

FORMULATION AND CHARACTERIZATION OF PICKERING EMULSIONS
STABILIZED WITH WHEY PROTEIN ISOLATE AND GUM ARABIC

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ARABIC**

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ABSTRACT

FORMULATION AND CHARACTERIZATION OF PICKERING EMULSIONS STABILIZED WITH WHEY PROTEIN ISOLATE AND GUM ARABIC

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Pickering emulsions, stabilized by Pickering particles, show considerable potential in various fields due to their stability and applicability. This study aims to formulate stable Pickering emulsions using whey protein isolate and gum arabic, which are widely used hydrocolloids in the food industry. Pickering particles were produced with varying gum arabic to whey protein isolate ratios (0.01, 0.02, 0.05, 0.10) and polysaccharide-protein complexes were formed by partial electrostatic interactions under the influence of heat and pressure at a neutral pH. The formation of Pickering particle solutions with distinct properties from both polymers was demonstrated through FTIR spectroscopy and TD-NMR analyses. The addition of sunflower oil (20% w/w) to the newly created Pickering particles, through the process of high-pressure homogenization, enabled the production of a Pickering emulsion where their stability is analyzed by particle size analysis and TD-NMR experiments over four weeks of storage. Confocal laser scanning microscopy imaging of Pickering emulsions successfully demonstrated that proteins in complex with gum arabic coated the oil droplets. Stability analysis showed that all formulations, except for

the Pickering particles with the lowest polysaccharide-to-protein ratio, remained stable for four weeks. Additionally, the fate of emulsions produced at neutral pH when added to food formulations was investigated through the *in vitro* digestion method. Acid-induced digestion was also used to study the effect of enzymes on digestion and stability of Pickering emulsions in acidic environments. The rheological analysis indicated that the emulsion with the highest polysaccharide-to-protein ratio notably increased bolus viscosity upon enzymatic digestion. Acid-induced digestion results showed that the presence of pepsin greatly contributes to digestion.

Keywords: Pickering emulsion, Pickering particles, *In vitro* digestion, Time Domain Nuclear Magnetic Resonance

ÖZ

PEYNİR ALTI SUYU PROTEİNİ İZOLATI VE ARAP ZAMKI İLE STABİLİZE EDİLEN PICKERING EMÜLSİYONLARININ FORMÜLASYONU VE KARAKTERİZASYONU

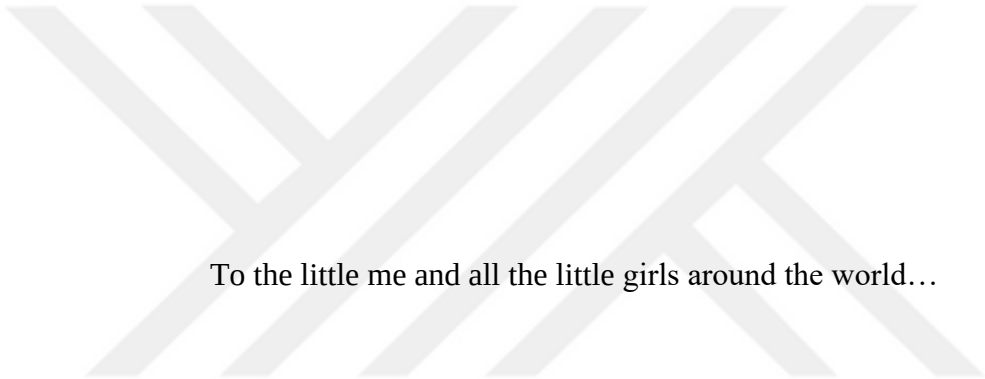
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Pickering partikülleri ile stabilize edilen Pickering emülsiyonları, stabiliteleri ve uygulanabilirlikleri nedeniyle çeşitli alanlarda büyük bir potansiyele sahiptir. Bu çalışmada, gıda endüstrisinde yaygın olarak kullanılan hidrokolloidler olan peynir altı suyu proteini izolatu ve arap zımkı kullanılarak stabil Pickering emülsiyonlarının formüle edilmesi amaçlanmıştır. Pickering partikülleri farklı arap zımkı-peynir altı suyu proteini izolatu oranlarında (0.01, 0.02, 0.05, 0.10) hazırlanmış ve nötr pH'da ısı ve basınç etkisi altında kısmi elektrostatik etkileşimlerle polisakkarit-protein kompleksleri oluşturulmuştur. Her iki polimerin farklı özelliklerine sahip Pickering partikül çözeltilerinin oluşumu FTIR spektroskopisi ve TD-NMR analizi ile gösterilmiştir. Yeni oluşturulan Pickering partiküllerine yüksek basınçlı homojenizasyon işlemiyle ayçiçek yağı (%20 w/w) eklenmesi, dört haftalık depolama süresince partikül boyutu analizi ve TD-NMR deneyleriyle stabilitelerinin analiz edildiği bir Pickering emülsiyonunun üretilmesini sağlamıştır. Pickering emülsiyonlarının konfokal lazer tarama mikroskobu ile görüntülenmesi, arap zımkı ile kompleks halindeki proteinlerin yağ damlacıklarını kapladığını başarılı bir şekilde göstermiştir. Stabiliteler sonuçları, en düşük polisakkarit-protein oranına sahip Pickering partikülleri hariç tüm formülasyonların dört hafta boyunca stabil kaldığını

göstermiştir. Ayrıca, nötr pH'da üretilen emülsiyonların gıda formülasyonlarına eklendiğinde sindirim sistemindeki akıbeti *in vitro* sindirim yöntemiyle araştırılmıştır. Pickering emülsiyonlarının asidik ortamdaki stabilitesini ve sindirimi üzerinde enzimlerin etkisini incelemek için asitle indüklenen sindirim de kullanılmıştır. Reolojik analizler, en yüksek polisakkarit-protein oranına sahip emülsiyonun enzimatik sindirim sonrasında bolus viskozitesini belirgin şekilde artırdığını göstermiştir. Asitle indüklenen sindirim sonuçları, pepsin varlığının sindirime büyük ölçüde katkıda bulunduğunu göstermiştir.

Anahtar Kelimeler: Pickering emülsiyonu, Pickering partikülleri, *In vitro* sindirim, Zamansal Alanda Nükleer Manyetik Rezonans



To the little me and all the little girls around the world...

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LIST OF ABBREVIATIONS

FTIR: Fourier-transform infrared spectroscopy

CPMG: Carr-Purcell-Meiboom-Gill

TD-NMR: Time Domain ^1H Nuclear Magnetic Resonance

WPI: Whey protein isolate

GA: Gum Arabic

pI: isoelectric point

IR: Infrared

RF: Radio frequency

NNLS: Non-Negative least squares

CLSM: Confocal Laser Scanning Microscopy

FITC: Fluorescein isothiocyanate

SGF: Simulated gastric fluid

SSF: Simulated salivary fluid

CHAPTER 1

INTRODUCTION

1.1 Pickering Emulsions

Most of the food products we consume regularly are composed of different immiscible phases, such as oil and water. These products often form emulsions, which are systems characterized by the dispersion of one liquid as droplets within another liquid, where the dispersed phase does not completely dissolve in the continuous phase. Due to the thermodynamic instability of emulsions, achieving the desired stability typically requires the addition of a surfactant along with energy input. Surfactants, which are generally amphiphilic, provide this stability through their hydrophilic and hydrophobic groups. These amphiphilic molecules adsorb at the water/oil interface, with the hydrophilic group interacting with water and the hydrophobic group interacting with oil, thereby effectively reducing the interfacial tension (Chen et al., 2020). In Pickering emulsions, stability is achieved through the irreversible adsorption of colloidal particles at the oil-water interface, as shown in Figure 1.1. The absence of surfactants in Pickering emulsions makes them appealing for food systems because they offer a safe and environmentally friendly encapsulation method, enabling targeted delivery and controlled release, thus improving the absorption of active compounds by the body (Cheon et al., 2023).

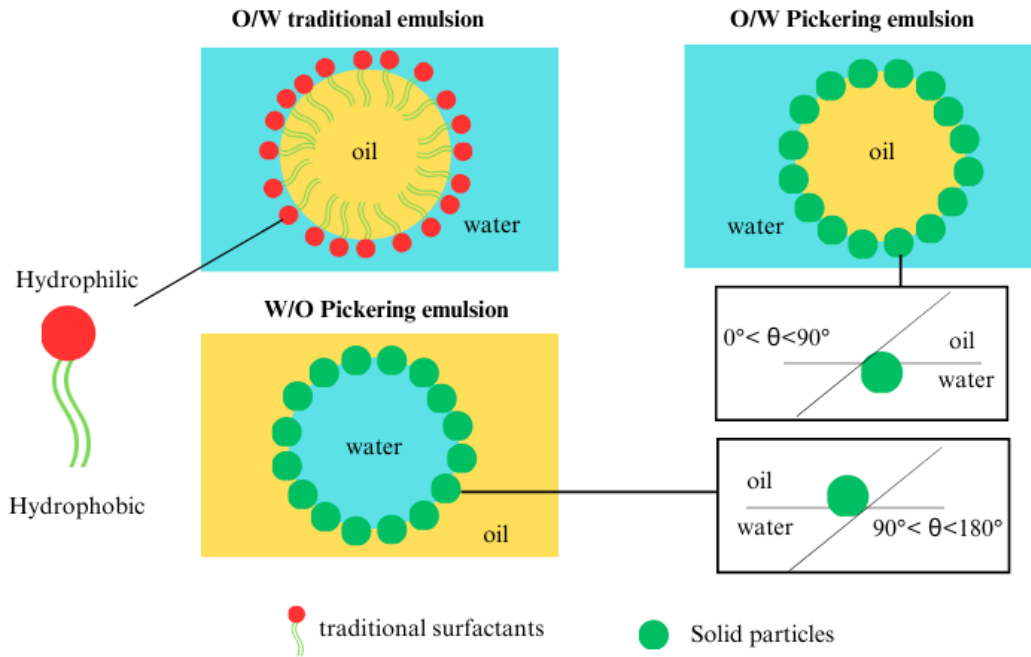


Figure 1.1 Illustration of conventional emulsions and Pickering emulsions (Li et al., 2022a)

Emulsions are generally considered stable if they do not undergo physical changes over a useful period of time. Figure 1.2 summarizes the different ways an emulsion can become unstable or break down. Figure 1.2(a) is an initially well-dispersed emulsion, and it may undergo phase separation (breaking) (Figure 1.2(f)) via various pathways such as flocculation (Figure 1.2(b)), sedimentation (Figure 1.2(c)), Oswald ripening (Figure 1.2(d)), coalescence (Figure 1.2(e)). In flocculation, the droplets cluster together to form aggregates without any change in the original size of the droplets. Meanwhile, gravity causes creaming and sedimentation, resulting in concentration gradients due to density differences between immiscible liquids (Dickinson, 1998). On the other hand, Ostwald ripening occurs when smaller droplets dissolve in the continuous phase and then regenerate into larger droplets due to the restricted solubility of a dispersed phase in the continuous phase. Additionally,

coalescence reduces the total interfacial area of the system by combining many droplets into fewer, larger droplets (Lopetinsky et al., 2006a).

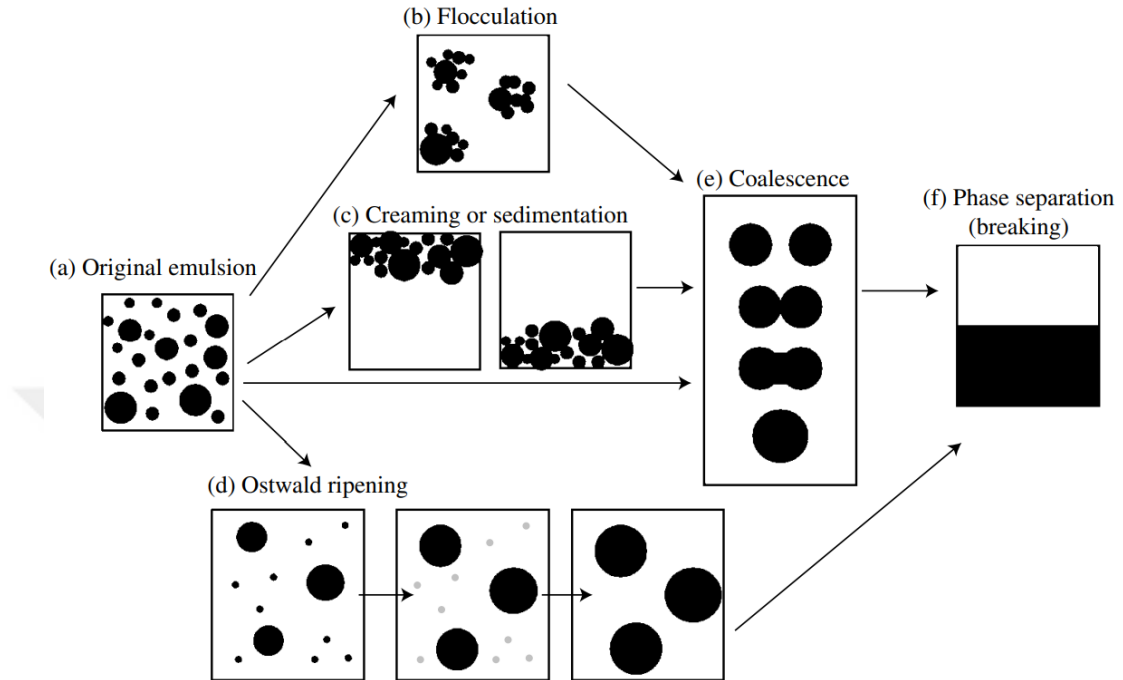


Figure 1.2 Destabilization mechanisms of emulsions (Lopetinsky et al., 2006b)

Numerous theoretical studies have been carried out to understand how solid particles enhance emulsion stabilization. Lopetinsky et al. have reported on possible underlying mechanisms and configurations of particles in Pickering stabilized emulsions (Lopetinsky et al., 2006a). Figure 1.3(a) demonstrates a bilayer stabilization process, where oil droplets are typically fully covered by Pickering particles, creating two protective layers that inhibit coalescence when the droplets come into contact. Another mechanism involves a single layer of particles between droplets, contributing to stability by creating “bridges” in the shown Figure 1.3(b). Bridges between particles result from the bridging phenomena and indicate the transformation from a bilayer to a monolayer, which happens when the capillary pressure overcomes the strength of the interface (Cheon et al., 2023). The formation of a two-dimensional network (Figure 1.3(c)) and clustering between particles (Figure 1.3(d)) are another configuration of the emulsion droplet surface. Finally,

Figure 1.3(e) represents systems in which bilayer stabilization exists, and a large number of Pickering particles overflow into the continuous phase to form a three-dimensional network (Lee et al., 2012; Li et al., 2022a).

All of the possible mechanisms for the stability of the particles are shown in the Figure 1.3(i-iv). In the majority of Pickering emulsions, more than one particle stabilization mechanism plays an active role. Therefore, most of the systems are dominated by more than one mechanism rather than systems where a single mechanism provides stabilization alone. Particles are restrained by steric hindrance that can prevent them from being displaced from the interface in both bilayer and single-layer stabilization processes, as shown in Figure 1.3(i) and (ii), respectively. Additionally, the stability of the thin film in the continuous phase is affected by the maximum capillary pressure that prevents film thinning (Figure 1.3(iii)) and the rheological properties that impact film drainage (Figure 1.3(iv)). Emulsions, where particles create two- or three-dimensional networks and clusters, usually include all four possible stabilization mechanisms (Lopetinsky et al., 2006b).

Overall, Pickering particles that adhere to the interface between oil and water experience partial wetting by both phases and their wettability can be evaluated by measuring contact angles (θ). The desorption energy of colloidal particles from the water/oil interface is high, resulting in the irreversible adsorption of particles at the interface and thereby stabilizing the emulsion (Li et al., 2022b). Colloidal particles, often referred to in the literature as nano or solid particles or Pickering particles, can be based on proteins, polysaccharides, or a combination of both (Ribeiro et al., 2021). In this study, Pickering particles are produced by combining protein and polysaccharides.

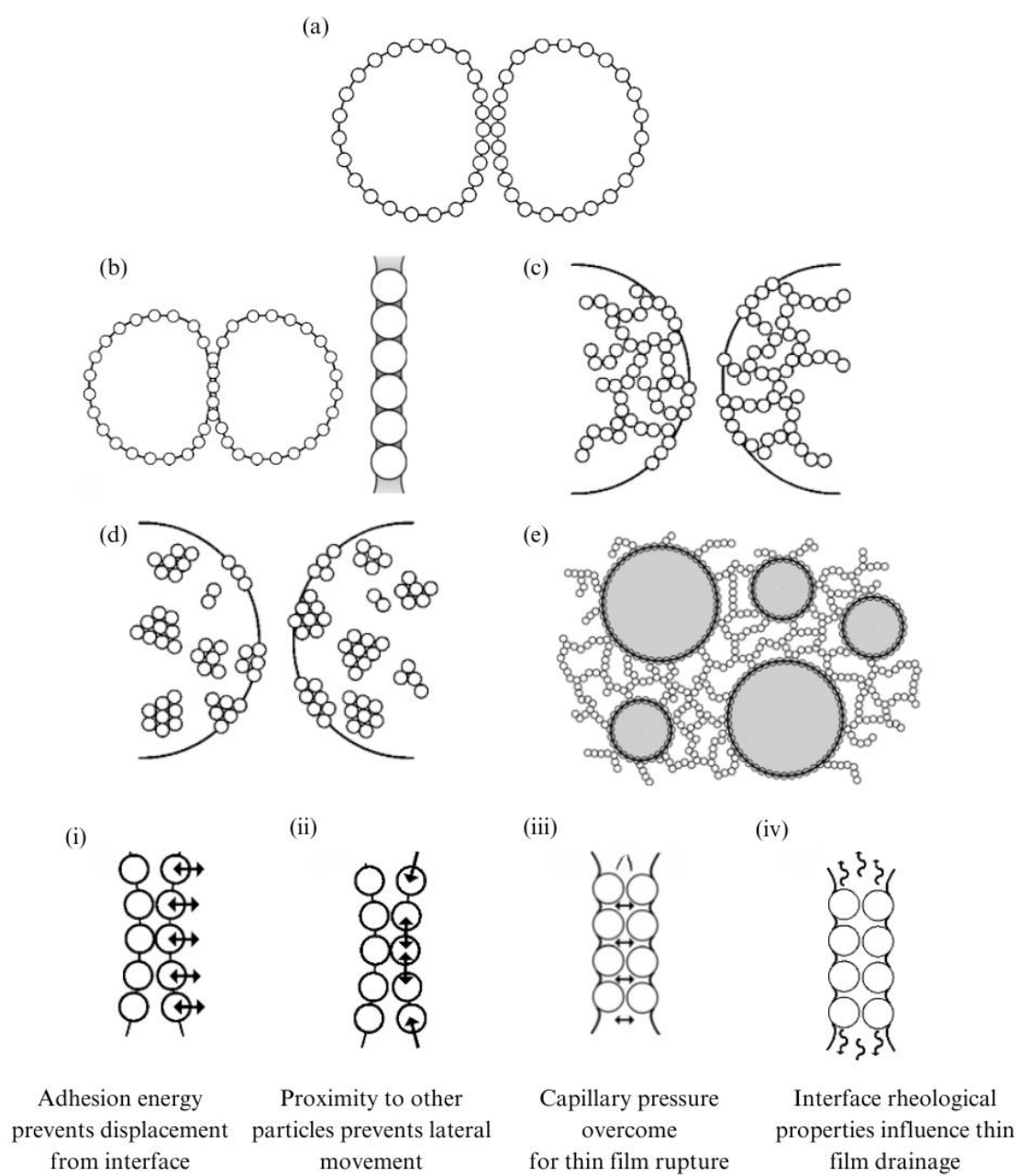


Figure 1.3 Possible configurations of the particles in the Pickering emulsions (a-e) and the mechanisms behind their stability (i-iv) (Lopetinsky et al., 2006a)

1.1.1 Pickering Particles

Interactions between proteins and polysaccharides, achieved through various physical or chemical mechanisms, lead to the formation of structures with improved functionalities. These composite particles are utilized in a range of applications, including the stabilization of Pickering emulsions, as well as in encapsulation and delivery systems, fat replacers, and the formation of films (Ribeiro et al., 2021). Protein-polysaccharide interactions primarily arise from a variety of non-covalent interactions, including electrostatic forces, hydrogen bonding, hydrophobic interactions, and steric effects (Li et al., 2022a). However, electrostatic interactions frequently emerge as the predominant driving force for complexation, but it is also stated that hydrogen bonding and hydrophobic interactions take a part in the stability of the protein-polysaccharide complexes (Doublier et al., 2000; Ghosh & Bandyopadhyay, 2012b). The complexation of proteins and polysaccharides has been studied to be a function of several parameters: pH, polysaccharide: protein mixing ratio, ionic strength, thermal processing conditions (Jones et al., 2009; Jones & McClements 2010a; Klein et al., 2010). Jones & McClements have characterized two main approaches that can be used to produce biopolymer particles based on the combination of globular protein-polysaccharide complexation and thermal treatment. The first of these was termed "Type-1" where particles were characterized by the addition of polysaccharide to denatured globular proteins at a specific pH. "Type-2" particles are defined as particles formed by heating protein and polysaccharide together above the denaturation temperature of the protein at a certain pH (Jones & McClements, 2011). Particles obtained by complexing proteins and polysaccharides can also be used as encapsulating agents, delivery systems, tissue modifiers, fat mimetics, and to encapsulate and protect chemically unstable bioactive substances such as ω -3 fatty acids, carotenoids or vitamins (Jones & McClements 2010b).

