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ORIGINAL RESEARCH PAPER

SODIUM CASEINATE OR SOY PROTEIN ISOLATE AND GELLAN GUM COMPOSITE EMULGELS INDUCED BY ACIDIFICATION: TEXTURAL CHARACTERIZATION

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Abstract

The textural characterization via compression, penetration, and extrusion of acid-induced emulgels was determined. Emulsions of sodium caseinate or soy protein isolate mixed with gellan gum in different proportions were acid-gelled with glucono-lactone. In the formulated emulgels, when the proportion of protein was higher, the samples were less cohesive or easy to compress (0.53 N and 9.26 for 100 and 0 sodium caseinate protein proportion, respectively), softer or easy to penetrate (5.13 N and 14.96 for 100 and 0 sodium caseinate protein proportion, respectively), and more extrudable (5.26 N and 36.20 for 100 and 0 sodium caseinate protein proportion, respectively). This was because the final pH of the emulgel was below both proteins' isoelectric point. As the gellan gum proportion increased, the emulgel became stiffer, since the negatively charged carboxyl groups of gellan gum were dissociated, retaining more water in the continuous phase of the emulgel. Since the oil phase was constant, the texture of acid-induced emulgel can be manipulated according to the protein/gellan gum ratio, depending on the application to replace saturated fat, focused on fermented dairy products as yogurt.

Keywords: emulgel, acid-induced gelation, sodium caseinate, soy protein isolate, gellan gum, texture

Introduction

Emulsification and gelling are the primary techno-functional properties of food proteins that contribute to the development of the characteristic texture, flavor, and yield of processed foods. In one hand, food proteins form an interfacial protein film to decrease the surface tension of dispersed oil droplets to stabilize emulsions (Damodaran, 2006). On the other hand, Food proteins can form a three-dimensional

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network when a denaturant agent provokes a sufficient degree of structural unfolding to achieve a different molecular rearrangement, resulting in an ordered protein matrix (Totosaus *et al*., 2002). When emulsified lipid droplets are entrapped within the gel matrix, in a sort of emulsion-filled gel, an emulgel is formed (Lu *et al*., 2019). Notwithstanding, in contrast with proteins, the role of polysaccharides in food emulsions is as a stabilizer agent, since their hygroscopic capacity can stabilize the emulsion by increasing the continuous phase viscosity (Dickinson, 2002). However, protein and polysaccharides can form complexes as a new type of stabilizer, more efficient than the individual components, where proteins are the emulsifier, and polysaccharides avoid coalescence and flocculation of oil droplets (Chassenieux and Nicolai, 2023). Emulgels could have applications in fat-reduced foods, owing to their adjustable textural properties and the reduced lipolysis rate of fat, due to the thicker interface formed by protein-polysaccharide composites (Lu *et al*., 2019).

As a denaturant agent, the most commonly employed acidulant to obtain acid-induced gels is glucono-γ-lactone (GDL). GDL hydrolysis progressively decreases the pH of the solution, approaching the proteins' isoelectric point, which leads to protein aggregation and gel formation. In this process predominate non-covalent interactions to gel network formation (hydrogen bonding, hydrophobic interactions, and van der Waals forces) (Grygorczyk and Corredig, 2013). At these acidic conditions, the association of proteins and polysaccharides is driven by electrostatic interactions, though hydrophilic and hydrophobic interactions play certain roles as well (Chassenieux and Nicolai, 2023).

Some protein/polysaccharide composites in model systems have been studied to determine their techno-functional properties, employing sodium caseinate or soy protein isolate with gellan gum. It has been reported that gellan gum enhances the emulsion properties (Zhang *et al*., 2022) and improves the gelling process (Nishinari *et al*., 2000) in the interaction with proteins. For instance, soy proteins with gellan emulsions (Ma *et al*., 2025), soy proteins acid-gels (Puppo *et al*., 1995; Wan *et al*. 2020), acid-induced caseinate emulsions (Chen *et al*., 1999), caseinate and gellan gum emulsions (Sosa-Herrera *et al*., 2008), caseinate and gellan acid-gels (Li *et al*., 2021a; 2021b), milk proteins with gellan acid gels (Picone and Cunha, 2010), and acid-induced gellan gum gels (Horinaka *et al*., 2004; Yamamoto and Cunha, 2007).

