.4±0.02^{de}	0.93±0.11^{c}
4UH200	93.4±0.6^{ab}	3.57±0.03^{b}	0.63±0.07^{c}	1.86±0.20^{a}	95.5±0.06^{cd}	0.96±0.11^{c}
7CH	94.0±0.2^{a}	3.12±0.03^{c}	1.33±0.10^{b}	-	95.8±0.02^{cd}	2.02±0.15^{b}
7UH100	93.4±0.3^{ab}	2.92±0.01^{d}	1.86±0.08^{a}	1.00±0.20^{b}	95.6±0.03^{de}	2.84±0.12^{a}
7UH200	93.6±0.1^{ab}	3.25±0.03^{e}	1.20±0.06^{b}	1.18±0.03^{b}	95.7±0.05^{cd}	1.84±0.11^{b}
4CH-7CH				1.57±0.78^{a}		
4UH100-7UH100				1.63±0.30^{a}		
4UH200-7UH200				0.85±0.26^{a}		

Means in each column with different superscript letters were significantly different ($p < 0.05$).
Values are mean and SD of three replications. L*, a*, b*: CIELab color coordinates;
ΔE: Color Difference between UHPH and CH treatments for each BM concentration formulation;
WI: Whiteness Index; YI: Yellowness Index.

Morphology of SDE examined by SEM are shown in Fig. 2. All the images always showed spherical particles, with apparent smooth surfaces without remarkable presence of fractures, although depression and superficial folds were observed in some microcapsules, probably due to the sudden contraction that occurs in the early stages of drying. The general aspect of all SDE were similar, regardless of the percentages of BM or homogenization treatments. Particle sizes were heterogenous, showing the presence of small and larger particles in the same sample, frequently aggregated to each other. In general, formulations containing 4% BM showed higher particle size (Table 2) than 7% BM formulations. However, it is difficult to establish any correlation since the variety observed in the particle size was often high. This aspect has also been observed in different studies (Benito-Román, de Paz, Melgosa, Beltrán, & Sanz, 2018; Carneiro et al., 2013) which is typical in spray-dried foods.

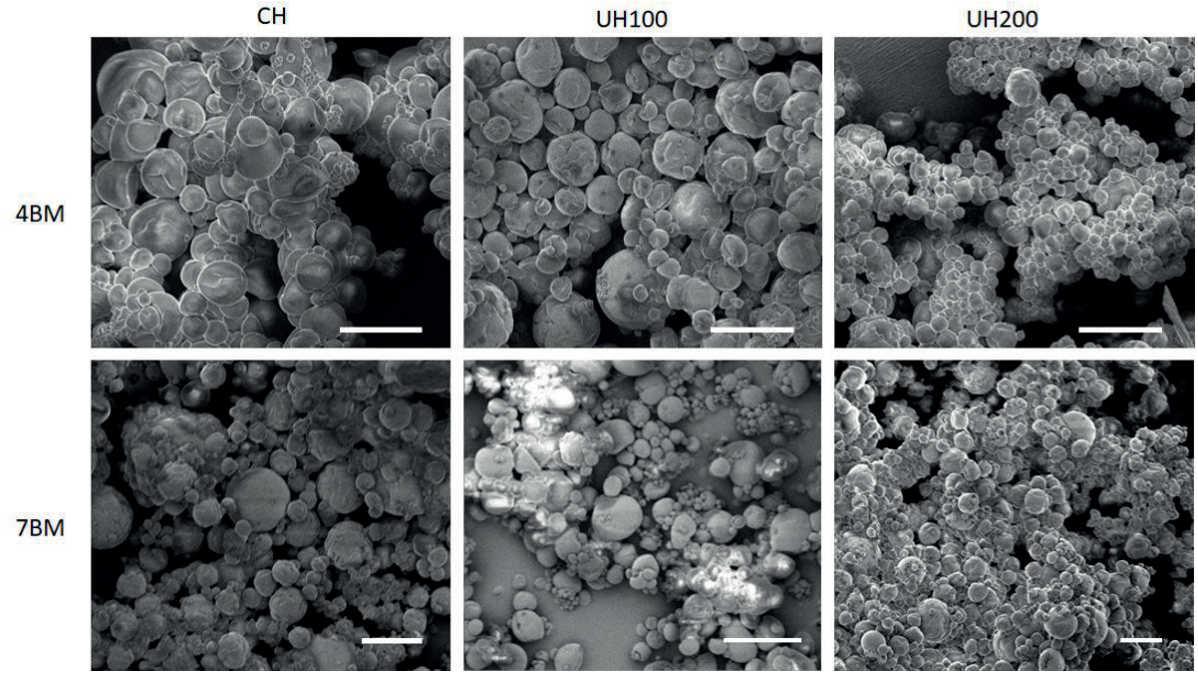

Fig. 2. SEM images of SDE containing 4 and 7% BM (lines) from feeding emulsions treated by CH and UHPH (columns). Scale bars correspond to 5 mm.

4.3.3. Oxidation stability of SDE

The stability of SDE against oxidation was evaluated in accelerated conditions of heating at 50 °C for one month, at days 1, 7, 14, and 31 of storage. The oxidation analysis was performed for primary and secondary oxidation. The effect of drying on primary oxidation developed in SDE samples was also estimated compared to the fresh emulsions as depicted in Fig. 3. As expected, the effect of drying caused an increase of hydroperoxide concentration in all samples, which varied in function of homogenization treatment and BM concentration. Regarding BM concentration, both fresh emulsions and SDE were more protected against oxidation with 7% BM. The effect of treatment was more accused in CH emulsions, which exhibited higher difference of hydroperoxides between fresh and SDE compared to those treated by UH. Probably, the fresh UHPH-treated emulsions were more exposed to pro-oxidant agents due to the high increase of oil surface droplets generated during UHPH treatment. However, the effect of BM concentration prevailed against treatment as the oxidative state of SDE on day 1 was quite similar in all the 7BM SDE series.

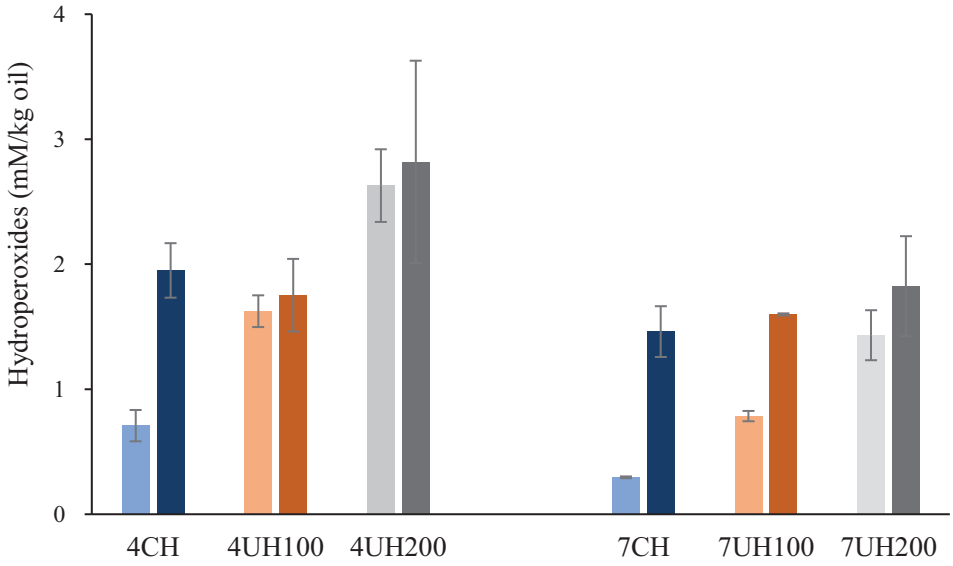

Fig. 3. Effect of drying process on primary oxidation in formulations containing 4 and 7 % BM. Clear color (feeding emulsions on day 1), dark color (SDE on day 1 after drying).

Fig. 4 shows the hydroperoxides concentration evolution during SDE storage. The pattern of the primary oxidation curves was similar in all samples. They were characterized by a subtle increase in the concentration between days 1 and 7, followed by a pronounced increase on day 14, and a noticeable decrease at day 31.

This observed evolution of hydroperoxides is consistent with the kinetics of development of primary lipid oxidation, in which a latency period appears at the beginning followed by a propagation or exponential increase of the oxidation products (Kamal-Eldin, McAkinen, & Lampi, 2003). The decrease observed after the peak is due to the secondary oxidation products being formed from the primary products' degradation (Papastergiadis et al., 2012). Considering BM concentration, hydroperoxide levels of 7% BM samples were lower during storage than those of 4% BM, exerting less protection by recovering the interface of small oil droplets and aggregates generated by UHPH treatment than 7% BM. Hebishy, Buffa, et al. (2017) in sodium caseinate emulsions treated by UHPH observed this dependence of protein concentration on the oil droplet protection against oxidation. Thus, at the lowest amount of emulsifier, the film formed is not dense enough to protect the oil from oxidation, allowing the diffusion of oxygen and pro-oxidants towards the oil droplets during storage (Sarkar, Arfsten, Golay, Acquistapace, & Heinrich, 2016).

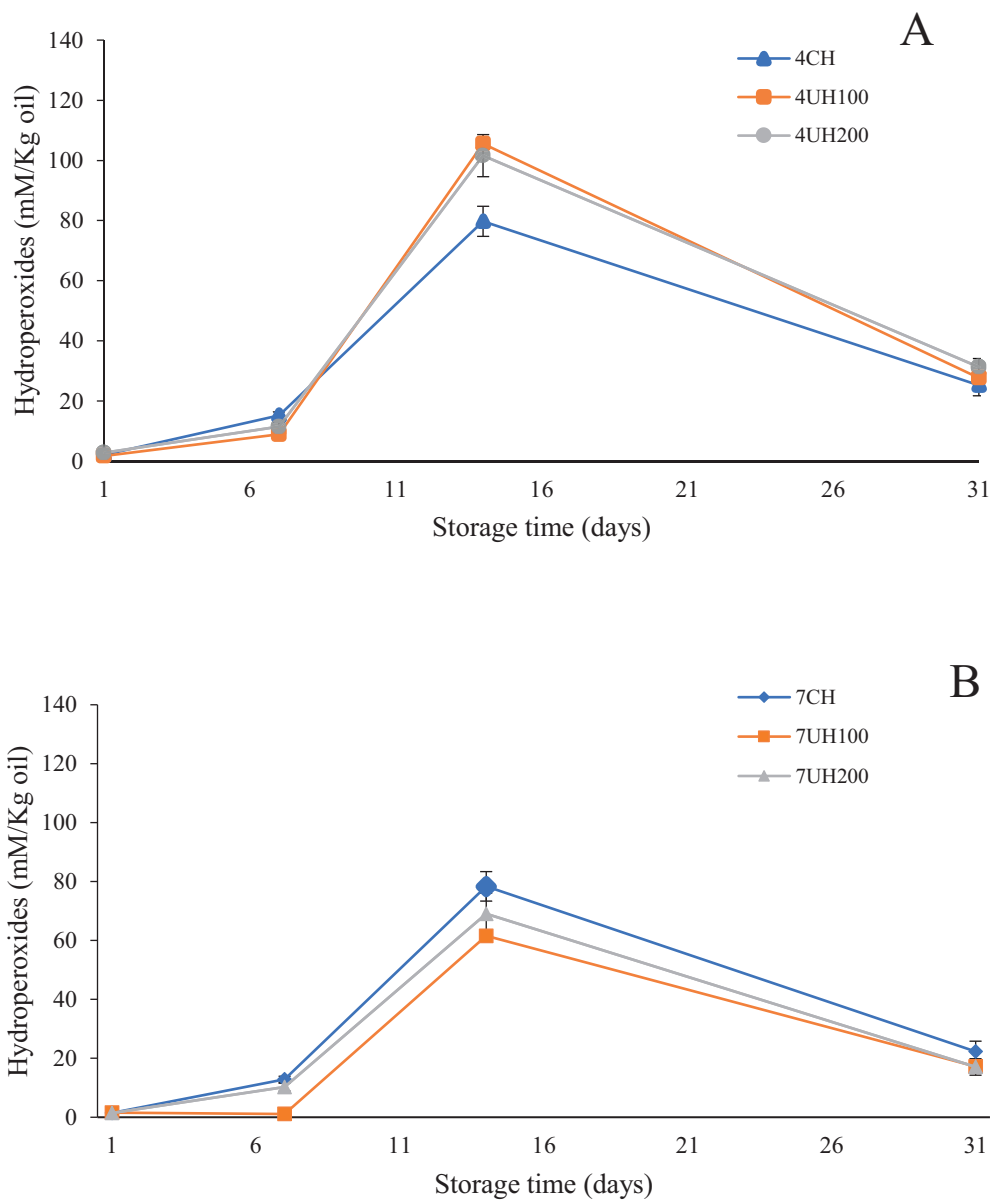

Fig. 4. Hydroperoxides concentration evolution during storage of SDE in accelerated conditions at 50 °C obtained from feeding emulsions treated by different homogenization systems (CH and UHPH) and BM concentration. **(A)** 4% BM; **(B)** 7% BM.

The effect of BM concentration and homogenization treatment was consistent with the EE observed (Table 2). EE was higher in UHPH-treated samples containing higher BM concentrations. Zhang et al. (2020) showed a similar tendency in spray-dried emulsions produced by homogenization at 30 MPa (3 cycles), and formulated with different concentrations of BM, maltodextrin, and seaweed oil, finding that the formulations with the highest percentage of BM showed the lowest hydroperoxides, attributing this effect to higher EE.

Fig. 5 shows the evolution of secondary oxidation through the determination of malondialdehyde (MDA) concentration in SDE over time. In general, low levels of MDA were observed in most of the stored SDE with an MDA increase on day 31, which was especially marked in SDE containing 7% BM. This MDA increase coincided with the decrease in hydroperoxides mentioned above, confirming that as the end of accelerated storage approaches, oxidation state continues to advance. Regarding the higher MDA concentrations observed in 7% BM formulations, it could be explained based on the presence of some compounds of BM that could behave as pro-oxidants, especially if they were present in excess (Berton-Carabin, Ropers, & Genot, 2014). BM contains phospholipids that may have antioxidant effects. However, when they are in dehydrated dairy products, they can also behave as pro-oxidants due to the unsaturation presented by MFGM phospholipids such as phosphatidylcholine and phosphatidylethanolamine (Cui & Decker, 2016). As can also be seen in Fig. 5, emulsions treated at 100 MPa were generally those that presented lower MDA within the storage, compared with CH and UH200 treated emulsions. This significant difference observed, especially at day 31 of storage, may be due to the bigger size of the oil droplet aggregates formed at 100 MPa compared to 200 MPa treated samples. Although at the two UH pressures applied in this study, a high size reduction of individual oil droplets was observed (Aghababaei et al., 2021); reaggregation was produced, giving more stable emulsions with smaller particle sizes than those obtained with conventional technology. However, as can be seen in Fig. 1, UH100 emulsions had bigger aggregates, thus the effective exposure of oil to the oxidation catalyzers were lower by limiting their access to lipids. Further study of oxidation at ambient temperature could clarify the real behavior of those dried emulsions, given that storage temperature greatly determines the oxidation kinetics as reported by other authors (Escalona-García et al., 2016), being highly reduced at 25 °C in chia oil encapsulated with whey protein concentrate.

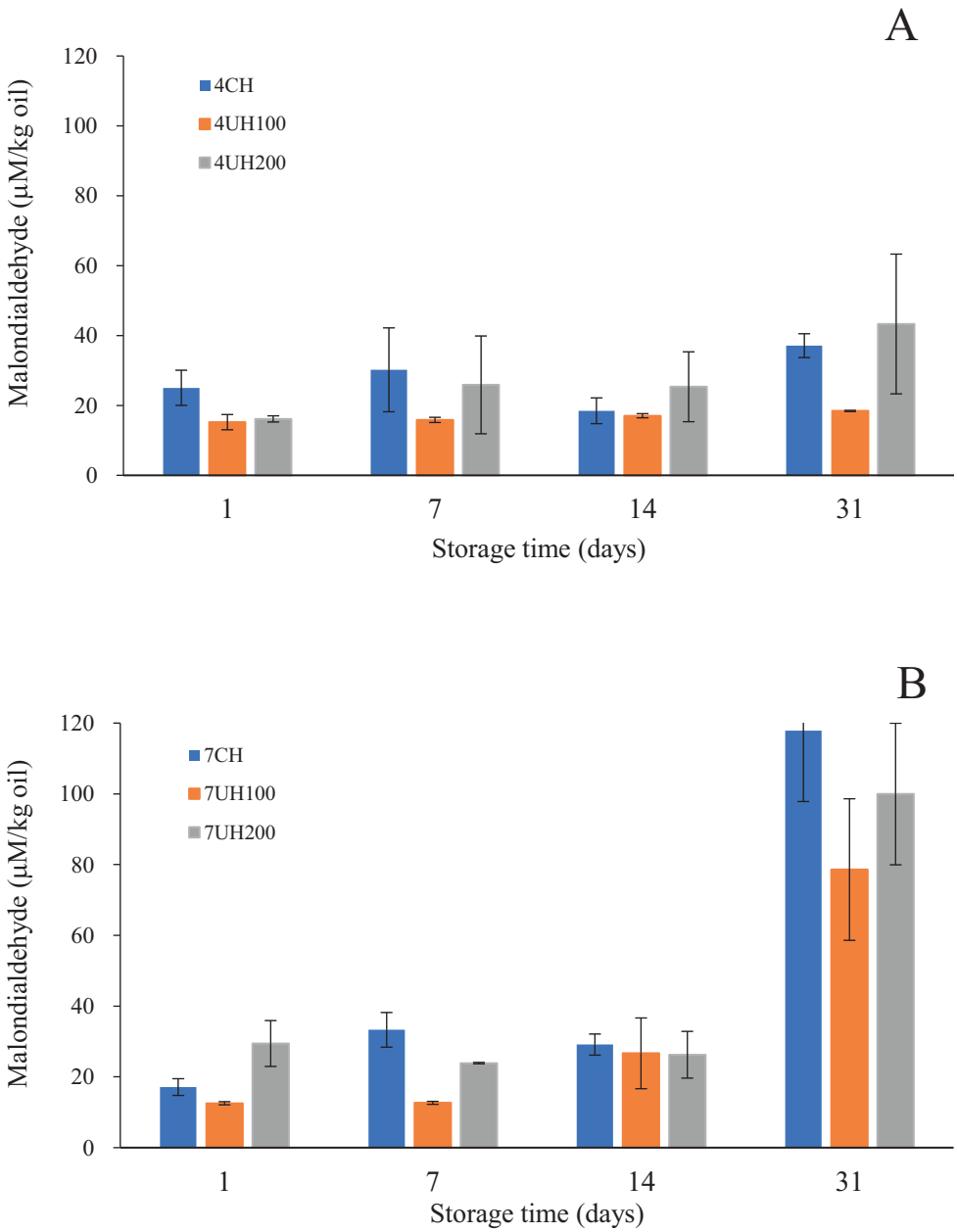

Fig. 5. Malondialdehyde concentration evolution during storage of SDE in accelerate conditions at 50 °C obtained from feeding emulsions treated by different homogenization systems (CH and UHPH) and BM concentration. **(A)** 4% BM; **(B)** 7% BM.

4.4. Conclusions

Incorporation of buttermilk (at 4 or 7%) has allowed the production of spray-dried products with good general properties such as optimal flowability and cohesiveness. In addition, when UHPH treatment was used compared to conventional homogenization, general properties of powders were similar or improved, such as the EE, which increased its performance as encapsulant in producing dried emulsions. Oxidative stability evolved in a similar direction regarding primary oxidation. However, the secondary products of oxidation were higher in 7% than in 4% buttermilk formulations, probably due to the higher concentrations of unsaturated phospholipids of buttermilk at the oil-water interface. This hypothesis is reinforced as the UHPH treatment at 100 MPa produced the best oxidative stability of spray-dried emulsions; probably it was caused by the microstructure of feeding emulsion obtained at these UHPH conditions, in which prevailed the oil droplets protected by buttermilk hidden inside the big aggregates observed in those emulsions.

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Chapter 5

***Experiment 3*:**

Encapsulating capacity of ultra-high-pressure homogenization (UHPH): replacement of milk fat by vegetable oils using buttermilk as functional ingredient in yogurt processing.

5.1. Introduction

Functional foods have gained significant popularity in international markets over the past two decades due to their diverse health benefits (Liu et al., 2019). Omega-3 fatty acids (FA) are essential for various biochemical processes, cell membranes, brain development, and physiological functions in the human body. The global market for omega-3 FA supplements reached a value of USD 5.580,000 in 2020, with an expected annual growth rate of 8.6% from 2020 to 2028 (Grandview Research, 2020).

To meet the demand for oils rich in polyunsaturated FA (PUFA), fortification of commonly consumed foods with these fatty acids has become prevalent in the food industry. However, during processing, distribution, and handling, PUFA are susceptible to oxidation, resulting in off-Flavors and a decrease in quality and health benefits (Uluata, McClements, & Decker, 2015). Encapsulation strategies have been extensively developed to protect PUFA from oxidation during processing and storage, focusing on the emulsification system and compounds employed to shield them. Various compounds, such as proteins, carbohydrates, and low molecular weight emulsifiers, are commonly used to create stable emulsions for further drying and encapsulation of oil droplets into microcapsules (Ruiz, Ortiz, & Segura, 2017).

Buttermilk (BM), a co-product of butter manufacturing, is an inexpensive and readily available ingredient in the food industry. It possesses emulsifying capacity and functionality, attributed to the polar lipids and proteins present in the milk fat globule membrane (MFGM) (Barry, Dinan, & Kelly, 2017). These compounds have been also associated with notable health benefits, including improved brain cognitive development and immune system support for optimal growth in infants (Hernell, Timby, Domellöf, & Lönnerdal, 2016; Singh & Gallier, 2017). Moreover, commercial BM has demonstrated excellent encapsulating properties, surpassing skim milk powder when used to encapsulate fish oil (Augustin et al., 2015). It also mimics the natural milk fat globule membrane due to its polar lipid composition, potentially enhancing the digestion and utilization of encapsulated omega-3 FA (Zhang et al., 2020).

The successful encapsulation of lipophilic compounds, such as PUFA-rich oils, requires the formation of stable emulsions prior to dehydration. Emulsions are thermodynamically unstable colloidal systems, prone to creaming, aggregation, and coalescence. High-energy mechanical devices like high-pressure homogenizers or ultrasonic generators are commonly employed in the industry to produce stable emulsions by reducing droplet size (Weiss, Takhistov, & Julian, 2006; Floury, Desrumaux, & Lardières, 2000).

Ultra-high-pressure homogenization (UHPH) is a versatile technology operating at pressures up to 350 MPa, compared to conventional homogenization (CH) typically ranging between 20 and 50 MPa. UHPH can inactivate microorganisms and enzymes, produce submicron emulsions with excellent physical stability, and induce changes in colloidal structures. The composition of emulsifying agents used in UHPH can influence the restructuring of the protective layer of droplets, impacting the techno-functional properties (Dumay et al., 2013; Sato, Matsumiya, Kaneko, Okazaki, & Matsumura, 2021).

Spray drying is a cost-effective method widely used in the food industry to convert emulsions into easy-to-handle, transport, and preserve powder form. Encapsulation through spray drying enables the production of small-size particles resistant to chemical and physical damage, ensuring protection against oxidation, light, and temperature (Geranpour, Assadpour, & Jafari, 2020; Di Giorgio, Salgado, & Mauri, 2019).

Yogurt, a popular dairy product, is known for its natural composition rich in nutrients and numerous health benefits attributed to fermentation (Donovan & Rao, 2019; Gumus & Gharibzahedi, 2021; Kok & Hutkins, 2018). It is an ideal candidate for nutrient fortification. Substituting milk fat with PUFA-rich oils emulsified with MFGM components from buttermilk presents an opportunity to develop a balanced functional food. The production of spray-dried emulsions (SDE) containing PUFA-rich oils stabilized by UHPH has been recently investigated (Varela et al., 2022), demonstrating superior properties compared to SDE produced by CH. This study aims to evaluate the influence of homogenization type (CH and UHPH) used to produce SDE and the concentration of SDE added to stirred yogurts on the relevant quality characteristics of the final product.

5.2. Material and Methods

5.2.1. Materials

Materials used for yogurt manufacturing, UHT skim milk, and skim milk powder (SMP), were purchased from a local supermarket. From the supplier information, SMP contained 32.5% protein, 1% fat, and 54.5% carbohydrate. Lyophilized yogurt culture, composed of *Lactobacillus delbruekii* subsp. *bulgaricus* and *Streptococcus thermophilus* Y0-MIX 300 LYO 10 DCU, was purchased from Danisco (Buxiéres, Francia). Refined sunflower was purchased from Gustav Heess Company (Barcelona, Spain).

The characteristics and composition according to the producer were: acid value = 0.1% (mg KOH/g); peroxide value (meq O_2/kg) = 0.02, fatty acid composition (15-85%C-18:1, 50-72% C-18:2). Crude Chia oil was as follows: acid value= 0.27% (mg KOH/g), peroxide value=1.5 (meq O_2/kg), Fatty acid composition (6.35%C-18:1, 19.29% C-18:2, 64.85% C-18:3) according to the specifications was obtained from Interfat Natural Oils (Barcelona, Spain). All other chemicals used were of analytical or better grade.

