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Development of biopolymer-based nanoparticles for use in food formulation

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Riassunto

Le attività agricole (produzioni vegetali e animali) e agroindustriali, come il comparto della trasformazione di ortaggi e frutta, trasformazione olive e semi oleaginosi, generano rilevanti quantità di scarti e sottoprodotti di natura organica di ottima qualità, in quanto costituiti da parti della stessa materia prima, che vengono allontanate durante la raccolta nel caso di prodotti agricoli, o nel percorso lungo la linea di trasformazione industriale.

I residui agricoli consistono principalmente in tutte le parti della pianta che non rappresentano il prodotto principale, destinato ad uso alimentare umano o animale, come steli, foglie, tutoli, raspi, rami, ramaglie e legno. I sottoprodotti dall'industria agroalimentare invece derivano dalle operazioni di lavorazione dei vegetali e dei frutti o loro preparati per il consumo alimentare.

L'aumento della produzione di semi oleosi per soddisfare il fabbisogno di olio vegetale biologico è inevitabile, con conseguente aumento di sottoprodotti agricoli e industriali con un alto potenziale di valorizzazione.

Dopo l'estrazione dell'olio dai semi oleosi, il sottoprodotto risultante è una farina sgrassata. L'olio è utilizzato nell'alimentazione umana e nella produzione di biodiesel. La farina sgrassata, costituita essenzialmente da proteine e carboidrati, ha un elevato valore nutritivo. Pertanto, è importante procedere alla sua valorizzazione, garantendo una migliore sostenibilità globale dell'industria del biodiesel da semi oleosi. L'estrazione, la concentrazione e l'isolamento delle frazioni proteiche dalle farine sgrassate è fondamentale per la valorizzazione dei prodotti.

Le proteine, in particolare quelle ottenute da fonti vegetali, rappresentano un gruppo di ingredienti alimentari con ampia applicazione nell'industria alimentare, per esempio come proteine testurizzate, concentrate, isolate o idrolizzate, . Agli estratti ottenuti viene assegnata la denominazione di concentrati o isolati proteici, in funzione della loro concentrazione proteica su base secca. Pertanto, rientrano nella categoria di concentrati o isolati proteici quando il loro livello di concentrazione raggiunge rispettivamente il 65% e il 90%.

Le interazioni proteina-polisaccaride non solo svolgono un importante ruolo strutturale nei sistemi biologici (p. es., per il mantenimento dell'integrità cellulare e della divisione cellulare), ma anche a livello macroscopico per il controllo delle proprietà macroscopiche degli alimenti perché possono modificare la consistenza e le proprietà reologiche.

Inoltre, questo tipo di interazioni possono anche portare ad una modifica delle proprietà funzionali delle proteine agendo sulla loro carica superficiale con conseguente aggregazione.

Questi aspetti sono fondamentali se si pensa che è possibile ampliare le applicazioni nel campo delle tecnologie alimentari.

Sebbene la ricerca sulla coacervazione non sia una novità, la maggior parte delle produzioni scientifiche si è concentrata sullo studio delle interazioni che coinvolgono i polisaccaridi con le proteine animali. Negli ultimi dieci anni le proteine vegetali hanno ricevuto sempre più attenzione da parte dei consumatori che cercano fonti proteiche alternative a quelle animali per ragioni dietetiche, religiose e culturali. Tuttavia, la solubilità delle proteine vegetali in generale è inferiore a quella delle proteine derivate da fonti animali. Pertanto, sono state

tentate varie strategie per migliorare le loro prestazioni nei sistemi alimentari. Un approccio consiste nell'adattare le loro interazioni con i polisaccaridi per creare nuove caratteristiche e strutture superficiali.

Alla luce di questi presupposti, la ricerca proposta consentirebbe l'introduzione di una tecnologia innovativa basata principalmente sul meccanismo di interazione tra proteine e polisaccaridi in ambiente acquoso a basso impatto ambientale ed economicamente redditizia. Lo scopo di questa tesi di dottorato era la produzione di bio-nanoparticelle a partire da proteine estratte da farina sgrassata di semi di zucca, da impiegare come stabilizzanti nelle emulsioni ad alta fase interna e di investigare la loro applicazione come sostituto dei grassi in creme da farcitura. Il primo caso studio ha riguardato l'estrazione e la caratterizzazione delle proteine di semi di zucca al fine di migliorare la solubilità e la loro carica di solvatazione., Successivamente mediante uno studio di coacervazione con polisaccaride, è stato determinato l'effetto del tempo di trattamento agli HUI sulla solubilità, lo zeta *potential* e il raggio idrodinamico medio delle proteine di zucca. Questo studio mirava a indagare l'effetto dei tempi di trattamento con ultrasuoni ad alta intensità (HIUS) su sospensioni di proteine isolate di semi di zucca (PsPI) in funzione del pH (da 2,5 a 10,5), valutando la dimensione delle particelle, il potenziale zeta, l'emulsione e proprietà della schiuma. Le sospensioni acquose di PsPI con diversi valori di pH sono state sonicate per 5, 10 e 20 minuti a 150 W (durata dell'impulso 0,5 s). A livello globale, i risultati hanno mostrato che il trattamento prolungato con HIUS ha favorito la formazione di aggregati molecolari. Infatti, il trattamento di 5 minuti con HIUS è stato sufficiente per ottenere un aumento significativo della capacità schiumogena ed emulsionante rispetto al controllo non trattato, specialmente nell'intervallo di pH di 2,5-6,5. L'uso di HIUS si è rivelato un eccellente metodo di trattamento dipendente dal tempo per migliorare le proprietà fisico chimiche di PsPI, pertanto, il trattamento HIUS a 5 minuti è stato scelto come pretrattamento per il successivo caso studio. Il secondo caso studio ha riguardato le interazioni elettrostatiche e intermolecolari durante la formazione della struttura tra l'isolato proteico di zucca (PsPI) e il chitosano a basso peso molecolare (Ch) per rivelare i loro meccanismi di coacervazione complessa. A tal fine, la formazione del complesso è stata valutata in funzione del pH (2,5-10,5) e del rapporto tra proteine e polisaccaridi (PsPI:Ch, R = 1:1-120:1 p/p) mediante dynamic light scattering (DLS) analisi, ζ-potenziometria e resa in coacervato. La più alta coacervazione, che si è verificata con un rapporto R = 8:1 e un pH elettricamente neutro di 7,795, è stata rappresentata dalla resa più alta (94%) e dalla dimensione più piccola (R8:1 664 nm), mentre le caratteristiche morfologiche dei coacervati PsPI-Ch liofilizzati, determinati mediante microscopia elettronica a scansione, hanno fornito ulteriori informazioni sui processi associativi durante la coacervazione complessa. Inoltre, le interazioni molecolari tra PsPI e Ch sono state confermate dalla spettroscopia infrarossa in trasformata di Fourier (FTIR), che ha rivelato principalmente interazioni elettrostatiche. Nel terzo caso studio, sono state studiate la capacità dell'isolato proteico di semi di zucca e del chitosano di produrre emulsioni Pickering e la stabilità di quest'ultime. I risultati hanno mostrato che i coacervati sono stati in grado di stabilizzare le emulsioni O/W, e il contenuto massimo di olio dell'81% è stato fornito dalle particelle separate a pH 5,5. La capacità emulsionante dei coacervati PsPI-Ch era strettamente dipendente dalle loro concentrazioni, con emulsioni di Pickering ad alta fase interna (HIPE) che si formavano solo a concentrazioni