The objective of this work was to establish the effect of two commercial proteins, sodium caseinate and soy protein isolate, mixed with different proportions of gellan gum, on the textural properties of acid-induced emulgels, determined by compression, penetration and extrusion.

Materials and methods

Protein and gellan gum acid-induced emulgels

The method reported by Li *et al*. (2021b) for glucono-δ-lactone induced alginate sodium/casein composite gels was adapted, employing gellan gum as a polysaccharide. A stock solution of sodium caseinate (FABPSA, Mexico City) was prepared by dissolving 6% (w/v) in 100 mL of distilled water with magnetic stirring

at a temperature below 50 °C for one hour until complete incorporation. The solution was cooled down and kept in refrigeration.

The stock soy protein isolate solution was prepared by dispersing 9% of soy protein isolate (Food Technologies Trading, Mexico City) in 100 mL of distilled water, heating to 80 °C for 30 min to achieve complete hydration with constant agitation. The solution was cooled down and kept in refrigeration (Ma *et al*., 2025).

The gellan gum stock solution was prepared by dispersing 1 % (w/v) of gellan gum (Jr Food, Mexico City) in 100 mL of distilled water, heating at 70 °C for 3 hours until complete hydration (water was added to compensate for evaporation). The solution was cooled down and kept in refrigeration (Yamamoto and Cunha, 2007).

The acid-induced emulgels were prepared by mixing different proportions of the protein stock and gellan gum stock solutions, at 100/0, 75/25, 50/50, 75/25, 0:100 (protein/gellan, v/v). 100 mL of emulsion was prepared by homogenizing 30 mL of edible oil in 70 mL of the different protein/gellan solutions, employing an Oster mixer (Sunbeam Mexicana, Mexico City) at ca. 12,000 rpm, for 2 min. Immediately, 2% of glucono-δ-lactone (Sigma-Aldrich, St Louis) was added and homogenized again for 2 more minutes. The different emulsions were poured into cellulose casings and stored at 4 °C overnight to allow gel formation.

Textural characterization

Compression test

The emulgels of the different treatments for both proteins were cut into 20 mm length cylindrical (20 mm diameter) samples and compressed 10 mm with a 50 mm diameter acrylic probe at a constant speed of 1 mm/s in the same texturometer. From the force-deformation curves, compression force (maximum peak force) and compression work (integral of the area under the curve) were calculated (Fig. 1a). Results are the average of at least 5 reproducible samples of two different batches.

Penetration test

The emulgels of the different treatments for both proteins were cut into 20 mm length cylindrical (20 mm diameter) samples and penetrated 10 mm with a 10 mm diameter acrylic probe at a constant speed of 1 mm/s in a Brookfield LFRA 4500 texturometer (Brookfield Engineering, Middleboro). From the force-deformation curves, penetration force (maximum peak force) and penetration work (integral of the area under the curve) were calculated (Fig. 1b). Results are the average of at least 5 reproducible samples of two different batches.

Extrusion test

For the extrusion test, 20 g of the different treatments for both proteins were cut into small pieces (0.5-0.7 mm long by 3-5 mm wide) and placed in the Ottawa cell (TA-OC additament, Brookfield Engineering, Middleboro) adapted to the same texturometer extruding the sample through the base at a constant speed of 0.6 mm/s to 90% of original sample height (Lima & Singh, 1993). From the force-deformation curves, extrusion force (maximum peak force) and extrusion work (integral of the

area under the curve) were calculated (Fig. 1c). Results are the average of at least 5 reproducible samples of two different batches.

