PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
SCHOOL OF ENGINEERING

DEVELOPMENT AND CHARACTERIZATION OF MICROGELS AS FOOD MATRIX FOR ELDERLY

ALICIA MAGALY LEON TACCA

Thesis submitted to the Office of Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences

Advisor:
José Miguel Aguilera

Santiago de Chile, September, 2018

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To my children, Fernanda Micaela and José Benjamin Alfonso, who are my strength and inspiration.

To my parents, Alfonso and Josefina.

To my dear sisters Candy and Betsabe.

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LIST OF CONTENTS

TABLE INDEX

FIGURE INDEX

ABSTRACT

The amount of elderly people is growing fast and will account for approximately 20% of the world population by 2050. The aging is a process that involves physiological changes such as the difficulty to feed and nourish properly. Elderlies need special foods, which must be soft to chew, easy to swallow and capable of carrying nutrients and proteins. The aim of the present thesis was to develop and characterize food matrices in the form of gelled microparticles (GMP) produced gels of whey protein isolate and sodium alginate (WPI/NaAlg) mixtures, and prepared by size reduction of their bulk gels previously made by the cold-gelation method in sizes < 100 μm. The mechanical properties and texture profile analysis (TPA) of GMP pastes were assessed in a back extrusion cell especially designed, and image analysis were employed to resolve their sizes ranges. Olive oil microdroplets were incorporated into GMP matrix by ultrasound (US) and high-speed blending (HSB) producing emulsion-gelled microparticles (EGM). Rheological tests at 20°C and 40°C showed that US-EGM and HSB-EGM exhibited a predominant elastic behavior, with G'>G" throughout all the frequency range evaluated. US-EGM pastes were quite stable when heated to 60°C. Additionally, five different sources of dietary fiber (DF) were incorporated into the GMP matrix, and their microstructural characteristics as well as the mechanical and rheological properties, were tested. Addition of soluble DF did not change the GMP matrix aggregated. Rheological tests at 20°C revealed that GMP with added DF exhibited a predominantly elastic behavior. TPA suggested that GMP with soluble DF have advantages over commercial thickeners in terms of adhesiveness and cohesiveness. Consequently, GMP are an alternative to complement elderly feed as carriers of bioactive compound and food texture modifiers. Sensory analysis of GMP and its applications in real foods and beverages will complement the results obtained until now.

RESUMEN

La cantidad de personas de tercera edad está creciendo rápidamente y representará aproximadamente el 20% de la población mundial para el año 2050. El envejecimiento es un proceso que implica cambios fisiológicos tales como la dificultad para alimentarse y nutrirse adecuadamente. Los ancianos necesitan alimentos especiales, suaves para masticar, fáciles de tragar y capaces de transportar nutrientes y proteínas. El objetivo de la presente tesis fue desarrollar y caracterizar matrices alimentarias en forma de micropartículas gelificadas (GMP) producidas a partir de la trituración de geles preparados previamente por gelificación en frío, a partir de mezclas de aislado proteico de suero de leche y alginato de sodio (WPI/NaAlg) en tamaños < a 100 μm. Las propiedades mecánicas de GMP y el análisis del perfil de texturas (TPA) se evaluaron en una celda de *back extrusion* especialmente diseñada y se empleó análisis de imágenes para determinar el rango de tamaño de las micropartículas. Se incorporó microgotas de aceite de oliva en la matriz de GMP utilizando ultrasonido (US) y ultraturrax (HSB) obteniéndose micropartículas gelificadas en emulsión (EGM). Las pruebas reológicas a 20 °C y 40 °C indican que los EGM producidos por US y HSB presentan un comportamiento elástico predominante, con valores de G' > G" a lo largo de todo el rango de frecuencias evaluado. Las pastas de US-EGM resultaron ser estables a temperaturas de 60 °C. Adicionalmente, se incorporó cinco diferentes fuentes de fibra dietética (DF) en la matriz de GMP y se evaluaron sus características microestructurales y propiedades mecánicas y reológicas. La adición de DF soluble no cambia la matriz de GMP. Las pruebas reológicas a 20 °C revelaron que GMP con adición de DF exhiben un comportamiento predominantemente elástico. El TPA sugiere que los GMP con DF insoluble tienen ventajas sobre los espesantes comerciales en términos de adhesividad y cohesión. En consecuencia, las matrices de GMP son una alternativa para la alimentación de las personas de tercera edad como transportadores de diferentes compuesto bioactivos y modificadores de textura. El análisis sensorial de los GMP y su empleo en alimentos y bebidas habituales complementarán los resultados obtenidos en este trabajo.

LIST OF PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals.

I. Leon, A. M., Medina, W. T., J Park, D. J., & Aguilera, J. M. (2016). Mechanical properties of whey protein/Na alginate gel micrcoparticles. *Journal of Food Engineering*, 188, 1-7.

II. Leon, A. M., Medina, W. T., J Park, D. J., & Aguilera, J. M. (2018). Properties of microparticles from a whey protein isolate/alginate emulsion gel. *Food Science and Technology International*. 24(5), 414-423

III. Leon, A. M., J Park, D. J., & Aguilera, J. M. (2018). Mechanical, rheological and structural properties of fiber-containing microgels. *Carbohydrate Polymers*. (Submitted)

1. INTRODUCTION

The growing rate of elderly population around the world is an actual demographical phenomenon in our society. The aging process entails many physiological changes that affect older people's ability to feed and nourish properly when consuming conventional foods. Among the main causes are chewing difficulties, swallowing disorders and gastrointestinal complications. Reducing and minimizing these causes and improving the nutritional requirements of the elderly, designing new food matrices with modified textures that are able to transport key components, have become a challenge for food scientists and technologists. Thus, the fundamental hypothesis for this work was to propose that gel microparticles (GMP) made with different proportions of whey protein (WPI) and sodium alginate (NaAlg), gelled by cold gelation and by incorporating oil and fiber to their microstructure, constitute a food matrix that has a range of mechanical, rheological and structural properties suitable for elderly feeding.

Consequently, the main objective of the thesis was to develop and characterize food matrices of WPI and NaAlg in the form of GMP that would have applications in the feeding of the elderly and, at the same time, would serve as carriers of the needed flavors and nutrients. In order to fulfill this purpose, the specific objectives were:

- To produce and physically characterize soft gel microparticles from WPI and NaAlg mixed solutions gelled at a low temperature (cold gelation), exhibiting a wide range of mechanical properties (Paper I).

- To produce and characterize emulsion-gelled microparticles (EGM) having olive oil microdroplets embedded in a gel matrix of WPI/NaAlg, in the form of an emulsion produced by ultrasound (US) and high-speed blending (HSB) (Paper II).

- To assess the incorporation of different sources of dietary fiber (DF) into gel microparticles and evaluate their microstructural characteristics as well as the mechanical and rheological properties of the resulting gels and pastes (Paper III).

1.1 The Elderly Demography around the World and by Region

The elderly population is defined as people aged 65 and over or 60 years (Rothenberg & Wendin, 2015). Nowadays, elderly are being very careful with their life style, consequently their life expectancy is increasing. Among the factors involved in the decrease of the overall mortality are: health status and well-being habits (He, Goodkind, & Kowal, 2016).

The World Health Organization estimates that by 2050, the elderly will constitute approximately 28% of the total world population (WHO, 2014) (Figure 1.1).

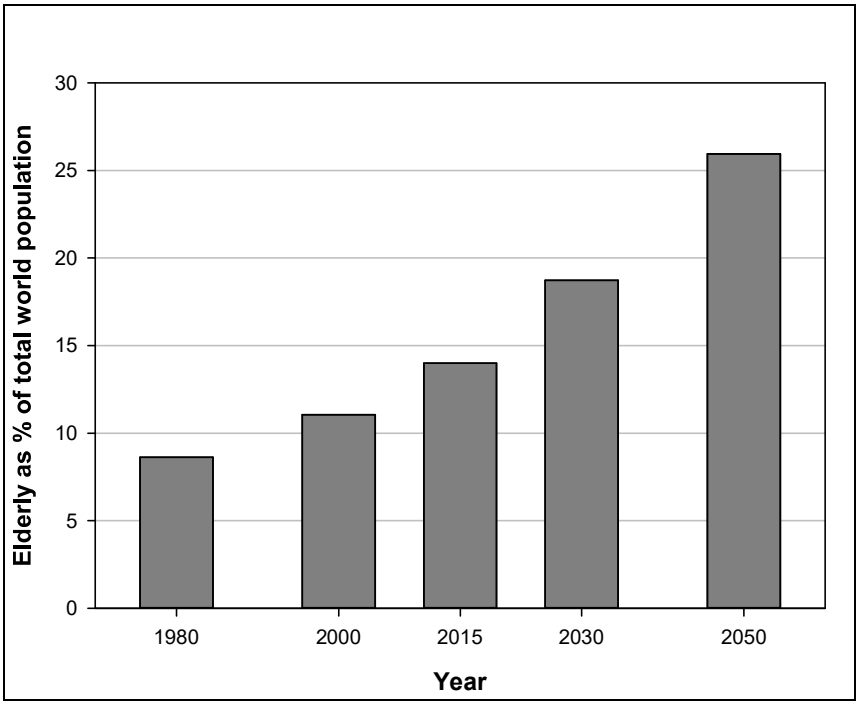

Figure 1.1. Percent of elderly people in relation to total world population between 1980 and 2015 and projection between 2030 and 2050 (United Nations, 2017, 2018).

Table 1.1 shows the increasing world population aged 60 and over, by region, between 2017 and 2050. Africa will be the region with the highest growth in the number of elderly people, where it is projected to increase more than threefold between 2017 and 2050; Latin America and the Caribbean is the region where the older population is projected to increase more than twofold between 2017 and 2050. Asia is also expected to undergo a twofold increase in the number of older persons, while Oceania, Northern America and Europe are expected to grow most slowly, around 1.6 times on average (United Nations, 2017).

Table 1.1. Elderly people in 2017 and projection by 2050 by world region (in millions of persons) (United Nations, 2017)

Region	2017	2050	Growth Factor
Africa	69	226	~ 3.3
Latin America and the Caribbean	76	198	~ 2.6
Asia	549	1273	~ 2.3
Oceania	7	13	~ 1.9
Northern America	78	123	~ 1.6
Europe	183	247	~ 1.3

As seen, the special nutrition requirements and foods aimed at this segment of the world population are a unique opportunity for the food industry.

1.2 Main Physiological and Pathophysiological Changes related to Aging

The people's aging process involves changes in all physiological systems that decline both their capacities and normal functions. Among these changes, we can point out the following: chewing capacity, swallowing difficulties and digestive problems, which affect their dietary habits and nutritional requirements, by further influencing the loss of muscle mass, loss of body water, loss of fat-free mass and bone mass phenomena.

1.2.1 Body Composition and Nutritional Status

The body composition offers important information about the person's nutritional status. The age-related body composition change starts in early middle age, and it is represented by a continuous loss of body water, bone content, fat-free mass, skeleton muscle mass and function, and a body fat increase, mainly visceral. (Rothenberg & Wendin, 2015). For example, sarcopenia is a chronic disease that is a progressive and irreversible reduction of muscle mass and strength. (Amarya, Singh, & Sabharwal, 2015; Rothenberg & Wendin, 2015).

Food protein plays an important role in elderly health and well-being (Shang, Meram, Bandara, & Wu, 2018). Hydrolyzed protein supplements (e.g., casein and whey protein hydrolysates) were employed, showing a better assimilation than their natural counterparts and less age-related losses of muscle mass (Pennings et al., 2011; Pennings et al., 2012), which offers the opportunity to do research about the use of other sources of protein matrices. GMP constitute an excellent alternative as source of protein (Paper I).

1.2.2 Chewing and Swallowing Disorders

During old age, the loss of teeth (edentulism), and consequently a reduced chewing efficiency, are due to dental problems in the elderly. This fact has a negative effect on their food intake, and makes it difficult for them to chew food properly (Amarya et al., 2015), particularly fruit, vegetables and meats, with subsequent malnutrition (Abd-El-Gawad & Rasheedy, 2016).

Among other age-related changes in the oral cavity are swallowing disorders (or dysphagia). Dysphagia is defined as the difficulty in swallowing or moving foods and liquids from the mouth to the stomach (Khan & Carmona, 2014). Dysphagia may result

in serious health complications including dehydration, important malnutrition risk, airway obstruction, aspiration pneumonia (infectious process) or pneumonitis (chemical injury caused by the inhalation of sterile gastric contents) (Sura, Madhavan, Carnaby, & Crary, 2012). Texture-modified foods are recommended for the elderly who have chewing or swallowing problems (Goldman, McKay, Mojet, & Kremer, 2014).

1.2.3 Gastrointestinal Disorders

Multiple physiological and pathological changes in the gastrointestinal tract interfere with food intake (Abd-El-Gawad & Rasheedy, 2016). Gastrointestinal disorders such as constipation, bloating, flatulence and abdominal pain are common physiological changes in older people (Valentini, Kahl, & Lindenau, 2016). These changes occur predominantly in persons over 80 years old and are rare in older people below 80 years (Lee et al., 2007).

Increasing DF along with the use of probiotic or prebiotic supplements or functional foods, have been suggested to improve digestion, relieve constipation and improve the immune system in older people (Dror, 2003). GMP has the capacity to incorporate soluble and insoluble dietary fiber into its microstructure (Paper III).

1.2.4 Gradual Loss in Chemosensory Perception and Appetite

With aging, another important change is the deterioration of the sensory system. The alterations on the perceptions of smell and taste decrease the enjoyment of food and alter their dietary habits (Song, Giacalone, Bølling Johansen, Frøst, & Bredie, 2016; Valentini et al., 2016). Consequently, foods for the elderly should be flavorsome and attractive, and their loss of flavor sensitivity should be compensated by adding extra tastants and odorants (Aguilera & Park, 2016). The senses of taste and smell are less

sharp among older people, which interfere with their appetite in relation to many foods (Amarya et al., 2015).

1.3 Nutritional Requirements

The main nutritional requirements for the elderly are proteins, carbohydrates, fats, dietary fiber, minerals such as calcium, and vitamin D. Table 1.2 summarizes the recommended nutritional requirements for the elderly.

1.4 Food Development for the Elderly

Eating is a vital and pleasurable process for everybody, but the aging population can show more serious complications, mainly due to the physiological and pathophysiological changes related to aging (Gettings, 2009). Aguilera and Park (2016) indicate that the main factors to be considered in the development of healthy foods for the elderly are: mastication and swallowing dysfunctions, loss of muscular body mass and bone mass, gradual loss in sensorial perception and appetite, and other specific nutritional needs.

Japan has developed different types of foods with special diets for the elderly. Representative foods are semi-solidified (either thickened or gelled) enteral nutrition, water-supply jelly, and nutrition-supply jelly as a ready-to-eat product, and instant gelling agent for pasted/pureed foods and dysphagia thickener as a dry mix powder working at a relatively low addition level in existing foods. The terms ‘jelly’, ‘gelling’ and ‘thickener’ all relate directly to the functions of polysaccharides (Funami, 2016).

Table 1.2. Recommended Nutritional Requirements for the Elderly

Nutrient	Recommendation	Products	References
Protein	1.0-1.2 g/kg for healthy older people; ≥1.2 g/kg for active and exercising older adults; 1.2-1.5 g/kg for older adults who have acute or chronic disease or 15–20% of total energy intake.	Milk and milk products, eggs, meat, fish, and chicken, as well as pulses and nuts	(Wham & Miller, 2016) (Amarya et al., 2015; Chernoff, 2016) Bauer et al., 2013; Pedersen and Cederholm, 2014
Fat	15% of total energy intake to ensure adequate consumption of total energy, essential fatty acids, and fat soluble vitamins a maximum of 30-35% of total energy for most individuals.	Fish oils,	(Wham & Miller, 2016)
Carbohydrates	55 - 60% of total energy intake	Whole cereals, pulses, fiber-rich fruits, and vegetables	(Amarya et al., 2015; Chernoff, 2016)
Dietary fiber	25 - 35 g day^{-1}	Fresh fruits, vegetables, legumes, and whole-grain products.	(Amarya et al., 2015; Chernoff, 2016)
Calcium and vitamin D	For vitamin D a level of 15 µg day^{-1} is recommended for those over 70 years old	Milk and milk products such as cottage cheese and curd, green leafy vegetables, and sesame seeds	(Brownie, 2009; Chernoff, 2016)

The Japan Care Food Association and Japanese Food Companies have created common standards for food hardness/smoothness, which make it convenient for older people to choose a food with a suitable texture; see Figure 1.2 (Heiniö, Pentikäinen,

Rusko, & Peura-kapanen, 2014). Figure 1.3 shows the Japanese food classification according to the hardness of texture standards.

Figure. 1.2. In Japan, foods have been classified into four categories according to the hardness of the texture. The same four texture categories are followed in foods that are served in care homes.

Classification	Standard Of Chew	Standard Of Swallow
ABLE TO CHEW EASILY	Contain hard and big ingredients, a little hard to swallow	Able to swallow commonly
ABLE TO SMASH WITH GUMS	Contain hard and big ingredients, not easy to swallow	Depends on ingredients, hard to swallowing
ABLE TO SMASH WITH TONGUE	Contain soft and small ingredients, easy to swallow	Have experience hard to swallow water or liquid ingredients
ABLE TO SWALLOW WITHOUT CHEW	Hard to swallow if contain solid food	Hard to swallow water or liquid ingredients

Figure 1.3. Japanese Food Classification according to the Hardness of their Texture Standards for Chewing and Swallowing.
https://earthink.trustpass.alibaba.com/product/145018803-106232639/Japan_Nursing_Care_Food_Diet_for_Elderly_Seniors_WAKODO.html

1.5 Texture-modified Foods (TM Foods)

Texture is an important property of food palatability and eating safety in recent aged society. The textural properties of food products should be modified regarding their rheological, colloidal and tribological aspects in order to make products friendlier for the elderly, particularly those who have trouble chewing or swallowing (Funami, 2016).

Rothenberg and Wendin (2015) indicate that the food solution for elderly people is the texture modification of conventional foods. Texture-modified foods (TM foods) is a term that refers to foods with soft textures and/or reduced particle size as well as thickened liquids (drinks) aimed at the market segment of elderly people with eating dysfunctions (Cichero, 2015).

The most common texture classifications for TM foods used in clinical practice consider: pureed foods, mashed or minced foods and soft foods (Table 1.3) (Cichero, 2015).

To further elucidate the physical and sensory meaning of these terms, objective rheological as well as analytical sensory measurements have been conducted. Results are shown in Table 1.4 (Rothenberg & Wendin, 2015).

Table 1.3. Texture Classification for TM Foods used in Clinical Practice

Classification	Required Chewing	Textural Features	Behavior during Eating	Special Features
Soft foods	Need some chewing	Soft-textured	Breaks down easily with a fork. Moist or served with sauce or gravy as appropriate to increase moisture content.	Australia and UK, size particles from 1.5 cm to 'bite-sized North America pieces'<2.5 cm.
Mashed or minced foods	Minimal chewing	Moist and soft-textured	Easily mashed with a fork. Tongue-to-palate shearing can be used to break the food down. Meats are ground or minced.	Acceptable particle sizes for these foods range from 0.2 cm to approximately 0.5 cm.
Pureed foods	No	Cohesive, homogenous in texture, smooth and moist	Eaten with a fork and the prongs of a fork make a clear pattern on the surface of a puree.	**Thin or runny puree** Cannot be piped, layered, molded, scooped, or eaten with a fork. They are best poured. **Thick puree** Can be layered, molded, scooped, or eaten with a fork.

Table 1.4. Texture Measurement: Summary of Sensory and Rheological Analyses

Category	Sensory Description	Rheological Parameter
Pâtés	Higher degree of chewing resistance and larger particles compared to timbales and jellied products.	Maximum load: 0.6–2.4 N Strain at max. load: 16–34% G': 11 000 - 20000 Pa △: 7.4 - 7.9°
Timbales	Moderate degree of chewing resistance, creaminess and wobbling. More porous, wobbly, creamy and melting than pâtés.	Maximum load: 0.5 - 0.8 N Strain at max load: 25 - 33% G': 15 000 - 17 000 Pa △: 6.6 - 7.2°
Jellied products	Wobbly, homogenized and creamy. Lower degree of chewing resistance and firmness but higher degree of melting and creaminess compared to timbales and pâtés.	Maximum load: 0.1 - 0.3 N Strain at max. load: 18 - 28% G': 800 - 16000 Pa △: 4.4 - 8.4°
Low viscosity fluids (soups)	Lower degree of chewing resistance, firmness, porosity and wobbling compared to high-viscosity fluids.	Consistence index in shear: 1.0 - 3.3 $Pa \times s^n$ In tension 120 - 520 $Pa \times s^n$ Shear thinning exponent: 0.4 - 0.5 Tension thinning exponent: 0.1 - 0.3
High viscosity fluids (thickened soups)	More melting, easier to swallow and creamy compared to low-viscosity fluids	Consistence index in shear: 7.6 - 2.0 $Pa \times s^n$ In tension 410 - 1260 $Pa \times s^n$ Shear thinning exponent: 0.3 - 0.4 Tension thinning exponent: 0.2 - 0.4

1.6 Measuring Methods for Mechanical, Viscoelastic and Textural Properties of TM Food

The main methods for measuring TM food's mechanical, viscoelastic and textural properties are: uniaxial compression, dynamic oscillatory rheometry, and both texture profile analysis (TPA) and compression-extrusion test, respectively.

1.6.1 Uniaxial Compression

The determination of compressive properties of materials requires a deformation-force curve, which is a diagram where the deformation values are plotted on the axis of the abscissa and the force values, on the axis of the ordinates. The deformation-force curve is obtained from the application of a uniaxial static load on a given sample at a certain speed of the force application. The speed of the test is chosen based on the sample's sensitivity to the loading speed (ASAE, 1991).

