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EVALUATION OF THE EFFECT OF OIL-FRACTION TYPE (OIL OR OLEOGEL) ON THE TEXTURAL PROPERTIES OF PROTEINS AND GELLAN GUM ACID-INDUCED EMULGELS BY A MIXTURE DESIGN APPROACH

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Abstract: *The textural properties (penetration, compression, and extrusion) of acid-induced emulgels composed of sodium caseinate, soy protein isolate, and gellan gum were determined employing a mixture design approach. The type of oil-fraction effect, edible oil or candelilla wax oleogel, in emulgels was also determined. In general, emulgels with candelilla wax oleogel as oil fraction presented a softer, less cohesive, and less firm texture, resulting from the higher emulsion stability provoked by candelilla wax, requiring less protein to form the emulsion. The relatively higher protein available affected the gel structure formation since, in the acidic conditions in the systems, the formation of ductile protein aggregates was disadvantaged, since pH was close to their isoelectric point. Gellan gum dominated the acid-induced gel, with certain interaction with acid-altered proteins. The acid-induced gelation of composite emulsions offers the possibility to develop different textures employing different types of oil-fractions. These systems can be employed to replace fat in processed foods like yogurts.*

Key words: *Emulgel, acid-induced gelation, oleogel, texture, mixture design approach.*

1. Introduction

Emulgels are composite systems where a hydrocolloid or a mixture of hydrocolloids is employed to emulsify an oil phase with a hydrophobic active agent or drug, which is then gelled. In food systems, emulgels, or emulsion-filled gels, are emulsions generally stabilized by proteins. In the formulation of composite emulsion systems, two types of main components can be distinguished, the emulsifier agent

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and the stabilizer agent. The emulsifier agent is one the responsible to form the interfacial film that covers dispersed fat globules, and the stabilizers are agents that provide long-term stability via increasing the viscosity of the aqueous phase of the emulsion system. Proteins are the most employed emulsifying agents, whereas polysaccharides are known for their capacity to increase viscosity. Both phenomena enhance the emulsion stability [11]. The capacity of proteins as surfactants is related to their conformational arrangement at the emulsion's interface to reduce interfacial tension, in the formation of a cohesive interfacial protein film [9]. In contrast, polysaccharides with a predominant hydrophilic large molecular polysaccharide chain favouring the formation of a thicker stabilizing layer to protect oil droplets in emulsions against flocculation or coalescence [12]. The proteins' gelation can be defined as the aggregation of denatured protein molecules with a certain degree of order to form a continuous network, and the denaturation could be by physical (heat) or chemical (acidity) means [34]. In the acid gelation, the mechanism of gel formation is elucidated by the fractal aggregation theory, where the denatured protein particles aggregate in fractal clusters to build blocks of the gel [23]. These imply that in the emulgel, first, the oil droplets must be stabilized through the formation of the interfacial film, and second, there must be denatured conditions to allow the formation of the gel matrix. Acid-induced gelation is important in many foods, where the proteins are responsible for the particular texture, as in the case of soy proteins in tofu elaboration, or caseins in yogurt elaboration. The acid-induced gelation of protein gels involves two steps: first, the pre-heating of the native protein solution at a pH far from the isoelectric point to enhance protein aggregates, and second, the addition of a compound that reduces intermolecular repulsion to promote gel formation, as a coagulant, and glucono-δ-lactone neutralizing the charge on protein surface promoting proteins to form a gel [22]. The acid-induced gelation of proteins resulted in weak gels with liquid-like behaviour and required shorter times to form a soft gel [34]. This softer but self-supporting gel structure can be employed as fat replacer in dairy foods, since already emulsion-filled gels or emulgels can be employed as well to replace saturated fats in processed foods, with the concomitant caloric reduction and the increase of protein content, as in the case of baked goods, as muffins [35]. In addition, since the preparation of a thermostable emulgel made with gellan gum has also been reported [17], the combination of proteins at a pH below their isoelectric point (carrying positive superficial charge) with gellan gum as an anionic polysaccharide could result in different textures, due to the surfactant proteins activity, plus the thickener capacity of gellan gum. This can be determined by a mixture design approach, that is the most suitable statistical tool in food engineering to determine the interaction among components in the formulation of food composite ingredients as food replacers [1, 40].