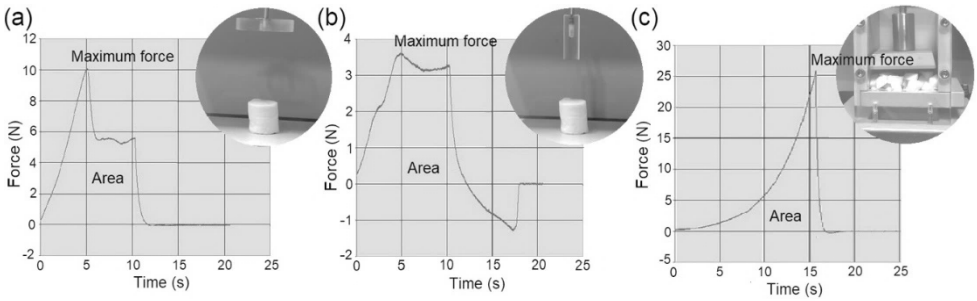

Figure 1. Maximum force and area under the curve of typical force-time deformation graphs for: (a) compression test, (b) penetration test, and (c) extrusion test.

pH monitoring

Changes in the different acid-induced emulgels due to GDL hydrolysis were observed with a PHS-3C potentiometer (Dpofirs, China). Mixtures of the different emulsions of sodium caseinate or soy protein isolate/gellan gum solutions (100/0, 50/50, and 0:100, protein/gellan, v/v) were prepared as previously described, measuring changes in pH before GDL addition, immediately GDL addition, and during 12 h.

Phase contrast microscopy

The microstructure of the acid-induced emulgels was observed by taking a sample of each treatment during the acidification period (monitored by measuring the pH of the mixture). Samples were carefully placed in between the microscope slide and the cover slip for optical observation with a contrast phase microscope Velab VE-B10 (Velab, Texas) at 20X magnification. The microscopy images were captured with a 64 MP cell phone digital camera adapted to the microscope ocular. Brightness and contrast of the most representative images were performed in Microsoft Power Point software (Bastos *et al*., 2016).

Experimental design and data analysis

Data analysis was performed by an analysis of variance using the R studio® platform (https://www.rstudio.com/), and the significant difference ($P<0.05$) between means was determined by Tukey's honestly significant difference in the same platform.

Results and discussion

All gels were self-supporting, indicating the development of a gel structure. With the different texture tests employed in this research, the whole emulgel structure characterization was determined, affected by the protein/gellan proportion, after the acid-induced gelation of the emulsified mixtures.

In the compression force, a softer texture was observed in mixtures with higher protein proportion, since these acid-induced emulgels presented significant (P<0.05) lower compression force values. Sodium caseinate acid-induced emulgels resulted in significant (P<0.05) harder texture The same tendency was observed for the compression work (energy required to compress), with significant (P<0.05) lower values for no gellan gum mixtures made with soy protein isolate (Table 1).

Table 1. Penetration test parameters determined in the different proportions of the protein/gellan gum composite acid-induce gels.

Mixture (protein/gellan gum)	Penetration force (N)		Penetration work (N s)	
	Sodium caseinate	Soy protein isolate	Sodium caseinate	Soy protein isolate
100/0	0.53±0.1 $^{d, A}$	0.86±0.1 $^{d, B}$	3.66±1.4 $^{c, A}$	6.00±0.4 $^{c, B}$
75/25	1.63±0.1 $^{d, A}$	1.30±0.0 $^{d, B}$	10.34±1.8 $^{c, A}$	8.59±0.3 $^{c, B}$
50/50	3.40±0.1 $^{c, A}$	2.40±0.0 $^{c, B}$	19.98±4.2 $^{c, A}$	16.50±0.9 $^{c, B}$
25/75	6.23±0.3 $^{b, A}$	4.10±0.4 $^{b, B}$	41.88±4.8 $^{b, A}$	29.20±4.0 $^{b, B}$
0/100	9.26±0.8 $^{a, A}$	8.20±1.0 $^{a, B}$	61.28±5.3 $^{a, A}$	39.54±2.3 $^{a, B}$

a, b, c, d… Means with the same letter across the column are not significantly different (P>0.05) for the mixtures.