The uniaxial compression is a simple compression in one plane (Figure 1.4). It is the simplest and most widely used mode of testing food mechanical properties and textures. It is usually performed on a universal testing machine or texturometer. This type of test is a commonly used test principle for solid foods. The sample is compressed in one direction and is unrestrained in the other two. For a true compression test, the platen that compresses the food should be larger in diameter than the food specimen. If the platen diameter is smaller than the diameter of the food it becomes a puncture test (Bourne, 2002).

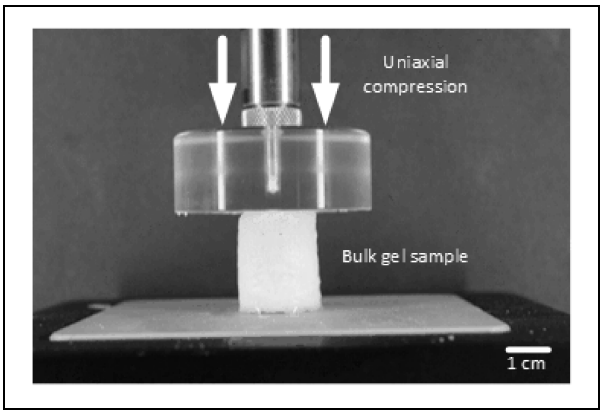

Figure 1.4. Picture of the uniaxial compression test of bulk gel of WPI/NaAlg

The conventional unit of force is Newton (N) and the deformation is expressed in distance (mm). Units obtained from force–deformation curve are: stress (σ), that is the force per unit area, expressed in Pa or N m^{-2}, and strain (γ) that is referred to the change in size or shape of a material when subjected to a stress. Strain does not have units because it is the ratio of mm to mm^{-1} and is expressed as percent (%). Figure 1.5 shows experimental data of the stress-strain curve for different concentrations of WPI and NaAlg for cylindrical bulk gels (extracted from Paper I).

1.6.2 Dynamic Oscillatory Rheometry

Dynamic oscillatory rheometry tests provide valuable information on the viscoelastic nature of materials, allow characterizing their viscoelastic behavior and measuring their degree of elasticity and viscosity (Rothenberg & Wendin, 2015; Steffe, 1996). Among others, they can be applied for studying the characteristics of gels, such as gelation and melting point (Rao, 2014) .

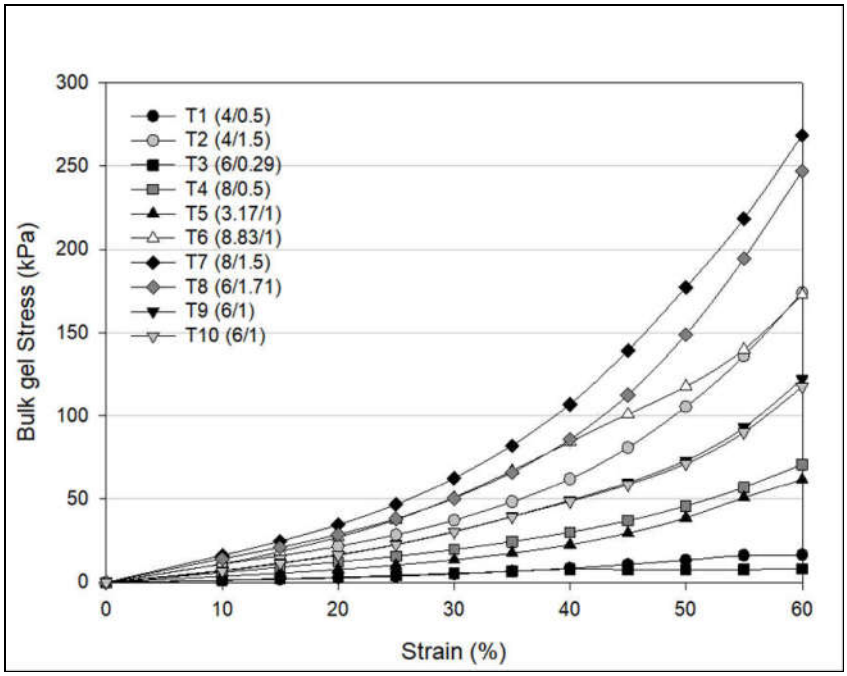

Figure 1.5. Influence of WPI/NaAlg concentration on the true surface stress–compression behavior of matrix bulk gels samples 20 mm high and 20 mm diameter tested at a speed of 1mm s^{-1} (Leon, et al., 2016).

During the dynamic oscillatory rheometry, a layer of test material is mechanically oscillated and the transmitted force is measured as a function of the displacement. Figure 1.6 illustrates the key parameters that define a wave in an oscillation. The amplitude is a measure of the degree of deformation and the wavelength defines the duration of the wave. The frequency is the number of wavelengths per second and it is a measure of the oscillation speed (Rosenthal, 2015).

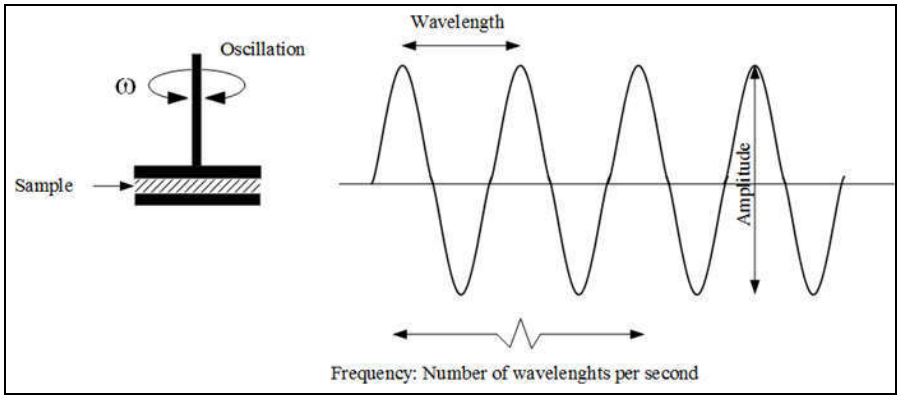

Figure 1.6. Key Parameters that Define a Wave in an Oscillation (Rosenthal, 2015)

Initially, in the dynamic oscillatory rheometry it is normal to explore the stability of a material over a range of amplitudes with an amplitude sweep. This allows identifying the range over which the material behaves in a linear viscoelastic manner (Figure 1.7A). Following the amplitude sweep, a frequency sweep is normally undertaken within the linear viscoelastic region. The frequency sweep shows how the viscous and elastic behavior of the material changes with the strain or stress application rate (Figure 1.7B). Another function used to describe viscoelastic behavior is the tangent of the phase shift or phase angle (called tan delta) (Figure 1.7C), which is also a function of frequency (Steffe, 1996).

$$\tan(\delta) = \frac{G''}{G'}$$

Where: G'' is the loss modulus or viscous; G′ is the storage modulus or elastic

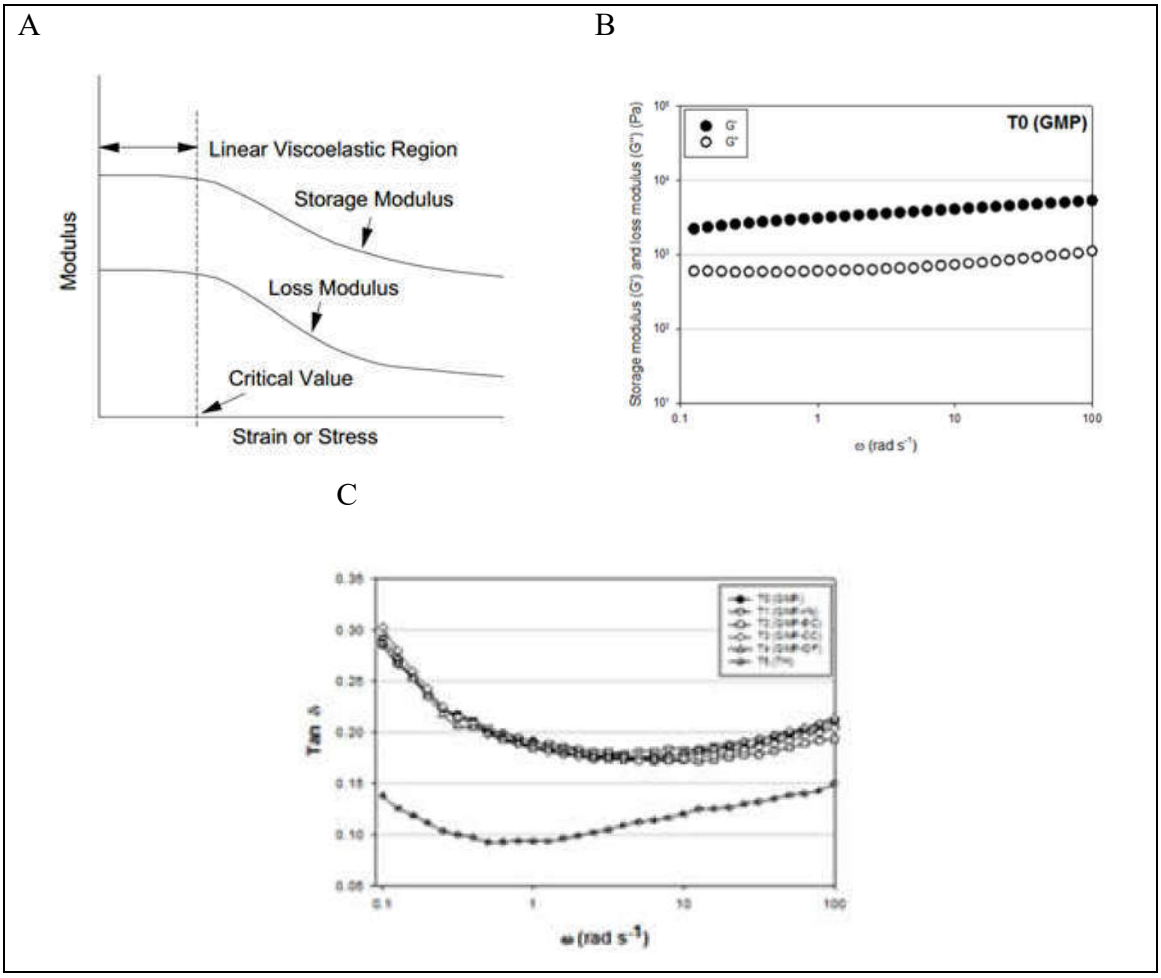

Figure 1.7. (A) Typical response to a strain or stress sweep showing the linear viscoelastic region defined by the critical value of the sweep parameter. **(B)** Mechanical spectra (G' and G'') as a function of angular frequency of GMP. **(C)** Tangent (δ) as a function of angular frequency for GMP and GMP with different dietary fiber pastes. (Figures 1.6 B and 1.6 C were extracted from Paper III).

1.6.3 Two-cycle Penetration Test

The two-cycle penetration test (TCPT) is a two-cycle compression test that is intended to mimic the first two bites during mastication (Rosenthal, 2015). Figure 1.8 shows a typical TCPT curve for GMP and GMP-Dietary fiber pastes generated in a

Texturometer TA.XT plus Texture Analyzer. The parameters derived from this curve are: hardness, cohesiveness, resilience and adhesiveness.

The height of the force peak on the first compression cycle (first bite) was defined as hardness; the ratio of the positive force areas under the first and second compressions ((A4+A5) / (A1+A2)) was defined as cohesiveness. The negative force area of the first bite (A3) represented the work needed to pull the compressing plunger away from the sample and was defined as adhesiveness (Bourne, 2004). Resilience is calculated by dividing the upstroke energy of the first compression by the down stroke energy of the first compression A2/A1. Resilience is how well a product "fights to regain its original height" (Texture Technologies Corp. and Stable Micro Systems, 2018).

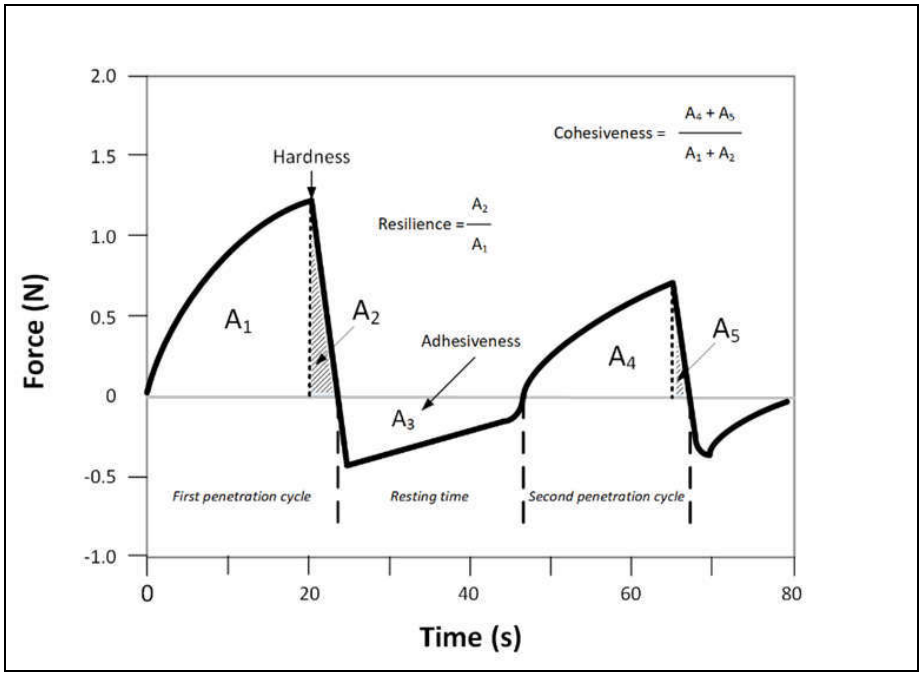

Figure 1.8. Scheme of the two-cycle penetration test (TCPT) to GMP and GMP-DF pastes and the starch thickener (Extracted from Paper III).

1.6.4 Compression-extrusion Test

The compression–extrusion test consists in forcing a loose-fitting plunger down into an extrusion cell containing the food of interest. This type of test is used on viscous liquids, gels, fats, and fresh and processed fruits and vegetables. Since extrusion requires that, the food flows under pressure. Figure 1.9 shows the process of the test. Figure 1.9A shows a cell with an open top. A loose-fitting plunger is then forced down into the cell (Figure 1.9B), and then the food flows up through the space between the plunger and the cell walls (Figure 9C); this space is called annulus (Bourne, 2004; Rosenthal, 2015; Steffe, 1996). The experimental back extrusion cell employed during the thesis research is shown in Figure 1.10.

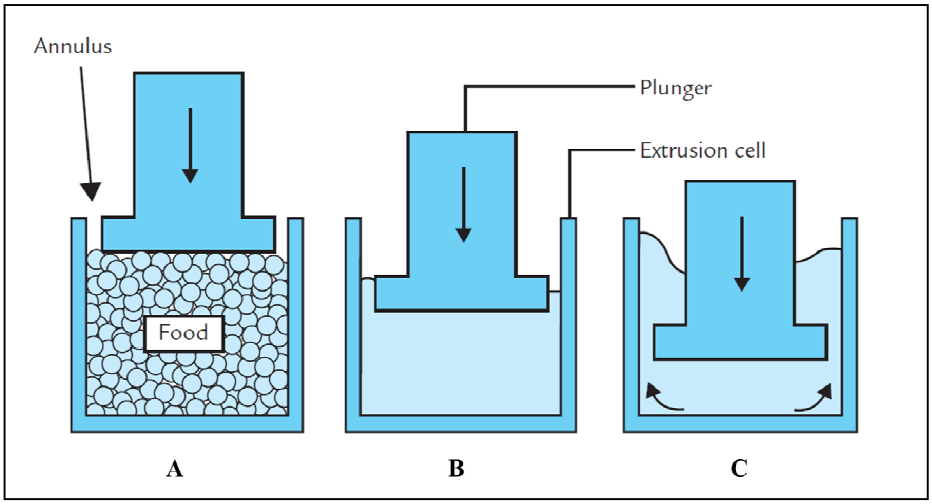

Figure 1.9. Schematic diagram of a simple cell for back extrusion tests. **(A)** The plunger goes down and starts touching the surface of the food; **(B)** The food is packed down and some liquid may be squeezed out; **(C)** The food is extruded through the annulus (Bourne, 2004).

Figure 1.10. Experimental back extrusion cell with GMP-FD paste sample

1.7 Role of Gel Microparticles in the Development of TM Foods

Nowadays, thickeners such as starch and gum play an important role in TM food development to reduce dysphagia symptoms, since they slow down the flow of liquids during swallowing and avoids their aspiration through the airway (Cichero et al., 2013; Zargaraan, Rastmanesh, Fadavi, Zayeri, & Mohammadifar, 2013). Gel microparticles constitute an interesting alternative, because they are 'soft' microscopic cross-linked polymeric particles that allow both rheology and texture control, as well as encapsulation and targeted delivery in the design of TM foods (Joye & McClements, 2014; Shewan & Stokes, 2013).

1.7.1 Materials for Producing Microgels

Microgels can be produced from a range of different ingredients, including biopolymers, lipids, surfactants, and minerals (Joye & McClements, 2014). Proteins, polysaccharides and lipids are the basic building-blocks for the design of most TM foods (Aguilera & Park, 2016).

a) Proteins

Proteins are natural polymers that are used extensively in order to modify the mechanical properties and, subsequently, the texture of foods (Ritzoulis & Karayannakidis, 2015). Globular proteins unfold and denature when heated, increasing the viscosity of liquids (e.g., in protein drinks). On further heating they may self-assemble into nano-sized aggregates and fibrils, eventually becoming the network chains of gels (Chen & Subirade, 2006).

Whey protein is one of the most commonly used food ingredients for forming biopolymer particles. It can be gelled by heat-set gelation (heating above the thermal denaturation temperature under appropriate pH and ionic strength conditions) or by cold-set gelation. Cold-set gelation has been used to form hydrogel particles from heat-denatured whey protein solutions, using both extrusion and phase separation methods (Chen & Subirade, 2006).

Proteins are not only valued for their structural roles, but also for some essential amino acids (e.g., leucine) whose high content of hydrolysates appear to favor muscle protein synthesis during aging (Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2006).

b) Polymers

Polysaccharides are often used as a texture modifier for the preparation of special elderly diets (Funami, 2016). They are employed to thicken aqueous food dispersions, stabilize emulsions and foams, and as gelling agents (Funami, Ishihara, Nakauma, Kohyama, & Nishinari, 2012).

Alginate is a biopolymer extracted from brown seaweed, which contains 1–4 linked α-L-guluronic (G) and β -D-mannuronic (M) acid residues. This is one of the most widely used polysaccharides for developing delivery systems. Alginate solutions rapidly form gels in the presence of different divalent cations, with calcium being the most widely used (Puguan, Yu, & Kim, 2014).

1.7.2 Method Employed for Producing Microgels

There are two methods for obtaining microgels, based on two types of approaches: top-down and bottom-up methods. These approaches are widely used in the industry to form biopolymer particles (Farjami & Madadlou, 2017; Merisko-Liversidge, Liversidge, & Cooper, 2003).

a) Top-down Approach

The top-down approach generally involves the breakdown of bulk solids or liquids (or large particles) into smaller particles. Typically, these approaches rely on five types of disruptive forces to break the particles down: shear, impact, compression, shear homogenization and extrusion (Merisko-Liversidge et al., 2003).

b) Bottom-up Approach

The bottom-up approach includes: liquid antisolvent precipitation, coacervation, inclusion complexation and fluid gel formation methods. Bottom-up approaches typically allow the production of very fine particles with an improved control over particle properties, such as size, morphology and physical state (David Julian McClements, 2017; Shewan & Stokes, 2013)

The method employed in the thesis is the principle of top-down approach and the scheme is shown in Figure 1.11.

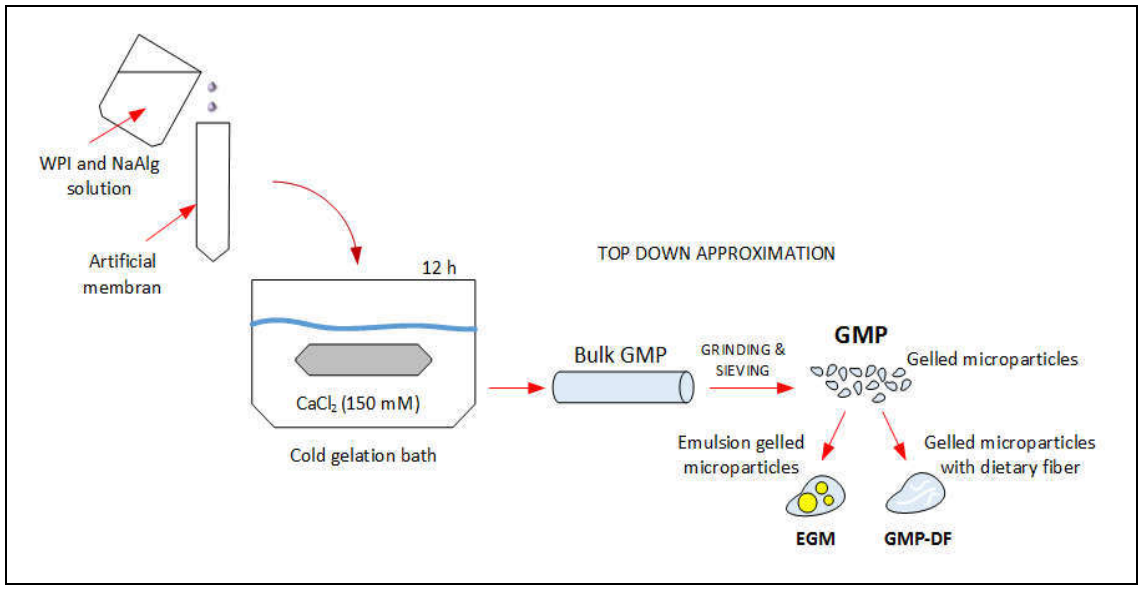

Figure 1.11. Experimental scheme employed to obtain gelled microparticles (GMP) according to the top-down approach method (Joye & McClements, 2014)

2. MECHANICAL PROPERTIES OF WHEY PROTEIN AND ALGINATE GEL MICROPARTICLES

2.1 Introduction

Ageing of our society is a phenomenon occurring at a fast pace in nearly all countries: old people are today the fastest growing demographic segment of the population. Worldwide, by 2020 more than 700 million people will have over 65 years of age and by 2045 life expectancy will reach 76 years. It is likely that by 2050 around 400 million people will be aged 80 years and over (United Nations, 2015). Amazingly, the potential market for food products aimed at senior consumers has not been given an adequate attention by the food industry (IUFoST, 2014). The ageing phenomenon imposes a challenge of feeding the elderly consumer and satisfying his/her special requirements (Bernstein & Schmidt Luggen, 2010; Costa & Jongen, 2010; Raats, De Groot, & van Asselt, 2008). For instance, a substantial number of old seniors suffer from poor mastication and swallowing dysfunctions (dysphagia). Thus, they have to be fed with "soft diets" which include mashed or pureed foods, mousses and jellies, as well as thin and thickened liquids (Cichero, 2015). This market for "texture-modified" foods for the elderly opens new opportunities to develop foods with small and soft particles that do not need chewing and can be swallowed safely (Loret, 2015).