The aim of this work was to compare the textural properties of acid-induced emulgels formulated with sodium caseinate, soy protein isolate, and gellan gum, employing two types of oil-phases: edible oil or candelilla wax oleogel, by a mixture design approach.

2. Materials and Methods

2.1. Protein and Gellan Gum Stock Solutions

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 1 hour until complete incorporation. 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 for complete hydration with constant agitation. 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, and adding water to compensate for the water evaporated. The solutions were cooled down at room temperature and kept in refrigeration until use.

2.2. Acid Induced Emulgels

First, mixtures of the different stock solutions were elaborated according to the Table 1 proportions, mixing for at least 30 min to ensure components dispersion, employing 0.30 of oil-fraction (ϕ= 0.30). Two different types of oil-fractions were employed, one of edible vegetable oil and one of candelilla wax oleogel. The oleogel was elaborated with 3% of candelilla wax plus 97% of edible vegetable oil, heating it to 95°C to dissolve wax with magnetic stirring, and cooled down at room temperature before use [33]. Second, the oil-phase was dispersed into different mixtures with an Ika T25 Ultra-turrax digital homogenizer (Ika Works, Staufen) for 2 minutes. To each emulsion 2% (w/v) of glucono-d-lactone (Sigma Aldrich, St Louis) was added and homogenized for two more minutes. Emulsions were placed into cellulose tubes to allow acid-gelation overnight.

2.3. Textural Characterization

2.3.1. 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.

2.3.2. 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.

2.3.3. Extrusion Test

For the extrusion test, 20 g of the sample of the different treatments for both proteins were cut in small pieces close to the size of a rice grain [19]. The sample was 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. From the force-deformation curves, extrusion force (maximum peak force) and extrusion work (integral of the area under the curve) were calculated.

Table 1

Stock solutions formulation in the three-component constrained simplex lattice mixture design in proportion (sum = 1.000) and concentration (%, w/v) for the emulgel elaboration

Run	Sodium caseinate		Soy protein isolate		Gellan gum	
	Proportion	%	Proportion	%	Proportion	%
1	1.000	6.00	0.000	0.00	0.000	0.00
2	0.000	0.00	1.000	9.00	0.000	0.00
3	0.000	0.00	0.000	0.00	1.000	1.00
4	0.333	2.00	0.333	3.00	0.333	0.33
5	0.500	3.00	0.500	4.50	0.000	0.00
6	0.000	0.00	0.500	4.50	0.500	0.50
7	0.500	3.00	0.000	0.00	0.500	0.50
8	0.667	4.00	0.167	1.50	0.167	0.17
9	0.167	1.00	0.667	6.00	0.167	0.17
10	0.167	1.00	0.167	1.50	0.667	0.67

2.4. Experimental Design and Data Analysis

A simple lattice design of three components where all the components have the same range, starting with the extreme vertices, centroid, and axial blends (10 points, runs) to estimate the polynomial Scheffe's quadratic equation regression (Eq. (1)):

$$\hat{y} = \sum_{i=1}^{q} \beta_i \cdot x_i + \sum_{i=1}^{q} \beta_i \cdot x_i^2 \qquad (1)$$

where:

$\hat{y}$ is the predicted response;
q – the number of components [3];
i – the i-component in the mixture;
β – the regression coefficient;
x – the proportion of the components.

The coordinate system for the mixture proportions is a simplex coordinate system (Eq. (2)), where each vertex relates to pure blends (sodium caseinate, soy protein isolate, or gellan gum), each side relates to binary mixtures, and interior points relate mixtures of the three components with the centroid point [25] (Table 1 and Figure 1).

$$\eta = \beta_1 \cdot x_1 + \beta_2 \cdot x_2 + \beta_3 \cdot x_3 + \beta_{12} \cdot x_{12} + \beta_{13} \cdot x_{13} + \beta_{23} \cdot x_{23} \qquad (2)$$

where:

η is a predictive dependent variable (textural properties) [N or N*s];
β_1, β_2, β_3, β_{12}, β_{13}, and β_{23} – the corresponding parameters estimates for each linear and cross-product term produced for the prediction models for sodium caseinate (β_1), soy protein isolate (β_2), and gellan gum (β_3).

Data was analysed with the Design Expert® Software version 13.0 (Stat-Ease, Inc., Minneapolis, Minnesota).