In this study, Pickering particles were produced from whey protein isolate, known for its widespread utility in the food industry, and gum arabic, characterized as a

natural exudate constituting a protein-polysaccharide complex. The following sections provide a detailed overview of these polymers.

1.1.1.1 Whey Protein Isolate

Whey protein isolate (WPI) is composed of α -lactalbumin, β -lactoglobulin, and various other minor proteins, serving as a commonly utilized natural emulsifying agent in food products (Chen et al., 2023; Singh & Dalgleish, 1998). In addition to the use of native WPI for its emulsification properties, the use of heat-treated WPI is also a commonly applied method for particle formation (Wu et al., 2015; Zhang & Zhong, 2010). Heating of WPI causes the protein to denature and form aggregates, which leads to the formation of micro- and nanoparticles (Zhang & Zhong 2010). Proteins undergo conformational changes when denatured, exposing their non-polar regions and disulfide bonds typically hidden interior. The nature of the resulting aggregates depends on heating conditions. Weak electrostatic repulsion between molecules, which occurs at pH levels near the isoelectric point or under high ionic strength, leads to the formation of particulate aggregates. Conversely, strong electrostatic repulsion exists when the pH deviates significantly from the isoelectric point (pI) and at low ionic strength, resulting in the formation of filamentous aggregates (Jones, 2009). WPI exhibits a positive charge when the pH is below the pI, and this value varies between 4.5 and 5.5 (Durham & Hourigan, 2007; Pelegriane & Gasparetto, 2005).

1.1.1.2 Gum Arabic

Gum Arabic (GA) is a natural exudate from the *Acacia* tree and is widely used in many industries due to its emulsifying properties. GA is composed of three primary fractions: arabinogalactan (approximately 90%), arabinogalactan protein (around 10%), and glycoprotein (roughly 1%). The arabinogalactan protein, characterized by hydrophilic polysaccharide blocks connected to hydrophobic polypeptide chains, is

recognized as the primary contributor to GA's emulsifying properties (Mahendran et al., 2008). The application of heat to GA has the potential to facilitate the formation of aggregates between arabinogalactan and galactan protein, leading to the enhancement of its amphiphilic properties (Yue et al., 2022). GA shows good emulsifying properties at low viscosity and it is not much affected by the pH of the environment. It becomes negatively charged above pH 2.2 as the dissociation of carboxyl groups is suppressed. Therefore, it is generally negatively charged in most food formulations (Weinbreck et al., 2003).

1.1.1.3 WPI-GA Complexes

The investigations generally focused on the complexation between WPI and various polysaccharides by electrostatic interactions via acidification at 20–25 °C or heating at a pH below the isoelectric point of the protein (Igartúa et al., 2022; Loveday et al., 2013). As previously stated, GA is an anionic polysaccharide with a pKa of 2.2. Studies have investigated two main methods for creating biopolymer particles by combining WPI and anionic polysaccharides. One method involves combining positively charged WPI with anionic polysaccharides at the isoelectric point. In the other method, WPI is first subjected to thermal treatment and then coated with anionic polysaccharides at pI (Ribeiro et al., 2021).

There is a notable lack of research on Pickering particle production, which involves applying heat to both polymers at neutral pH due to both polymers being negatively charged at pH 7.0. This is not limited to WPI and GA, but studies on the formation of soluble complexes between proteins and anionic polysaccharides at neutral pH are generally limited (Khalesi et al., 2016; Wang et al., 2024). Generally, complex coacervation (insoluble complexes) occurs when proteins and anionic polysaccharides are mixed at neutral pH (Stone et al., 2013).

Klein et al. have shown that solutions consisting of combinations of WPI and GA form weak interactions between pH 5.0 and 7.0. It was stated that charge interactions

occur only when the protein is neither completely negative nor completely positive. (Klein et al., 2010). Co-solubility is possible at low concentrations of similarly charged polymers, whereas repulsive phase separation happens at higher concentrations (Han et al., 2023; Stone et al., 2013). Besides, solution parameters are not only crucial factors for controlling various modes of interaction between proteins and polysaccharides, but factors such as the type of protein/polysaccharides, molecular weight, charge density, and hydrophobicity of biopolymers also significantly affect the extent of complexation between two biopolymers under constant environmental conditions (Ghosh & Bandyopadhyay, 2012a). Briefly, studies have explained that soluble complexes are formed through weak interactions between partially positively charged proteins and negatively charged polysaccharides.

The characteristics of the particles produced affect the overall physicochemical properties of the system, such as its stability and rheology. Therefore, it is possible to create particle systems with different properties by adjusting the solution and heating conditions (Jones, 2009). In this study, different ratios of GA to WPI were allowed to complex in the presence of heat at neutral pH, and they were used to form emulsions containing 20% sunflower oil.

1.2 Digestive Behaviour of Pickering Emulsions

Pickering particles are not only significant for their ability to increase emulsion stability but also for their ability to reduce digestion rate (Chen et al., 2006; Mouécoucou et al., 2004). Studies have shown that whey protein and polysaccharide complex formation can create a physical barrier for the digestion of protein by pepsin and that different polysaccharides have a protective effect on the in vitro digestibility of casein (El Kossori et al., 2000; Larsen et al., 1994). Under simulated stomach conditions, it is hypothesized that some portion of GA carries a negative charge and whey protein is positively charged, resulting in the formation of microgel particles. The presence of microgel structures can form a protective barrier on the surface of

droplets in the gastric environment. This barrier makes it difficult for digestive enzymes such as pepsin to reach the droplets, which can slow down the digestion process (Sarkar et al., 2016; Zhang et al., 2023). Thus, microgel particles are believed to enhance the residence time of food in the stomach, as opposed to their absence, consequently extending the transit time of the bolus to the intestine and thereby prolonging the digestion duration (Madadlou et al., 2016). Current literature highlights that the rheology of foods plays a role in influencing digestion and nutrient release by influencing flow properties during digestion (Fischer & Windhab, 2011). In this study, the digestion behavior of foods was characterized by examining the rheological properties of emulsions. Reviewing the literature on Pickering emulsion digestion, studies found that acid-induced digestion systems were also used to characterize digestion behavior (Le et al., 2020; Xiao et al., 2015; Zhang et al., 2021). Some studies have also been conducted without the addition of enzymes to determine the pH stability of particles produced during the gastric digestion process (Cheng et al., 2019). Therefore, digestion experiments were extended on formulated Pickering emulsions using simulated gastric fluids without pepsin and amylase to understand the role of pepsin and amylase in digestion.

1.3 Characterization of Pickering Particles

1.3.1 Turbidity

Turbidity is a measure of the transparency of a liquid that expresses the amount of light absorbed or scattered by particles in suspension (Bozorg-Haddad et al., 2021). Suspended particles can scatter the light depending on the concentration and shape. Therefore, it may provide valuable information about the presence and concentration of suspended particles. This method has been used to determine particle size in colloidal suspensions in dilute systems for a long time (Apfel et al., 1994; Kleizen et al., 1995).

Turbidity measurement can be divided into two basic methods, and the techniques used are shown in Figure 1.4. One of these methods determines the degree of light transmission, while the other evaluates the degree of light scattering. This division is based on the mathematical descriptions used to model phenomena. In the first case, the corresponding theories are due to Beer and Lambert. In the second case, many theories and models have been derived to describe many scattering processes, and these models are mainly based on Mie theory (Kitchener et al., 2017; Omar & MatJafri, 2009).

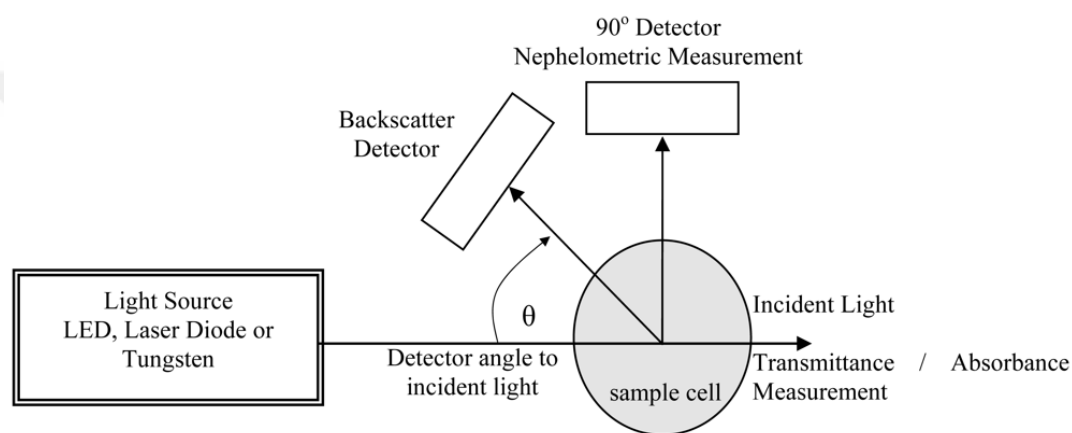


Figure 1.4 Techniques of turbidity measurement(Omar & MatJafri, 2009)

In the literature, turbidity measurements of protein and polysaccharide mixtures were performed as it provides information on the concentration, size, and shape of the biopolymer particles. Changes in turbidity of biopolymer particles produced under different ionic strength and pH conditions or in the presence of heat treatment are believed to be related to complexation, and increased turbidity has been associated with particle aggregation (Jones, 2010a; Ru et al., 2012; Wan et al., 2023; J. Xia et al., 1993). Turbidity has been complemented by numerous studies using different methods, where it has provided important information about protein-polysaccharide complexation.

1.3.2 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy is a technique that operates in the mid-infrared range ($400\text{--}4000\text{ cm}^{-1}$) and provides insights into the absorption and emission characteristics of molecular structures (Berthomieu & Hienerwadel, 2009). The working mechanism of FTIR involves using an interferometer, as shown in Figure 1.5, where infrared light is directed toward a beam splitter positioned at a 45° angle to obtain the infrared (IR) spectrum. One portion of the light passes through the beam splitter, while another is reflected at a right angle. The light that is reflected hits a mirror that is at a standstill, while the transmitted light hits a mirror that is moving at a steady speed. After reflecting off the mirrors, the beams recombine at the beam splitter. Due to the varying distances traveled by the beams, they merge to form an interference pattern known as an interferogram (Wade, 2008).

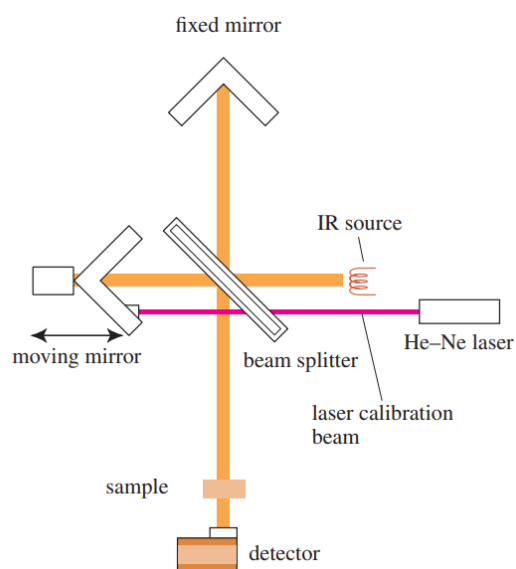


Figure 1.5 Block diagram of an interferometer in an FTIR spectrometer (Wade, 2008)

The interferogram, representing the energy detected as the mirror moves through the signal, carries information about the molecule in the time domain. A Fourier transform is applied to convert this data into the frequency domain. This transformation helps us to observe the absorption intensity as a function of frequency

or wavelength (Ismail et al., 1997). The resulting IR spectrum is used to determine which functional groups are present in the molecule by providing information about the transition energies corresponding to the changes in the vibrational energy state for the functional groups. According to this, the FTIR spectrum can be divided into 4 main regions: single bond (O-H, N-H, C-H) is visible in 2500-3500 cm^{-1} , triple bond ($\text{C}\equiv\text{C}$, $\text{C}\equiv\text{N}$) is present in the 2000-2200 cm^{-1} and double bond ($\text{C}=\text{C}$, $\text{C}=\text{O}$, $\text{C}=\text{N}$) is detectable in the relatively lower wavenumber which is 1500-2000 cm^{-1} as can be shown in Figure 1.6. In addition, the 1400-500 cm^{-1} region is called the "fingerprint region" and it is generally used for molecular identification (Mohamed et al., 2017; Wade, 2008).

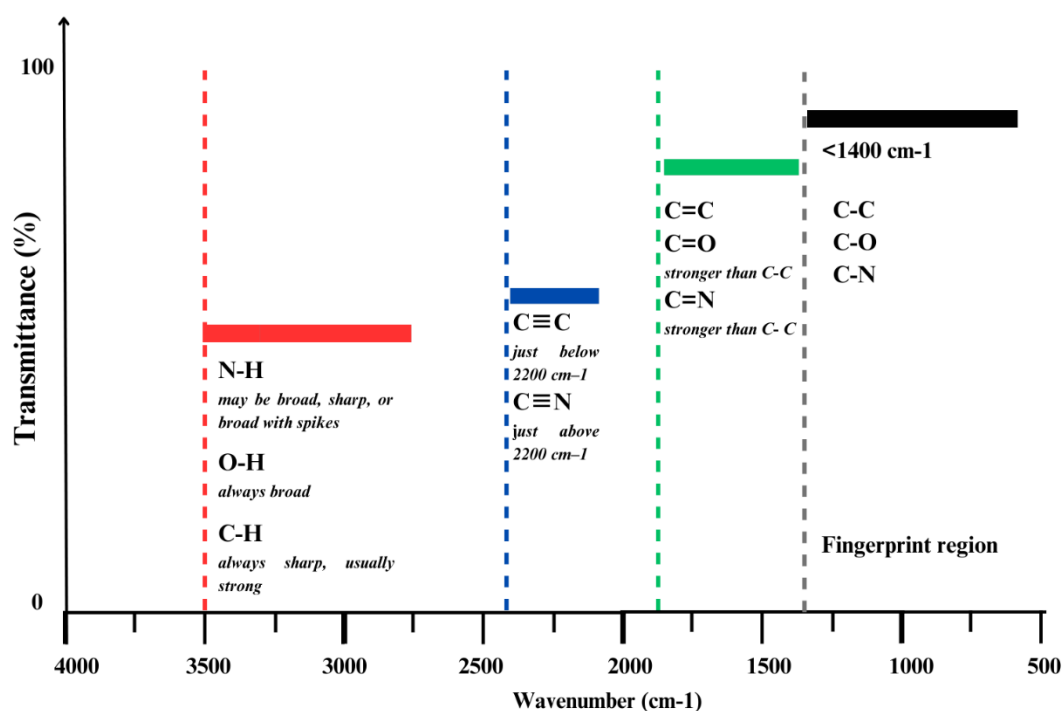


Figure 1.6 Summary of the FTIR spectrum, reproduced from (Wade, 2008)

In summary, an FTIR spectrum gives an idea of the functional groups present and reveals the absence of other functional groups that would otherwise produce strong absorptions. Lastly, it can be used as a tool to verify the identity of a compound by comparing its spectrum with that of a known reference sample (Wade, 2008).

1.3.3 Time Domain ^1H Nuclear Magnetic Resonance

Time Domain ^1H Nuclear Magnetic Resonance is a noninvasive tool for investigating the molecular dynamics of various material matrices (Ozel & Oztop, 2021). NMR was originally developed to investigate protons, specifically the nuclei of hydrogen atoms. "Nuclear Magnetic Resonance" typically refers to "proton magnetic resonance" unless another nucleus is indicated. Any nucleus with either an odd atomic number or an odd mass number possesses a nuclear spin that can be detected by an NMR spectrometer (Wade, 2008).

Figure 1.7 shows the four main components of an NMR spectrometer. The first component is a magnet, which creates a stable magnetic field and a temperature controller that regulates the magnet's temperature. The second component is a radio-frequency (RF) transmitter that emits a specific frequency. The third component is a detector, which measures the sample's absorption of RF energy. The last component is software, which plots the data from the detector against the applied magnetic field (Zia et al., 2019).