Gels are soft solids which immobilize large amounts of water (e.g., >90%) within a biopolymer network, whose microstructure may be adjusted within a wide range of physical properties and textures (Aguilera, 1995). Food gels are usually made from proteins, polysaccharides or their combinations (de Jongh, 2007; Morris, 2007). Whey proteins are largely globular proteins present in the serum fraction of milk exhibiting several advantageous physicochemical and nutritional properties as a food ingredient (Jovanović, Barać, & Maćej, 2005). Solutions of whey protein isolate (WPI) above a

critical concentration form gels when heated over 70°C for prolonged time (Aguilera, 1995), or at low temperature by a cold-setting mechanism. In this latter case after an initial heating step to form protein aggregates, gelation occurs at ambient temperature, usually adding calcium ions (Barbut & Foegeding, 1993). Alginate belongs to a family of linear copolymers of (1→4)-linked b-D-mannuronic acid (M) and a-L guluronic acid (G) residues, with M and G residues present in varying proportions and sequences depending on the alginic acid source. Alginate is an excellent gelling agent among hydrocolloids because it gels easily with calcium ions at room temperature (Belitz, Grosch, & Schieberle, 2004). Hence, mixed solutions of WPI and sodium alginate (NaAlg) are ideal starting materials to structure soft gels since both ingredients undergo gelation with calcium at room temperature.

Food-grade gel microparticles are small-sized gels usually formed by proteins or polysaccharides and their combinations. They are finding increasing application in foods due to their unique physicochemical characteristics and functional attributes (Joye & McClements, 2014; Li & Ngai, 2015). The subject of manufacturing food-grade gel microparticles has been reviewed by Sağlam, Venema, van der Linden, and de Vries (2014) and Shewan and Stokes (2013). Basically, a biopolymer solution is shaped into microparticles by various techniques (emulsification, spraying, coacervation, droplet break-up, microfluidics, etc.) followed by a gelation step. Alternatively, small gel particles may be generated by mechanical comminution of bulk (macro) gels. Modern gastronomy has exploited the concept to form “artificial caviars” or small spheres of Ca-gelled alginate loaded with many flavors (Vega & Castells, 2012). Gelled microparticles of whey proteins have been used to immobilize a variety of bioactives and drugs (Beaulieu, Savoie, Paquin, & Subirade, 2002; T. Egan, O’Riordan, O’Sullivan, & Jacquier, 2014; Oh, Lee, & Park, 2008) and to encapsulate lipids and fat-soluble vitamins (Abbasi, Emam-Djomeh, Mousavi, & Davoodi, 2014 ; Thelma Egan, Jacquier, Rosenberg, & Rosenberg, 2013). Spherical whey protein microgels (100 nm - 1 mm in size) with different internal microstructures may be formed under controlled conditions

of concentration, pH, temperature, heating time and ionic environment (Nicolai, 2016). Pertinent to this work, gel beads made of WPI and alginate have been proposed to immobilize nutraceuticals (Chen, Remondetto, & Subirade, 2006) and as carriers for vitamin B2 added to a model beverage (Wichchukit, Oztop, McCarthy, & McCarthy, 2013). In this latter example, beads (2.6-2.8 mm in diam.) were formed by dropping the biopolymer solution into an agitated CaCl2 bath using a syringe.

Mechanical breakdown is a common technique to produce small-particle soft gels. Among many apparatus to effect size reduction the Ultra-Turrax benchtop homogenizer has been widely utilized because it yields gel particles in an ample range of sizes, i.e., 20-200 mm (Shimojo, Pires, de la Torre, & Santana, 2013). Capela, Hay, and Shah (2007) obtained calcium alginate beads around 40 mm and a high survival rate of microencapsulated microorganisms when using this equipment. Even a simple food processor has been used to make calcium alginate microparticles of diameter around 200 μm (Yu, Jia, Cheng, Zhang, & Zhuo, 2010).

The objective of this work was to fabricate and physically characterize soft gel micro-particles from WPI and NaAlg mixed solutions gelled at a low temperature (cold gelation), exhibiting a wide range of mechanical properties. These soft particles could be used as texture-modified foods for the elderly (e.g., caviar-type products), added to provide consistency to fluid foods (juices, milks) or mixed into traditional foods (e.g., soups, purees, sauces, etc.).

2.2 Materials and methods

2.2.1 Materials

Whey protein isolate (WPI) with a moisture content of 4.7% ± 0.3 and protein content of 97.7% ± 0.7 (d.b.) was purchased from BIPRO (Davisco Foods Intl., MN, USA). The pH of a WPI dispersion in distilled water was 7.0 ± 0.3. Sodium alginate (NaAlg) was obtained from Gelymar Natural Extracts (Chile) with a composition of 38% mannuronic acid, 16% guluronic acid and 46% alternate units. Artificial membranes VISCOFAN of corrugated cellulose with a caliber of 20 mm diameter and 30-60 μm of thickness were acquired from Filter Print Ltda. (Santiago, Chile). Calcium chloride was from Sigma Chemical Co. (St. Louis, MO, USA).

2.2.2 Preparation of gel microparticles (GMP)

The composition and pH of WPI/NaAlg solutions are reported in Table 2.1. WPI/NaAlg powders were dissolved in distilled water at room temperature under mild agitation avoiding foam formation and left at 5°C overnight for proper hydration. WPI/NaAlg mixed solutions were placed in the artificial membranes, sealed on both sides and immersed in water at 80°C for 30 min to denature and aggregate the whey proteins. Then, the contents were cooled to room temperature overnight. Suspensions of aggregated WPI/NaAlg in artificial membranes were next immersed in a bath of 200 mM calcium chloride for 12 hours at 5°C in order to gel the WPI and NaAlg. Gel microparticles (GMP) of WPI/NaAlg mixtures were obtained by mechanical size reduction of the respective bulk gels, first by crushing for one minute in a mini pimer and then by homogenization with a high-speed blender (Ultra Turrax digital T25, IKA-Werke, Germany) at a speed of 10,000 rpm for 3 minutes, until a uniform paste was obtained. Large particles were removed with a sieve (0.5 mm openings) from a suspension with distilled water. The filtered particle dispersion was centrifuged at 6,000 rpm for 5 minutes to remove the excess liquid and obtain a paste of GMP.

Table 2.1. Composition and properties of gelled microparticles (GMP) and bulk gels

Treatment	WPI (%)	NaAlg (%)	pH	Bulk gels Stress (kPa) @ 30% strain	GMP Stress (kPa) @ 30% strain	GMP Equivalent diameter (μm)
T1	4.00	0.50	6.97	5.1 ± 0.3	169.6 ± 15	15.8 ± 17.8
T2	4.00	1.50	6.90	37.4 ± 0.5	1014.7 ± 854	12.4 ± 4.1
T3	6.00	0.29	6.99	5.5 ± 0.3	459.8 ± 140	16.8 ± 13.7
T4	8.00	0.50	7.03	19.9 ± 0.9	476.3 ± 57	22.1 ± 17.7
T5	3.17	1.00	7.00	13.7 ± 0.6	694.8 ± 409	14.9 ± 6.7
T6	8.83	1.00	6.94	51.0 ± 0.6	771.3 ± 180	12.2 ± 3.5
T7	8.00	1.50	6.94	62.5 ± 2.0	914.8 ± 170	14.6 ± 6.0
T8	6.00	1.71	6.89	50.5 ± 0.2	585.2 ± 125	13.8 ± 4.5
T9	6.00	1.00	6.96	30.6 ± 3.0	1050.9 ± 75	12.2 ± 3.6
T10	6.00	1.00	6.96	30.5 ± 1.3	1239.7 ± 217	12.4 ± 3.6

2.2.3 Imaging and image analysis

GMP were observed using an optical microscope model SMZ-2T 2B (Nikon Corp., Tokyo Japan) and images were acquired with a ToupCam model UCMOS 08000 digital camera (ToupTek Photonics, Zhejiang, China) attached to the microscope. Images were first enhanced using contrast-limited adaptive histogram equalization to a pre-defined pixel distribution (Zuiderveld, 1994) and borders of particles were attenuated by means of a low-pass Butterworth frequency filter (Gonzalez & Woods, 1992). Segmentation consisted in building a binary mask to distinguish the position, area and perimeter of particles from the background using threshold segmentation and morphologic image operations (e.g., elimination of small isolated groups of pixels and the suppression of objects at image borders). Mean values and standard deviations of equivalent diameter were determined for each object (Haralick & Shapiro, 1992). Relation of pixels to size was accomplished acquiring the image of a 1000 mm

microscopy ruler under the same conditions as the samples. 10 images were processed per sample of GMP and at least 100 objects were measured per image. Equivalent diameters (D_{eq}) were calculated as:

$$D_{eq} = \sqrt{\frac{4 \times A}{\pi}}$$

Where A is the area of a microparticle in mm^2.

2.2.4 Mechanical evaluation of GMP

Mechanical properties of gels are usually assessed by uniaxial compression of cylindrical samples. Soft foods are often preferred by the elderly because they are easily compressed between the tongue and the palate avoiding mastication (Ishihara et al., 2013). A special test cell was constructed to evaluate the GMP (Figure 2.1). Samples were placed in the sample holder of the acrylic back-extrusion test cell. The moisture content of the GMP pastes was 10-18 g H20/g DM, depending on the solid content of each sample. The cylindrical hole holding the sample (3.9 mm in diameter and 25 mm in depth) was filled to the top with paste and compressed with a cylindrical metal plunger (3.75 mm in diameter) up to 60% strain. The test cell was attached to a texture analyzer model TA.XT2 Plus (Stable Micro System Ltd., Godalming, UK) and the contents compressed at a speed of 1 mm/s, after verifying a good alignment of the plunger and the hole. Mechanical properties were also determined in the bulk gels. Cylindrical samples (20 mm diameter x 20 mm height) were subjected to uniaxial compression in the same texture analyzer using a round plate 75 mm in diameter and a constant speed of 1 mm/s until reaching 60% strain. All measurements were done in triplicate. Results from mechanical tests were expressed as response surfaces of the stress at 30% strain as a function of WPI and NaAlg concentrations. This deformation value is important

because texture for timbales and jellied products for the elderly are defined at strains of 25-33% and 18-28%, respectively (Rothenberg & Wendin, 2015).

2.2.5 Experimental desing and statistical analysis

A central composite design was used to plan the experiments (Table 2.1). Runs were performed randomly and experimental data were analyzed to fit polynomial models as response surfaces of stress at 30% deformation using an analysis of variance (ANOVA). Statistical significance was determined using the Statgraphics Plus software (Statistical Graphics Corporation, version 5.1, Rockville, USA) at a probability level of 0.05 ($p<0.05$).

Principal component analysis (PCA) is a multivariate statistical technique that identifies those characteristics that vary most between individuals and uses a scoring system (the principal components) which can be treated like new types of measurements to compare populations with similar features. The PCA method was used to analyze the dataset of 24 averaged measurements obtained from the 10 treatments corresponding to bulk gels and GMP. The 24 averaged measurements are composed of 22 strain values (from 10% to 60% at 5% intervals) for bulk gels and GMP extracted from Figure 2.2, WPI and NaAlg concentration. Data were arranged in a matrix 10 by 24. The correlation matrix was used to calculate eigenvalues, loadings (eigenvectors) and the principal components (PC1 and PC2) related to the original variables. The two principal components were plotted to show and underline similar data in a scatter graph (Medina, Skurtys, & Aguilera, 2010; Mohammadi & Prassanna, 2003; Townend, 2002).

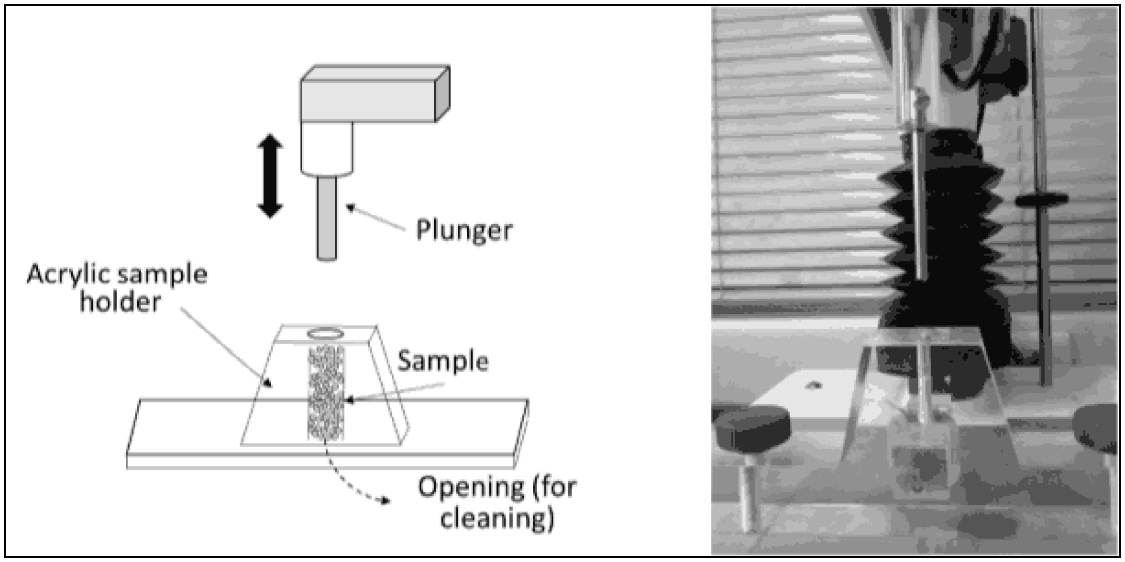

Figure 2.1. Back extrusion testing cell for the evaluation of mechanical properties of gelled microparticles (GMP). Right, picture of the device mounted in the texturemeter.

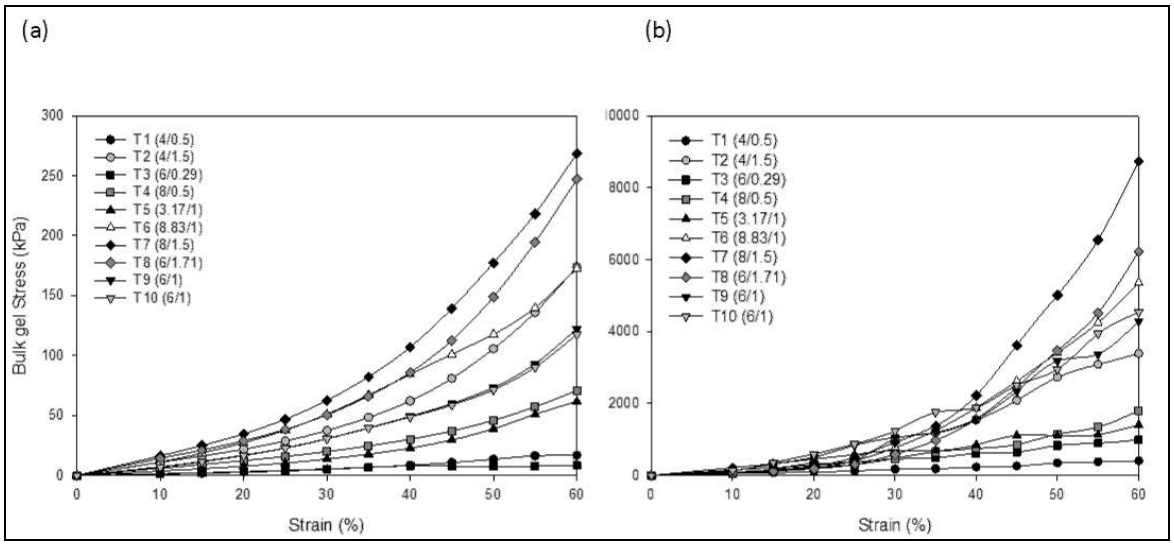

Figure 2.2. Stress-strain curves of 2-cm cylinders of bulk gels assayed by compression (a) and of GMP assayed in the back extrusion cell (b).

2.3 Results and discussion

2.3.1 Structural features of gelled microparticles

Figure 2.3 presents a gallery of photomicrographs of GMP for the different treatments. The mean equivalent diameter of GMP as determined by image analysis was between 12µm and 22 µm (Table 2.1). However, the size distribution curves (not shown) suggest that large sizes extended to 40 µm. This result is in agreement with findings of (Capela et al., 2007) who reported a mean size of alginate gel particles of 39.2 µm when using the Ultra-Turrax homogenizer at 13,500 rpm for 4 min. Larger particle dimensions can be achieved by reducing the rpm and/or the time of the homogenization process. GMP obtained for high concentrations of WPI and low concentrations of NaAlg did not have a definite structure (e.g., GMP with 6% WPI and 0.29% NaAlg). However, in the presence of high concentrations of NaAlg, GMP were more defined, homogeneous and smaller in size (e.g., 6% WPI and 1.71% NaAlg). This feature coincided with the lowest pH value (6.89) suggesting that even small variations in pH may influence the size and shape of the particles. Furthermore, an increase in size of the GMP was observed when the concentration of WPI increased (e.g., 8% WPI and 0.5% NaAlg) whose pH 7.03 was the highest recorded for all solutions.

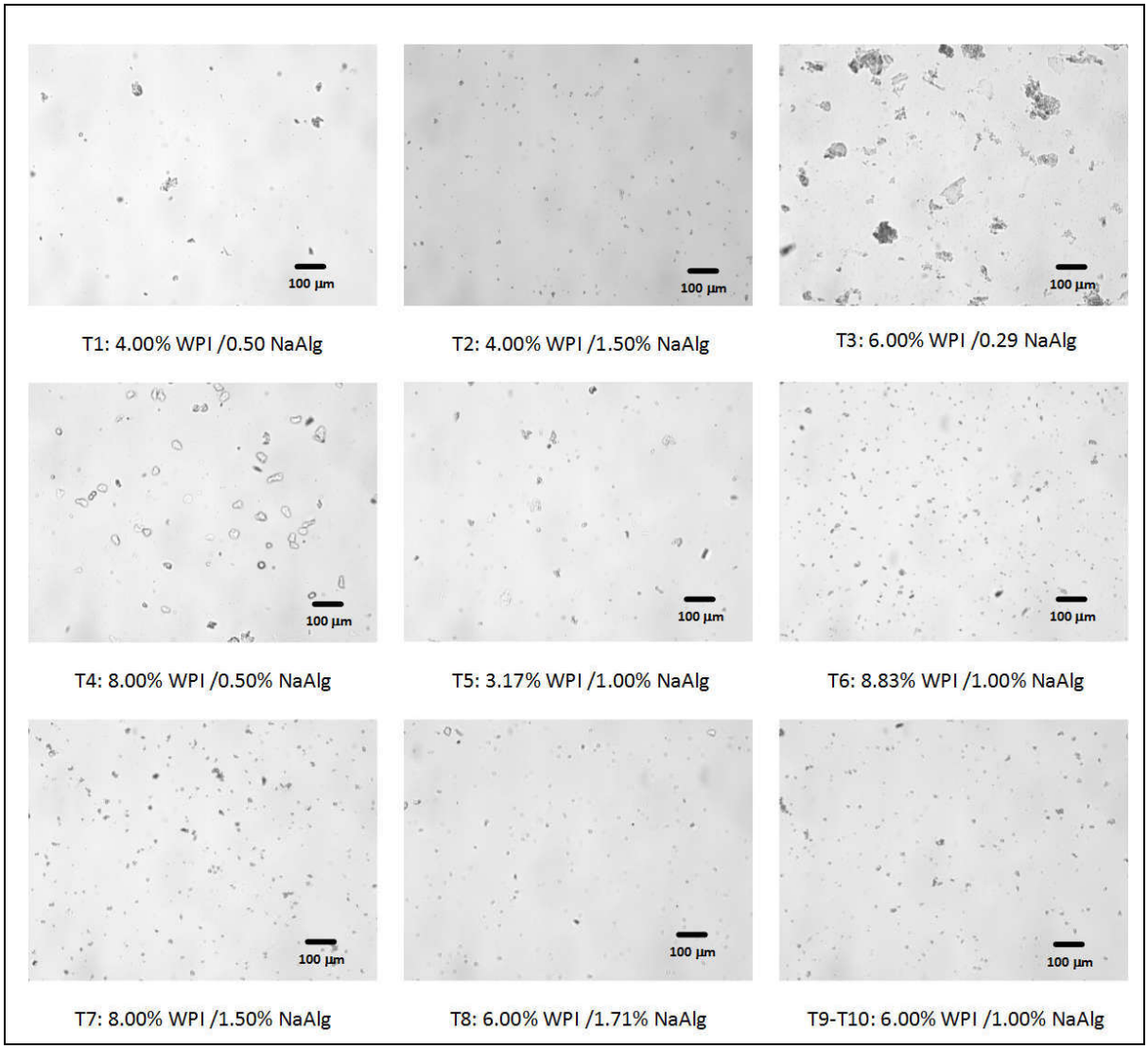

Figure 2.3. Images of gelled microparticles (GMP) prepared at different concentrations of WPI and NaAlg (marker 100 µm).

