








Congreso Iberoamericano de Ingeniería de los Alimentos

Segregative phase separation in aqueous systems of whey protein and carboxymethylcellulose under isoionic conditions

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Abstract

Whey, a byproduct of cheese production, contains valuable proteins such as whey protein (WP), which are key in the food industry due to their functional properties, especially the ability to form gels. When combined with polysaccharides like carboxymethylcellulose (CMC), WP can undergo phase separation, affecting properties like viscosity and texture in food products. This separation can be segregative, when the macromolecules are incompatible, or associative, when oppositely charged macromolecules attract each other and form networks. This study focuses on phase separation in WP and CMC mixtures under isoionic conditions, evaluating the effect of the molecular weight of CMC and the rheological properties of the mixtures. The tests included measurements of electrokinetic potential, microscopic analysis, and rheology of individual solutions and mixtures of WP with low- and high-viscosity CMC. It was found that mixtures with high-viscosity CMC tend to form segregated phases more easily. The flow and gelation properties also depended on the concentration of WP and the type of CMC, with higher concentrations of WP resulting in stronger gels. The results provide key insights for controlling the structure and functionality in food systems.

Keywords: whey protein, carboxymethylcellulose, rheology, macromolecules, phase separation

Editor

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Received 31 Oct 2024
Accepted 03 Feb 2025
Published 24 Apr 2025

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Separación de fases segregativa en sistemas acuosos de proteína de suero y carboximetilcelulosa en condiciones isoiónicas

Resumen

El suero de leche, subproducto de la producción de queso, contiene proteínas valiosas como la proteína de suero (WP), que es clave en la industria alimentaria por sus propiedades funcionales, especialmente la capacidad de formar geles.



Baldor S, Enatariaga-Scull MN, Genna-Coronel AG, Torres PB, Nambuena CF, Boeris V. Segregative phase separation in aqueous systems of whey protein and carboxymethylcellulose under isoionic conditions. Agrocienza Uruguay [Internet]. 2025 [cited dd mmm yyyy];29(NE1):e1602. Doi: 10.31285/AGRO.29.1602.

En combinación con polisacáridos, como la carboximetilcelulosa (CMC), la WP puede experimentar separación de fases, lo que afecta propiedades como la viscosidad y la textura de los alimentos. Esta separación puede ser segregativa, cuando las macromoléculas son incompatibles, o asociativa, cuando macromoléculas de cargas opuestas se atraen y forman redes. Este estudio se enfoca en la separación de fases en mezclas de WP y CMC bajo condiciones isoiónicas, evaluando el efecto del peso molecular de la CMC y las propiedades reológicas de las mezclas. Las pruebas incluyeron mediciones del potencial electrocinético, análisis microscópico y reología de soluciones individuales y mezclas de WP con CMC de baja y alta viscosidad. Se encontró que las mezclas con CMC de alta viscosidad tienden a formar fases segregadas más fácilmente. Las propiedades de flujo y gelación también dependieron de la concentración de WP y el tipo de CMC, con mayores concentraciones de WP resultando en geles más fuertes. Estos resultados proporcionan información clave para el control de la estructura y la funcionalidad en sistemas alimentarios.

Palabras clave: proteína de lactosuero, carboximetilcelulosa, reología, macromoléculas, separación de fases

Separação de fases segregativa em sistemas aquosos de proteína de soro e carboximetilcelulose sob condições isoiónicas

Resumo

O soro de leite, subproduto da produção de queijo, contém proteínas valiosas (WP), que são fundamentais na indústria alimentícia devido às suas propriedades funcionais, especialmente a capacidade de formar geis. Em combinação com polissacarídeos, como a carboximetilcelulose (CMC), a WP pode passar por separação de fases, o que afeta propriedades como a viscosidade e a textura dos alimentos. Essa separação pode ser segregativa, quando as macromoléculas são incompatíveis, ou associativa, quando macromoléculas de cargas opostas se atraem e formam redes. Este estudo foca na separação de fases em misturas de WP e CMC sob condições isoiónicas, avaliando o efeito do peso molecular da CMC e as propriedades reológicas das misturas. Os testes incluíram medições do potencial eletrocinético, análise microscópica e reologia de soluções individuais e misturas de WP com CMC de baixa e alta viscosidade. Foi encontrado que misturas com CMC de alta viscosidade tendem a formar fases segregadas mais facilmente. As propriedades de fluxo e gelificação também dependeram da concentração de WP e do tipo de CMC, com concentrações mais altas de WP resultando em geis mais fortes. Esses resultados fornecem informações chave para o controle da estrutura e funcionalidade em sistemas alimentares.