A, B… Means with the same letter across the row are not significantly different (P>0.05) for the type of protein.

The penetration test parameters are listed in Table 2. The mixtures containing 25/75 of protein/gellan gum ratio presented significant (P<0.05) higher penetration force values. Sodium caseinate acid-induced emulgels presented significant (P<0.05) higher force values. For the penetration force, a similar pattern was observed, where higher gellan gum proportions resulted in significant (P<0.05) firmer acid emulgels, being more difficult to penetrate. Mixtures with sodium caseinate presented significant (P<0.05) higher penetration work values.

Table 2. Compression test parameters determined in the different proportions of the protein/gellan gum composite acid-induce gels.

Mixture (protein/gellan gum)	Compression force (N)		Compression work (N s)	
	Sodium caseinate	Soy protein isolate	Sodium caseinate	Soy protein isolate
100/0	5.13±0.1 $^{e, A}$	2.00±0.1 $^{e, B}$	24.47±1.9 $^{e, A}$	16.39±0.7 $^{e, B}$
75/25	9.73±2.0 $^{d, A}$	2.13±0.1 $^{d, B}$	54.47±5.7 $^{d, A}$	16.60±0.4 $^{d, B}$
50/50	10.67±0.6 $^{c, A}$	5.20±0.0 $^{c, B}$	62.07±5.4 $^{c, A}$	35.08±0.3 $^{c, B}$
25/75	24.00±1.0 $^{a, A}$	11.63±0.1 $^{a, B}$	130.87±6 $^{a, A}$	70.06±4.3 $^{a, B}$
0/100	14.96±1.6 $^{b, A}$	10.90±0.6 $^{b, B}$	74.63±7.5 $^{b, A}$	51.54±2.9 $^{b, B}$

a, b, c, d… Means with the same letter across the column are not significantly (P>0.05) for the different for the mixtures.

A, B… Means with the same letter across the row are not significantly (P>0.05) for the different for the type of protein.

The acid-induced emulgels formulated 25/75 of protein/gellan gum proportions presented significant (P<0.05) higher extrusion force values. Sodium caseinate resulted in significant (P<0.05) higher extrusion force values. For the extrusion work, the same trend was observed, with significant (P<0.05) higher extrusion force values at higher gellan gum concentration. Sodium caseinate mixtures presented significant (P<0.05) higher extrusion force values than soy protein isolate mixtures (Table 3).

Table 3. Extrusion test parameters determined in the different proportions of the protein/gellan gum composite acid-induce gels.

Mixture (protein/gellan gum)	**Extrusion force (N)**		**Extrusion work (N s)**	
	Sodium caseinate	Soy protein isolate	Sodium caseinate	Soy protein isolate
100/0	5.26±0.4 $^{d, A}$	10.88±2.4 $^{d, B}$	36.20±9.8 $^{d, A}$	69.47±7 $^{d, B}$
75/25	16.67±0.6 $^{c, A}$	14.53±3.1 $^{c, B}$	148.88±6.1 $^{c, A}$	69.56±4 $^{c, B}$
50/50	26.23±2.1 $^{b, A}$	26.97±3.0 $^{b, B}$	202.30±0.9 $^{b, A}$	156.03±8 $^{b, B}$
25/75	33.37±2.4 $^{a, A}$	30.77±6.7 $^{a, B}$	280.40±0.6 $^{a, A}$	195.64±3 $^{a, B}$
0/100	37.63±1.4 $^{ab, A}$	24.63±1.2 ^{ab,B}	302.34±4 $^{ab, A}$	141.18±6 $^{ab, B}$

a, b, c, d… Means with the same letter across the column are not significantly (P>0.05) for the different for the mixtures.