di stabilizzante pari a1,5% (p/v) e superiori. Gli HIPE hanno mostrato un'eccellente stabilità alle variazioni di pH da 2,0 a 10,0, al riscaldamento a 100 °C e alla centrifugazione, ma collassavano facilmente al congelamento e allo scongelamento; una maggiore concentrazione di stabilizzante ha comportato una migliore stabilità. Questo studio ha dimostrato l'applicabilità di coacervati PsPI-Ch nell'industria alimentare come stabilizzanti HIPE. L'ultimo caso studio aveva come obiettivo quello di sviluppare una crema da farcitura utilizzando un'emulsione Pickering, ad alta fase interna, stabilizzata dal complesso (PsPI-Ch) dove lo spesso strato che ricopre le gocce d'olio forma una barriera fisica, che impedisce la fuoriuscita di olio dalla struttura. In particolare, lo studio ha riguardato l'applicazione dell'emulsione Pickering (HIPE) al 3%, come sostituto del grasso in una crema da farcitura dolce. La crema era composta da amido, zucchero, acqua ed emulsione come fonte di grasso per la crema con emulsione (C) e olio come per la crema di controllo (CTR). Sono stati valutati i parametri fisico-chimici delle due formulazioni, le proprietà reologiche, in particolare la viscoelasticità con la determinazione del modulo elastico G' e del modulo viscoso G", e mediante test dinamici, per stimare la viscosità apparente (η). Infine, la stabilità fisica con l'utilizzo di un Turbiscan. I risultati ottenuti hanno mostrato che la crema di controllo è più strutturata e richiede maggiori sollecitazioni di taglio per scorrere rispetto alla crema con emulsione, in quanto presenta valori di modulo elastico inferiori rispetto a quest'ultima. Inoltre, entrambe le creme mostrano un comportamento pseudo-plastico simile a un gel di media forza e una leggera dipendenza dalla frequenza, ma la crema con emulsione mostra valori di viscosità a taglio zero significativamente più alti rispetto alla crema di controllo. Per quanto riguarda la stabilità fisica, espressa come ΔBS e TSI, la crema con emulsione, dopo 21 giorni, mostra valori di ΔBS e TSI significativamente inferiori e, soprattutto, raggiunge un plateau a differenza della crema di controllo che continua a subire forti fenomeni migratori all'interno della struttura. La maggiore stabilità è dovuta sia alla minore granulometria sia alla maggiore viscosità dell'intera struttura, oltre che al chitosano, presente nell'emulsione Pickering, che migliora la qualità degli alimenti.

Abstract

Agricultural activities (crop and livestock production) and agro-industrial activities, such as fruit and vegetable processing, olive and oilseed processing, generate significant amounts of organic waste and by-products of excellent quality, since they are initially composed of parts of the same material that, in the case of agricultural products, are removed during harvesting or industrial processing. Agricultural residues mainly include all plant parts that are not the main product and are intended for human or animal consumption, such as stems, leaves, cobs, stalks, branches, twigs and wood. Moreover, food agro-industrial by-products derive from the processing of vegetables and fruits or their preparations for food consumption. The increase in oilseed production to meet the demand for organic vegetable oil is inevitable and leads to an increase in agricultural and industrial by-products with high valorization potential. After extracting the oil from oilseeds, the by-product is a defatted flour. The oil is used in human nutrition and in the production of biodiesel. The defatted flour, which consists mainly of proteins and carbohydrates, has a high nutritional value. Therefore, it is important to advance its valorization to ensure better global sustainability of the oilseed biodiesel industry. Extraction, concentration and isolation of protein fractions from defatted flours is essential for product upgrading. Proteins represent a group of food ingredients that find wide application in the food industry as texturized, concentrated, isolated or hydrolyzed proteins, especially from plant sources. The extracts obtained are called protein concentrates or isolates, depending on their protein concentration in dry matter. They are protein concentrates or isolates when their concentration reaches 65% or 90%, respectively. Protein-polysaccharide interactions play an important structural role in biological systems (e.g., in maintaining cellular integrity and cell division) and in controlling the macroscopic properties of foods (e.g., flowability, stability, texture, and mouthfeel). They can also be used to control the functionality of proteins in foods by altering the surface chemistry of proteins and their aggregation behaviour. Controlling the interactions between plant proteins and polysaccharides can lead to the development of new complex electrostatic structures that can confer unique functionalities. This in turn may expand the variety of applications for which they are suitable. While coacervation research is not new, most work has focused on studying the interactions of polysaccharides with animal proteins. Plant proteins have attracted tremendous attention from both consumers and industry in the last decade due to the increasing focus on the safety of animal-based foods, religious and cultural-based diets, and lower costs than animal protein. However, the solubility of plant-based protein products is generally lower than that of proteins from animal sources. Therefore, several strategies have been explored to improve their performance in food systems. One approach is to engineer their interactions with polysaccharides to create new surface features and structures. Considering these assumptions, the proposed research would allow the introduction of an innovative technology based mainly on the mechanism of interaction between proteins and polysaccharides in an aqueous environment, with low environmental impact and economically viable. The aim of this PhD was to produce bio-nanoparticles from proteins extracted from defatted pumpkin seed flour to be used as stabilizers in high internal phase emulsions and to

study their application as substitutes for fats in creams from fillings. The first case study involved the extraction and characterization of pumpkin seed proteins to improve the solubility and solvation charge of pumpkin proteins. For the subsequent study of coacervation with polysaccharide, the effect of HUI treatment time on the solubility, zeta potential, and mean hydrodynamic radius of pumpkin proteins was determined. The aim of this study was to investigate the effect of high-intensity ultrasound (HIUS) treatment times on suspensions of pumpkin seed protein isolate (PsPI) as a function of pH (from 2.5 to 10.5) by evaluating particle size, zeta potential, and emulsion and foam properties. Aqueous PsPI suspensions with different pH values were sonicated at 150 W (pulse duration 0.5 s) for 5, 10, and 20 min. Overall, the results showed that prolonged treatment with HIUS promoted the formation of molecular aggregates. In fact, a 5-min treatment with HIUS was sufficient to achieve a significant increase in foam formation and emulsification capacity compared to the untreated control, especially in the pH range of 2.5-6.5. The use of HIUS proved to be an excellent time-dependent treatment method to improve the physicochemical properties of PsPI, and therefore the 5-min HIUS treatment was chosen as the pretreatment for the next case study. In the second case study, the electrostatic and intermolecular interactions during the structure formation between pumpkin protein isolate (PsPI) and low molecular weight chitosan (Ch) were investigated to reveal their complex coacervation mechanisms. To this end, the formation of the complex as a function of pH (2.5-10.5) and protein to polysaccharide ratio (PsPI:Ch, R = 1:1-120:1 w/w) was evaluated by dynamic light scattering (DLS) analysis, ζ-potentiometry, and coacervate yield. The highest coacervation, which occurred at a ratio of R = 8:1 and an electrically neutral pH of 7.795, was characterized by the highest yield (94%) and the smallest size (R8:1 664 nm), while the morphological features of the lyophilized PsPI-Ch coacervates determined by scanning electron microscopy provided further insight into the associative processes during complex coacervation. In addition, molecular interactions between PsPI and Ch were confirmed by Fourier transform infrared spectroscopy (FTIR), which revealed mainly electrostatic interactions. In the third case study, the ability of pumpkin seed protein isolate (PsPI) and chitosan (Ch) to prepare Pickering emulsions and the stability of the resulting emulsions were investigated. The results showed that the coacervates were able to stabilize the O/W emulsions and that the separated particles had a maximum oil content of 81% at pH 5.5. The emulsifying ability of the PsPI-Ch coacervates was strictly dependent on their concentrations, with emulsions with high internal phase pecking (HIPE) forming only at stabilizer concentrations of 1.5% (w/v) and higher. The HIPEs showed excellent stability to pH changes from 2.0 to 10.0, heating to 100°C, and centrifugation, but collapsed easily upon freezing and thawing; higher stabilizer concentration resulted in better stability. This study demonstrated the applicability of PsPI-Ch coacervates in the food industry as HIPE stabilizers. The final case study aimed to develop a filling cream using a Pickering emulsion with a high internal phase stabilized by pumpkin chitosan-protein complex (PsPI-Ch), with the thick layer covering the oil droplets forming a physical barrier to prevent oil from leaking from the structure. The cream consisted of starch, sugar, water, and emulsion as the fat source for the emulsion cream (C) and oil as in the control cream (CTR). The physicochemical parameters of the two formulations were evaluated. The viscoelastic properties were evaluated through oscillatory dynamic tests to determine the elastic modulus G' and the viscous modulus G".and