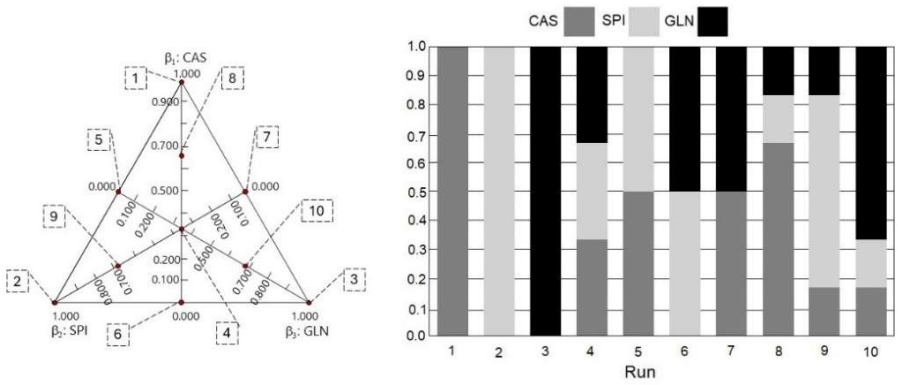

Fig. 1. *Simplex coordinate system for three components (left) and the respective proportions of each one (right)*

3. Results and Discussion

3.1. Penetration Test

For the acid-induced emulgels with oil as oil-fraction, the linear terms for sodium caseinate (*CAS*, β_1), soy protein isolate (*SPI*, β_2), and gellan gum (*GLN*, β_3) presented a highly significant ($P<0.01$) effect on penetration force, and a significant ($P<0.05$) effect of the sodium caseinate and soy protein isolate interaction (β_{12}) but with negative sign ($R^2= 0.9953$) (Table 1). In the contour plot for acid-induced emulgel with oil as oil-fraction, the penetration force increased with the gellan gum proportion, irrespective of the protein type, where both protein combinations resulted in lower penetration forces (Figure 2a), as indicated by the negative sign in their interaction. The linear terms and both protein interactions with gellan gum (β_{13} and β_{23}) presented a highly significant ($P<0.01$) effect on the penetration work, with a significant ($P<0.05$) effect but with a negative sign ($R^2= 0.9926$) (Table 2). In the contour plot, this effect can be observed since the higher penetration forces were observed perpendicularly to the gellan gum vertex (Figure 2b).

In the penetration work for samples with oil as oil-fraction, linear terms presented a highly significant ($P<0.01$) effect, and a significant negative effect ($P<0.05$) due to the proteins' interaction (β_{12}) ($R^2= 0.9781$) (Table 2). In the contour plot, this can be observed, since the predicted values for this parameter were the lowest detected close to the sodium caseinate and the soy protein isolate vertex, and higher values were observed when the gellan gum proportion was increased in the acid-induced emulgel formulation (Figure 2c).

Table 2

Regression coefficients and correlation for the adjusted model to the experimental data in the mixture design approach for acid-induced emulgels textural tests. Penetration test

Parameter	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	P<	R^2
Force w/oil	0.790**	0.957**	14.41**	-18.04*	0.462•	1.47•	<0.0001	0.9953
Force w/oleogel	1.32**	1.30**	5.13**	-37.88**	23.12**	28.42**	0.0002	0.9926
Work w/oil	5.26**	6.38**	49.91**	-161.01*	81.36	100.66	0.0021	0.9781
Work w/oleogel	6.91**	9.68**	26.83**	-174.9**	128.79**	134.20**	0.0002	0.9930

Note: β_1: sodium caseinate, β_2: soy protein isolate, β_3: gellan gum. ** highly significant (P<0.01), * significant (P<0.05), • no significant (P>0.05)