The variation in size is important if gelled microparticles are supposed to contain fillers (e.g., fiber particles or oil microdroplets). Higher values for samples with concentrations WPI high and low concentrations of NaAlg not have a definite structure (for example, GMP 6% and 0.29% WPI NaAlg). However, in the presence of high concentrations of NaAlg, GMP defined, homogeneous and smaller (for example, 6% and

1.71% WPI NaAlg). This feature coincided with the lowest pH value (6.89) suggesting that even small changes in pH can affect the size and shape of the particles. Furthermore, an increase in size of the GMP concentration was observed when WPI increased (for example, 8% and 0.5% WPI NaAlg) whose pH 7.03 was the highest recorded for all solutions. The variation in size is important if it is assumed that the gelled microparticles containing fillers (e.g., fiber particles or oil droplets). An advantage of the microparticulation technology presented here is that GMP can be adapted to almost any size by controlling the speed of the Ultraturrax and grinding time.

2.3.2 Mechanical properties of bulk gels and GMP

The pH of solutions varied in the range between 6.89 and 7.03 (Table 2.1), a small variation to not likely to induce any significant structural difference between gels (Nicolai, 2016). Bulk gels were formed by diffusion of Ca ions into the solutions contained in the artificial membranes. To assure a uniform gel formation the membranes and their contents were kept in the calcium bath for up to 72 hr, concluding that a 24-hr immersion was sufficient to achieve a homogeneous gel.

Due to the minute size of the gelled droplets (approximately 10-40 μm) a special back extrusion testing cell had to be constructed to assay the mechanical properties of a paste of GMP. Similar test cells have been constructed to evaluate the mechanical properties of small grains (Cagampang, Kirleis, & Marks, 1984; Reyes & Jindal, 1990) and slurries (Brusewitz & Yu, 1996). This testing method determines a combination of mechanical properties including elastic behavior, rupture strength, yield stress and the viscosity of the escaping liquid (Rosenthal, 2015). In fact, although the plunger fitted snugly in the hole containing the GMP paste, some liquid was forced upwards through the annulus gap during its downward travel.

Stress-deformation curves of cylindrical WPI/NaAlg bulk gels are presented in Figure 2.2A. Overall, an ample range of textures was obtained within the combinations selected for WPI and NaAlg concentrations. Table 2.1 shows values of stresses at 30% compression for the different treatments in the case of bulk gels and GMP. We selected this strain value as reference based on the recommendations of the guide for texture-modified foods used in the management of dysphagia (Rothenberg & Wendin, 2015). Soft gels were obtained for low concentrations of both biopolymers (e.g., Treatment 3, 6% WPI and 0.29% NaAlg) while strong gels resulted when intermediate combinations of WPI and/or NaAlg were present (e.g., Treatment 7, 8% WPI and 1.5% NaAlg). All GMP samples seemed to have a linear behavior up to around 30% compression and a departure towards a non-linear increase occurred at a strain of about 35-40%. A large standard deviation of mean stress values (at 30% deformation) of GMP shown in Table 1 is probably due to the uneven packing of particles during the test, a matter that needs further consideration. The strongest GMP were those having 1.5% NaAlg, emphasizing the role that this hydrocolloid has on structure formation and that adding protein to alginate solutions generates gels that are stronger than pure alginate gels (Wichchukit et al., 2013).

A second order surface response equation was estimated for the stress of GMP at 30% strain as a function of composition (Figure 2.4). The correlation coefficient (R2) was equal to 0.82. Statistical analysis shows that the stress was significantly dependent on %NaAlg in the formulation. The response surface suggests that % WPI and % NaAlg strongly influenced the stress value and a maximal stress point was found at about 6.2% WPI and 1.3% NaAlg. More important, the interval of concentrations of the biopolymers resulted in GMP with stress values (a proxy of firmness) from approximately 200 to 1200 kPa. This ample range of mechanical properties is highly desirable to tailor the GMP for different aplications.

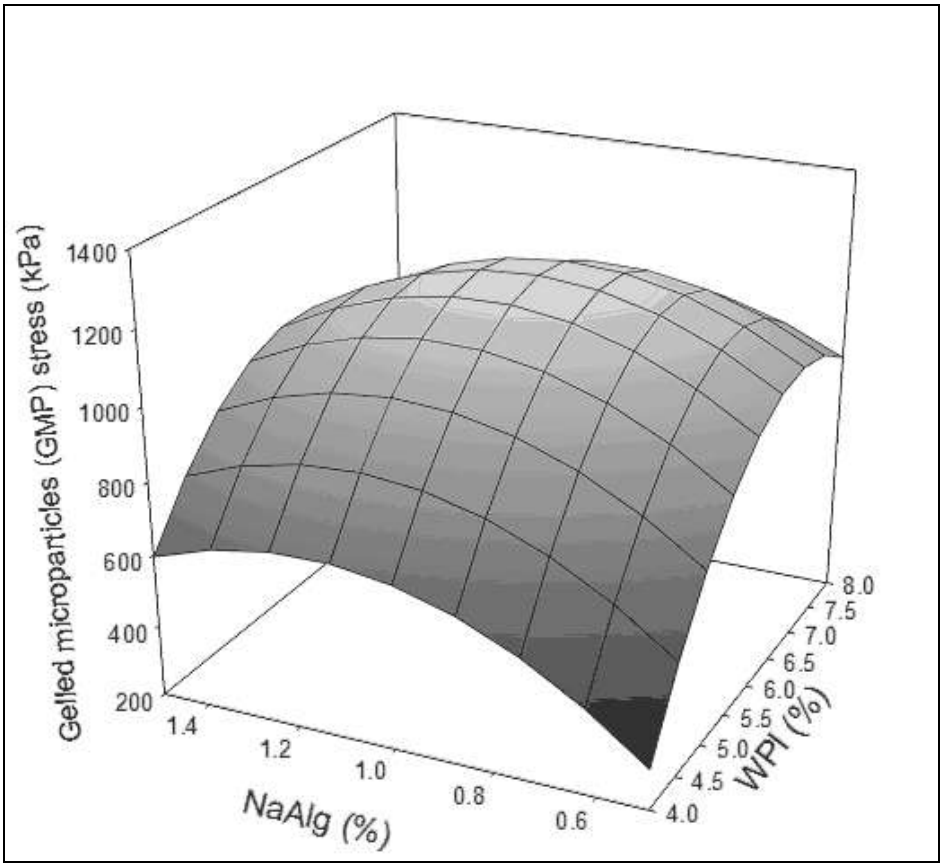

Figure 2.4. Response surface of stress (at 30% strain) for gelled microparticles (GMP).

2.3.3 Principal component analysis

The calculated eigenvalues from the matrix indicated that it was possible to describe 92.8% of the total variation between bulk gels and GMP for all treatments when the first and second principal components (PC1 and PC2) were considered. A scatter plot of PC1 and PC2 is presented in Figure 2.5. Samples exhibiting low stress values (T1, T3, T4 and T5) corresponded to low concentrations of NaAlg and were grouped on the left part of the Figure, next to the PC1 axis. Samples with high stress values were associated with high NaAlg concentrations and were located in the lower right quadrant of the

graph (T2, T6, T7 and T8). Samples representing the central point became positioned in the upper central part of the scatter plot (T9 and T10).

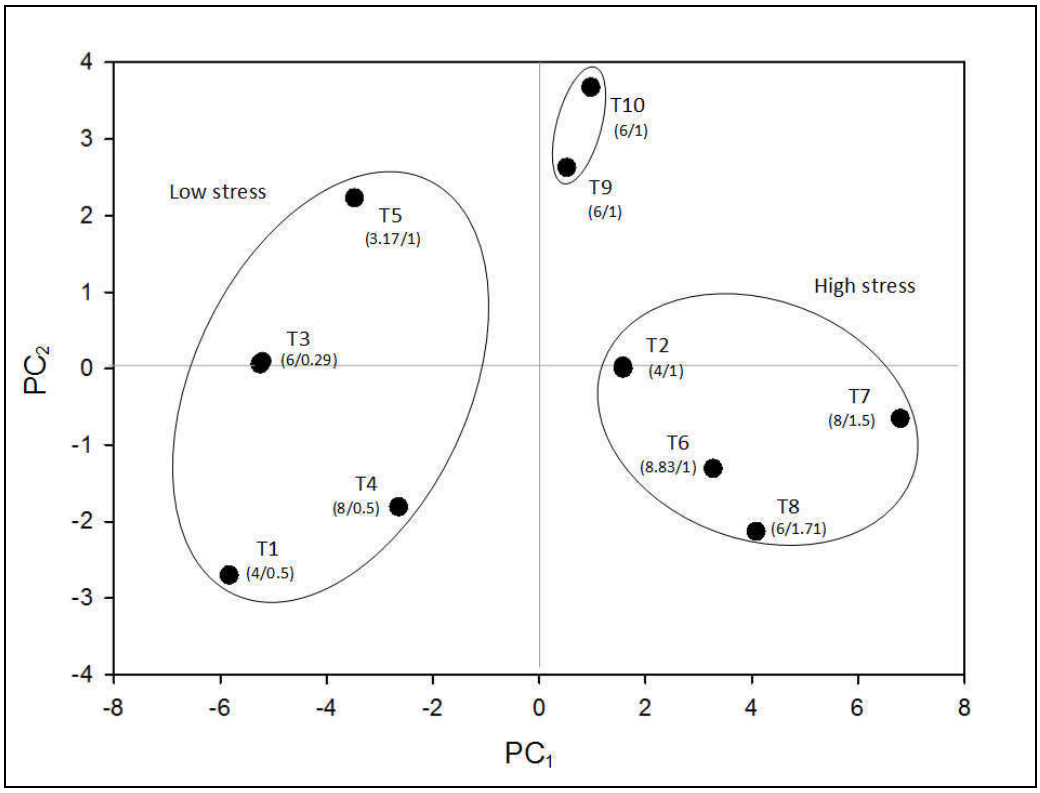

Figure 2.5. Two dimensional scatter plot of the scores of PC1 and PC2 for all samples (bulk gels and GMP) demonstrating the grouping based on stress values.

2.3.4 Comparison of mechanical properties of bulk gels and GMP

A good linear correlation (R^2 = .887) existed between the stress values at 60% strain for bulk gels and GMP (Figure 2.6). This relationship suggests that the grinding process maintained the structural characteristics of bulk gels in the GMP to a great extent. Dispersion of values may be due to the fact that the back extrusion test determines a combination of mechanical properties other than just the force-deformation

under uniaxial compression (Rosenthal, 2015). For example, during testing it is quite likely that packing of particles and rearrangement of the medium had occurred.

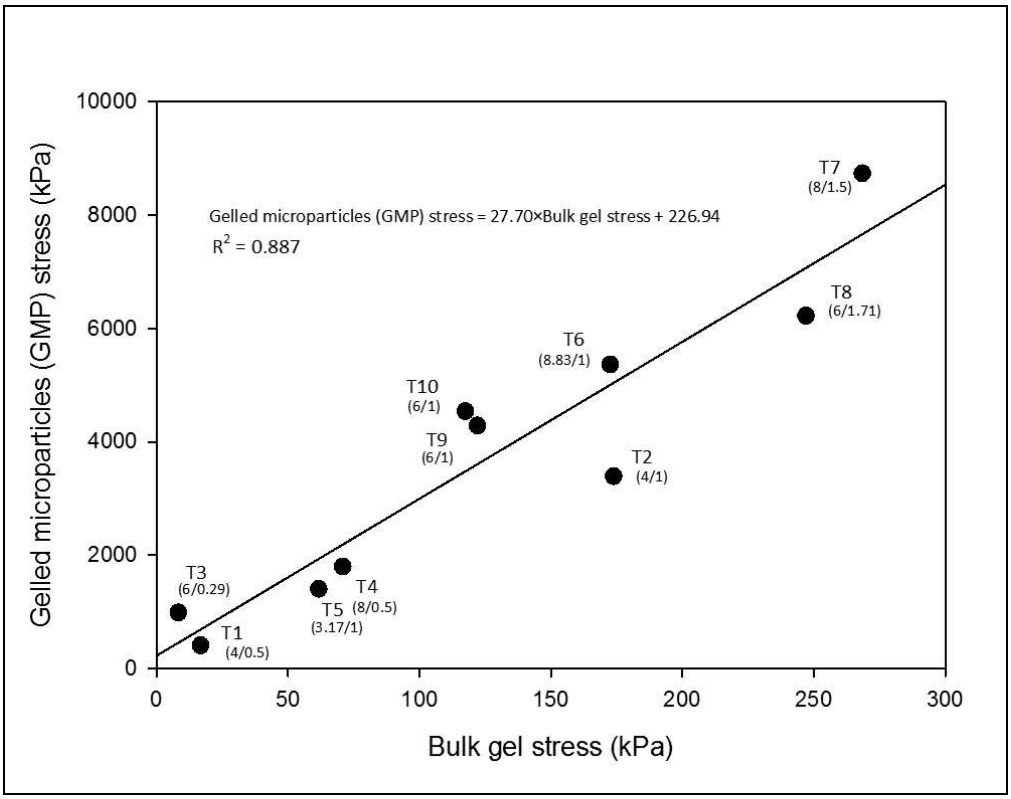

Figure 2.6 Relationship between the stress (at 60% strain) of bulk gels and gelled microparticles (GMP) for different treatments (WPI/NaAlg concentrations).

2.4 Conclusions

WPI/alginate gel microparticles (GMP) were prepared by homogenization of bulk gels produced by cold gelation and diffusion of calcium ions into the mixed solutions. If larger particles were needed, GMP could be obtained by reducing the intensity of the grinding method. The size of particles was determined using image

analysis and a specially derived software which has the advantage that the objects can be seen in the photographs. A novel back-extrusion test cell was constructed to assess mechanical properties of microgels. Stress of bulk gels and GMP exhibited a good correlation, meaning that the back-extrusion method is an appropriate alternative to directly assess the mechanical properties of a paste of microparticles. Principal component analysis (PCA) allowed to distinguish softer GMP with low content of NaAlg from firmer GMP having a high concentration of the polysaccharide, thus, corroborating the positive role of alginate in structure formation of the mixed gel. We expect that these gelled microparticles may find applications as texture-modified foods for elderly people with mastication and swallowing dysfunctions. Future work is underway to use GMP as carriers of lipid droplets and fiber nanoparticles.

3. PROPERTIES OF MICROPARTICLES FROM A WHEY PROTEIN ISOLATE/ALGINATE EMULSION GEL

3.1 Introduction

Designing soft, palatable and healthy texture-modified foods for the elderly, particularly those with masticatory/swallowing dysfunctions or needing special nutrition, is a major challenge for food technologists and the food industry (Aguilera & Park, 2016; Cichero, 2016). Recently, Aguilera and Park (2017) discussed the many alternatives available to design soft texture-modified foods for the elderly highlighting their impact on taste, flavor, rheology and the delivery of bioactives. Gel microparticles due to their small size (e.g., < 100 μm) and adjustable physical properties are an excellent alternative to tailor the rheological properties of these type of foods (Leon et al., 2016; Stokes, 2012). For example, gel microparticles may be added to thin liquids and purees to modify their flow behavior and texture perception. Moreover, the fragile structure of gel microparticles is functional to elicit strong flavor intensities during breakdown in the mouth and to deliver nutrients and bioactive compounds during digestion (L. Chen & Subirade, 2006; Kalvianen, Roininen, & Tuorila, 2000).

In addition to providing a soft texture, texture-modified foods should also cover some nutritional requirements of the elderly. Lipids (e.g., those containing omega-3 fatty acids) are important in many diseases associated with aging, such as Alzheimer's disease, which is the most common form of dementia in the elderly (Florent-Béchard et al., 2009; Hooijmans & Kiliaan, 2008; Úbeda, Achón, & Varela-Moreiras, 2016; van der Beek & Kamphuis, 2008). Furthermore, lipids also provide mouth feel, impart flavor, and contribute to the feeling of satiety after eating (Emadzadeh & Ghorani, 2015; Khetarpaul, Mutneja, & Khetarpaul, 2014). Likewise, texture-modified foods may be

used as carriers for oil soluble vitamins A, D, E and K and to control their delivery and proper absorption (Khetarpaul et al., 2014; Kravchenko, 2017).

Whey protein isolate (WPI) is extensively used in various functional food applications due to its emulsification, gelation, thickening, foaming and water-binding properties, and excellent nutritional value, as well as the ability to carry hydrophobic substances (Bryant & McClements, 1998; Kimpel & Schmitt, 2015). Gelation of WPI can be induced by a two-step cold-set method which involves the preparation of a heat-denatured WPI solution at a concentration below the critical concentration for gelation, followed by the gelation of the denatured protein solution at ambient temperature under saline conditions (Bryant & McClements, 2000; Hongsprabhas & Barbut, 1997). Since cold gelation occurs at low temperatures (e.g., 5°C) gel formation by WPI is advantageous over heat-set gelation when thermolabile substances are incorporated in the gel. Calcium can induce cold-set gelation of whey proteins, and has been exploited in the production of WPI microgels (Ni et al., 2015). Alginate is an attractive polymer as a component of a delivery matrix because it gels quite easily from aqueous solutions and at room temperature in the presence of calcium ions (Kikuchi, Kawabuchi, Watanabe, & Sugihara, 1999). Additionally, it has been proposed that alginate gels may be used as carriers for lipophilic actives in pharmaceutical and food products (Ching, Bansal, & Bhandari, 2015). Hence, mixed WPI and NaAlg solutions not only undergo gelation with calcium at room temperature but also their combination may result in gels with specific physicochemical properties as support for food ingredients, nutrients and bioactive substances (L. Chen & Subirade, 2006, 2007; Mession et al., 2013; Wichchukit et al., 2013).

Emulsions are unique microstructures that may hold chemical compounds (i.e. taste compounds) partitioned between an aqueous and a lipid phase (Dumont, 2006). Gel-like materials containing emulsified fat include several manufactured food products, for example, yoghurt, some cheeses, thick sauces, and meat products (frankfurters and

spreadable pastes). The physical result of incorporating emulsified fat droplets into gel matrices is either a reinforcement of the structure, if the gel network attaches to the interfacial layer of oil droplets, or a weaker structure if this interaction occurs to a limited extent (Aguilera & Kessler, 1989; Dickinson, 2012; Mession et al., 2013). Although emulsions are generally formed by mechanical means, a simple and energy saving procedure to generate oil droplets is to use ultrasound energy (Awad, Moharram, Shaltout, Asker, & Youssef, 2012; Ching et al., 2015). Emulsion-gelled microparticles (EGM) are a relatively new class of soft solid particles of small size and unique functional properties (Lesmes & McClements, 2009). These particles are characterized by emulsion droplets entrapped inside a soft semi-solid matrix which provides control of the mechanical properties and delivery mechanisms (Beaulieu et al., 2002; Ching et al., 2015; Dickinson, 2015; Thelma Egan et al., 2013; Garrec & Norton, 2012; Sung, Xiao, Decker, & McClements, 2015). Additionally, EGM protect entrapped lipids and lipophilic compounds such as polyunsaturated fatty acids against oxidation (L. Chen & Subirade, 2006; Torres, Murray, & Sarkar, 2016). The aim of this work was to produce and characterize emulsion-gelled microparticles (EGM) having olive oil microdroplets embedded in a gel matrix of WPI/NaAlg with properties needed to improve food texture for people with masticatory problems.

3.2 Materials and methods

Figure 3.1 summarizes the main steps in the preparation of emulsion-gelled microparticles and the procedures employed to characterize bulk emulsion gels and EGM pastes.

3.2.1. Materials

A 6% WPI and 1% NaAlg (Leon, Medina, Park, & Aguilera, 2016) solution was prepared by dissolving both ingredients in distilled water at room temperature under mild agitation to avoid foam formation. The mixed solution was left overnight at 4 °C for hydration of the components and then immersed in a temperature controlled water bath at 80 °C for 30 min to denature and aggregate the WPI. This procedure facilitates the formation of stable whey protein emulsion structures when oil is added due to the excellent surface activity of denature whey proteins (de Araujo Mantovania, Cavallieria, & da Cunhaa, 2011; Hu, McClements, & E.A., 2003; Wijayanti, Bansal, & Deeth, 2014). Olive oil was added at room temperature to obtain 5, 15 and 25% oil concentration in the WPI/NaAlg dispersion, and emulsified in an ice bath by two different mechanisms: a) with a high-speed blender (Ultra Turrax digital equipment T25, IKA-Werke, Germany) at 15,000 rpm for 5 min, and; b) with an ultrasonic sonifier (Branson Ultrasonic Corporation, Model 450L, Danbury, USA). Ten mL of sample were placed in a beaker and the sonicator's tip (diameter of 1.905 cm) was centrally fixed at half of the sample height. The sonifier had a maximum power output of 400 W at 20 kHz and was operated for 60 s delivering a power of approximately 40 W (Cucheval & Chow, 2008; Gaikwad & Pandit, 2008; Saleh, Annuar, & Simarani, 2017). The emulsified biopolymer mixtures were placed in the synthetic membranes, sealed on both sides and immersed in a 200 mM calcium chloride bath for 12 h at 5 °C to induce gelation of the food biopolymers (Leon et al., 2016). Cylindrical bulk emulsion gels (10 mm height and 20 mm diameter) were obtained after removing the cellulosic casings.

