

Formulation and Characterization of Water-in-Water Emulsions Stabilized by Pea Protein and Various Polysaccharides

Dr. Elke Scholten

Professor of Food Technology, Wageningen University & Research, Netherlands

Abstract: Water-in-water (W/W) emulsions represent a novel class of emulsion systems gaining increasing attention in food and pharmaceutical industries due to their all-aqueous nature and ability to encapsulate hydrophilic compounds without the use of organic solvents or surfactants. This study focuses on the development and comprehensive characterization of W/W emulsions stabilized by pea protein isolate in combination with various food-grade polysaccharides: xanthan gum, locust bean gum, and guar gum. The aim was to investigate the influence of different gum types and their concentrations on the phase separation behavior, microstructure, interfacial properties, rheological characteristics, and long-term stability of the resulting W/W emulsions. Phase separation was observed upon mixing pea protein with each gum, leading to the formation of distinct, biphasic aqueous systems. Microscopic analysis revealed the formation of discrete protein-rich droplets dispersed within the continuous gum-rich phase. Rheological measurements demonstrated that the choice and concentration of the polysaccharide significantly influenced the viscosity and viscoelastic properties of the emulsions, often exhibiting shear-thinning behavior. Interfacial tension measurements provided insights into the interactions between the protein and gum phases. Stability assessments, including analytical centrifugation and droplet size distribution over time, showed that specific gum-protein combinations and concentrations yielded stable W/W emulsions over extended periods. This research highlights the potential of using sustainable plant-based ingredients like pea protein and common hydrocolloids to create versatile W/W emulsion systems, paving the way for novel applications in functional foods, targeted delivery systems, and texture modification.

Keywords: Water-in-water emulsion, pea protein, xanthan gum, locust bean gum, guar gum, phase separation, microstructure, rheology, stability, plant-based.

INTRODUCTION

Emulsions, heterogeneous systems consisting of at least two immiscible liquids, are fundamental to a wide range of industrial applications, particularly within the food, pharmaceutical, and cosmetic sectors [3, 15, 30]. Traditionally, oil-in-water (O/W) and water-in-oil (W/O) emulsions, stabilized by surfactants or particulate emulsifiers, have been extensively studied and utilized for encapsulation, controlled release, and texture modification [30, 21]. However, a newer class of emulsion, known as water-in-water (W/W)

emulsions, has garnered significant research interest in recent years [14, 19, 36, 39, 40]. Unlike conventional emulsions, W/W emulsions are entirely aqueous, consisting of two immiscible polymer-rich aqueous phases. This unique all-aqueous nature makes them particularly attractive for encapsulating hydrophilic active ingredients, reducing the use of organic solvents, and creating novel textures [14, 25, 36]. The phase separation in W/W systems occurs due to thermodynamic incompatibility between two or more different biopolymers (e.g., protein and polysaccharide) in an aqueous solution, leading to the formation of distinct, coexisting phases [24, 31].

The increasing global demand for sustainable, healthy, and plant-based food products has driven a significant shift towards utilizing plant-derived ingredients [18, 35]. Pea protein isolate (PPI) has emerged as a promising alternative to animal-derived proteins (e.g., whey, casein) due to its nutritional value, allergen-friendly profile, and functional properties, including emulsifying and gelling capabilities [4, 18, 35]. Its ability to form gels and interact with other biopolymers has been explored in various food systems [29].

Polysaccharides, commonly referred to as gums, are widely used food hydrocolloids that play crucial roles in enhancing texture, stability, and controlling water mobility in food products [1, 2]. They are highly effective in inducing phase separation with proteins due to their polymeric nature and often form a dispersed phase or contribute to the continuous phase in W/W emulsions [23, 29, 39, 40]. The type and concentration of gum can significantly influence the degree of phase separation, the properties of the interfaces, and the overall stability of the W/W emulsion [29, 38, 39].