5.2.2. Emulsion and yogurt preparation

Preparation of emulsion and spray dried emulsion (SDE) were previously and fully described (Aghababaei et al., 2021). The SDE composition used for yogurt manufacturing were: 7% (w/w) BM, 30% (w/w) MD, 10% (w/w) oil, 47% total solids, and 3% (w/w) moisture. Four different recombined milks (RM) to produce yogurts were prepared by varying the percentage of SDE added as well as the type of homogenization used to obtain the SDE. Thus, the nomenclature of yogurts indicates these two variables as follows: CH4 (4% SDE treated by CH); CH6 (6% SDE treated by CH); UH4 (4% SDE treated by UHPH); and UH6 (6% SDE treated by UHPH). To prepare the RM for further preparation of yogurts, UHT skimmed milk was mixed with 3% SMP and 4 or 6% SDE. RM were stirred with a blade stirrer for 10 min and were further pasteurized in batch at 82 °C for 20 min. After cooling of the mixes, they were stored at 4 °C overnight to complete powder hydration. The next day, RM were heated to 45 °C, inoculated with 0.02% of the starter culture Y0-MIX 300 LYO 10 DCU (Danisco, Buxiéres, Francia) containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus,* and incubated at 43 °C until pH 4.6 was reached. Yogurts were stored at 4 °C for 24 h and further stirred with a Jata FP500P rod blender (Tudela, Navarra, Spain) for 1 min clockwise to breakdown the gels. Then, stirred yogurts were transferred to sterile media bottles and stored at 4 °C until analysis.

5.2.3. Evaluation of coagulation properties

The coagulation process was monitored on inoculated RM (10 mL) at 43±2 °C using an Optigraph System (Ysebaert Dairy Division, Frepillon, France) which measure is based on the attenuation of near-infrared region (NIR) signal. From coagulation curves (optical signal *vs* time), three parameters were obtained (Fig. 1): onset of gelation (OG) obtained from the maximum of the second derivative; aggregation rate (AR), calculated from the slope of linear part of the curve; and final gel firmness (FGF) at the end of the coagulation, obtained by the difference between the final and initial values of the optical signals.

The acidification curves of RM during the fermentation process were performed with a Cinac32 equipment (Ysebaert Dairy Division, Frépillon, France), by continuous monitoring of pH. Inoculated RM (100 mL) were poured in 250 mL in Erlenmeyer flasks at 43 °C water bath until yogurts reached pH 4.6.

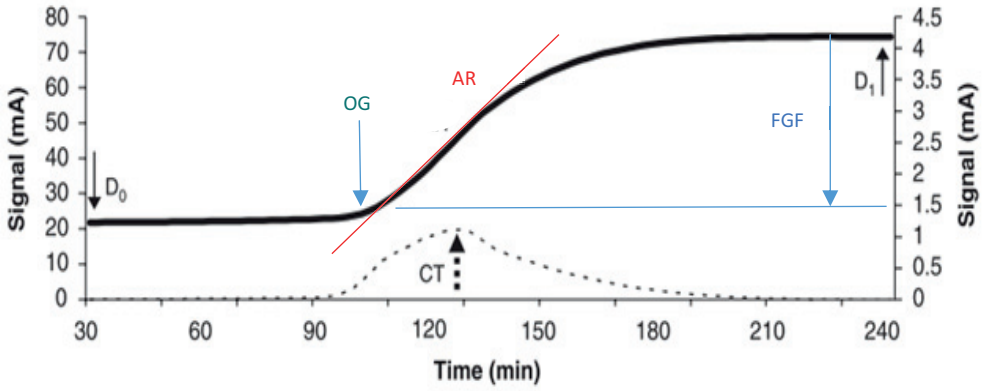

Fig. 1. Coagulation curves as a function of time. CT is the time at the maximum of first derivative value (dotted line). AR is the aggregation rate, which is the slope of the plot over the coagulation period. Final gelation firmness (FGF) was calculated as D1–D0.

5.2.4. Texture, rheology, and microstructure

Textural characteristics were evaluated by using a Texture Analyzer TA.TX2 (Stable Micro Systems, Surrey, UK) equipped with a flat cylindrical probe 35 mm diameter, applying a constant speed of 1 mm/s until to a depth of 5 mm. A back-extrusion test was performed to determine firmness (maximum force in compression) and consistency (compression work) (Liu et al., 2007). Briefly, 60 g of yogurt at 4 °C was poured into an 80 mm height, 45 mm diameter plastic container. The analysis was conducted in yogurts at the refrigeration temperature, about 4 ºC.

Dynamic oscillatory testing of stirred yogurts was performed using a rheometer Thermo Haake RS1 (Thermo Electron Corporation, Karlsruhe, Germany) with 20 mm diameter parallel serrated plates probe with 2 mm gap at 4 °C. One spoon of the sample was carefully loaded in the plate of the rheometer, and once the measure position was reached, sample was leaved to relax for 5 min before the test started. Frequency sweep was performed in the range of 0.1 to 10 Hz, in the viscoelastic linear region previously determined. The viscoelastic parameters, storage modulus (G') and loss modulus (G'') were recorded.

Confocal laser scanning microscopy (CLSM) observations were performed in a Leica TCS SP2 AOBS microscopy (Leica, Heidelberg, Germany). The protein matrix was stained using 10 mg of FITC (Fluka, Sigma- Aldrich, Germany) completely dissolved in ethanol. Then, it was added to 5 mL of pre-heated and inoculated RMs. After incubation at 43 ºC for 4 h, yogurts were kept overnight at refrigeration temperature and further stirred. Microscope observations were made with ×63 oil immersion objective at wavelength of 488 nm and with 1024×1024 format.

5.2.5. Physico-chemical characteristics of yogurts: color, pH, total acidity, and water holding capacity (WHC).

The color of yogurts was assessed with a colorimeter Konica Minolta CR-410 (Konica Minolta, Osaka, Japan), with a reference D65 light source and a 10° observer angle. The CIE L* a* and b* color coordinates were obtained. The following formulas were utilized to determine the Yellowness Index (YI), Whiteness Index (WI), respectively.

$$YI = 142.86 \times b^* \times L^{*-1}$$

$$WI = 100 - [(100 - L^*) + a^{*2} + b^{*2}]^{0.5}$$

The pH of yogurts was measured using Crison Basic 20 pH-meter (Crison Instruments S.A., Alella, Spain). Titratable acidity (TA) was determined according to the ISO/TS 11869:2012 method.

The WHC was determined by centrifuging 40 g of yogurt at 5000 *g* for 20 min at 22 °C to separate the supernatant (whey) in triplicate on days 1, 14, and 28. The following equation was used to calculate WHC%:

$$WHC\% = (W_1 - W_2)/W_1 \times 100$$

Where: W_1 =Initial yoghurt weight, W_2 = Weight of whey after centrifugation

5.2.6. Stability to oxidation

Quantification of hydroperoxides and malondialdehyde in yogurts, the methods proposed respectively by Hu et al. (2003) and Papastergiadis et al. (2012) were carried out, with slight modification, which was fully described previously (Varela et al., 2022).

5.2.7. Fatty acid composition of yogurts

Oleic, linoleic, and α-linolenic acid content of yogurts fortified with 4 and 6% of SDE was determined on days 1 and 28 of storage by using a gas chromatograph HP-6890 Series GC System (Hewlett-Packard, Waldbronn, Germany) equipped with a flame ionization detector and a HP-6890 Series Injector. The procedure reported by Bondia-Pons et al. (2007) with a slight modification. General chromatography conditions were as follow: separation of FAMEs was carried out on a capillary column (100 m × 250 μm × 0.20 μm), the carrier gas was helium with a head pressure of 220 kPa, the split ratio was 50:1 and the injection volume were 1 μL. The oven and detector temperature were 250 °C and 260 °C, respectively. The temperature program was as follows: initial temperature 100 °C, increased at 8 °C/min to 180 °C and held at this temperature for 9 min and increased at 1 °C/min to 230 °C and held at this temperature for 10 min (total run time: 84 min). Detector gas flow: H_2, 40 mL/min; make-up gas (He), 25 mL/min; air, 400 mL/min.

5.2.8. Microbial analysis

Mesophilic aerobic bacteria, aerobic spores, coliform bacteria, and *E. coli* were analyzed on RM samples. Samples were serially diluted in peptone water (Oxoid, Basingstoke, UK) and plated on Plate Count Agar (PCA, Oxoid) to enumerate mesophilic aerobic bacteria. Viable counts of spores were enumerated after the serial dilutions of samples were heated at 80 °C for 10 minutes, quickly cooled, poured in PCA plates, and incubated at 30 °C for 48 h. For the enumeration of coliforms/*E. coli*, serial dilutions were plated on the surface of Chromogenic Coliform Agar (CCA) and incubated at 37 °C for 24 h. *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* were enumerated according to ISO 7889:2003. For each treatment, samples were analyzed during storage at 4 °C on days 1, 14 and 28. Yogurt samples were serially diluted from 10^{-1} to 10^{-6} in peptone water. *L. bulgaricus* and *S. thermophilus* were counted in the selective media MRS and M17, under anaerobic conditions, at 37 °C for 72 h and 37 °C for 48 h, respectively. All samples were analyzed in duplicate.

5.2.9. Sensory evaluation

A panel of 20 university faculty and staff members who were familiar with yogurt (mean age = 31 years, with an age range between 22 and 61 years), were asked to identify differences. The sensory evaluation consisted of triangular, descriptive, and preference tests. Two triangular tests were carried out on days 7 and 14 after yogurt manufacture to assess whether the judges

could find significant differences between the two treatments of CH4 *vs* UH4 and CH6 *vs* UH6 or not. Once the results of the first triangular test had been analyzed, the effect of the concentration incorporated into the yogurts (UH4 versus UH6) was compared using another triangular test on day 14. A descriptive test based on a 7-point hedonic scale (1: dislike extremely; 7: like extremely) was conducted to identify the different attributes (creaminess, consistency, acidity, strange aroma, and lactic aroma) in the stirred yogurt. Finally, they were asked to rank the samples of yogurt between treatments UH4 and UH6 in order of preference.

5.2.10. Statistical analysis

Effect of different levels of SDE (treated with CH and UHPH) addition, and storage time on physical, chemical, and sensory parameters were analyzed based on one-way and two-way analysis of variance (ANOVA) test using the Minitab Express™ version 1.5.3 (Minitab, State College, PA, USA). Significant differences between means were determined by the Tukey test. A confidence level of 95% ($p < 0.05$) was used. At least three individual productions of each formulation and treatment were performed.

5.3. Results and discussion

5.3.1. Acid coagulation process

During the acidification process of yogurt, the milk caseins become unstable and coagulate, forming a protein matrix that entraps the aqueous phase and oil droplets, with varying degrees of interaction between them (Alexander & Dalgleish, 2004).

Table 1 shows the acid coagulation parameters of samples incubated at 43 °C, and the pH evolution during the coagulation process is depicted in Figure 2. The onset of gelation (OG) occurred significantly earlier in UHPH recombined milks compared to CH recombined milks (RMs). Additionally, increasing the percentage of added SDE (spray dried emulsion) resulted in earlier OG. This could be attributed to the smaller size of oil droplet aggregates in UHPH-RMs, which had a larger effective surface area compared to the individual oil droplets in CH-RMs (Varela et al., 2022). The smaller aggregates in UHPH-RMs contribute to the earlier onset of gelation, as observed in previous studies (Serra, Trujillo, Quevedo, Guamis, & Ferragut, 2007). However, once gelation started, the aggregation rate (AR) was higher in CH-RMs, as network formation primarily relies on protein-protein interaction. This could be due to the lower number of oil droplets per unit volume in CH-RMs, resulting in less steric hindrance. The symmetric shape of fat globules in CH-RMs compared to the asymmetry of aggregates in UHPH-RMs may also contribute to steric hindrance in casein network formation.

Consequently, UHPH yogurts exhibited a more porous network, leading to lower firmness at 43 °C compared to CH yogurts, aligning with the observations for the AR parameter.

Table 1. Coagulation parameters of yogurts containing different content (4 and 6 %) of CH and UH SDE.

Sample	OG (min)	AR (mA min$^{-1)}$	FGF (mA)
CH4	115±1^{a}	0.35±0.01^{b}	41.3±0.4^{b}
CH6	110.4±0.2^{b}	0.372±0.001^{a}	45.3±0.3^{a}
UH4	106.5±0.3^{c}	0.332±0.001^{c}	37.8±0.5^{c}
UH6	102±1^{d}	0.346±0.003^{b}	40.5±0.4^{b}

Means in each column with different superscript letters were significantly different ($P < 0.05$).
OG: onset of gelation; AG: aggregation rate; FGF: gel firmness at the end of gelation.

The acidification curves of the yogurts did not show significant differences between treatments, indicating similar kinetics of fermentation (pH < 0.05). The overlapping acidification curves in Figure 2 demonstrate that pH 4.6 was reached after approximately 198±3 minutes. This aligns with the findings of previous studies, which reported incubation times of about 183-240 minutes to reach the desired pH (Horiuchi et al., 2009; Soukoulis et al., 2007).

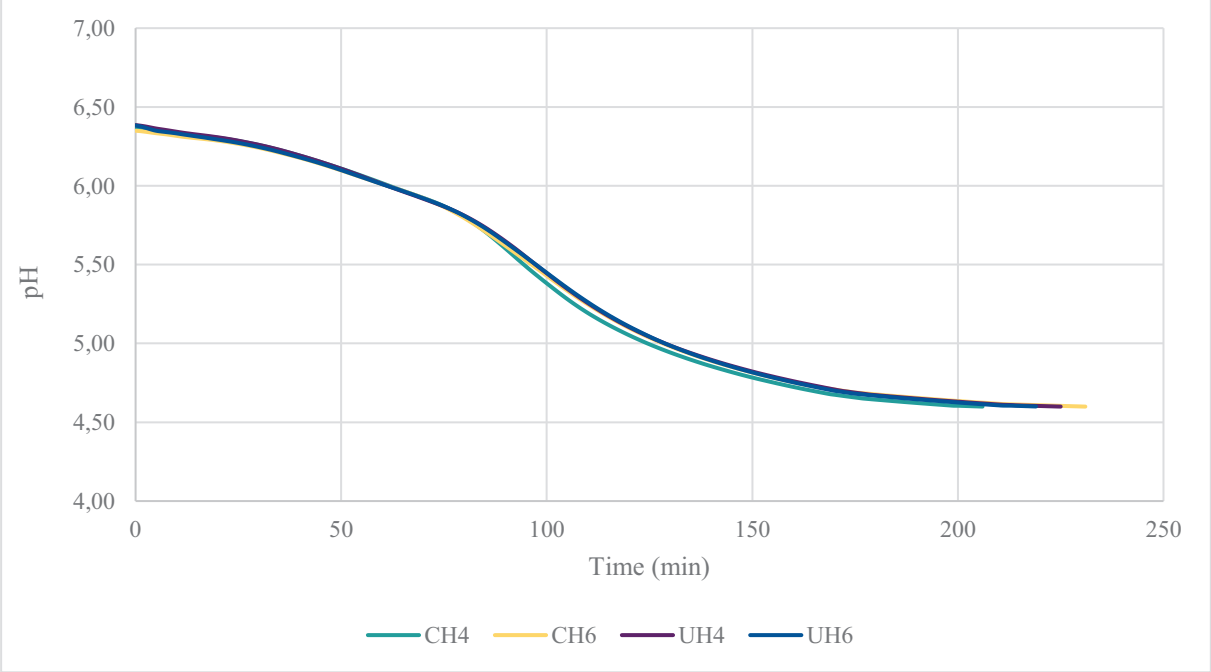

Fig. 2. pH curves as a function of time during fermentation of the different types of yogurts.

5.3.2. Texture, rheology, and microstructure

Texture parameters, specifically firmness and consistency, of the yogurts were assessed using back extrusion testing during storage at 4 °C. Firmness refers to the maximum force applied during compression, while consistency indicates the thickness of the sample (Ciron, Gee, Kelly, & Auty, 2010). Back extrusion testing provides insights into the behavior of the sample as it flows back under applied compression, leading to the destruction of the gel microstructure.

On the first day, there were no significant ($P > 0.05$) differences in texture parameters among the various yogurt samples (Table 2). At this early stage, when the stirred gel has not settled yet, the main factor influencing yogurt texture is likely the crystallized fat under refrigerated conditions (Serra et al., 2007), which was consistent across all yogurts. However, as the gel structure settled on days 14 and 28, some similarities and differences emerged among the yogurts during storage. CH6 and UH4 yogurts appeared to exhibit similar firmness and consistency, aligning with the microstructure observed in these yogurts (Fig. 3).

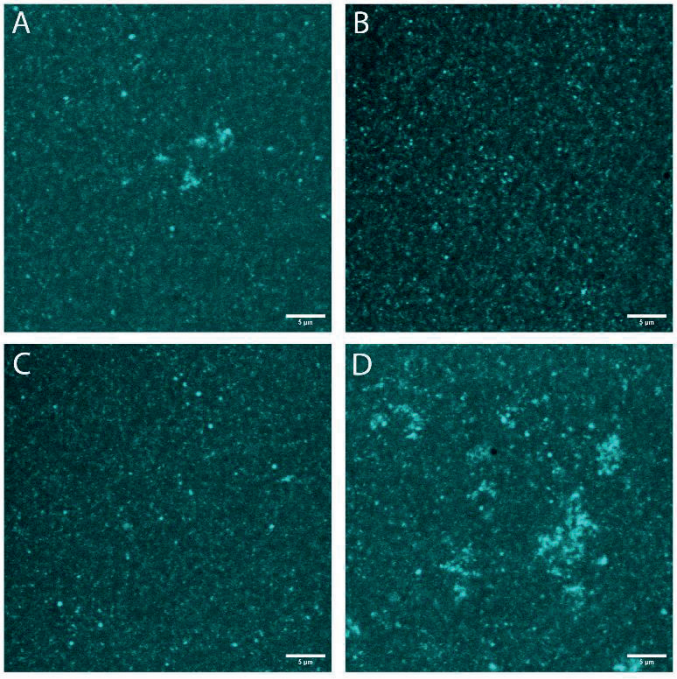

Fig. 3. CLSM images of the different types of yogurts: **(A)** CH4, **(B)** CH6, **(C)** UH4, and **(D)** UH6.

Both types of yogurts displayed a comparable integration of fat within the casein network: UH4 contained 4% SDE with small fat aggregates, while CH6 contained 6% SDE with larger individual droplets. This similarity suggests that the flow and compression of the gel structure

resulted in the formation of yogurt flocs of similar size. Previous studies on emulsions and SDEs have highlighted the formation of small aggregates of protein-bound oil droplets in UHPH emulsions (Aghababaei et al., 2021; Varela et al., 2022). In contrast, UH6 exhibited the lowest values of texture parameters. Although it may seem contradictory that the yogurt formulation with 6% SDE displayed the lowest texture parameter values, this can be explained by the higher amount of fat droplet aggregates acting as disruptive particles, interrupting the continuity of the casein network. As a result, more open spaces were present in the network of the stirred yogurts, as depicted in Fig. 3, which ultimately led to reduced texture parameter values. Similar findings were reported by Ciron et al. (2010) in low-fat stirred yogurts when comparing conventional homogenization and microfluidization at 150 MPa. Microscopic observations revealed that microfluidized milk-based yogurts exhibited more open spaces compared to those made with conventionally treated milk, impacting the texture parameters obtained through back extrusion testing.

Dynamic oscillatory testing with small amplitudes within the linear viscoelastic region allowed the assessment of interaction strength responsible for the network formation in semi-solid and solid foods without disrupting their structure. G' (storage modulus) represents the intensity and/or number of interactions contributing to network formation, while G" (loss modulus) relates to the viscous nature and interactions that do not directly affect the three-dimensional network (Tabilo-Munizaga & Barbosa-Cánovas, 2005). All yogurts exhibited characteristic solid behavior, with higher G' values than G" values, as shown in Table 2. CH yogurts consistently displayed significantly higher ($P < 0.05$) G' values than UHPH yogurts throughout the storage period. Moreover, the increase in viscoelastic parameters with the addition of higher percentages of SDE (4% and 6%) aligned with this difference, as expected. Furthermore, all yogurts showed a significant increase in both G' and G" from day 1 to day 28 of cold storage, indicating the settling of the gel structure and increased interactions among yogurt particles.

Table 2. Mean values ± SD of textural and rheological parameters of yogurts during storage at 4 °C.

Sample	d1	d14	d28
Firmness (N)			
CH4	$0.23\pm0.04^{a,A}$	$0.214\pm0.004^{ab,B}$	$0.183\pm0.003^{b,C}$
CH6	$0.24\pm0.03^{a,A}$	$0.22\pm0.01^{ab,AB}$	$0.200\pm0.005^{b,B}$
UH4	$0.26\pm0.03^{a,A}$	$0.233\pm0.007^{a,A}$	$0.22\pm0.01^{a,A}$
UH6	$0.21\pm0.02^{a,A}$	$0.190\pm0.008^{b,C}$	$0.183\pm0.004^{b,C}$
Consistency (N*s)			
CH4	$0.9\pm0.1^{a,A}$	$0.82\pm0.02^{a,AB}$	$0.78\pm0.01^{a,B}$
CH6	$0.9\pm0.1^{a,A}$	$0.84\pm0.04^{a,A}$	$0.80\pm0.01^{a,AB}$
UH4	$1.0\pm0.1^{a,A}$	$0.87\pm0.03^{a,A}$	$0.85\pm0.03^{a,A}$
UH6	$0.8\pm0.1^{a,A}$	$0.77\pm0.01^{a,B}$	$0.76\pm0.03^{a,B}$
G´ (Pa)			
CH4	$67\pm1^{c,B}$	$99\pm1^{b,B}$	$128.5\pm0.5^{a,B}$
CH6	$87.1\pm0.9^{c,A}$	$120.9\pm0.8^{b,A}$	$158\pm1^{a,A}$
UH4	$60.7\pm0.9^{c,D}$	$66\pm1^{b,D}$	$97.2\pm0.5^{a,D}$
UH6	$65\pm1^{c,C}$	$86.8\pm0.08^{b,C}$	$100\pm1^{a,C}$
G´´ (Pa)			
CH4	$18.7\pm0.6^{c,B}$	$25.7\pm0.3^{b,B}$	$33\pm1^{a,B}$
CH6	$24,7\pm0.2^{c,A}$	$32.0\pm0.5^{b,A}$	$40\pm1^{a,A}$
UH4	$17.2\pm0.4^{b,C}$	$17.4\pm0.3^{b,D}$	$26\pm1^{a,C}$
UH6	$18.6\pm0.3^{c,B}$	$22.8\pm0.2^{b,C}$	$26.1\pm0.3^{a,C}$

Different capital letters in each column indicate significant differences ($P < 0.05$).
Different small letters in each row indicate significant differences ($P < 0.05$) (n=3).

5.3.3. Evaluation of color, pH, acidity and WHC

Consumer preference is influenced by the immediate perception of color, which is a fundamental characteristic. The color coordinates (L*, a*, b*) of the yogurts were measured during storage, with L* values representing luminosity, the white achromatic attribute. L* values for all yogurts and storage periods were similar, ranging from 82.9 to 83.9. Stirred yogurt, characterized by a rough surface due to the gel particles formed during beating, exhibited a slightly lower diffuse reflection than expected. However, neither the type nor the content of SDE added to the RM had a significant influence on luminosity. The a* (green-red) and b* (blue-yellow) coordinates indicated positive values, with red and yellow contributing to the chromatic component of the color, respectively. The a* values ranged from 1.51 to 1.83, while the b* values ranged from 2.55 to 3.34, with the latter indicating a predominant yellow

contribution to the yogurt color. The calculated values of whiteness index (WI) and yellowness index (YI) (Table 3) demonstrated the prevalence of whiteness over yellowness, with a slightly higher yellowness observed in UHPH yogurts.

Table 3. Color parameters (WI, YI), WHC, TA and pH values of different yogurt samples during storage at 4 °C.

Sample/days	1	14	28
WI			
CH4	$94.86\pm0.01^{ab,B}$	$94.89\pm0.03^{a,A}$	$94.81\pm0.01^{b,B}$
CH6	$94.81\pm0.01^{b,B}$	$94.72\pm0.01^{c,C}$	$94.916\pm0.005^{a,A}$
UH4	$94.74\pm0.01^{b,C}$	$94.776\pm0.004^{a,B}$	$94.68\pm0.01^{c,C}$
UH6	$94.50\pm0.01^{b,D}$	$94.488\pm0.005^{b,D}$	$94.64\pm0.02^{a,D}$
YI			
CH4	$4.68\pm0.05^{a,D}$	$4.45\pm0.06^{b,D}$	$4.39\pm0.07^{b,B}$
CH6	$4.83\pm0.04^{b,C}$	$4.91\pm0.01^{a,C}$	$4.365\pm0.009^{c,B}$
UH4	$5.05\pm0.02^{a,B}$	$5.018\pm0.007^{b,B}$	$5.08\pm0.01^{a,A}$
UH6	$5.68\pm0.03^{b,A}$	$5.85\pm0.01^{a,A}$	$5.14\pm0.05^{c,A}$
pH			
CH4	$4.50\pm0.04^{a,A}$	$4.30\pm0.02^{b,A}$	$4.25\pm0.01^{b,A}$
CH6	$4.47\pm0.06^{a,A}$	$4.29\pm0.01^{b,A}$	$4.23\pm0.01^{b,A}$
UH4	$4.52\pm0.01^{a,A}$	$4.28\pm0.03^{b,A}$	$4.226\pm0.005^{c,A}$
UH6	$4.52\pm0.01^{a,A}$	$4.30\pm0.03^{b,A}$	$4.23\pm0.02^{b,A}$
TA (mmol/100g)			
CH4	$16.6\pm0.1^{b,A}$	$17.1\pm0.6^{ab,A}$	$17.5\pm0.2^{a,A}$
CH6	$16.8\pm0.1^{a,A}$	$17.1\pm0.2^{a,A}$	$17.1\pm0.2^{a,AB}$
UH4	$16.01\pm0.07^{c,B}$	$16.7\pm0.2^{b,A}$	$17.0\pm0.1^{a,AB}$
UH6	$15.81\pm0.07^{b,B}$	$16.4\pm0.4^{a,A}$	$16.6\pm0.3^{a,B}$
WHC (%)			
CH4	$31.0\pm0.2^{b,C}$	$31.1\pm0.1^{b,D}$	$31.8\pm0.3^{a,C}$
CH6	$35.20\pm0.07^{a,A}$	$35.2\pm0.4^{a,B}$	$35.9\pm0.7^{a,A}$
UH4	$33.1\pm0.1^{a,B}$	$33.1\pm0.3^{a,C}$	$33.5\pm0.4^{a,B}$
UH6	$35.7\pm0.5^{a.A}$	$36.0\pm0.1^{a,A}$	$36.3\pm0.6^{a,A}$

Different capital letters in each column indicate significant differences ($P < 0.05$). Different small letters in each row indicate significant differences
($P < 0.05$). Each value is expressed as mean ± SD.