3.2.2 Preparation of WPI/NaAlg emulsion-gelled microparticles (EGM)

3.2.2.1. Bulk emulsion gels of WPI/NaAlg

A 6% WPI and 1% NaAlg solution was prepared by dissolving both ingredients in distilled water at room temperature under mild agitation to avoid foam formation. The mixed solution was left overnight at 4 °C for hydration of the components and then

immersed in a temperature controlled water bath at 80 °C for 30 min to denature and aggregate the whey proteins. Olive oil was added at room temperature to obtain 5, 15 and 25% oil concentration in the WPI/NaAlg dispersion, and emulsified in an ice bath by two different mechanisms: a) with a high-speed blender (Ultra Turrax digital equipment T25, IKA-Werke, Germany) at 15,000 rpm for 5 min, and; b) with an ultrasonic sonifier (Branson Ultrasonic Corporation, Model 450L, USA). Ten mL of sample were placed in a beaker and the sonicator's tip (diameter of 1.905 cm) was centrally fixed at half of the sample height. The sonifier had a maximum power output of 400 W at 20 kHz and was operated for 60 s delivering a power of approximately 40 W•cm-2 (Cucheval & Chow, 2008; Gaikwad & Pandit, 2008; Saleh, Annuar, & Simarani, 2017). The emulsified biopolymer mixtures were placed in the synthetic membranes, sealed on both sides and immersed in a 200 mM calcium chloride bath for 12 h at 5 °C to induce gelation of the food biopolymers (Leon et al., 2016). Cylindrical bulk emulsion gels (10 mm height and 20 mm diameter) were obtained after removing the cellulosic casings.

3.2.2.2. Pastes of WPI/NaAlg emulsion-gelled microparticles (EGM)

EGM in the form of pastes were obtained by mechanical size reduction of the respective bulk emulsion gels, using first a hand-held blender and then a high-speed blender (Ultra Turrax digital T25, IKA-Werke, Germany) at 5000 rpm for 5 minutes. Large agglomerates were removed by sieving through a 1 mm screen (Leon et al., 2016). Emulsion-gelled microparticles are referred to as HSB-EGM if they contained oil emulsified with a high-speed blender and as US-EGM when ultrasound was employed.
All EGM pastes obtained were stable and no phase separation was observed during the time of the experiment

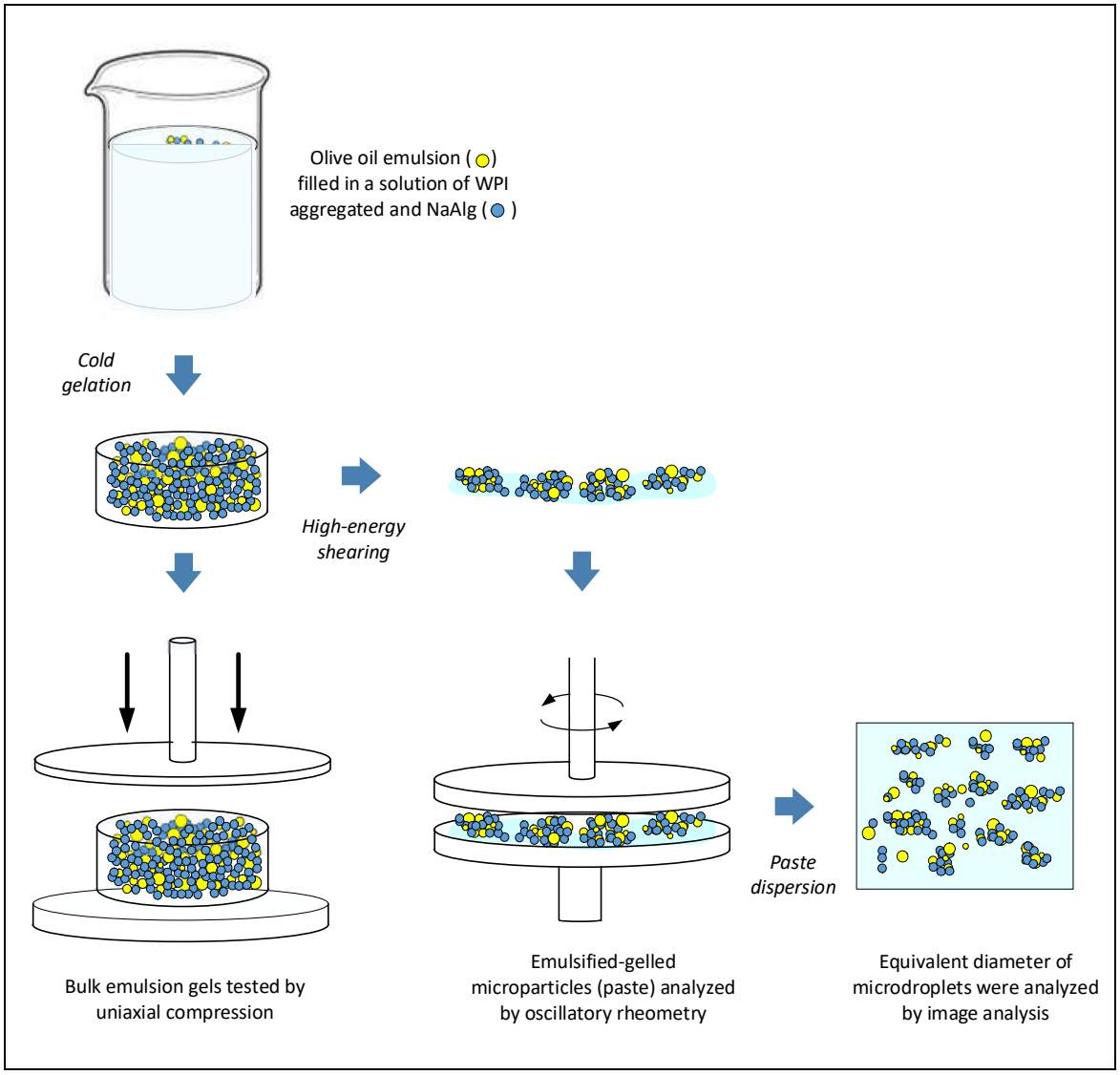

Figure 3.1. Diagram of the main steps in sample preparation and testing procedures for bulk emulsion gels and EGM pastes.

3.2.3. Microstructural analysis of EGM

Microstructure of both HSB-EGM and US-EGM pastes were examined at a magnification of 40× objective lens and 10× eyepiece, with an optical microscope (model SMZ-2T 2B, Nikon Corp., Tokyo, Japan). Images were acquired with a digital camera Toup Tek model UCMOS 08000 (Photonics, Zhejiang, China) with a resolution of 2540 × 1744 pixels. A small aliquot of each sample was placed on a glass slide, gently dispersed with one drop of distilled water using a spatula, and covered with a coverslip. Equivalent diameter of oil microdroplets (OMD) in EGM pastes were calculated from digital images using a program developed in MatLab 6.0 (Mathworks, Inc., Natick, MA, USA) as reported in Leon et al. (2016). Ten images were processed per treatment and at least 100 objects were measured per image.

3.2.4 **Mechanical properties of bulk emulsion** gels

Cylindrical samples (10 mm height x 20 mm diameter) of bulk emulsion gels were subjected to uniaxial compression in a texture analyzer model TA.XT2 Plus (Stable Micro System Ltd., Godalming, UK) to acquire stress-deformation curves and determine the maximum compressive stress. A round metal plate 75 mm in diameter was used to compress the samples up to 80% strain and a constant speed of 1 $mm \cdot s^{-1}$ and at room temperature (Leon et al., 2016). All measurements were done in duplicate.

3.2.5 Rheological properties of EGM pastes

Viscoelastic properties of EGM pastes were studied by small amplitude dynamic oscillatory measurements using a Discovery hybrid rheometer (model RH-2, TA Instruments, New Castle, DE) with a 40 mm diameter parallel plate geometry and a 1 mm gap. About 2 g of sample was employed in all measurements. The linear

viscoelastic range was determined after a strain sweep from 0.01 to 100% and a strain of 0.1% was chosen for all subsequent frequency sweep tests (0.1 to 100 rad s-1). Storage modulus (G'), loss modulus (G"), and tan (δ) = (G"/ G') values were obtained (in duplicate) at 20°, 40° and 60°C (Funami et al., 2012; Steffe, 1996; Tarrega, Ramírez-Sucre, Vélez-Ruiz, & Costell, 2012).

3.2.6 Size distribution analysis of EGM

The particle size distribution of both HS-EGM and US-EGM pastes with 25% of oil content were measured using a laser diffraction particle size analyzer (Mastersizer Hydro 2000, Malvern Instruments, Ltd., Worcestershire, UK). The microparticles sizes were measured directly after dispersing both samples in distilled water as fluid carrier at a speed of 2000 rpm. The percentage of laser obscuration was 11.3% with a relative refractive index of 1.52 for GMP and 1.33 for distilled water. A particle adsorption of 0.1 was used to calculate the size distribution. The particle size distribution was calculate with Mie theory. An average from five readings was taken for each sample.

3.2.7 Statistical analysis

Experiments were planned as a randomized complete design with a 2 × 3 factorial arrangement. The factors were the emulsification methods (high-speed blending and ultrasound), and the three concentrations of olive oil: 5, 15 and 25% (Table 3.1). Treatments T1 to T6 (see Table 3.1) were performed randomly and experimental data analyzed by analysis of variance (ANOVA). Statistical significance using the Statgraphics Plus software (Statistical Graphics Corporation, version 5.1, Rockville, USA) was set at a probability level of 0.05 ($p<0.05$)

Table 3.1. Factors and treatments (T1 to T6) of the experimental design

Olive oil concentration (%)	**Method used to emulsify the oil**	
	High-speed blending (HSB)	Ultrasound (US)
5	T1	T4
15	T2	T5
25	T3	T6

3.3 Results and discussion

3.3.1 Microstructural features of EGM

Micrographs of HSB-EGM, US-EGM and of samples from intermediate steps in the preparation of EGM are shown in Figure 3.2. At the scale of the image, the dispersion of aggregated WPI and NaAlg appeared as a two-phase system of dispersed globules in a continuous milieu, some of them fused together (Figure 3.2A). Phase separation is not uncommon in globular protein-polysaccharide mixtures (van den Berg, Rosenberg, van Boekel, Rosenberg, & van de Velde, 2009). In a pre-gelled emulsion, oil microdroplets (OMD) were clearly identified as bright round objects (Figure 3.2B). In turn, views of gelled biopolymer microparticles without oil (Figure 3.2C) were instrumental to recognize the gel matrix in emulsified gel microparticles (Leon et al., 2016). Mession et al. (2013) also observed large, irregularly shaped agglomerates with sizes that extended to several microns in gels produced from pea protein/alginate mixtures. This arrangement is typical of the process of aggregation during cold gelation of denatured whey proteins (Donato, Kolodzieccyk, & Rouvet, 2011).

Most oil droplets in EGM were trapped within the WPI/NaAlg network or attached to it (Figures 3.2D to 3.2I). Since the oil was emulsified into a dispersion of

denatured whey protein and NaAlg prior to cold gelation, it is reasonable to assume that an interfacial layer of these biopolymers surrounded the oil droplets (Dickinson, 2012). This biopolymeric interface may have acted as an anchor between the droplets and the gel matrix (Dickinson, 2012; McClements, Monahan, & Kinsella, 1993; Sala, van Aken, Stuart, & van de Velde, 2007). Images suggest that OMD in US-EGM were smaller than in HSB-EGM, with an average equivalent diameter assessed by image analysis varying between 2.0-3.2 μm and 4.5-6.7 μm, respectively (Figure 3.3). This difference confirms that emulsification by ultrasound generally results in droplet sizes smaller than those achieved with mechanical shearing (Abismaïl, Canselier, Wilhelm, Delmas, & Gourdon, 1999).

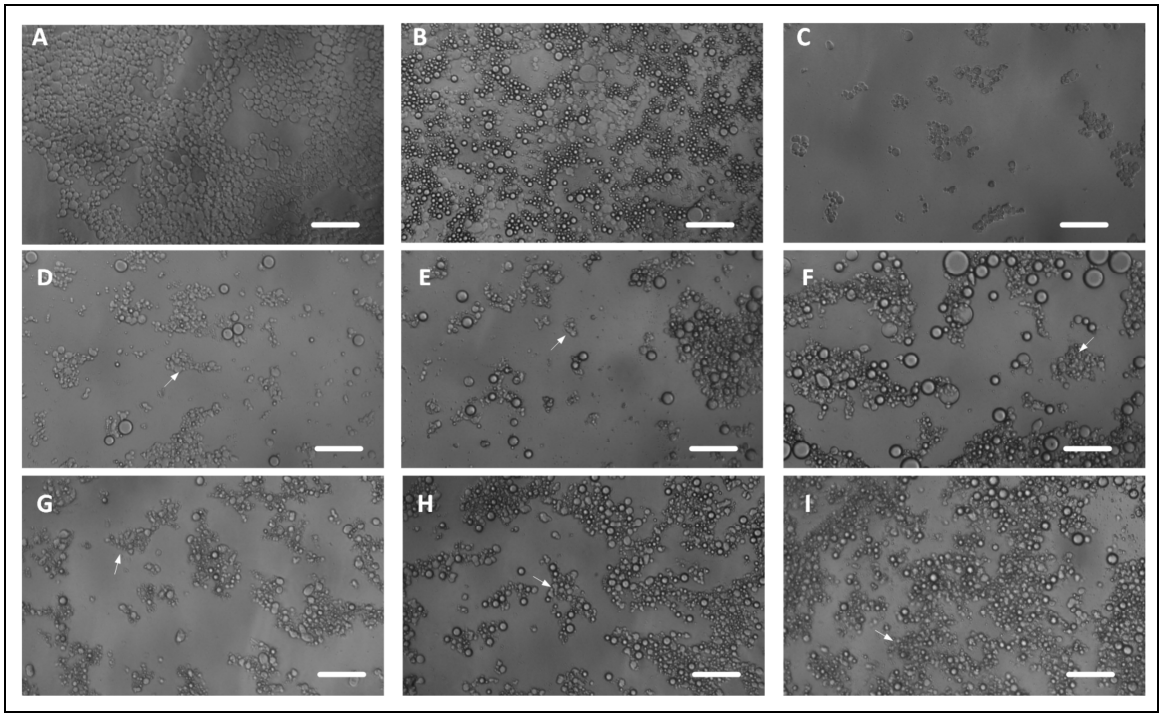

Figure 3.2. Photomicrographs of: **A)** Solution of aggregated WPI and NaAlg; **B)** Emulsion prior to cold gelation, and; **C)** WPI/NaAlg gelled microparticles (with no oil added). Emulsion-gelled microparticles (EGM) with different oil contents (in parentheses) and prepared by different emulsification methods. High-speed blending

(HSB-EGM): **D)** T1 (5%), **E)** T2 (15%), **F)** T3 (25%); ultrasound (US-EGM): **G)** T4 (5%), **H)** T5 (15%) and **I)** T6 (25%). Arrows point at the gel matrix in EGM. Scale bar = 50 µm.

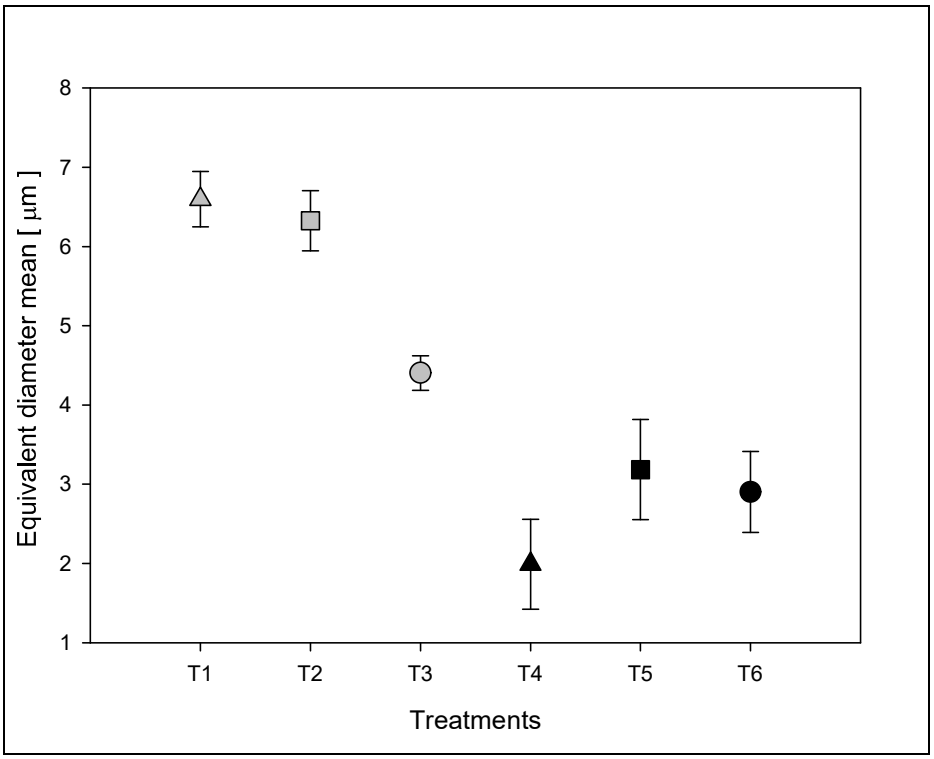

Figure 3.3. Equivalent diameter of oil droplets in EGM at 20°C as determined by image analysis. HSB-EGM (gray symbols), US-EGM (black symbols).

The contour, hence, the size of EGM in the pastes was difficult to assess by image analysis due to clumping of particles and their irregular shapes. Particle analysis by laser scattering for samples containing 25% oil (T3 and T6) resulted in a bimodal size distribution curve with two peaks (Figure 3.4): one around 6 to 12 µm and a second one between 200 and 500 µm. The first peak occurred at a size slightly larger than that of oil droplets (Figure 3.3) while the second one may be associated to the EGM themselves, some of which are distinguished in Figures 3.2D to 3.2I. Bimodal size distributions have

been reported for gelled beads containing alginate and WPI (Zhang, Zhang, Zou, & McClements, 2016). Also, when a bulk gel is fractionated by shearing forces, both coarse and fine particles are simultaneously formed, causing that the particle size distribution curve exhibits two peaks (Zhao, Dai, Zhao, You, & Chen, 2013).

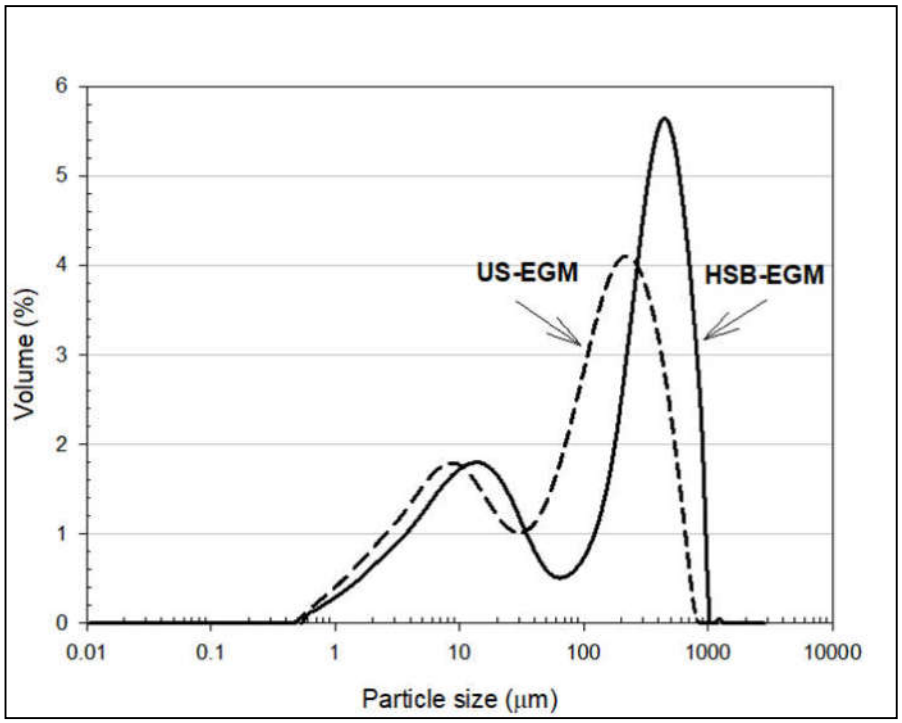

Figure 3.4. Particle size distribution curve of EGM with a concentration of 25% of olive oil prepared by ultrasound (US-EGM) and high-speed blending (HSB-EGM).

3.3.2 Mechanical properties of bulk emulsion gels

Stress-deformation curves and maximum compressive stress for bulk emulsion gels are shown in Figures 3.5A and 3.5B, respectively. All bulk gels containing emulsified oil exhibited a maximum compressive stress at some intermediate point

between 60-80% strain, while the stress of the bulk gel devoid of oil increased continuously with deformation (Figure 3.5A). Interestingly, at small deformations (e.g., < 68% strain), bulk gels containing emulsified oil had higher stresses than the control no-oil gel but at some intermediate point this trend was opposed. This feature suggests that when oil-filled gels are compressed at high strains (e.g., over around 68%) the biopolymer matrix itself is no longer the structural element resisting to the load. It has been reported that mechanical properties of gels filled with emulsions depend on the interactions between oil droplets and the gel matrix (Sala and others 2007), the oil content and oil droplet size (Aguilera & Kinsella, 1991; Kim, Renkema, & van Vliet, 2001).

All bulk emulsion gels produced by US and HSB exhibited a reduction in the maximum compressive stress as the oil content increased (Figure 3.5B), with the lowest stress corresponding to samples with 25% oil (T3 and T6 in Figure 3.5B). However, no significant differences were found between bulk gels containing the same proportion of US or HSM emulsions, which means that the difference in the size of fat droplets was not determinant. In general, lipid-filled gels showed an increase in stiffness upon addition of smaller and more polydispersed fat globules compared to those with larger oil droplets droplets (Kim et al., 2001; Rosa, Sala, van Vliet, & van de Velde, 2006).