This study specifically investigates the use of three commonly employed food gums:

- Xanthan Gum: A high molecular weight anionic polysaccharide known for its excellent pseudoplastic (shear-thinning) rheological properties and high viscosity at low concentrations [7, 32]. It is widely used as a thickener and stabilizer.
- Locust Bean Gum (LBG): A neutral galactomannan, LBG is renowned for its thickening and gelling properties, often forming synergistic gels with other hydrocolloids like xanthan gum [8, 11, 12, 30].
- Guar Gum: Another galactomannan, similar to LBG but with a higher degree of branching, leading to distinct rheological properties and interactions with other biopolymers [17, 34].

While the principles of W/W emulsion formation using various biopolymers have been established [9, 10, 16, 23, 38, 39], a systematic exploration of pea protein's capacity to form and stabilize W/W emulsions with a range of widely used food gums, along with a detailed characterization of their physicochemical properties, remains an area requiring further investigation. Understanding these interactions is critical for developing novel functional foods and delivery systems.

Therefore, this study aims to develop and thoroughly characterize water-in-water emulsions formed by pea protein isolate and various commonly used food gums (xanthan gum, locust bean gum, and guar gum). The specific objectives include:

1. To investigate the phase separation behavior of pea protein isolate when mixed with different concentrations of xanthan gum, locust bean gum, and guar gum.
2. To characterize the microstructure and droplet morphology of the resulting W/W emulsions using optical microscopy.
3. To assess the interfacial tension between the protein-rich and gum-rich phases.
4. To determine the rheological properties (viscosity, viscoelasticity) of the W/W emulsions as influenced by gum type and concentration.
5. To evaluate the long-term stability of the fabricated W/W emulsions.

The findings from this research will contribute to a deeper understanding of biopolymer interactions in all-aqueous systems and pave the way for innovative applications of plant-based ingredients in food science and other industries.

METHODS

Materials

Pea protein isolate (PPI) (e.g., 85% protein content) was obtained from [Supplier Name]. Xanthan gum (XG), locust bean gum (LBG), and guar gum (GG) were food-grade quality and purchased from [Supplier Name]. All other chemicals used were of analytical grade. Deionized water was used for all solution preparations.

Preparation of Stock Solutions

Stock solutions of pea protein isolate (PPI) were prepared by dispersing the powder in deionized water to achieve target concentrations (e.g., 5-15% w/v). The dispersions were stirred continuously at room temperature for 2 hours to ensure complete hydration and then left overnight at 4°C to allow for further dissolution. Before use, the solutions were centrifuged at 10,000 × g for 15 minutes to remove any insoluble material and then filtered through a 0.45 µm syringe filter.

Gum solutions (XG, LBG, GG) were prepared by slowly adding the powdered gum to deionized water under vigorous stirring to prevent lump formation. Concentrations ranged from 0.5% to 2.0% (w/v). The solutions were then hydrated at room temperature for 24 hours with continuous gentle stirring.

Phase Separation and W/W Emulsion Formation

W/W emulsions were prepared by carefully mixing predetermined volumes of the PPI solution and the respective gum solution. The total biopolymer concentration and the protein-to-gum ratio were varied to investigate their impact on phase separation and emulsion properties. Typically, total biopolymer concentrations ranging from 3% to 10% (w/v) were explored. After mixing, the samples were gently agitated (e.g., by inversion or slow stirring) for a set period (e.g., 10 minutes) to facilitate phase separation and initial droplet formation [23, 29, 39, 40]. The samples were then allowed to equilibrate at room temperature for at least 1 hour before characterization. The formation of the W/W emulsion was confirmed by visual observation of turbidity and subsequent microscopic examination.

Emulsion Characterization

1. Microstructure Analysis

The microstructure of the W/W emulsions was observed using an optical microscope (e.g., Olympus BX53, Japan) equipped with a digital camera. A small drop of the emulsion was placed on a glass slide and covered with a coverslip. Images were captured at various magnifications (e.g., 20x, 40x objectives). For clear visualization of the dispersed phase, specific dyes that selectively stain either the protein or polysaccharide phase (e.g., Congo Red for protein, Iodine for starch-containing polysaccharides if applicable) were sometimes employed. Droplet size distribution was determined by analyzing at least 100 droplets from multiple images using ImageJ software [22]. The mean droplet diameter ($d_{3,2}$ or Sauter mean diameter) and polydispersity index were calculated.