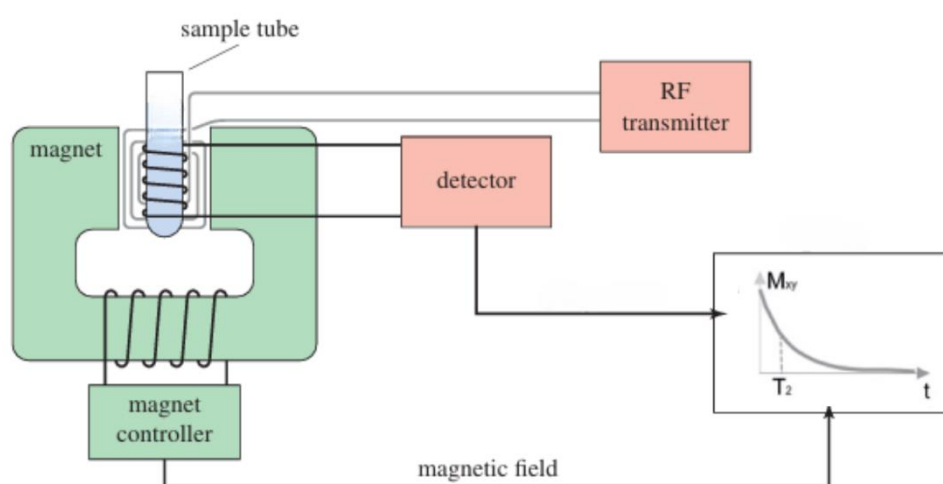


Figure 1.7 Block diagram of a nuclear magnetic resonance (NMR) spectrometer (Zia et al., 2019)

The operating principle of NMR is based on the magnetic moment of atomic nuclei, which arises from the intrinsic spin of the nuclei. The magnetic moment occurs due to the magnetic field created by the rotating particles within the nucleus. When the sample is placed on the NMR magnet, the magnetic moment of the protons in the sample is aligned or positioned in the opposite direction under the influence of the magnetic field originating from the NMR magnet, as shown in Figure 1.8 (Rashid et al., 2021).

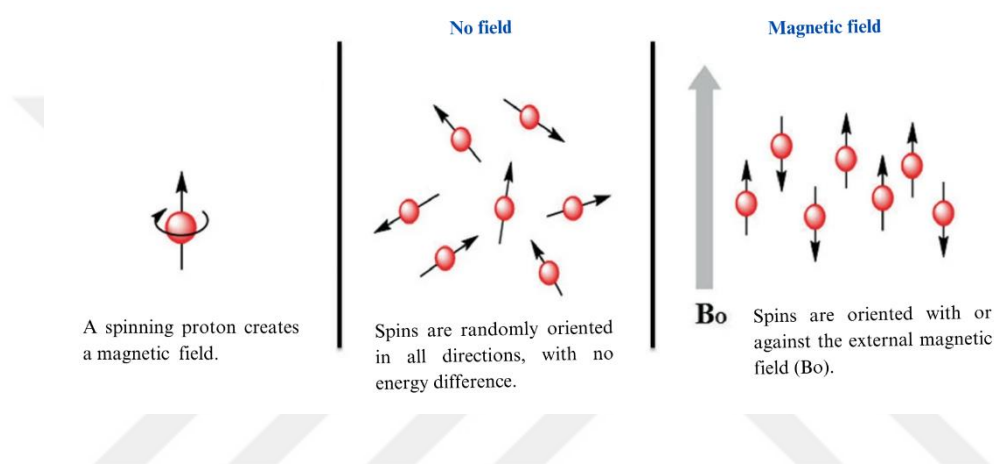


Figure 1.8 Orientations of spinning protons, with and without external magnetic field, reproduced from (Rashid et al., 2021)

The number of protons aligned parallel and anti-parallel to the external magnetic field vector is not equal. However, this difference is not as large as one might think and varies depending on the strength of the magnetic field (B_0). The net magnetization vector, which aligns parallel to the strong magnetic field vector, is referred to as longitudinal magnetization (T_1). At the same time, under the influence of the strong magnetic field, the protons begin to exhibit a random oscillatory motion called precession. RF is required to obtain a signal from the sample by utilizing the net magnetization vector. RF pulse causes some protons undergoing precession to absorb energy from the radio waves and change their orientation. After the application of a 90° RF pulse, the net magnetization vector shifts, and the newly formed magnetization vector is referred to as transverse magnetization (T_2) (Konez,

1995). The plots of transverse and longitudinal relaxation against time are illustrated in the Figure 1.9.

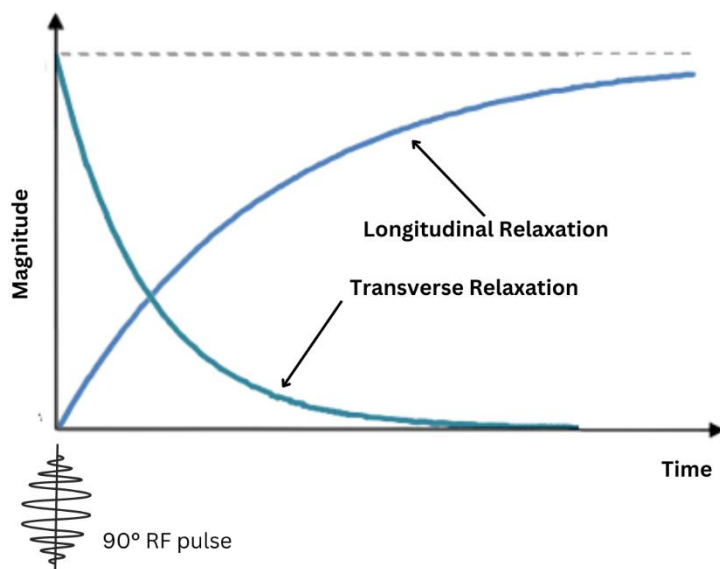


Figure 1.9 T_1 and T_2 relaxation curves, reproduced from (Konez, 1995)

In short, transverse relaxation time (T_2), also known as spin-spin relaxation time, is the duration it takes for transverse magnetization (T_1) to decay to zero at equilibrium (Alacik Develioglu et al., 2020). T_2 offers insights into the mobility of hydrogen molecules, indicating an immobile protons when T_2 is short and a more mobile structure when T_2 is long (M. Han et al., 2014; Sánchez-Alonso et al., 2012).

In this study, TD-NMR is used for the characterization of both Pickering particles and emulsions. Non-Negative Least Square (NNLS) was applied to the T_2 decay curves of Pickering particle solutions to obtain a relaxation spectrum. The relaxation spectrum contains peaks, in other words, proton pools that can be an indication of water distributions, such as solid-water interactions, solid-solid interactions, and water entrapped within the matrix (Kilercioglu et al., 2016; Ozel et al., 2017; Oztot et al., 2010).

1.4 Characterization of Pickering Emulsions

1.4.1 Confocal Laser Scanning Microscopy

Confocal Laser Scanning Microscopy (CLSM) operates on the principle that the light source is focused at a specific depth within the sample. This approach was developed to address the limitations of traditional wide-field fluorescence microscopy. CLSM eliminates out-of-focus light caused by pinholes, resulting in high resolution by reducing the noise-to-signal ratio in the image. In addition, it can create detailed three-dimensional images and surface profiles of samples (Blonk & van Aalst, 1993; Takeuchi & Frank, 2001).

Food microstructures can be observed with CLSM by selectively labeling components such as carbohydrates, lipids, and proteins. Component-specific stains, in other words, fluorescent dyes, can be used for labeling. These stains are either adsorbed or can be covalently coupled to the microstructure of specific components. This allows for clear differentiation of components in the emulsion when viewed under confocal fluorescent microscopy (Hu et al., 2017). CLSM reveals the interfacial structures and location of solid particles adsorbed at the interface of Pickering emulsions. The morphology and positioning of solid particles at the interface with CLSM have been well-reported in the literature (Kwok & Ngai, 2016; Mwangi et al., 2016; Yusoff & Murray, 2011).

1.4.2 Rheology

Rheology is the science that studies how materials flow and deform. The rheology of foods plays an important role in determining quality characteristics of food processing and meeting consumer expectations (McKenna & Lyng, 2003). The science of rheology generally studies the relationship between stress (σ) and strain (γ), using the parameters derived from this correlation to characterize foods. Rheological measurements are made by applying stress to the food with the help of

specific geometries suitable for the textural properties of the food and measuring the strain under applied stress (Zhong & Daubert, 2013). Food emulsions generally are in a liquid state, and important parameters to determine for liquid foods are viscosity (μ), yield stress (σ_0) if present, and flow behavior. Liquids in which the shear stress is directly proportional to the shear rate are Newtonian liquids; their viscosity is constant, and the relationship between them is shown in equation 1.1 (Ofoli et al., 1987)

$$\sigma = \mu\gamma \quad (1.1)$$

Water, oil, fruit juice, and milk are examples of Newtonian liquids. However, most liquid foods have non-newtonian flow behavior. The equation used to describe non-Newtonian fluids is the Hershel-Bulkley model (1.2):

$$\sigma = K\gamma^n + \sigma_0 \quad (1.2)$$

where K is the consistency coefficient, n is the flow behavior index, and σ_0 is the yield stress. Many factors influence the choice of rheological model used to describe how a given fluid flows. Hershel-Bulkley model is suitable for modeling the flow behavior of many liquid foods. The equation is very useful because it obeys Newtonian fluids when there is no yield stress and $n=1$, a power law (shear thinning) when $0 < n < 1$, and shear thickening when $1 < n < \infty$. The Bingham plastic behavior can be considered as a distinct case. For Newtonian and Bingham plastic behavior, K is generally called viscosity (μ) or plastic viscosity (μ_{pl}), respectively. The Table 1.1 summarizes this constant for each flow behavior model (Steffe, 1996).

Many factors are known to affect the rheology of emulsions. These include volume fraction, viscosity of each phase, size of the droplets, interfacial tension (Tatar et al., 2017). Additionally for the Pickering emulsions rigid colloidal particle structures may exist at the interface and in each bulk phase, which significantly affects the rheological properties of the Pickering emulsion. Therefore, Pickering emulsions can exhibit characteristic rheological properties and the studies summarizing the rheological properties of Pickering emulsions are categorized into dilute,

intermediate and concentrated regimes. The stored energy of isolated droplets can be easily dissipated by the continuous phase in dilute regimes where the amount of dispersed phase is relatively small. The shear-thinning behavior in emulsion systems in dilute regime is particularly evident for various reasons, including droplet alignment in the direction of flow and droplet breakup under significant external forces. In comparison, conventional surfactant stabilized emulsions exhibit lower viscosity and a much slower reduction in viscosity with increasing shear rate compared to Pickering emulsions, likely due to the flexible nature of surfactant-coated droplets (Chevalier & Bolzinger, 2013; Lee et al., 2022).

Table 1.1 Special cases of the Herschel-Bulkley model: Newtonian, Power Law and Bingham Plastic fluids (Steffe, 1996)

Fluid	K	n	σ_0
Herschel Bulkley	>0	$0 < n < \infty$	>0
Newtonian	>0	1	0
Shear thinning (pseudoplastic)	>0	$0 < n < 1$	0
Shear thickening (dilatant)	>0	$1 < n < \infty$	0
Bingham plastic	>0	1	>0

Examining the rheology of Pickering emulsions provides insights into their flow behavior and stability, while also helping to identify potential mechanisms of instability (Dickinson, 2003; Xia et al., 2021). Moreover, rheology is crucial in understanding how foods behave within the digestive system. When a solid food is masticated, textural properties change, or when a liquid food passes through the digestive system, the flow behavior of food is affected by rheological properties (Liu et al., 2021). In view of the fact that most of the foods that we consume on a daily basis are emulsions, the study of their fate in the digestive system is very important. It is well known that a liquid food generally passes through the digestive system

without being chewed. Its flow behavior as it passes from the stomach to the intestines is influenced by different digestive environments (Bornhorst & Singh, 2014; Marciani et al., 2001). A method to prolong the time food remains in the stomach, thereby extending digestion time, is to slow down the rate at which food passes from the stomach to the intestines. It is believed that the rate of passage is slow down by gelation in the gastric environment or by increasing the viscosity of the continuous phase (Guo et al., 2020; Jin et al., 2023). Modifying the rheological properties of foods through engineering techniques is considered effective in managing non-communicable diseases like obesity, which arises from excessive food intake and its associated health conditions (Madadlou et al., 2016).

1.4.3 Time Domain ^1H Nuclear Magnetic Resonance

As previously mentioned, TD-NMR was used for the characterization of both Pickering particles and emulsions, as well as for analyzing the microstructural changes during digestion. The transverse magnetization is often used in analyzing colloidal systems to provide detailed information regarding the mobility of the water and oil phases. It is linked to the energy exchange between nearby spins and is described by the spin-spin relaxation time constant (T_2). TD-NMR experiments can detect changes in T_2 , which reflect variations in water content, the molecules surrounding the water, and the physical state of the water (Akbas et al., 2019; Kirtil et al., 2017). The interaction of water molecules with their environment can generally be explained by fitting the exponential decay signal to the bi or exponential curves. The parameters obtained from fitted curves are associated with the component that decays in a longer time (T_{2a}) and the component that decays in a shorter time (T_{2b}). In emulsion systems, the shorter (T_{2b}) component typically corresponds to the portion interacting with oil, while the longer (T_{2a}) component generally represents the portion interacting with water (Alacik Develioglu et al., 2020; Kirtil & Oztop, 2016c). Thus, T_2 times of emulsions are helpful in understanding the mobility of

water and oil protons during the shelf life of emulsions by observing changes in the microstructure (Akbas et al., 2019; Pocan et al., 2019).

During the digestion process with and without enzymes is also analyzed by applying NNLS to the T_2 decay curves of digesta. Analyzing the digestion behavior of Pickering emulsions using the T_2 relaxation spectrum offers valuable insights into how enzymes and pH influence emulsion stability and helps identifying the various proton pools within the digestive bolus.

1.4.4 Laser Diffraction Analysis

Laser diffraction determines particle size distributions of the systems by analyzing how the intensity of light scattered by a dispersed sample changes with the scattering angle as a laser beam passes through it. The operating principle of laser diffraction is displayed in Figure 1.10. Large particles scatter light at narrower angles, in contrast to small particles, which scatter light at broader angles relative to the laser beam. This scattering pattern is analyzed using Mie theory to determine the sizes of the particles (Silva, 2022; Wriedt, 2012).

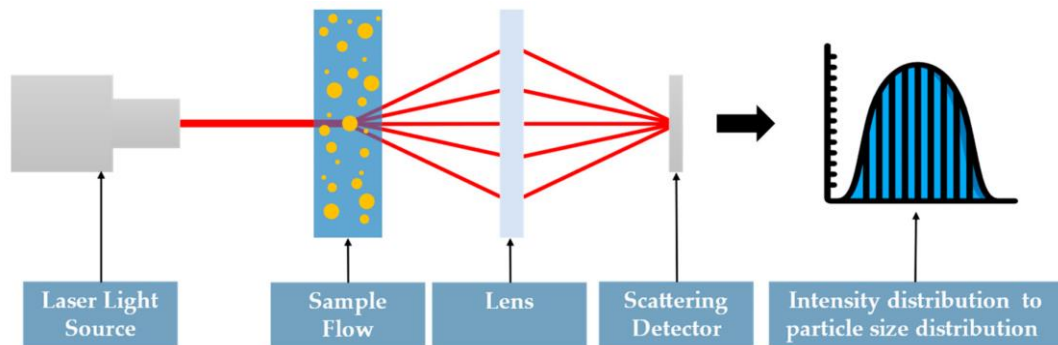


Figure 1.10 Operating principle of laser diffraction analysis

Particle size of the oil droplets in the emulsions significantly affects stability and rheological properties (L'Estimé et al., 2023). Typically, smaller particle sizes provide better stability against instability mechanisms such as flocculation, gravitational separation and coalescence. The growth of oil droplets during storage

generally leads to instability in emulsions. Additionally, emulsions with smaller particle sizes are generally observed to exhibit increased viscosity and elastic properties (Dickinson, 1994; Garrett, 1965; Pal, 1996a). Furthermore, the size of the particles also has an impact on other properties, including optical, sensory, and quality. Sensory properties such as mouthfeel and creaminess are strongly influenced by particle size (L'Estimé et al., 2023). Optical properties are significantly affected by particle size and have an impact on the visual characteristics of emulsions (Wang et al., 2021).

1.5 Objective of the Study

The main objective of this study is to formulate stable Pickering emulsions stabilized with whey protein isolate and gum arabic, which are widely used as protein and polysaccharides, respectively, in the food industry. This thesis aims:

- To analyze the complexation of Pickering particles formed by negatively charged polymers at neutral pH
- To investigate the formulation parameters and rheological properties of Pickering emulsions by varying the ratio of gum arabic and whey protein isolate
- To evaluate the stability of the formulated Pickering emulsions over time, by using Laser Diffraction and TD-NMR analysis.
- To assess the digestion behavior of the formulated Pickering emulsions using *in vitro* methods.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Whey protein isolate containing 96% protein (WPI) was purchased from Hardline Nutrition (Hipro Isowhey, Kavi Food Ltd. Co., Istanbul, Turkey). Gum arabic (GA) obtained from *Acacia senegal* was used (Smart Chemistry Ltd. Sti., Izmir, Turkey). Sunflower oil (Yudum, Balikesir, Turkey) was used for emulsion preparation and purchased from a local grocery store in Ankara, Turkey. Mucin from porcine stomach was used to simulate the composition of saliva and gastric environments. (Sigma-Aldrich Chemical Co.), along with chemicals such as NaCl (Sigma-Aldrich Chemical Co.), KCl (Sigma-Aldrich Chemical Co.), and NaHCO₃ (Sigma-Aldrich Chemical Co.). Additionally, α -amylase enzyme derived from *Bacillus subtilis* with the activity of 160,000 BAU g⁻¹ (MP Biomedicals, USA) and pepsin from porcine gastric mucosa with the activity of 242 U mg⁻¹ (MP Biomedicals, USA) were utilized for the digestion experiments. Sodium azide was used to inhibit microbial growth during the storage period of the emulsions (Merck KGaA, Germany). All solutions were prepared using distilled water. Sodium hydroxide (NaOH, Sigma-Aldrich Chemical Co., USA) and hydrochloric acid (HCl, Sigma-Aldrich Chemical Co., USA) were used for pH adjustments.

Simulated saliva and gastric fluids were prepared with relevant chemicals for *in vitro* pepsin characterization. To simulate the saliva (SSF), a solution was prepared using deionized water containing mucin (1g L⁻¹), α -amylase (1.180g L⁻¹), NaCl (0.117g L⁻¹), KCl (0.149g L⁻¹), and NaHCO₃ (2.100g L⁻¹). For simulated gastric juice (SGF), mucin (1.5g L⁻¹), NaCl (7.8g L⁻¹), and pepsin (1g L⁻¹) were mixed in deionized water.

2.2 Methods

2.2.1 Preparation of Pickering Particles

WPI (10% w/w) and GA stock solutions (2% w/w) were prepared by applying high shear at 17.000 rpm (Ultraturrax, IKA T18, Germany) for 3 minutes using distilled water and stored overnight at 4 °C for complete hydration. The experimental approach described in the work of Zhang et al. was followed during the preparation of Pickering particle solutions (Sha Zhang et al., 2014). Hydrated WPI and GA solutions were mixed by applying high shear for 2 minutes at 14.000 rpm (Ultraturrax, IKA T18, Germany) to produce Pickering emulsion with PR: PS ratio (0.01, 0.02, 0.05, 0.10) as shown in Table 2.1. To investigate the complexation behavior of WPI and GA under heat, the pH of the protein-polysaccharide solutions was adjusted to 7.0 using 1 M HCl or NaOH (Mettler Toledo, Switzerland). Distilled water was used to eliminate the effect of ionic strength, ensuring that both pH and ionic strength remained stable throughout the complexation process. The mixture was placed in a water bath at 85°C for 30 minutes to facilitate the complexation of protein and polysaccharide. Subsequently, the solutions were cooled using cold water and then processed using a high-pressure homogenizer (PandaPLUS 2000, GEA, Parma, Italy) operating at two stages, of 250 and 50 bar pressures with 2 passes.