dynamically to estimate the apparent viscosity (η), whereas the Turbiscan was used to determine the physical stability The results obtained show that the control cream is more structured and requires larger shear stresses to flow than the emulsion cream, having lower elastic modulus values than the latter. In addition, both creams exhibit medium-strength, gel-like, pseudoplastic behaviour and a slight frequency dependence, but the emulsion cream exhibits significantly higher zero shear viscosity values than the control cream. As for physical stability, expressed as ΔBS and TSI, the emulsion cream shows significantly lower ΔBS and TSI values after 21 days and, most importantly, reaches a plateau in contrast to the control cream, which continues to show strong migration phenomena within the structure. The greater stability is due both to the smaller particle size and the higher viscosity of the whole structure and to the chitosan contained in the Pickering emulsion, which improves the quality of the food.

Chapter 1

1. Introduction

1.1 Protein-polysaccharide interactions

A great variety of food ingredients and products is composed of proteins and polysaccharides, both contributing to the system structure through their thickening, gelling, stabilizing, or interfacial activity. The functional properties of the individual polymers are already well characterized at the molecular level but, on the other hand, the role of protein:polysaccharide interactions concerning their functionality in complex multiphase systems, such as dispersions, emulsions, or food gels, is still subject of extensive research to explore properties arising from these mixtures(Telis, 2012) Protein and polysaccharide mixtures that, under certain circumstances, result in phase separation are useful for creating new structures; the addition of polysaccharides, even at low concentrations, can make a great difference in both the structural and rheological properties, causing great changes in viscosity, affecting consistency or texture, with only small effects on the other organoleptic properties (Renard et al, 2006). The use of protein:polysaccharide electrostatic complexes as emulsifiers has received considerable attention, particularly in the encapsulation of active compounds (Cooper et al, 2005)

Proteins can interact with polysaccharides through a variety of physical or chemical mechanisms, inducing structural transformation that provide new functions. Proteins have a net negative charge above their isoelectric point (pI) and a net positive charge below this pH. Proteins with a high net negative charge (pH > pI) exert a repulsive force on anionic polysaccharides. However, if the pH is adjusted around the pI of the protein (pH ≅ pI), the cationic segments on the protein interact with the anionic groups on the polysaccharide, resulting in weak electrostatic complex formation. Further decrease of the pH (pH < pI) leads to increased interaction between the protein and the polysaccharide, resulting in phase separation of the fully neutralized complex, also called coacervate (Figure 1.1). Complex coacervation is known as associative, liquid-liquid phase separation that occur in solution of oppositely-charged macromolecular species. This leads to complexation between the polymers, which may result in soluble complexes or separation of associated phases. The last case is characterized by two coexisting phases, one of them rich in solvent with a very low concentration of biopolymers, and a biopolymer-rich phase that is called coacervate (Doublier et al, 2000). The coacervation process is governed by the enthalpic contribution to the Gibbs energy of the mixture (Perrechil et al, 2008, Spagnuolo et al, 2005)

De Jong (1949) verified that an aggregative phase separation depends on the biopolymers concentration, molecular weight, ratio of polyelectrolytes, ionic strength, pH and temperature. This study was the basis for most of the research on the coacervation phenomenon. In general, miscibility occurs at low polymer concentrations, while incompatibility or coacervation are observed at high concentrations. There are few cases – for example, serum albumin, and pectin mixtures (Semenova et al, 1991) – in which a genuine

and complete miscibility is observed in concentrated dispersions of two biopolymers. Most cases show some type of soluble complexation, complex coacervation, or amorphous coprecipitation, depending on the biopolymer structure and the conditions of the solution (pH, ionic strength, etc.)

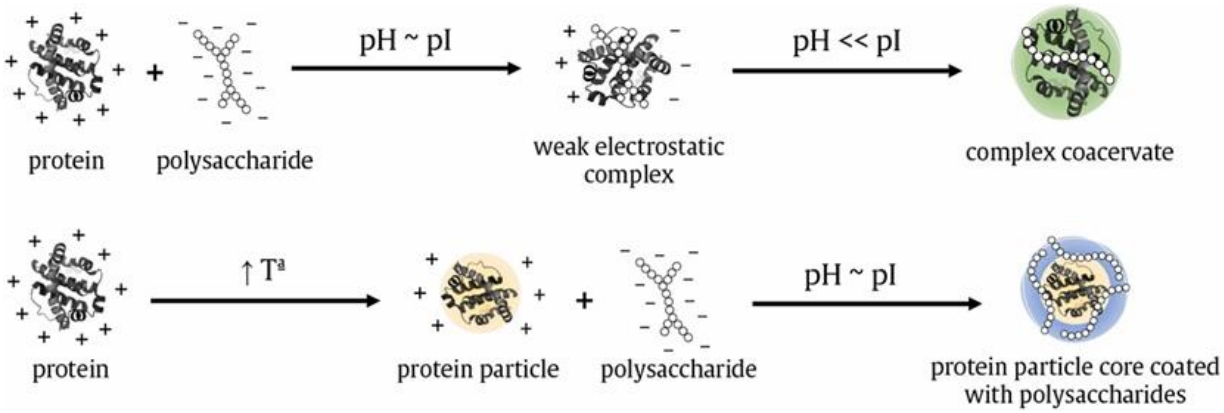

Figure 1.1 - Schematic representation of the two non-covalent protein-polysaccharide interactions (Ribeiro et al., 2021).