The pH values did not differ significantly among all types of yogurts. A significant decrease ($P < 0.05$) in pH was observed from day 1 to day 14 of storage due to the high metabolic activity of the yogurt starters during the initial stages. The pH remained constant from day 14 until day 28 when yogurt production ceased in refrigeration (Lucey, 2004).

The titratable or total acidity of yogurts should ideally be within the range of 0.5 - 1.6% according to quality standards for fermented milks (WHO/FAO, 2003). Table 3 displays the total acidity values for each type of yogurt during the 28-day storage at 4 °C. Initially, CH yogurts exhibited higher total acidity compared to UHPH yogurts. This initial difference persisted during storage, although it was not significantly different ($P > 0.05$) from day 14 onwards. A study by Serra et al. (2009) on physicochemical characteristics of yogurts made from CH and UHPH-treated milks also reported higher total acidity in CH yogurts during storage. In this study, lactose primarily derived from UHT milk and skim milk powder used in RM formulations remained constant across all yogurts. Therefore, there were no significant differences ($P > 0.05$) in total acidity between yogurts with 4% or 6% added SDE. Additionally, the perceived sensory acidity among samples was found to be irrelevant, as will be shown in the corresponding section. It is worth noting that the total acidity in this study was into the at the higher end of the recommended range. This may be attributed to an over-acidification experienced on day 0, as the fermentation process was stopped below pH 4.6.

Syneresis, the expulsion of the aqueous phase from the yogurt gel, is a common issue that affects product quality (Lucey, 2004). The amount of expelled aqueous phase is inversely related to water-holding capacity (WHC). Hence, greater expulsion results in lower WHC. To minimize syneresis, skim milk powder (SMP) and/or milk proteins are commonly added in yogurt production. In this study, 3% SMP was added to all RMs. Centrifugation was used to induce syneresis and compare the WHC of the different yogurt types produced. It is important to note that centrifugation-induced syneresis does not fully represent the real network contraction of yogurts but rather indicates the gel structure ability to retain water. Results of WHC (Table 3) showed that CH6 and UH6 yogurts had significantly ($P < 0.05$) higher WHC compared to CH4 and UH4 yogurts. Furthermore, UHPH yogurts exhibited significantly higher WHC compared to CH yogurts. Thus, in this study, the SDE content and UHPH treatment were responsible for increasing water retention in yogurts. The positive contribution of UHPH treatment may be attributed to the higher water retention inside and at the interface of the oil-protein aggregates in UHPH-treated yogurts, as observed by Varela et al. (2022) in SDE obtained through UHPH treatment. It has been reported that acid-induced gelation of protein-based emulsions with high fat content leads to higher WHC (Gyawali and Ibrahim, 2016). This

could be due to the oil droplets, bound by proteins at the oil-water interface, acting as protein particles in the case of UHPH SDE, thereby increasing immobilized water.

Moreover, the WHC remained stable during the storage of each sample. Although water expulsion is a time-dependent phenomenon due to progressive compaction of the yogurt network over time (Li, Ye, & Singh, 2021; Gilbe & Turgeon, 2021), the values obtained through centrifugation also included water expelled by spontaneous syneresis, which was observed in small quantities and could not be measured accurately.

5.3.4. Stability to oxidation

Chia and sunflower oils are rich in PUFA, thus they are prone to oxidation during processing and storage, which may produce off-flavors (Estrada et al., 2011). Oxidation of yogurts containing chia and sunflower oils (50:50) were evaluated for primary (hydroperoxide concentration) and secondary oxidation (malondialdehyde, MDA, concentration) at days 1 and 28. The effect of type of homogenization system to obtain SDE and the percentage added to RMs (4 and 6%) on the primary and secondary oxidation of yogurts is shown in Fig. 4. Hydroperoxide concentration (Fig. 4A) of CH yogurts was significantly higher ($P < 0.05$) than UHPH yogurts on day 1 as well as on day 28 of storage. Moreover, the increase of those values of UHPH-yogurts from day 1 to day 28 was much less pronounced than those observed in CH yogurts. Varela et al. (2022) observed similar results in SDE containing 7% BM, like in the present study, i. e. 7CH-SDE was more prone to primary oxidation than 7UHPH-SDE. However, they performed the study of oxidation stability in forced conditions at 50 ºC for one month and observed a maximum value of hydroperoxide concentration at day 15, which was followed by a reduction in day 31, meaning that the secondary oxidation was in progress by the degradation of hydroperoxides. In the present study, the oxidation was analyzed in yogurts at 4 ºC, thus the further degradation of hydroperoxides was not produced.

One of the final products of polyunsaturated fatty acid oxidation is MDA, which is indicative of the secondary oxidation (Fig. 4B). This parameter showed a similar pattern as primary oxidation, with higher MDA values in CH compared to UHPH-yogurts, both on days 1 and 28. As was observed in 7SDE by Varela et al. (2022), the encapsulation efficiency of UHPH-SDE, in general and, in 7UHPH-SDE, in particular, were higher than those SDE obtained by CH. Probably, the small droplets well covered by phospholipids and proteins forming aggregates were responsible to keep well retained the oil in those colloidal structures, and therefore, protecting oil against oxidation.

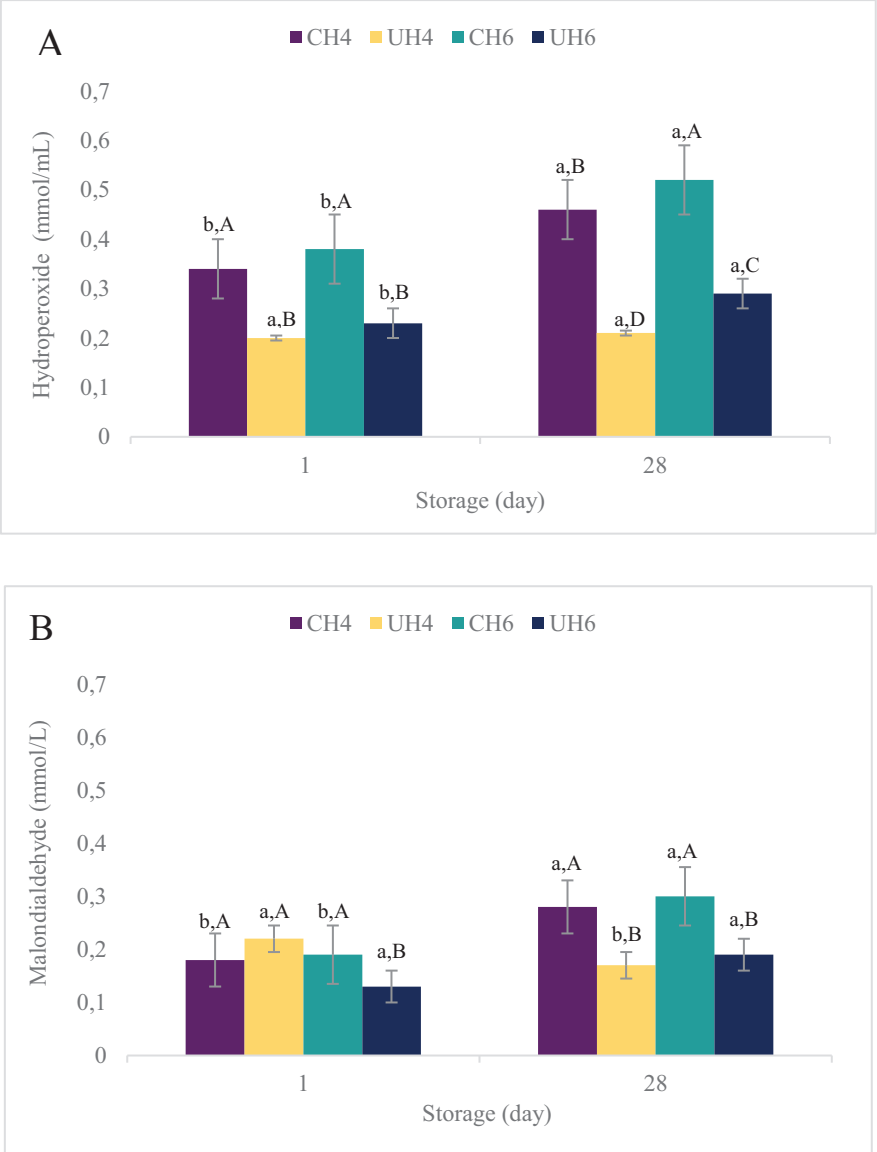

Fig. 4. (A) Hydroperoxides and **(B)** malondialdehyde concentration of yogurts at days 1 and 28 of storage at 4 ºC.

5.3.5. Fatty acids content

The main composition of unsaturated fatty acids (oleic, linoleic, and α-linolenic acids) in yogurts on days 1 and 28 of storage, analyzed using gas chromatography, is presented in Table 4. Linoleic acid was found to be the most abundant polyunsaturated fatty acid (PUFA) in all yogurts due to the 50:50 ratio of chia and sunflower oils in their composition. This oil mixture was selected to mitigate the strong green aroma of pure chia oil and contribute to a balanced ratio of omega-6 to omega-3 fatty acids in yogurts. Sunflower oil is rich in oleic and linoleic

fatty acids, while chia oil primarily contributes to the α-linolenic acid content of the mixture, with a lesser extent of linoleic acid. As anticipated, increasing the amount of SDE added to RMs resulted in higher levels of fatty acids in yogurts. In all cases, UHPH treatment significantly increased the content of unsaturated fatty acids in yogurts compared to CH. UHPH yogurts contained higher amounts of oleic, linoleic, and α-linolenic acids. As mentioned earlier (Aghababaei et al., 2021; Varela et al., 2022), the encapsulation efficiency of UHPH-treated SDE was higher than that of CH, providing better protection against oxidation, as observed in section 3.4. During storage, a significant decrease in FA concentrations was observed in yogurts. In addition to the high susceptibility of unsaturated FA to oxidation, hydrolysis of triglycerides with the liberation FA, which was observed in the over-acidification during storage, may have also contributed to the oxidation (Table 3). Despite the reduction in FA levels during storage, the PUFA content of yogurts makes a valuable contribution to the diet since yogurt is a daily food consumed by many people, enriching the intake of these essential fatty acids for a balanced diet.

Table 4. Fatty acid composition (mg/100g) of the different yogurts on days 1 and 28 of storage at 4 ºC.

Sample/day	D1	D28
Oleic acid		
CH4	$107.80\pm3.87^{a,B}$	$84.16\pm4.36^{b,B}$
CH6	$185.61\pm5.73^{a,A}$	$149.53\pm7.85^{b,A}$
UH4	$118.61\pm6.81^{a,B}$	$94.47\pm3.16^{b,B}$
UH6	$194.91\pm2.05^{a,A}$	$155.463\pm1.420^{b,A}$
Linoleic acid		
CH4	$201.92\pm2.99^{a,C}$	$146.89\pm2.03^{b,C}$
CH6	$256.26\pm8.29^{a,A}$	$233.84\pm2.34^{b,A}$
UH4	$216.77\pm2.97^{a,B}$	$189.86\pm3.81^{b,B}$
UH6	$268.167\pm1.419^{a,A}$	$242.23\pm6.38^{b,A}$
Linolenic acid		
CH4	$190.01\pm3.12^{a,C}$	$112.30\pm3.74^{b,D}$
UCH6	$227.73\pm4.10^{a,A}$	$174.80\pm3.64^{b,B}$
UH4	$210.79\pm8.08^{a,B}$	$158.66\pm1.99^{b,C}$
UH6	$240.73\pm4.94^{a,A}$	$193.573\pm1.615^{b,A}$

Different capital letters in each column indicate significant differences for each sample and for each FA in the same day ($P < 0.05$).

Different lowercase in each row for each FA indicate significant differences for each sample in days 1 and 28 ($P < 0.05$).

5.3.6. Microbiology

Initial microbial counts of RMs for further production of yogurts were performed in selective media for coliforms/*E. coli*, mesophilic aerobes, and their spores. No coliform/*E. coli* colonies were detected in any of the samples, and, in the case of mesophilic aerobes and their spores, the mean values were 1.70 and 1.34 log CFU/mL, respectively.

European legislation (WHO/FAO, 2003) states that the lactic acid bacteria in yogurt (*L. bulgaricus* and *S. thermophilus*) must be viable and present in the finished product in a minimum amount of 1×10^7 CFU/g or mL. Table 5 shows the microbial counts of each microorganism in yogurts after 0, 1, 14, and 28 days in MRS and M17 media (selective for *L. bulgaricus* and *S. thermophilus*, respectively). Counts after yogurt production corresponded to the RM inoculated and analyzed prior to the fermentation process, which explains the significant difference between the counts on day 0 and those on subsequent days. Once the fermentation process was completed, in general, bacterial growth was around 7 log CFU/g, as required by legislation.

Table 5. Microbiological counts (mean log CFU/g ± SD) of different yogurt samples during storage at 4 °C.

Sample/days	d0	d1	d14	d28
L. bulgaricus				
CH4	$4.56\pm0.22^{b,A}$	$7.01\pm0.62^{ab,A}$	$7.85\pm1.74^{a,A}$	$7.53\pm0.49^{a,A}$
CH6	$4.60\pm0.30^{b,A}$	$6.91\pm0.42^{a,A}$	$6.96\pm1.50^{a,A}$	$7.28\pm0.56^{a,A}$
UH4	$4.55\pm0.23^{b,A}$	$7.24\pm0.30^{a,A}$	$6.78\pm0.81^{a,A}$	$7.41\pm0.70^{a,A}$
UH6	$4.58\pm0.21^{b,A}$	$7.36\pm0.40^{a,A}$	$6.81\pm0.86^{a,A}$	$7.45\pm0.50^{a,A}$
S. thermophilus				
CH4	$4.17\pm1.63^{b,A}$	$8.50\pm0.71^{a,A}$	$8.44\pm0.96^{a,A}$	$9.15\pm0.00^{a,B}$
CH6	$3.69\pm0.65^{b,A}$	$8.58\pm0.65^{a,A}$	$8.41\pm0.96^{a,A}$	$9.19\pm0.00^{a,A}$
UH4	$3.17\pm0.39^{b,A}$	$8.79\pm0.47^{a,A}$	$8.51\pm0.98^{a,A}$	$9.14\pm0.00^{a,C}$
UH6	$3.79\pm0.59^{b,A}$	$8.58\pm0.67^{a,A}$	$8.46\pm0.92^{a,A}$	$9.13\pm0.00^{a,C}$

Different capital letters in each column indicate significant differences ($P < 0.05$). Different small letters in each row indicate significant differences ($P < 0.05$).

Yogurts made with SDE treated by CH or UHPH showed no significant differences ($P > 0.05$) between them and no effect related to the percentage of SDE added to yogurts was observed.

Counts of *L. bulgaricus* remained constant during the storage of yogurts while *S. thermophilus* showed a significant increase ($P < 0.05$) in counts on day 28, which could be related to a greater adaptation of S. thermophilus to the formulations used.

5.3.7. Sensory evaluation

Sensory evaluations were conducted using triangular testing, followed by descriptive and preference testing. Twenty judges participated in different sets of triangular tests to determine if the addition of different percentages of SDE (4% or 6%) and the type of homogenization (CH or UHPH) were detectable in the yogurts. The sets of triangular tests were carried out sequentially, comparing two different samples in each set, based on the results of the previous tests. The first set of triangular testing (Fig. 5A) compared CH4 vs UH4 yogurts, and no significant differences were detected. Therefore, the next set of triangular tests (Fig. 5B) compared CH6 vs UH6 yogurts, and again, no significant differences were observed. Subsequently, a third set of triangular tests (Fig. 5C) compared UH4 vs UH6 yogurts, based on the superior quality characteristics observed in this study compared to CH yogurts. The results of this set of triangular testing did not show any significant differences ($P < 0.05$) between UH4 and UH6, indicating that none of the pairs of samples were distinguished by the judges. This is particularly important because the addition of 6% SDE, which contains a higher concentration of PUFA, did not result in any unusual flavors in the yogurts.

For the descriptive test, five terms associated with the sensory profile of the yogurts were evaluated: consistency, creaminess, acidity, lactic aroma, and off-flavors. Fig. 5D illustrates that UH6 obtained higher scores than UH4 in all parameters except "lactic aroma" and "acidity." "Consistency" and "creaminess" were the only parameters that showed a significant difference ($p < 0.05$) between the two samples. UH6 yogurts obtained scores of 4.3 ± 0.4 and 4.43 ± 0.43, respectively, on a scale of 0-5, while UH4 yogurts obtained scores of 3.8 ± 0.4 and 3.75 ± 0.35, respectively, for those attributes. Additionally, although not significantly different ($P > 0.05$), the preference test conducted between UH4 and UH6 showed that yogurts with 6% SDE received higher scores, likely due to the better texture perceived in the descriptive test.

Fig. 5. Sensory analysis of yogurts. Triangular testing of the different pairs of yogurts: **(A)** CH4 vs UH4, **(B)** CH6 vs UH6, **(C)** UH4 vs UH6. **(D)** Descriptive test of yogurts UH4 and UH6.

5.4. Conclusions

The choice of homogenization system (CH or UHPH) influenced the quality parameters of the yogurts. CH resulted in larger, well-protected droplets that favored casein-casein interaction and provided higher firmness. In contrast, UHPH created smaller droplet aggregates coated with caseins and phospholipids, leading to interrupted casein networks but improved water-holding capacity (WHC). UHPH also offered better protection against oxidation, preserving PUFA content during storage compared to CH yogurts. Importantly, sensory evaluation indicated that neither the homogenization system nor the SDE content significantly impacted yogurt flavor, allowing for the use of higher SDE concentrations without altering sensory attributes.

UHPH technology, with its ability to simultaneously homogenize and modify colloidal structures, has broad applications in the food industry. It offers opportunities for improved emulsion stability and interaction between emulsifiers and the oil phase.

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Chapter 6

General discussion

General discussion

The primary objective of this PhD research is to comprehensively investigate the distinctive properties and attributes of functional emulsions produced through the utilization of Ultra-High-Pressure Homogenization (UHPH) technology, which will subsequently undergo spray drying. These emulsions were formulated by combining varying concentrations of buttermilk with polyunsaturated fatty acid-rich vegetable oils, such as chia oil, rich in α-linolenic acid, and sunflower oil, rich in linoleic acid. The ultimate purpose of these emulsions is to serve as a substitute for milk fat in stirred yogurt, and to assess their overall quality.

This thesis has been divided into three parts which have been studied sequentially. Each one corresponds to the specific objectives and which have been the subject of publication. Previous studies were conducted to establish the formulations of emulsions that would later undergo dehydration and be used for the experimental design of emulsions in this thesis (Annex II). The stability of these emulsions was evaluated through various techniques including particle size measurements, rheological studies, calculation of the stability index using optical measurements, and, in some instances, spray drying to observe the drying aptitude and morphology of the dried emulsions through scanning electron microscopy observations.

These previous experiments were conducted to formulate emulsions by varying the type of wall material, specifically maltodextrin (MD) and octenyl succinyl anhydride modified starch (OSA), as well as their proportion in the formulation. The choice of these materials was based on studies published by different authors. However, the use of OSA, either alone or in combination with MD, was discarded due to the high viscosity of the resulting emulsions, rendering them unsuitable for subsequent drying. Furthermore, OSA's emulsifying capacity, resulting from the presence of hydrophobic groups, could mask the emulsifying capacity of the buttermilk, which is a significant focus of study in this thesis.

In addition, experiments were performed by varying the oil and buttermilk content to determine the optimal combination that would ensure good flowing ability with the 10% oil content for achieving satisfactory emulsion stability. Based on the results obtained, it was decided to maintain a constant oil content of 10% and a constant MD content of 30%, while varying the concentration of buttermilk between 4% and 7%.

In the first part of this thesis, the objective was to achieve a fine and stable emulsion for further spray drying, four different formulations were produced (Chapter 4). O/W emulsions formulated with 10% chia: sunflower oil (50:50); 30% MD, and 4 to 7% BM were obtained by UHPH at 100 and 200 MPa and CH at 30 MPa (all treatments 30 ºC inlet temperature). According to this study, UHPH significantly reduced the particle size of the oil droplets with the different BM concentrations and UHPH treatments used compared to the CH. The values of CH-processed emulsions exhibited a decrease in oil droplet size as the concentration of BM was increased from 4 to 7%. However, the differences were only found to be significant between BM concentrations of 4-5% and 6-7%. In UHPH-treated emulsions, an increase in pressure from 100 to 200 MPa resulted in a reduction of particle size at the same BM concentration. The most notable decrease was observed in UH200-treated samples as the BM concentration rose from 4 to 7%. However, despite the smaller size of the particles produced by the UHPH treatment compared to the conventional one, it was observed that the particles from the former showed a higher number of colloidal structures in the form of small droplet aggregates. When particle size analysis was performed in 0.05% SDS media, aggregates were dispersed, showing individual oil droplets to be significantly lower than those dispersed in water. The presence of aggregates appears to be a pertinent factor in the rheological behavior of all studied emulsions, exhibiting a slight thixotropic character. However, the equilibrium viscosity observed at relatively low deformations demonstrated adequate values for the purpose of spray drying. The formation of aggregates can be attributed to the partial denaturation caused by the combined effect of high pressure and temperature increases in the high-pressure valve during UHPH treatment. Moreover, depletion flocculation could be the most possible mechanism of aggregation due to the effective reduction of individual droplets in the UHPH treatments, concomitant with the presence of 30% MD in the formulations. Probably the thermodynamic incompatibility between carbohydrates and proteins contributed to the aggregate formation.

Zeta potential, which is a measure of the particle charge in the dispersed phase, is an important factor affecting emulsion stability. It provides information about the electrostatic interactions between the dispersed droplets. In the case of the emulsions under study, regardless of the applied treatment, the measured zeta potential values ranged from -22 to -29 mV, indicating a delicate dispersion threshold. The observed low zeta potential values in the emulsions, particularly in the ones treated with UHPH, suggest

that the treatment process has an impact on the charge properties of the particles. It is worth noting that this effect was consistent across different concentrations of the BM and applied pressures. The lower zeta potential values in the UHPH-treated emulsions can potentially be attributed to a reduced exposure of proteins and phospholipids at the interface. Proteins and phospholipids that are typically present at the interface may interact more with each other within these aggregates. This internal interaction within the aggregates could result in a lower availability of charged species at the droplet interfaces, leading to a decrease in the measured zeta potential.

In terms of emulsion stability, it seems that particle size combined with the effect of coverage of oil droplets by protein-phospholipid were the main factors that contributed to stability. Dynamic stability was evaluated by using Turbiscan® equipment during 8 days of storage at 20 ºC. The total stability index (TSI) was calculated from backscattering curves. CH emulsions resulted in higher TSI values than those of UHPH samples, indicating that the former was less stable than the latter. UHPH samples showed little differences in stability between 100 and 200 MPa. On the other hand, there was a significant impact of BM concentration on stability. At 4 and 5% BM concentration, the difference in TSI was more significant between CH and UHPH samples, compared to 6 and 7% BM emulsions, which demonstrate the important contribution of BM concentration to emulsion stability. In addition to TSI, creaming layer thickness (CL) was measured at day 1 and 8 of storage. UHPH significantly showed a small CL in compared to CH emulsions. Although the influence of BM concentration on CL in UHPH treatments was not significant, there was a correlation of this parameter with the type and degree of homogenization applied, which in turn was in line with the particle size values observed.

From results in the previous study (chapter 5), the second experiment aimed to characterize and assess the oxidative stability of the spray-dried emulsions (SDE). The selected formulations for this experiment were those containing the minimum (4%) and the maximum (7%) BM content. Notably, the formulations with 4% and 5% BM exhibited similar characteristics, as did the formulations with 6% and 7% BM.

The characterization of SDE involved determining various parameters relevant to powders, such as water content, water activity, flowing properties, color parameters, and solubility. Encapsulation efficiency and morphology by SEM was also evaluated. To assess the oxidation stability of SDE, accelerated oxidation conditions at 50 °C were

employed, and the analysis was conducted on days 1, 7, 14, and 31 of storage, focusing on primary and secondary oxidation evolution.

The drying conditions for the SDE were chosen based on data from the literature (Table 5 in the introduction) concerning dried o/w emulsions containing PUFAs. The optimization process was conducted to ensure that the spray-dried emulsions (SDE) exhibited good flowability and were not sticky, thus yielding satisfactory results.