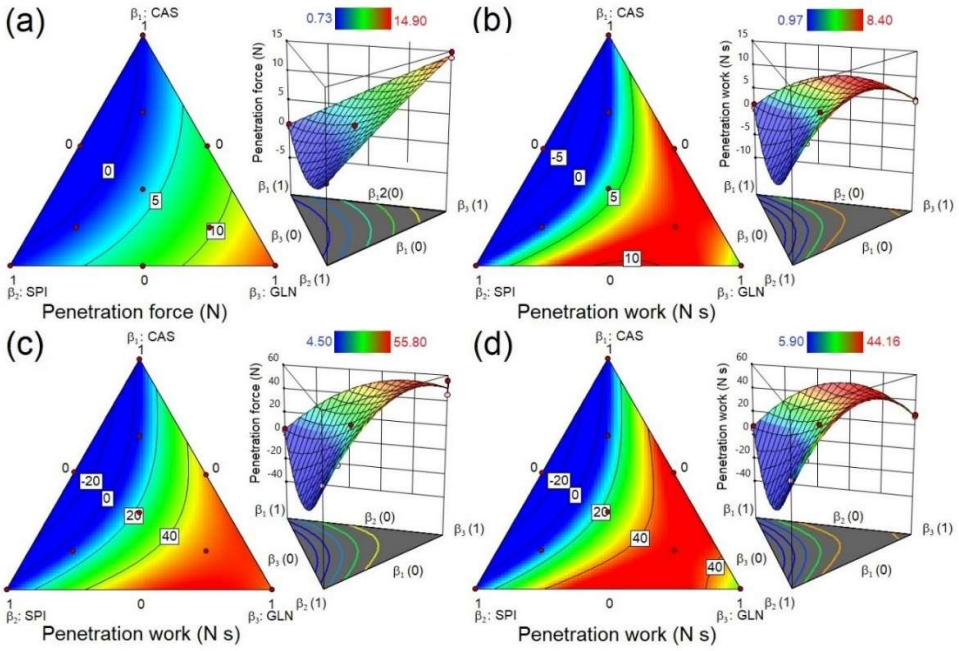

Fig. 2. *Iso-response contour plots and three-dimensional response surface for the regression equation adjusted model for emulgels penetration force with oil (a) or oleogel (b) as oil-fraction, and emulgel penetration work with oil (c) or oleogel (d) as oil-fraction*

In samples containing oleogel as oil-fraction, the linear terms and both protein interactions with gellan gum presented a highly significant (P<0.01) effect on the penetration work, with a significant but negative (P<0.05) effect of the proteins' interaction (R^2= 0.9930) (Table 2). In the contour plot, it can be observed that the higher values are close to the gellan gum vertex (1.00 proportion) (Figure 2d).

The lower texture detected during penetration is the result of the different oil-fraction dispersion during the emulsifying and acid gelation processes, as well as the interaction between components, particularly candelilla wax in oleogel. Actually, the proteins' interaction with gellan gum was statistically significant in oleogel samples. Penetration force in oil-containing samples presented the highest values close to the gellan gum vertex (10 N).

In oleogel-containing samples, the penetration force values were lower (half), although when soy protein isolate was employed in the mixture with gellan gum (50-50), the penetration force values were the same. This implies an interaction between soy protein isolate and oleogel in the acid-induced gelation of the emulsion. For the penetration work, this interaction was not observed. The penetration work values decreased to half in oleogel-containing samples when the gellan gum proportion increased to close to 1.0. The penetration test measures the force required to penetrate the sample by probe at a specific depth, involving both shear and compression of the sample [21]. Only the cross-sectional area of the employed probe in the sample is subject to puncture, affected by the sample matrix uniformity and density, measuring the degree of solidity of the samples, that is, hardness [16]. In this view, emulgel samples with oil as the oil fraction were harder than the samples with oleogel as the oil fraction.

3.2. Compression Test

For the compression force of the acid-induced emulgels with oil as oil-fraction, the linear terms for sodium caseinate (*CAS*, β_1), soy protein isolate (*SPI*, β_2), and gellan gum (*GLN*, β_3) presented a highly significant ($P<0.01$) effect, with the proteins' interactions quadratic term (β_{12}) with a significant ($P<0.05$) but negative effect ($R^2= 0.9667$). The sodium caseinate and soy protein interaction presented a negative sign (Table 3).

Table 3

Regression coefficients and correlation for the adjusted model to the experimental data in the mixture design approach for acid-induced emulgels textural tests. Compression test

Parameter	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	P<	R^2
Force w/oil	2.48**	2.81**	19.36**	-65.13*	25.58•	38.37•	0.0047	0.9667
Force w/oleogel	2.28**	2.98**	8.39**	-87.12**	55.58**	63.38**	<0.0001	0.9964
Work w/oil	13.11**	16.01**	100.76**	-388.55•	204.00•	261.00•	0.0105	0.9498
Work w/oleogel	14.46**	16.89**	48.62**	-421.1**	302.07**	324.70**	0.0006	0.9885

Note: β_1: sodium caseinate, β_2: soy protein isolate, β_3: gellan gum. ** highly significant ($P<0.01$), * significant ($P<0.05$), • no significant ($P>0.05$)