1.2 Protein-polysaccharide complexes as emulsion stabilizers

The protein component plays a key role in the formation of small droplets during homogenization, while the polysaccharide component contributes to better stability to environmental stresses (temperature, pH and ionic strength) (Ma et al., 2020). The complex exhibits the best properties of both components and has a synergistic effect, as their combination confers better kinetic stability and a thicker boundary layer to the Pickering O/W emulsions (Araiza-Calahorra et al., 2018). The formation of the protein-polysaccharide complex is often the result of an electrostatic interaction between two molecules with opposite charges, and the strength of the interaction determines the solubility of the complex (Yousefi & Jafari, 2019). Emulsion stabilization as a result of protein:polysaccharide soluble conjugates may be at least partly achieved by mechanisms related to steric effects. In order to provide steric stabilization, it is desirable that, in addition to the hydrophobic groups that will remain permanently attached to the oil droplets' surface, the macromolecular structure formed at the oil–water interface presents a large fraction of hydrophilic chain segments that would be able to protrude from the interface, thus increasing the thickness of the stabilizing layer (Dickinson et al., 2008).

Furthermore, if this macromolecular structure contains charged groups that contribute to increasing the net repulsive electrostatic interactions, it would help to prevent aggregation of adjacent droplets caused by the attractive van der Waals forces. Proteins alone may not be able to fulfil all these requirements, which could otherwise be provided by the supramolecular structures established from the associative interactions of polysaccharides with the stabilizing protein layer after the emulsion formation.

Wang & Heuzey (2016a) established a complex through the electrostatic interaction between chitosan and gelatin. They demonstrated that only at pH 5 < pH < 6.5 the two biopolymers had

opposite charges and formed insoluble complex particles. They also found that the Pickering emulsions stabilized with insoluble particles had smaller droplet sizes and lower long-term stability than the emulsions stabilized with soluble particles. The protein-polysaccharide complex can not only form stable emulsions, but also stabilize Pickering emulsions with high internal phase.

Huang et al. (2019a) formulated chitosan-caseinophosphopeptide nanocomplexes by electrostatic interactions, and the final result was a 3D network with high internal phase and ideal structure. The particles formed a dense interfacial layer around the droplets, inhibited the oxidation of linseed oil, and improved the gastrointestinal bioaccessibility of encapsulated curcumin by 28.72% in vitro.

The core of Pickering emulsions for food use consists of edible particles. The particles used to stabilize high internal phase Pickering emulsions fall into three categories:

- polysaccharides, such as nanocrystalline cellulose, chitin and starch.
- proteins, e.g., gluten proteins, soy.
- complex particles, e.g., polysaccharide-polysaccharide, polysaccharide-protein complexes (Hao et al., 2020).

1.3 Polysaccharides

Polysaccharides are biopolymers consisting of one or more types of monosaccharide units linked together by glycosidic bonds. They can vary in structure (linear or branched), degree of branching, molecular mass, polarity, electrical charge and degree of hydrophobicity (Li Haiming, et al., 2019). However, very often their native structure is not stable and chemical modifications are required to use them as pickering particles (Yang et al., 2018).

1.3.1 Chitosan

Chitosan is a linear cationic polymer composed of 2-amino-2-deoxy-d-glycopyranose and 2-acetamido-2-deoxy-d-glycopyranose units linked by β-1,4-glycosidic bonds (Figure 1.3.1). This polymer is obtained by partial deacetylation of chitin and plays a structural role in the exoskeleton of crustaceans, mollusks, annelids, coelenterates, and insects. It is also found in the cell walls of some fungi, mainly in the Mucorales (phylum Zygomicota).

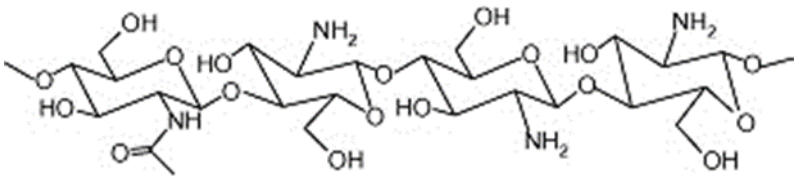

Figure 1.3.1- Chemical structure of chitosan (Mourya et al., 2008).

Chitosan is commercially produced from chitin by treatment with alkali. The exoskeleton of crustaceans is currently the main industrial biomass source for the production of chitin.

Crustacean shell wastes consist of proteins (20-40%), calcium and magnesium salts (mainly carbonate and phosphate, 30-60%), chitin (20-30%), and lipids (0-14%). These proportions vary according to species and season.

Being a polycationic compound (pKa = 6.2-6.8), a very rare property in nature, it can easily interact with negatively charged substances such as proteins, anionic polysaccharides (e.g., alginate, carrageenan, pectin, etc.), fatty acids, bile acids, and phospholipids, due to the high density of amino groups present in the polymer (Agullò et al., 2003).

The study of chitin, chitosan and derivatives is of great interest in the food industry due to the potential of their use. So far, the most important and widely used applications in the food industry are the production of edible films (mainly for the controlled transfer of moisture, for the release of antimicrobial and antioxidant substances, or as reverse osmosis membranes), antimicrobial agents (bactericides and fungicides), additives (fining and deacidification of fruits and beverages, emulsifiers, thickeners and stabilizers, color stabilization), nutritional quality (dietary fiber) and other applications (immobilization of enzymes, encapsulation of nutraceuticals). An interesting property from a food technology perspective is that chitosan has been shown to have good emulsifying properties. In a study conducted by De Blanco et al. (1999), the effects of the degree of deacetylation of chitosan on the properties of emulsifiers were investigated. All chitosan emulsions were very stable to temperature changes and aging. They were also stable to flocculation and coalescence. This could be due to the formation of a highly hydrated steric-mechanical barrier on the surface of the droplet, which is also positively charged. This barrier prevented the contact of the oil droplets due to mechanical and electrical constraints. The best degree of deacetylation for the emulsification of the oil was between about 80 and 89%, with two optimal values, 81 and 88%, where the system was a homogeneous emulsion without free oil (De Blanco et al., 1999).

Wang & Heuzey (2016b) also studied the effect of ultrasonic treatment on chitosan and chitosan-stabilized Pickering emulsions. Ultrasound treatment reduces the molecular weight of chitosan, which promotes the formation of more stable emulsions. Moreover, complexation of chitosan with other biopolymers is considered as a simple alternative to the currently used chemical surface modification strategies to improve its functionality.

A promising protein-polysaccharide complex between gliadin and chitosan has been developed. Li et al. (2019) investigated Pickering emulsions stabilized with gliadin-chitosan nanoparticles (GCNPs). Three types of GCNP structures, including primary complexes, soluble complexes, and coacervates, were obtained by simply changing the pH to produce different interaction levels. The enhancement of gliadin-chitosan interactions decreased the surface charges of the particles and improved their wettability to be absorbed at the O/W interface. The soluble complexes and coacervates of gliadin-chitosan stabilized multiple oil droplets by binding them together and forming a strong network structure against coalescence. Moreover, the Pickering emulsion stabilized with coacervates exhibited the highest viscoelasticity and solid-like behaviour. The results showed that such interactions can regulate both stability and rheological stability.

1.4 Protein

Proteins are amphiphilic molecules composed of anionic, cationic, and neutral amino acids that regulate electrical properties at different pH and salt concentrations (Damodaran, 2005). Proteins can stabilize highly concentrated emulsions by forming a physical barrier between droplets thanks to steric and electrostatic interactions (Hao et al., 2020). To date, plant proteins are the cheapest and most abundant source and, most importantly, an excellent alternative to animal proteins in various food formulations.