Palavras-chave: proteína do soro de leite, carboximetilcelulose, reologia, macromoléculas, separação de fases

1. Introduction

Whey, a byproduct of cheese production, accounts for approximately 85-90% of the total milk volume processed, retaining a significant portion of the original nutrients from milk, including lactose, fats, mineral salts, and soluble proteins. Whey proteins (WP), which constitute around 20% of the protein content in bovine milk, are recognized for their high nutritional value and versatile functional properties, making them a key ingredient in the food industry. One of their most important functional characteristics is their ability to form gels, particularly through thermal treatment, which has been extensively studied and applied in food product development⁽¹⁾.

Protein-polysaccharide colloidal systems are commonly used to modify the texture, viscosity, and other essential properties of food products. These systems can undergo phase separation, which is a key phenomenon in determining their functionality. Phase separation in protein-polysaccharide systems can be classified into two main types: segregative and associative⁽²⁾. Segregative phase separation occurs when the two macromolecules—such as proteins and polysaccharides—are thermodynamically incompatible, leading to their separation into distinct phases, each enriched in one of the components⁽³⁾. In contrast, associative phase separation involves the attraction between oppositely charged macromolecules, resulting in the formation of complexes or

networks that remain in a mixed phase⁽⁴⁾. The type of phase separation that occurs depends on various factors, including the charge, concentration, and molecular characteristics of the biopolymers, as well as the environmental conditions, such as pH, ionic strength, and temperature⁽⁵⁾.

Segregative phase separation generally occurs when the macromolecules involved carry similar charges, or when one of them is uncharged. In this case, repulsion between the molecules leads to the formation of two immiscible phases, one enriched in protein and the other in polysaccharide. This type of separation is typically favored at higher macromolecule concentrations and under conditions where the macromolecules do not interact strongly with each other⁽⁶⁾. Associative phase separation, on the other hand, occurs when the macromolecules carry opposite charges, resulting in electrostatic attraction between them. This leads to the formation of either two liquid phases (coacervation) or a combination of a liquid and solid phase (precipitation), with one phase rich in both macromolecules and the other rich in solvent. This type of separation is typically favored at lower ionic strengths and concentrations, and is largely determined by the isoelectric point of the protein and the pKa of the polysaccharide⁽⁷⁾.

The functional properties of protein-polysaccharide mixtures, such as their ability to form gels or influence viscosity, are highly dependent on the type of phase separation that occurs. Whey protein concentrate is a common functional ingredient in food products⁽⁸⁾, whereas carboxymethylcellulose (CMC), a water-soluble anionic polysaccharide, is widely used in combination with proteins to form mixed systems with specific structural and functional properties⁽⁹⁾. Understanding the interactions between WP and CMC is essential for predicting and controlling the phase behavior of these systems, which in turn determines the texture, water-holding capacity, and sensory properties of the final food product.

In recent years, research has focused on the role of phase separation in mixed protein-polysaccharide systems and its implications for food structure and stability⁽¹⁰⁾⁽¹¹⁾. Studies have shown that the phase behavior of such systems is influenced by a variety of factors, including the molecular weight and concentration of the macromolecules, the ionic strength of the medium, and the presence of co-solutes such as sugars and salts⁽¹²⁾⁽¹³⁾. Similarly, changes in pH can alter the charge of the macromolecules and thus affect their interactions and phase behavior. The type and extent of phase separation also influence the rheological properties of the system, such as its viscosity and gel strength, which are critical for the development of food products with desirable textures and stability⁽¹⁴⁾.

The objective of this study is to investigate the phase behavior of WP-CMC mixtures under isoionic conditions, evaluating the effect of the molecular weight of the polysaccharide. In addition, by assessing the rheological properties of these mixtures, this research aims to provide a deeper understanding of how WP and CMC interact under isoionic conditions.

2. Materials and Methods

2.1 Materials

Whey protein concentrate (Star Nutrition, Argentina) with a protein content of 82% was purchased in a local market. Carboxymethylcellulose (CMC) sodium salt was obtained from Sigma-Aldrich (USA), with two different samples utilized: low-viscosity CMC (CMC_l) and high-viscosity CMC (CMC_h).

2.2 Electrokinetic potential

The electrokinetic potential of individual systems (WP, CMC_h, and CMC_i) and mixed systems (WP-CMC_h and WP-CMC_i) was measured by dynamic light scattering using a particle size analyzer (Horiba NanoPartica SZ100, Japan)⁽¹⁵⁾. To determine the ζ -potential, samples were prepared at the pH range from 2 to 6. The concentration of WP was maintained at 0.82%, while the concentration of CMC was set at 0.5%.

2.3 Phase behavior evaluation

The phase behavior of WP and CMC in aqueous solutions was evaluated using systems with varying concentrations of both components. Two experiments were conducted: one with CMC_h and the other with CMC_i. Stock solutions of WP at 16.4% and CMC at 1% were prepared at isoionic pH and mixed thoroughly to achieve the desired concentrations, with the appropriate amount of water added. Phase separation was assessed after 24 and 72 h of incubation by visually inspecting the upper and lower layers formed in each system⁽¹⁶⁾.