In the contour plot, the penetration force increased with the gellan gum proportion, irrespective of the protein type, where both protein combinations resulted in lower penetration forces (Figure 3a), as indicated by the negative sign in their interaction. In the samples with oleogel as oil-fraction, the linear, as well as the quadratic terms related to components' interaction, presented a highly significant ($P<0.01$) effect. The sodium caseinate and soy protein interaction presented a negative sign ($R^2= 0.9964$) (Table 3). In the contour plot, both protein combinations resulted in lower penetration forces, as indicated by the negative sign in their interaction, and the force values increased with the gellan gum proportion, with higher values in the soy protein isolate and gellan gum side (Figure 3b).

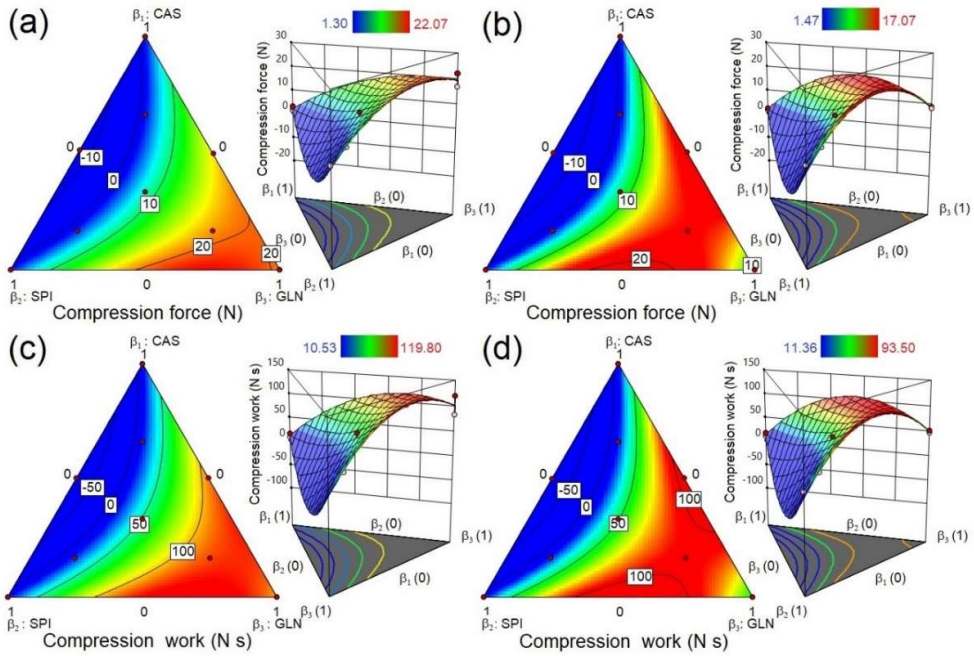

Fig. 3. *Iso-response contour plots and three-dimensional response surface for the regression equation adjusted model for emulgels compression force with oil (a) or oleogel (b) as oil-fraction, and emulgel compression work with oil (c) or oleogel (d) as oil-fraction*

For the compression work in samples with oil as oil-fraction, only the linear terms presented a highly significant ($P<0.01$) effect ($R^2= 0.9498$) (Table 3). In the contour plot, this effect can be observed since the higher penetration forces were observed perpendicularly to the gellan gum vertex, and the lower ones towards the protein vertices (Figure 3c). When oleogel was employed as oil-fraction, there was a highly significant ($P<0.01$) effect of both linear and quadratic terms on the compression

force, with a negative sign in the proteins' interaction terms (R^2= 0.9930) (Table 3). In the contour plot, higher values were observed when the gellan gum proportion increased with one protein or the other, and the lower values at the lowest gellan gum proportions (Figure 3d).

In the compression test, since the probe is larger than the sample, the entire sample is subjected to compressive, tensile, and frictional stresses due to the compression load, reflecting the resistance to failure, related to sample cohesiveness [16]. Samples are subjected to uniaxial compression and often have a cylindrical shape [21]. Gellan gum seems to be responsible for the emulgels cohesiveness, since the highest values were observed close to this vertex in samples with oil as oil-fraction, with no effect of the interaction of gellan gum with proteins. The compression force values were practically twice as much when oil was employed as oil-fraction in the acid-induced gelation, as compared to oleogel samples, mainly near to the gellan gum vertex (higher gellan gum proportion, 20 and 10 N, respectively). In compression work, the energy required to compress the sample, there was also no effect of proteins' interaction with gellan gum, besides these values decreased to the half in samples with oleogel as oil-fraction.

