# INTERACTIONS BETWEEN INGREDIENTS IN THE FORMULATION OF FOOD GELS BASED ON FORAGE PALM MUCILAGE: Texture and rheological analyses using whey protein, egg albumin, and salts

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## ABSTRACT

This study investigates how different ingredients (whey protein, egg albumin, NaCl, and $CaCl_2$) affect the texture and viscosity of food gels, using a Simplex Centroid Mixture Design. Texture parameters (firmness, cohesiveness, consistency, and viscosity index) and rheological properties (ascending area, descending area, hysteresis, and chewing viscosity at 10 $s^{-1}$) were analyzed. The results reinforce the importance of texture in consumer acceptance of food products, demonstrating that it is directly related to ingredient selection and processing. It was observed that whey protein and egg albumin contribute to the formation of structural networks that enhance gel firmness and viscosity. Additionally, salts such as NaCl and $CaCl_2$ were found to influence protein interactions, resulting in a more cohesive structure. However, certain ingredient combinations were shown to produce undesirable effects, compromising the product's consistency and stability. The mathematical model developed allowed for a better understanding of these interactions, supporting the formulation of foods with optimal instrumental texture. The optimal point was composed of 0.98 egg albumin, 0.02 NaCl and 0.00 $CaCl_2$, in coded levels.


**Keywords:** Hydrogels; Molecular structure; Protein functionality; Rheological parameters.

## INTRODUCTION

Hydrocolloids are compounds that, when mixed with water, form colloidal solutions capable of altering the texture and viscosity of food products. These polysaccharides play a crucial role in the formation of food gels, contributing to characteristics such as water retention, elasticity, and stability (Dickinson, 2020).

When hydrocolloids interact with proteins, gels with enhanced properties may be formed. Factors such as pH, ionic strength, and the ratio between these biopolymers directly influence this interaction. For instance, the addition of natural gums to proteins has been shown to increase water retention and improve gel firmness, thereby expanding their potential applications in the food industry (Hidalgo *et al.*, 2021). Moreover, different types of proteins, such as whey proteins and casein micelles, can be combined to produce gels with superior mechanical characteristics. According to Larajim (2014), when denatured proteins aggregate, the gel network is strengthened, becoming more homogeneous and reducing liquid release (syneresis).

According to Yang *et al*. (2021), gels are semi-rigid three-dimensional structures capable of retaining large amounts of water or other liquids. Gel formation involves interactions among carbohydrates, proteins, salts, and water. In recent years, food gels have attracted growing interest due to their biodegradability, biocompatibility, and nutritional and sensory properties (Liu *et al.*, 2022).

In this context, the objective of these study was to analyze the rheological and textural properties of gels by employing a Simplex-Centroid Mixture Design, investigating the effects of different concentrations of proteins (egg albumin and whey protein) and salts (NaCl and $CaCl_2$) at pH 6.0 and a temperature of 25 °C.

## MATERIAL AND METHODS

### Raw material

The research project involving forage palm was conducted by the Integrated Laboratory of Cereals and Lipids (LICEL) and has been registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) of the Ministry of the Environment of the Federative Republic of Brazil under protocol AF2C488.

Cladodes of the forage palm, variety Sertânia, were donated by the Agricultural Research Company of Minas Gerais (EPAMIG) and harvested in December 2023 at the Acauã experimental field (District of Leme do Prado, State of Minas Gerais), located at 17°07'37"S latitude and 42°46'02"W longitude. In this study, the primary focus was the extraction of mucilage from the cladodes. The extracted mucilage was composed of 96.06% moisture and 3.94% total solids, which were comprised of proteins (6.78%), ether extract (2.26%), ash (26.29%), and total carbohydrates (64.67%).

The other ingredients used were xanthan gum (Soldiers Nutrition), egg albumin with a minimum protein content of 78% (Naturovos), commercial sucrose (Guarani), iodized sodium chloride (Cisne), analytical-grade calcium chloride (Perfyl Tech), whey proteins with a minimum protein content of 90% (Growth Supplements), analytical-grade citric acid (Dinâmica), and analytical-grade sodium carbonate (Dinâmica).

### Standard formulation

The standard formulation for the study was based on previous investigations conducted by our research group (unpublished data). It was composed of forage cactus mucilage (36.25%), xanthan gum (1.5%), sucrose (3.66%), and distilled water (42.59%), resulting in a mass fraction of 0.84. The remaining fraction (0.16) consisted of egg albumin, whey proteins, calcium chloride, and sodium chloride, according to the experimental design.

### Experimental design

The experimental method adopted for the present study was the Response Surface Methodology (RSM), using the Simplex-Centroid Mixture Design as a tool, with four independent variables. In this model, $X_1$ represents whey protein, $X_2$ represents egg albumin, $X_3$ represents NaCl, and $X_4$ represents $CaCl_2$, as shown in Tables 1 and Table 2. The mathematical model (Equation 1) proposed by Rodrigues and Iemma (2014) was followed, where: Y represents the response variable under study; $\beta_i$ and $\beta_{ij}$ are the regression coefficients of the individual components and the binary and ternary interactions, respectively; $x_i$ and $x_j$ are the coded values of the independent variables; and ε corresponds to the experimental error.

$$Y = \beta_{1x1} + \beta_{2x2} + \beta_{3x3} + \beta_{4x4} + \beta_{12x1x2} + \beta_{13x1x3} + \beta_{14x1x4} + \beta_{23x2x3} + \beta_{24x2x4} + \beta_{123x1x2x3} + \beta_{124x1x2x4} + \beta_{234x2x3x4} + \varepsilon \quad \text{(Eq. 1)}$$