The microstructure of six WP-CMC systems after 24 h incubation was assessed using confocal laser scanning microscopy (CLSM), labelling the WP with Rhodamine B. Fluorescence excitation was achieved with a laser light source at a wavelength of 488 nm, while fluorescent emission was recorded at 568 nm. The systems were observed using a 60 \times oil immersion objective on a Nikon Eclipse TE-2000-E confocal microscope (Japan). Images were captured with a pixel resolution of 1024 \times 1024 using Nikon's EZ-C1 software⁽¹⁷⁾.

2.4 Rheological measurements

The flow behavior of the individual stock solutions and the apparent viscosity of the selected mixed samples were measured. A dynamic rheometer (TA-Discovery HR30 series, USA) was used to perform flow tests by conducting a flow ramp with increasing shear up to 100 s⁻¹ at constant temperature (25 °C)⁽¹⁸⁾.

To analyze the gelation process of the selected mixed systems, samples were prepared by mixing the appropriate quantities of each stock solution to achieve the following WP and CMC concentrations: 14.76% WP - 0.1% CMC, 8.2% WP - 0.5% CMC, and 16.4% WP - 0.9% CMC. The samples were subjected to a controlled temperature ramp, and gelation was evaluated using parallel plate geometry, by monitoring the viscoelastic properties during heating and cooling. A rheometer operated in oscillatory mode at a frequency of 0.5 Hz and a strain of 0.1% was used for this analysis. The mixed solutions were maintained at 25 °C for 3 min before being heated to 90 °C at a rate of 5 °C/min. The temperature was held at 90 °C for 300 s, followed by cooling from 90 °C to 25 °C at the same rate of 5 °C/min. The storage modulus (G') and loss modulus (G'') were recorded, with the crossover of these moduli serving as the criterion for gelation⁽¹⁸⁾. The gel reinforcement (Gr), representing the strengthening of the gel network, was calculated as the difference in G' at 25 °C (after cooling) and G' at 90 °C (before cooling)⁽¹⁹⁾.

2.5 Statistical analysis

Two or more independent trials were carried out on each sample. The average and standard errors were then calculated from this data. ANOVA (post hoc Holm-Sidak test) was employed to ascertain significant differences between samples ($p < 0.05$).

3. Results

3.1 Rheological characterization of the biopolymer suspensions

The flow behavior of the stock solutions of WP and both types of CMC was evaluated at 25 °C, as shown in **Figure 1**. Both WP and CMC_l showed Newtonian flow, whereas the CMC_h solution demonstrated pseudo-plastic behavior under the tested conditions. The CMC_h solution exhibited a significantly higher viscosity, approximately one order of magnitude greater than that of WP, while the viscosity of the CMC_l solution was slightly lower than that of WP.

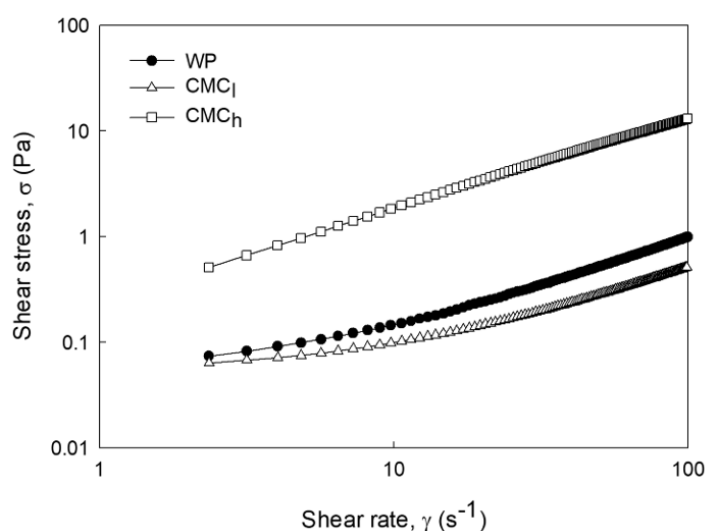


Figure 1. Flow behavior curves of the stock solutions of WP 16.4% and both types of CMC, 1%, measured at 25 °C