3.3. Extrusion Test

For the acid-induced emulgels with oil as oil-fraction, the linear terms for sodium caseinate (*CAS*, β_1), soy protein isolate (*SPI*, β_2), and gellan gum (*GLN*, β_3) presented a highly significant (P<0.01) effect and a significant (P<0.05) effect of the soy protein isolate and gellan gum interaction (β_{23}) on the extrusion force (R^2= 0.9407) (Table 4). In the contour plot, the increase in gellan gum proportion resulted in higher force values, and in the gellan gum with soy protein mixtures, the higher values were observed (Figure 4a).

Table 4

Regression coefficients and correlation for the adjusted model to the experimental data in the mixture design approach for acid-induced emulgels textural tests. Extrusion test

Parameter	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	P<	R^2
Force w/oil	6.21**	7.88**	41.32**	-201.36•	58.50•	119.67*	0.0145	0.9407
Force w/oleogel	6.41**	7.04**	37.64**	-69.63*	-10.93•	155.30*	0.0019	0.9792
Work w/oil	9.99**	26.17**	118.12**	-914.71•	589.96•	764.09*	<0.0001	0.9991
Work w/oleogel	19.11*	28.64*	112.71*	-508.5*	199.76•	543.44*	0.1061	0.8296

Note: β_1: sodium caseinate, β_2: soy protein isolate, β_3: gellan gum. ** highly significant (P<0.01), * significant (P<0.05), • no significant (P>0.05)

The linear terms in oil-containing samples presented a highly significant (P<0.01) effect, besides a significant (P<0.05) effect of the soy protein isolate

and gellan gum interaction (R^2= 0.9792). In the contour plot, it can be observed that the higher force values were detected as long as the gellan gum and soy protein isolate were combined, particularly in the 0.5-0.5 proportion (Figure 4b).

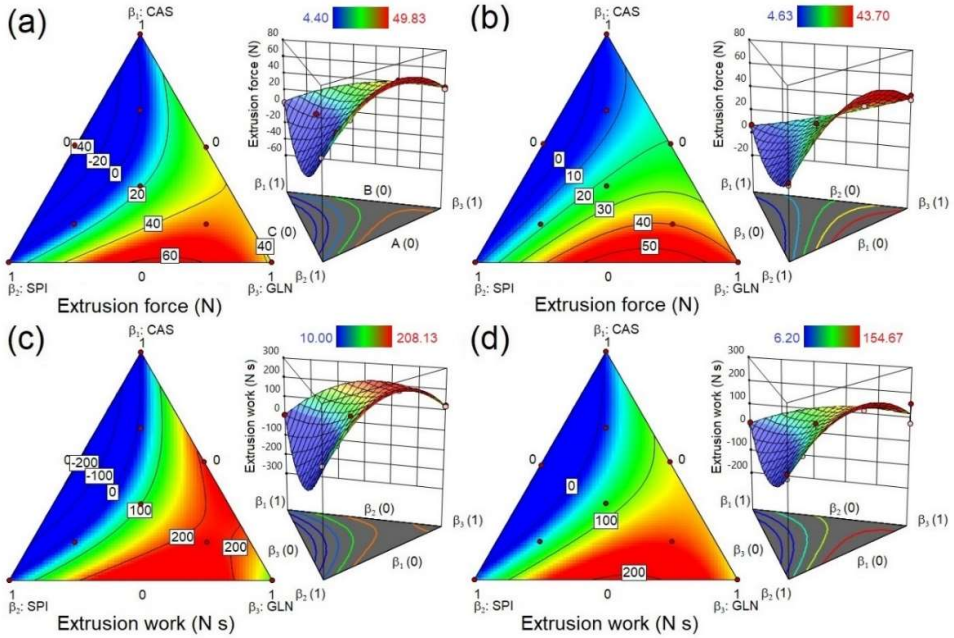

Fig. 4. *Iso-response contour plots and three-dimensional response surface for the regression equation adjusted model for emulgels extrusion force with oil (a) or oleogel (b) as oil-fraction, and emulgel extrusion work with oil (c) or oleogel (d) as oil-fraction*