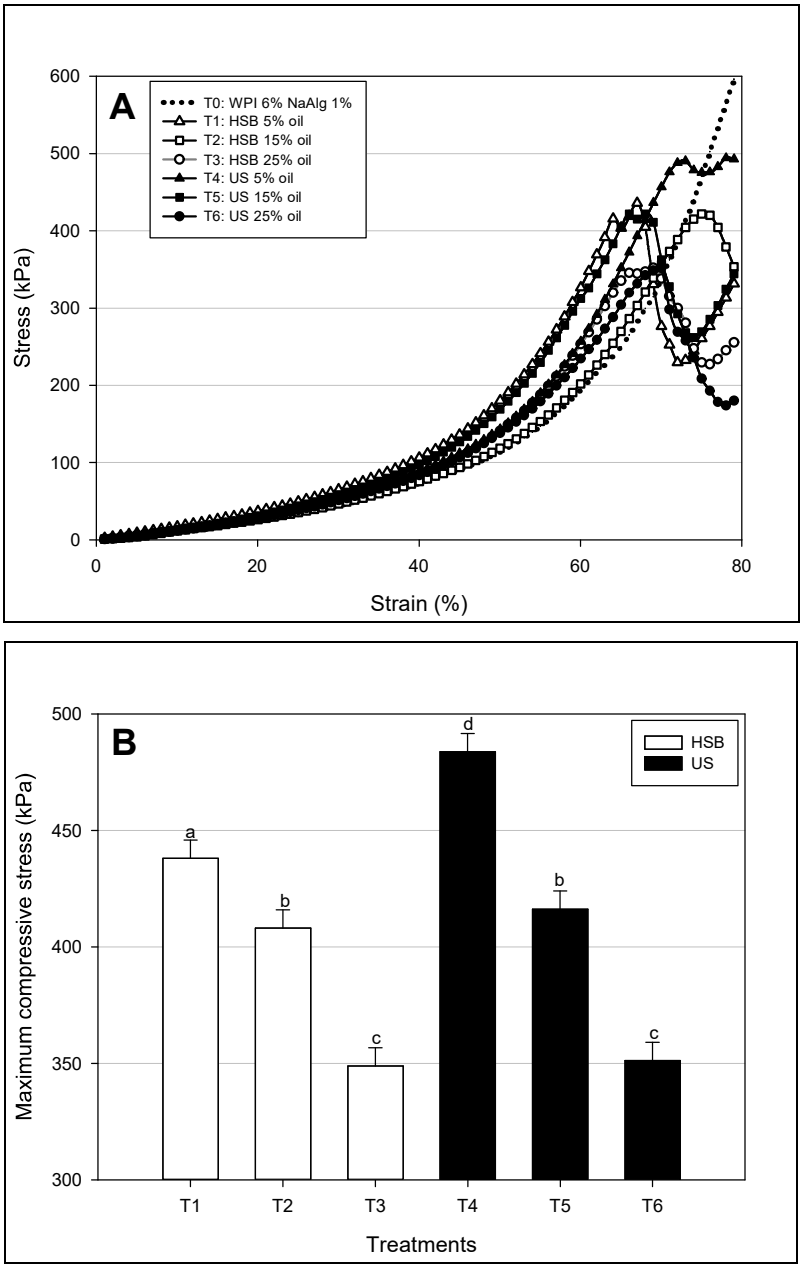

Figure 3.5. (A) Stress-deformation curves of bulk emulsion gels. Control gel with no oil (dotted line). Emulsions in T1 (△-5%), T2 (□-15%) and T3 (○-25%) were prepared by high-speed blending (white symbols) and in T4 (▲-5%), T5 (■-15%) and T6 (●-25%) by ultrasound (black symbols). **(B)** Maximum compressive stress values of the same gels.

3.3.3 Rheological properties of EGM pastes

Figure 3.6 shows the mechanical spectra of EGM pastes at 20°C as a function of frequency (ω). Ideal gels exhibit an elastic response where the elastic or storage modulus G' is much higher than the viscous or loss modulus (G'') and is independent of frequency. As seen in Figure 3.6 A and 3.6 B, G' was always higher than G'' for HSB-EGM and US-EGM, so these pastes showed a predominant elastic behavior (Erçelebi & Ibanoğlu, 2009). In gel networks with imperfections both moduli increase with frequency, as was the case for EGM pastes. At high frequencies (short times) the gel network does not have time to rearrange and physical entanglements persist longer than the oscillation frequency, thus, elastic energy is stored and contribute to viscous dissipation (Grillet, Wyatt, & Gloe, 2012). US-EGM exhibited higher G' values than HSB-EGM for all frequencies, meaning that samples containing oil emulsified by ultrasound had a more solid-like behavior than those produced by high shear blending. US-EGM pastes with higher oil contents exhibited larger G' values, coinciding with the results obtained by Mao, Roos, and Miao (2014) for emulsion-filled protein gels, and Gunasekaran and Ak (2000) for salad dressings. Probably olive oil microdroplets at 25% (T6) were more stabilized by WPI and incorporated into the gel structure reinforcing the gel network, than treatments with 15% (T5) and 5% (T4). D J McClements, Monahan, and Kinsella (1993) indicate that protein molecules adsorbed at oil droplet surface can therefore interact with those in the gel network due to a combination of covalent (disulfide) bonds and non-covalent interactions. Conversely, in emulsions prepared by HSB T1 (5%) was more stable than T2 (15%) and T3 (25%) possibly because mechanical emulsification energy was reduced with oil concentration, even though equivalent diameter of oil droplets were minor in T3 (~ 4.2 μm) than T2 and T1 (~ 6.5 μm). In turn, Matsumura, Kang, Sakamoto, Motoki, and Mori (1993) reported that emulsion gels made from a fine emulsion (i.e., US-EGM) exhibited higher G′ and G′′ values than gels made from coarse emulsions containing larger oil droplets.

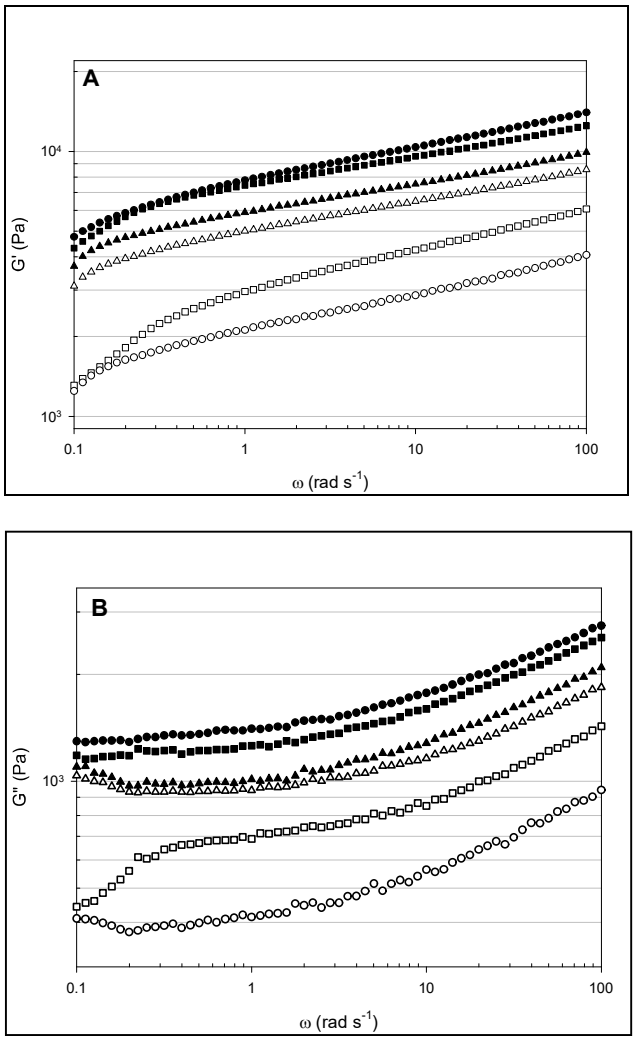

Figure 3.6. Mechanical spectra of EGM-pastes at 20 °C. **(A)** Storage modulus G', **(B)** Loss modulus G''. T1 (△: 5%), T2 (□: 15%), and T3 (○: 25%), T4 (▲: 5%), T5 (■: 15%), and T6 (●: 25%).

For all experimental treatments and temperatures, the values of the tan (δ) were in the range of 0.16-0.76 (Figure 7). These values are between those typical of an elastic solid (δ = 0) and a fully viscous material (δ = 1.57). Tan (δ) showed minor variation

when pastes were heated from 20°C to 40°C (Figures 3.7A and 3.7B). However, runs performed at 60°C (Figure 3.7C) showed a marked transition for HSB-EGM from an elastic to a more viscous behavior, particularly in samples with a high oil content, as has been also reported for oil-filled gels (Kim et al., 2001). The temperature of 60 °C is considered as a reference for foods consumed hot, such as soups and hot beverages.

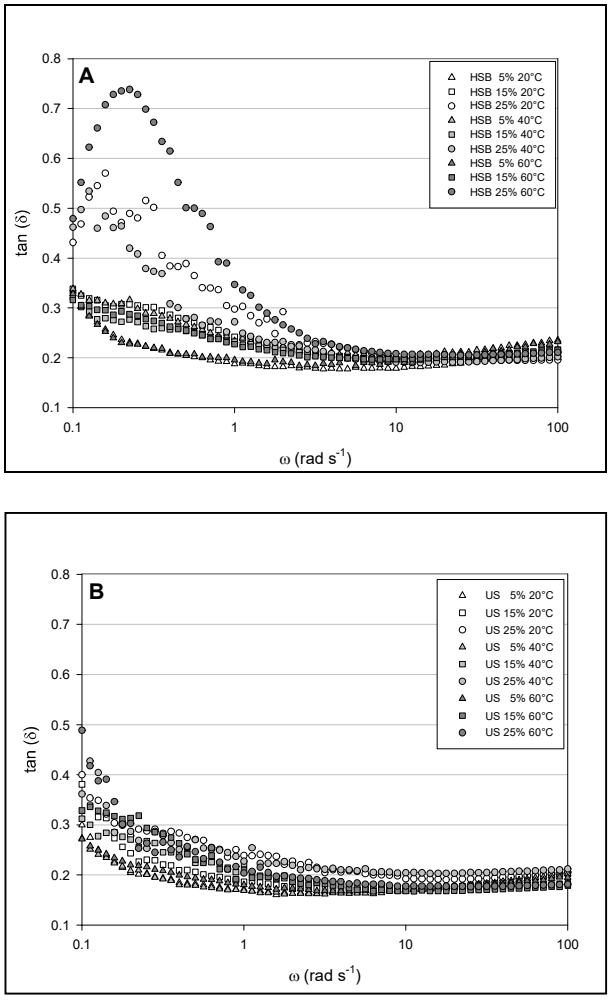

Figure 3.7. Effect of temperature on the tan (δ) of selected EGM pastes with different oil contents and three temperatures: 20°C, 40°C, and 60°C. **(A)** HSB-EGM; **(B)** US-EGM

Values of tan (δ) at 2π rad s^{-1} and 20°C between 0.07 and 0.27, have been reported for some soft pastes and purees as well as for texture-modified foods for the elderly (Figure 3.8): fruit cocktail, bean pâté, beef pâté, broccoli timbale, beef timbale, fruit mousse, jellied vegetables, jellied meat (Wendin et al., 2010), fine, standard and coarse mustard (Aguilar, Rizvi, Ramirez, & Inda, 1991), tomato paste (Rao & Cooley, 1992), blueberry pie filling (Steffe, Castell-Perez, Rose, & Zabik, 1989). Hence, EGM may be used to formulate texture-modified foods such as jellies, pates and timbales while maintaining their rheological properties.

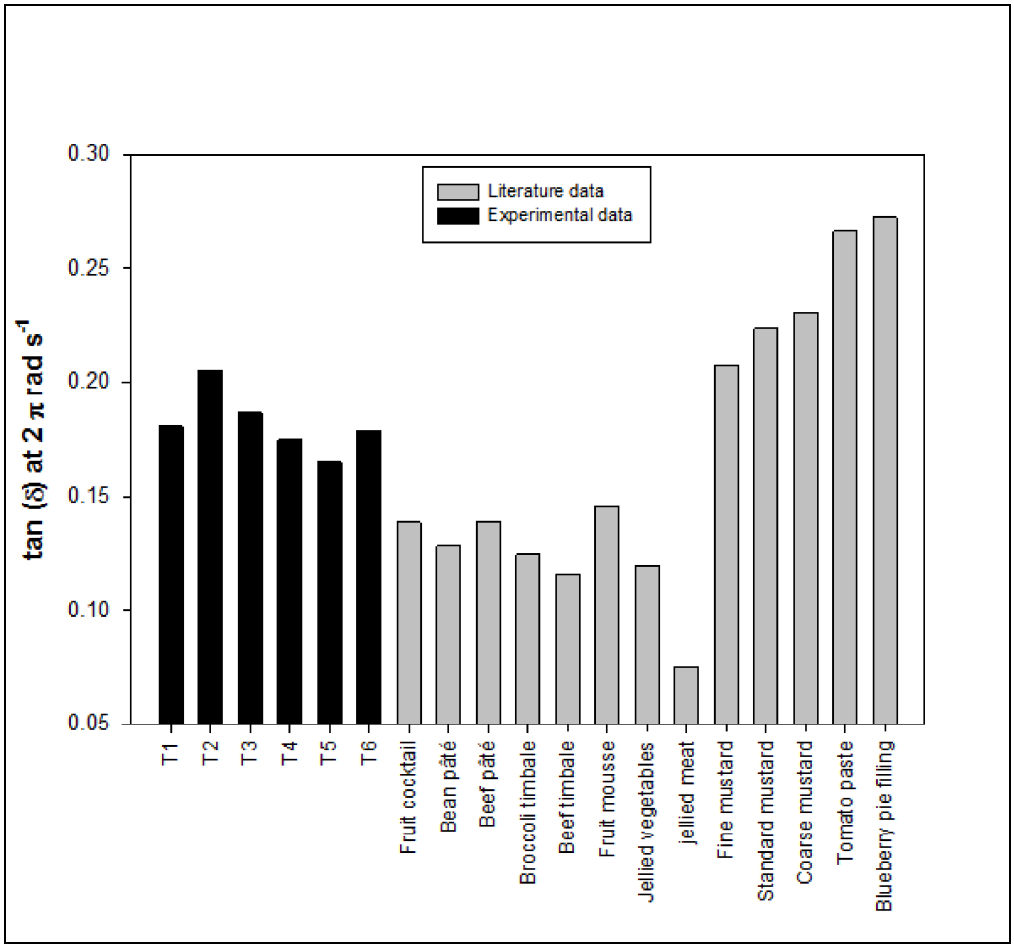

Figure 3.8. Experimental data of tan (δ) at 2π rad s^{-1}, 20°C and comparison with data from the literature.

3.4 Conclusions

Emulsion-gelled microparticles (EGM) consisting of a WPI/NaAlg gel network and encasing olive oil microdroplets were obtained using emulsification by sonication and high-shear homogenization, followed by cold gelation. Optical microscopy was instrumental in identifying oil droplets and aggregates forming the gel network. Oil microdroplets emulsified by ultrasound were smaller than those produced by high-shear blending. A wide range of mechanical and rheological properties of EGM was attained through the investigated formulations. US-EGM pastes containing oil droplets emulsified by ultrasound were quite stable when heated to 60 °C and would be appropriate to formulate heated foods such as soups. EGM are potential texture modifiers for thin liquids fed to elderly people or as formulated pastes. Further work should include the effect of formulation (e.g., wider range of biopolymer concentration and other types of lipid phase) on mechanical and rheological properties and a precise characterization of the structural features of EGM.

Nomenclature

EGM	Emulsified gelled microparticles
US-EGM	EGM containing oil emulsified by ultrasound
HSB-EGM	EGM containing oil emulsified by high-speed blending
OMD	Oil microdroplets in the EGM

4. MECHANICAL, RHEOLOGICAL AND STRUCTURAL PROPERTIES OF FIBER-CONTAINING MICROGELS

4.1 Introduction

Aging of the world's population occurs at a rapid pace; the elderly are now the fastest growing demographic segment. This situation imposes the challenge of providing appropriate foods that satisfy some of their particular needs, among them, easy mastication and safe swallowing, as well as special nutrition supplements (Aguilera & Park, 2016). For example, in old people with dysphagia, liquids have to be thickened with starch and gums to nectar- or pudding-like consistencies to moderate the flow rate during swallowing (Moret-Tatay, Rodríguez-García, Martí-Bonmatí, Hernando, & Hernández, 2015; Quinchia et al., 2011). Due to mastication and swallowing problems some old people tend to avoid foods with a high content of fiber (e.g., fruits or vegetables), precluding their modulatory effect on the bowel function and making constipation a common disorder (Chen & Huang, 2003; Donini, Savina, & Cannella, 2009; Schuster, Kosar, & Kamrul, 2015). Thus, soft foods with added fiber supplements have been recommended for elderly patients with constipation (Gallegos-Orozco, Foxx-Orenstein, Sterler, & Stoa, 2012).

Dietary fiber (DF) is that part of edible plant material which is resistant to enzymatic digestion by humans. DF components include cellulose, non-cellulosic polysaccharides such as hemicellulose, pectic substances, gums, mucilages, and the non-carbohydrate component lignin (DeVries et al., 2001; Dror, 2003; Prosky, 2000). DF sources are usually classified into soluble in water (pectins, inulin, gums and mucilages) and insoluble ones (e.g., cellulose, hemicellulose and lignin) (Dhingra, Michael, Rajput, & Patil, 2012). Major physiological functions of DF are to increase the fecal weight by retaing water and the promotion of bacterial growth in the intestine (prebiotic action) (Spiller, 2001). Additionally, DF may have a protective effect against cardiovascular

diseases, diabetes, obesity and intestinal disorders (Anderson et al., 2009; Nsor-Atindana, Chen, Goff, & Zhong, 2017). Technologically, DF imparts hydrocolloidal properties as a food ingredient and it is used as a fat and sugar replacer, bulking agent and texture modifier (Dhingra et al., 2012). Cell wall material in fruits, vegetables, cereals and nuts as well as gums are the main sources of DF in human diets (Dhingra et al., 2012). Inulin and other fructo-oligosaccharides are water soluble sources of DF which can induce a range of health benefits as well as functional properties to several foods (Shoaib et al., 2016). Bacterial cellulose is naturally present in several fermented foods as well as produced from agricultural wastes (Castro et al., 2011; Iguchi, Yamanaka, & Budhiono, 2000).

Soft, cold-gelled microparticles have been obtained from whey protein isolate (WPI) and sodium alginate (NaAlg), and proposed to modify the rheological behavior of foods for the elderly (Leon et al., 2016). Gel microparticles (with sizes < 100 μm) can provide consistency to liquid foods (juices and beverages, milk) or be mixed with traditional meals such as soups and sauces to increase their viscosity (Ellis & Jacquier, 2009). Aguilera and Park (2016) have suggested that beyond their texture-imparting properties, these microgels may also be used as carriers for lipids, fiber and other nutrients.

The use of fillers in gel matrices (e.g., addition of a small proportion of particulate inclusions) usually results in composites with properties different than those of the parent material (Banerjee & Bhattacharya, 2012). Physical properties of texture-modified foods for the elderly are usually assessed by mechanical compression and penetration testing and small amplitude oscillatory shear rheometry (Wendin et al., 2010). Shi, Zhang, Phillips, and Yang (2014) reported that fiber particles obtained from sugar cane contributed to a firmer gel structure in foods. Alakhrash, Anyanwu, and Tahergorabi (2016) suggested that surimi gels filled with oat bran (up to 8 g/100 g) exhibited enhanced gel texture (measured as Kramer shear force and by texture profile

analysis), and provided a higher water retention after cooking. Recently, insoluble DF particles (i.e., microcrystalline cellulose, oat fiber and walnut shell flour) have been used as potential fillers and texture modifiers of myofibrillar gel systems (Gravelle, Barbut, & Marangoni, 2017). The objective of the present study was to assess the incorporation of different sources of DF into gelled microparticles and evaluate the microstructural characteristics as well as the mechanical and rheological properties of the resulting gels and pastes.

4.2 Materials and methods

4.2.1 Materials

Whey protein isolate (WPI) with a moisture content of 4.7% ± 0.3 and protein content of 97.7% ± 0.7 (dry basis) was purchased from Davisco (BiPRO, Davisco Foods Intl., MN, USA). Sodium alginate (NaAlg) was obtained from Gelymar Natural Extracts (Chile) with a composition of 38% mannuronic acid, 16% guluronic acid and 46% alternate units. Added dietary fiber (DF) sources were: a commercial inulin (IN) soluble fiber (Fibruline® Instant; Cosucra Groupe Warcoing S.A., Belgium); a colloidal product derived from microcrystalline cellulose (CC) (Avicel® cellulose gel, FMC Corporation Health and Nutrition, USA) having an average particle size of around 75 μm; a commercial oat fiber (OF) (Canadian Harvest® Oat Fiber 780 (SunOpta Grains and Foods Inc., MN, USA) with particle sizes between 75 and 100 μm; and, a bacterial cellulose (BC) supplied by the School of Engineering, Universidad Pontificia Bolivariana, Medellin, Colombia, and produced by fermentation (Castro et al., 2011). Additionally, a commercial maize starch-based thickener (TH) (Thick & Easy, Hormel Health Labs, Austin, MN), specially designed for the management of dysphagia, was used as control. The artificial VISCOFAN corrugated cellulose membranes with a caliber of 20 mm in diameter and 30-60 μm in thickness were purchased from Filter

Print Ltda. (Santiago, Chile), and calcium chloride obtained from Sigma Chemical Co. (St. Louis, MO, USA).