3.2 Electrokinetic potential determination

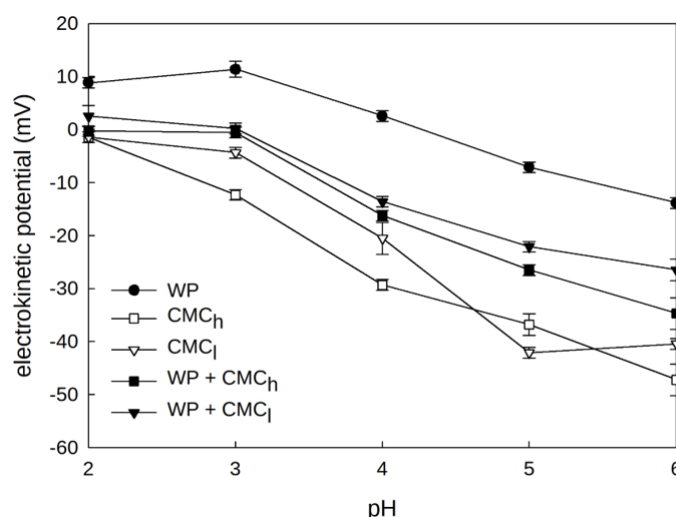


Figure 2. Electrokinetic potential of aqueous solutions of WP 0.82%, CMC_h 0.5%, CMC_l 0.5%, and mixtures WP 0.82% - CMC 0.5%, measured at 25 °C

The electrophoretic mobility of proteins and polysaccharides in aqueous dispersions is influenced by their intrinsic properties, such as amino acid or carbohydrate composition, shape, and size, as well as external fac-

tors like pH and ionic strength. The colloidal suspensions were analyzed under acidic conditions, with pH values ranging from 6 (isoionic pH) to 2, to assess the behavior around the isoelectric point of the main whey proteins—reported as 5.4 for β -lactoglobulin and 4.3 for α -lactalbumin. As shown in **Figure 2**, the WP exhibited a positive ζ -potential below pH 4 and a negative ζ -potential above that pH, consistent with previous findings⁽²⁰⁾. Both CMC samples showed negative ζ -potential between pH 3 and 6, becoming nearly neutral around pH 2. The magnitude of the ζ -potential was higher for CMC_h than for CMC_i. In WP-CMC mixed systems, only one population was detected, i.e., the distribution resulted monomodal, with the ζ -potential values intermediate between those corresponding to WP or CMC alone.

3.3 Phase behavior evaluation

Figure 3 presents the phase behavior of aqueous systems composed by WP and CMC at isoionic pH, incubated for 24 or 72 h. Each graph highlights two distinct regions: one where systems remained homogeneous and another where phase separation occurred. At low concentrations of WP and CMC, the systems were homogeneous, indicating the co-solubility of these biopolymers in that condition; however, at increasing biopolymer concentration, segregative phase separation is induced. Both phases are liquid: the upper is concentrated in CMC, and the lower is enriched in WP.