The total solid content of the SDE formulations containing 4% BM was 44%, while those with 7% BM had a total solid content of 47%. The moisture content of the SDE ranged from 2.06% to 3.19. The 4CH SDE had the lowest water content, while the 7UH100 sample had the highest water content. It was evident that both the homogenization treatment and the BM concentration influenced the water content. In CH treatments, water was likely easier to eliminate during spray drying due to the lower particle volume fraction observed in these samples. This trend of CH samples compared to UH samples was observed for both 4% and 7% BM concentrations. Furthermore, within the series of SDE with different BM concentrations, the 7% BM SDE exhibited higher water content, which could explain the challenges faced during water evaporation in the drying process. Additionally, the different colloidal structures observed, such as larger droplets in CH or smaller aggregates in UH SDE, might indicate variations in protein distribution between the continuous and oil-water interface of the feeding emulsions, thereby impacting water elimination during the spray-drying process. However, it is worth noting that, in terms of powder stability during storage, dry foods with moisture content ranging from 3% to 10% have been reported to exhibit good behavior. This suggests that the observed moisture content variations within the SDE formulations in this study fall within an acceptable range.

The water activity (Aw) values of the spray-dried emulsions (SDE) ranged from 0.130 to 0.191, and no significant differences were observed among them. However, it is worth noting that the SDEs with lower BM content tended to exhibit slightly higher Aw values, which aligns with expectations. Despite these minor variations, all the Aw values obtained in this study are considered suitable for dry foods, as they ensure microbiological stability.

Bulk density is a significant characteristic of powders, and in this study, it exhibited a narrow range, with values from 430 to 455 kg/m3 for all samples. No significant differences were observed between the bulk density values of the different spray-dried emulsions (SDE). It is worth noting that these values fall within the range reported in the literature for most spray-dried emulsions, which can vary widely depending on the composition and drying conditions employed. The bulk density values obtained in our study are consistent with those reported by other authors (Table 5). The flowing properties of the spray-dried emulsions (SDE), represented by Carr's Index (CI) and Hausner ratio (HR), demonstrated significantly different behaviors, primarily influenced by the concentration of BM, regardless of the homogenization system employed. Specifically, all SDEs with 4% BM exhibited fair flowing properties, which were different from those of the 7% BM formulations that most of them showed near excellent flowing properties.

Regarding color, the L* value did not exhibit significant variations between the applied treatments or BM concentrations. However, the a* value displayed a slight range in the red tonality, while the b* value primarily contributed to the observed color differences, resulting in near-white samples as indicated by yellowness (YI) and whiteness (YI) values. It was noted that the formulations containing 7% BM showed the highest WI values, indicating improved whiteness.

The encapsulation efficiency (EE) serves as an indicator of the ability of the wall material to retain oil within microcapsules. In this study, the EE values demonstrated effective oil encapsulation, ranging from 77.1% to 91.5% across the different SDE. Both the concentration of BM and the homogenization system employed influenced the results obtained. In formulations containing 4% BM, the application of UHPH treatment, at either 100 or 200 MPa, significantly improved the oil retention capacity of the microcapsules compared to the conventionally homogenized (4CH) formulation. Notably, the highest EE values were observed in the 7UH100 and 7UH200 SDEs, which exhibited similar values. This suggests that the emulsifying properties of the BM, attributed to its composition rich in casein, whey proteins, and phospholipids, enable its adsorption at the oil-water interface, thereby facilitating the desired coverage of oil droplets. Consequently, increasing the BM content can lead to improved effective oil coverage and, consequently, enhance the encapsulation efficiency.

All the SDEs produced in this study displayed high solubility in water at room temperature.

The oxidation stability of the SDEs was assessed through the analysis of primary and secondary oxidation products. The primary oxidation curves exhibited a similar pattern in all samples, characterized by a subtle increase in concentration between days 1 and 7, a pronounced increase on day 14, and a noticeable decrease on day 31. This observed evolution of hydroperoxides aligns with the kinetics of primary lipid oxidation, where an initial latency period is followed by exponential propagation of oxidation products. The subsequent decrease is attributed to the formation of secondary oxidation products resulting from the degradation of hydroperoxides and other products.

When considering the BM concentration, the hydroperoxide levels in the 7% BM samples were lower during storage compared to the 4% BM samples. This suggests that the 7% BM formulation provided greater protection in recovering the interface of small oil droplets and aggregates generated by UHPH treatment, in comparison to the 4% BM formulation. The influence of BM concentration and homogenization treatment on oxidation stability correlates with the encapsulation efficiency (EE) results observed. The samples treated with UHPH and containing higher BM concentrations exhibited higher EE values.

Secondary oxidation was evaluated by measuring the concentration of malondialdehyde (MDA) in the SDE over time. Generally, low levels of MDA were observed in most of the stored SDE, with an increase observed on day 31. This increase was particularly notable in the SDE containing 7% BM. The rise in MDA concentration coincided with the decrease in hydroperoxides, confirming that as the accelerated storage period approaches its end, the oxidation state continues to advance. The higher MDA concentrations observed in the 7% BM formulations can be attributed to the presence of certain compounds in BM that may act as pro-oxidants, especially if they are present in excess. BM contains phospholipids, which can exhibit antioxidant effects. However, in dehydrated dairy products, these phospholipids may also behave as pro-oxidants due to the unsaturation present in milk fat globule membrane (MFGM) phospholipids such as phosphatidylcholine and phosphatidylethanolamine.

It is important to note that the oxidation stability study was conducted under accelerated conditions, which may not precisely reflect the oxidative behavior of the SDE under normal storage conditions.

In the subsequent study, 7% spray-dried emulsions (SDEs) treated with both CH and UHPH at 200 MPa were utilized in the production of yogurt. Recombined milks (RMs) were formulated by adding two different concentrations (4% and 6%) of SDE to UHT skim milk with 3% skim milk powder (SMP) and 2% starter culture, followed by fermentation (Chapter 6). Gelation process and yogurt characteristics were assessed during cold storage, encompassing various parameters such as texture and rheology, microstructure, physicochemical characteristics (color, pH, total acidity, and water holding capacity), oxidative stability, main fatty acid profile, microbial assessment, and sensory evaluation.

During the monitoring of the gelation process, it was observed that the onset of gelation (OG) occurred significantly earlier in UHPH-RMs compared to CH-RMs. Additionally, an increase in the percentage of SDE added resulted in a further advancement of OG. This phenomenon can be attributed to the smaller size of oil droplet aggregates in UHPH-RMs, which possess a larger effective surface area compared to individual oil droplets in CH-RMs. However, once gelation began, the aggregation rate (AR) was found to be higher in CH-RMs, as network formation primarily relies on casein-casein interactions. Thus, the lower number of oil droplets per unit volume in CH-RMs, could facilitate the protein network formation. The symmetric shape of fat globules in CH-RMs, in contrast to the asymmetry of the high content of aggregates in UHPH-RMs, may also contribute to steric hindrance in casein network formation in UHPH yogurts. Consequently, UHPH yogurts exhibited a more porous gel, resulting from aggregate interruption of the casein network. These hypotheses were supported by microstructural observations through confocal microscopy and results from mechanical evaluations such as back extrusion and viscoelasticity testing. CH yogurts exhibited higher firmness, consistency, and viscoelastic parameters (G' and G") compared to UHPH yogurts at the same SDE concentration. On the other hand, all yogurts displayed a significant increase in both G' and G" from day 1 to day 28 of cold storage, indicating the settling of the gel structure and increased interactions among yogurt particles.

However, yogurts produced by UHPH treatment, improved the water holding capacity (WHC). This parameter is crucial for yogurt quality. The results indicated that the incorporation of 6% SDE in both CH and UHPH yogurts significantly increased the WHC compared to the 4% yogurts. Moreover, UHPH yogurts exhibited superior WHC compared to CH yogurts. The positive contribution of UHPH treatment to WHC may be attributed to the higher water retention inside and at the interface of the oil-protein aggregates in UHPH-treated yogurts.

The titratable or total acidity of yogurts should ideally be within the range of 0.5 - 1.6% according to quality standards for fermented milks. All yogurts in this study were into the stablished range. Although initially CH yogurts displayed higher acidity during the first 14 day, from that moment on, the acidity did not show significant differences between the samples.

Oxidation of yogurts containing chia (rich in α-linolenic) and sunflower (rich in oleic FA) oils (50:50) were evaluated for primary (hydroperoxide concentration) and secondary oxidation (malondialdehyde, MDA, concentration) at days 1 and 28.

The hydroperoxide concentration in UHPH yogurts was significantly lower than in CH yogurts, both on day 1 and day 28 of storage. Additionally, the increase in hydroperoxide levels from day 1 to day 28 was much less pronounced in UHPH yogurts compared to CH yogurts. The trend observed for secondary oxidation, measured by MDA values, was consistent with primary oxidation. Hence, UHPH provided superior protection against oxidation, thereby preserving PUFA content during storage when compared to CH yogurts. Probably, the small droplets well covered by phospholipids and proteins forming aggregates were responsible to keep well retained the oil in those colloidal structures, and therefore, protecting oil against oxidation. In line with these results, in all cases, UHPH yogurts showed higher content of oleic, linoleic, and α-linolenic acids compared to CH yogurts.

Apart from these quality parameters, microbial analysis of the yogurts demonstrated that, yogurts made with SDE treated by CH or UHPH ere similar, and no effect related to the percentage of SDE added to yogurts was observed. Counts of L. bulgaricus remained constant during the storage of yogurts, while S. thermophilus showed a significant increase in counts on day 28, which could be related to a greater adaptation of S.

thermophilus to the formulations used. All yogurts showed viable acid bacteria counts in the finished product around 7 log CFU/g, as required by legislation.

From the sensory point of view, the successive sets of triangular testing performed led to compare UH4 vs UH6, which neither produced significative differences in the panel of judges. In descriptive test between this pair of samples, the two parameters "consistency" and "creaminess" were the only that showed a significant difference. Finally, yogurt with higher scores was 6UH, likely due to the better texture perceived in the descriptive test.

Chapter 7

Conclusion

Conclusion

1. The application of UHPH technology in the production of feeding emulsions (O/W) containing 4-7% buttermilk, 30% maltodextrin, and 10% chia oil and sunflower oil (50:50) has proven to be highly effective in achieving a more stable emulsion compared to conventional homogenization. The key factor behind this improved stability is the generation of smaller particle sizes through UHPH. The emulsions treated with UHPH at 100 and 200 MPa exhibited high physical stability, particularly in the case of 7% buttermilk, showing significant resistance against creaming in the formulations.

2. Microstructure analysis revealed great differences between UHPH and conventional homogenization emulsions in the interaction of oil droplets en the disperse phase. UHPH-treated emulsions showed small tightly joined droplets forming aggregates, which were smaller than the mean value of the individual droplets of the conventionally treated emulsions. Furthermore, the application of 200 MPa during UHPH resulted in smaller and more regular-shaped droplets, leading to a more homogeneous disperse phase compared to emulsions treated at 100 MPa. Conventionally treated emulsions presented low content of big aggregates which seemed to exhibit weaker interaction within droplets.

3. Comparison between conventional homogenization and UHPH technologies, as well as concentrations of buttermilk, did not demonstrate a clear correlation between those variables and the rheological behavior of the samples. This lack of correlation may be attributed to the irregular particle size generated in the homogenization process and /or when subjected to shear stress during the rheological evaluation. Despite this, all samples exhibited non-Newtonian behavior with a slight thixotropic character. However, in all cases the equilibrium viscosity was attained at low shear rate values, which were suitable for further drying of emulsions.

4. Spray-dried emulsions demonstrate that the use UHPH treatment, as compared to conventional homogenization, yields similar or improved general properties of the powders. Notably, the encapsulation efficiency was enhanced when UHPH treatment

was employed. This improvement states the effectiveness of buttermilk as encapsulant in the production of dried emulsions and highlight the potential of UHPH technology to enhance the performance and quality of dried emulsions products, offering opportunities for the development of improved encapsulation processes in various applications.

5. Flowing properties of the spray-dried emulsions were significantly influenced by the concentration of buttermilk, irrespective of the homogenization system utilized. Notably, dried emulsions containing 4% buttermilk displayed fair flowing properties, while those with 7% BM demonstrated near excellent flowing properties for most of the formulations. This indicates the ability for handling and transportation of powders.

6. No significant differences were observed in UHPH and conventionally treated dried emulsions in terms of water solubility, and water activity. All dried emulsions produced in this study showed high solubility in water at room temperature. The range of water activity values between 0.13 and 0.19 obtained in this study are considered suitable for dry foods, as they ensure microbiological stability.

7. The oxidation stability of spray-dried emulsions is influenced by buttermilk concentration and homogenization technology. Emulsions with 7% buttermilk exhibit greater stability against primary oxidation compared to 4% buttermilk formulations. The use of UHPH provides enhanced protection for maintaining the integrity of small oil droplets and aggregates compared to conventional homogenization. Most emulsions showed low levels of malondialdehyde indicating secondary oxidation. However, the 7% buttermilk formulations displayed increased malondialdehyde levels towards the end of storage, possibly due to the pro-oxidant effect of phospholipids at the oil-water interface, particularly under accelerated storage conditions.

8. The gelation process of yogurts prepared with 4% or 6% dried emulsion containing 7% buttermilk revealed different characteristics between UHPH and conventional

homogenization treated samples. UHPH samples exhibited an earlier onset of gelation compared to conventional treatment. This can be attributed to the smaller size of oil droplet aggregates in UHPH samples, which have a larger effective surface area compared to individual oil droplets in conventional samples. However, values of the aggregation rate and final optical density of yogurts at the end of fermentation suggest that network formation in conventionally treated yogurts primarily relies on casein-casein interactions, while in UHPH yogurts, the high content of aggregates in protein network interrupted those association, thus producing a more porous gel, which could be confirmed by microstructural observations.

9. During cold storage, the conventional yogurts exhibited higher textural parameters such as firmness and consistency, as well as viscoelastic parameters including G' and G", compared to the UHPH yogurts at the same dried emulsion concentration. These findings are aligned with the different network formation mechanisms observed.

10. Yogurts produced from UHPH showed improved water holding capacity, a crucial parameter for yogurt quality. Moreover, incorporating 6% spray-dried emulsion significantly increased water holding capacity in both UHPH and conventional homogenization yogurts compared to 4% formulations. The higher water retention within and at the interface of oil-protein aggregates in UHPH yogurts is likely responsible for the enhanced response of this parameter.

11. The choice of homogenization technique and the percentage of spray-dried emulsion added formulations (4% and 6%) influenced the primary and secondary oxidation of yogurts. Yogurts treated with UHPH showed lower hydroperoxide levels compared to those treated with conventional homogenization. Similarly, the higher concentration of malondialdehyde in CH yogurts indicated a similar pattern to primary oxidation.

12. The different types of homogenizations applied and the percentages of SDE used, did not significantly affected the microbial counts lactic acid bacteria of yogurts during the cold storage, which was in the range required by legislation.

13. In accordance with the higher oxidation stability exhibited by UHPH yogurts, total polyunsaturated fatty acids, particularly oleic, linoleic, and α-linolenic acids content showed higher levels than conventional homogenization yogurts.

14. Sensory evaluation showed that both the homogenization system and the spray-dried emulsion content did not have a significant impact on the flavor of the yogurt. This suggests that the highest concentrations of dried emulsion in this study can be used without compromising the sensory attributes of the yogurt.

15. Notably, a sensory evaluation revealed that neither the homogenization system nor the SDE content significantly affected the flavor of the yogurt. This enables the use of higher SDE concentrations without altering the sensory attributes of the yogurt.

Appendix I

Article 1

foods

MDPI

Article

Buttermilk as Encapsulating Agent: Effect of Ultra-High-Pressure Homogenization on Chia Oil-in-Water Liquid Emulsion Formulations for Spray Drying

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Abstract: Functional foods are highly demanded by consumers. Omega-3 rich oil and commercial buttermilk (BM), as functional components, used in combination to produce emulsions for further drying may facilitate the incorporation to foods. Ultra-high-pressure homogenization (UHPH) has a great potential for technological and nutritional aspects in emulsions production. The present study aimed to examine the potential improvement of UHPH technology in producing buttermilk-stabilized omega-3 rich emulsions (BME) for further drying, compared with conventional homogenization. Oil-in-water emulsions formulated with 10% chia: sunflower oil (50:50); 30% maltodextrin and 4 to 7% buttermilk were obtained by using conventional homogenization at 30 MPa and UHPH at 100 and 200 MPa. Particle size analysis, rheological evaluation, colloidal stability, zeta-potential measurement, and microstructure observations were performed in the BME. Subsequent spray drying of emulsions were made. As preliminary approximation for evaluating differences in the homogenization technology applied, encapsulation efficiency and morphological characteristics of on spray-dried emulsions (SDE) containing 21.3 to 22.7% oil content (dry basis) were selected. This study addresses the improvement in stability of BME treated by UHPH when compared to conventional homogenization and the beneficial consequences in encapsulation efficiency and morphology of SDE.

Keywords: buttermilk; chia oil; high-pressure homogenization; oil-in-water emulsions; spray-dried emulsions

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1. Introduction

Consumer tendency to make food choice is greatly based on their healthy characteristics. Those tailored functional foods contain added bioactive ingredients, with a physiological function in the human organism [1]. Within the functional foods offered in the market, there are protective foods for preventing chronic diseases and/or improving some natural protective systems' development. The functional ingredients of interest include a wide range of bioactive components. Among those, omega-3 and some phospholipids have health-promoting activities.

Buttermilk (BM) is the liquid fraction obtained from butter production. It is largely considered as a by-product in the dairy industry, usually incorporated into feed. However, when processed by concentration and spray drying, it is used as an ingredient in food formulations as a partial substitute of milk solids. BM is rich in milk fat globule membrane (MFGM) residues. The MFGM is the natural encapsulating system of milk fat, consisting in a triple layer of phospholipids and proteins, so that it has a great potential as a functional

ingredient. In food emulsions, the proteins and phospholipids content of BM has a techno-functional role as stabilizing agents due to their surface-active properties, which adsorbed at the interface oil-water create a viscoelastic layer preventing from coalescence [2]. From a bio-functional point of view, gangliosides, polar lipids and proteins of the MFGM have been described as health-promoting components by preventing infections, improving immunity, protecting and supporting adequate growth in healthy infants, and improving brain and cognitive system development [3–5]. At the same time, the use of BM as encapsulating agent, gives a great opportunity for revaluating this by-product of the dairy industry and also providing benefits from an environmental point of view by reducing its waste.

It has been confirmed the potential of BM as a new ingredient for encapsulation in atomized emulsions compared to milk proteins, and as a delivery system for bioactive compounds, such as rich fatty acids (FA) omega-3 oils [6,7]. The use of whole BM stream is an opportunity for higher value applications in functional food by capitalizing the dual functionality of BM, i.e., techno-functional and physiological functionality [6]. In this sense, the commercial BM could be a valuable ingredient for multiple applications.

Chia (*Salvia hispanica* L.) seed oil is a vegetable source with a high content of omega-3 FA, with up to 67.8% of the total lipid fraction [8]. Consumption of omega-3 FA must be incorporated to the human diet since they are essential, and are involved in multiple physiological regulations and protection against heart disease, among others [9]. In the last revision of FAO/WHO report [10] for consuming recommendation of fats and fatty acids, omega-3 FA dietary intake recommendations were established between 0.5 and 2% of the total fat intake to prevent coronary heart diseases. The use of oils high in linolenic acid such as chia oil is an interesting tool to increase the contribution of omega-3 FA to the diet.

The most common way to incorporate a lipophilic compound to food products is by emulsions encapsulation [11]. Therefore, emulsions consisting in omega-3 FA rich oils encapsulated by buttermilk surface active compounds is a potential way to incorporate those bioactive substances to produce functional foods.

Emulsions are unstable thermodynamic colloidal systems, which exhibit destabilization phenomena such as creaming, aggregation, and coalescence. These phenomena, despite an adequate formulation, take place to some extent during storage. In addition to the quality of emulsifiers, one of the most effective mechanisms to stabilize emulsions is the reduction of the droplet size in terms of both thermodynamic stability, by decreasing the attractive interaction of droplets, and kinetics, by delaying the creaming destabilizing process. To produce stable emulsions, high-energy mechanical devices, such as high-pressure homogenizers or sonication equipment, are used at industry. These homogenizers create intense breakdown forces to reduce the size of the pre-emulsion oil droplets. Ultra-high-pressure homogenization (UHPH) is a versatile technology that has the ability to inactivate microorganisms and enzymes, to give rise to submicron emulsions of great physical stability, and to produce some modifications in colloidal structures, due to the high pressures applied ranged from 100 to 350 MPa [12,13]. UHPH have a potential to produce restructuring of the protective layer of the droplets according to the composition of the emulsifying agents used, with repercussions on the techno-functional properties. Several studies [13–16] performed in emulsions using this technology have demonstrated the improvement of colloidal stability when compared to conventional homogenization.

To make easier handling, transport, and preservation for a longer time of emulsions with encapsulated functional compounds, a common practice is dehydration. Spray drying is one of the most used techniques for the microencapsulation of oils due to the high availability of equipment and low production costs compared to the other methods. Obtaining a homogeneous and stable BME previously to spray drying is decisive [17], and it is directly related to a reduced droplet size distribution, ideally submicronic [18].

This study aims to investigate the ability of commercial BM to produce BME suitable for further spray drying. It is intended to compare the emulsion characteristics of conventional high-pressure homogenization with UHPH treatment for elucidating the ability of this technology for encapsulating omega-3 oil with BM. The drying of emulsions previously processed by UHPH has not been studied yet, thus it is hypothesized that those BME could lead to improved characteristics of powders obtained. For this purpose, this study was mainly focused in the BME characteristics and, in less extent, in those obtained by spray drying.

2. Materials and Methods

2.1. Materials

Maltodextrin (MD) Glucidex® 19-Maltodextrin was purchased from Roquette Freres (Lestrem, France) with 19 DE. Buttermilk powder (BM) had the following composition provided by the company: 30% protein, 7% fat, 52% lactose, less than 4% moisture and 7% ash, and was purchased from Activa Food-Tech, S. A. (Girona, Spain). Crude Chia oil (20% C-18:2, >56% C-18:3 according to the specifications) was obtained from Interfat Natural Oils (Barcelona, Spain). Crude Sunflower oil (4–9% C-16:0, 1–7% C18:0, 15–85% C18:1, 50–72% C18:2) was purchased from Gustav Heess Oils (Barcelona, Spain). All other chemical used were of analytical or better grade.

2.2. Emulsion Preparation

Four formulations of oil-in-water emulsions were prepared with an oil mixture of chia and sunflower (50:50), MD as wall material and BM as emulsifier. Continuous phase of emulsions was prepared by dispersing individually MD (50% *w*/*w*) and BM (30% *w*/*w*) with a Thermomix (Vorwek, Wuppertal, Germany) at 2000 rpm for 5 min. The resulting aqueous dispersions were stored overnight at 4 °C for complete hydration. To prepare pre-emulsions, the corresponding weights of MD dispersion to final concentration of 30% (*w*/*w*), BM dispersions (to 4–7% *w*/*w*) and water were mixed. Oil was slowly added to the pre-warmed (40 °C) aqueous phase and stirred by using a conventional rotor-stator mixer (Charles Ross & Son Company, Hauppauge, NY, USA) at 15,000 rpm for 5 min. The final solid content of the emulsions varied from 44 to 47% (*w*/*w*). Coarse emulsions were further homogenized using conventional or UHPH treatments at 40 °C inlet temperature. Conventional homogenization (CH) of pre-emulsions was performed in a benchtop Homolab (FBF Italia, Sala Baganza PR, Italy) at 30 MPa. Subsequently, heat treatment of emulsions was made at 65 °C, 30 min. UHPH treatments at 100 and 200 MPa were processed in an Ypsicon equipment Model A-60, which is a high-pressure continuous device (60 L/h) (Ypsicon Advance Technologies, S.L., Barcelona, Spain) that works up to 300 MPa. Working temperatures of samples were 40 °C inlet, 60 ± 2 and 80 ± 3 °C, respectively to 100 and 200 MPa at the high-pressure valve, and 25 °C outlet temperature, reached after a quick cooling by a heat exchanger connected to the UHPH equipment. Residence time in the high-pressure valve was less than one second. Emulsions were collected in Pyrex bottles for further sampling and analysis. In Table 1, the designation and composition of emulsions produced and analyzed are detailed.

Table 1. Name of samples and formulation composition of initial emulsions.

Sample Name	H (MPa)	Oil% (*w*/*w*)	MD% (*w*/*w*)	BM% (*w*/*w*)	TS% (*w*/*w*)
4CH	30	10	30	4	44
4UH100	100	10	30	4	44
4UH200	200	10	30	4	44
5CH	30	10	30	5	45
5UH100	100	10	30	5	45
5UH200	200	10	30	5	45
6CH	30	10	30	6	46
6UH100	100	10	30	6	46
6UH200	200	10	30	6	46
7CH	30	10	30	7	47
7UH100	100	10	30	7	47
7UH200	200	10	30	7	47

CH (emulsions processed with conventional homogenizer); UH (emulsions processed with ultra-high-pressure homogenizer); H (homogenization pressure); Oil (50:50, chia:sunflower); MD (maltodrextrin); BM (buttermilk); TS (total solids).

2.3. Emulsion Characterization

2.3.1. Particle Size Analysis

Particle size and distribution of emulsions were measured in the fresh samples after UHPH or CH treatments using a Mastersizer laser diffraction 2000 analyzer (Malvern Instruments Ltd., Worcestershire, UK). Emulsion were added directly to the recirculating measuring cell containing distilled water or 0.5% SDS solution until 5–9% obscuration was achieved. The optical model based on the Mie theory of light scattering by spherical particles was used. The optical model used were a refractive index of 1.460 for BM, a refractive index of 1.332 for water, and absorption of 0.01. Results were expressed as the volume-weighted mean diameter ($d_{4,3}$, μm) and Span index. Measurements were made separately for water and SDS as dispersing media. Span is a parameter which indicates the homogeneity of the particle size distribution and was calculated using Equation (1).

$$\text{Span} = (d_{90} - d_{10})/d_{50} \quad (1)$$

where d_x (μm) is the size point below which x% of the particles is contained.