For the extrusion work in samples with oil as oil-fraction, there was a highly significant (P<0.01) effect of the linear and quadratic terms on this textural parameter R^2= 0.9991). In contour plots for the extrusion work, the higher the proportion of gellan gum in the mixtures, the higher the values of this parameter moreover, in the gellan gum vertex (Figure 4c). In the acid-induced emulgels with oleogel as oil-fraction, the work of extrusion presented a significant (P<0.05) effect only for the linear terms and the soy protein isolate interaction with gellan gum (R^2= 0.8296). In the contour plot, the compression work was higher when gellan gum was combined with soy protein isolate and drastically decreased at lower gellan gum proportions (Figure 4d).

In the Ottawa cell, during the extrusion test, the force applied to the sample resulted in a simultaneous combination of shearing, extrusion, and adhesion, forcing the sample to flow through the outlet cavities [2]. The Ottawa cell measuring system has been employed to determinate the firmness in particulate acid gelled systems such as cottage cheese [6] and as an indication of product integrity of a soy protein and corn meal extruded system [4].

In the regression equation, the extrusion force was also dominated by the gellan gum component. In oil-containing samples, the firmness values increased towards the gellan gum vertex, and the highest ones were in the interaction of gellan gum with soy protein isolate (60 N). In the oleogel containing samples, a strong interaction between soy protein isolate and gellan gum resulted in higher extrusion force (firmness) values, although lower than oil-containing samples (40 N). The extrusion work was also higher due to the gellan gum effect in the acid-induced emulgel systems, with values twice as high as in samples with oil as oil fraction, as compared to samples with oleogel as oil-fraction, with an interaction with soy protein isolate in oleogel-containing samples.

Comprehensively, the emulgels with oil as oil fraction were harder, more cohesive, and firmer than those samples containing oleogel as oil fraction. On one hand, in the acid-induced gelation, soy proteins first polymerize by forming cross-linking aggregates to form the gel network, where the proteins undergo a pre-aggregation state before finally creating the gel structure [36]. The aggregation of soy proteins is favoured when the pH is close to the isoelectric point, about 4.5, since the proteins' charge is neutralized progressively by the acidic conditions [29, 30], indicating that the protein aggregation was enhanced at this pH as a result of the reduction of repulsive electrostatic forces of negatively charged protein groups by gluconic acid protons, promoting hydrophobic interactions of denatured proteins to form aggregated [5]. For its part, caseins tend to aggregate at a pH value around its electronic point, ca. pH 4.6 [37]. The presence of other protein molecules and aggregates during acidification resulted in steric interference affecting the casein gel network formation [15]. Besides, soy proteins have an isoelectric point similar to caseins, and under more acidic conditions, soy proteins do not participate in the active gel net formation of casein acid gels [31]. The range of pH in the final acid-induced emulgels irrespective of the type of oil-fraction was in the range of 4.1-4.4. However, Chen et al. [8] reported that there is an important structural difference between acid-induced caseinate emulsion gels and caseinate gels, since in the former there are two different structural components, the emulsion droplets with the protein interfacial film and the casein acid-aggregates, resulting in a more viscous character than caseinate gels, since protein-covered oil droplets were incorporated into the gel network. The same phenomenon could occur in soy protein isolate emulgels. The proteins and their interactions resulted in lower (or negatively predicted) force and work values, but a stronger interaction among gellan gum and soy protein isolate stands out in the regression analysis. These indicate that both proteins were incompatible under the experimental conditions employed, since softer, less cohesive, and weaker structures were formed.

On the other hand, the pH affects the conformation of gellan gum as an anionic polyelectrolyte, provoking a shielding effect or an electrostatic repulsion [39], and these pH variations change the anionic nature of the gellan gum, affecting the degree of dissociation of the carboxyl groups, resulting in lower charge density of gellan gum chains [13]. When the pH of a gellan solution is higher than the gellan gum pKa (3.5), there is an increase in the

molecular electrostatic repulsion between molecules [10]. At relatively higher concentrations, the gellan gum chains are closer, enhancing the aggregation, forming junction zones [28], and a macroscopic gel formation resulted when, after thermal coil-helix transition, the gellan double helix aggregates by hydrogen bonds between junction zones induced by lowering the pH or adding salts [39]. Anyhow, gellan gum dominates the texture of the acid-induced emulsions.