2.2.2 Preparation of Pickering Emulsions

Sunflower oil was incorporated into the emulsion at a concentration of 20%(w/w), subjected to high shear at 14.000 rpm for two minutes, and subsequently processed through a high-pressure homogenizer (PandaPLUS 2000, GEA, Parma, Italy) operating at two stages, of 250 and 50 bar pressures with 2 passes. The ratio of polysaccharide to protein (PS:PR) in the resulting Pickering emulsions was varied (0.01, 0.02, 0.05, 0.10) by solely adjusting the concentration of gum arabic (GA),

while maintaining a constant ratio of protein and oil. The emulsions were tested to be oil in water by dropping water into them, and it was observed that they dissolved in the continuous phase when water was added. Emulsions were stored by adding 0.01% sodium azide to prevent microbial growth. Each week of storage, particle size and TD-NMR measurements of emulsions were carried out in order to investigate the stability of emulsions during storage.

Table 2.1 Pickering Emulsion Formulations Stabilized with Whey Protein Isolate (WPI) and Gum Arabic (GA)

Sample Name	PS:PR Ratio	Protein (%)	Polysaccharide (%)
WG_0.10	0.10	5%	0.50%
WG_0.05	0.05	5%	0.25%
WG_0.02	0.02	5%	0.10%
WG_0.01	0.01	5%	0.05%

2.2.3 *In vitro* digestion of Pickering Emulsions

The static *in vitro* digestion method was performed using a method similar to that of Mennah Govea & Bornhorst (Mennah-Govea & Bornhorst, 2016) altering the INFOGEST 2.0 static digestion simulation method (Minekus et al., 2014). To simulate the saliva (SSF), a solution was prepared using deionized water containing mucin (1.0 g L⁻¹), α -amylase (1.180 g L⁻¹), NaCl (0.117 g L⁻¹), KCl (0.149 g L⁻¹), and NaHCO₃ (2.100 g L⁻¹). Subsequently, the pH of the solution was adjusted to 7.0 using 1.0 M HCl. For simulated gastric juice (SGF), mucin (1.5 g L⁻¹), NaCl (7.8 g L⁻¹), and pepsin (1 g L⁻¹) were mixed in deionized water. PH of SGF was adjusted to 1.8 using 3.0 N HCl for simulation of fasting state of the stomach.

Briefly, in this *in vitro* digestion simulation procedure, the experimental conditions are constant at all stages of oral and gastric digestion. The oral phase begins with diluting the food 1:1(w/w) with simulated salivary fluid (SSF). The oral bolus is then diluted 1:1(vol/vol) with simulated gastric fluid (SGF) and gastric enzyme (pepsin) and incubated under shaking at pH 3.0 for 20 min. The dilution factors were made with reference to this procedure.

2.2.4 Characterization of Pickering Emulsions

2.2.4.1 Rheology

The rheological properties of the emulsions before and after digestion were measured using the Kinexus Pro Rheometer (Malvern Instruments Ltd., Worcestershire, UK). Emulsion characterization experiments were performed at room temperature (25 °C) and a shear rate range of 0.1 to 100 s⁻¹ using a concentric cylinder system (cup and bob geometry). Measurements were performed in triplicate. Flow behaviour of emulsions was modeled using the power-law model with the following formula;

$$\tau = K \cdot \dot{\gamma}^n \quad (2.1)$$

2.2.4.2 Turbidity

The absorbance of colloidal surfactant solutions used to stabilize the emulsions was measured at 600 nm using a microplate reader (FlexA-200/FlexA-200HT, Allsheng, China). Colloidal particle solutions were loaded on the microplate and shaken for 2-3 seconds before measurement. Distilled water was used as a blank reference. All measurements were recorded in triplicate for each sample and mean values are reported.

2.2.4.3 Confocal Laser Scanning Microscopy

Confocal Laser Scanning Microscope (Zeiss, LSM 510, Germany) was used to observe the microstructure of the emulsions. Pickering emulsions with the highest protein-polysaccharide ratio (0.1) were dyed on the second day of production, with reference to the work of Li et al. (2019). One ml emulsion was mixed by taking 20 μ L of Nile Red and fluorescein isothiocyanate (FITC) dyes separately prepared in 1mg/mL ethanol and kept in the dark for 30 minutes. The aim was to dye the oil in emulsions with Nile Red and the protein with FITC dye. The dyed emulsions wrapped with aluminum foil were viewed on the coverslip with a 10x objective. Nile Red and FITC were excited at wavelengths of 514 and 488 nm, respectively.

2.2.4.4 Particle Size Measurements

Particle size of Pickering emulsions was measured using a laser diffraction particle size analyzer based on Mie Scattering Theory (Mastersizer 3000; Malvern Instruments, Worcestershire, UK). Particle size of emulsions were stated using the Sauter mean diameter $D[3,2]$ formula shown in equation $d_{3,2} = \frac{\sum_{i=1}^n n_i d_i^3}{\sum_{i=1}^n n_i d_i^2}$ (2.2). Refractive index of sunflower oil was taken as 1.46 and absorbance was chosen 0.01 in all measurements. The limit of the obscuration range was set as 6-10%. Emulsions were stored at 25 °C and measured in triplicate for each week.

$$d_{3,2} = \frac{\sum_{i=1}^n n_i d_i^3}{\sum_{i=1}^n n_i d_i^2} \quad (2.2)$$

2.2.4.5 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectra of freeze-dried colloidal surfactant solutions, WPI and GA stock solutions were obtained using an IR Affinity-1 spectrometer (Shimadzu Corporation, Kyoto, Japan). The measurements were collected within a 600-4000 cm^{-1} spectral range, and 10 scans were applied at a resolution of 4 cm^{-1} . The baseline-normalized

spectrum was analyzed using LabSolutions IR software (Shimadzu Corporation, Kyoto, Japan). FTIR peaks were found by using OriginLab (version 2024) statistical software and plotted using Excel.

2.2.4.6 Time Domain ¹H Nuclear Magnetic Resonance (TD-NMR)

TD NMR experiments were performed using a 0.5 T (20.34 MHz) time domain NMR System (Spin Track, Resonance Systems GmbH, Kirchheim/Teck, Germany) with a 10 mm radiofrequency (RF) coil and were carried out to investigate the interactions between oil and polymer solution. Transverse relaxation time (T_2) of the emulsions were obtained using Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. CPMG pulse sequence was applied, with acquisition parameters set at an echo time of 2000 ms, a range of 800–1200 ms for the number of echoes, and 16 for the number of scans. The relaxation times were calculated using MATLAB (R2019b, The MathWorks Inc., USA) by considering a bi-exponential behavior.

$$M_{xy}(t) = M_0 \left(e^{-\frac{t}{T_2}} \right) \quad (2.3)$$

2.2.4.7 Statistical Analysis

The measurements were conducted in replicates, and the results are presented as means along with their corresponding standard errors. The data underwent analysis of variance (ANOVA) to identify significant differences at a 5% significance level, and the Tukey test was applied using Minitab (ver. 21.1.1, Minitab Inc., UK). Ryan-Joiner test was checked for normality, and transformations (i.e., square root, logarithmic, and Box-Cox) were performed on the dataset if assumptions were not met.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Characterization of Pickering Particles

3.1.1 Turbidity

Turbidity provides information about biopolymer aggregation in the systems and gives an idea regarding the stability of the solutions. The turbidity of solutions is related not only to the droplet size of the polymers but also to droplet shape and distribution. An increase in turbidity indicates an increase in biopolymer aggregation and a decrease in stability (Jones, 2009; Linke & Drusch, 2016; Wang et al., 2022). As shown in Table 3.1, the PS:PR ratio of 0.10, exhibits lower turbidity among other particles ($p < 0.05$), which can be correlated with a more uniform and well-distributed particle solution while the PS:PR ratio of 0.01 is the most turbid ($p < 0.05$) Pickering particle solution indicating non-uniform droplet size and distribution.

Table 3.1 Turbidity of formulated particles at 600 nm

Particle Name	Turbidity
WG_0.01	$11.255 \pm 0,0003^a$
WG_0.02	$9.2300 \pm 0,0002^c$
WG_0.05	$10.4800 \pm 0,0021^b$
WG_0.10	$8.54000 \pm 0,0001^d$

**Different letters in the same column represents a significant difference ($p < 0.05$)*

It is known that GA is a proteinous polysaccharide whose structure can be represented by the Wattle Blossom model, where carbohydrate units, arabinogalactans, are attached to arabinogalactan protein complex (AGP/GP) through serine, hydroxyproline, and proline groups. The denaturation of proteins

causes exposure of hydrophobic sites which result in altered electrostatic interactions that proteins can undergo. Proteins typically possess charged groups on their surface, which can interact electrostatically with other charged particles in the solution. The turbidity trend caused by the PS: PR ratio change can be explained through two basic mechanisms. First, the increase in concentration in GA may lead to an increase in the number of local positive charges on arabinogalactan units. This may enable a more uniform electrostatic interaction with the negatively charged groups in the whey protein isolate (Sabet et al., 2021), resulting in the formation of more similar particles in terms of size and shape by providing less turbidity. Secondly, pressure might induce protein denaturation and interdigitation of the carbohydrate chains of GA by reducing the hydrodynamic volume and resulting in a more branched structure due to partial or full interdigitation of arabinogalactan units (Ma et al., 2015; Tan et al., 2004). Therefore, it is thought that the reorientation of hydrophobic groups of both WPI and GA as a result of heat and high pressure causes more electrostatic interaction around the polypeptides, producing particles with less hydrodynamic volume but branched structure with the increase in GA concentration. For this reason, it was thought that particles produced with a PS: PR ratio of 0.10 may have less turbidity. It was concluded that the turbidity of Pickering particle solutions could be used as a tool to demonstrate differences in the adsorption behavior of oil droplets while stabilizing the emulsion. It is suggested that due to smaller hydrodynamic diameter and branched structure, particles with PS: PR ratio of 0.10 showed better surface adhesion properties and more surface for interaction with other molecules may have been produced.

3.1.2 Time Domain ^1H Nuclear Magnetic Resonance

A relaxation spectrum is obtained by Inverse Laplace Transformation of relaxation curves by providing a one-dimensional spectra, which makes it easier to follow the molecular diffusion and physiochemical changes in proton populations over time or during a process (Duval et al., 2006; Kirtil & Oztop, 2016a). With the T_2 relaxation spectra, it is possible to obtain information about the water content of the sample, the physical properties of water, and the interaction of water with surrounding macromolecules. T_2 relaxation times of the Pickering solutions were measured in the study to examine the properties of newly formed particles. As can be seen in Figure 3.1, while WPI solution (10 % w/w) was measured to have a relaxation time of 0.69 s, GA (2% w/w) had a relaxation time of 1.44 s respectively. It is expected that WPI and GA would show different relaxation times due to their different structural and interaction properties. This difference was explained by the structured protein matrix that was created by WPI and the flexible polysaccharide structure of GA. On the other hand, the complexation of these biopolymers resulted in new proton pools having relaxation times of 0.334 and 0.386 s for different PS: PR ratios (Figure 3.1). It is known that T_2 relaxation time decreases as intermolecular affinity increases (Kirtil & Oztop, 2016a; Ozel et al., 2017). Based on this, the difference in the relaxation times can be directly interpreted as a change in the relationship of the molecules with water and even an increase in the affinity between the molecules. This supports the claim that new Pickering particles might have been produced, which aligns with the results to be discussed later. The particles obtained with a PS: PR ratio of 0.10 had shorter T_2 values (0.334 s) Figure 3.1(f), indicating that particles most effectively restricted the molecular mobility of water. This was attributed to their smaller hydrodynamic diameter and branched structure, which provided a greater surface area for interaction with water molecules.

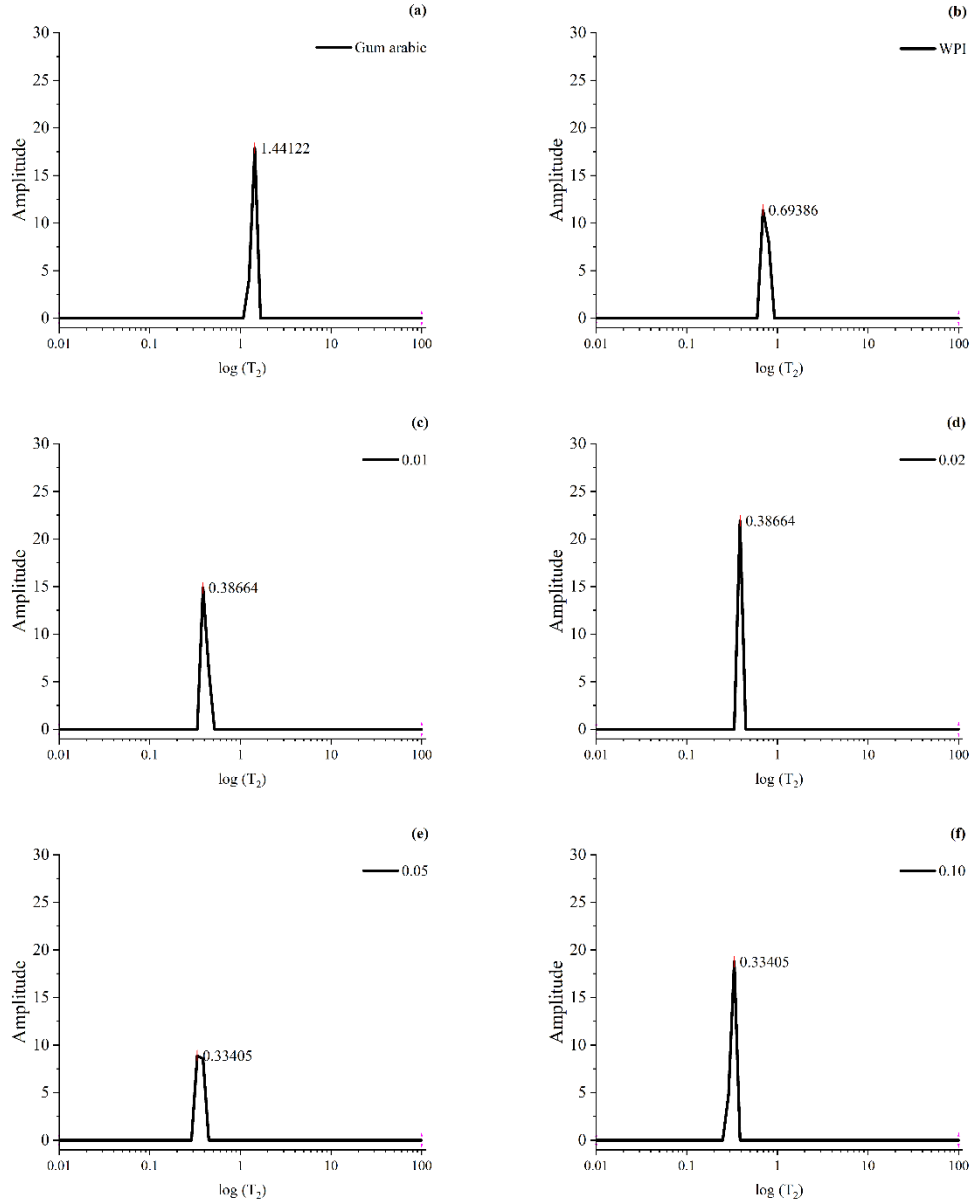


Figure 3.1 NNLS spectrum of particle solutions (a) GA (2%), (b) WPI (10%), (c) PS: PR=0.01, (d) PS: PR=0.02, (e) PS: PR=0.05, (f) PS: PR=0.10

When the system is analyzed from a thermodynamic perspective, two primary configurations emerge within aqueous solutions of proteins and polysaccharides: (1) a uniform, stable single-phase system where the molecules coexist harmoniously, and (2) a biphasic system where both types of molecules share the same concentrated phase (Laneuville et al., 2000; Sun et al., 2018). In the context of the Pickering

particle solutions being investigated, water serves as the solvent, with the anticipated interactions leading to phase formation primarily involving water. Should the system exhibit biphasic characteristics, it would result in the observation of two distinct proton populations within the T_2 spectra. In complex food systems, it is common to encounter multiple proton pools that reflect changing interactions and mobility within the system. Therefore, it can be assumed that by using varying PS:PR ratios, new particles that exhibit different water binding abilities but have a uniform one proton pool are produced. In addition, the fact that more than one proton pool is not dominant in the spectra suggests that all WPI and GA are used for the production of new particles and that there is no uninteracted native polymer.

3.1.3. FTIR Spectra of Whey Protein and Gum Arabic Complex

The Fourier-transform infrared spectroscopy spectra of the WPI, GA, and WG Pickering particles with different concentrations of PS: PR are shown in Figure 3.2. It is seen that the spectrum of the Pickering particles is more similar to the native WPI structure, which may indicate no change in the main backbone of the WPI structure where GA is attached. Two notable and distinct peaks observed in the spectra correspond to the primary amide group of proteins at 1630 cm^{-1} (Amide I) and the secondary amide group (Amide II) at 1530 cm^{-1} (Figure 3). These bands are indicative of the protein's secondary structure and are susceptible to changes in C-N stretching, C-O in-plane bending, C-C stretching along with the N-H bending on Amide II band (Saxton & McDougal, 2021) or C=O, C-N stretching and C-C-N deformation vibrations of the Amide I band (Ertugrul et al., 2021) of the protein backbone. However, a decrease in peak intensity without a shift in position may indicate that the attachment of new molecules affecting the local environment around the protein's functional groups, and thereby leading to reduced absorbance (Barth, 2007). When the FTIR spectra of newly created Pickering particles examined, it can be seen that the intensity of the characteristic absorption bands of Amide I and Amide II bands of Pickering particles decreases as the PS:PR ratio increases while the

positions of these peaks remain unchanged. This suggest that while local environment or interactions of functional groups may be affected more with the increased GA ratio, but overall secondary structure or the backbone conformation of the protein was not altered by the attachment of GA.

The GA spectrum draws attention with the medium strength peak associated with the galactan structure characterized by C-O-C and C-O stretching at 1024 cm^{-1} and the broad OH band observed around 3320 cm^{-1} (Hou et al., 2022). The broad peak around 3320 cm^{-1} is primarily attributed to O-H stretching vibrations. It can be seen that peak is quite broad in GA spectra, and gradually shift to 3256 cm^{-1} or disappeared for the Pickering particles with increasing PS:PR ratio. The width of the peak is correlated with the intense hydrogen bonds between the hydroxyl groups found in the polysaccharide chains of GA where disappearance indicates that the hydroxyl groups are no longer free and are likely involved in hydrogen bonding with the protein molecules in WPI. This phenomenon can be attributed to the decreased interaction of Pickering particles with water as the PS: PR ratio increases. It can be concluded that the hydrophilic behavior of particles decreases with the increase in PS: PR ratio. Therefore, it was hypothesized that an increased PS: PR ratio would lead to improved absorption of Pickering particles to the oil interface.