The functional properties of proteins, such as solubility, gelling ability, rheological properties, water and oil holding capacity, emulsifying ability, and foaming, are influenced by molecular size and charge distribution (Shevkani et al., 2015), which is why various techniques are used to prepare plant proteins as Pickering emulsifiers: centrifugation, pH regulation, heat treatment, ultrasonic treatment.

1.4.1 Pumpkin seed protein

In a circular economy, it is very important to try to recycle all the by-products of the business. Even more interesting is the possibility of giving economic and nutritional value to the latter. The remaining cake after the extraction of pumpkin seed oil, which is considered a by-product and is mainly used as a food supplement and for animal feed, has a protein content of up to 60-65%. The high protein content of pumpkin seeds has also led to an increase in demand for these products among health-conscious consumers who maintain a healthy lifestyle. Protein can be incorporated into products as a concentrate or isolate and used as an emulsifier, foaming agent, or to fortify foods (Dotto and Chachab, 2020).

Studies characterising pumpkin seed proteins have shown that the major protein fraction consists of a 12S globulin called cucurbitin, which is homologous to proteins found in legume seeds. The cucurbitin molecule has a molecular weight of 325 kDa and consists of six subunits that are similar to each other. These subunits in turn contain two disulfide-linked polypeptide chains with molecular weights of approximately 33 kDa and 22 kDa (Rezig et al., 2013).

Bucko et al. (2015) found by tensiometric analysis that PsPI proteins (pumpkin seeds protein isolate) adsorb at the oil-water interface over a wide range of pH values and ionic strengths, indicating the formation of protein films around the oil droplets emulsified with them. However, pumpkin seed protein ingredients have not yet been widely introduced into food applications. This is probably due to limited information on their structural and functional properties.

1.5 Pickering Emulsions

Many of the foods we consume every day consist of several immiscible phases, such as oil and water. The resulting droplets of one liquid phase dispersed in the other are called emulsions, which may be oil-in-water (O/W) (e.g., milk, salad dressing, or mayonnaise) or water-in-oil (W/O) emulsions (e.g., butter).

The use of solid particles as stabilizers for oil droplets in water dates back over a century (Binks, 2002). As reported by Berton-Carabin & Schroën (2015), the first studies documenting

particle-stabilized emulsions, also called Pickering emulsions, were published by Ramsden and Pickering and have attracted increasing interest in recent years. Since an emulsion stabilized by solid particles is called a Pickering emulsion, a high internal phase emulsion stabilized by solid particles is also called a "Pickering high internal phase emulsion" (Weiss et al., 2020).

A high internal phase emulsion, i.e., a highly concentrated emulsion system, has a minimum dispersed phase volume fraction greater than 74% (Kim et al., 2017). Unlike conventional emulsions, which tend to destabilize over time due to gravity separation, droplet aggregation, and Ostwald ripening, high internal phase emulsions have shown excellent stability to physical (coalescence), chemical (oxidation and hydrolysis) and environmental (temperature, pH, and ionic strength) conditions (Ma et al., 2020).

In the past, high internal phase emulsions have been stabilized by inorganic particles in combination with synthetic surfactants, but some health side effects have been noted with the use of such stabilizers, such as impairment of normal gastrointestinal tract, gut microbiota, and cellular toxicity (Mcclements & Xiao, 2017).

In multiphase food systems, protein and polysaccharide particles in the form of nanoparticles or microparticles can act as stabilizers and thickeners (Dickinson, 2012).

Recently, particles based on biopolymers such as proteins and polysaccharides have been proposed as an alternative for highly concentrated edible emulsions that also meet consumer demand for "natural" products and the industry is geared towards to supply "clean label" foods. The wettability, size, and surface charge of Pickering particles play a critical role in the formation of high internal phase Pickering emulsions, as the particles can readily adsorb and irreversibly anchor to the oil-water interface to prevent particle aggregation droplets, which form a steric barrier (Wang et al., 2020).

The main advantages of using biopolymers (Figure 1.5) for stabilizing Pickering emulsions are: well-controlled droplet size distribution and robust internal network, low toxicity, superior stability with improved shelf life of months to even years.

In recent years, researchers have found that high internal phase Pickering emulsions can be prepared with even higher internal phase volume (ϕ disp ≥ 0.80) at lower particle concentration (Jin et al., 2017).

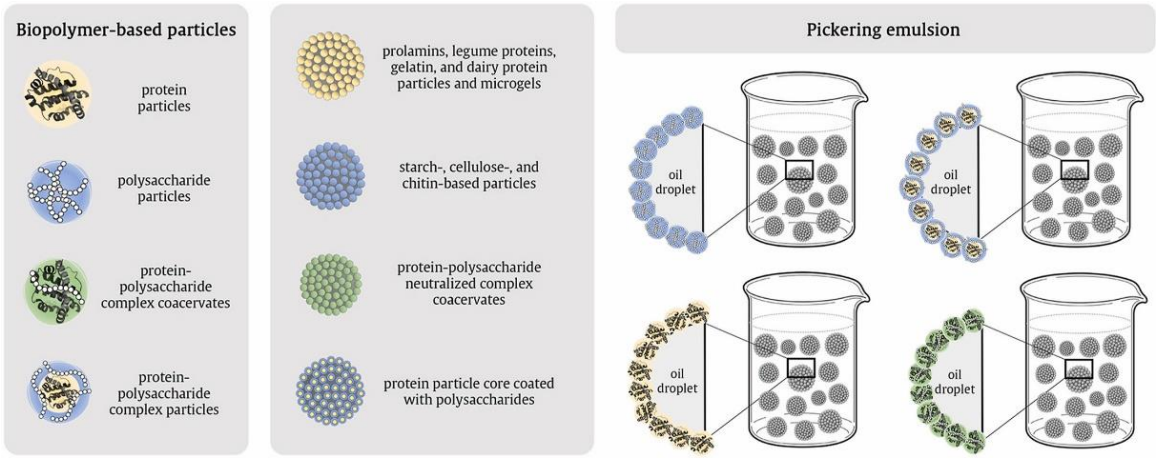

Figure 1.5 - Biopolymers used as stabilizers for high internal phase Pickering emulsions (Ribeiro et al., 2021)

1.6 Properties of Pickering particles

The most important properties of a particle to be used as a Pickering emulsifier are:

- dual wettability to achieve sufficient absorption at the oil-water interface;
- the particle size should be at least one order of magnitude smaller than that of the oil droplets to form a protective layer that prevents coalescence;
- surface charge (Xiao et al., 2016).

1.6.1 Wettability of particles

Pickering emulsions generally consist of three parts: Particles as stabilizers, oil phase and water phase. Therefore, the properties of each part affect the stability of the emulsions. Particle wettability is a parameter that regulates the distribution of particles at the oil-water interface (Weiss et al., 2020). The three-phase contact angle θ, used to define wettability, is thermodynamically related to the separation energy required to detach the particles from the oil-water interface and is expressed by the following equation:

$$E = \gamma\pi r2\ (1 - |\cos\theta|)2$$

Where E is the desorption energy, r is the particle radius, γ is the surface tension of the oil-water interface, θ is the three-phase contact angle (Xia, et al., 2021).
If a particle forms a contact angle of less than 90°, it preferentially stabilizes oil-in-water emulsions (hydrophilic particles). If a particle forms a contact angle of more than 90°, it preferentially stabilizes water-in-oil emulsions (hydrophobic particles). To achieve firm anchorage at the oil-water interface, the particle should form a θ-value close to 90° (Figure 1.6.1) so that it can be immersed equally in the dispersed and continuous phases.