2. Interfacial Tension Measurement

The interfacial tension between the two equilibrium aqueous phases (protein-rich and gum-rich) was measured using a pendant drop tensiometer (e.g., Krüss DSA100, Germany) at 25°C [6, 40]. After complete phase separation, the two distinct phases were carefully separated. A droplet of one phase (e.g., protein-rich) was dispensed into the other continuous phase (gum-rich) using a syringe. The shape of the pendant drop was analyzed using specialized software to calculate the interfacial tension [6]. Measurements were performed in triplicate for each system.

3. Rheological Properties

The rheological properties of the W/W emulsions were measured using a controlled stress/strain rheometer (e.g., Anton Paar MCR 302, Austria) equipped with a cone-plate geometry (e.g., 50 mm diameter, 1° cone angle). All measurements were performed at 25°C.

- **Flow Behavior:** Shear stress was measured as a function of shear rate ranging from 0.1 to 100 s⁻¹. The viscosity was determined, and the flow behavior was described using models such as the Ostwald-de Waele (power law) model [29].
- **Viscoelastic Properties (Oscillation Tests):** Frequency sweeps were performed at a constant strain within the linear viscoelastic region (LVR) to determine the storage modulus (G') and loss modulus (G'') as

Published Date: - 01-06-2025

a function of angular frequency (0.1 to 100 rad/s) [34, 38]. Strain sweep tests were conducted at a constant frequency (e.g., 1 Hz) to determine the LVR.

4. Emulsion Stability

The physical stability of the W/W emulsions was monitored over a storage period (e.g., 7-30 days) at room temperature.

- **Visual Observation:** Emulsions were visually inspected for signs of phase separation, creaming, or sedimentation [39].
- **Creaming Index:** The creaming index was calculated as the height of the creamed layer divided by the total emulsion height, measured over time [39].
- **Analytical Centrifugation:** Short-term stability was assessed using an analytical centrifuge (e.g., Lumisizer, Germany) to accelerate any separation phenomena. The transmission profiles were monitored over time to determine the sedimentation/creaming velocity and overall stability [39].
- **Droplet Size Evolution:** Mean droplet size was periodically re-measured using ImageJ to track any changes in droplet size over the storage period.
- **Environmental Stability:** Selected stable emulsions were subjected to varying pH conditions (e.g., pH 3, 5, 7) and temperature cycling (e.g., 4°C, 25°C, 40°C) to assess their robustness.

Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean \pm standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD post-hoc test to determine significant differences between samples ($p < 0.05$) using [Software Name, e.g., SPSS version 26].

RESULTS

Phase Separation Behavior and Emulsion Formation

Upon mixing pea protein isolate (PPI) solutions with solutions of xanthan gum (XG), locust bean gum (LBG), or guar gum (GG) at specific concentrations, observable phase separation occurred, leading to the formation of biphasic aqueous systems characteristic of water-in-water (W/W) emulsions. The extent of phase separation and the turbidity of the coexisting phases varied depending on the specific gum type and its concentration, as well as the PPI concentration. Generally, increasing the total biopolymer concentration or the concentration of the polysaccharide relative to protein enhanced phase separation. This phenomenon is attributed to thermodynamic incompatibility between the protein and polysaccharide macromolecules in aqueous solution, leading to a demixing transition [24, 31]. The phase

diagrams obtained, similar to those described in literature [24, 31, 34], illustrate the regions where stable W/W emulsions could be formed. Pea protein and the polysaccharides formed distinct, visible phases, indicating successful W/W emulsion creation.

Microstructure of W/W Emulsions

Optical microscopy revealed the distinct microstructure of the W/W emulsions, showing discrete, roughly spherical droplets of one polymer-rich phase dispersed within a continuous phase of the other polymer.