4.2.2 Preparation of gelled microparticles with dietary fiber

The general procedure to prepare gelled microparticles (GMP) is described in Leon et al. (2016). A solution of 6% WPI in distilled water was heated in a temperature controlled water bath at 80 °C for 30 min to denature and aggregate the whey proteins. Subsequently, NaAlg and the individual DF were incorporated to the WPI solution at a 5% level, except the oat fiber (2.5%) due to its high water holding capacity. Cold gelation of the mixed solutions of WPI, NaAlg and DF took place in artificial membranes, sealed on both sides and immersed in a 150 mM calcium chloride bath for 12 h at 5 °C, as previously reported by Leon et al. (2016). Cylindrical gels obtained after removal of the artificial membranes are referred to as bulk gels. The GMP with or without DF of different sources (referred to as GMP-DF, see Table 4.1) were obtained by mechanical size reduction of the respective bulk gels, first by triturating for one minute using a hand-held blender, and then homogenized into a paste with a high-speed blender (Ultra Turrax digital T25, IKA-Werke, Germany) for 5 min at 8000 rpm (Leon et al., 2016). The starch-based commercial thickener used as control was dissolved in distilled water at 0.08 g/mL to a pudding-like consistency.

4.2.3 Microstructural features of GMP with DF

Microstructural characteristics of the BC dispersion, GMP, GMP-IN and GMP-BC were evaluated with a scanning microscope (model TM 3000, SEM Hitachi, Tokyo, Japan). Samples were critical point dried, coated with gold/palladium and observed under a voltage of 15 kV. Micrographs were taken at 7000 × and selected images from at least three specimens per sample are reported. Additionally, small aliquots of GMP and GMP-DF samples were placed on glass slides, gently dispersed with one drop of

distilled water, covered with a coverslip and observed in an optical microscope (model SMZ-2T 2B, Nikon Corp., Tokyo, Japan), attached to a digital camera Toup Tek, Photonics (model UCMOS 08000, Zhejiang, China) with a resolution of 2540 × 1744 pixels.

4.2.4 Mechanical properties of gelled bulk gels

Cylindrical samples cut from bulk gels (20 mm diameter × 10 mm height) were subjected to uniaxial compression up to 80% strain in a texture analyzer (model TA.XT2 Plus, Stable Micro System Ltd., Godalming, UK) using a round plate 75 mm in diameter and a constant crosshead speed of 1 mm s^{-1}. All measurements were done in triplicate.

4.2.5 Rheological properties of GMP and GMP-DF pastes

GMP, GMP-DF with the consistency of a paste, and the starch thickener were subjected to small amplitude dynamic oscillatory measurements at 20°C using a Discovery hybrid rheometer (model RH-2, TA Instruments, New Castle, DE) with a 40 mm diameter parallel plate geometry and a 1 mm gap. To determine the linear viscoelastic region, stress sweeps were performed at 1 Hz. Then, frequency sweeps were performed over the range between 0.1 to 100 rad s^{-1} and the values of the storage or elastic modulus (G'), loss or viscous modulus (G'') and loss tangent (tan δ = G"/G') were calculated using the equipment software. All measurements were done at least in triplicate using fresh samples. Data were expressed as a power law model using a linear least squares fitting method:

$$G' = G'_1 \times \omega^m \qquad (1)$$

where G'_1 is defined as an effective coefficient (Pa × s^m), m is effective flow behavior index, and ω is the angular velocity (rad s^{-1})) (Quinchia et al., 2011; Steffe, 1996).

4.2.6 Two-cycle back extrusion test (TCBET)

Samples (around 20 g) of GMP, GMP-DF and the starch thickener were subjected to two consecutive back extrusion cycles using a TA.XTplus Texture Analyzer (Stable Micro System Ltd, Goodling, Surrey, UK). The sample holder (22 mm diameter and 60 mm height) and compression probe (15 mm diameter) were made in acrylic (Figure 4.1). Testing was done at a speed of 1 mm s^{-1} and a down stroke to 20 mm from the bottom of the cell (approximately 50% of the initial height of GMP) (James et al., 2011).

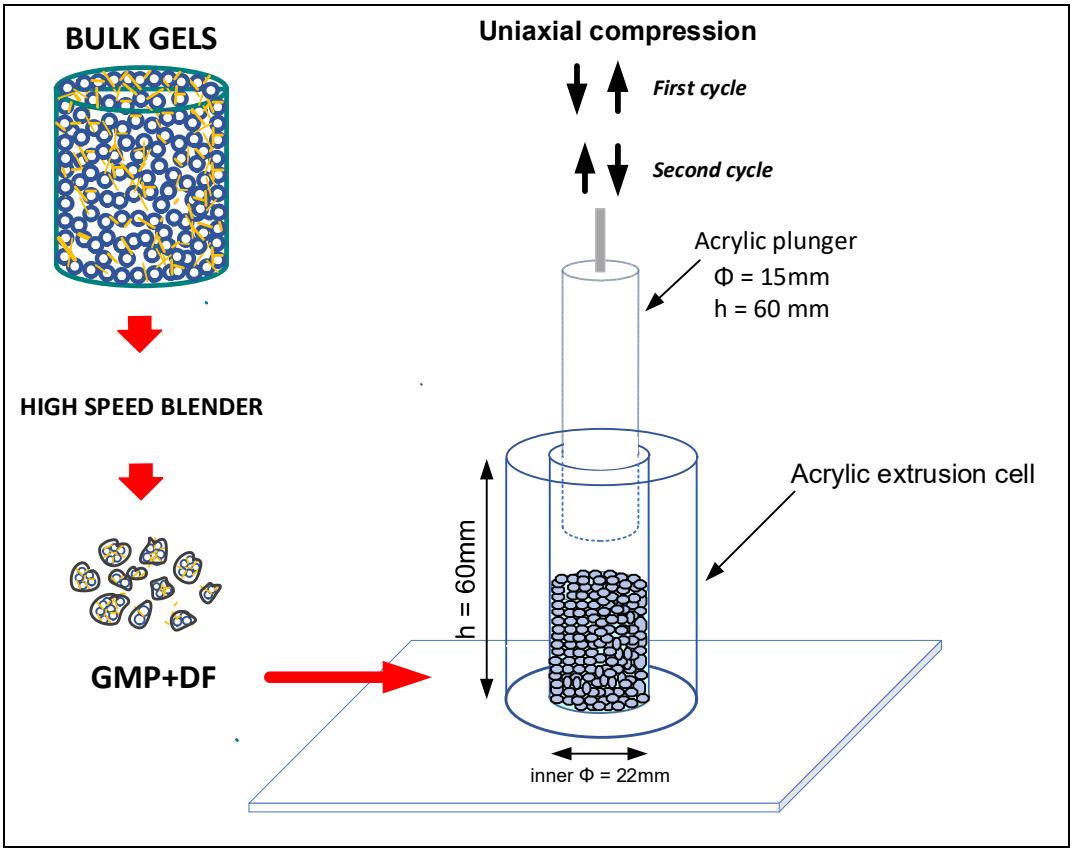

Figure 4.1 Scheme of the back extrusion cell used in the two-cycle compression test (TCBET) of pastes of GMP.

Results of the TCBET were interpreted as in the texture profile analysis (TPA), and they provided a proxy of the mechanical properties and textural attributes during oral processing of foods (Bourne, 2002; Liu, Deng, Gong, Han, & Wang, 2017; Wilkinson, Dijksterhuis, & Minekus, 2000). Parameters derived from the TPA (e.g., hardness, resilience, adhesiveness and cohesiveness) of semi-solid food gels have been used to evaluate foods for dysphagic patients (Devezeaux de Lavergne, van de Velde, van Boekel, & Stieger, 2015; Momosaki, Abo, & Kobayashi, 2013). Figure 4.2 shows a scheme of a typical TCBET curve and the parameters derived. Hardness is the force corresponding to the maximum height of the first curve. Resilience is the ratio of the upstroke and downstroke work of the first cycle (A2/A1). Adhesiveness is the area of the negative force curve (A3) and represents the adhesion of the sample to the plunger after the first cycle. Cohesiveness is the total area under the curve for the second cycle divided by the total area during the first cycle [(A4 + A5)/(A1 + A2)] and represents the degree of structure breakdown after the first cycle (Devezeaux de Lavergne et al., 2015; Liu et al., 2017; Marfil, Anhe, & Telis, 2012 ; Texture Technologies Corp. and Stable Micro Systems, 2018).

4.2.7 Experimental design and statistical analysis

Experiments were carried out following a complete randomized design with five treatments and three repetitions (Table 4.1). Statistical significance was determined using Statgraphics Plus software (Statistical Graphics Corporation, version 5.1, Rockville, USA) at a probability level of 0.05 ($p < 0.05$).

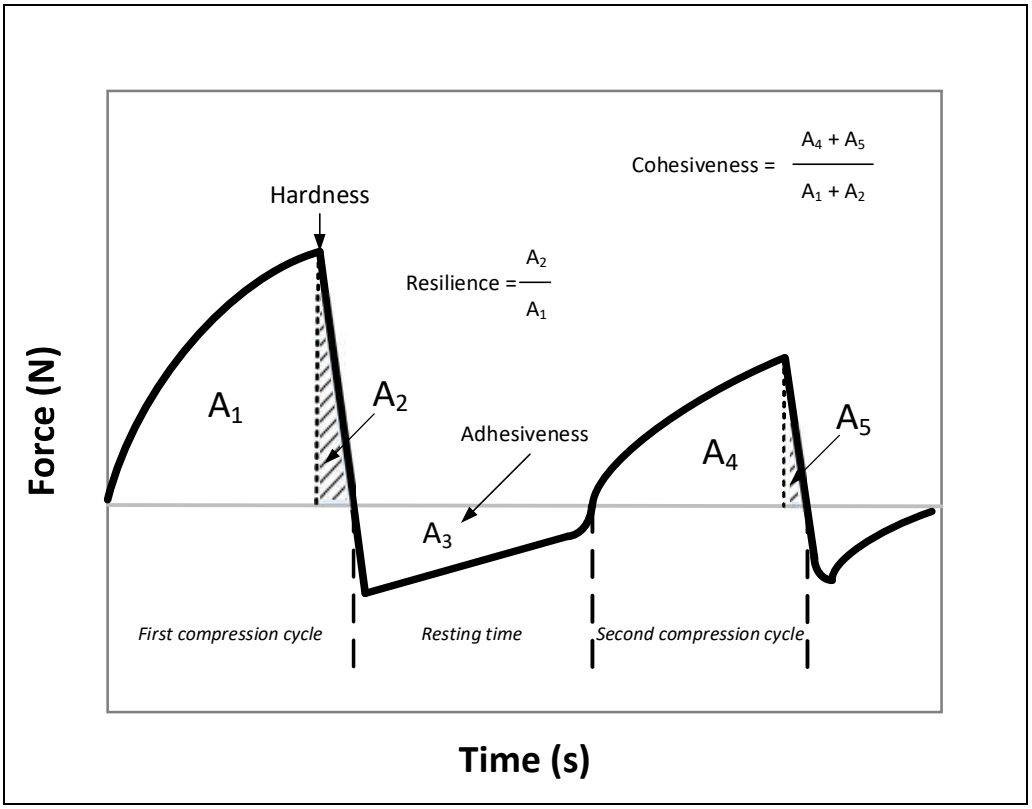

Figure 4.2 Scheme of two cycle penetration test (TCPT) to GMP and GMP-DF pastes and the starch thickener

Table 4.1 Complete randomized design of experiments and nomenclature

TREATMENT	Sample name	SYMBOL
T0	WPI/NaAlg	GMP
T1	WPI/NaAlg + soluble fiber	GMP-IN
T2	WPI/NaAlg + insoluble fiber	GMP-BC
T3		GMP-CC
T4		GMP-OF
T5	Commercial thickener	TH

4.3 Results and discussion

4.3.1 Microstructural features of GMP

Light microscopy images obtained for the four types of GMP-DF are shown in Figure 4.3. The gel matrix of all GMP-DF consisted of irregularly shaped clusters of agglomerates, most of them under 100 μm in size. These structures are typical of the process of aggregation during cold gelation of denatured whey proteins (Donato et al., 2011; Leon et al., 2016). GMP containing inulin were similar in size and structure to the GMP of pure WPI/NaAlg (Leon et al., 2016) which was expected since inulin is dispersed at the molecular level in the gel matrix (Figure 4.3A). The addition of bacterial cellulose was not appreciated at this scale of resolution, but apparently it brought clusters closer together as shown by some dark areas within the clusters (Figure 4.3B). Insoluble fiber particles with cylindrical shapes seemed to adhere to the surface of the GMP matrix in in GMP-CC. However, oat fiber particles protruded into the gel matrix in GMP-OF probably due to their good water holding capacity (Steenblock, Sebranek, Olson, & Love, 2011; Yamazaki, Murakami, & Kurita, 2005).

Scanning electron microscoscopy (SEM) permited a more detailed view of the microstructure of gel matrices, although they had undergone water removal by critical point drying, a process that usually induces changes from the fully hydrated state (Figure 4.4). Figures 4.4A and 4.4B, confirmed the microstructural similarity between GMP and GMP-IN matrices, and the predominant presence of aggregates as reported for mixed whey protein-polysaccharide cold-set gels (de Jong, Klok, & van de Velde, 2009). Figure 4.4C displays the microstructure of an entaglement of thin nanofibers of bacterial cellulose (BC). Figure 4.4D shows how cellulose nanofibers penetrated into the GMP matrix segregating it into fragments “glued” together by a separated network of

nanofibers. Similar microstructures were observed in synthetic gels to which BC had been added (Numata, Sakata, Furukawa, & Tajima, 2015).

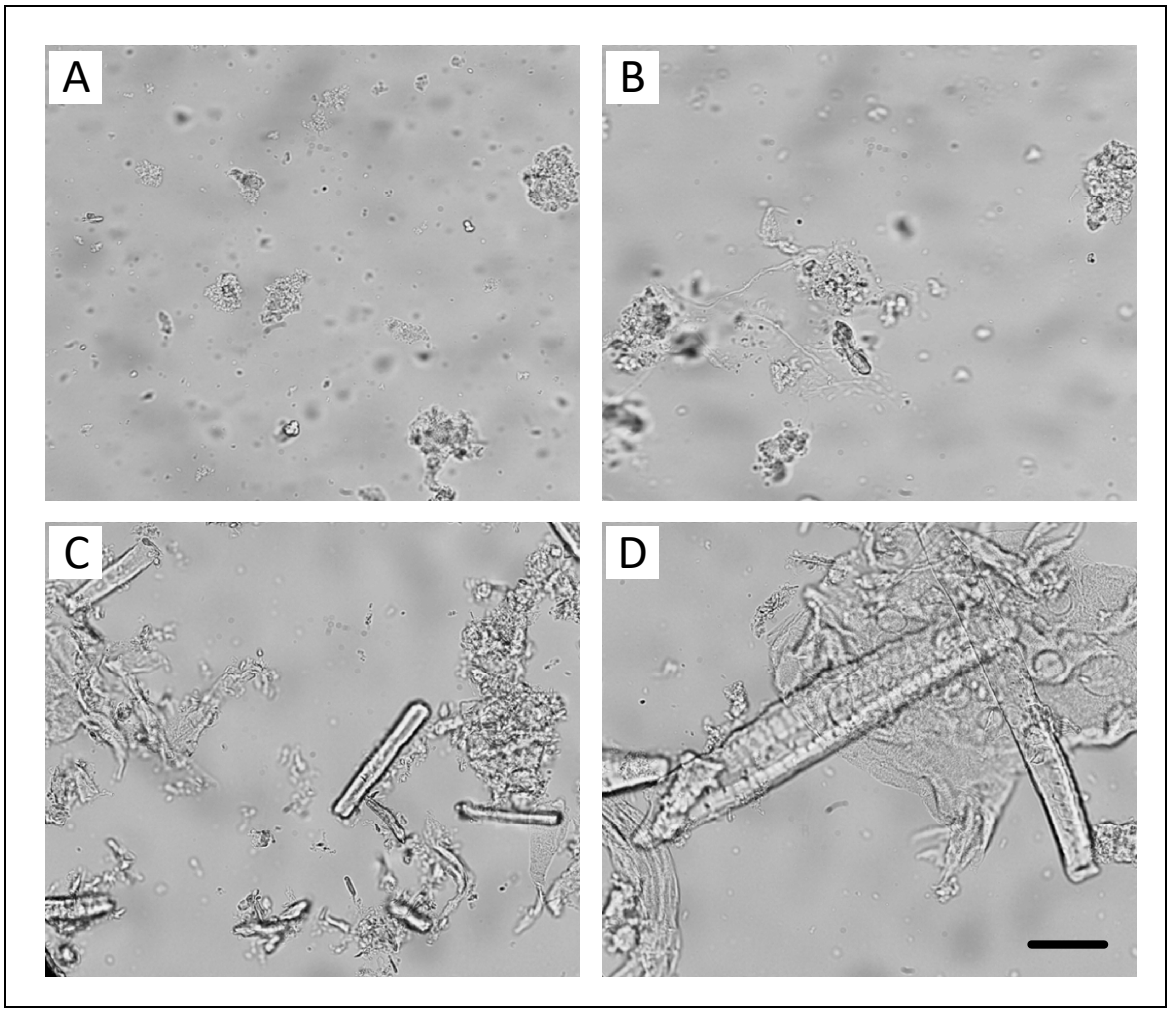

Figure 4.3 Light photomicrographs of GMP-DF. **(A)** GMP with 5% of IN, **(B)** GMP with 5% BC, **(C)** GMP with 5% of CC and **(D)** GMP with 2.5% OF. Marker = 50 µm (all figures).

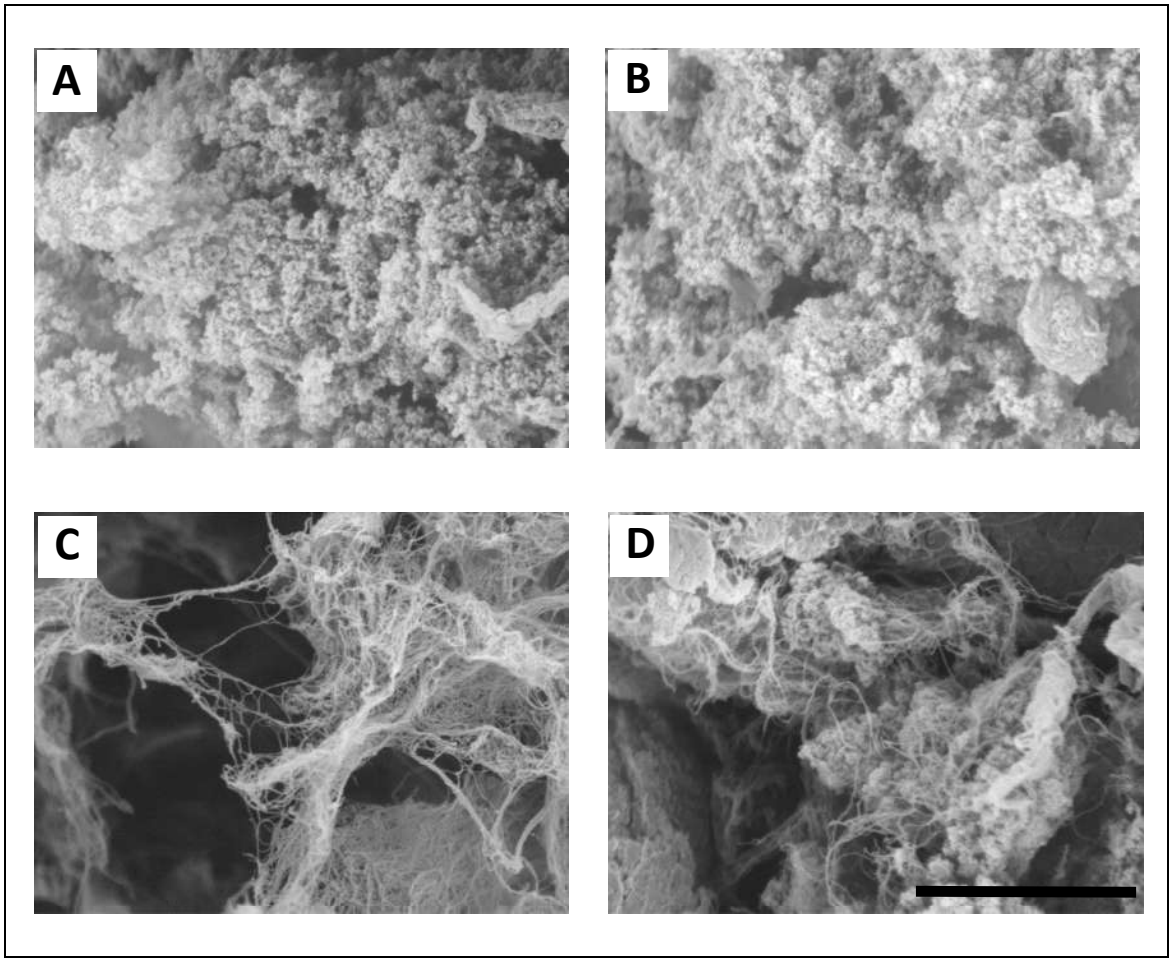

Figure 4.4 Scanning electron micrographs of GMP and DF. **(A)** WPI/NaAlg GMP; **(B)** WPI/NaAlg GMP with added inulin; **(C)** Bacterial cellulose fibers, and; **(D)** WPI/NaAlg GMP with added bacterial cellulose. Marker = 10 μm (all figures).

4.3.2 Mechanical properties of bulk gels

Stress-deformation curves of cylindrical bulk gels during uniaxial compression are shown in Figure 4.5. All treatments exhibited a similar behavior up to a strain of around 45%. Addition of insoluble fiber in the form of microcrystalline cellulose and oat fiber did not produced a notorious change in the mechanical behavior during

compression with respect to the fiber-less control (GMP) in the entire range of deformation. On the other hand, addition of inulin (GMP-IN) reduced the gel stress beyond a strain of about 45%. Evageliou, Tseliou, Mandala, and Komaitis (2010) also found that the strength and firmness of gellan gels decreased in presence of inulin and attributed it to the interference with the gelling mechanism. However, addition of bacterial cellulose fibers (GMP-BC) increased the strength of the gel up to about 60% strain, in fact, even reinforced the structure, as was also observed by Numata et al. (2015). Beyond this strain value a series of fracture events started to occur that weakened the gel. Based on the microstructure of GMP-BC in Figure 4D it can be surmised that beyond the 60% strain, the network of cellulose nanofibers gluing together the gel fragments started to collapse and the assembly of fragments was not as strong as the homogeneous (unbroken) gel matrix of the other GMP.