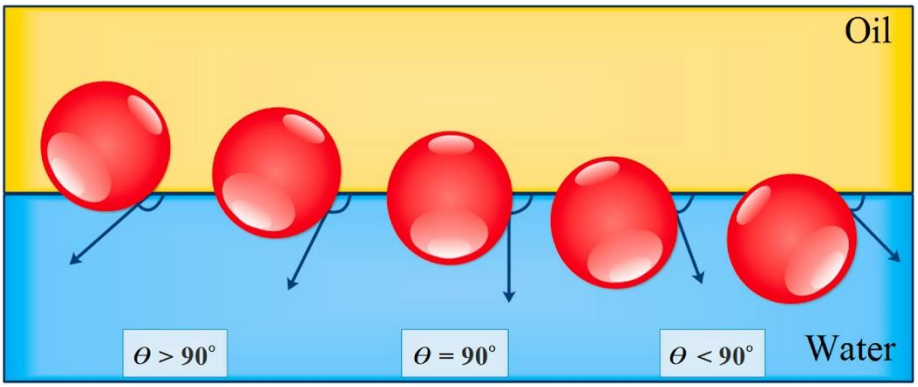

Figure 1.6.1 - Adsorption of a Pickering particle at the oil-water interface based on wettability (Weiss et al., 2020).

1.6.2 Particle size

Particle size is important for the formation of the emulsion structure. Smaller Pickering particles are preferable to larger ones because they take longer to adsorb at the oil-water interface, resulting in emulsions with large droplets. Therefore, emulsions are usually systems formed by the application of intense shear for dispersing one phase into another, aiming at an increase in the interfacial area and thus an exposition of this interface to the stabilizing components. For this reason, particular interest has been given to ultrasonic and high-pressure homogenization methods, due to their potential for obtaining stable emulsions.

A higher concentration of particles promotes stability at the interface and limits the aggregation process. When the particle concentration is greater than the maximum concentration they can adsorb at the interface, the remaining particles form a steric barrier to water, making the emulsion more stable. However, extremely high particle concentration leads to particle aggregation and deposition (Xia et al., 2021).

1.6.3 Surface charge

Surface charge is the distribution of ions on the particle surface, which can lead to electrostatic attractions with the opposite charge of the adjacent surface. Therefore, the surface charge of a Pickering particle is considered as the equilibrium point of Pickering stabilizers at the oil-water interface (Hu et al., 2016). It should be noted that the particle charge behavior is determined by the pH and ionic strength, which affect the stability and performance of high internal phase Pickering emulsions throughout their shelf life. The pH has a strong influence on the continuous aqueous phase and thus regulates the interaction between the solid particles.

Alison et al. (2018) studied Pickering emulsions stabilized by chitosan, and the results showed that the greatest stability occurred at neutral pH. When the pH was increased from 4 to 5.5, the Pickering emulsion adopted a network structure, which improved the stability of the emulsions. As the pH decreased, the electrostatic repulsion between the particles increased, leading to demulsification. The stabilization mechanism of the emulsions was different at different pH values.

The surface charge of Pickering particles can also be altered by the addition of salts, which can promote adsorption of the particles at the oil-water interface by forming a physical barrier in the form of a film. When the film is thicker, it leads to greater repulsion between droplets, improving the stability of the emulsion (Rayner et al., 2014).

1.7 Emulsion stabilization

All emulsions are thermodynamically unstable and tend to breakage with time, resulting in two separated liquid phases. Food emulsions may become unstable due to many different physicochemical mechanisms, (McClements et al, 2005, Dickinson et al, 1992) including gravitational creaming, sedimentation, flocculation, coalescence, and Ostwald ripening (Dickinson et al, 1992), (Figure 1.7.1). Flocculation involves the association of the emulsified droplets into flakes without the individual droplets being destroyed. When the droplet interface is destroyed and the formation of larger droplets takes place, the process is known as coalescence. Creaming occurs when the dispersed phase is less dense than the continuous phase, but there is no coalescence. In sedimentation, the droplets are denser than the continuous medium. Ostwald ripening is the process that occurs from the difference in solubility and/or chemical potential between small and large particles (Friberg et al, 2004); larger droplets grow at the expense of smaller droplets due to mass transport of dispersed phase material through the continuous phase.

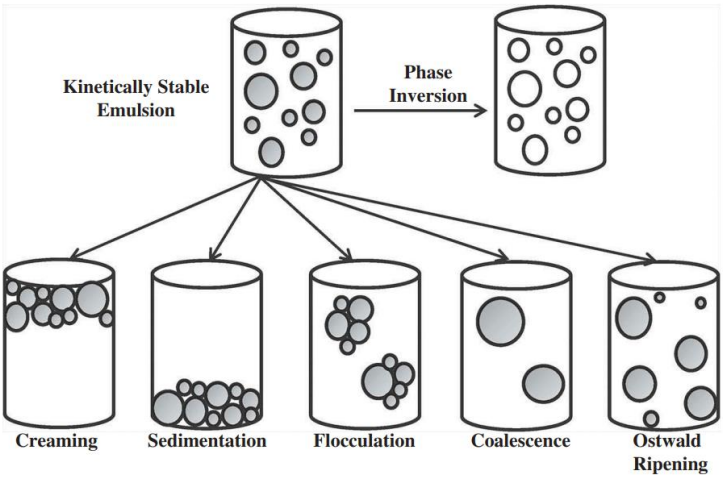

Figure 1.7.1. Schematic representation of the mechanisms of O/W emulsion destabilization. (Weiss et al., 2020).

Phase inversion is the event in which an O/W emulsion changes into a water-in-oil emulsion, or vice versa (Meinders et al, 2003, Kourniatis et al, 2010). It is important to note that these various physicochemical instability mechanisms are often interrelated; for instance, increasing particle sizes due to flocculation, coalescence or Ostwald ripening will generally lead to a lower stability of the droplets against gravitational separation, finally resulting in creaming or sedimentation (McClements et al, 2007). Being thermodynamically unstable systems, information on the kinetic stability of emulsions is important for developing products with desirable properties that will last a sufficiently long period of time. Emulsions that remain stable for considerable periods of time (weeks to years) are said to be kinetically stable and require the addition of chemical substances acting as emulsifiers and/or stabilizers, in addition to the application of mechanical forces (homogenization process) to promote the droplet size reduction (Dickinson et al, 1992). Emulsifiers are molecules with a surface activity that are adsorbed on the surface of the dispersed droplets formed during the homogenization process (McClements et al, 2005), (Figure 1.7.2). Once present in the droplet surface, such molecules act by reducing interfacial tension and forming a protective membrane on their surface, preventing a further aggregation of the droplets. There are two major classes of emulsifiers used in food processing: low molar mass surfactants (monoacylglycerides, polysorbates, lecithin, etc.) and macromolecular emulsifiers (usually proteins, especially from milk and egg) (McClements et al, 2005). However, there are other possibilities of interface stabilization as observed in Pickering emulsions (Pickering 1907), which rely on solid particles that are partly wetted by oil and by water and accumulate at the O/W interface to stabilize droplets against coalescence.