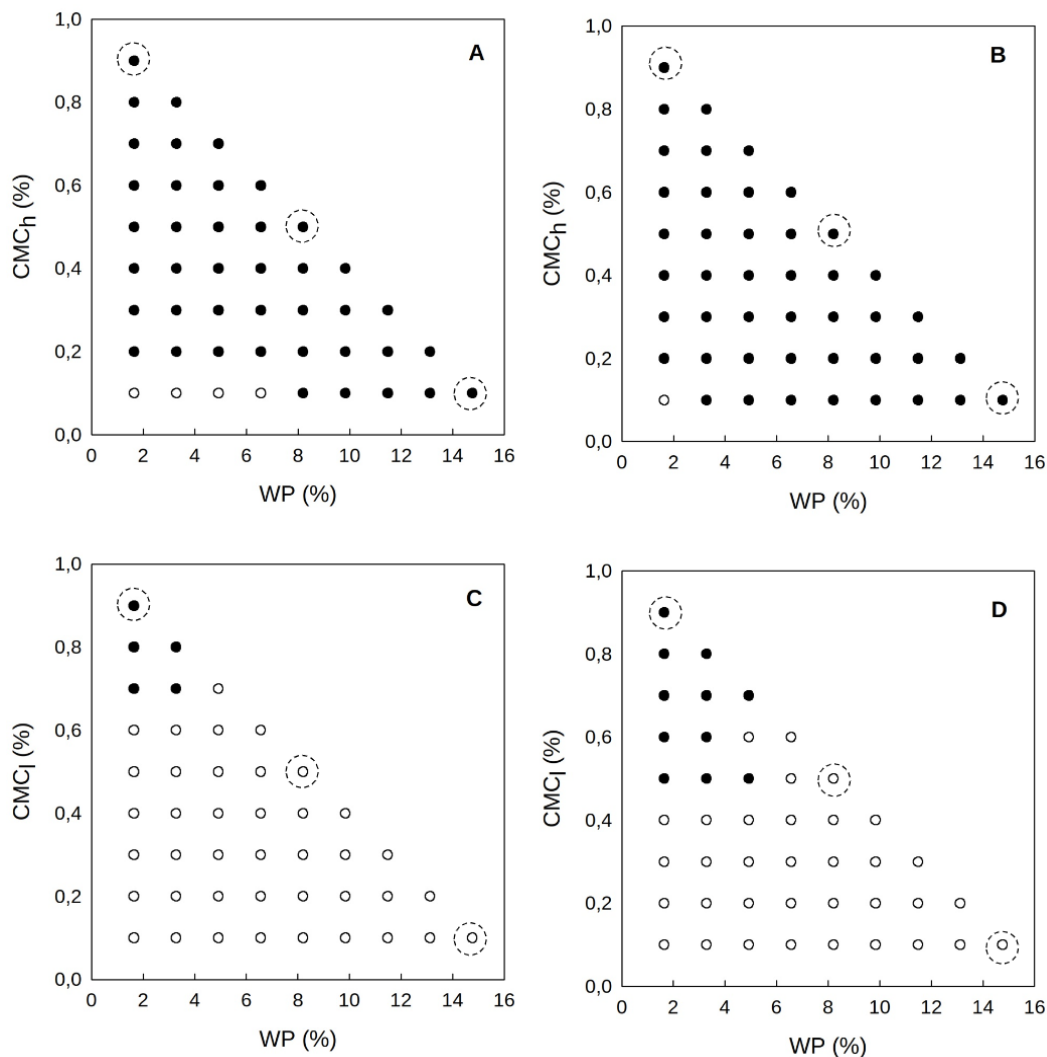


Figure 3. Bulk phase separation in aqueous mixtures of WP and CMC_h (A and B) or CMC_i (C and D), as determined by visual inspection after 24 h (A and C) or 72 h (B and D) of incubation

Black symbols represent phase-separated systems, whereas white symbols represent homogeneous systems. Dashed circles indicate the systems that were selected for further study.

To further deepen the understanding of these systems, a selection of them was studied in greater detail, as indicated by the dashed circles in **Figure 3**. One of these systems is characterized by having the highest WP-to-CMC ratio ($R = 14.76/0.1 = 174.6$), another by having the lowest ($R = 1.64/0.9 = 1.82$), and an additional one with an intermediate ratio ($R = 8.2/0.5 = 16.4$) was also selected.

Confocal laser scanning microscopy (CLSM) was employed to elucidate the microstructure of the WP-CMC aqueous samples, as shown in **Figure 4**. WP are stained with Rhodamine B and appear as bright areas in the micrographs.

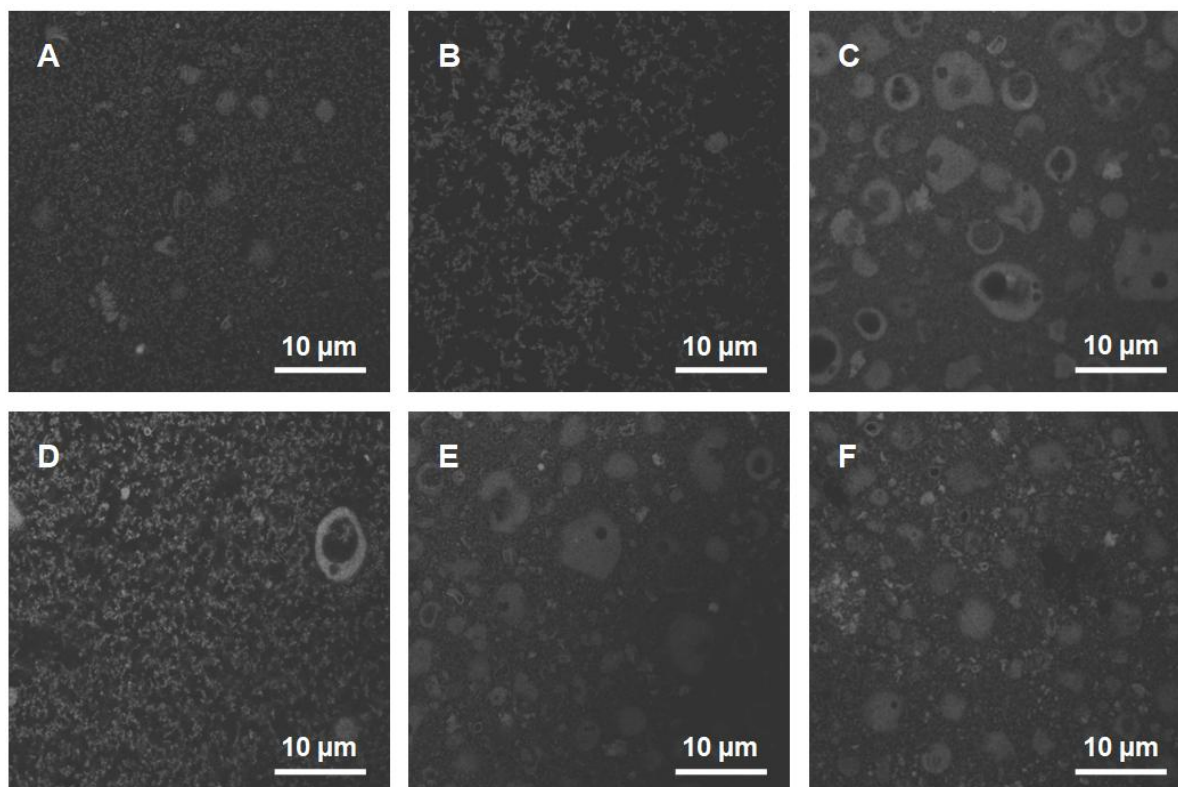


Figure 4. CLSM micrographs for aqueous mixtures of WP and CMC_h (A, B and C) or WP and CMC_l (D, E and F), as determined after 24 h of incubation