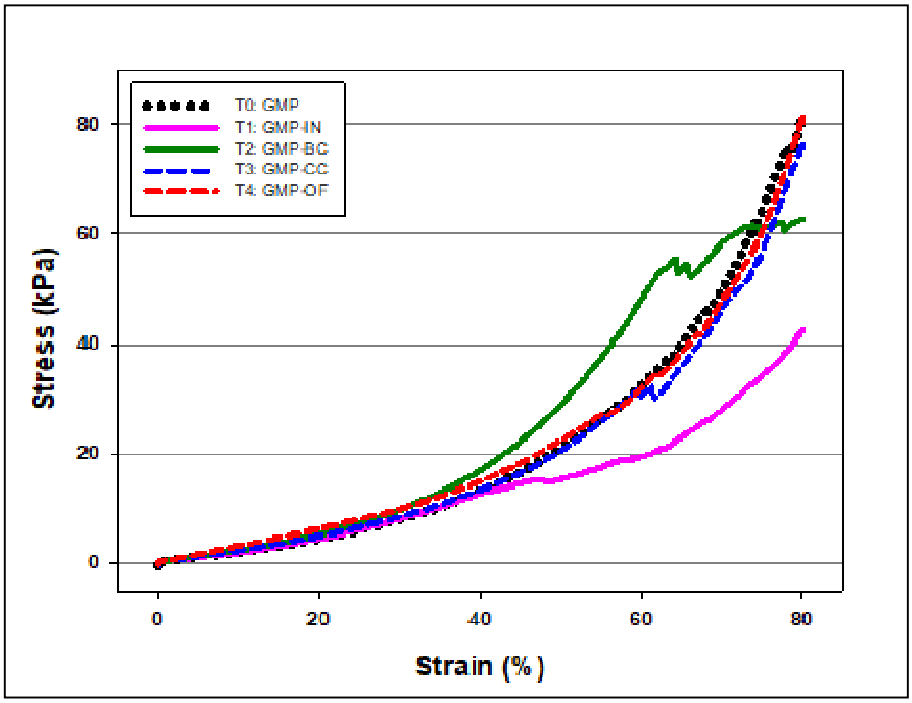

Figure 4.5 Stress-strain curves for uniaxial compression of cylindrical bulk gels.

4.3.3 Rheological properties of GMP, GMP-DF and commercial thickener

Mechanical spectra representing storage modulus (G') and loss modulus (G'') as a function of frequency (ω) for GMP, GMP-DF and the commercial thickener are shown in Figure 4.6. G' (Figure 4.6A) was higher than G" (Figure 4.6B) throughout the frequency range, indicating that there was a predominance of the elastic over the viscous behavior in all samples, as is typically observed for gelled structures (Nazir, Asghar, & Aslam Maan, 2017). Also, there was only a slight dependence of both moduli with frequency as it is often reported for structured liquids.

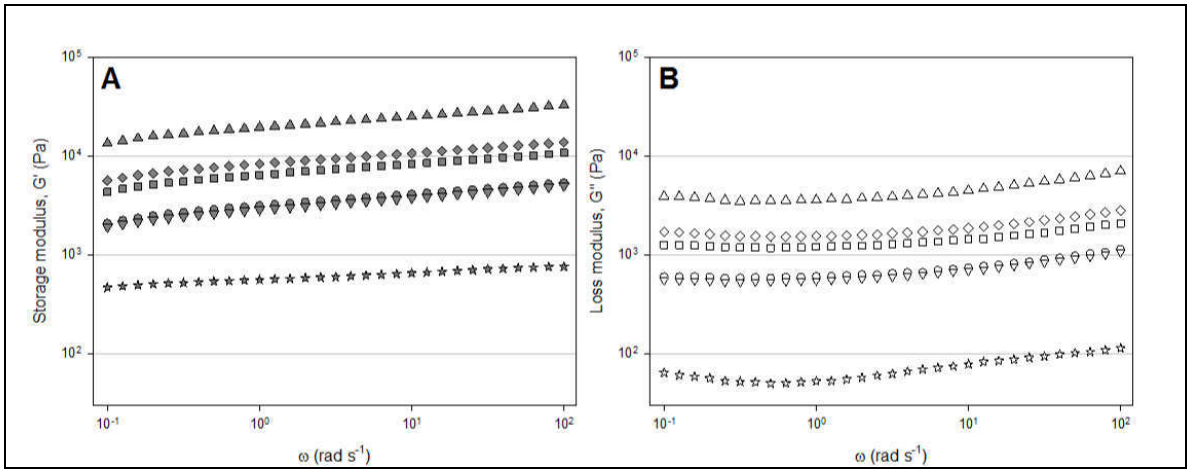

Figure 4.6 Mechanical spectra as a function of angular frequency of GMP with different DF and a commercial thickener. (○) T0 (GMP), (▽) T1 (GMP-IN), (□) T2 (GMP-BC), (◇) T3 (GMP-CC), (△) T4 GMP-OF and (☆) T5 (TH). A. G 'storage modulus (Pa) and B. G'' loss modulus (Pa).

Values of the viscoelastic moduli G' and G" for GMP-OF, GMP-CC and GMP-CB were higher than those of the control GMP and GMP-IN, suggesting that the

presence of insoluble DF in the GMP matrix provided a reinforcement effect (Le Goff, Gaillard, Helbert, Garnier, & Aubry, 2015; Wang & Chen, 2017). The presence of inulin, however, did not weaken the gel matrix. (Glibowski, 2009) indicated that a firm gel is formed between whey proteins and inulin due to their synergistic interactions. In summary, all GMP pastes can be described as weak gel-like systems, because G′ and G″ were almost parallel, G′ > G″ and both moduli varied with frequency (Ikeda & Nishinari, 2001; Picout & Ross-Murphy, 2003; Seo & Yoo, 2013). These characteristics suggest that GMP-DF pastes could contribute to form a bolus with a weak gel consistency, easy to masticate and swallow (Sayaka Ishihara, Nakauma, Funami, Odake, & Nishinari, 2011).

Mechanical spectra curves of GMP, GMP-DF and the commercial thickener were modelled according to a power law equation to describe the variation of the storage modulus G' with frequency (Quinchia et al., 2011). Parameters of the resulting equations are shown in Table 4.2. RMSE values were low for all G' data and adjusted R2 were superior and equal to 0.96.

Table 4.2 Parameters of the power law model for GMP, GMP-DF and commercial thickener

	Power law parameters of storage modulus (G')			
Treatments	G'_1 ($Pa \times s^m$)	m	R^2-Adj	RMSE ($Pa \times s^m$)
GMP	3831.33^{c}	0.1205^{a}	0.99	161.20
GMP-IN	3514.67^{c}	0.1204^{a}	0.96	292.90
GMP-BC	7758.67^{bc}	0.1207^{a}	0.98	353.67
GMP-CC	10008.30^{b}	0.1227^{a}	0.98	520.73
GMP-OF	23550.00^{a}	0.1286^{a}	0.98	995.90
TH	628.83^{c}	0.0695^{b}	0.98	12.23
Pudding[1]		0.15-0.16		

[1](Quinchia et al. 2011)

The effective flow behavior index (m) for all GMPs was similar (around 0.12; $P<0.05$) and significantly different from that of TH (0.07), probably because it was not a gelled matrix. The n value reported for a pudding (0.15-0.16) was slightly larger than that of GMP samples (Quinchia et al., 2011). The effective coefficient G'_1 of GMP-OF was much higher than those of other GMP, which only reflects the larger experimental values of G' of this sample.

Tan δ values for all samples were < 1 in the studied frequency range (Figure 4.7), suggesting that they exhibited predominantly elastic properties (Funami, 2016). Tan δ values were similar for GMP and GMP-DF (values between 0.17 and 0.28) and different from that of TH. Lower values of tan δ for TH could indicate that it was more "structured" (at the concentration used) than GMP and GMP-DF samples.

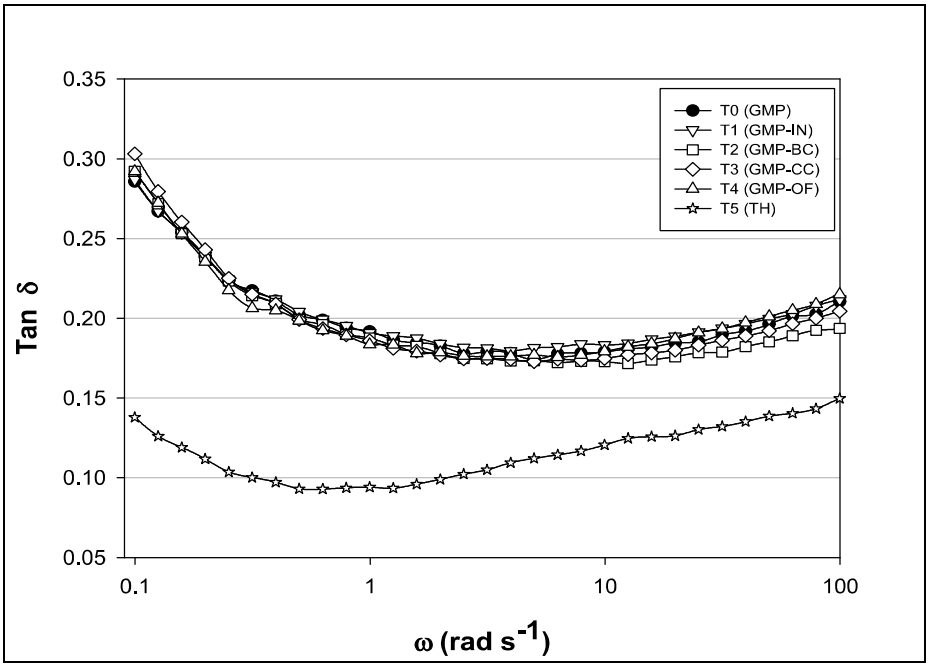

Figure 4.7 Loss tangent as a function of angular frequency for GMP pastes and the commercial thickener.

Values of tan (δ) in the range of 0.1–1 have been suggested as rheological criterion for safe-swallow foods destined for dysphagia patients (Sayaka Ishihara et al., 2011). Funami (2011) suggested that gels could be a good material for dysphagia diets due their viscoelastic character. Thus, GMP and GMP-DF not only may be used to modify the viscosity of liquid foods as suggested by Ellis and Jacquier (2009), but also to be consumed directly as food carriers.

4.3.4 Two-cycle back extrusion test (TCBET)

Main textural parameters determined for texture modified (TM) foods are hardness (hard to soft), adhesiveness (i.e., tendency of particles to adhere to surfaces) and cohesiveness (ability to form a swallow-safe bolus in the mouth) (Aguilera & Park, 2016). Determination of mechanical properties of wet particulate materials had been resolved using a back extrusion cell (Brusewitz & Yu, 1996; Reyes & Jindal, 1990). A two-cycle compresion test similar to the texture profile analysis (TPA) has been performed in a back extrusion cell to evaluate soft gels such as yogurt (James et al., 2011; Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004). Results of the TCBET are shown in Figure 4.8.

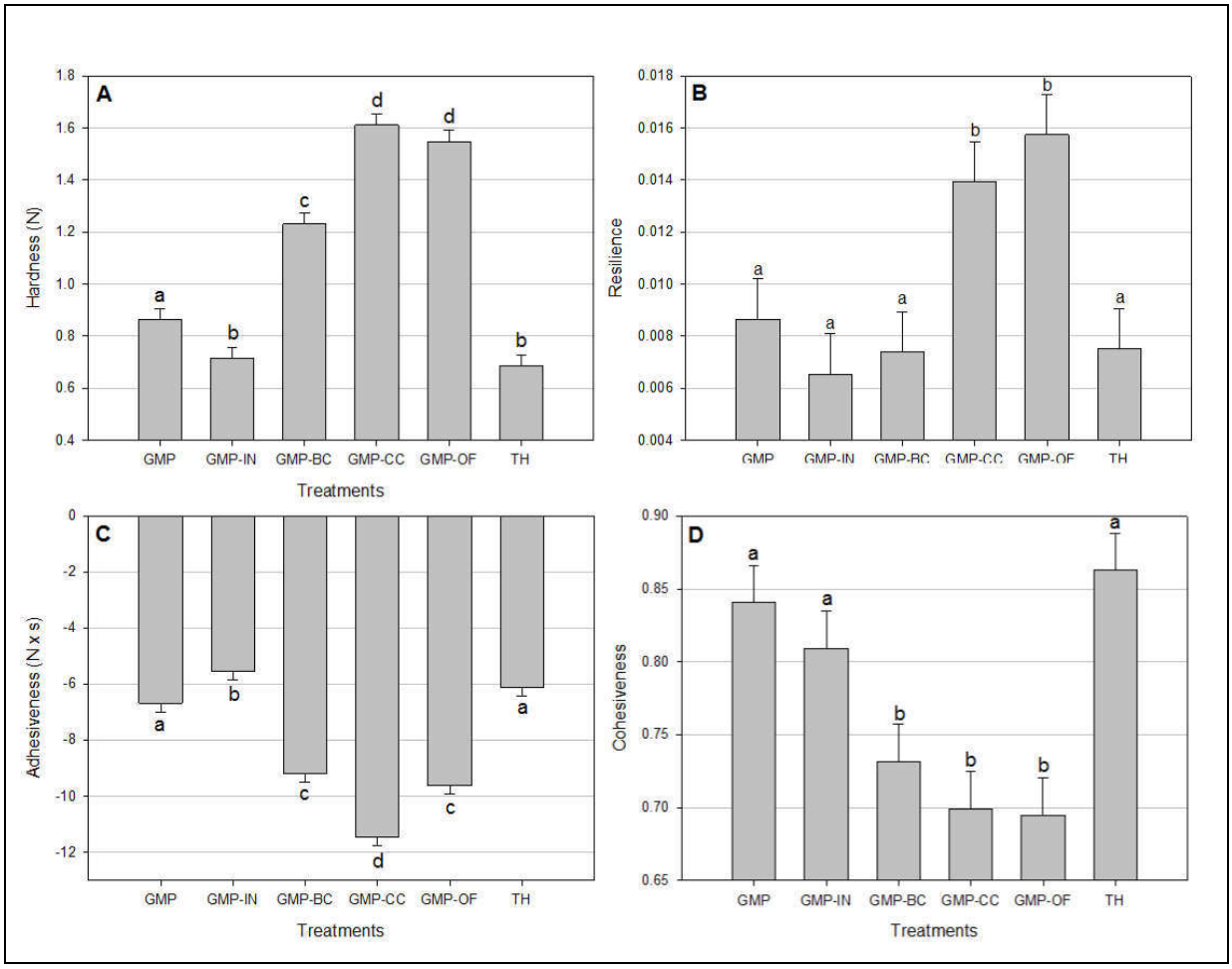

Figure 4.8 Two cycle back-extrusion test (TCBET) parameters for GMP, GMP-DF and the starch thickener. **(A)** Hardness; (B) Resilience; (C) Adhesiveness, and; (D) Cohesiveness.

Figure 4.8A shows that incorporation of the soluble DF inulin reduced significantly ($P<0.05$) the hardness of GMP from 0.87 to 0.72 N, a value similar to that of the commercial thickener TH (around 0.69 N). These results coincide with those obtained by Delgado and Bañón (2018) who reported that inulin decreased the gel strength of jellies when used unheated and at a low concentration. Conversely, addition of insoluble DF increased significantly the hardness of the matrix of GMP-CC and

GMP-OF, to values of ~1.57 N. Gravelle et al. (2017) found that microcrystalline cellulose and oat fiber increased the hardness of myofibrillar gels when they were used as filler particles due to their crowding properties. GMP-BC exhibited an intermediate value of hardness between that of GMP and the GMP-CC and GMP-OF samples.

Resilience or the capacity of pastes to spring back to their initial state and shape after the first cycle of compression (Figure 4.8B) was highest for GMP-CC and GMP-OF (~0.015), coincidental with their high hardness values (Figure 4.8A). GMP, GMP-IN, GMP-BC and TH had similar ($P<0.05$) and lower values (~0.009). Structural recovery is a determinant factor among rheological properties of the bolus in texture-modified foods for the elderly (Zargaraan et al., 2013). Addition of DF to GMP resulted in a wide range of adhesiveness values, from -5.53 to -11.45 N × s (Figure 4.8C). Adhesiveness of GMP and GMP-IN were similar to that of the commercial thickener TH (~ -6.1 N × s), suggesting ample possibilities to tune this parameter in texture modified foods by changing the fiber source or its concentration. Cohesiveness is an important characteristic of foods for the elderly. Cohesive TM-foods will not break easily into pieces in the mouth, thus, avoiding the scatter of small particles in the pharyngeal phase (Sayaka Ishihara et al., 2011). Average cohesiveness values of all samples studied varied between 0.69 and 0.87 (Figure 4.8D), however, values of treatments containing insoluble fiber particles were significantly lower than for GMP, GMP-IN and TH, yet, higher than recommended. Houjaij, Dufresne, Lachance, and Ramaswamy (2009), who also used TPA evaluate the cohesiveness of pureed therapeutic cakes, reported values of cohesiveness between 0.391 and 0.568. In particular, the low adhesiveness and high cohesiveness of GMP containing soluble DF as inulin are quite promising for the design of food matrices for the elderly (Ishihara et al., 2011; Momosaki et al., 2013). In summary, hardness, resilience, cohesiveness and adhesiveness of GMP and GMP-IN were quite similar or superior to those of the commercial thickener.

4.4 Conclusions

There is an urgent need of foods that combine good nutritional properties as well as adequate textural properties to feed the frail elderly. Fiber is an important nutrient due to its prebiotic as well as the anti-constipation effect. Different sources of dietary fiber (DF) were incorporated into the matrix of whey protein isolate/sodium alginate gelled microparticles (GMP) and their properties compared to those of a commercial thickener. Stress-strain curves for bulk gels were similar up to around 40% deformation, and beyond this value GMP containing DF differed in their mechanical behavior. Microstructural analysis demonstrated the effective incorporation of DF in the gel matrix while mechanical and rheological properties of GMP were influenced by the intrinsic physicochemical (e.g., water binding capacity, solubility, etc.) and physical properties (e.g., size and shape of particles, fibrousness, network formation) of DF. All GMP pastes and the control thickener exhibited a viscoelastic behavior typical of weak gels, thus, they were suitable as texture modifiers in foods for the elderly. Adhesiveness and cohesiveness values derived from the texture profile analysis performed in a back extrusion cell, in particular, those for GMP and GMP containing inulin were similar to those of the commercial thickener. These results must be complemented with sensory testing and actual applications of GMP in real foods and beverages, as well as validated by studies of the clinical efficacy against constipation.

5. GENERAL CONCLUSIONS

The most important findings are:

- WPI/NaAlg gel microparticles (GMP) were prepared by homogenization of bulk gels produced by diffusion of calcium ions into the mixed solutions and cold gelation. If larger particles were needed, GMP could be obtained by reducing the intensity of the size reduction method. A new back-extrusion test cell was built to assess the mechanical properties of GMP. The stress of bulk gels and GMP exhibited a good correlation, meaning that the back-extrusion method is an appropriate alternative to directly assess the mechanical properties of a paste of microparticles. The principal component analysis (PCA) allowed distinguishing softer GMP, with low content of NaAlg from firmer GMP having a high concentration of polysaccharide, thus corroborating the positive role of alginate in the structural formation of the mixed gel.

- Emulsion-gelled microparticles (EGM) consisting of a WPI/NaAlg gel network and encasing olive oil microdroplets were obtained using emulsification by sonication and high-shear homogenization, followed by cold gelation. Oil microdroplets emulsified by ultrasound were smaller than those produced by high-shear blending. A wide range of mechanical and rheological properties of EGM was attained through the investigated formulations. US-EGM pastes containing oil droplets emulsified by ultrasound were quite stable when heated to 60 °C and would be appropriate to formulate heated foods such as soups.

- There is an urgent need of foods that combine good nutritional properties as well as adequate textural properties to feed the frail elderly. Fiber is an important nutrient due to its prebiotic as well as the anti-constipation effect. Different sources of dietary fiber (DF) were incorporated into the matrix of whey protein

isolate/sodium alginate gelled microparticles (GMP) and their properties compared to those of a commercial thickener. Stress-strain curves for bulk gels were similar up to around 40% deformation, and beyond this value GMP containing DF differed in their mechanical behavior. Microstructural analysis demonstrated the effective incorporation of DF in the gel matrix while mechanical and rheological properties of GMP were influenced by the intrinsic physicochemical (e.g., water binding capacity, solubility, etc.) and physical properties (e.g., size and shape of particles, fibrousness, network formation) of DF. All GMP pastes and the control thickener exhibited a viscoelastic behavior typical of weak gels, thus, they were suitable as texture modifiers in foods for the elderly. Adhesiveness and cohesiveness values derived from the texture profile analysis performed in a back extrusion cell, in particular, those for GMP and GMP containing inulin were similar to those of the commercial thickener. These results must be complemented with sensory testing and actual applications of GMP in real foods and beverages, as well as validated by studies of the clinical efficacy against constipation.

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6. FUTURE OUTLOOK

This work on production and characterization of gelled microparticles contribute in this respect. However, much remains to be done. Suggestions for further research include the following:

- To assess the effect of the formulation (e.g., wider range of biopolymer concentration and other types of lipid phase) on the mechanical and rheological properties and to make an accurate characterization of the structural features of EGM.

- To assess how gelled microparticles can find applications as texture-modified foods for elderly people with mastication and swallowing dysfunctions, including the assessment of GMP-DF additions at different volume fractions on the viscosity of food liquids.

- To assess the capacity of GMP matrices to incorporate aromas and flavors into its microstructure and perform sensory tests of its acceptability in elderly people.

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