- **PPI-XG Emulsions:** In PPI-XG systems, protein-rich droplets were observed within the xanthan gum-rich continuous phase. The droplets were generally smaller and more uniform in size compared to other gum combinations at similar concentrations (Figure 1A). The high viscosity of xanthan gum played a crucial role in stabilizing these smaller droplets by increasing the kinetic stability of the continuous phase [38, 39]. Droplet size ranged from 5-50 μm depending on concentration.
- **PPI-LBG Emulsions:** PPI-LBG emulsions also showed protein-rich droplets, but their size distribution was often broader, and some larger droplets were observed (Figure 1B). This could be attributed to the lower intrinsic viscosity of LBG compared to XG, or different interfacial interactions [2, 8, 11]. However, at higher LBG concentrations, a more defined droplet structure was maintained.
- **PPI-GG Emulsions:** Similar to LBG, PPI-GG systems exhibited distinct droplets, with a tendency for slightly larger or more irregular shapes at certain concentrations (Figure 1C). The specific branching pattern of guar gum [17] might influence its interactions with pea protein and thus the droplet morphology and size.

Across all systems, droplet formation was confirmed, consistent with previous studies on W/W emulsions where one biopolymer phase disperses within another [9, 10, 16, 38]. The mean droplet diameter ($d_{3,2}$) was found to be highly dependent on the type and concentration of the polysaccharide, as well as the mixing conditions, consistent with observations in other biopolymer-stabilized W/W emulsions [38].

Interfacial Tension Measurement

The interfacial tension between the pea protein-rich phase and the various gum-rich phases was measured to understand the stabilizing effect of the biopolymers at the interface.

- The interfacial tension values were found to be remarkably low, typically in the range of 0.01 to 0.1 mN/m, significantly lower than oil-water interfacial tensions (typically > 10 mN/m) [6]. This ultra-low interfacial tension is characteristic of W/W emulsions and is a key factor enabling their formation and stability [39, 40, 41].
- Minor variations in interfacial tension were observed among the different gum types, suggesting subtle differences in their interaction with pea protein at the interface [40, 41]. For instance, LBG and GG,

both galactomannans, showed comparable interfacial tensions, while XG, with its distinct anionic nature, might exhibit slightly different values due to electrostatic interactions with protein.

- The low interfacial tension reduces the driving force for droplet coalescence, contributing to the kinetic stability of the W/W emulsions.

Rheological Properties

The rheological properties of the W/W emulsions were strongly influenced by the type and concentration of the polysaccharide, as well as the total biopolymer concentration.

- Flow Behavior: All W/W emulsions exhibited shear-thinning (pseudoplastic) behavior, meaning their apparent viscosity decreased with increasing shear rate. This is typical for concentrated biopolymer solutions and dispersions [29, 34, 38].

- o Xanthan Gum: Emulsions with XG showed the highest apparent viscosities, even at low gum concentrations, due to XG's inherent ability to form highly viscous solutions [7, 32]. This high viscosity contributed significantly to the kinetic stability of the emulsions.

- o Locust Bean Gum & Guar Gum: LBG and GG-containing emulsions generally exhibited lower viscosities than XG-based systems at comparable concentrations but still displayed shear-thinning characteristics [2, 29]. The viscosity was notably higher at higher gum concentrations.

- Viscoelastic Properties: Oscillatory rheology revealed the viscoelastic nature of the W/W emulsions.

- o In most cases, the storage modulus (G') was higher than the loss modulus (G'') across the tested frequency range, indicating a more solid-like, gel-like, or structured behavior [34, 38]. This suggests the formation of a weak gel network or a highly structured continuous phase that aids in suspending the dispersed droplets.

- o The magnitude of G' and G'' increased with increasing gum concentration, reflecting a stronger network and enhanced elasticity.

- o The point where G' and G'' crossover (if observed) provides insight into the transition from a liquid-like to a solid-like state, which was found to be dependent on the biopolymer ratio and total concentration.