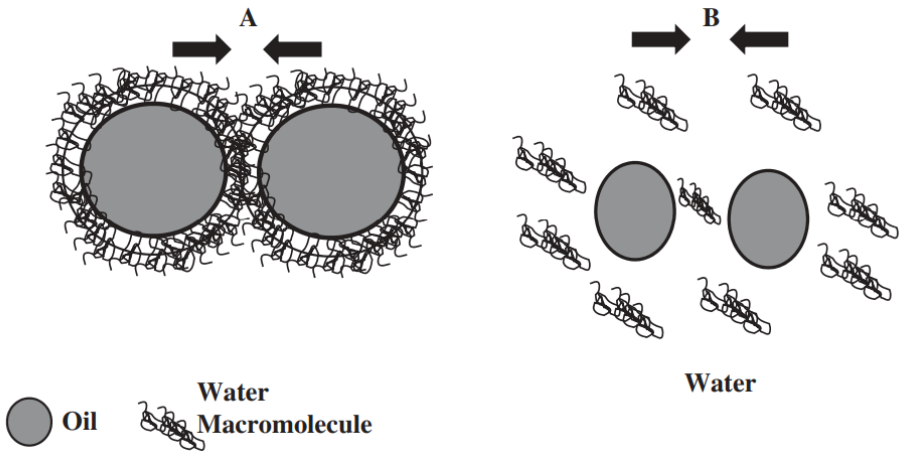

Figure 1.7.2- Schematic illustration of oil droplets in water stabilized by (A) amphiphilic molecules adsorbed on the oil–water interface (e.g., proteins) and (B) non adsorbed macromolecules increasing the continuous phase viscosity (e.g., polysaccharide). John Wiley and Sons (2001).

1.8 Applications in the food sector

Potential applications of so-called Pickering emulsions in food have only recently begun to be considered. Dickinson (2010, 2012) provides two reviews on this topic. In the first, he concludes that "the portion of this research that is directly applicable to food is necessarily small" (Dickinson 2010). Only two years later, however, he concluded that "for Pickering stabilization of food-grade O/W emulsions, various types of dispersed particles of biological origin have recently proven useful" (Dickinson et al, 2012). This reflects the growing interest in this area, and many more studies have been published since 2012, and indeed the number of scientific papers on Pickering emulsions for food use has increased exponentially, but it is unsatisfactory that their applications in food are almost nil.

Pickering emulsions should have a place in food applications because of their stability, rheology and health. As we all know, the shelf life of milk and yogurt is short. If Pickering emulsions were used as stabilizers, the shelf life of dairy products could be extended. Another improvement could be made in the packaging of baked goods (Xia et al., 2021). Fasihi et al. (2019) produced thin films with excellent antioxidant and antibacterial properties by incorporating Pickering emulsions. In addition, the results showed that these films have excellent anti-UV properties, suggesting that this is a strong point for application in food packaging.

Nowadays, a striking number of foods are consumed in the form of emulsions, e.g., creams, desserts, condiments, and spreadable creams (sweet and savory), and the source of their semisolid consistency is both animal fats (the use of which, however, has greatly decreased over time as consumers prefer vegetable sources) and partially hydrogenated oils (PHO).

Hydrogenated oils are usually produced by heating vegetable oils high in unsaturated fatty acids in the presence of hydrogen and a metal catalyst (such as nickel, copper, or chromium). This causes the double bonds in the unsaturated fatty acids to bond with the hydrogen atoms, ultimately increasing the melting point and hardness of the fat.

Since it is known that animal fats are high in saturated fats, PHOs are a source of harmful trans fatty acids, and it is recognized that both saturated fats and trans fatty acids are risky for the

human body as their consumption increases the risk of cardiovascular disease, there is growing interest in finding alternative strategies to form semi-solid food emulsions without the use of PHO, and the use of Pickering emulsions seems to be the best solution. Zeng et al. (2017) showed that Pickering emulsions with high internal phase of oil-in-water type can be used as PHO substitutes.

In addition to the health aspect, fatty foods are particularly susceptible to lipid oxidation during processing and storage, especially if they contain high levels of unsaturated fatty acids. The volatile compounds formed during lipid oxidation are the main reason for the deterioration of the sensory and nutritional quality of fatty foods. The main cause of lipid oxidation in many emulsion-based foods is the interaction between lipid hydroperoxides and transition metal ions near the droplet surface. In Pickering emulsions stabilized by protein-polysaccharide complexes, the thick layer coating the oil droplets forms a physical barrier that prevents contact between the lipid hydroperoxides in the droplets and the transition metal ions in the aqueous phase, thus retarding "oxidation." (Mcclements & Decker, 2000).

However, these applications are limited to small-scale production in the laboratory, and there is still a long way to go before large-scale industrial production.

1.9 Filling creams

Many commonly consumed foods are complex multiphase materials containing a mixture of different types of particles. For example, many sauces, desserts, and condiments consist of a mixture of starch granules and fat droplets (Sikora et al., 2008). Starch granules are generally used as thickeners because they swell when heated and form a highly viscous or gelatinous material (Tattiyakul et al., 2009). Fat droplets impart a variety of desirable physical and sensory properties to these foods: they result in a creamy appearance; they increase viscosity due to their ability to alter the flow profile; they provide a desirable mouthfeel due to their interactions with the tongue; they enhance the flavour profile thanks to their ability to solubilize nonpolar aromatic molecules (Bellamy et al., 2009).

The most commonly used fats in bakery and confectionery products are butter, margarine, or hydrogenated oils. Filling creams usually contain 30-35% fat, but this percentage can be as high as 60% and has a major impact on rheological and sensory properties (Miele et al., 2015). The amount of fat used, and its properties influence the structural properties of the final product. Rheological properties are among the most important physical properties to define the structure of a food system and can be used as a tool to develop new or improve existing formulations (Liu, et al., 2007). Different flow and temperature conditions as well as formulation changes (omission or addition of new ingredients) affect rheological behaviour (Lončarević et al., 2016).

In general, filler creams are shear-thinning liquids with viscoelastic behaviour typical of weak gels (Tárrega et al., 2012).

Excessive fat consumption is associated with obesity and other diseases, so consumers demand low-fat foods with a healthier lipid profile but with similar physical and sensory properties to conventional products. The use of vegetable fats, such as sunflower oil, improves the lipid profile of the final product, but the low level of saturated fat in the composition

cannot achieve the semi-solid structure associated with solid fat. One way to achieve these properties is to use hydrocolloids as stabilizers in an oil-water emulsion, which can serve as a substitute for saturated fats (Tarancón, Salvador, and Sanz, 2013). The semi-solid consistency given by the hydrocolloid is suitable to mimic the rheological properties of fat, leading to good sensory acceptability (Martínez-Cervera et al., 2015).
Consumers like the taste of fatty foods; in fact, the greatest stimuli are perceived when consuming high-fat foods, and this sensation can be generated by the use of stabilizers or thickeners that improve the perceived creaminess of the reformulated product (Drewnowski, 1990).