Also, it is observed that the peak at 1024 cm^{-1} in GA diminishes consistently across all PS: PR ratios and a peak is observed at 1070 cm^{-1} , which comes from WPI. This disruption suggests that glycosidic linkage and hydroxyl groups present in GA are involved in new interactions or bonding with WPI, altering their vibrational environment and proving the complexation of WPI and GA. Although both biopolymers are negatively charged at pH 7.0, successful complexation with a lack of precipitation is thought to be due to partial charge neutralization or partial charge-charge interactions between the two biopolymers (Loveday et al., 2013).

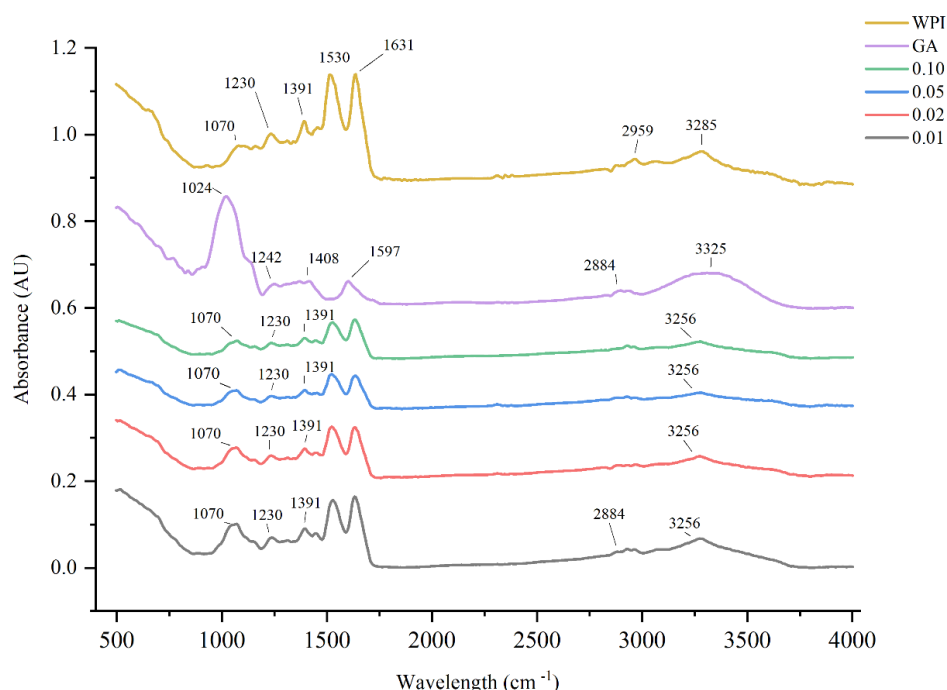


Figure 3.2 FTIR spectra of WPI, GA and WG colloidal particles with different PS:PR ratio

3.2 Characterization of Pickering Emulsions

3.2.1 Microstructure of Pickering Emulsions

Confocal Laser Scanning Microscopy (CLSM) experiments were performed, drawing on findings from Xiao et al. (2016) and Wei et al. (2019), to verify the formation of emulsion droplets mediated by Pickering particles (Wei et al., 2019; Xiao et al., 2016). The study specifically focused on a Pickering emulsion stabilized by Pickering particles with the highest PS: PR ratio of 0.10 to examine its morphology. The CLSM images revealed four distinct components, including a light microscope image positioned in the lower left corner, as depicted in Figure 3.3. Light microscopy confirmed the precise localization of oil droplets. The fluorescence from the green spectrum highlighted the proteins tagged with Fluorescein Isothiocyanate

(FITC), interacting with GA. Simultaneously, the fluorescence from the red spectrum signified sunflower oil tagged with Nile Red. The integration of both images in the lower right quadrant allowed for a comprehensive visualization. The confocal imaging demonstrated that proteins, in complex with gum arabic and exhibited in green fluorescence, encase the oil droplets, which are excited in red, confirming the successful formation of Pickering emulsions

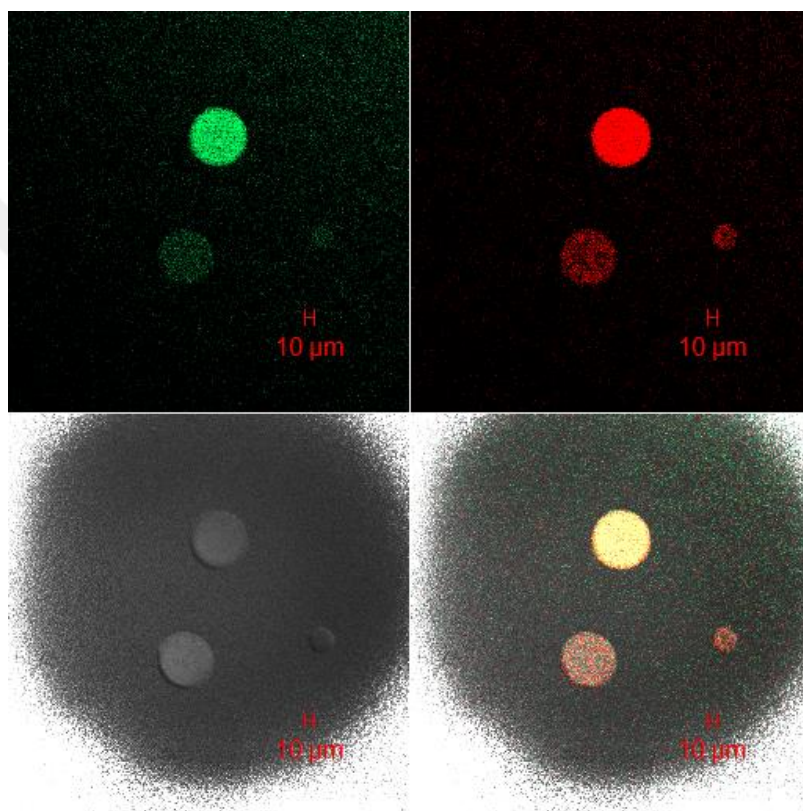


Figure 3.3. Confocal images of 20 wt % O/W Pickering emulsions stabilized by Whey Protein Isolate and Gum Arabic

3.2.2 Time Domain ^1H Nuclear Magnetic Resonance

T_2 relaxation times of Pickering emulsions were measured right after sample preparation and at the end of the 4th week. Emulsions are complex food systems where more than one mobile proton pool exists in the environment, which results in an exponentially decaying signal represented by a bi-exponential fit. In this study,

(T_{2a}) and (T_{2b}) are addressed to transverse relaxation time constants of fast (water) and slow (oil) relaxing components (Figure 3.4 a & b). According to the results, PS:PR ratio significantly affects the T_{2a} relaxation times ($p < 0.05$) which can be associated with the decreased mobility of water due to interaction with particles present in the continuous phase. The protons within macromolecules can be categorized into two types: labile and non-labile. Labile protons are those that can readily undergo chemical exchange with the protons in water. In contrast, non-labile protons are associated with groups that exchange with water protons more slowly, reflecting the inherent mobility of the macromolecules themselves (Duval et al., 2006). Heat treatment of GA has been shown to promote the formation of arabinogalactan (AG) and galactan protein (GP) into surface-active molecules known as arabinogalactan protein (AGP), through the establishment of covalent bonds (Klein, Aserin, Ishai, et al., 2010; Yue et al., 2022). Similarly, whey protein isolates, predominantly composed of β -lactoglobulin, undergo increased mobility upon heating, facilitating the formation of covalent bonds with adjacent molecules (Nicolai et al., 2011). Consequently, this process leads to the formation of WPI-GA particles characterized by an abundance of labile proton groups. These groups exhibit enhanced interactions with water, contributing to a reduction in the T_{2a} relaxation times.

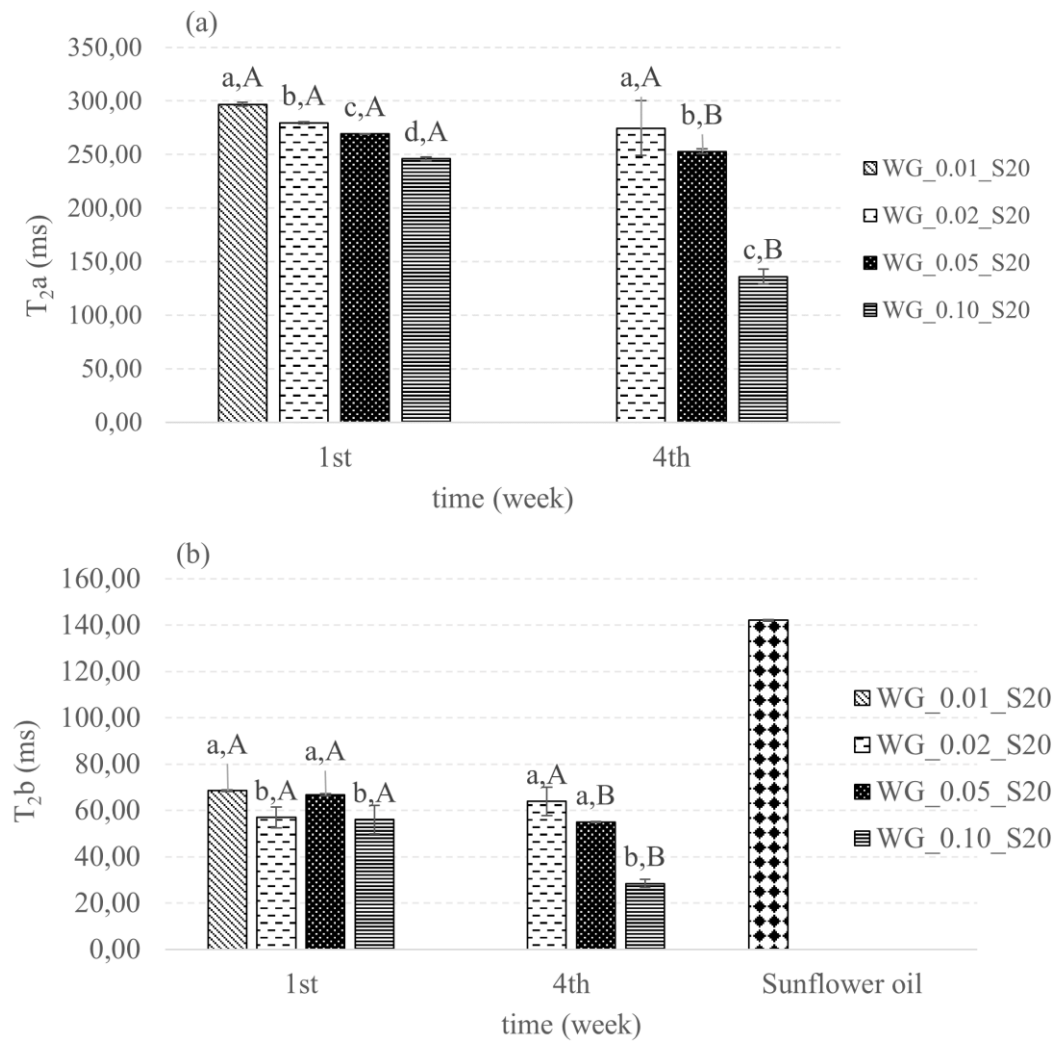


Figure 3.4. T_2 relaxation times were obtained from bi-exponential fitting of NMR relaxation data of Pickering emulsions (a) T_{2a} (b) T_{2b} .

Means within the same week, followed by the different small letters (a–d) are significantly different for each sample ($p < 0.05$). Means within the storage, followed by the different capital letters(A–B) are significantly different ($p < 0.05$).

Oil protons move at a frequency close to the Larmor frequency of ^1H by exhibiting a shorter relaxation time. Research conducted by Peng (2021) on the T_1 - T_2 maps showed that T_1 and T_2 relaxation times of sunflower oil are 228 ms and 168 ms respectively (Peng, 2021). It is clearly seen that in Figure 3.4b, the relaxation time of pure sunflower oil used in the preparation is measured as 148 ms, while T_{2b} times of each emulsion are much lower than that value. These lower relaxation times could

be associated with the presence of surface active agents adsorbed on the oil droplets droplets (Kirtil & Oztop, 2016b) by indicating the emulsification ability of the newly created WPI-GA particles. It is crucial to highlight that those emulsions prepared with Pickering particles at PS: PR ratios of 0.02 and 0.10 exhibit lower T_{2b} times in comparison to other emulsions in the first week ($p < 0.05$). As previously mentioned, the turbidity values of these particles, which were attributed to the production of more uniform and smaller particles, were lower than the other particles. When the relaxation times are analyzed, it might be evident that corresponding particles reduce the oil mobility more than the other particles, indicating that smaller and more numerous particles bind to the oil droplets. A striking result emerges here, which can be suggested as one of the most prominent results of this study relaxation times T_{2a} & T_{2b} , which are expected to increase over time due to deterioration of stability, decrease. It is mentioned that particles with specific interfacial electric potential can spontaneously adsorb at the oil-water interface without the need for additional energy (Ridel et al., 2016; Salari et al., 2014). Therefore, the results were interpreted with the presence of an excessive number of particles that show high affinity to adsorb oil droplets and complex with each other and further reduce the mobility of the oil phase during storage.

3.2.3 Particle Size

Particle size measurement is crucial for understanding the stability, quality, and performance of emulsions during storage since the distribution and size of the droplets significantly affect the system thermodynamically. Figure 3.5 shows the surface-weighted mean diameters ($D[3,2]$) of the emulsions. The results show that the particle size of the designed Pickering emulsions varies in the range of 0.756-0.789 μm for the freshly produced samples. It is important to note that formulation with a PS: PR ratio of 0.01 underwent phase separation during storage, so that it is not included on 4th week of measurements. According to the results, there is no

statistical difference in the particle size of the oil droplets between the 1st and 4th weeks ($p > 0.05$).

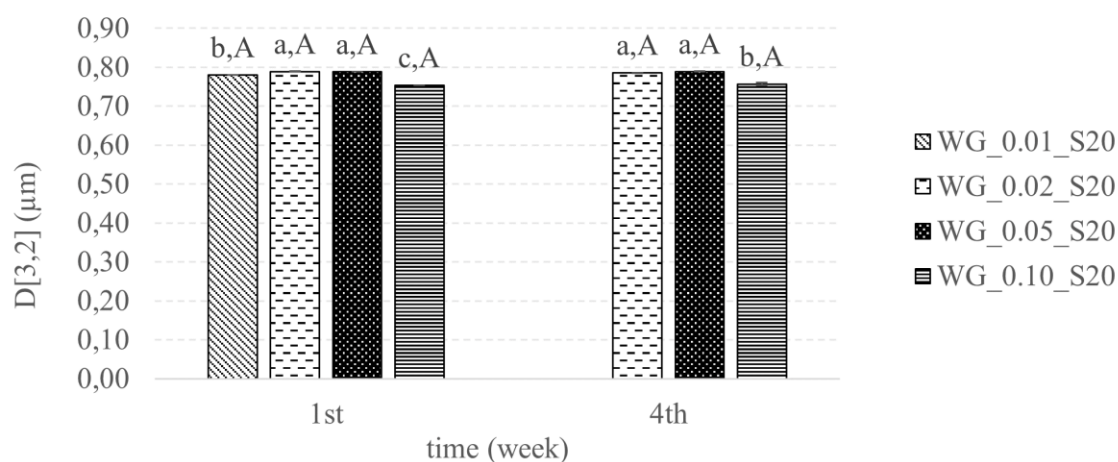


Figure 3.5 Surface-weighted mean diameter $D[3,2]$ values of emulsions.

Means within the same week, followed by the different small letters (a–d) are significantly different for each sample ($p < 0.05$). Means within the storage, followed by the same capital letter(A) is not significantly different ($p > 0.05$).

The stability of Pickering emulsions, characterized by the irreversible adsorption of particles, is closely correlated with particle concentration (Benyaya et al., 2024). This leads to better coverage of droplets and a reduction in the tendency of phase separation by creating a more stable structure. Therefore, the particle concentration must have a high ability to cover the oil-water interface. At sufficiently high surface coverage, physically stable emulsions can be obtained. It was inferred that a sufficient concentration was achieved in emulsions with particle concentrations exceeding 0.01. The occurrence of phase separation for WG_0.01_S20 has been attributed to insufficient particle concentration. While a particle having a PS: PR ratio of 0.01 did not produce a stable emulsion, doubling the GA ratio used to produce particles to 0.02, the number of particles to cover oil droplets is sufficient to obtain a stable emulsion. Furthermore, it was observed that a tenfold increase in the ratio of GA from 0.01 to 0.10 resulted in a significant decrease in the particle size of the emulsions ($p < 0.05$) indicating (i) a larger surface area relative to volume and more surface area for particles to adsorb (Iyer et al., 2015) or (ii) oil droplets covered

by high number of particles (Benyaya et al., 2024) which both can enhance the stability of emulsions. Based on this, it might be concluded that the increase in the GA ratio can also be related to the number of particles produced.

Research has shown that a decrease in droplet size leads to an increase in the viscosity of both W/O and O/W emulsions and on the shear thinning effect of fine emulsions (L'Estimé et al., 2024; Pal, 1996b). As stated previously WG_0.10_S20 has lower particle size compared to other formulations which may result in increased surface area, enhanced interaction between droplets and surrounding medium contributing to the shear thinning behavior. The increased surface area of smaller droplets enhances particle-particle interactions, leading to a more pronounced shear-thinning behavior in the emulsion, as will be discussed in the latter section. This observation underscores the physical behavior of the particles observed in turbidity and chemical behavior suggested by FTIR experiments.

3.2.4 Rheological Characterisation of Pickering Emulsions

The rheological properties of the developed emulsions are given in Figure 3.6 and revealing that all emulsions, regardless of their PS: PR ratios, demonstrate shear-thinning behavior, also known as pseudoplastic behavior ($n < 1$). This observation aligns with the existing literature, which frequently reports pseudoplastic behavior in oil-in-water (O/W) emulsions modified with various polysaccharides (Coia & Stauffer, 1987). The emulsion with the lowest PS:PR ratio of 0.01, corresponding to the minimal polysaccharide concentration Table 2.1, had the least pronounced pseudoplastic behavior ($n=0.384$), while the one with the highest polysaccharide concentration (0.50%), PS:PR ratio of 0.10, exhibited the highest pseudoplastic behavior ($n=0.253$) (Table 3.2).