2.3.2. Zeta Potential

The zeta-potential of emulsions was measured using a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK). Emulsions were diluted 1:100 with ultrapure water and allowed to equilibrate at 25 °C for 120 s in the cuvette prior to analysis. The measurement was performed on the same day of homogenization using an automatic voltage selection. Zeta-potential was calculated using the Smoluchowski model using the software provided by Malvern Instruments.

2.3.3. Rheological Evaluation

Flow curves were performed on fresh emulsions (24 h after treatment) at 20 °C with a controlled stress rheometer (Haake Rheo Stress 1, Thermo Electron Corporation, Karlsruhe, Germany). A concentric cylinders probe was used. Samples were loaded into the probe for 5 min before starting the test in order to reach equilibrium.

Flow curves were obtained in ascending and descending shear rates in the range of 0.1 and 100 s^{-1} for 60 s, respectively. Ostwald de Waele rheological model (Equation (2)) were fitted for descending curves, and the rheological parameters (K, n) were obtained. From the difference between the area under the ascendant and descendant curves, the hysteresis was calculated as indicative of thixotropic behavior.

$$\sigma = K\gamma^n \quad (2)$$

where s is the shear stress (Pa), K is the consistency index (Pa.s^n), g is the shear rate s^{-1}, and n is the flow behavior index (n = 1 indicates Newtonian behavior n $\neq$ 0 indicates non-Newtonian behavior).

2.3.4. Physical Stability

Colloidal stability of the emulsions was evaluated by using Turbiscan MA 2000 optical analyzer device (Formulaction, Toulouse, France). Emulsions were transferred into borosilicate glass tubes of 27.5 mm dimeter up to 40 mm height, and sodium azide (0.04%) was added to prevent microbial growth. Three tubes of each sample were prepared and stored at 20 °C for 8 days. The evolution of stability was analyzed at 0, 1, 4, 6, and 8 days. This equipment provides a powerful technique for characterization of dispersions, detecting variations in stability phenomena by measuring backscattering (BS) along the sample tubes. Stability index (TSI, Formula 3) and creaming layer thickness evolution was determined by using the software (Turbisoft 2.3.1.125 version) provided by the manufacturer.

$$TSI = \frac{\sum h[scan_i(h) - scan_{i-1}(h)]}{H} \quad (3)$$

where $scan_i$ (h) is mean BS for each i of measurement, $scan_{i-1}$ (h) is mean BS for i−1 measurement, and H is the height of a sample. Higher TSI indicates stronger destabilization caused by particle aggregation and/or dynamic migration.

2.3.5. Confocal Observations

A confocal laser-scanning microscope (Leica TCS SP5, Leica Microsystems GmHB, Mannheim, Germany) was used to observe the structure of fresh emulsions (24 h after production). The protein and oil were fluorescently labelled together and separately from phospholipid components of the samples. Proteins were labelled by Fast Green FCF (Sigma-Aldrich, St. Louis, MO, USA), prepared at concentration of 1% in distilled water and subsequent addition of 10% to the emulsion, excited by a 633 nm laser, and detected at 650–750 nm. Oil was labelled by Nile Red (5H-Benzo-phenoxazine-5-one, 9-diethylamino; Sigma-Aldrich, St. Louis, MO, USA), by dissolving 1 mg/mL in acetone. About 100 µL of Nile Red solution was added to 1 mL of emulsion. Excitation was made at a 488 nm laser and detected at 500–600 nm. Phospholipids were labelled by Liss Rhod PE (1,2-dioleoyl-sn-glycero-3- phosphoethanolamine-N-lissamine rhodamine B sulfonyl; 1 mg/mL; Avanti Polar Lipids Inc., St. Louis, MO, USA), by adding 40 µL to 1 mL emulsion, excited by a 561 nm laser and detected at 575–630 nm. All fluorescently labelled samples were mounted on cavity plates and examined at room temperature with a 100× oil immersion objective.

2.4. Spray Drying

BME were dried in a Mini Spray-Dryer B-290 (Büchi Labortechnik AG, Flawil, Switzerland). The samples were tempered at 25 °C, and the drying working conditions were 150 °C inlet temperature, 80% aspiration, and 30% feed flow. To improve conservation and reduce possible oxidative damage, the solid samples were collected in aluminum bags that were heat-sealed and stored at –80 °C for further analysis.

2.5. Microstructure of SDE

The morphology of the solid emulsions was observed by SEM, using the Quanta ™ 650 FEG scanning electron microscope (FEI Company, Hillsboro, OR, USA), with an accelerating beam voltage (HV) of 5 kV. Samples were prepared by fixing a small amount of powder on metal discs with double-sided carbon tapes, which were then platinum-plated in a Leica EM ACE600 vacuum chamber (Leica Microsystems, Wetzlar, Germany).

2.6. Encapsulation Efficiency

Extraction of free oil from SDE was carried out as described by Gonzalez et al. [19]. Dried emulsions (2.00 ± 0.01 g) were weighed and transferred to a beaker containing

30 mL of petroleum ether, stirred for 1 min and filtered. The filter paper was washed with 10 mL of petroleum ether through a pre-weighed flask to evaporate organic solvent under vacuum. Finally, the flask was heated at 105 °C in an oven to constant weight for 1 h. Encapsulation efficiency (EE) was determined according to Equation (4).

$$EE = \frac{[TO - SO]}{TO} \times 100 \quad (4)$$

where, TO is the total oil contained in the microcapsules, and SO is the free oil on surface.

2.7. Statistical Analysis

Results are presented as mean ± standard deviation. All data were subjected to a one-way analysis of variance (ANOVA) test using the Minitab Express™ version 1.5.3 (Minitab, State College, PA, USA). Significant differences between means were determined by Tukey test. A confidence level of 95% ($p < 0.05$) was used. At least two individual productions of each formulation and treatments were performed. All analysis were replicated three times.

3. Results and Discussion

3.1. BME Characterization

3.1.1. Particle Size and Distribution

The particle size distribution of BM stabilized emulsions is shown in Figure 1. In Table 2, the volume weighted ($d_{4,3}$) mean values and Span index for emulsions dispersed in water or 0.5% SDS, respectively, are shown.

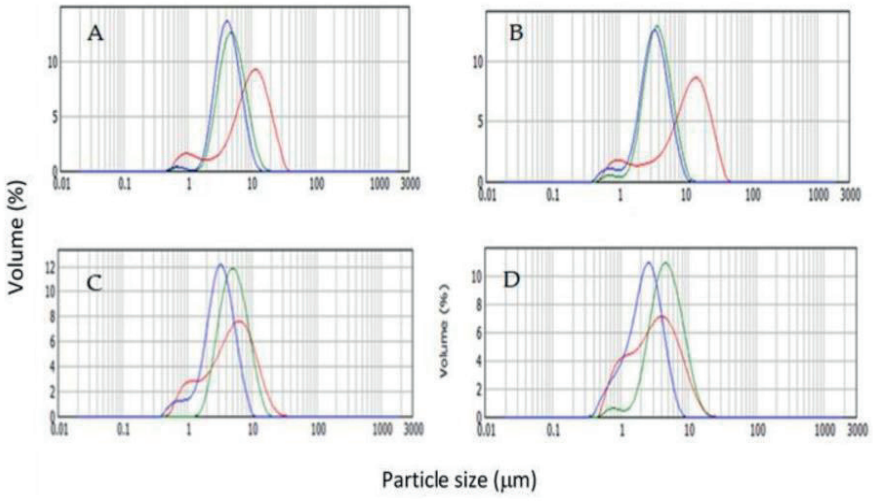

Figure 1. Particle size distribution curves measured by laser diffraction of emulsions containing different BM concentration: (**A**) 4%, (**B**) 5%, (**C**) 6% and (**D**) 7%, processed by CH (red), UHPH at 100 MPa (green) and UHPH at 200 MPa (blue).

Table 2. Droplet size parameters ($d_{4,3}$ and Span index) of fresh emulsions (24 h after production) dispersed in water (w) and SDS.

Emulsions	$d_{4,3}$ (w) (μm)	Span (w)	$d_{4,3}$ (SDS) (μm)	Span (SDS)
4CH	13.1 ± 2.50^{a}	1.8 ± 0.100^{bcd}	2.60 ± 0.500^{b}	1.9 ± 0.300^{b}
4UH100	5.45 ± 0.02^{bc}	1.27 ± 0.01^{e}	0.69 ± 0.070^{d}	1.00 ± 0.04^{cd}
4UH200	4.64 ± 0.03^{bcd}	1.19 ± 0.02^{e}	0.495 ± 0.008^{d}	1.00 ± 0.04^{cd}
5CH	11.5 ± 0.90^{a}	2.04 ± 0.02^{bc}	2.60 ± 0.400^{b}	1.7 ± 0.100^{b}
5UH100	4.16 ± 0.05^{bc}	1.2 ± 0.100^{e}	0.56 ± 0.020^{d}	1.15 ± 0.01^{c}
5UH200	3.65 ± 0.04^{de}	1.35 ± 0.02^{de}	0.40 ± 0.010^{d}	0.89 ± 0.06^{cd}
6CH	5.93 ± 0.10^{b}	2.21 ± 0.01^{b}	1.92 ± 0.010^{c}	1.7 ± 0.100^{b}
6UH100	5.08 ± 0.10^{bcd}	1.39 ± 0.04^{de}	0.42 ± 0.040^{d}	0.9 ± 0.100^{cd}
6UH200	3.44 ± 0.03^{de}	2.0 ± 0.600^{bc}	0.32 ± 0.010^{d}	0.75 ± 0.03^{d}
7CH	4.5 ± 0.500^{bcd}	2.7 ± 0.300^{a}	3.9 ± 0.8000^{a}	2.5 ± 0.400^{a}
7UH100	4.4 ± 0.900^{bcd}	1.59 ± 0.09^{cde}	0.43 ± 0.010^{d}	0.83 ± 0.03^{cd}
7UH200	2.48 ± 0.08^{e}	1.61 ± 0.09^{cde}	0.30 ± 0.010^{d}	0.73 ± 0.03^{d}

Means with different letters in the same column are significantly different at $p < 0.05$.

A bimodal distribution was observed in most of samples represented by a small population of particles in the range of 0.5–1 μm and a big population, which varied in function of BM concentration and homogenization conditions applied. The exception to these distribution curves were the UHPH processed emulsions with 7% BM at 200 MPa, which shifted to a unimodal distribution. The span values indicated narrower peaks of UHPH-treated emulsions compared to CH, indicating a higher homogeneity in the particle size of the former emulsions. In most of BM concentrations formulations, the CH processed emulsions showed higher particle size than UHPH emulsions. $D_{4,3}$ values of CH-processed emulsions decreased as BM concentration increased from 4 to 7%, although differences only were significant ($p < 0.05$) between 4–5 and 6–7% BM concentrations. In UHPH-treated emulsions, increasing pressure from 100 to 200 MPa caused a reduction in $d_{4,3}$ within the same BM concentration, although not always significant. The most accused reduction of $d_{4,3}$ in UHPH treatments was observed in UH200-treated samples as BM concentration increased from 4 to 7%.

When comparing $d_{4,3}$ values of emulsions dispersed in water and in 0.5% SDS, droplet aggregation phenomena was revealed. Values of $d_{4,3}$ emulsions dispersed in SDS were considerably lower than those dispersed in water in all cases, which was caused by the breakdown of aggregates by the dispersing agent. In the case of UHPH-processed emulsions, particle size of individual droplets (SDS dispersed droplets) is in agreement with those reported by Fernandez-Avila et al. [20], who studied UHPH-treated emulsions prepared with soy protein at 4% and 10% soy oil. In this study, particle size of oil droplets in emulsions processed at 100 and 200 MPa were of 0.3 and 0.4 μm, respectively, which correspond to values found in the present study for formulations with 6 and 7% BM processed at 100 and 200 MPa.

The aggregate formation in UHPH-treated emulsions [15] prepared with sodium caseinate and sunflower oil, and several vegetable beverages, such as tigernut, almond, and soy [21–23], have also been described. In the mentioned studies, the presence of these structures were bigger and more abundant as pressure increased from 100 to 300 MPa, and increasing inlet temperature of samples. Authors attributed the aggregation formation to partial protein denaturation caused by the combined effect of high pressure and temperature increase in the high-pressure valve during UHPH treatment. Inlet temperature of emulsions in UHPH treatments in this study was of 40 ± 3 °C, which rise to 60 ± 4 and 80 ± 3 °C at 100 and 200 MPa, respectively, at the pressure valve, for less than one second. Samples were quickly cold down at 25 ± 2 °C in the heat exchanger connected to the UHPH equipment. Thus, these UHPH conditions were not especially conducive to the aggregate formation. Apart from forces acting in UHPH treatments, responsible for partial denaturation and disintegration of whey proteins and casein micelles, respectively, aggregate

formation could be explained by a combined effect of factors. On the one hand, depletion flocculation could be the most possible mechanism of aggregation due to the effective reduction of individual droplets in the UHPH treatments [24]. Moreover, the presence of 30% of MD in the formulations could have contributed to droplet aggregation, probably due to the thermodynamic incompatibility between carbohydrates and proteins [25].

3.1.2. Microstructure

BM composition provides techno-functional components involved in the formation and stability of emulsions. Milk proteins, especially caseins, and polar lipids from MFGM are the surface-active components of this ingredient. Polar lipids contain ionic groups, exerting a repulsive force between oil droplets. Proteins stabilize emulsions by forming a viscoelastic layer at the oil–water interphase. The effect of this adsorbed layer on emulsion stability combines electrostatic and steric repulsion, preventing from coalescence. In foods containing both polar lipids and proteins, binding each other through electrostatic and hydrophobic interactions [26] may take place. Thus, combined layers of polar lipids and proteins from BM are the most likely oil droplets protection of these emulsions.

The CLSM images (Figure 2) of freshly prepared emulsions were obtained with selective staining. On the one hand preparations stained to visualize the lipid (red) and protein (green) structures together were made. On the other hand, preparations were stained to observe the polar lipids (cyan). Images showed the difference in microstructure between CH and UHPH emulsions. A background of protein (green) was observed in CH emulsions, which had a lower surface area of droplets to be protected by surface-active components compared to UHPH-treated emulsions. The presence of aggregates in UHPH-processed emulsions was observed in all formulations independently of BM concentration (not shown). In those emulsions, proteins can be seen located as part of aggregates formed by small oil droplets and acting as bonding material between them.

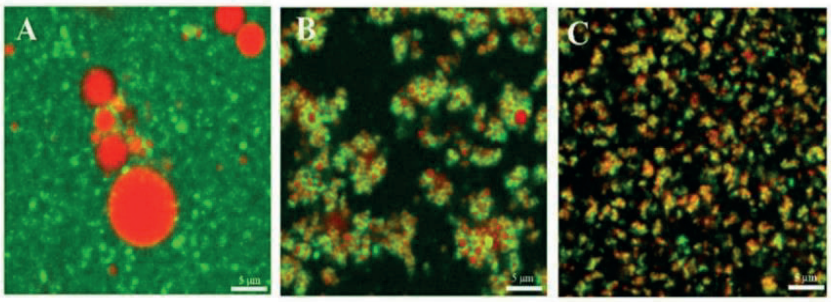

Figure 2. CLSM images of emulsions containing 5% BM showing the oil droplets aggregates: (**A**) 5CH, (**B**) 5UH100 and (**C**) 5 UH200. Scale bar 5 μm. Oil (red) stained with Nile Red; protein (green) stained with Fast Green FCF.

The difference in aggregates size and arrangement between droplets produced in emulsions is noticeable (Figure 3). While in CH emulsions the attachment within droplets appeared weak, in UHPH-treated emulsions, there were considerably lower sizes than the previous ones, and the attachment of droplets into the aggregates appeared tightly joined. At 100 MPa, those structures were bigger with irregular shape, while at 200 MPa, a smaller size and regular shape were observed, constituting a more homogeneous disperse phase than those emulsions treated at 100 MPa. This pattern was observed in all BM concentration emulsions.

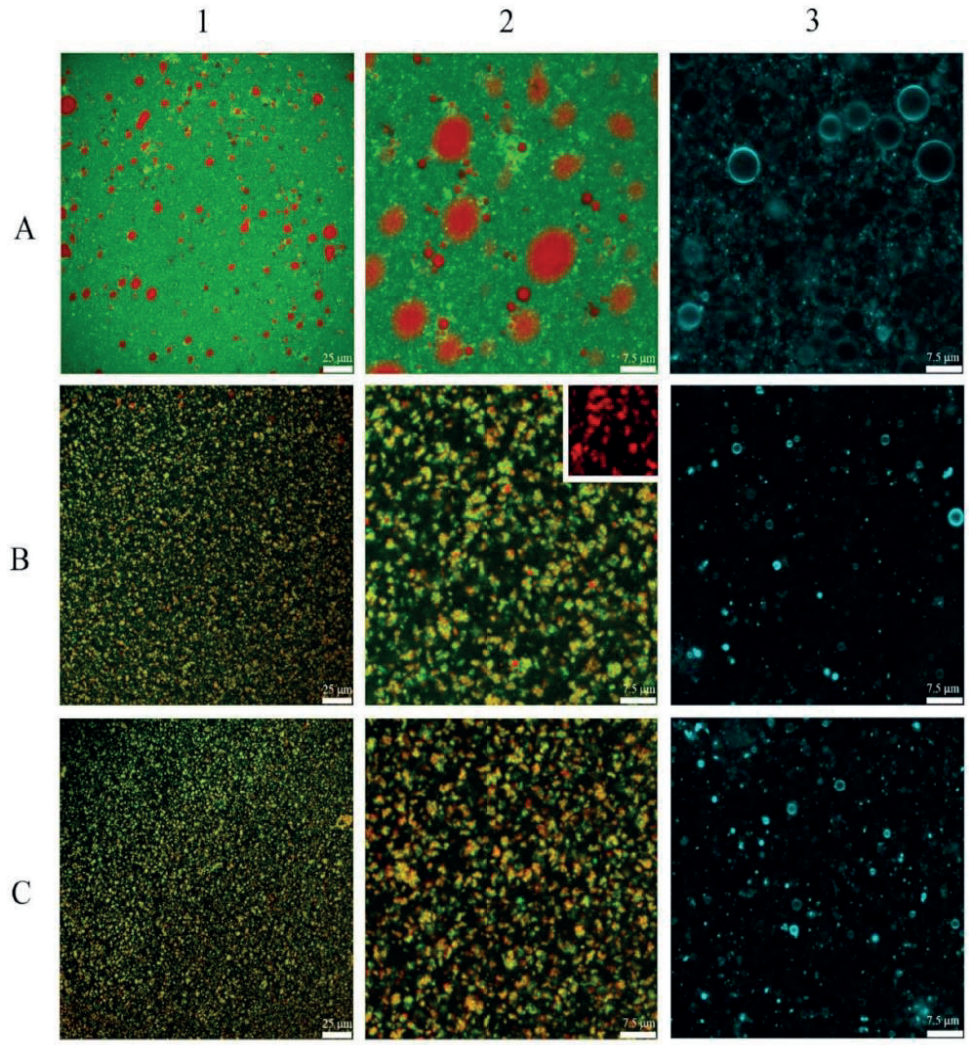

Figure 3. CLSM images of emulsions containing 7% BM. Columns 1 (scale bar 25 µm) and 2 (scale bar 7.5 µm) are images of the overlay of green labelled protein (stained with Fast Green FCF) and red labelled fat (stained with Nile Red). Column 3 (scale bar 7.5 µm) corresponds to images of cyan labelled polar lipids labelled images (stained with Liss Rhod PE). (**A**) 7CH, (**B**) 7UH100, and (**C**) 7UH200. (**B2**) Detail of fat labelled aggregates.

Images of column 3 in Figure 3, polar lipids distribution around oil droplets can be observed. All images showed a good and homogeneous coverage of droplets. From images, it seems that at least a high percentage of oil droplets are well protected by polar lipids, which probably interacted with proteins to create a combined oil droplets protection.

3.1.3. Rheological Behavior

Flow curves of emulsions were fitted to the Power law and rheological parameters K (consistency index) and n (flow behavior index) of emulsions for descendant curves are shown in Table 3. Flow curves and viscosity curves (Supplementary Materials Figures S1 and S2, respectively) analysis revealed a non-Newtonian behavior with slight thixotropic character in all emulsions. All samples, to a greater or lesser extent, ex-

hibited a low variation of apparent viscosity values as shear rate increased during shearing. However, time dependence of shearing varied from one to another samples, especially depending on homogenization treatment applied, and to a lesser extent, according to BM concentration. This behavior demonstrates a certain degree of structuration of emulsions due to interactions between colloidal particles, i.e., oil droplets and BM proteins. The no coincidence of ascendant and descendant shearing curves observed were especially evident at low deformations values in CH and UH200 (from 0 to approximately 30 s^{-1}) independently of BM concentration of formulation. UH100 samples showed a more marked thixotropic character, with hysteresis area being detected nearly in all range of shear rate applied in the flow curves. Hebishy et al. [27], found a Newtonian behavior in emulsions with 5% sodium caseinate and 10% oil when treated by UHPH or conventional homogenization. The presence of aggregates in the present formulations, and probably the contribution of 30% MD in the continuous phase are responsible of the thixotropic character of emulsions, contributing to a certain degree of structuration of the samples.

Table 3. Rheological parameters (consistency index, K, and flow behavior index, n) of descendant flow curves, and the hysteresis area from flow curves of fresh emulsions (24 h after production).

Emulsions	K (Pa.s ^{n})	n	Hysteresis (Pa/s)
4CH	0.07 ± 0.010^{abc}	0.96 ± 0.0100^{cd}	5.9 ± 1.20^{cd}
4UH100	0.07 ± 0.020^{abc}	0.96 ± 0.0100^{cd}	10.7 ± 1.1^{b}
4UH200	0.045 ± 0.007^{c}	0.980 ± 0.007^{bcd}	3.4 ± 0.10^{d}
5CH	0.042 ± 0.003^{e}	0.970 ± 0.009^{cd}	4.4 ± 0.60^{d}
5UH100	0.06 ± 0.0100^{abc}	0.98 ± 0.0200^{bcd}	7.8 ± 2.10^{bc}
5UH200	0.04 ± 0.0100^{de}	1.002 ± 0.001^{ab}	3.5 ± 0.20^{d}
6CH	0.045 ± 0.007^{de}	0.985 ± 0.004^{abc}	5.1 ± 0.60^{cd}
6UH100	0.083 ± 0.003^{ab}	0.95 ± 0.0100^{d}	10.1 ± 0.2^{b}
6UH200	0.05 ± 0.0100^{de}	0.97 ± 0.0100^{cd}	4.9 ± 0.10^{cd}
7CH	0.057 ± 0.006^{cde}	0.981 ± 0.002^{abcd}	6.2 ± 0.90^{cd}
7UH100	0.09 ± 0.0100^{a}	0.974 ± 0.003^{bcd}	14.7 ± 3.5^{a}
7UH200	0.043 ± 0.003^{e}	1.00 ± 0.0100^{a}	5.5 ± 0.10^{cd}

Means with different letters in the same column are significantly different at $p < 0.05$.

Consistency index and flow behavior index were calculated only in the descendant flow curve to assimilate the flow conditions during most of the operations at industry. K values of samples ranged from 0.02 to 0.09 Pa.s in all formulations. Although results did not reveal a clear pattern within samples in relation to homogenization treatment and BM concentration, some general behavior could be observed attributable to the presence of aggregates. Since viscosity manifestation depends to a great extent not only on particle size, but also on asymmetry of particles, aggregates could be the reason for the observed results. Aggregate formation and destruction do not follow a pattern since they are formed randomly. Thus, the particles shape and their asymmetry may vary to some extent in each individual emulsion production and during shearing. This could be the reason that caused a variability of frictional response during rheological measurements of emulsions with a partial disintegration during shearing. It could be hypothesized that aggregates formed in CH emulsions are easily disintegrated during shearing since those are weakly packed, as can be seen in Figure 3. Thus, in the descendent curves, K value of CH and UH200 emulsions were closer in most of cases. It could be attributed to particle size. In UH200 emulsions, aggregate sizes were closer to the individual droplets of CH emulsions, as shown in $d_{4,3}$ (SDS) in Table 1. We assumed that the shearing of UH200 emulsions was not sufficient to breakdown those aggregates, as hysteresis was observed in UH200 samples which showed the lowest values. Thus, viscosity manifestation in these emulsions could be attributed to the friction of small and tightly packed homogeneous aggregates. However, UH100 emulsions exhibited the highest K and hysteresis values. As mentioned previously, these samples had bigger aggregate sizes than UH200. Probably the pressure

difference of UHPH from 100 to 200 MPa is responsible for different degrees in casein micelles disintegration and conformation [12] in both 100 and 200 UHPH treatments, producing the observed differences in aggregate size and interaction forces. In this sense, during shearing of UH100 emulsions, the manifestation of higher K values and hysteresis may be due to a partial destruction of aggregates, which provoked a higher structuration degree and internal friction during flowing.

In any case, the viscosity values in all emulsion formulations ranged in values far from the maximum suitable for spray dried, being the final objective of these emulsions. Di Battista et al. [28] confirmed that to obtain a good atomization, viscosity of emulsion has to be lower than 0.3 Pa.s.

3.1.4. Physical Stability

In this study, the influence of homogenization treatments and BM concentration was evaluated by using Turbiscan® equipment during 8 days of storage at 20 °C. The backscattering (BS) at t = 0 was considered as reference to analyze the stability evolution of the system. The evolution curves during monitoring of samples by obtaining the difference of backscattering (ΔBS) gives information about the destabilization phenomena such as creaming, flocculation and coalesce, which are the common phenomena occurring in emulsions during storage. The reduction of BS in the bottom of the vial containing the sample and the increase of BS values in the top area were observed in all the ΔBS profiles (Supplementary Materials Figure S3) of samples, indicating that creaming was the main destabilization mechanism in these emulsions.