A, B… Means with the same letter across the row are not significantly (P>0.05) for the different for the type of protein.

During the compression test, the whole sample is under compressive, frictional, and tensile stresses, where the entire specimen is subjected to compression load, reflecting the overall failure response to compression, and this resistance to failure during compression is related to cohesiveness (Lee and Chung, 1989). In this context, it seems that as the acid-induced emulgel is formed, both proteins are close to their respective isoelectric point, affecting the gel matrix, resulting in a less cohesive texture, easier to compress. In the penetration test, only the area of the probe cross-section is subjected to penetration, affected by the density and uniformity of the sample matrix, and this resistance to penetration thus measures the degree of compactness or density of the sample, *i.e*., hardness (Lee and Chung, 1989). In respect to compression, the gelling capacity of gellan gum at acidic conditions seems to be responsible of emulgel hardness when protein proportion decreased. Apropos of the extrusion test, the applied force to the sample makes it flow through the outlet cavities in the Ottawa cell, where a combination of shearing, extrusion, and adhesion can occur simultaneously (Bourne, 1982), and hence the less cohesive (easy to compress) and less hard (easy to penetrate) emulgels with higher proportion of sodium caseinate or soy protein isolate presented less resistance to pass through the cell openings.

The acid-induced gelation of the formulated emulsion seems to be dominated by the gellan gum presence, since higher proportions of gellan gum resulted in stronger and more ductile structures. Li *et al*. (2021a) reported that when a polysaccharide

(alginate) was added to a casein solution, the emulgel hardness increased, forming a continuous biphasic composite system of both protein and polysaccharide. In the same manner, increasing the concentration of gellan gum augmented gel strength in glucono-lactone-induced casein gels (Li *et al*., 2021b). During emulgel formation, the polysaccharide did not affect the aqueous phase structure or the gelation process, affecting instead the interfacial composition and structures due to oil droplets distribution (Lu *et al*., 2019).

The change of pH due to gluconolactone hydrolysis during acid-gel formation for the different proportions of proteins (sodium caseinate, CAS, or soy protein isolate, SPI) and the corresponding micrography are depicted in Fig. 2. In the acid-emulgel samples with only protein (100/0 protein/gellan gum), the pH drop was gradual, reaching the lowest values after 12 h., with close values between both proteins employed, although lightly higher in sodium caseinate samples. In micrographs, a granular texture (dispersed oil) can be appreciated, as a consequence of the dispersion of the oil into the protein solution. As the pH falls from 6.7-6.8 to 5.2, the coarse texture seems to be compacted, due to the dispersion phase acidification that resulted in the acid gelation of the protein, which does not participate in the interfacial protein film formation (Figure 2a). When the protein and gellan gum proportion was the same (50/50), the pH drop was markedly faster, with lower pH for both proteins after 12 h. The emulgel structure presented noticeable changes, since bigger and translucent globules were observed, with no perceptible changes in emulsion structure (Figure 2b). In the no-protein samples, only gellan gum emulsion, the pH decreased faster in a shorter time, reaching values below 4 within the first 10 min. The emulsion structure presented bigger fat globules, which seem to coalesce during this time (Figure 2c).

The changes in pH gradual decrease due to the incorporation of glucono lactone are due to the presence of proteins in the formulated emulgels. Food proteins have buffering capacity, and protein content impacts on physicochemical and enzymatic reactions during digestion (Mennah-Govela *et al*., 2020). Proton binding of proteins accounts for the buffer capacity (Olthuis *et al*., 1994). Purified caseins exhibit a maximal buffering capacity in the range of approximately pH 5.0-5.5, as some ionizable groups become accessible after a pH change and protein denaturation (Salaün *et al*., 2004). Buffering capacity of soybean whey proteins has also been reported (Zhang *et al*., 2024). In this view, with the diminution of the protein proportion in the composite, the buffer capacity of the samples was limited, allowing a faster pH decline, reducing the time to reach the isoelectric point of proteins. In consideration of this, sodium caseinate, as a milk protein, presented a slight buffer capacity, reflected in the higher pH values after GDL addition during the gelling process.