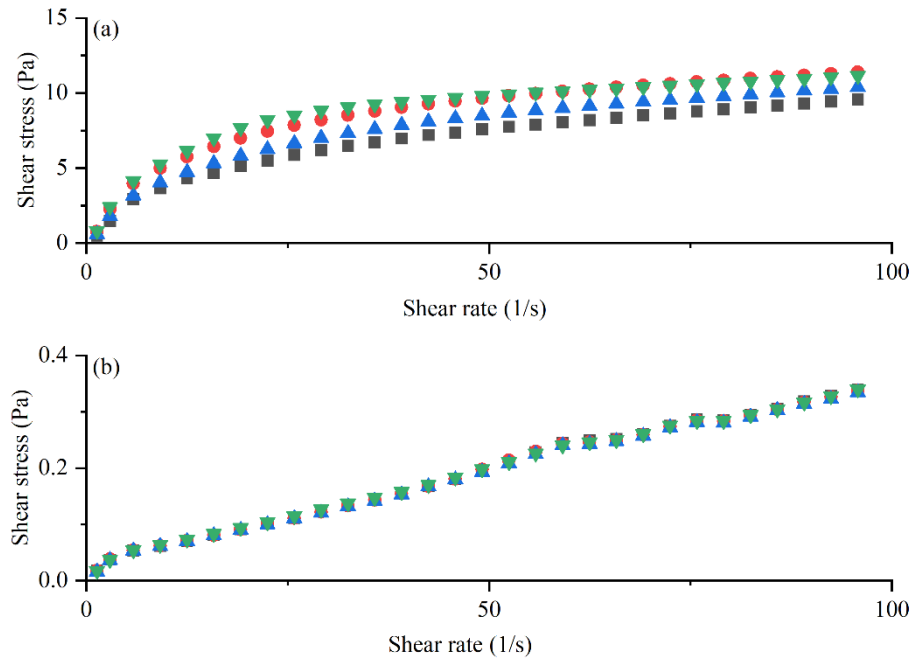


Figure 3.6 Shear stress and shear rate graphs of the emulsions before digestion (a) and after 20 minutes of digestion (b) (▼): WG_0.10_S20, (▲): WG_0.05_S20, (●):WG_0.02_S20, (■):WG_0.01_S20.

Table 3.2 Rheological model constants before digestion

Sample Name	K	n	R ²
WG_0.01_S20	1.67±0.201 ^a	0.384±0.0195 ^a	0,99
WG_0.02_S20	2.807±0.016 ^b	0.3115±0.00174 ^b	0,98
WG_0.05_S20	2.01±0.054 ^a	0.363±0.0051 ^a	0.98
WG_0.10_S20	3.6±0.48 ^b	0.253±0.0271 ^c	0.94

Means in the same column followed by different lowercase letters (a–d) are significantly different for each sample ($p < 0.05$).

The consistency index (K), a measure of the system's viscosity, indicates that emulsions with higher polysaccharide concentrations are more viscous and exhibit stronger pseudoplastic characteristics (Erçelebi & Ibanoglu, 2009). However, the anticipated increase in viscosity and shear stress with rising polysaccharide

concentration did not have a consistent trend at all ratios. Surprisingly, the emulsions with PS: PR ratios of 0.01 and 0.05 showed similar rheological profiles, and there was no significant difference ($p > 0.05$) in the consistency index and flow behavior index. This trend diverges from previous findings suggesting that increasing GA concentration in whey protein-based emulsions typically enhances viscosity while diminishing pseudoplastic properties (İbanoğlu, 2002). Contrary to the expectations, emulsions at PS: PR ratios of 0.02 and 0.10 were significantly different ($p < 0.05$) consistency indexes from the formulations at 0.02 and 0.10 ratios, as presented in. The emulsion with a PS:PR ratio of 0.01 has the highest shear thinning property compared to the others, followed by the emulsion with a PS:PR ratio of 0.02 ($n=0.311$). This deviation is expected, given that the factors influencing the rheological behavior of the Pickering emulsions are not yet fully understood. Depending on the system examined, some studies indicate that the viscous response of the emulsion increases with higher concentrations of particles used as emulsifiers (Hohl et al., 2016; Xiao et al., 2016), while other studies suggest that high particle concentrations do not have a significant effect (Benyaya et al., 2024; Griffith & Daigle, 2018; Wei & Huang, 2019). It is important to emphasize the relationship between the turbidity of the particle solutions and the rheological behavior of emulsions prepared with corresponding particles. Table 3.1 shows that the particles having the lower turbidity (PS: PR 0.02 and 0.10) (Table 3.2) have higher consistency index and more pseudoplastic behavior. The decreased turbidity observed at the 0.02 and 0.10 PS:PR ratios support our hypothesis that these ratios would exhibit superior emulsification properties due to smaller and uniform particle distribution which enhances the stability of the emulsion by creating a denser particle network. Moreover, the NMR relaxation findings (decrease in T_{2b}) corroborate our hypothesis that these particles have a higher tendency to adsorb to the droplets, as indicated by the shorter relaxation times. This demonstrates stronger particle-droplet interactions and a more robust interfacial layer, further stabilizing the emulsion. Thus, these results collectively confirm the proposed hypotheses regarding the emulsifying properties and adsorption tendencies at the corresponding ratios.

3.2.5 Digestive Properties

Extensive research has established that the rheological characteristics of foods can significantly influence gastric emptying through various mechanisms (Jin et al., 2023; Marciani et al., 2006; Ménard et al., 2018; Shang et al., 2020). These mechanisms include slowing down enzymatic reaction kinetics, extending intestinal transit time, decreasing intestinal motility, delaying nutrient diffusion in the intestines, and lowering the absorption rate of macronutrients. Consequently, modifying food rheology, such as altering viscosity, may slow down the digestion process (Ishida et al., 2019; Villemejane et al., 2016). Therefore, understanding the change in the flow behavior of emulsions, characterized by their consistency (K) and flow behavior index (n) values, is crucial for predicting their digestibility.

3.2.5.1 Rheological Characteristics of *In Vitro* Pepsin Digestion of Pickering Emulsions

The emulsions, denoted as AD (after digestion) (Table 3.3) and W/E (after digestion without pepsin, representing acid-induced digestion) (Table 3.4), maintained their pseudoplastic nature observed before digestion. However, there was a notable decrease in both the consistency (K) and pseudoplastic property for AD and W/E. This reduction is more pronounced when viewed in Figure 3.6 and Figure 3.8. The diminished viscosity and pseudoplasticity of the emulsions under both digestion scenarios (AD and W/E) compared to their pre-digestion states can be attributed to the 1:1 dilution factor applied to the solutions during each stage of digestion, encompassing both the oral and gastric phases.

Table 3.3 Rheological model constants after digestion (AD)

Sample Name	K	n	R ²
AD_WG_0.01_S20	0.0088 ± 0.00065 ^b	0.796 ± 0.0074 ^a	0.99
AD_WG_0.02_S20	0.0088 ± 0.00067 ^b	0.7983 ± 0.0010 ^a	0.99
AD_WG_0.05_S20	0.0087 ± 0.00062 ^b	0.79 ± 0.0637 ^a	0.99
AD_WG_0.10_S20	0.0096 ± 0.00060 ^a	0.7757 ± 0.0070 ^b	0.99

Means in the same column followed by different lowercase letters (a–b) are significantly different for each sample ($p < 0.05$).

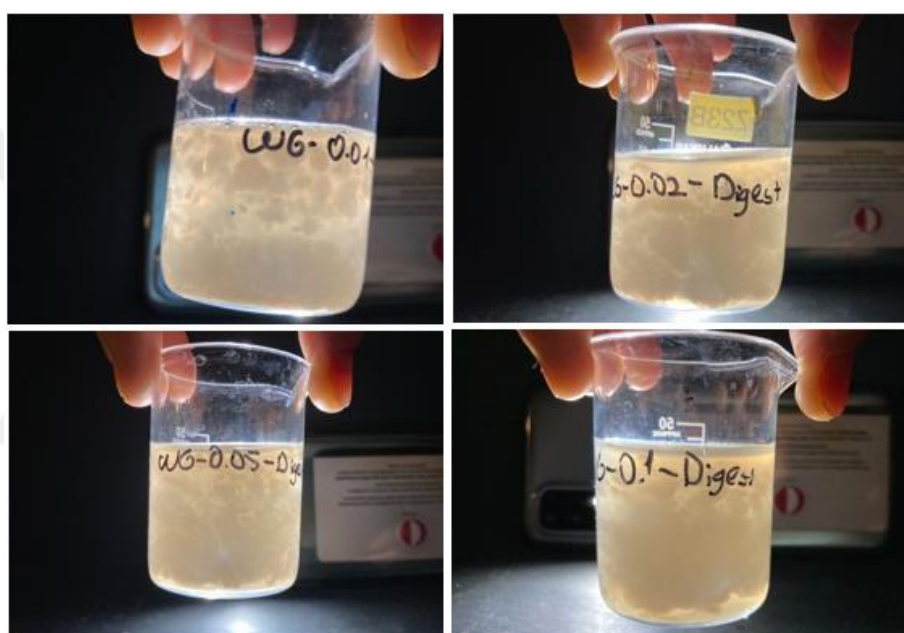


Figure 3.7 Emulsions after digestion

Post-digestion (AD) all emulsions had a cloudy, gel-like structures (Figure 3.7). The emulsion with a PS:PR ratio of 0.10 uniquely exhibited a different flow behavior and consistency index, showing the most shear thinning behavior and most viscous characteristics, as seen in Table 3.3. A higher consistency index means that the fluid resists deformation and maintains its structure better at different shear conditions, which can contribute to the stability of emulsions by indicating more stability against digestion conditions. This result aligns with expectations when considering that the Pickering particles bear structural similarity to WPI and local environment or

functional groups are affected with the number of GA attached with the increased PS:PR ratio, as inferred and discussed in FTIR spectral analysis. As given in Table 2.1, while protein concentration is same for all particles, they differ in GA content. Consequently, it can be hypothesized that presence of more gum arabic in complex with the protein might impede its interaction with pepsin, compared to the emulsions with lower PS:PR ratios. This suggests that pepsin may not have access to the hydrophobic moieties in the WPI-GA complex at high GA concentration. It is concluded that, in the 0.10 PS:PR formulation, a larger fraction of particles to which pepsin cannot effectively bind and degrade may cling to the oil phase, elevating the viscosity of the digested mass. Hence, it is hypothesized that Pickering particles in the 0.10 PS:PR ratio decrease protein digestion relative to other ratios, underscoring the complex interplay between emulsion composition and enzymatic digestion dynamics.

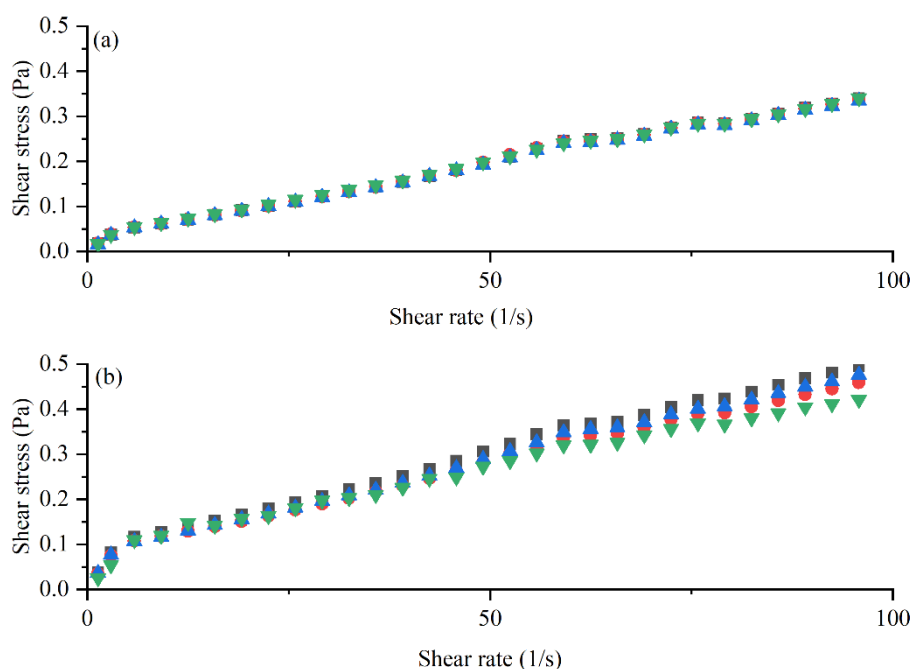


Figure 3.8 Shear stress and shear rate graphs of emulsions after digestion (a) and digestion without enzyme (b) (∇): WG_ 0.10_ S20, ($\blacktriangle$): WG_ 0.05_ S20, ($\bullet$): WG_ 0.02_ S20, ($\blacksquare$): WG_ 0.01_ S20.

3.2.5.2 Rheological Characteristics of *In Vitro* Acid Induced Digestion of Pickering Emulsions

On the other hand, considering the acid-induced (W/E) digestion results (Table 3.4), the one with the lowest viscosity belongs to the one with 0.10 PS:PR ratio ($p < 0.05$), which is different from enzyme-assisted digestion (AD). The flow curve of acid-induced digestion is different from that of enzyme-assisted, as shown in Figure 3.8. The fact that the digestion behavior in the presence of enzymes approaches each other for all the ratios was explained in the investigation of the digestion of the emulsions with the TD-NMR section. The absence of amylase in the oral phase (at pH 7.0), compared to enzymatic digestion, allows us to think that GA attached to WPI does not affect very much while entering the gastric environment. Results show that WG_0.10, which is the highest amount of GA attached, was significantly affected by the gastric environment (without pepsin, pH 1.8) Based on this, it hypothesized that increasing the amount of polysaccharide in emulsions increases pH sensitivity in the absence of amylase and pepsin enzymes. Ai et al. demonstrated that in the encapsulation of curcumin using GA as a wall material, changes in pH can lead to changes in intermolecular and intramolecular interactions, thereby changing the molecular configuration of polysaccharides (Ai et al., 2023). The absence of this phenomenon during enzyme digestion can be attributed to the dominance of pepsin in the system. The significant effect observed with increasing GA concentration is explained by the sensitivity of GA to pH changes.

As a result of examining the digestion (W/E) in simulated gastric fluid (SGF) without pepsin, it is seen that the presence of pepsin significantly affects the rheological properties ($p < 0.05$). Overall digestion W/E result in to be more viscous and more pseudoplastic than AD, implying that the presence of pepsin contributes the digestion greatly. All results suggest that the emulsion with the highest PS:PR ratio significantly increased bolus viscosity in the enzymatic digestion. Thus, it can be concluded that the significant increase in viscosity achieved by changing the particle

properties and extended time spent in stomach and slowed down rate of digestion can be achieved.

Table 3.4 Rheological model constants digestion without enzyme (W/E)

Sample Name	<i>K</i>	<i>n</i>	<i>R</i> ²
W/E_WG_0.01_S20	0.0239 ± 0.00187 ^a	0.661 ± 0.0093 ^b	0.98
W/E_WG_0.02_S20	0.0212 ± 0.00174 ^b	0.6701 ± 0.0005 ^b	0.98
W/E_WG_0.05_S20	0.0207 ± 0.00170 ^b	0.6807 ± 0.0016 ^b	0.98
W/E_WG_0.10_S20	0.0149 ± 0.00183 ^c	0.707 ± 0.0043 ^a	0.99

Means in the same column followed by different lowercase letters (a–c) are significantly different for each sample (*p* < 0.05).

3.2.5.3 Investigation of Digestion of Emulsions with TD-NMR

Studying the digestion behavior of Pickering emulsions through the *T*₂ relaxation spectrum can provide detailed insights into the effect of enzymes and pH on emulsion stability and understanding the different proton pools in the digestive bolus. Figure 3.9 shows representative 1D NMR *T*₂ relaxation spectra of digestion of Pickering emulsions with (c-d) and without enzyme addition (a-b). Two proton pools with different *T*₂ (spin-spin) relaxation times were observed for the oral phase depending on the formulations.

Figure 3.9a illustrates that the sample with a polysaccharide content of 0.01 exhibits distinct behavior with longer relaxation times during the oral phase in the absence of enzymes. However, when enzymes are introduced, the differences among the emulsions diminish, making their behaviors more parallel. Nonetheless, the sample containing particles PS:PR ratio of 0.10 indicative of a high number of GA attached to WPI —displays increased relaxation times during the oral phase, aligning with expectations based on mucin's interaction with polysaccharides. Transitioning to the gastric phase results in longer relaxation times, a predictable effect attributed to

dilution. Remarkably, the sample with a 0.02 PS:PR ratio demonstrates a greater change than the others, suggesting a diminished association and an enhanced tendency for instability in the absence of digestive enzymes.

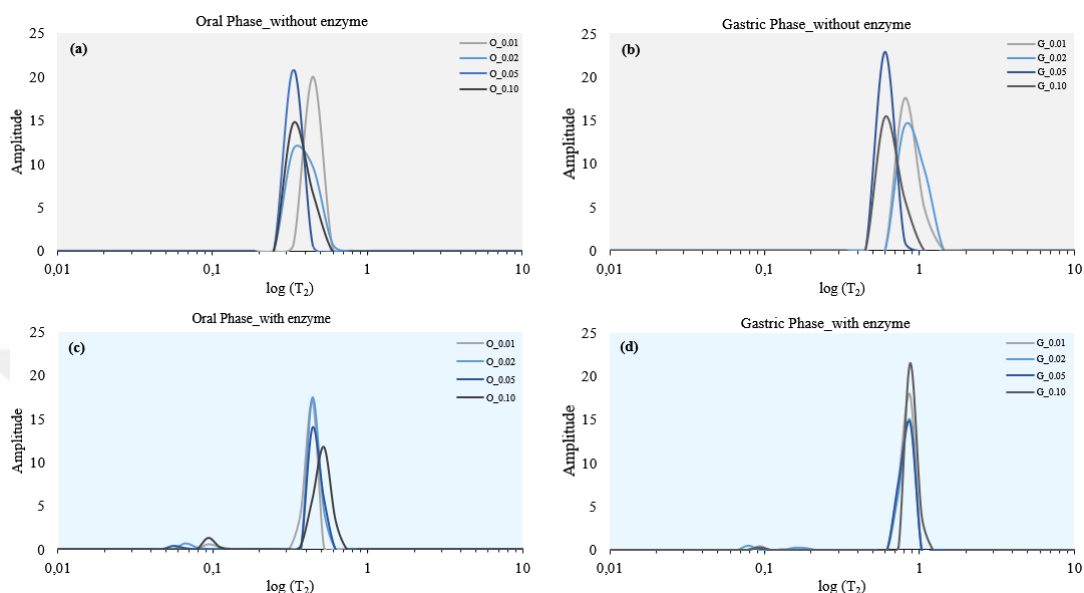


Figure 3.9 Representative T2 Relaxation Spectrum of (a)-(b) oral and gastric digestion of Pickering emulsions without enzyme and (c)-(d) oral and gastric digestion of Pickering emulsions in the presence of enzyme.



CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this study, Pickering particle formation was achieved by complexing GA and WPI at neutral pH with the effect of heat and high pressure. The FTIR spectrum of the Pickering particles more similar to the native WPI structure, which may indicate little change in the main backbone of the WPI structure to which GA is attached. Upon examination of Pickering particle solutions by TD-NMR, it was observed that particles with distinct properties were produced from both polymers. The oil absorption and water interaction behaviors of Pickering particles changed as the PS:PR ratio increased. In fact, the particles obtained with a PS:PR ratio of 0.10 had a shorter T_2 value from others, indicating that particles with the highest PS:PR ratio most effectively limited the molecular mobility of water and oil.

Building on the unique properties of Pickering particles, their application and contribution to stabilizing emulsions are also explored. Results showed that Pickering emulsions prepared with different PS:PR ratios remained stable for 4 weeks, except for 0.01 PS:PR. It was concluded that T_{2a} & T_{2b} relaxation times, which were expected to increase due to deterioration of stability over time, decreased and stable emulsions were produced during the 4-week storage period when sufficient particle concentration was provided which was above 0.01. Furthermore, it was observed that the emulsion with a PS:PR ratio of 0.10 had the most viscous and pseudoplastic properties. Rheological properties of emulsions represent that nature of pickering particles of WPI and GA was not linearly correlated in terms of affecting the viscous behavior. As observed in all other analyzes, particles with 0.02 and 0.10 ratios exhibited similarities, indicating that the observed effects are more complex and multifactorial, rather than being directly correlated with an increase in PS:PR ratio.