The velocity of migration of the oil droplets and aggregates toward the surface is mainly determined by their particle size, the viscosity of the continuous phase, and by the density difference between the continuous and the disperse phases [26]. From the evolution of ΔBS profiles during eight days, a global stability index (TSI, Figure 4) and creaming layer thickness (CL) at day 1 and day 8 (Table 4) weret calculated.

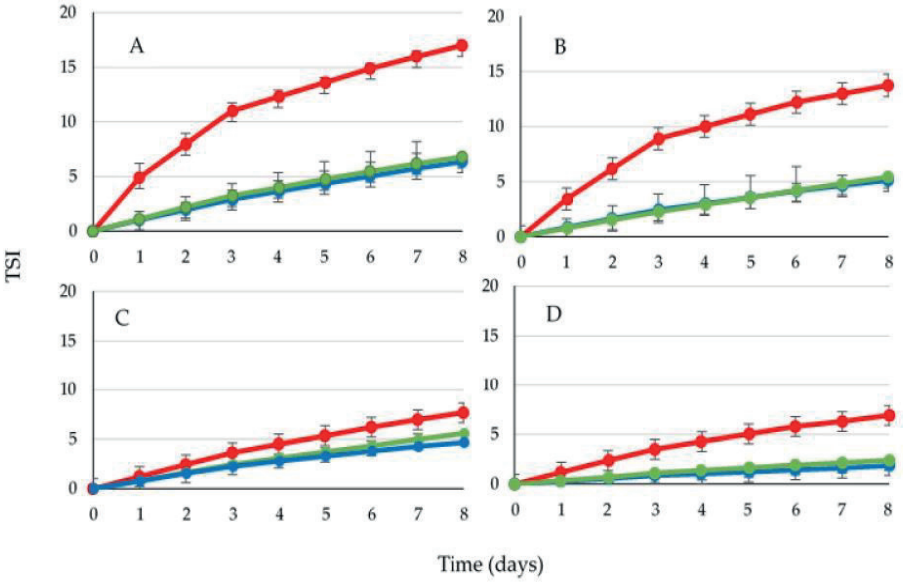

Figure 4. Evolution during 8 days storage of stability index (TSI) of emulsions with different BM concentration and homogenization treatments: (**A**) 4% BM; (**B**) 5% BM; (**C**) 6% BM; (**D**) 7% BM. CH (red line); UH100 (green line); UH200 (blue line).

Table 4. Stability parameters (creaming layer, CL) of fresh (1 day) and stored emulsions (8 days) from backscattering measurements curves, and zeta-potential of fresh emulsions (1 day).

Emulsions	CL-d1 (mm)	CL-d8 (mm)	Zeta-Potential (mV)
4CH	3.8 ± 0.4^{a}	9.7 ± 0.9^{a}	-26 ± 1^{bcd}
4UH100	0.8 ± 0.3^{c}	3.3 ± 0.6^{b}	-29 ± 2^{d}
4UH200	0.7 ± 0.3^{c}	2.8 ± 0.4^{b}	-22 ± 1^{a}
5CH	4.2 ± 1.0^{a}	12 ± 3.0^{a}	-28 ± 1^{cd}
5UH100	0.8 ± 0.4^{c}	4.4 ± 0.8^{b}	-23 ± 1^{ab}
5UH200	0.7 ± 0.2^{c}	4.2 ± 0.5^{b}	-25 ± 1^{abcd}
6CH	1.3 ± 0.8^{bc}	5.1 ± 2.0^{b}	-27 ± 2^{bcd}
6UH100	1.0 ± 0.1^{c}	4.4 ± 1.0^{b}	-23 ± 1^{abc}
6UH200	0.7 ± 0.2^{c}	3.1 ± 0.4^{b}	-28 ± 1^{abcd}
7CH	2.2 ± 0.400^{b}	4.8 ± 2.0^{b}	-27 ± 4^{cd}
7UH100	0.53 ± 0.05^{c}	2.3 ± 0.4^{b}	-25 ± 1^{abc}
7UH200	0.38 ± 0.08^{c}	2.1 ± 0.6^{b}	-25 ± 1^{abc}

Means with different letters in the same column are significantly different at $p < 0.05$.

CH emulsions always presented higher values of TSI than UHPH samples, indicating that the former were less stable than the latter. UHPH samples barely showed differences in stability between 100 and 200 MPa. With the same tendency, the effect of BM concentration, was significant. At 4 and 5% of BM, the TSI difference was more marked between CH and UHPH samples than in 6 and 7% BM emulsions. Related to the CL values (Table 4), the results were concomitant with TSI values. No significant differences ($p < 0.05$) were found between UHPH treatments, while they were significant as compared to the CH emulsions. Although the influence of BM concentration on CL in UHPH treatments was not significant statistically ($p < 0.05$), there was a correlation of creaming layer thickness with the type and degree of homogenization applied, which in turn was in line with the particle size values observed (Table 2). The presence of big aggregates in the CH samples and the flocculation phenomenon observed during the storage, which was observed in the ΔBS curves (Supplementary Materials Figure S3), explain the high tendency of these samples towards creaming compared to UHPH-treated emulsions. In the ΔBS curves, the flocculation phenomenon is observed if there is separation of the profiles in the middle of the vial and the different days of monitoring. In UHPH emulsions, the aggregation phenomena was found in all formulations from the beginning; however, aggregates behaved as small particles with limited mobility to the top. Flocculation was not observed during the storage in any of UHPH samples as the ΔBS profiles showed (Supplementary Materials Figure S3).

In addition to the type of homogenization applied, the BM concentration appeared as the main factor in TSI index and CL values. Increasing BM from 4–5 to 6–7% might increase the amount of adsorbed protein at the interface, with a closer packing layer formation resulting in higher stability [29].

Zeta-potential is the charge that develops at the interface between particles and the continuous, and it is used to evaluate the colloidal stability in dispersed systems. Zeta-potential is also related to the ionized groups from protein layers that surround oil droplets, when the pH was above the isoelectric point [30]. Values of zeta-potential were in the range from −22 to −29, which are near, but not in the optimum range values considered to have good stability due to electrostatically repulsion (i.e., higher/lesser than ±30 mV). Thus, the stability of these emulsions by electrostatic repulsion was not the most relevant contribution. There was no correlation of zeta-potential with homogenization procedure, nor with BM concentration. This variability in zeta-potential values might be related to the heterogeneous distribution of proteins into the aggregates observed, especially in UHPH emulsions, in which, zeta-potential values had less negative charge. Probably, the protein was occluded inside the aggregates, hindering the exposure to the surface of those colloidal structures.

3.2. Spray-Dried Emulsions

3.2.1. Encapsulation Efficiency

The degree to which wall material can prevent the internal oil extraction is given by the encapsulation efficiency (EE) [31]. This is an important parameter taken into consideration for the quality of dried emulsions. In Figure 5, comparison of the SDE with different formulations and homogenization treatments applied to BME can be seen.

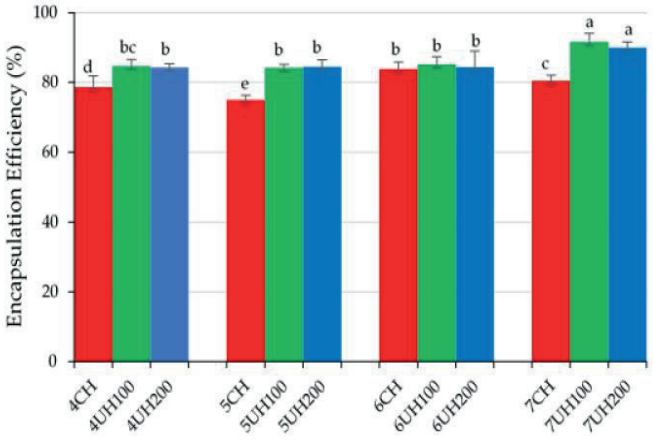

Figure 5. Encapsulation efficiency of the SDE from the different homogenization treatments (CH, UHPH at 100 MPa and UHPH at 200 MPa) applied to the BME, containing different percentages (*w*/*w*) of BM (4, 5, 6 and 7%). Different letters indicate significant differences ($p < 0.05$).

The main formulation factors affecting encapsulation efficiency are total solid content and the ratio of oil to wall material and oil content. In the present emulsions, total solid content varied from 44 to 47%, and oil content was from 21.3 to 22.7% (dry basis) corresponding to formulations with 4 to 7% BM. MD content remained constant (30%) in all emulsions. The corresponding ratio of oil to wall material varied from 1: 3.4 to 1: 3.7 according to the increase of BM content. In agreement to previous studies reported by Botrel et al. [32], the optimum for encapsulation efficiency in terms of mass ratio of oil to wall material could be 1:3 and less than 1:4 (*w*/*w*). Thus, the present formulations are in range. Values of free oil on the surface of powder particles decreased, in general, as total BM concentration increased for all samples. Within the same BM content, the homogenization technology applied significantly ($p < 0.05$) affected the free oil on the surface, which was always higher in CH emulsions, indicating the positive effect of UHPH technology on the encapsulation efficiency (Figure 6). However, the pressure intensity applied in UHPH produced similar results. A significant improvement of encapsulation efficiency was observed in formulations with 7% BM, which had around 90% EE. Especially in those emulsions, the difference between CH and UHPH was more marked, indicating that probably the colloidal structures created in the UHPH processing could be responsible for maintaining a better oil encapsulation. The small oil droplets attached by proteins in the aggregates would create a protective system for oil migration to the surface. BM containing casein, whey protein, and phospholipids has good emulsifying properties contributing to oil protection. Zhang et al. [7] reported encapsulation efficiency values between 89.6 and 94.3% in SDE formulated with BM, with or without MD as wall materials. Although the formulations differed substantially in BM content from that of the present study, those authors attributed to BM a great oil encapsulating capacity and found that increasing MD from 33 to 66.7% considerably decreased the encapsulation efficiency.

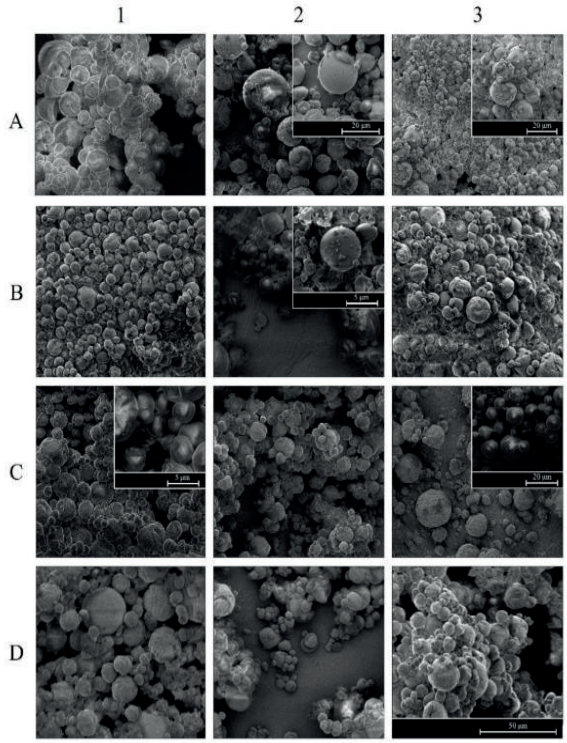

Figure 6. SEM images (2691×) of emulsions obtained by the different homogenization treatments: (**1**) CH; (**2**) UHPH at 100 MPa; (**3**) UHPH at 200 MPa, with different BM concentration: (**A**) 4% BM; (**B**) 5% BM; (**C**) 6% BM; (**D**) 7% BM. Magnification of overlapped images are: (**A2**) 6177×; (**A3**) 4974×; (**B2**) 9767×; (**C1**) 20614×; (**C3**) 5267×.

3.2.2. Morphology

The morphological structure of the obtained microcapsules after the spray-drying of emulsions was examined by scanning electron microscopy. The SEM images of the dried emulsions are illustrated in Figure 6. All the images showed microcapsules with similar aspect, always being spherical, without apparent fractures, and with more or less rough surfaces without noticeable differences between the powders with different percentages of BM or homogenization treatments.

In all the SDE, heterogeneity in particle sizes could be observed, ranging from 5 to 20 μm, clearly distinguishing at least two populations of different sizes. This size variety has also been observed in different studies [33,34], and is a typical characteristic presented by particles that have been spray dried.

Depression and superficial folds were observed in some microcapsules; this may be due to the sudden contraction that occurs in the early stages of drying. The viscoelastic properties of emulsifiers influence the appearance of these folds [32], which may be due, in this study, to the presence of MD in the formulation of the emulsions. Korma et al. [35] observed that emulsions formulated with whey proteins and maltodextrin had a higher number of dents than those formulated with whey proteins alone.

In dry emulsions obtained by conventional homogenization [6], formulated with MD and BM at different concentrations, the integrity of the microcapsules observed in the SEM images of this study indicated that BM had good properties to form a film, which efficiently protected seaweed oil contained in capsules.

4. Conclusions

From this study, it can be concluded that commercial BM could be applied to stabilize emulsions with omega-3 oil for further spray drying. The application of UHPH technology improved the global quality characteristics of BME in terms of stability. These formulations, especially the UHPH-processed emulsions, induced the aggregation of oil droplets when combining MD and BM. However, the particle size of the colloidal structures in the UHPH-treated emulsions was similar or lower than the individual oil droplets produced by conventional homogenization. The presence of aggregates seems to be relevant in the rheological behavior of all emulsions studied, with a slight thixotropic character, although the equilibrium viscosity at relative low deformations showed adequate values for spray drying purposes. Results of SDE obtained to evaluate the influence of UHPH technology applied to the BME showed good behavior in terms of powder morphology and encapsulating efficiency. The formulation containing 7% BM presented the best general characteristics in both BME and SDE.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/foods10051059/s1, Figure S1: Flow curves of emulsions (4 to 7% BM, 10% oil and 30% MD) processed by CH and UHPH. Figure S2: Viscosity curves of emulsions (4 to 7% BM, 10% oil and 30% MD) processed by CH and UHPH. Figure S3: Backscattering profiles of emulsions (4 to 7% BM, 10% oil and 30% MD) processed by CH and UHPH during storage.

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Article 2

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Characterization and oxidation stability of spray-dried emulsions with omega-3 oil and buttermilk processed by ultra-high-pressure homogenization (UHPH).

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ABSTRACT

Integrating functional ingredients, such as buttermilk and omega-3 rich oils, in spray-dried emulsions (SDE) is a suitable way to incorporate these ingredients in dairy products to substitute dairy fat and increase their added value. Ultra-high-pressure homogenization (UHPH) processing of liquid emulsions considerably improves stability compared to the conventional homogenization (CH) process. With this premise, SDE were produced while comparing CH (30 MPa) and UHPH (100 or 200 MPa) processing of feeding emulsions. Emulsions were formulated with (50:50 chia:sunflower) oil, whole commercial buttermilk (BM), and maltodextrin (MD) as wall materials. Further spray drying of emulsions was then conducted. Obtained SDE were characterized in terms of water content, A_w (water activity), flowing properties, water solubility, encapsulation efficiency (EE), color, and microstructure. Oxidation stability of SDE was analyzed in accelerated oxidation conditions at 50 °C for one month for primary and secondary oxidation analysis evolution on days 1, 7, 14, and 31 of storage. Results showed better ability of BM as encapsulating agent in UHPH-processed emulsions with 7% of BM. This improvement was especially observed in the flowing properties and encapsulating efficiency. Seven percent BM UHPH-treated SDE showed the best primary oxidation stability during storage, while the 4% BM-UHPH-treated SDE exhibited better secondary oxidative stability.

1. Introduction

Substituting or enriching the lipid fraction of foods with oils rich in polyunsaturated omega-3 offers the opportunity to consumers to increase the daily intake of these components, which in general is insufficient in the diet of most of the population (Kris-Etherton, Harris, & Appel, 2002). In addition, if buttermilk (BM) is also incorporated in the emulsion as an emulsifier, which is also recognized as a biofunctional compound (Ali, 2019; Hernell, Timby, Domellöf, & Lönnerdal, 2016; Singh & Gallier, 2017; Vanderghem et al., 2010), the potential advantages of the obtained emulsion are multiple. BM is a by-product of butter production, with a market maintaining an average annual growth rate of 1.16% from 2013 to 2016 (Ali, 2019). Its use in the food industry includes the production dry mixes, bakery products, and dairy products such as cheese or yoghurt (Ali, 2019). Thus, BM may increase the added value of food products to which it is supplemented, such as, infant formulae. BM is rich in milk fat globule membrane (MFGM), composed mainly by phospholipids, sphingolipids, and glycoproteins, all of them responsible for giving BM functional value (He et al., 2017; Lopez et al., 2017). These compounds have shown beneficial health effects, such as lowering of cholesterol levels and improved brain development and cognitive function in infants, within others (He et al., 2017; Lopez et al., 2017; Spitsberg, 2005). The use of oil-in-water emulsions is common in the pharmaceutical and food industries for encapsulating bioactive lipids as delivery systems of lipophilic functional components. Spray drying is the most widely used technology in the food sector for the preparation of solid emulsions due to its wide availability, versatility, and low cost.

Microencapsulation of oil in SDE requires droplets to be surrounded by a coating in a homogeneous or heterogeneous matrix to give small capsules providing a physical barrier between the core compound and

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Abbreviations

a*	red-green color parameter
Aw	water activity
b*	yellow-blue color parameter
BM	butter milk
CH	conventional homogenization
CI	Carr's index
HR	Hausner Ratio
MD	maltodextrin
MDA	malondialdehyde
MFGM	milk fat globule membrane
EE	encapsulation efficiency
L*	luminosity
SDE	spray dried emulsion
UHPH	ultra-high-pressure homogenization
YI	yellowness index
WI	whiteness index

other components of the product. Thus, the combination of an emulsifying agent to adsorb at the oil-water interface and a polysaccharide with high solid content and low viscosity is required to create the matrix in which droplets are embedded. Both types of compounds, emulsifiers, and polysaccharides, are commonly called wall materials. Maltodextrin (MD) is one of the most widely used polysaccharides in SDE combined with an emulsifying material, in this case BM.

Although drying of emulsions is a good system for the protection and release of bioactive compounds, unsaturated fatty acids are susceptible to oxidation. However, as reported by Augustin et al. (2015) whole BM powder was found to be better to high heat skim milk powder as an encapsulant to produce recombined omega-3 oil powders, founding a lower oxidation in BM emulsions. Similarly, another study (Zhang et al., 2020) using algal oil encapsulated with 100% or 50% buttermilk mixed with 50% maltodextrin showed good EE and oxidation stability of spray dried emulsions.

Homogenization systems produce fine emulsions, which show different degrees of oil protection against oxidation. UHPH processing may work up to 400 MPa (Hebishy, Buffa, Juan, Blasco-Moreno, & Trujillo, 2017). Currently, the UHPH technology capable of reaching up to 400 MPa is under development. Therefore, it is considered an emerging technology with great potential for the food, pharmaceutical and other industries in which colloidal products require great physical stability, as well as a microbial reduction. Mechanisms acting in UHPH are great intensity physical forces on dispersed particles such as friction, compression, acceleration, and shear resulting in great size reduction of particles, including microorganisms. All this result in high physical stability of dispersions, and microorganisms' inactivation when compared with conventional high-pressure homogenization systems (Dumay et al., 2013; Fernandez-Avila, Arranz, Guri, Trujillo, & Corredig, 2015; Hebishy, Buffa, Guamis, Blasco-Moreno, & Trujillo, 2015). On the other hand, protein material usually used as emulsifiers in food emulsions, may also experience structural modifications to some extent, depending on the type of protein incorporated (Fernandez-Avila & Trujillo, 2016; Hebishy, Buffa, et al., 2017; Hebishy, Zamora, Buffa, Blasco-Moreno, & Trujillo, 2017), thus creating a thick interfacial layer in the oil-water interface which prevents against lipid oxidation (Fernandez-Avila & Trujillo, 2016). These studies showed that oil-in-water emulsions made with whey protein isolate and sodium caseinate and treated by UHPH exhibited a greater physical and oxidative stability than emulsions produced with conventional homogenization. With this premise and considering the good results in emulsion stability observed in a previous study (Aghababaei et al., 2020) with a similar formulation to the present study, it is expected that improved characteristics of SDE-UHPH-treated emulsions will be found compared to conventional homogenized ones. The effect of UHPH and its potential protection of spray-dried against oxidation, as well as the general characteristics of powders, could contribute to find new opportunities for this emerging technology, which has not yet been studied for this purpose. Hence, the main objective of this work was to characterize and evaluate the oxidative stability of spray-dried emulsions obtained from UHPH (100 and 200 MPa) and conventional (30 MPa) homogenized feeding emulsions formulated with 4 and 7% BM, 10% oil and 30% MD.

2. Material and methods

2.1. Materials

Glucidex® 19-Maltodextrin (MD) was purchased from Roquette Freres (Lestrem, France) with 19 DE. Buttermilk powder (BM) was obtained from Activa Food-Tech, S. A. (Girona, Spain) with the following composition: 30% protein, 7% fat, 52% lactose, less than 4% moisture and 7% ash. Crude chia oil (20% C-18:2, >56% C-18:3 according to the specifications) was obtained from Interfat, S. A. (Barcelona, Spain). Crude sunflower oil (4–9% C-16:0, 1–7% C18:0, 15–85% C18:1, 50–72% C18:2) was purchased from Gustav Heess (Barcelona, Spain). All other chemicals used were of analytical or better grade.

2.2. Emulsion preparation and analysis

Six different samples (Table 1) of emulsions were obtained. The preparation procedure is fully described elsewhere (Aghababaei, Cano-Sarabia, Trujillo, Quevedo, & Ferragut, 2021). After mixing of ingredients, coarse emulsions were further homogenized using conventional (CH) or UHPH treatments. CH was performed with a Homolab (FBF Italia, Sala Baganza PR, Italy) at 30 MPa. Subsequently, heat treatment of emulsions was made at 65 °C, 30 min. UHPH treatments at 100 and 200 MPa were processed in an Ypsicon equipment Model A-60, which is an ultra-high-pressure continuous device (60 L/h) (Ypsicon Advance Technologies, S.L., Barcelona, Spain) that works up to 300 MPa. Working temperatures of samples were the following: 40 °C inlet temperature; 60 ± 2 and 80 ± 3 °C, at the high-pressure valve, corresponding to 100 and 200 MPa respectively; and 25 °C outlet temperature, which was reached after a quick cooling by a heat exchanger connected to the UHPH equipment. Emulsions were collected in Pyrex bottles for further sampling and analysis. Emulsions were analyzed as fully described by Aghababaei et al. (2021) for particle size distribution, using a Mastersizer laser diffraction 2000 analyzer (Malvern Instruments Ltd., Worcestershire, UK) and confocal laser-scanning microscope (Leica TCS SP5, Leica Microsystems GmHB, Mannheim, Germany) was used to observe the structure of fresh emulsions (24 h after production).

2.3. Spray drying of emulsions

Emulsions were dried in a Mini Spray-Dryer B-290 (Büchi

Table 1
Emulsion formulations and treatments.

Sample name	H (MPa)	Oil % (w/w)	MD % (w/w)	BM % (w/w)	TS % (w/w)
4CH	30	10	30	4	44
4UH100	100	10	30	4	44
4UH200	200	10	30	4	44
7CH	30	10	30	7	47
7UH100	100	10	30	7	47
7UH200	200	10	30	7	47

H: homogenization conditions; CH: conventional homogenization treatment. UH: ultra-high-pressure homogenization treatment; Oil: 50:50, chia:sunflower. MD: maltodextrin; BM: buttermilk; TS: total solids.

Labortechnik AG, Flawil, Switzerland). The samples were tempered at 25 °C, and the drying working conditions were 150 °C inlet temperature, 80% aspiration (32 m^3/h), and 30% feed flow (9 mL/min). Spray-dried emulsions (SDE) were collected in aluminum bags for further analysis.

2.4. Spray-dried emulsion characteristics

2.4.1. Moisture content and Aw

Moisture content of SDE was determined gravimetrically by drying 2 g of powder until constant weight (AOAC standard method no. 990.20). Aw was determined using Aqualab equipment, Model Series 3 TE (Decagon Devices, Pullman, WA).

2.4.2. Flowing properties

The bulk (ρ_{bulk}) and tapped (ρ_{tapped}) densities were determined as described by Tatar, Tunç, Dervisoglu, Cekmecelioglu, and Kahyaoglu (2014), with minor modifications. About 5 mL of SDE was added into a 25 mL glass graduated cylinder, measured, and weighed. Then, the cylinder was gently tapped by hand fifty times and the volume was read directly from the cylinder. The weight of SDE divided by the volume was used to calculate respectively the bulk (non-compacted powder) and tapped (compacted) densities. The powder flowability was evaluated using Carr's Index (CI) and the Hausner Ratio (HR) (Turchiuli et al., 2005), which were calculated following equations (2) and (3), respectively:

$$CI = (\rho_{tapped} - \rho_{bulk})/\rho_{tapped} \times 100 \quad (2)$$

$$HR = \rho_{tapped}/\rho_{bulk} \quad (3)$$

2.4.3. Water solubility

Solubility of SDE was evaluated as described by Botrel, Borges, Fernandes, and Lourenço Do Carmo (2014) with minor modifications. The sample (1 ± 0.01 g) was weighed and added to a beaker with 20 mL of distilled water, while stirring at 200 rpm. When all the sample had been added, the stirring speed was increased to 1200 rpm for 3 min. Subsequent centrifugation at 1600 rpm for 15 min at 20 °C was performed. Then, 5 mL of the supernatant was shifted to a preweighed capsule and dried at 110 °C for 4 h until constant weight. The water solubility (WS) was calculated by equation (4):

$$WS\ (\%) = (s \times 4/m) \times 100 \quad (4)$$

where: s is the grams of solids in supernatant and m is the grams of sample.