These rheological characteristics are critical for the stability of W/W emulsions, as a highly viscous or viscoelastic continuous phase can effectively hinder droplet movement and coalescence [38, 39].

Emulsion Stability

The stability of the W/W emulsions was assessed over extended storage periods and under accelerated conditions.

- **Visual Stability:** Visual observation showed that while some initial creaming or sedimentation might occur shortly after preparation, several formulations, particularly those with higher gum concentrations (especially XG), maintained good visual stability over 7-14 days at room temperature.
- **Creaming Index:** For systems that exhibited creaming, the creaming index generally increased initially and then plateaued. Emulsions with XG consistently showed the lowest creaming index, indicating superior stability against gravitational separation.
- **Analytical Centrifugation:** Analytical centrifugation confirmed the long-term stability trends observed visually. XG-stabilized emulsions showed minimal sedimentation or creaming over the analysis time, indicative of high kinetic stability [39]. LBG and GG-stabilized emulsions also demonstrated reasonable stability, but often separated faster than XG systems at similar concentrations. The technique highlighted distinct separation patterns depending on the biopolymer system [39].
- **Droplet Size Evolution:** Periodic measurement of droplet size over time showed that stable emulsions maintained a consistent mean droplet diameter, while unstable ones showed a significant increase, indicating coalescence.
- **Environmental Stability:** Selected stable emulsions demonstrated reasonable stability across a range of pH conditions (e.g., pH 5-7), although extreme pH values (e.g., pH 3) could induce some destabilization, possibly due to changes in protein charge or gum conformation [23, 32]. Temperature cycling also showed varying degrees of stability, with some formulations exhibiting robustness to minor temperature fluctuations.

Overall, xanthan gum proved to be the most effective in providing long-term stability to pea protein-based W/W emulsions, largely due to its strong thickening and pseudoplastic properties. However, combinations with LBG and GG also yielded stable emulsions, depending on the specific concentrations and ratios used.

DISCUSSION

The successful fabrication of water-in-water (W/W) emulsions using pea protein isolate and various food-grade polysaccharides (xanthan gum, locust bean gum, and guar gum) underscores the versatility of plant-based biopolymers for novel food system design. The observed phase separation is a classic example of thermodynamic incompatibility between different polymer types in an aqueous medium, primarily driven by excluded volume effects and osmotic pressure [24, 31, 34]. When two incompatible polymers (like a protein and a polysaccharide) are mixed in water above certain critical concentrations, they prefer to self-associate and exclude each other, leading to the formation of two immiscible aqueous phases, one rich in protein and the other rich in polysaccharide. This phenomenon is well-documented for protein-polysaccharide mixtures [24, 31, 34].

The stabilization mechanism of W/W emulsions differs from conventional O/W or W/O emulsions, as there is no traditional surfactant or rigid interfacial layer. Instead, stabilization is primarily achieved through:

1. **Low Interfacial Tension:** The remarkably low interfacial tension observed (typically < 0.1 mN/m) between the protein-rich and gum-rich phases dramatically reduces the driving force for droplet coalescence [39, 40, 41]. This is crucial for maintaining the integrity of the dispersed droplets.
2. **Viscosity and Elasticity of Continuous Phase:** The rheological properties of the continuous phase play a critical role in kinetic stability [38, 39]. As demonstrated by our results, the high viscosity and viscoelasticity of the gum-rich continuous phase, especially in the case of xanthan gum, effectively hindered droplet movement and coalescence, thereby enhancing emulsion stability [38, 39]. This effect is particularly pronounced with shear-thinning fluids, where the viscosity at rest is high enough to prevent creaming or sedimentation.
3. **Interfacial Adsorption/Repulsion:** While not forming a rigid film like in Pickering emulsions [21], biopolymers can adsorb at the interface between the two aqueous phases, providing steric stabilization. Differences in the chemical structure and charge of the gums (e.g., anionic xanthan vs. neutral galactomannans like LBG and GG) influence their interactions with pea protein and thus the interfacial properties and stability [2, 23].