Finally, digestion without enzymes resulted in to be more viscous and more pseudoplastic than enzyme assisted digestion, implying that the presence of pepsin contributes the digestion greatly. Rheological characterization of the emulsions showed that with the highest PS:PR ratio significantly increased bolus viscosity in the enzymatic digestion. Thus, it can be concluded that the significant increase in viscosity can be achieved by the complexation of GA and WPI with different ratios, which are promising for changing the extended time spent in the stomach and slowing down the rate of digestion.

Further studies can be extended to better understand the stability and rheological properties of Pickering emulsion by investigating the surface coverage of the Pickering particles. This would help to determine how effectively the particles stabilize the emulsion and resist destabilizing processes. In addition, the size of the Pickering particles plays a significant role in determining their effectiveness at adsorbing to the water-oil interface. Understanding the relationship between the size of the Pickering particles and their adsorption efficiency at the interface is crucial for optimizing the formulation of Pickering emulsions, as it directly influences both their structural integrity and long-term stability. To gain deeper insights into the underlying stability mechanisms, it can be advisable to include measurements of interfacial tension and microrheology, which can provide valuable data on the interactions at the molecular level that contribute to the emulsion's overall stability and performance.

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APPENDICES

A. Statistical Analysis

Table A. 1 Analysis of variance of Turbidity values of Pickering Particles

(1) General Linear Model: Turbidity of Pickering Particles

TURBIDITY

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Particle_Turbidity	Fixed	4	0.01; 0.02; 0.05; 0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Particle_Turbidity	3	0,000894	0,000298	253,27	0,000
Error	4	0,000005	0,000001		
Total	7	0,000898			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0010846	99,48%	99,08%	97,91%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,098762	0,000383	257,57	0,000	
Particle_Turbidity					
0.01	0,013787	0,000664	20,76	0,000	1,50
0.02	-0,006462	0,000664	-9,73	0,001	1,50
0.05	0,006038	0,000664	9,09	0,001	1,50
0.10	-0,013362	0,000664	-20,12	0,000	*

Regression Equation

Absorbance_Turbidity = 0,098762 + 0,013787 Particle_Turbidity_0.01
 - 0,006462 Particle_Turbidity_0.02 + 0,006038 Particle_Turbidity_0.05
 - 0,013362 Particle_Turbidity_0.10

Grouping Information Using the Tukey Method and 95% Confidence

Particle_Turbidity	N	Mean	Grouping
0.01	2	0,11255	A
0.05	2	0,10480	B
0.02	2	0,09230	C
0.10	2	0,08540	D

Means that do not share a letter are significantly different.

Table A. 2 Analysis of variance of T₂ Measurements. Effect of formulations at (1) week 1 (2) week 4

(1) General Linear Model: T₂ Measurements of emulsions at week 1

TD-NMR T_{2a} (week 1)

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Formulation	Fixed	4	0,01; 0,02; 0,05; 0,10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	2859,74	953,246	435,63	0,000
Error	4	8,75	2,188		
Total	7	2868,49			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,47927	99,69%	99,47%	98,78%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	273,290	0,523	522,54	0,000	
Formulation					
0,01	25,070	0,906	27,68	0,000	1,50
0,02	6,280	0,906	6,93	0,002	1,50
0,05	-4,045	0,906	-4,47	0,011	1,50
0,10	-27,305	0,906	-30,14	0,000	*

Regression Equation

T_{2a} = 273,290 + 25,070 Formulation_0,01 + 6,280 Formulation_0,02
 - 4,045 Formulation_0,05
 - 27,305 Formulation_0,10

Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
0,01	2	298,360	A
0,02	2	279,570	B
0,05	2	269,245	C
0,10	2	245,985	D

Means that do not share a letter are significantly different.

TD-NMR T_{2b} (week 1)

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Formulation	Fixed	4	0,01; 0,02; 0,05; 0,10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation	3	251,4	83,81	2,81	0,172
Error	4	119,5	29,86		
Total	7	370,9			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5,46479	67,79%	43,63%	0,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	62,02	1,93	32,10	0,000	
Formulation					
0,01	6,46	3,35	1,93	0,126	1,50
0,02	-5,09	3,35	-1,52	0,203	1,50
0,05	4,66	3,35	1,39	0,236	1,50
0,10	-6,03	3,35	-1,80	0,146	*

Regression Equation

$$\begin{aligned} T_{2b} = & 62,02 + 6,46 \text{ Formulation}_{0,01} - 5,09 \text{ Formulation}_{0,02} \\ & + 4,66 \text{ Formulation}_{0,05} \\ & - 6,03 \text{ Formulation}_{0,10} \end{aligned}$$

Grouping Information Using the Tukey Method and 95% Confidence

Formulation	N	Mean	Grouping
0,01	2	68,475	A
0,05	2	66,680	A
0,02	2	56,930	A
0,10	2	55,985	A

Means that do not share a letter are significantly different.

(2) General Linear Model: T₂ Measurements of emulsions at week 4

TD-NMR T_{2a} (week 4)

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Formulation_1	Fixed	4	0,01; 0,02; 0,05; 0,10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation_1	3	30768	10256	9,19	0,029
Error	4	4465	1116		
Total	7	35233			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
33,4114	87,33%	77,82%	49,31%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	240,1	11,8	20,32	0,000	
Formulation_1					
0,01	56,7	20,5	2,77	0,050	1,50
0,02	34,6	20,5	1,69	0,166	1,50
0,05	12,7	20,5	0,62	0,567	1,50
0,10	-104,0	20,5	-5,08	0,007	*

Regression Equation

$$T2a_1 = 240,1 + 56,7 \text{ Formulation}_1_{0,01} + 34,6 \text{ Formulation}_1_{0,02} + 12,7 \text{ Formulation}_1_{0,05} - 104,0 \text{ Formulation}_1_{0,10}$$

Grouping Information Using the Tukey Method and 95% Confidence

Formulation_1	N	Mean	Grouping
0,01	2	296,720	A
0,02	2	274,650	A
0,05	2	252,795	A B
0,10	2	136,065	B

Means that do not share a letter are significantly different.

TD-NMR T_{2b} (week 4)

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Formulation_1	Fixed	4	0,01; 0,02; 0,05; 0,10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Formulation_1	3	1973,3	657,76	18,03	0,009
Error	4	146,0	36,49		
Total	7	2119,2			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6,04056	93,11%	87,95%	72,45%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	54,11	2,14	25,34	0,000	
Formulation_1					
0,01	15,17	3,70	4,10	0,015	1,50
0,02	9,74	3,70	2,63	0,058	1,50
0,05	0,80	3,70	0,22	0,840	1,50
0,10	-25,71	3,70	-6,95	0,002	*

Regression Equation

$$T2b_1 = 54,11 + 15,17 \text{ Formulation}_1_{0,01} + 9,74 \text{ Formulation}_1_{0,02} + 0,80 \text{ Formulation}_1_{0,05} - 25,71 \text{ Formulation}_1_{0,10}$$

Grouping Information Using the Tukey Method and 95% Confidence

Formulation_1	N	Mean	Grouping
0,01	2	69,285	A
0,02	2	63,855	A
0,05	2	54,910	A
0,10	2	28,405	B

Means that do not share a letter are significantly different.

Table A. 3 Analysis of variance of T₂ Measurements. Effect of storage weeks within the formulations

TD-NMR (Effect of storage weeks in the formulations)

T_{2a} WG_0.01_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_0.01	Fixed	2	1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_0.01	1	2,69	2,69	0,00	0,970
Error	2	3071,97	1535,99		
Total	3	3074,66			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
39,1917	0,09%	0,00%	0,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	297,5	19,6	15,18	0,004	
Ratio_0.01					
1	0,8	19,6	0,04	0,970	1,00
28	-0,8	19,6	-0,04	0,970	*

Regression Equation

$$T2a_2 = 297,5 + 0,8 \text{ Ratio_0.01_1} - 0,8 \text{ Ratio_0.01_28}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_0.01	N	Mean	Grouping
1	2	298,36	A
28	2	296,72	A

Means that do not share a letter are significantly different.

T_{2b} WG_0.01_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_0.01	Fixed	2	1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_0.01	1	0,6561	0,6561	0,02	0,900
Error	2	64,4965	32,2483		
Total	3	65,1526			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5,67875	1,01%	0,00%	0,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	68,88	2,84	24,26	0,002	
Ratio_0.01					
1	-0,40	2,84	-0,14	0,900	1,00
28	0,40	2,84	0,14	0,900	*

Regression Equation

$$T2b_2 = 68,88 - 0,40 \text{ Ratio_0.01_1} + 0,40 \text{ Ratio_0.01_28}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_0.02	N	Mean	Grouping
28	2	63,855	A
1	2	56,930	A

Means that do not share a letter are significantly different.

T_{2a} WG_0.02_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_0.02	Fixed	2	1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_0.02	1	24,21	24,21	0,04	0,864
Error	2	1287,58	643,79		
Total	3	1311,78			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
25,3730	1,85%	0,00%	0,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	277,1	12,7	21,84	0,002	
Ratio_0.02					
1	2,5	12,7	0,19	0,864	1,00
28	-2,5	12,7	-0,19	0,864	*

Regression Equation

$$T2a_2_2 = 277,1 + 2,5 \text{ Ratio_0.02_1} - 2,5 \text{ Ratio_0.02_28}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_0.02	N	Mean	Grouping
1	2	279,57	A
28	2	274,65	A

Means that do not share a letter are significantly different.

T_{2b} WG_0.02_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_0.02	Fixed	2	1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_0.02	1	47,96	47,96	0,82	0,461
Error	2	117,11	58,55		
Total	3	165,06			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7,65210	29,05%	0,00%	0,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	60,39	3,83	15,78	0,004	
Ratio_0.02					
1	-3,46	3,83	-0,90	0,461	1,00
28	3,46	3,83	0,90	0,461	*

Regression Equation

$$T2b_2_2 = 60,39 - 3,46 \text{ Ratio_0.02_1} + 3,46 \text{ Ratio_0.02_28}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_0.02	N	Mean	Grouping
28	2	63,855	A
1	2	56,930	A

Means that do not share a letter are significantly different.

T_{2a} WG_0.05_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_0.05	Fixed	2	1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_0.05	1	270,60	270,603	53,90	0,018
Error	2	10,04	5,021		
Total	3	280,64			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2,24073	96,42%	94,63%	85,69%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	261,02	1,12	232,98	0,000	
Ratio_0.05					
1	8,23	1,12	7,34	0,018	1,00
28	-8,23	1,12	-7,34	0,018	*

Regression Equation

$$T2a_2_3 = 261,02 + 8,23 \text{ Ratio_0.05_1} - 8,23 \text{ Ratio_0.05_28}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_0.05	N	Mean	Grouping
1	2	269,245	A
28	2	252,795	B

Means that do not share a letter are significantly different.

T_{2b} WG_0.05_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_0.05	Fixed	2	1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_0.05	1	138,533	138,533	601,01	0,002
Error	2	0,461	0,230		
Total	3	138,994			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,480104	99,67%	99,50%	98,67%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	60,795	0,240	253,26	0,000	
Ratio_0.05					
1	5,885	0,240	24,52	0,002	1,00
28	-5,885	0,240	-24,52	0,002	*

Regression Equation

$$T_{2b_2_3} = 60,795 + 5,885 \text{ Ratio_0.05_1} - 5,885 \text{ Ratio_0.05_28}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_0.05	N	Mean	Grouping
1	2	66,68	A
28	2	54,91	B

Means that do not share a letter are significantly different.

T_{2a} WG_0.10_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_0.10	Fixed	2	1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_0.10	1	12082,4	12082,4	231,37	0,004
Error	2	104,4	52,2		
Total	3	12186,8			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7,22637	99,14%	98,71%	96,57%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	191,03	3,61	52,87	0,000	
Ratio_0.10					
1	54,96	3,61	15,21	0,004	1,00
28	-54,96	3,61	-15,21	0,004	*

Regression Equation

$$T2a_2_4 = 191,03 + 54,96 \text{ Ratio_0.10_1} - 54,96 \text{ Ratio_0.10_28}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_0.10	N	Mean	Grouping
1	2	245,985	A
28	2	136,065	B

Means that do not share a letter are significantly different.

T_{2b} WG_0.10_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_0.10	Fixed	2	1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_0.10	1	760,66	760,66	18,25	0,051
Error	2	83,34	41,67		
Total	3	844,00			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6,45533	90,13%	85,19%	60,50%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	42,20	3,23	13,07	0,006	
Ratio_0.10					
1	13,79	3,23	4,27	0,051	1,00
28	-13,79	3,23	-4,27	0,051	*

Regression Equation

$$T2b_2_4 = 42,20 + 13,79 \text{ Ratio_0.10_1} - 13,79 \text{ Ratio_0.10_28}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_0.10	N	Mean	Grouping
1	2	55,985	A
28	2	28,405	A

Means that do not share a letter are significantly different.

Table A. 4 Analysis of variance of rheological constants (n and K) before digestion

General Linear Model: Rheological Characterization Before Digestion

WG_S20_N

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
emulsion	Fixed	4	wg_0.01_s20; wg_0.02_s20; wg_0.05_s20; wg_0.10_s20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
emulsion	3	0,030823	0,010274	35,80	0,000
Error	8	0,002296	0,000287		
Total	11	0,033119			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0169413	93,07%	90,47%	84,40%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,32831	0,00489	67,13	0,000	
emulsion					
wg_0.01_s20	0,05607	0,00847	6,62	0,000	1,50
wg_0.02_s20	-0,01678	0,00847	-1,98	0,083	1,50
wg_0.05_s20	0,03548	0,00847	4,19	0,003	1,50
wg_0.10_s20	-0,07477	0,00847	-8,83	0,000	*

Regression Equation

n = 0,32831 + 0,05607 emulsion_wg_0.01_s20
 - 0,01678 emulsion_wg_0.02_s20
 + 0,03548 emulsion_wg_0.05_s20 - 0,07477 emulsion_wg_0.10_s20

Fits and Diagnostics for Unusual Observations

Obs	n	Fit	Resid	Std Resid
10	0,22469	0,25354	-0,02885	-2,09 R

R Large residual

Grouping Information Using the Tukey Method and 95% Confidence

emulsion	N	Mean	Grouping
wg_0.01_s20	3	0,384377	A
wg_0.05_s20	3	0,363783	A
wg_0.02_s20	3	0,311530	B
wg_0.10_s20	3	0,253537	C

Means that do not share a letter are significantly different.

WG_S20_K

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
emulsion	Fixed	4	wg_0.01_s20; wg_0.02_s20; wg_0.05_s20; wg_0.10_s20

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
emulsion	3	0,107848	0,035949	43,48	0,000
Error	8	0,006615	0,000827		
Total	11	0,114463			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0287548	94,22%	92,05%	87,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,65136	0,00830	78,47	0,000	
emulsion					
wg_0.01_s20	0,1235	0,0144	8,59	0,000	1,50
wg_0.02_s20	-0,0545	0,0144	-3,79	0,005	1,50
wg_0.05_s20	0,0532	0,0144	3,70	0,006	1,50
wg_0.10_s20	-0,1221	0,0144	-8,49	0,000	*

Regression Equation

transformed K = 0,65136 + 0,1235 emulsion_wg_0.01_s20 - 0,0545 emulsion_wg_0.02_s20
+ 0,0532 emulsion_wg_0.05_s20 - 0,1221 emulsion_wg_0.10_s20

Fits and Diagnostics for Unusual Observations

Obs	transformed K	Fit	Resid	Std Resid
1	0,7239	0,7748	-0,0509	-2,17 R

R Large residual

Grouping Information Using the Tukey Method and 95% Confidence

emulsion	N	Mean	Grouping
wg_0.01_s20	3	0,774823	A
wg_0.05_s20	3	0,704535	A
wg_0.02_s20	3	0,596829	B
wg_0.10_s20	3	0,529273	B

Means that do not share a letter are significantly different.

Table A. 5 Analysis of variance of rheological constants (n and K) after digestion

General Linear Model: Rheological Characterization After Digestion

AD_S20_N

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio	Fixed	4	AD_0.01; AD_0.02; AD_0.05; AD_0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio	3	0,000630	0,000210	29,32	0,003
Error	4	0,000029	0,000007		
Total	7	0,000659			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0026761	95,65%	92,39%	82,60%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,793223	0,000946	838,36	0,000	
Ratio					
AD_0.01	0,00629	0,00164	3,84	0,018	1,50
AD_0.02	0,00505	0,00164	3,08	0,037	1,50
AD_0.05	0,00396	0,00164	2,41	0,073	1,50
AD_0.10	-0,01530	0,00164	-9,34	0,001	*

Regression Equation

$$n_{AD} = 0,793223 + 0,00629 \text{ Ratio_AD_0.01} + 0,00505 \text{ Ratio_AD_0.02} + 0,00396 \text{ Ratio_AD_0.05} - 0,01530 \text{ Ratio_AD_0.10}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio	N	Mean	Grouping
AD_0.01	2	0,799515	A
AD_0.02	2	0,798275	A
AD_0.05	2	0,797180	A
AD_0.10	2	0,777920	B

Means that do not share a letter are significantly different.

AD_S20_K

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio	Fixed	4	AD_0.01; AD_0.02; AD_0.05; AD_0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio	3	0,000001	0,000000	77,20	0,001
Error	4	0,000000	0,000000		
Total	7	0,000001			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0000658	98,30%	97,03%	93,21%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,009012	0,000023	387,61	0,000	
Ratio					
AD_0.01	-0,000203	0,000040	-5,03	0,007	1,50
AD_0.02	-0,000187	0,000040	-4,66	0,010	1,50
AD_0.05	-0,000223	0,000040	-5,52	0,005	1,50
AD_0.10	0,000612	0,000040	15,21	0,000	*

Regression Equation

K_AD = 0,009012 - 0,000203 Ratio_AD_0.01 - 0,000187 Ratio_AD_0.02 - 0,000223 Ratio_AD_0.05 + 0,000612 Ratio_AD_0.10

Grouping Information Using the Tukey Method and 95% Confidence

Ratio	N	Mean	Grouping
AD_0.10	2	0,009625	A
AD_0.02	2	0,008825	B
AD_0.01	2	0,008810	B
AD_0.05	2	0,008790	B

Means that do not share a letter are significantly different.