2.4.4. Encapsulation efficiency

The EE, total oil content (TO), and surface oil content (SO) of SDE were determined as described by González, Martínez, Paredes, Leónn, and Ribotta (2016). Briefly, for TO determination, 4.0 ± 0.1 g of sample were extracted in a Soxhlet apparatus for 24 h, with 200 mL of n-hexane solvent. After complete evaporation of the solvent, the oil extracted was weighed and expressed as a percentage of oil (dry basis) of the SDE.

For SO determination, 2 ± 0.01 g of SDE were mixed with 30 mL of petroleum ether followed by shaking for 1 min at room temperature and filtered through Whatman no. 1 paper. Solids in the filter were washed with 10 mL hexane and organic phases were combined. The filtrated solution was then transferred to an oven at 105 °C for the complete evaporation of hexane. EE was determined by calculating the ratio of the total oil contained in the SDE (TO) and the free oil (SO) located on its surface, according to equation (5).

$$EE = (TO - SO) \times 100/TO \quad (5)$$

2.4.5. Color evaluation

Color parameters were assessed with a colorimeter Konica Minolta CR-410 (Konica Minolta, Osaka, Japan), using D_{65} light source and angle of 10° observer as references. ESD samples were placed in a 50 mL clear optical glass container filled up to 10 mm followed by a white stopper disk to standardize measurements. The bottom external surface was measured. Results were expressed in the CIE L*a*b* color space, where L* is the lightness, a* is the greenness-redness, and b* is the blueness-yellowness. The Whiteness Index (WI), Yellowness Index (YI), and total color difference (ΔE) were calculated using the following equations (6)–(8), respectively.

$$WI = 100 - [(100 - L^*) + a^{*2} + b^{*2}]^{0.5} \quad (6)$$

$$YI = 142.86 \times b^* \times L^{*-1} \quad (7)$$

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \quad (8)$$

2.4.6. Morphology and size

The morphology of SDE was observed by SEM, using the Quanta ™ 650 FEG scanning electron microscope (FEI Company, Hillsboro, OR, USA), with an accelerating beam voltage (HV) of 5 kV. Samples were prepared by fixing a small amount of powder on metal discs with double-sided carbon tapes, which were then platinum-plated in a Leica EM ACE600 vacuum chamber (Leica Microsystems, Wetzlar, Germany). Mean diameter of particle size of SDE samples were analyzed by measuring 100 particles per sample on the SEM photographs using the microscope software.

2.5. Oxidation stability

The oxidative stability of SDE was determined by the peroxide value and by quantification of malondialdehyde as secondary oxidation product. SDE were stored at 50 °C for 31 days to accelerate the oxidation process. Primary and secondary oxidation were determined in SDE on days 0, 7, 15, and 31. Feeding emulsions of the corresponding SDE were also analyzed on day 0 to evaluate the effect of the drying process on primary oxidation.

2.5.1. Primary oxidation

The hydroperoxide concentration was determined according to the previously described method by Hu, McClements, and Decker (2003). SDE (1 ± 0.01 g) were reconstituted in 10 mL distilled water. A sample of 300 μL emulsion was taken in triplicate and mixed with 1.5 mL of an isooctane: 2-propanol solution (3:1) in glass tubes, and vortexed for 30 s (10 s × 3). The organic phase of the mixtures was separated by centrifugation at 1000 rpm at 20–25 °C for 2 min. 200 μL of the organic phase was added to 2.8 mL of a methanol: 1-butanol solution (2: 1). Then, 1 μL of 3.97 M ammonium thiocyanate and 15 μL of iron solution prepared with 0.132 M BaCl2 and 0.144 M FeSO4 were added continuously. Finally, the test tubes were vortexed (10 s), and, after 20 min, the absorbance of the solution was measured at a wavelength of 510 nm in a spectrophotometer Dinko UV 2310 (Dinko Instruments, Barcelona, Spain). Hydroperoxide concentrations were determined by means of a standard curve, made from cumene hydroperoxide, in a concentration range of 0.002–2 mM.

2.5.2. Secondary oxidation

Quantification of malondialdehyde was made according to the method proposed by Papastergiadis, Mubiru, Van Langenhove, and De Meulenaer (2012) by HPLC. For sample preparation, 5 mL of emulsion were taken and mixed with 15 mL of 7.5 g/100 mL TCA containing 0.1 g/100 mL EDTA and 0.1 g/100 mL propyl gallate into 50 mL conical centrifuge tubes, and shaken horizontally for 15 min. Subsequently, they were centrifuged at 3500 rpm for 15 min and then, 1 mL of the

supernatant was taken and added to glass tubes (with cap and screw) together with 3 mL of 40 mM TBA. The tubes were boiled for 40 min, then cooled to room temperature, and 1 mL of methanol was added to each one and vortexed for 10 s. Samples were placed in Eppendorf tubes and centrifuged at 3500 rpm for 15 min to avoid the presence of precipitates. The separation and quantification of malondialdehyde was carried out in an HPLC equipment composed of an automatic injector (Waters 717 Plus, Milford, Massachusetts, USA), a Perkin Elmer model 515 booster pump (Waltham, Massachusetts, USA), and a serial fluorescence detector 200 (Perkin Elmer). Aliquots (20 μL) of the sample were injected onto an Agilent Pursuit 3 C18 column (5 μm, 150 × 4.6 mm) at 40 °C analysis temperature. The mobile phase consisted of a 50 mM KH_2PO_4 buffer solution, methanol, and acetonitrile (72:17:11, pH 5.3) pumped isocratically with a flow of 0.8 mL/min. The fluorometric excitation and emission wavelengths were set at 525 and 560 nm, respectively. To quantify the amount of malondialdehyde, a standard curve was made from 1.1 to 3.3 tetraethoxypropane in a concentration range of 0.25–25 μM. Peak quantification was performed using Turbochrom TC6 software (Perkin Elmer).

2.6. Statistical analysis

Results are presented as mean ± standard deviation. SDE characterization was subjected to a one-way analysis of variance (ANOVA) test using the Minitab Express™ version 1.5.3 (Minitab, State College, PA, USA). Significant differences between means were determined by the Tukey test. A confidence level of 95% ($p < 0.05$) was used. At least two individual productions of each formulation and treatment were performed. All analysis were replicated three times.

3. Results and discussion

3.1. Characteristics of emulsions

Processing of feeding emulsion determines the characteristics of microcapsules produced by spray drying (González et al., 2016). BM concentration as well as homogenization treatment influenced the particle size distribution and microstructure of feeding emulsions (Fig. 1). In CH emulsions, mean particle size ($d_{4,3}$) was about 13 and 4.5 μm corresponding to 4CH and 7CH, respectively. The particle size distribution curves were bimodal, which corresponded to individual and aggregated oil droplets. This fact was especially present in 4CH emulsions. UHPH-processed emulsions showed a particle size reduction in comparison to CH emulsion in both 4UH and 7UH. In Fig. 1 the CLSM micrographs are inserted, in which the structure of oil droplets can be observed. The most remarkable characteristic of UHPH-processed emulsions, independently of the BM concentration, was the formation of oil droplets aggregates which were smaller in particle size than the individual oil droplets of the CH emulsions, which was associated to a higher emulsion stability of UHPH-processed emulsions (Aghababaei et al., 2021). The strong physical forces acting in UHPH have been described to cause partial protein denaturation (Fernandez-Avila et al., 2015; Floury, Desrumaux, & Legrand, 2002; Serra, Trujillo, Guamis, & Ferragut, 2009) and casein micelle disintegration (Sharma, Jana, & Chavan, 2012), which facilitates the aggregation by means of protein-oil and protein-protein interactions. This phenomenon has also been described in other emulsions treated by UHPH such as those formulated with 1, 3 and 5% sodium caseinate (Hebishy, Buffa, et al., 2017), in which aggregates were only observed at 3 and 5% of that emulsifier. In our emulsions, which were formulated for further spray drying, the presence of 30% of MD could lead to depletion flocculation, promoting the aggregation of individual oil droplets in presence of protein and carbohydrates, by their partial thermodynamic incompatibility (De Kruif & Tuinier, 2001).

3.2. Characteristics of spray-dried emulsions

In this study, spray-drying conditions were kept constant according to preliminary trials to make sure that the SDE were not sticky and flowability was good enough for an acceptable yield. Total solid content of SDE was 44% for formulations containing 4% BM and 47% for formulations with 7% BM. In Table 2, characteristics of SDE are shown. Moisture content varied from 2.06 to 3.19% in all SDE with few differences ($p < 0.05$) being observed among them. 4CH sample was the SDE with the lowest water content and 7UH100 that which showed the highest water content of SDEs. It appeared that both homogenization treatment and BM concentration influenced water content. Probably, in CH treatments, water was easier to eliminate by spray drying because of the lower particle volume fraction observed in these sample (Fig. 1). This behavior of CH compared to UH samples was observed for both BM concentrations, 4 and 7%. In addition, when comparing the BM concentration SDE series, 7% SDE showed higher water content, which could explain certain difficulty for water evaporation in the drying process. On the other hand, the different colloidal structures, big droplets in CH or small aggregates in UH SDE, may indicate a different protein distribution between the continuous and oil-water interface of the feeding emulsions, modifying water elimination during the spray drying process. However, in terms of powder stability during storage, as reported by Klaypradit and Huang (2008), dry foods with moisture content between 3 and 10% have a good behavior.

Aw values ranged from 0.130 to 0.191 with no significant differences ($p < 0.05$) between them, although the highest values observed corresponded to those with lower BM content, as expected. These values are considered adequate for dry foods since they guarantee microbiological stability (Comunian et al., 2019).

One relevant characteristic of powders is bulk density, which varied in a narrow range, from 430 to 455 kg/m^3 for all samples with no significant differences ($p < 0.05$) observed between them. These values are in the range of most of the spray-dried emulsions reported in literature, which may vary broadly depending on composition and drying conditions used. Our SDE bulk density values were similar to those found by other authors (Sarkar, Arfsten Golay, Acquistapace, & Heinrich, 2016;

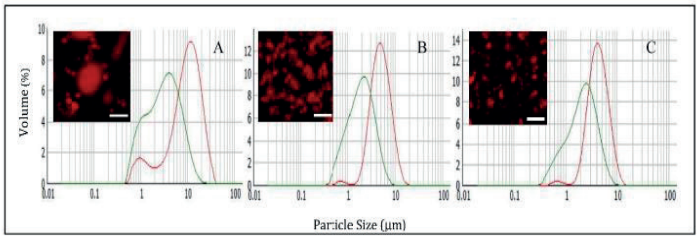

Fig. 1. Particle size distribution of 4% BM (red line) and 7% BM (green line) of emulsions processed by CH (A), UH100 (B) and UH200 (C). CLSM images correspond to oil droplets and aggregates observed in 7% BM at the different homogenization treatments applied, labelled with Nile Red (scale bar 7.5 mm). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Characteristics of SDE from feeding emulsions containing 4 and 7 (%) BM and processed by different homogenization systems (CH and UHPH).

Sample	Moisture (%)	A_w	r_b (kg/m^3)	r_t (kg/m^3)	CI	HR	WS (%)	EE (%)	Size (μm)
4CH	2.06 ± 0.04^{c}	0.191 ± 0.01^{a}	455 ± 17^{a}	551 ± 11^{a}	17.3 ± 2.6^{a}	1.21 ± 0.03^{a}	94.1 ± 3.4^{a}	77.1 ± 2.6^{c}	10.3 ± 3.6
4UH100	2.64 ± 0.10^{b}	0.192 ± 0.01^{a}	438 ± 12^{a}	526 ± 17^{ab}	16.6 ± 1.1^{a}	1.20 ± 0.06^{a}	93.6 ± 2.9^{a}	83.0 ± 2.1^{b}	11.4 ± 5.5
4UH200	2.82 ± 0.30^{ab}	0.185 ± 0.01^{a}	454 ± 12^{a}	534 ± 10^{ab}	15.1 ± 0.7^{ab}	1.17 ± 0.01^{ab}	92.1 ± 3.3^{a}	84.1 ± 0.8^{b}	7.4 ± 6.3
7CH	2.76 ± 0.08^{ab}	0.130 ± 0.01^{a}	434 ± 09^{a}	470 ± 06^{c}	7.70 ± 0.7^{c}	1.08 ± 0.01^{cd}	96.8 ± 2.2^{a}	81.2 ± 1.3^{bc}	8.8 ± 5.2
7UH100	3.19 ± 0.06^{a}	0.151 ± 0.01^{a}	445 ± 13^{a}	506 ± 15^{b}	11.9 ± 1.1^{b}	1.13 ± 0.01^{bc}	91.7 ± 4.5^{a}	91.5 ± 2.5^{a}	6.3 ± 2.9
7UH200	3.07 ± 0.10^{ab}	0.131 ± 0.01^{a}	430 ± 07^{a}	465 ± 10^{c}	7.50 ± 1.0^{c}	1.08 ± 0.01^{d}	91.5 ± 1.3^{a}	89.4 ± 0.9^{a}	11.0 ± 4.5

Means in each column with different superscript letters were significantly different ($p < 0.05$). Values are mean and SD of three replications.
A_w: water activity; r_b: Bulk density; r_t: Tapped Density; CI: Carr Index; HR: Hausner Ratio; WS: Water Solubility; EE: Encapsulation Efficiency; Size: mean diameter of particle size analyzed.

Carneiro, Tonon, Grosso, & Hubinger, 2013) who also reported oil content similar to those used in this study. Some powders parameters related to bulk and tapped densities (eqs. (2) and (3)), were calculated: Carr's Index (Carr, 1965) is a scale of flowability (values ≤ 10 are excellent and values > 38, are awful). The Hausner Ratio (Hausner, 1967) is a scale of cohesiveness from 1 (excellent) to > 1.6 (awful). Thus, both parameters, CI and HR, can be used to determine powder properties during processing and storage conditions (Quispe-Condori, Saldaña, & Temelli, 2011; Sanchez-Reinoso & Gutiérrez, 2017). Flowing properties of the present SDE showed a significantly different ($p < 0.05$) behavior, mainly depending on BM concentration regardless of the homogenization system used; i. e., all 4% BM SDE were similar but different from 7CH and 7UH200, which exhibited lower values of CI and HR. As shown in Table 2, SDE with 4% BM had values in the range corresponding to a fair flowability and cohesiveness (CI = 16–20 and HR = 1.19–1.25, are considered as fair on their respective reference scales). In these samples, although no statistical differences were observed, CI and HR values decreased as homogenization pressure increased, indicating a certain influence of this technological treatment. SDE with 7% BM had values mostly in the range of excellent flowability and cohesiveness, thus exhibiting very good handling properties, and showing that BM is a good encapsulating agent capable of producing powder emulsions with high quality characteristics of handling and transport.

Solubility in water is a relevant quality parameter of dry powders which mostly depends on its chemical composition and physical state (Dhanalakshmi, Ghosal, & Bhattacharya, 2011). All SDE produced in this study showed high solubility in water at room temperature. They did not exhibit significant variations in their solubility ($p > 0.05$), which ranged between 91.5 and 96.8%. This parameter is strongly influenced by the nature of the wall materials (carbohydrates and proteins), which in this work were constituted by 30% MD and 4 or 7% BM, both with high solubility in water. Solubility values observed in this study were similar to those reported by other authors (Korma et al., 2019) in powder emulsions formulated with WPI and MD or inulin with 30% total solids.

The EE reflects the degree to which wall material can retain oil in microcapsules. Values of this parameter showed good encapsulation of oil, ranging between 77.1 and 91.5% in the different SDE. Both BM concentration and homogenization system influenced the results obtained. In formulations containing 4% BM, the UHPH treatment (100 or 200 MPa) improved significantly ($p < 0.05$) the capacity of oil retention of microcapsules compared to 4CH. On the other hand, SDE containing 4% BM and treated by UHPH showed similar values of this parameter than formulation 7CH, indicating the positive influence of BM concentration. The best EE was observed in 7UH100 and 7UH200 SDE, which showed similar values. Emulsifying properties of BM are attributed to its composition, rich in casein, whey proteins and phospholipids, which adsorbs at the oil-water interface exerting the desired covering of oil droplets. Thus, increasing BM content could improve the effective oil coverage, and in consequence, the EE as reported also by other authors (Wang, Che, Fu, Chen & Selomulya, 2016). On the other hand, as observed in the feeding emulsions, UHPH promoted the aggregation of oil droplets though protein-protein interactions. In consequence, oil could be hidden in those colloidal structures keeping better EE.

Color attributes are important characteristics for consumer acceptance. Table 3 summarizes the results of color measurements: CIE Lab parameters, color difference between homogenization treatments, and color difference between different BM concentrations for the same treatments of SDE. As well, two indexes used for near-white opaque materials (WI and YI) were calculated. L* did not show differences between treatments or BM concentration, ranging from 92.6 to 94.0. Red – green (a*) varied in a short range into the red tonality, while b* (yellow - blue), was the parameter that most contributed to the color differences and YI within samples, being formulations containing 7% BM those that showed the highest WI values ($p < 0.05$). Regarding color differences, by comparing the effect of homogenization treatment as well as the BM content, most of values were >1, which means that they can be classified as slightly noticeable (0.5–1.5) by the human eye (Sanchez-Reinoso & Gutiérrez, 2017).

Morphology of SDE examined by SEM are shown in Fig. 2. All the

Table 3
Color characteristics of SDE from feeding emulsions containing 4 and 7% BM processed by different homogenization systems (CH and UHPH).

Samples	L*	a*	b*	ΔE	WI YI	
4CH	92.9 ± 0.5^{ab}	3.70 ± 0.02^{a}	0.47 ± 0.22^{c}	–	95.4 ± 0.04^{e}	0.72 ± 0.34^{c}
4UH100	92.6 ± 0.1^{b}	3.60 ± 0.02^{b}	0.60 ± 0.07^{c}	1.60 ± 0.07^{a}	95.4 ± 0.02^{de}	0.93 ± 0.11^{c}
4UH200	93.4 ± 0.6^{ab}	3.57 ± 0.03^{b}	0.63 ± 0.07^{c}	1.86 ± 0.20^{a}	95.5 ± 0.06^{cd}	0.96 ± 0.11^{c}
7CH	94.0 ± 0.2^{a}	3.12 ± 0.03^{c}	1.33 ± 0.10^{b}	–	95.8 ± 0.02^{cd}	2.02 ± 0.15^{b}
7UH100	93.4 ± 0.3^{ab}	2.92 ± 0.01^{d}	1.86 ± 0.08^{a}	1.00 ± 0.20^{b}	95.6 ± 0.03^{de}	2.84 ± 0.12^{a}
7UH200	93.6 ± 0.1^{ab}	3.25 ± 0.03^{a}	1.20 ± 0.06^{b}	1.18 ± 0.03^{b}	95.7 ± 0.05^{cd}	1.84 ± 0.11^{b}
4CH–7CH				1.57 ± 0.78^{a}		
4UH100–7UH100				1.63 ± 0.30^{a}		
4UH200–7UH200				0.85 ± 0.26^{a}		

Means in each column with different superscript letters were significantly different ($p < 0.05$). Values are mean and SD of three replications. L*, a*, b*: CIELab color coordinates; ΔE: Color Difference between UHPH and CH treatments for each BM concentration formulation; WI: Whiteness Index; YI: Yellowness Index.

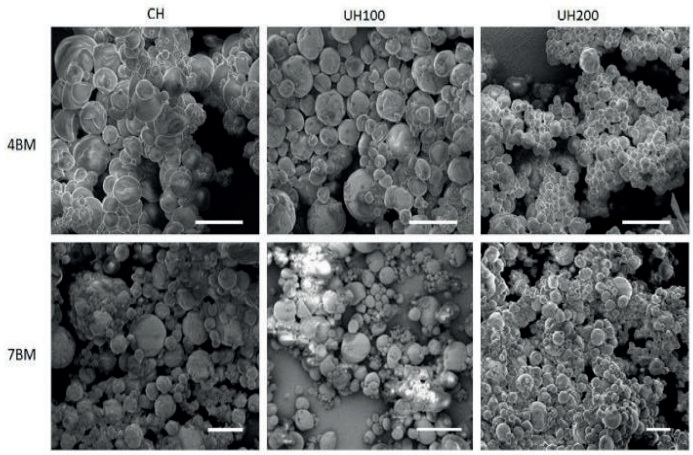

Fig. 2. SEM images of SDE containing 4 and 7% BM (lines) from feeding emulsions treated by CH and UHPH (columns). Scale bars correspond to 5 μm.

images always showed spherical particles, with apparent smooth surfaces without remarkable presence of fractures, although depression and superficial folds were observed in some microcapsules, probably due to the sudden contraction that occurs in the early stages of drying. The general aspect of all SDE were similar, regardless of the percentages of BM or homogenization treatments. Particle sizes were heterogenous, showing the presence of small and larger particles in the same sample, frequently aggregated to each other. In general, formulations containing 4% BM showed higher particle size (Table 2) than 7% BM formulations. However, it is difficult to establish any correlation since the variety observed in the particle size was often high. This aspect has also been observed in different studies (Benito-Román, de Paz, Melgosa, Beltrán, & Sanz, 2018; Carneiro et al., 2013) which is typical in spray-dried foods.

3.3. Oxidation stability of SDE

The stability of SDE against oxidation was evaluated in accelerated conditions of heating at 50 °C for one month, at days 1, 7, 14, and 31 of storage. The oxidation analysis was performed for primary and secondary oxidation. The effect of drying on primary oxidation developed in SDE samples was also estimated compared to the fresh emulsions as depicted in Fig. 3. As expected, the effect of drying caused an increase of hydroperoxide concentration in all samples, which varied in function of homogenization treatment and BM concentration. Regarding BM concentration, both fresh emulsions and SDE were more protected against oxidation with 7% BM. The effect of treatment was more accused in CH emulsions, which exhibited higher difference of hydroperoxides between fresh and SDE compared to those treated by UH. Probably, the fresh UHPH-treated emulsions were more exposed to pro-oxidant agents due to the high increase of oil surface droplets generated during UHPH treatment. However, the effect of BM concentration prevailed against treatment as the oxidative state of SDE on day 1 was quite similar in all the 7BM SDE series.

Fig. 4 shows the hydroperoxides concentration evolution during SDE storage. The pattern of the primary oxidation curves was similar in all samples. They were characterized by a subtle increase in the

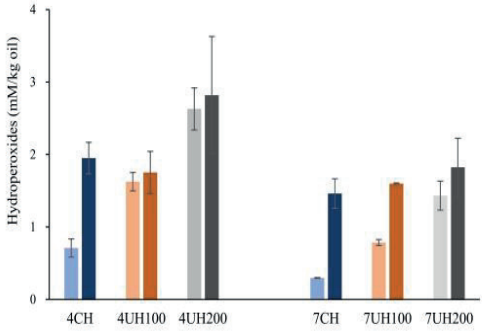

Fig. 3. Effect of drying process on primary oxidation in formulations containing 4 and 7% BM. Clear color (feeding emulsions on day 1), dark color (SDE on day 1 after drying). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

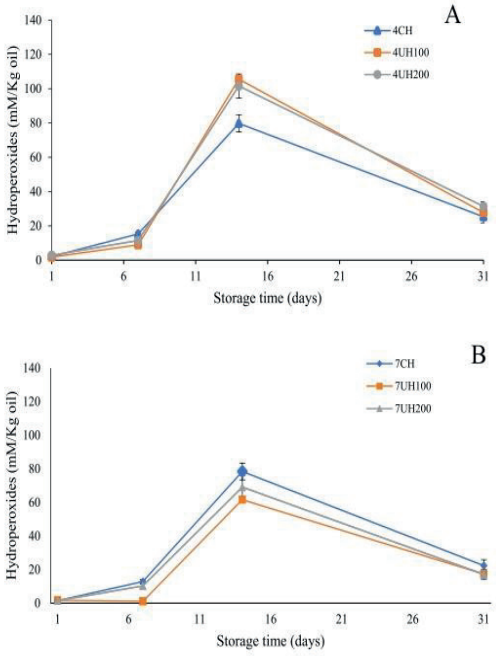

Fig. 4. Hydroperoxides concentration evolution during storage of SDE in accelerated conditions at 50 °C obtained from feeding emulsions treated by different homogenization systems (CH and UHPH) and BM concentration. 4% BM (A); 7% BM (B).

concentration between days 1 and 7, followed by a pronounced increase on day 14, and a noticeable decrease at day 31. This observed evolution of hydroperoxides is consistent with the kinetics of development of primary lipid oxidation, in which a latency period appears at the beginning followed by a propagation or exponential increase of the oxidation products (Kamal-Eldin, McAkinen, & Lampi, 2003). The decrease observed after the peak is due to the secondary oxidation products being formed from the primary products' degradation (Papastergiadis et al., 2012).

Considering BM concentration, hydroperoxide levels of 7% BM samples were lower during storage than those of 4% BM, exerting less protection by recovering the interface of small oil droplets and aggregates generated by UHPH treatment than 7% BM. Hebishy, Buffa, et al. (2017) in sodium caseinate emulsions treated by UHPH observed this dependence of protein concentration on the oil droplet protection against oxidation. Thus, at the lowest amount of emulsifier, the film formed is not dense enough to protect the oil from oxidation, allowing the diffusion of oxygen and pro-oxidants towards the oil droplets during storage (Sarkar, Arfsten, Golay, Acquistapace, & Heinrich, 2016).