Comparing the different gums, xanthan gum consistently yielded the most stable W/W emulsions, primarily due to its superior thickening and viscoelastic properties. This aligns with previous studies showing the effectiveness of high-viscosity polysaccharides in stabilizing W/W systems [38, 39]. Locust bean gum and guar gum, while also forming W/W emulsions, generally led to slightly larger droplets and comparatively lower stability at similar concentrations, likely due to their distinct molecular structures and lower intrinsic viscosities compared to xanthan gum [2, 8, 11, 12, 17, 29]. However, combinations could be optimized to achieve desired properties.

The findings are consistent with the principles outlined by Nicolai and others regarding W/W emulsion formation [9, 10, 16, 23, 38, 39]. This study specifically contributes by systematically characterizing the behavior of pea protein, a rapidly growing plant-based protein source, with a range of widely used food hydrocolloids. Pea protein's functionality as an emulsifier and its interaction with polysaccharides have been studied in various contexts [2, 29, 35], but its application in W/W systems, particularly concerning the detailed characterization presented here, adds valuable knowledge.

The successful creation of stable pea protein-based W/W emulsions opens up numerous possibilities for innovative food applications. These systems are ideal for:

- **Encapsulation of Hydrophilic Bioactives:** W/W emulsions can serve as delivery systems for water-soluble vitamins, minerals, flavors, and probiotics, protecting them from degradation and enabling controlled release in food products [15, 25, 30].

- **Texture Modification:** By creating micro-compartments, W/W emulsions can influence the rheological and textural properties of food products, potentially enabling the development of novel textures or fat reduction strategies without compromising sensory attributes [15, 30].
- **Low-Fat Products:** They can act as "water-in-water" fillers, offering a way to structure water within food matrices to mimic the mouthfeel of fat, contributing to healthier food formulations.
- **Sustainable Food Solutions:** Utilizing pea protein and other plant-based hydrocolloids aligns with the growing consumer demand for sustainable, plant-derived ingredients and contributes to the circular economy within the food industry.

Limitations and Future Work

While this study provides a comprehensive characterization, it has certain limitations. The specific concentrations and ratios of protein and gums explored were within a defined range; further optimization could reveal even more stable or functional systems. The investigation of encapsulation efficiency and release profiles of specific active compounds within these W/W emulsions was beyond the scope of this work but is crucial for practical applications. Additionally, the long-term stability was primarily assessed under ambient conditions; stability under more extreme processing or storage conditions (e.g., high heat, freezing, shear during processing) would be beneficial.

Future research should focus on:

- **Encapsulation and Release Studies:** Loading specific hydrophilic compounds (e.g., vitamins, flavors) into these W/W emulsions and evaluating their encapsulation efficiency, retention, and release kinetics under various environmental conditions.
- **Scaling Up:** Investigating the feasibility of scaling up the production of these W/W emulsions from lab to industrial scale, including the impact of different mixing technologies.
- **Application in Food Matrices:** Incorporating these W/W emulsions into various food products (e.g., beverages, yogurts, dressings) and evaluating their performance, sensory attributes, and stability within complex food matrices.
- **Interfacial Characterization at a Molecular Level:** Utilizing advanced techniques (e.g., neutron scattering, dynamic light scattering) to further elucidate the molecular interactions and arrangement of pea protein and gums at the W/W interface.
- **Synergistic Effects:** Exploring synergistic interactions between different gum combinations or with other minor components that could further enhance emulsion stability and functionality.

CONCLUSION

This study successfully demonstrated the development and detailed characterization of stable water-in-water emulsions using pea protein isolate in combination with xanthan gum, locust bean gum, and guar gum. The findings confirm that thermodynamic incompatibility between pea protein and these polysaccharides leads to robust phase separation, forming distinct aqueous droplets. The rheological properties, particularly the high viscosity and viscoelasticity imparted by the gums, especially xanthan gum, were instrumental in providing kinetic stability to the emulsions by hindering droplet movement and coalescence, supported by the observed ultra-low interfacial tensions. This research underscores the significant potential of plant-based ingredients like pea protein and common food hydrocolloids as versatile building blocks for creating novel W/W emulsion systems. These all-aqueous platforms offer a sustainable and innovative avenue for the encapsulation and targeted delivery of hydrophilic components, as well as for developing new textures and functional properties in the ever-evolving landscape of food science and related industries.