Table A. 6 Analysis of variance of rheological constants (n and K) before and after digestion

General Linear Model: Effect of Digestion (WG_S20 versus AD_WG_S20)

WG&AD_S20_N

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_1	Fixed	2	0.01; AD_0.01

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_1	1	0,136612	0,136612	4213,64	0,000
Error	2	0,000065	0,000032		
Total	3	0,136676			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0056940	99,95%	99,93%	99,81%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,61471	0,00285	215,92	0,000	
Ratio_1					
0.01	-0,18480	0,00285	-64,91	0,000	1,00
AD_0.01	0,18480	0,00285	64,91	0,000	*

Regression Equation

$$n_WG\&AD_1 = 0,61471 - 0,18480 \text{ Ratio_1_0.01} + 0,18480 \text{ Ratio_1_AD_0.01}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_1	N	Mean	Grouping
AD_0.01	2	0,799515	A
0.01	2	0,429905	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_2	Fixed	2	0.02; AD_0.02

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_2	1	0,191100	0,191100	276216,12	0,000
Error	2	0,000001	0,000001		
Total	3	0,191102			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0008318	100,00%	100,00%	100,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,579700	0,000416	1393,89	0,000	
Ratio_2					
0.02	-0,218575	0,000416	-525,56	0,000	1,00
AD_0.02	0,218575	0,000416	525,56	0,000	*

Regression Equation

$$n_WG\&AD_2 = 0,579700 - 0,218575 \text{ Ratio_2_0.02} + 0,218575 \text{ Ratio_2_AD_0.02}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_2	N	Mean	Grouping
AD_0.02	2	0,798275	A
0.02	2	0,361125	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_3	Fixed	2	0.05; AD_0.05

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_3	1	0,157379	0,157379	294827,32	0,000
Error	2	0,000001	0,000001		
Total	3	0,157380			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0007306	100,00%	100,00%	100,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,598825	0,000365	1639,23	0,000	
Ratio_3					
0.05	-0,198355	0,000365	-542,98	0,000	1,00
AD_0.05	0,198355	0,000365	542,98	0,000	*

Regression Equation

$$n_WG\&AD_3 = 0,598825 - 0,198355 \text{ Ratio_3_0.05} + 0,198355 \text{ Ratio_3_AD_0.05}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_3	N	Mean	Grouping
AD_0.05	2	0,79718	A
0.05	2	0,40047	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_4	Fixed	2	0.10; AD_0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_4	1	0,198582	0,198582	2014,00	0,000
Error	2	0,000197	0,000099		
Total	3	0,198779			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0099298	99,90%	99,85%	99,60%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,55511	0,00496	111,81	0,000	
Ratio_4					
0.10	-0,22281	0,00496	-44,88	0,000	1,00
AD_0.10	0,22281	0,00496	44,88	0,000	*

Regression Equation

n_WG&AD_4 = 0,55511 - 0,22281 Ratio_4_0.10 + 0,22281 Ratio_4_AD_0.10

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_4	N	Mean	Grouping
AD_0.10	2	0,777920	A
0.10	2	0,332295	B

Means that do not share a letter are significantly different.

WG&AD_S20_K

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_1	Fixed	2	0.01; AD_0.01

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_1	1	1,80816	1,80816	4183,32	0,000
Error	2	0,00086	0,00043		
Total	3	1,80903			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0207902	99,95%	99,93%	99,81%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,6811	0,0104	65,53	0,000	
Ratio_1					
0.01	0,6723	0,0104	64,68	0,000	1,00
AD_0.01	-0,6723	0,0104	-64,68	0,000	*

Regression Equation

K_WG&AD_1 = 0,6811 + 0,6723 Ratio_1_0.01 - 0,6723 Ratio_1_AD_0.01

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_1	N	Mean	Grouping
0.01	2	1,35349	A
AD_0.01	2	0,00881	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_2	Fixed	2	0.02; AD_0.02

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_2	1	5,21433	5,21433	1082203,41	0,000
Error	2	0,00001	0,00000		
Total	3	5,21434			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0021951	100,00%	100,00%	100,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1,15057	0,00110	1048,33	0,000	
Ratio_2					
0.02	1,14174	0,00110	1040,29	0,000	1,00
AD_0.02	-1,14174	0,00110	-1040,29	0,000	*

Regression Equation

$$K_WG\&AD_2 = 1,15057 + 1,14174 \text{ Ratio_2_0.02} - 1,14174 \text{ Ratio_2_AD_0.02}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_2	N	Mean	Grouping
0.02	2	2,29231	A
AD_0.02	2	0,00883	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_3	Fixed	2	0.05; AD_0.05

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_3	1	2,99053	2,99053	16767,19	0,000
Error	2	0,00036	0,00018		
Total	3	2,99089			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0133550	99,99%	99,98%	99,95%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,87345	0,00668	130,80	0,000	
Ratio_3					
0.05	0,86466	0,00668	129,49	0,000	1,00
AD_0.05	-0,86466	0,00668	-129,49	0,000	*

Regression Equation

$$K_WG\&AD_3 = 0,87345 + 0,86466 \text{ Ratio_3_0.05} - 0,86466 \text{ Ratio_3_AD_0.05}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_3	N	Mean	Grouping
0.05	2	1,73810	A
AD_0.05	2	0,00879	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_4	Fixed	2	0.10; AD_0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_4	1	6,52874	6,52874	428,09	0,002
Error	2	0,03050	0,01525		
Total	3	6,55924			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,123495	99,53%	99,30%	98,14%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1,2872	0,0617	20,85	0,002	
Ratio_4					
0.10	1,2776	0,0617	20,69	0,002	1,00
AD_0.10	-1,2776	0,0617	-20,69	0,002	*

Regression Equation

$$K_{WG\&AD_4} = 1,2872 + 1,2776 \text{ Ratio_4}_{0.10} - 1,2776 \text{ Ratio_4}_{AD.0.10}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_4	N	Mean	Grouping
0.10	2	2,56476	A
AD_0.10	2	0,00963	B

Means that do not share a letter are significantly different.

Table A. 7 Analysis of variance of rheological constants (n and K) without enzyme digestion

General Linear Model: Without Enzyme Digestion

WE_WG_S20_N

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio	Fixed	4	WE_0.01; WE_0.02; WE_0.05; WE_0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio	3	0,002406	0,000802	29,70	0,003
Error	4	0,000108	0,000027		
Total	7	0,002514			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0051969	95,70%	92,48%	82,81%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,68001	0,00184	370,10	0,000	
Ratio					
WE_0.01	-0,01849	0,00318	-5,81	0,004	1,50
WE_0.02	-0,00989	0,00318	-3,11	0,036	1,50
WE_0.05	0,00075	0,00318	0,24	0,825	1,50
WE_0.10	0,02762	0,00318	8,68	0,001	*

Regression Equation

$$n_WE = 0,68001 - 0,01849 \text{ Ratio_WE_0.01} - 0,00989 \text{ Ratio_WE_0.02} + 0,00075 \text{ Ratio_WE_0.05} + 0,02762 \text{ Ratio_WE_0.10}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio	N	Mean	Grouping
WE_0.10	2	0,707635	A
WE_0.05	2	0,680765	B
WE_0.02	2	0,670125	B
WE_0.01	2	0,661525	B

Means that do not share a letter are significantly different.

WE_WG_S20_K

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio	Fixed	4	WE_0.01; WE_0.02; WE_0.05; WE_0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio	3	0,000083	0,000028	1163,18	0,000
Error	4	0,000000	0,000000		
Total	7	0,000083			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0001544	99,89%	99,80%	99,54%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,020154	0,000055	369,21	0,000	
Ratio					
WE_0.01	0,003541	0,000095	37,46	0,000	1,50
WE_0.02	0,001136	0,000095	12,02	0,000	1,50
WE_0.05	0,000561	0,000095	5,94	0,004	1,50
WE_0.10	-0,005239	0,000095	-55,41	0,000	*

Regression Equation

$$K_WE = 0,020154 + 0,003541 \text{ Ratio_WE_0.01} + 0,001136 \text{ Ratio_WE_0.02} + 0,000561 \text{ Ratio_WE_0.05} - 0,005239 \text{ Ratio_WE_0.10}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio	N	Mean	Grouping
WE_0.01	2	0,023695	A
WE_0.02	2	0,021290	B
WE_0.05	2	0,020715	B
WE_0.10	2	0,014915	C

Means that do not share a letter are significantly different.

Table A. 8 Analysis of variance of rheological constants (n and K) after digestion and acid-induced digestion

General Linear Model: Effect of Enzyme on Digestion (AD_WG_S20 versus WE_WG_S20)

AD&WE_S20_N

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_1	Fixed	2	AD_0.01; WE_0.01

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_1	1	0,019041	0,019041	384,43	0,003
Error	2	0,000099	0,000050		
Total	3	0,019140			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0070378	99,48%	99,22%	97,93%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,73052	0,00352	207,60	0,000	
Ratio_1					
AD_0.01	0,06899	0,00352	19,61	0,003	1,00
WE_0.01	-0,06899	0,00352	-19,61	0,003	*

Regression Equation

$$n_AD\&WE_1 = 0,73052 + 0,06899 \text{ Ratio_1_AD_0.01} - 0,06899 \text{ Ratio_1_WE_0.01}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_1	N	Mean	Grouping
AD_0.01	2	0,799515	A
WE_0.01	2	0,661525	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_2	Fixed	2	AD_0.02; WE_0.02

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_2	1	0,016422	0,016422	21047,64	0,000
Error	2	0,000002	0,000001		
Total	3	0,016424			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0008833	99,99%	99,99%	99,96%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,734200	0,000442	1662,37	0,000	
Ratio_2					
AD_0.02	0,064075	0,000442	145,08	0,000	1,00
WE_0.02	-0,064075	0,000442	-145,08	0,000	*

Regression Equation

$$n_AD\&WE_2 = 0,734200 + 0,064075 \text{ Ratio_2_AD_0.02} - 0,064075 \text{ Ratio_2_WE_0.02}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_2	N	Mean	Grouping
AD_0.02	2	0,798275	A
WE_0.02	2	0,670125	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_3	Fixed	2	AD_0.05; WE_0.05

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_3	1	0,013552	0,013552	10512,91	0,000
Error	2	0,000003	0,000001		
Total	3	0,013555			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0011354	99,98%	99,97%	99,92%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,738973	0,000568	1301,70	0,000	
Ratio_3					
AD_0.05	0,058208	0,000568	102,53	0,000	1,00
WE_0.05	-0,058208	0,000568	-102,53	0,000	*

Regression Equation

$$n_AD\&WE_3 = 0,738973 + 0,058208 \text{ Ratio_3_AD_0.05} - 0,058208 \text{ Ratio_3_WE_0.05}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_3	N	Mean	Grouping
AD_0.05	2	0,797180	A
WE_0.05	2	0,680765	B

Means that do not share a letter are significantly different.

Method

Factor coding [-1; 0; +1]

Factor Information

Factor	Type	Levels	Values
Ratio_4	Fixed	2	AD_0.10; WE_0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_4	1	0,004940	0,004940	295,12	0,003
Error	2	0,000033	0,000017		
Total	3	0,004973			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0040913	99,33%	98,99%	97,31%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,74278	0,00205	363,10	0,000	
Ratio_4					
AD_0.10	0,03514	0,00205	17,18	0,003	1,00
WE_0.10	-0,03514	0,00205	-17,18	0,003	*

Regression Equation

$$n_AD\&WE_4 = 0,74278 + 0,03514 \text{ Ratio_4_AD_0.10} - 0,03514 \text{ Ratio_4_WE_0.10}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_4	N	Mean	Grouping
AD_0.10	2	0,777920	A
WE_0.10	2	0,707635	B

Means that do not share a letter are significantly different.

AD&WE_S20_K

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_1	Fixed	2	AD_0.01; WE_0.01

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_1	1	0,000222	0,000222	4420,21	0,000
Error	2	0,000000	0,000000		
Total	3	0,000222			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0002239	99,95%	99,93%	99,82%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,016252	0,000112	145,19	0,000	
Ratio_1					
AD_0.01	-0,007442	0,000112	-66,48	0,000	1,00
WE_0.01	0,007442	0,000112	66,48	0,000	*

Regression Equation

K_AD&WE_1 = 0,016252 - 0,007442 Ratio_1_AD_0.01 + 0,007442 Ratio_1_WE_0.01

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_1	N	Mean	Grouping
WE_0.01	2	0,023695	A
AD_0.01	2	0,008810	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_2	Fixed	2	AD_0.02; WE_0.02

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_2	1	0,000155	0,000155	40621,24	0,000
Error	2	0,000000	0,000000		
Total	3	0,000155			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0000618	100,00%	99,99%	99,98%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,015057	0,000031	486,93	0,000	
Ratio_2					
AD_0.02	-0,006232	0,000031	-201,55	0,000	1,00
WE_0.02	0,006232	0,000031	201,55	0,000	*

Regression Equation

K_AD&WE_2 = 0,015057 - 0,006232 Ratio_2_AD_0.02 + 0,006232 Ratio_2_WE_0.02

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_2	N	Mean	Grouping
WE_0.02	2	0,021290	A
AD_0.02	2	0,008825	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_3	Fixed	2	AD_0.05; WE_0.05

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_3	1	0,000142	0,000142	1137645,00	0,000
Error	2	0,000000	0,000000		
Total	3	0,000142			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0000112	100,00%	100,00%	100,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
------	------	---------	---------	---------	-----

Constant	0,014752	0,000006	2639,01	0,000	
Ratio_3					
AD_0.05	-0,005962	0,000006	-1066,60	0,000	1,00
WE_0.05	0,005962	0,000006	1066,60	0,000	*

Regression Equation

$$K_AD\&WE_3 = 0,014752 - 0,005962 \text{ Ratio_3_AD_0.05} + 0,005962 \text{ Ratio_3_WE_0.05}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_3	N	Mean	Grouping
WE_0.05	2	0,020715	A
AD_0.05	2	0,008790	B

Means that do not share a letter are significantly different.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Ratio_4	Fixed	2	AD_0.10; WE_0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Ratio_4	1	0,000028	0,000028	12437,38	0,000
Error	2	0,000000	0,000000		
Total	3	0,000028			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0000474	99,98%	99,98%	99,94%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,012270	0,000024	517,35	0,000	
Ratio_4					
AD_0.10	-0,002645	0,000024	-111,52	0,000	1,00
WE_0.10	0,002645	0,000024	111,52	0,000	*

Regression Equation

$$K_AD\&WE_4 = 0,012270 - 0,002645 \text{ Ratio_4_AD_0.10} + 0,002645 \text{ Ratio_4_WE_0.10}$$

Grouping Information Using the Tukey Method and 95% Confidence

Ratio_4	N	Mean	Grouping
WE_0.10	2	0,014915	A
AD_0.10	2	0,009625	B

Means that do not share a letter are significantly different.

Table A. 9 Analysis of variance of particle size (d [3,2]) values, effect of formulations on storage weeks at (1) week 1 (2) week 4

(1) General Linear Model: Effect of formulation at week 1

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ratio_1week	Fixed	4	0.01; 0.02; 0.05; 0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ratio_1week	3	0,001579	0,000526	4211,67	0,000
Error	4	0,000001	0,000000		
Total	7	0,001580			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0003536	99,97%	99,94%	99,87%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,777625	0,000125	6221,00	0,000	
ratio_1week					
0.01	0,002375	0,000217	10,97	0,000	1,50
0.02	0,010875	0,000217	50,23	0,000	1,50
0.05	0,010375	0,000217	47,92	0,000	1,50
0.10	-0,023625	0,000217	-109,12	0,000	*

Regression Equation

$$d_{3,2,1} = 0,777625 + 0,002375 \text{ ratio_1week_0.01} \\ + 0,010875 \text{ ratio_1week_0.02} \\ + 0,010375 \text{ ratio_1week_0.05} - 0,023625 \text{ ratio_1week_0.10}$$

Fits and Diagnostics for Unusual Observations

Obs	d3,2_1	Fit	Resid	Std Resid
3	0,789000	0,788500	0,000500	2,00 R

R Large residual

Grouping Information Using the Tukey Method and 95% Confidence

ratio_1week	N	Mean	Grouping
0.02	2	0,7885	A
0.05	2	0,7880	A
0.01	2	0,7800	B
0.10	2	0,7540	C

Means that do not share a letter are significantly different.

(2) General Linear Model: Effect of formulation at week 4

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ratio_4week	Fixed	3	0.02; 0.05; 0.10

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ratio_4week	2	0,000890	0,000445	38,71	0,007
Error	3	0,000035	0,000012		
Total	5	0,000925			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0033912	96,27%	93,78%	85,08%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,77817	0,00138	562,08	0,000	
ratio_4week					
0.02	0,00733	0,00196	3,75	0,033	1,33
0.05	0,00983	0,00196	5,02	0,015	1,33
0.10	-0,01717	0,00196	-8,77	0,003	*

Regression Equation

$$\begin{aligned} d3,2_4 = & 0,77817 + 0,00733 \text{ ratio_4week_0.02} \\ & + 0,00983 \text{ ratio_4week_0.05} \\ & - 0,01717 \text{ ratio_4week_0.10} \end{aligned}$$

Grouping Information Using the Tukey Method and 95% Confidence

ratio_4week	N	Mean	Grouping
0.05	2	0,7880	A
0.02	2	0,7855	A
0.10	2	0,7610	B

Means that do not share a letter are significantly different.

Table A. 10 Analysis of variance (A for effect of stage weeks on formulation (1) 0.02, (2) 0.05, (3) 0.10

(1) General Linear Model: Effect of storage day on WG_0.02_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels Values
week_0.02	Fixed	2 1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
week_0.02	1	0,000009	0,000009	18,00	0,051
Error	2	0,000001	0,000001		
Total	3	0,000010			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0007071	90,00%	85,00%	60,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,787000	0,000354	2225,97	0,000	
week_0.02					
1	0,001500	0,000354	4,24	0,051	1,00
28	-0,001500	0,000354	-4,24	0,051	*

Regression Equation

particle size_0.02 = 0,787000 + 0,001500 week_0.02_1 - 0,001500 week_0.02_28

Grouping Information Using the Tukey Method and 95% Confidence

week_0.02	N	Mean	Grouping
1	2	0,7885	A
28	2	0,7855	A

Means that do not share a letter are significantly different.

(2) General Linear Model: Effect of storage day on WG_0.05_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels Values
week_0.05	Fixed	2 1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
week_0.05	1	0,000000	0,000000	0,00	1,000
Error	2	0,000002	0,000001		
Total	3	0,000002			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,001	0,00%	0,00%	0,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,788000	0,000500	1576,00	0,000	
week_0.05					
1	0,000000	0,000500	0,00	1,000	1,00
28	0,000000	0,000500	0,00	1,000	*

Regression Equation

particle size_0.05 = 0,788000 + 0,0 week_0.05_1 + 0,0 week_0.05_28

Grouping Information Using the Tukey Method and 95% Confidence

week_0.05	N	Mean	Grouping
1	2	0,788	A
28	2	0,788	A

Means that do not share a letter are significantly different.

(3) General Linear Model: Effect of storage day on WG_0.10_S20

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
week_0.10	Fixed	2	1; 28

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
week_0.10	1	0,000049	0,000049	3,06	0,222
Error	2	0,000032	0,000016		
Total	3	0,000081			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,004	60,49%	40,74%	0,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,75750	0,00200	378,75	0,000	
week_0.10					
1	-0,00350	0,00200	-1,75	0,222	1,00
28	0,00350	0,00200	1,75	0,222	*

Regression Equation

particle size_0.10 = 0,75750 - 0,00350 week_0.10_1 + 0,00350 week_0.10_28

Grouping Information Using the Tukey Method and 95% Confidence

week_0.10	N	Mean	Grouping
28	2	0,761	A
1	2	0,754	A

Means that do not share a letter are significantly different.