In the acid-induced emulgels, as the pH decreased, gellan gum dominates the texture due to the proximity of the gum side chains that enhance junction zones aggregation, as an anionic polyelectrolyte (Picone and Cunha, 2010), in addition to the aggregation via hydrogen bonds of gellan gum double helices at lower pH that resulted in conformational changes due to pH, resulting in electrostatic repulsion and

shield effect (Yamamoto and Cunha, 2007). The pH lowering decreased the gellan gum density charge due carboxyl groups dissociation (Horinaka *et al*., 2004).

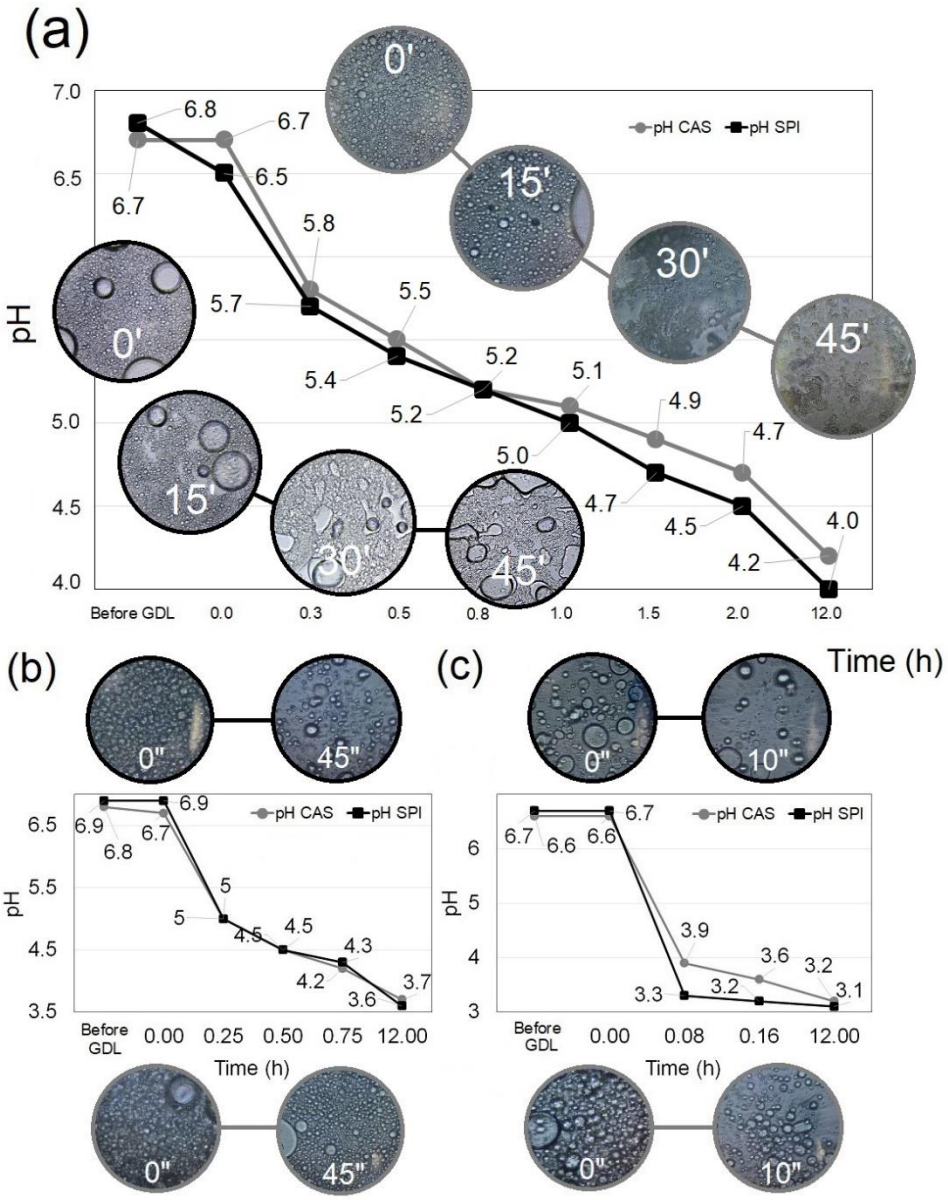

Figure 2. Changes in pH during the acid-induced gel formation in the different proportions of the protein/gellan gum composite acid-induce gels, and phase contrast micrography (at 20X) for protein/gellan gum proportions: (a) 100/0, (b) 50/50, and (c) 0/100 (CAS: sodium caseinate, SPI: soy protein isolate).

The increase in polysaccharide concentration resulted in more solid particles adsorbed and attached to the oil-water interface, forming a denser structure (Ma *et al*., 2025), and since the oil droplets remain in the protein-rich phase, the rigidity of this phase increases (Li *et al*., 2021b), these two phenomena results in a denser three-dimensional network formation related to higher gel strength. Under certain conditions, gellan gum is not only a thickening compound but also interacts with sodium caseinate to form a complex structure to increase gel strength (Nag *et al*., 2010; Picone and Cunha, 2010). Anionic polysaccharides generated abundant carbonyl and carboxyl groups, contributing to pH decrease, besides during the gradual release of H^+ from GDL during gel induction, and at higher polysaccharide concentrations, the electrostatic interactions between positively charged protein groups (below or close to the isoelectric point) and negatively charged carboxyl groups in polysaccharide reduce loss of water (Li *et al*., 2025).