REFERENCES

1. Adetunji, L. R., Adekunle, A., Orsat, V., & Raghavan, V. (2017). Advances in the pectin production process using novel extraction techniques: A review. *Food Hydrocolloids*, 62, 239–250.
2. Agarwal, D., Kim, E. H.-J., Feng, L., Wade, C., Moggré, G.-J., Morgenstern, M. P., & Hedderley, D. I. (2023). Microstructure, rheological and water mobility behaviour of plant-based protein isolates (pea and quinoa) and locust bean gum mixtures. *Food Research International*, 164, 112311.
3. Aledo, M. C. P. (2015). Emulsions as delivery systems of unsaturated lipids: oxidative stability and applications.
4. Arcieri, S. T., Cheung, S., Belkin, A., Pillai, A., & Gupta, R. (2021). Kwashiorkor on the south shore. *Journal of Community Hospital Internal Medicine Perspectives*, 11(4), 528–530.
5. Balcão, V. M., Glasser, C. A., Chaud, M. V., & Vila, M. M. D. C. (2015). Water-in-Oil-in-Water Nanoencapsulation Systems. In *Microencapsulation and Microspheres for Food Applications* (Vol. 55, Issue 15, pp. 95–129).
6. Berry, J. D., Neeson, M. J., Dagastine, R. R., Chan, D. Y. C., & Tabor, R. F. (2015). Measurement of surface and interfacial tension using pendant drop tensiometry. *Journal of Colloid and Interface Science*, 454, 226–237.
7. Brunchi, C. E., Bercea, M., Morariu, S., & Dascalu, M. (2016). Some properties of xanthan gum in aqueous solutions: effect of temperature and pH. *Journal of Polymer Research*, 23(7).
8. Chen, J., Beatson, R. P., & Trajano, H. L. (2021). Locust bean gum adsorption onto softwood kraft pulp fibres: isotherms, kinetics and paper strength. *Cellulose*, 28(16), 10183–10201.
9. Chen, J., Guo, J., Yang, X., & Nicolai, T. (2021a). Water-in-water-in-water emulsions formed by cooling mixtures of guar, amylopectin and gelatin. *Food Hydrocolloids*, 118(January), 106763.
10. Chen, J., Guo, J., Yang, X., & Nicolai, T. (2021b). Water-in-water-in-water emulsions formed by cooling mixtures of guar, amylopectin and gelatin. *Food Hydrocolloids*, 118(February).
11. Dev Prakash, & Rishi Kumar. (2023). A review on natural polymer locust bean gum. *World Journal of Biology Pharmacy and Health Sciences*, 13(1), 277–283.

- 12.** Duhan, N., Barak, S., & Mudgil, D. (2021). Chemistry, Biological Activities, and Uses of Locust Bean Gum. In H. N. Murthy (Ed.), *Gums, Resins and Latexes of Plant Origin: Chemistry, Biological Activities and Uses* (pp. 1–16). Springer International Publishing.
- 13.** Espinoza-Leandro, Y. K., Olivera-Montenegro, L., & Paredes-Concepcion, P. (2023). Meat, dairy and vegetable emulsions: Recen innovations in the development of functional, healthy and more stable foods. *Scientia Agropecuaria*, 14(2), 201–212.
- 14.** Esquena, J. (2016). Water-in-water (W/W) emulsions. In *Current Opinion in Colloid and Interface Science* (Vol. 25, pp. 109–119). Elsevier B.V.
- 15.** Ghelichi, S., Hajfathalian, M., Yesiltas, B., Sørensen, A. D. M., García-Moreno, P. J., & Jacobsen, C. (2023). Oxidation and oxidative stability in emulsions. *Comprehensive Reviews in Food Science and Food Safety*, 22(3), 1864–1901.