Regarding the gellan gum interaction with sodium caseinate and/or soy protein isolate, interaction between these components resulted in different textures. Polysaccharides can interact with sodium caseinate during acid-gelation, affecting protein-water and protein-protein interactions, reducing gel firmness [20]. Sosa-Herrera et al. [32] reported the formation of an intermediate complex of caseinate-gellan gum formed at pH 5.4, with both components carrying negative charge, forming an electrostatic interaction-mediated coacervate [26]. In sodium caseinate-gellan gum acid emulsions, oil droplets were dispersed onto the casein-rich phase, acting as an emulsifier, increasing the rigidity of the casein phase, which dominantly contributed to the overall texture of emulsion-gels [18]. The gellan gum does not simply result into higher viscosity but also forms a complex structure when added to the sodium caseinate solution, contributing to increase gel strength [26] and fracture stress and strain [28]. With the increase of polysaccharide concentration, the number of solid particles adsorbed and anchored at the oil-water interface increased, thus covering a larger interface area and forming a dense three-dimensional network preventing the agglomeration or emulsification of emulsion droplets [24]. This is the reason why the higher textural parameter values were observed closer to the gellan gum vertex.

Now, the acid-induced emulgel textural differences related to the oil-fraction type refer to the physical state and the use of candelilla wax as a structuring agent, which gives the oleogel a gel-like consistency. The particular hydrocarbons and lipid composition of candelilla wax as an organogelator in the restructured oil elaboration confers a characteristic crystallization and melting behaviour that enables the entrapment of oil by the wax crystals [14]. In this view, the crystalline network entrapping oil will interact in different ways with the protein matrix during and after the emulsion process, as compared to the oil, with no components that affect its hydrophobic profile. Employing hemp seed oil and rice bran wax to elaborate an oleogel to compare the performance of oil/water and oleogel/water emulsions, Wang et al. [37] reported that the shape of oleogel/water emulsions was irregular but smoother and with a more uniform distribution, and with less surface sediment (free protein molecules and their aggregates in the system), while indicating that the oleogel/water emulsion system presented a higher stability. At a low pH (3-5), close to the isoelectric point of hemp seed proteins, a partial collapse of the space layer and slight polymerization of the coated droplets occur. Still, the oleogel/water emulsion presented lower delamination (interfacial protein film deterioration) since the rice bran wax in oleogel enhanced emulsion stability. During the acid-induced gelation, the higher emulsion stability in oleogel/water emulsions was reflected in a

softer and less resistant gel structure, since with more protein available (sodium caseinate and soy protein isolate) both proteins carry a low electrostatic charge, provoking the inhibition of gel network rearrangement due to protein interactions [15]. At this point of acidic conditions (pH from 5.3 to 3.5), where proteins are occupied stabilizing the oil phase, gellan gum gels resulted in less deformable and stiffer structure due to an increase in the molecular aggregation provoking and increase in the strands mobility [28], same behaviour having been observed by Norton et al. [27] and by Bradbeer et al [3]. Cassanelli et al. [7] reported that the pH decrease of the gel to the pKa of gellan gum resulted in an increase in the gel strength and Young's modulus, where the gel structure aggregation and the formation of junction zones were related to the electrostatic interactions.

4. Conclusions

The gradual pH decline provoked by the glucono lactone resulted in softer gels when the protein proportion in the ternary mixture was higher, since as the pH approached the sodium caseinate's or the soy protein isolate's isoelectric points (both with close isoelectric point values, 4.6 and 4.5, respectively) the gel formed by the free protein, that is, the one which not in change of forming the interfacial protein film, didn't result in an order protein gel matrix, since no ordered aggregation was present, decreasing the textural properties of emulgels for both oil fractions, oil or oleogel. In this case, the gellan gum dominates the enhancement of the textural properties related to the emulsified system's hardness (penetration), cohesiveness (compression) and firmness (extrusion). Acid-induced gelation is also an alternative to developing different food structures since the environmental conditions such as the pH, modify the interaction between the components in relation to the proteins' isoelectric point or to the polysaccharides' pK. The acid-induced gelation of composite emulsions offers the possibility to develop different textures employing different types of oil-fractions, edible oils or oleogels.

The complete large deformation textural characterization allowed to determinate the degree of interaction of proteins and gellan gum at the experimental conditions employed related to hardness (penetration), cohesiveness (compression) and firmness (extrusion).

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