WP:CMC ratio @ equals 174.6 (A and D), 16.4 (B and E), or 1.82 (C and F). Clear areas correspond to fluorescence of labelled proteins with Rhodamine B

3.4 Rheological determinations

Further insights into the phase separation phenomenon were gained by measuring the apparent shear viscosity (**Figure 5A**). All biopolymer solutions behaved as pseudoplastic fluids within the shear rate range of 0.1-100 s⁻¹, where the apparent shear viscosity decreased with increasing shear rate. Rheograms were generated for dispersions by varying WP:CMC ratio, CMC type, and incubation time. To clarify the effects of these variables, the apparent shear viscosity at a fixed shear rate (100 s⁻¹) is presented in **Table 1**.

The initial apparent viscosity of the system containing 4.76% WP - 0.1% CMC ($R = 147.6$) is similar for systems with either CMC_l or CMC_h. However, as incubation time increases, the effect differs: the system with CMC_h shows an increase in viscosity over time, indicating greater structural rearrangements. In contrast, systems with lower WP concentrations display a decrease in viscosity over incubation time, suggesting that structural changes occur, weakening inter- and intramolecular interactions or reducing their extent.

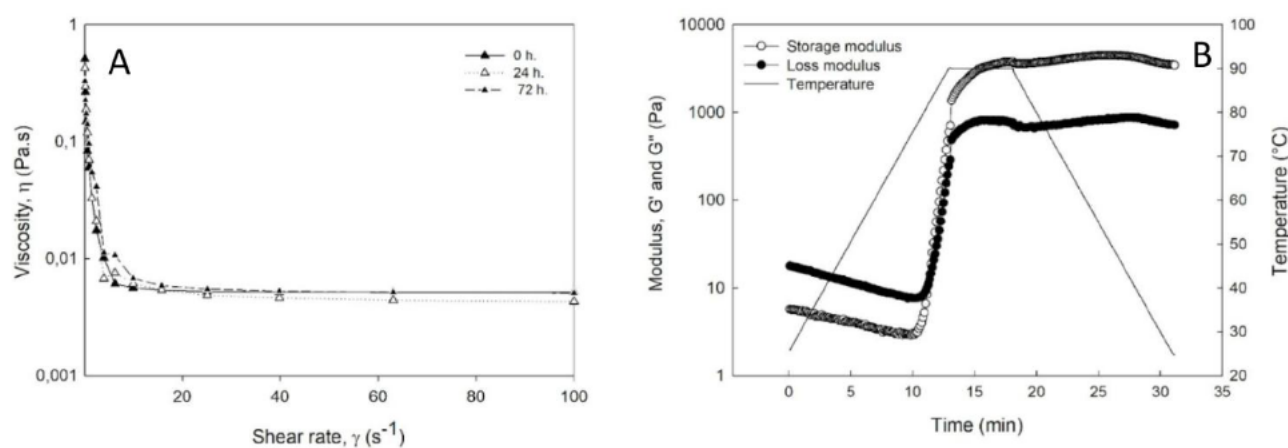


Figure 5. (A) Dynamic flow behavior at different incubation times. (B) Storage (G') and loss (G'') moduli as a function of time during the temperature ramp. Both graphs correspond to the system composed of 14.76% WP - 0.1% CMC_i ($R = 147.6$)

Table 1. Apparent shear viscosity (mPa.s) at 100 s⁻¹ of different WP-CMC aqueous systems incubated up to 72 h

Type	WP:CMC	0 h incubation	24 h incubation	72 h incubation
CMC _h	174.6	5.1 ± 0.1 ^{b, A}	6.3 ± 0.1 ^{d, B}	7.1 ± 0.1 ^{d, C}
	16.4	21.6 ± 0.8 ^{d, C}	14.9 ± 0.5 ^{e, B}	10.3 ± 0.3 ^{e, A}
	1.82	30 ± 1 ^{e, C}	28 ± 1 ^{f, B}	17.0 ± 0.7 ^{f, A}
CMC _i	174.6	5.14 ± 0.02 ^{b, B}	4.29 ± 0.08 ^{c, A}	5.10 ± 0.05 ^{c, B}
	16.4	5.60 ± 0.06 ^{c, B}	3.08 ± 0.09 ^{b, A}	3.15 ± 0.02 ^{b, A}
	1.82	2.52 ± 0.02 ^{a, B}	1.8 ± 0.1 ^{a, A}	2.80 ± 0.07 ^{a, C}

Note: Values are expressed as mean ± standard deviation. Uppercase letters indicate significant differences among incubation time for the same WP:CMC ratio ($p < 0.05$). Lowercase letters indicate significant differences among WP:CMC ratios within the same incubation time ($p < 0.05$).