For the sodium caseinate with gellan gum, as the pH decreased, a coacervation between proteins and gellan gum was not possible since, for example, at pH 5.4 both proteins and gellan gum are negatively charged (Sosa-Herrera *et al*., 2008). This is because, for caseins, at pH close to 5.1, there is an electrostatic repulsion with gellan gum, and even at lower pH values (ca. 4.3) the intermolecular attraction between the gellan and the caseins exists (Li *et al*., 2021b). At pH close to their isoelectric point, close to 4.6, the casein aggregates are formed (Wang *et al*., 2016). In caseinate acid-induced emulgels, the increase in viscosity is due to two structural components: protein emulsified oil droplets and casein acid-aggregates (Chen *et al*., 1999). Similarly, for soy protein isolates, at pH close to the soy proteins' isoelectric point (4.5), there is a higher protein aggregation that gradually neutralizes the surface charge (Puppo *et al*., 1995), forming cross-linking aggregates in the acid gel network (Wan *et al*., 2020).

Consequently, tailoring techno-functional and hence textural properties of protein-polysaccharide composite gels induced by acidification by adjusting coagulants concentration (like GDL) or polysaccharide proportions enable the gel characteristics diversification, allowing the control of products' texture and nutritional value to develop of innovative food products such as low-calorie and/or improved nutritional value (Li *et al*., 2025).

Conclusions

The development of acid-induced emulgels employing sodium caseinate or soy protein isolate, both with an isoelectric point close to 4.5, mixed in different proportions with an anionic polysaccharide as gellan gum, allows, firstly, to stabilize the dispersed oil phase before pH decline by the formation of the interfacial protein film; and, secondly, the increase of the disperse phase viscosity by gellan gum hydration. Since the oil phase was constant, the texture of acid-induced emulgel can be manipulated according to the protein/gellan gum ratio, depending on the application to replace saturated fat, focused on fermented dairy products as yogurt.

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