The effect of BM concentration and homogenization treatment was consistent with the EE observed (Table 2). EE was higher in UHPH-treated samples containing higher BM concentrations. Zhang et al. (2020) showed a similar tendency in spray-dried emulsions produced by homogenization at 30 MPa (3 cycles), and formulated with different concentrations of BM, maltodextrin, and seaweed oil, finding that the formulations with the highest percentage of BM showed the lowest hydroperoxides, attributing this effect to higher EE.

Fig. 5 shows the evolution of secondary oxidation through the determination of malondialdehyde (MDA) concentration in SDE over time. In general, low levels of MDA were observed in most of the stored SDE with an MDA increase on day 31, which was especially marked in SDE containing 7% BM. This MDA increase coincided with the decrease in hydroperoxides mentioned above, confirming that as the end of accelerated storage approaches, oxidation state continues to advance. Regarding the higher MDA concentrations observed in 7% BM formulations, it could be explained based on the presence of some compounds of BM that could behave as pro-oxidants, especially if they were present in excess (Berton-Carabin, Ropers, & Genot, 2014). BM contains phospholipids that may have antioxidant effects. However, when they are in dehydrated dairy products, they can also behave as pro-oxidants due to the unsaturation presented by MFGM phospholipids such as phosphatidylcholine and phosphatidylethanolamine (Cui & Decker, 2016). As can also be seen in Fig. 5, emulsions treated at 100 MPa were generally those that presented lower MDA within the storage, compared with CH and UH200 treated emulsions. This significant difference observed, especially at day 31 of storage, may be due to the bigger size of the oil droplet aggregates formed at 100 MPa compared to 200 MPa treated samples. Although at the two UH pressures applied in this study, a high size reduction of individual oil droplets was observed (Aghababaei et al., 2021); reaggregation was produced, giving more stable emulsions with smaller particle sizes than those obtained with conventional technology. However, as can be seen in Fig. 1, UH100 emulsions had bigger aggregates, thus the effective exposure of oil to the oxidation catalyzers were lower by limiting their access to lipids. Further study of oxidation at ambient temperature could clarify the real behavior of those dried emulsions, given that storage temperature greatly determines the oxidation kinetics as reported by other authors (Escalona-García et al., 2016), being highly reduced at 25 °C in chia oil encapsulated with whey protein concentrate.

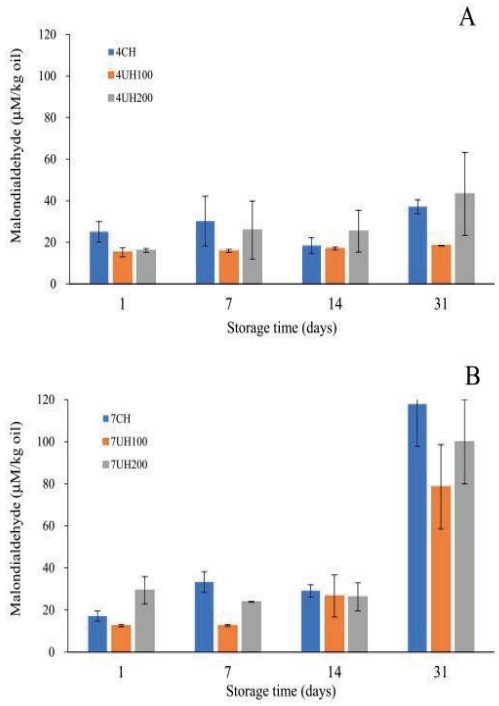

Fig. 5. Malondialdehyde concentration evolution during storage of SDE in accelerate conditions at 50 °C obtained from feeding emulsions treated by different homogenization systems (CH and UHPH) and BM concentration. 4% BM (A); 7% BM (B).

4. Conclusions

Incorporation of buttermilk (at 4 or 7%) has allowed the production of spray-dried products with good general properties such as optimal flowability and cohesiveness. In addition, when UHPH treatment was used compared to conventional homogenization, general properties of powders were similar or improved, such as the EE, which increased its performance as encapsulant in producing dried emulsions. Oxidative stability evolved in a similar direction regarding primary oxidation. However, the secondary products of oxidation were higher in 7% than in 4% buttermilk formulations, probably due to the higher concentrations of unsaturated phospholipids of buttermilk at the oil-water interface. This hypothesis is reinforced as the UHPH treatment at 100 MPa produced the best oxidative stability of spray-dried emulsions; probably it was caused by the microstructure of feeding emulsion obtained at these UHPH conditions, in which prevailed the oil droplets protected by buttermilk hidden inside the big aggregates observed in those emulsions.

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CRediT authorship contribution statement

Carolina Varela: Investigation, Data curation, Methodology, Writing – original draft, Formal analysis. **Fatemeh Aghababaei:** Investigation, Data curation, Formal analysis. **Mary Cano-Sarabia:** Visualization, Supervision, Resources. **Libni Turitich:** Investigation, Data curation. **Antonio J. Trujillo:** Project administration, Supervision, Conceptualization. **Victoria Ferragut:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

Authors declared that they have no conflicts of interest to this work.

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Appendix II

Preliminary experiments to determine emulsion formulations for further drying.

Experiment 1. Varying oil and buttermilk (BM) compared with skim milk powder (SMP).

Table 1. Emulsions formulation varying oil and buttermilk content

Sample	Oil (%)	MD (%)	SMP/MZ (%)
SMP2/10	10	30	2
SMP2/15	15		
MZ2/10	10		
MZ2/15	15		
MZ3/10	10		3
MZ3/15	15		

Table 2. Values (±SD) of $D_{4.3}$ and $D_{3.2}$ of the emulsion particles at day 1.

Muestras	$D_{3.2}$ (μm)	$D_{4.3}$ (μm)
SMP2/10	$7.17^{b} \pm 0.50$	$32.13^{a} \pm 6.36$
SMP2/15	$15.10^{d} \pm 2.81$	$62.76^{c} \pm 10.41$
MZ2/10	$8.88^{c} \pm 0.39$	$43.74^{b} \pm 9.17$
MZ2/15	$16.27^{d} \pm 1.13$	$57.05^{c} \pm 5.60$
MZ3/10	$5.47^{a} \pm 0.15$	$31.43^{a} \pm 2.89$
MZ3/15	$9.39^{c} \pm 1.35$	$45.35^{b} \pm 7.55$

[a-b-c-d] For each parameter. different letters in the same column indicate significant differences ($p<0.05$).

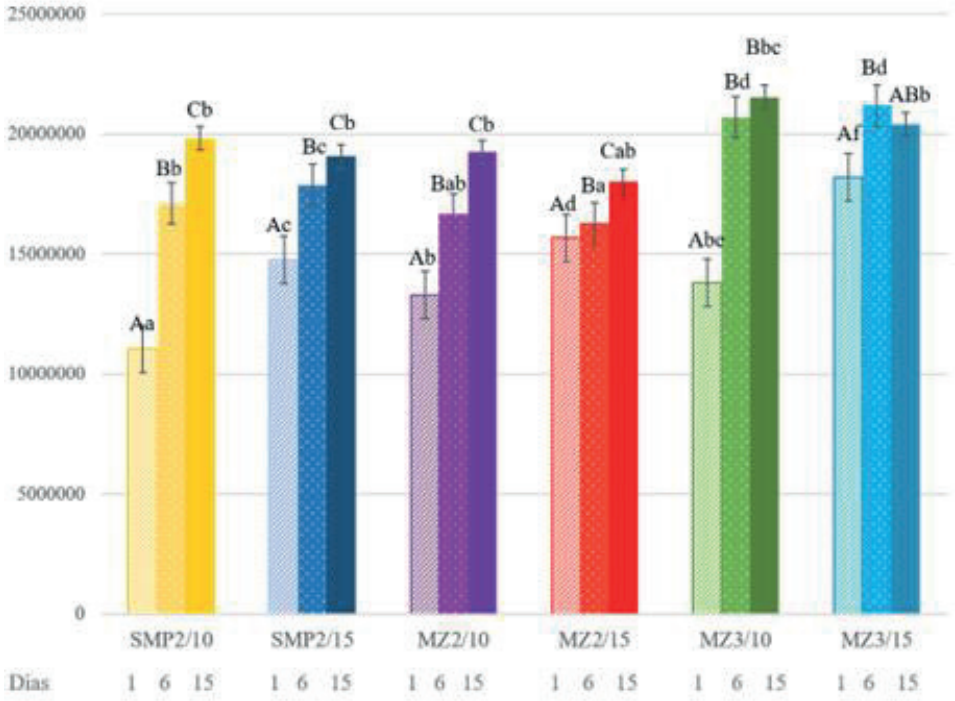

Figure 1. TSI of emulsions. Lowercase letters (a-b-c-d-e-f) represent significant differences between samples and uppercase letters (A, B, C) represent significant differences between days ($p<0.05$).

Table 3. Rheological parameters K and n (mean ± SD) of the emulsions (day 1).

Muestras	K (mPa x s)^n	n	r^2
SMP2/10	$0.0255^b \pm 0.0061$	$0.938^b \pm 0.014$	0.999 ± 0.0001
SMP2/15	$0.0189^a \pm 0.0038$	$0.950^b \pm 0.014$	0.999 ± 0.0003
MZ2/10	$0.0157^a \pm 0.0022$	$0.980^c \pm 0.008$	0.999 ± 0.0002
MZ2/15	$0.0246^b \pm 0.0050$	$0.946^b \pm 0.021$	0.999 ± 0.0001
MZ3/10	$0.0168^a \pm 0.0013$	$0.971^c \pm 0.012$	0.999 ± 0.0005
MZ3/15	$0.0354^c \pm 0.0022$	$0.933^{ab} \pm 0.015$	0.999 ± 0.0002

$^{a\text{-}b\text{-}c}$ Para cada parámetro. las diferentes letras en cada columna indican que existen diferencias significativas ($p<0.05$).

Table 4. Mean diameter (± SD) of dehydrated capsules.

Muestras	**Diámetro medio (µm)**
SMP2/10	1.24 ± 0.48
SMP2/15	2.12 ± 0.56
MZ2/10	5.42 ± 2.04
MZ2/15	3.79 ± 0.98
MZ3/10	2.51 ± 0.33
MZ3/15	2.63 ± 0.29

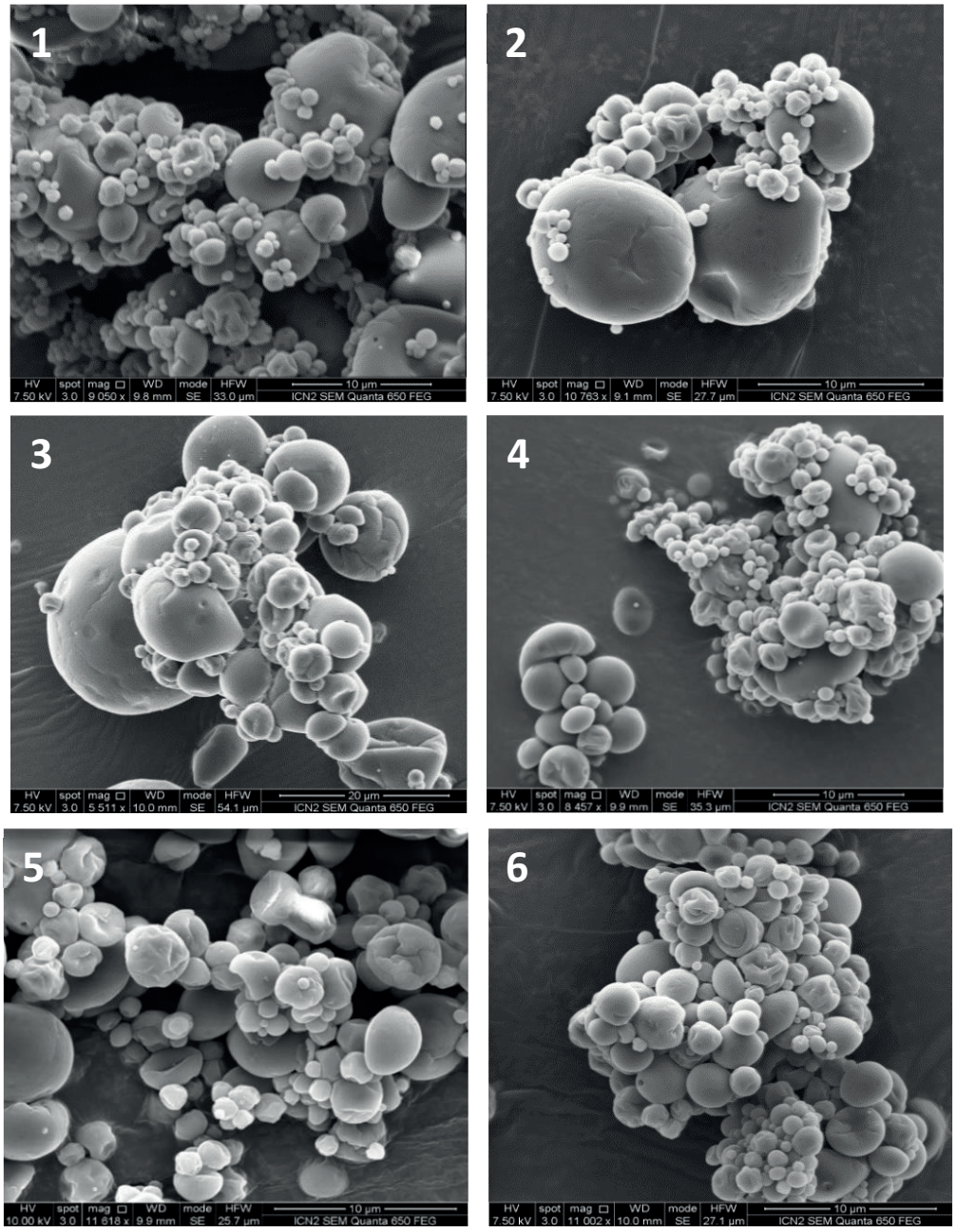

Figure 2. Morphology of the dehydrated emulsion capsules by SEMobservation. 1. SMP2/10, 2. SMP2/15 3. MZ2/10 4. MZ2/15 5. MZ3/10 6. MZ3/15

Experiment 2. Varying oil, buttermilk, and wall materials (OSA and MD) content.

Table 1. Formulations by combining wall materials (OSA and MD).

Oil-BM-WM	Oil (%)	BM (%)	OSA (%)	MD (%)	Water (%)
8-3-20	8	10	30	10	42
10-3-20	10	10	30	10	40
8-3-30	8	10	45	15	22
10-3-30	10	10	45	15	20
8-4-20	8	13.3	30	10	38.7
10-4-20	10	13.3	30	10	36.7
8-4-30	8	13.3	45	15	18.7
10-4-30	10	13.3	45	15	16.7
8-5-20	8	16.6	30	10	35.4
10-5-20	10	16.6	30	10	33.4
8-5-30	8	16.6	45	15	15.4
10-5-30	10	16.6	45	15	13.4

Table 2. formulations with MD as wall material

Oil-BM-WM	Oil ()	BM (g)	MD (g)	Water (g)
8-4-20	8	13.3	40	38.7
10-4-20	10	13.3	40	36.7
8-4-30	8	13.3	60	18.7
10-4-30	10	13.3	60	16.7
8-6-20	8	19.9	40	32.1
10-6-20	10	19.9	40	30.1
8-6-30	8	19.9	60	12.1
10-6-30	10	19.9	60	10.1

Table 3. Stability index (TSI) of emulsions formulated with OSA and MD on days 1 and 4.

Day 1						
	b1.1	**b1.2**	**b2.1**	**b2.2**	**mean**	**sd**
HC 8-3-20	36.9	27.3	38.6	36.9	34.93	5.146
HC 10-3-20	43.2	42.9	40.2	36.3	40.65	3.198
HC 8-3-30	33	32.9	27.9	29.7	30.88	2.506
HC 10-3-30	15	17.2	8.2	7.7	12.03	4.795
HC 8-4-20	1.1	1.3	26.6	22.3	12.83	13.54
HC 10-4-20	8.8	8.3	37.4	34	22.13	15.74
HC 8-4-30	7.8	7.6	7.6	6.9	7.475	0.3948
HC 10-4-30	9.5	9.5	5.1	5.2	7.325	2.512
HC 8-5-20	2.8	2.8	15.1	14.3	8.750	6.878
HC 10-5-20	4.4	3.5	11.9	12.5	8.075	4.784
HC 8-5-30	2.2	2.9	2.4	2.4	2.475	0.2986
HC 10-5-30	4.4	5.9	1.7	2.5	3.625	1.893
Day 4						
	b1.1	**b1.2**	**b2.1**	**b2.2**	**mean**	**sd**
HC 8-3-20	47.4	33.5	49.5	47.4	44.45	7.367
HC 10-3-20	54.6	55	61.3	58.3	57.30	3.140
HC 8-3-30	46.8	46.5			46.65	0.2121
HC 10-3-30	27.3	30.1			28.70	1.980
HC 8-4-20	8.1	8.3	69.1	64.3	37.45	33.83
HC 10-4-20	36.2	34.7	65.6	55	47.88	15.00
HC 8-4-30	25.6	25.6			25.60	0.0000
HC 10-4-30	24	21.8			22.90	1.556
HC 8-5-20	24.1	24	50.1	50.4	37.15	15.13
HC 10-5-20	24.1	16.6	41.4	43.7	31.45	13.21
HC 8-5-30	17.7	13			15.35	3.323
HC 10-5-30	22.9	22.8			22.85	0.07071

Table 4. Stability index (TSI) of emulsions formulated with MD on days 1 and 4.

Day1						
	p1.1	**p1.2**	**p2.1**	**p2.2**	**mean**	**sd**
MD 8-4-20	4.9	5.1	4.4	4.5	4.725	0.3304
MD 10-4-20	3.6	3.5	2.7	2.8	3.150	0.4655
MD 8-4-30	1.7	2	1.4	1.4	1.625	0.2872
MD 10-4-30	1.6	2	1.3	1.7	1.650	0.2887
MD 8-6-20	1.8	2.6	1.4	1.8	1.900	0.5033
MD 10-6-20	1.6	1.6	1.3	1.2	1.425	0.2062
MD 8-6-30	1	1.1	0.9	1.2	1.050	0.1291
MD 10-6-30	1	1.2	0.9	1.2	1.075	0.1500
Day 4						
	p1.1	**p1.2**	**p2.1**	**p2.2**	**mean**	**sd**
MD 8-4-20	12.8	13.2	11.7	11.7	12.35	0.7681
MD 10-4-20	10.2	10.2	8.2	8.2	9.200	1.155
MD 8-4-30	5.5	5.8	4.4	4.6	5.075	0.6801
MD 10-4-30	5.2	5.8	4.1	4.6	4.925	0.7365
MD 8-6-20	5.5	7.3	4.4	4.8	5.500	1.283
MD 10-6-20	5.5	5.4	3.9	4	4.700	0.8679
MD 8-6-30	2.7	2.8	2.4	3.2	2.775	0.3304
MD 10-6-30	3	3.4	2.5	3.1	3.000	0.3742

Table 5. Rheological parameters (K and n) of emulsion containing OSA and MD combination on day 1.

	1.1		**1.2**		**1.3**		**1.4**		**2.1**	
	K	**n**	**K**	**n**	**K**	**n**	**K**	**n**	**K**	**n**
HC 8-3-20	0.009603	0.9734	0.009412	0.9758	0.009355	0.9804	0.009543	0.9750	0.08801	0.8267
HC 10-3-20	0.2475	0.7018	0.2176	0.7219	0.2817	0.6688	0.2133	0.7249	0.3294	0.6722
HC 8-3-30	0.1563	0.8455	0.1528	0.8530	0.1476	0.8591	0.1437	0.8622	0.1327	0.8456
HC 10-3-30	0.2813	0.8063	0.3147	0.8046	0.3005	0.8117	0.2985	0.8118	0.2943	0.8143
HC 8-4-20	0.08432	0.7940	0.0951	0.7872	0.08441	0.8060	0.07936	0.8186	0.1134	0.7865
HC 10-4-20	0.2873	0.6944	0.2688	0.7055	0.2572	0.7135	0.2385	0.7262	0.3534	0.6745
HC 8-4-30	0.3406	0.7896	0.3162	0.8003	0.2926	0.8033	0.327	0.7967	0.4025	0.7597
HC 10-4-30	0.5194	0.7743	0.515	0.7722	0.5046	0.7737	0.4947	0.7782	0.6510	0.7218
HC 8-5-20	0.1738	0.7381	0.1732	0.7458	0.1624	0.7571	0.1475	0.7695	0.1762	0.7463
HC 10-5-20	0.08535	0.8043	0.08164	0.8130	0.07933	0.8162	0.07368	0.8276	0.3961	0.6639
HC 8-5-30	0.5516	0.7437	0.6025	0.7322	0.5864	0.7358	0.5516	0.7437	0.9680	0.6443
HC 10-5-30	0.5681	0.7287	0.5645	0.7283	0.5599	0.7290	0.5560	0.7293	1.069	0.6851
	2.2		**2.3**		**2.4**					
	K	**n**	**K**	**n**	**K**	**n**	**mean K**	**sd K**	**mean n**	**sd n**
HC 8-3-20	0.09403	0.8285	0.09127	0.8389	0.09152	0.8415	0.05034	0.04372	0.9050	0.07621
HC 10-3-20	0.2860	0.6914	0.1834	0.7517	0.1746	0.7548	0.2417	0.05422	0.7109	0.03304
HC 8-3-30	0.1279	0.8489	0.1334	0.8418	0.1227	0.8555	0.1396	0.01220	0.8515	0.007193
HC 10-3-30	0.3746	0.7958	0.3911	0.7886	0.3836	0.7929	0.3298	0.04526	0.8033	0.009673
HC 8-4-20	0.1221	0.7741	0.1149	0.7841	0.1159	0.7827	0.1012	0.01720	0.7917	0.01429
HC 10-4-20	0.2110	0.7424	0.2019	0.7500	0.2022	0.7473	0.2525	0.05167	0.7192	0.02715
HC 8-4-30	0.4273	0.7547	0.4410	0.7481	0.4396	0.7511	0.3734	0.06063	0.7754	0.02410
HC 10-4-30	0.5931	0.7452	0.5906	0.7435	0.5828	0.7475	0.5564	0.05572	0.7571	0.02039
HC 8-5-20	0.1812	0.7431	0.1799	0.7438	0.1740	0.7492	0.1710	0.01107	0.7491	0.009885
HC 10-5-20	0.3986	0.6622	0.3763	0.6725	0.3741	0.6717	0.2331	0.1640	0.7414	0.07928
HC 8-5-30	0.9578	0.6435	0.7991	0.6741	0.8151	0.6696	0.7290	0.1777	0.6984	0.04473
HC 10-5-30	1.028	0.6937	1.050	0.6900	1.048	0.6904	0.8054	0.2604	0.7093	0.02099

Table 6. Rheological parameters (K and n) of emulsion containing OSA and MD combination on day 1.

	1.1		**1.2**		**1.3**		**2.1**		**2.2**	
	K	**n**	**K**	**n**	**K**	**n**	**K**	**n**	**K**	**n**
MD 8-4-20	0.006503	1.026	0.006528	1.028	0.006508	1.029	0.006579	1.025	0.006685	1.007
MD 10-4-20	0.01232	1.006	0.0119	1.010	0.01188	1.008	0.01196	0.9873	0.01222	0.9851
MD 8-4-30	0.03068	0.9956	0.02952	0.9979	0.03699	0.9513	0.03607	0.9630	0.03535	0.9650
MD 10-4-30	0.03632	0.9895	0.03563	0.9905	0.03420	0.9965	0.02986	0.9714	0.02904	0.9909
MD 8-6-20	0.01437	1.006	0.01382	1.010	0.01373	1.010	0.01302	0.9926	0.01291	1.005
MD 10-6-20	0.01501	1.006	0.01483	1.007	0.01455	1.007	0.01483	1.005	0.01470	1.005
MD 8-6-30	0.04950	0.9910	0.04778	0.9950	0.04762	0.9934	0.03702	0.9646	0.03462	0.9760
MD 10-6-30	0.05245	0.9854	0.05144	0.9860	0.05128	0.09854	0.04347	0.9694	0.04146	0.9884

	2.2		**2.3**		**2.4**					
	K	**n**	**K**	**n**	**K**	**n**	**mean K**	**sd K**	**mean n**	**sd n**
MD 8-4-20	0.006685	1.007	0.006315	1.016	0.006447	1.013	0.006509	0.000114	1.021	0.008541
MD 10-4-20	0.01222	0.9851	0.01211	0.9869	0.01164	1.004	0.01200	0.00023	0.9982	0.01116
MD 8-4-30	0.03535	0.9650	0.03601	0.9644	0.03699	0.9614	0.03452	0.003089	0.9712	0.01804
MD 10-4-30	0.02904	0.9909	0.03087	0.9664	0.02896	0.9759	0.03213	0.003174	0.9830	0.01157
MD 8-6-20	0.01291	1.005	0.01291	1.008			0.01346	0.000605	1.005	0.006532
MD 10-6-20	0.01470	1.005	0.01482	1.004	0.01459	1.007	0.01476	0.000159	1.006	0.001215
MD 8-6-30	0.03462	0.9760	0.03399	0.9913	0.03428	0.9817	0.04069	0.007213	0.9847	0.01121
MD 10-6-30	0.04146	0.9884	0.04306	0.9687	0.04316	0.9685	0.04662	0.004833	0.8521	0.3324

Life is full of challenges

Up and down, good days and bad days

One day you are smiling to the world, one day you are sad

To reach the peak of success, you must continue

If you fall, get up, cry, try…

But never give up.

Fatemeh (Afagh) Aghababaei

July 2023