The process of gelation during heating, as indicated by changes in the storage modulus (G') while heating (at 5 °C/min) and holding at 90 °C for 5 minutes, is shown in **Figure 5B**. The development of the storage modulus follows the typical aggregation/gelation behavior of globular proteins. An increase in the elastic modulus relative to the loss modulus during heating signals the formation of a gel network, which was observed near 90 °C for all gels. Elasticity continued to increase throughout the heating and holding phases, reflecting a gradual strengthening of the gel network due to the incorporation of additional molecules or changes within the network structure. Upon cooling, G' increased further, driven by the formation of additional non-covalent interactions among the denatured proteins.

The results from the gelation analysis of the WP systems containing CMC_h or CMC_i (**Table 2**) show that the WP concentration significantly affects the thermal and mechanical properties of the resulting gels. At higher WP concentrations, gelation is reached before, and higher storage and loss moduli were observed, indicating stiffer and more resistant gels. Conversely, at lower WP concentrations, the gelation time increased, and the modulus values decreased, suggesting that these gels are weaker. A notably higher G_r value was found in gels with higher WP concentrations, with significant differences observed compared to the other samples. This suggests that attractive forces, such as van der Waals interactions and hydrogen bonding, were more prominent in systems with higher WP concentrations, leading to the formation of stronger gels.

Table 2. Thermal gelation parameters

Type	WP:CMC	Gelation temperature (T_{gel} , °C)	Storage modulus at T_{gel} (G' , Pa)	Gel reinforcement (G_r , Pa)
CMC _h	174.6	83.4 ± 0.5 ^{a, A}	9 ± 2 ^{a, A}	3021 ± 4 ^{c, A}
	16.4	89.8 ± 0.4 ^{b, A}	3.1 ± 0.3 ^{a, A}	398 ± 5 ^{b, B}
	1.82	90.01 ± 0.01 ^{b, A}	5.7 ± 0.2 ^{a, A}	28.8 ± 0.4 ^{a, A}
CMC _i	174.6	84.6 ± 0.5 ^{a, B}	14 ± 3 ^{b, B}	3431 ± 5 ^{c, B}
	16.4	90.04 ± 0.09 ^{c, A}	7 ± 1 ^{a, A}	259 ± 3 ^{b, A}
	1.82	89.1 ± 0.7 ^{b, A}	5.0 ± 0.5 ^{a, A}	13.8 ± 0.6 ^{a, A}

Note: Values are expressed as mean ± standard deviation. Uppercase letters indicate significant differences between CMC_h and CMC_i for the same WP:CMC ratio ($p < 0.05$). Lowercase letters indicate significant differences among WP:CMC ratios within the same type of CMC ($p < 0.05$).

4. Discussion

The CMC_h solution exhibited a much higher viscosity than both WP and CMC_i, suggesting CMC_h forms more entangled and larger macromolecular networks in aqueous solution. This behavior influences the overall flow properties of WP-CMC mixtures, as viscosity differences between the components affect their phase separation tendencies and stability. The CMC_h solution demonstrated pseudoplastic behavior, which aligns with existing literature for this shear rate range⁽²¹⁾. In contrast, both WP and CMC_i showed Newtonian flow under the tested conditions, as previously reported⁽²²⁾⁽²³⁾.

The ζ -potential measurements across a pH range from 6 to 2 provided critical information about the charge properties of WP and CMC. WP displayed a negative ζ -potential above pH 4 and positive values below this pH, consistent with the isoelectric points of its major protein components (β -lactoglobulin and α -lactalbumin). Both CMC types maintained a negative ζ -potential over the entire pH range, though CMC_h had a greater magnitude of charge than CMC_i, in agreement with Daubert and others⁽²⁴⁾, who found a direct relationship between ζ -potential and colloidal diameter in particles with the same chemical composition. This difference is crucial for understanding the interactions between these biopolymers, as the higher magnitude of negative charge of CMC_h could lead to stronger electrostatic repulsion between WP and CMC, promoting segregative phase separation. In contrast, the weaker charge on CMC_i may favor more subtle interactions, potentially affecting the extent of phase separation or even leading to partial associative interactions under specific conditions.