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Chapter 2

Modification of physico-chemical and functional properties of Pumpkin seeds Protein Isolate (PsPI) by high-intensity ultrasound: Effect of treatment time

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Chapter 3

Complex Coacervation of Pumpkin Seeds Protein Isolate with Low Molecular Weight Chitosan

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Chapter 4

Pumpkin Seeds Proteins Isolate - low molecular weight chitosan coacervate as novel stabilizers in oil/water Pickering emulsions

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Paper in preparation

Chapter 5

Application of high internal phase Pickering emulsion in preparation of a filling cream: study of physical stability and rheological properties

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Conclusions and future perspectives

In the present dissertation, complex coacervation was investigated as a top-down technique for the preparation of coacervates based on pumpkin seed protein -chitosan low molecular weight complex (PsPI-Ch) by electrostatic interaction to evaluate its potential application as a high internal phase Pickering emulsifier and the application of the prepared Pickering emulsion as a fat substitute in a filling cream. In particular, to improve the solubility, solvation charge of pumpkin proteins and reduce the size of protein aggregates, for the subsequent study of coacervation with polysaccharide, the effect of treatment time on high-intensity ultrasound (HIUS) on the chemical-physical and technological properties of pumpkin proteins were investigated.

The results showed that protein particle size and zeta potential, as well as emulsifying and foaming properties of pumpkin samples were significantly affected by HIUS treatment. PsPI powders dispersed at different pH values and treated with HIUS exhibited nanometric dimensions at all pH values regardless of HIUS exposure time.

These results may be related to the ability of HIUS waves to break some of the physical forces that hold protein molecules together into larger aggregates, releasing smaller soluble proteins. Principal component analysis (PCA) showed that our processing method affected the different indices. Specifically, it showed that as the pH increased and also as the HIUS treatment time increased from 10 minutes (C) and 20 minutes (D), the zeta number decreased; thus, there is a negative correlation between time and pH and zeta potential. However, when we kept the times low (5 minutes), the emulsifying and foaming ability (EAI and FC) as well as their stability (CI and FS) were more or less constant at each pH, so the latter was mainly affected by the treatment time. These results indicate that 5-min HIUS treatment is sufficient to achieve a significant improvement in the size, solvation charge, and functional properties of PsPI. Therefore, it was decided to pretreat the pumpkin seed proteins with HIUS for 5 min before adding the polysaccharide in the subsequent complex coacervation study.

Subsequently, the behavior of the associative phase of the pumpkin protein isolate (PsPI) and a cationic polysaccharide (chitosan, Ch) was studied by examining the effects of pH and the mixing ratio of the biopolymers on the formation of soluble and insoluble complexes. The properties of the coacervates were studied by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The results showed that the ability of PsPI molecules to interact with Ch molecules to form soluble and insoluble complexes strongly depends on the pH and the ratio of proteins to polysaccharides. The major role of electrostatic interactions in the formation of PsPI-Ch coacervates was confirmed by the stabilizing effect of bound hydrogen by FTIR analysis. Optimal associative phase separation was found at a biopolymer mixing ratio of 8:1 (w/w) and a pH of 7.5, resulting in a yield of 94%. Regarding their morphology, the coacervates showed an irregular, scaly shape and a compact and irregular surface morphology, which was consistent with the optimal biopolymer mixing ratio and could be characterized as a porous microstructure dominated by vacuoles of heterogeneous size. Once the parameters were optimized, the application of these PsPI-Ch particles generated by complex coacervation was investigated as a stabilizer of a high internal phase Pickering

emulsion (HIPE) for food. The coacervation pH had a large effect on the emulsifying ability of the resulting PsPI-Ch particles, and particles with a pH of 5.5 produced a HIPE with an oil content as high as 74%. This was attributed to the small size of the nanoparticles and a contact angle of 86.5 ± 0.9 at pH 5.5. It was found that the emulsifying ability of the PsPI-Ch coacervates was very sensitive to their concentration, so that HIPE could only be prepared with a stabilizer concentration of 1.5% (w/v) or higher, and the highest oil content was obtained with a tuberculosis concentration of 3%. The HIPEs showed a decrease in droplet size with increasing coacervate concentration from 1254.2 nm (at 2.25% PsPI-Ch) to 583 nm (at 3% PsPI-Ch). HIPEs stabilized by PsPI-Ch coacervates were stable only in solutions with a pH of 4.0 or less and resisted centrifugation and heating, but collapsed readily on freezing and thawing. Moreover, a higher concentration of stabilizer resulted in better stability. Applications of Pickering emulsions in foods have only recently been considered because they were formulated with only inorganic molecules as stabilizers. Now that organic molecules are coming into play, Pickering emulsions should have a place in food applications because of their stability, rheology and health. Nowadays, an impressive number of foods are offered in the form of emulsions, such as creams, desserts, condiments and spreads (sweet and savory), and the source of their semi-solid consistency is fats, which are particularly sensitive to lipid oxidation during processing and preservation, especially if they contain a high proportion of unsaturated fatty acids.

Pickering emulsions can also replace hydrogenated or animal fats to make a cake, and Pickering emulsion cakes can reduce caloric intake and extend shelf life without changing their color and texture. Therefore, in the last part of this PhD project, a Pickering emulsion stabilized by the protein complex of pumpkin seeds and chitosan (PsPI-Ch) was used as a fat substitute for the preparation of a filling cream with reduced fat content (19%). Therefore, the composition of both the cream with emulsion was studied to evaluate its feasibility and possible applications, and that of the control cream to see the differences; the rheology in both viscoelastic and dynamic range, and it was found that the control cream is more structured and requires a greater shear force to flow than the emulsion cream, having lower elastic modulus values than the latter.

Moreover, they show a medium-strength, gel-like, pseudoplastic behavior and a slight frequency dependence; the stability to Turbiscan, where the emulsion cream has lower ΔBS and TSI values than the control cream, is due to both the smaller particle sizes and the higher viscosity of the overall structure, and the contribution of the chitosan present in the Pickering emulsion, which, independently of its antimicrobial activity, improves food quality by delaying lipid oxidation. In conclusion, the results obtained show that the Pickering emulsion based on PsPI-Ch is a very versatile semi-finished product characterized by a high degree of technological innovation, but further studies on the sensory acceptance of this product by consumers would be required.

The results obtained made it possible to understand the mechanisms underlying the electrostatic interaction between the two biopolymers (PsPI and Ch) and to study the operating conditions suitable not only for the preparation of highly stable nanoparticles, but also for their application as a high-phase Pickering emulsifier, which has shown high stability over time and excellent performance as a fat substitute in the preparation of filling creams.

In conclusion, the results of this doctoral work contribute, even though partially, to the realization of a new method that guarantees the production of a semi-finished product as a fat substitute with healthy properties.

The unique technological properties associated with the high coalescence stability of Pickering emulsions are undisputed. Although several studies have been conducted on these functional colloidal structures in recent years, understanding their digestibility is still a challenge. The high stability of Pickering emulsions implies their application in the control of satiety, as they clearly limit the digestion of lipids. However, the transfer and delivery of bioactive compounds in these colloidal structures needs further research. Second, studies on the digestion of PE are limited and are also performed with in vitro models. Finally, for successful commercialization of foods containing Pickering emulsions, the inclusion of compounds that impart the desired flavor and aroma must be considered. HIPPEs are high fat/oil products, similar to cream or other high oil/fat products currently on the market. HIPPEs need to be developed so that they can become a healthier fat substitute. Future research should focus on overcoming the challenges associated with the production and application of PE, HIPPE in the food industry.