When WP and CMC were combined, the ζ -potential of the mixed system was intermediate between the values of the individual components, indicating a partial interaction between WP and CMC. This is consistent with the findings of Huan and others⁽¹⁷⁾, who reported similar trends. This intermediate ζ -potential suggests that, at the assayed diluted conditions, WP and CMC may exhibit weak attractive interactions, suggesting some degree of association. As was previously reported, the electrostatic interaction between WP and CMC gives place to soluble WP-CMC complexes in acid media, since they carry opposite electrical charges. Although at isoionic pH each isolated macromolecule possesses net negative charge, it has been proposed that the charge regulation phenomenon allows the association of WP with CMC⁽²⁵⁾. The negative ζ -potential of the soluble complexes resulted in higher magnitude for WP-CMC_h than for WP-CMC_i, as was previously observed for heated WP-CMC complexes at neutral pH⁽¹⁷⁾.

According to the Flory–Huggins theory, the segregative phase separation appears when the interaction parameter between WP and CMC (the biopolymers composing the system) is positive, indicating a net repulsion between them⁽²⁶⁾. In this study, the pH of the systems was 6, and, according to the ζ -potential values, negative for both biopolymers, it can be assumed that the interaction parameter is always positive. Notably, CMC_h ex-

tends the phase separation region compared to CMC_l , meaning that higher concentrations of CMC_l are required to induce phase separation in WP-CMC aqueous systems, while much lower concentrations of CMC_h are sufficient to trigger segregation. For phase separation to occur, the enthalpic advantage of segregation must outweigh the entropic advantage of mixing. The higher the molecular weight of CMC, the lower the entropy of mixing; thereby promoting phase separation. Additionally, longer incubation times resulted in a greater number of systems exhibiting phase separation. It is well established that phase separation in protein-polysaccharide systems is a kinetic process driven by local fluctuations in biopolymer concentrations⁽²⁷⁾. Although not all the systems present macroscopic phase separation, the mixed systems exhibit microdomains containing WP and a continuous phase without WP. The spatial arrangement of WP in these systems seems to correspond to a CMC-continuous phase with WP aggregates dispersed there.

The shear-thinning behavior of the assayed systems can be attributed to the progressive disruption, disentanglement, and elongation of biopolymer molecules, aggregates, or particles at higher shear rates⁽²⁸⁾. As the shear rate increases sufficiently to overcome Brownian motion, the particles become more aligned in the flow direction. Consequently, the flow resistance of the biopolymer dispersions decreases, leading to a reduction in shear viscosity. The increase in viscosity over incubation time in systems with high WP concentrations and CMC_h can be attributed to the formation of denser and more resilient viscoelastic networks, driven by stronger WP-WP and WP-CMC interactions. In other cases, where WP concentrations are lower, the interactions are insufficient to maintain a stable structure, and phase separation may lead to a redistribution that reduces viscosity⁽²⁹⁾.

The gelation experiments provided further insights into the thermal and mechanical properties of the WP-CMC mixtures. As expected, the gels formed at higher WP concentrations were stronger, with lower gelation time and higher storage and loss moduli, indicating stiffer networks. The observed increase in the elastic modulus during heating and cooling phases reflects the progressive formation and reinforcement of the gel network. Systems with higher WP concentrations exhibited significantly higher gel reinforcement (Gr), suggesting that stronger intermolecular interactions, such as van der Waals forces and hydrogen bonds, were responsible for the formation of more robust gels⁽³⁰⁾.

5. Conclusions

The rheological and phase separation behavior of WP and CMC aqueous systems, both high and low viscosity, were evaluated under isoionic conditions. The results demonstrated that systems with higher WP concentrations and CMC_h exhibited increased viscosity over time due to the formation of denser, viscoelastic networks facilitated by stronger protein-protein and protein-CMC interactions. Conversely, systems with lower WP concentrations or those containing CMC_l showed a decrease in viscosity, indicating weaker structures.

Phase separation was induced in systems with lower total biopolymer concentrations containing CMC_h compared to CMC_l . This is attributed to the lower entropy of mixing in the case of CMC_h , which promotes segregation. Incubation time also played a critical role in extending the phase separation region, further demonstrating the kinetic nature of the process in protein-polysaccharide systems.

Rheological measurements highlighted the pseudoplastic behavior of all biopolymer solutions, with shear-thinning characteristics driven by biopolymer alignment at higher shear rates. Systems with higher WP concentrations exhibited stronger thermal gelation properties, forming stiffer, more resistant gels with lower gelation times and higher storage moduli. This suggests that, during heat treatment, attractive interactions, such as van der Waals forces and hydrogen bonding, were more prevalent in systems with higher protein content, leading to enhanced gel strength and stability.

Acknowledgements

This work was supported by grants from CONICET (PIP 2022-2024 GI 11220210100490CO) and UNR (80020210200112UR).

Transparency of data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

SB: Formal analysis; Investigation; Methodology; Visualization; Writing – original draft

MNES: Investigation

AGGC: Investigation

PBT: Formal analysis; Validation; Writing – review & editing

CFN: Data curation; Funding acquisition; Supervision; Writing – review & editing

VB: Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Writing – review & editing

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