**Table 1 –** Coded levels and restricted coded levels for the evaluation of gel texture and rheology.

| Trials | Coded levels | | | | Restricted coded levels | | | |
|---|---|---|---|---|---|---|---|---|
| | $x_1$ | $x_2$ | $x_3$ | $x_4$ | $x_1$ | $x_2$ | $x_3$ | $x_4$ |
| 1 | 1.000 | 0.000 | 0.000 | 0.000 | 0.994 | 0.000 | 0.003 | 0.003 |
| 2 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.994 | 0.003 | 0.003 |
| 3 | 0.000 | 0.000 | 1.000 | 0.000 | 0.405 | 0.405 | 0.188 | 0.003 |
| 4 | 0.000 | 0.000 | 0.000 | 1.000 | 0.405 | 0.405 | 0.003 | 0.188 |
| 5 | 0.500 | 0.500 | 0.000 | 0.000 | 0.497 | 0.497 | 0.003 | 0.003 |
| 6 | 0.500 | 0.000 | 0.500 | 0.000 | 0.541 | 0.361 | 0.095 | 0.003 |
| 7 | 0.500 | 0.000 | 0.000 | 0.500 | 0.541 | 0.361 | 0.003 | 0.095 |
| 8 | 0.000 | 0.500 | 0.500 | 0.000 | 0.361 | 0.541 | 0.095 | 0.003 |
| 9 | 0.000 | 0.500 | 0.000 | 0.500 | 0.361 | 0.541 | 0.003 | 0.095 |
| 10 | 0.000 | 0.000 | 0.500 | 0.500 | 0.405 | 0.405 | 0.095 | 0.095 |
| 11 | 0.333 | 0.333 | 0.333 | 0.000 | 0.468 | 0.468 | 0.061 | 0.003 |
| 12 | 0.333 | 0.333 | 0.000 | 0.333 | 0.468 | 0.468 | 0.003 | 0.061 |
| 13 | 0.333 | 0.000 | 0.333 | 0.333 | 0.501 | 0.376 | 0.061 | 0.061 |
| 14 | 0.000 | 0.333 | 0.333 | 0.333 | 0.376 | 0.501 | 0.061 | 0.061 |
| 15 | 0.625 | 0.125 | 0.125 | 0.125 | 0.795 | 0.159 | 0.023 | 0.023 |
| 16 | 0.125 | 0.625 | 0.125 | 0.125 | 0.159 | 0.795 | 0.023 | 0.023 |
| 17 | 0.125 | 0.125 | 0.625 | 0.125 | 0.431 | 0.431 | 0.115 | 0.023 |
| 18 | 0.125 | 0.125 | 0.125 | 0.625 | 0.431 | 0.431 | 0.023 | 0.115 |
| 19 | 0.250 | 0.250 | 0.250 | 0.250 | 0.454 | 0.454 | 0.046 | 0.046 |
| 20 | 0.250 | 0.250 | 0.250 | 0.250 | 0.454 | 0.454 | 0.046 | 0.046 |
| 21 | 0.250 | 0.250 | 0.250 | 0.250 | 0.454 | 0.454 | 0.046 | 0.046 |

Where: $x_1$, $x_2$, $x_3$, and $x_4$ are the coded levels for whey protein, egg albumin, NaCl, and $CaCl_2$, respectively.

**Table 2** – Restricted coded levels and actual levels for the evaluation of gel texture and rheology.

| Trials | Restricted coded levels | | | | Real levels | | | |
|---|---|---|---|---|---|---|---|---|
| | $x_1$ | $x_2$ | $x_3$ | $x_4$ | $X_1$ | $X_2$ | $X_3$ | $X_4$ |
| 1 | 0.994 | 0.000 | 0.003 | 0.003 | 15.90 | 0.00 | 0.05 | 0.05 |
| 2 | 0.000 | 0.994 | 0.003 | 0.003 | 0.00 | 15.90 | 0.05 | 0.05 |
| 3 | 0.405 | 0.405 | 0.188 | 0.003 | 6.48 | 6.48 | 3.00 | 0.05 |
| 4 | 0.405 | 0.405 | 0.003 | 0.188 | 6.48 | 6.48 | 0.05 | 3.00 |
| 5 | 0.497 | 0.497 | 0.003 | 0.003 | 7.95 | 7.95 | 0.05 | 0.05 |
| 6 | 0.541 | 0.361 | 0.095 | 0.003 | 8.66 | 5.77 | 1.53 | 0.05 |
| 7 | 0.541 | 0.361 | 0.003 | 0.095 | 8.66 | 5.77 | 0.05 | 1.53 |
| 8 | 0.361 | 0.541 | 0.095 | 0.003 | 5.77 | 8.66 | 1.53 | 0.05 |
| 9 | 0.361 | 0.541 | 0.003 | 0.095 | 5.77 | 8.66 | 0.05 | 1.53 |
| 10 | 0.405 | 0.405 | 0.095 | 0.095 | 6.48 | 6.48 | 1.53 | 1.53 |
| 11 | 0.468 | 0.468 | 0.061 | 0.003 | 7.48 | 7.48 | 0.98 | 0.05 |
| 12 | 0.468 | 0.468 | 0.003 | 0.061 | 7.48 | 7.48 | 0.05 | 0.98 |
| 13 | 0.501 | 0.376 | 0.061 | 0.061 | 8.02 | 6.01 | 0.98 | 0.98 |
| 14 | 0.376 | 0.501 | 0.061 | 0.061 | 6.01 | 8.02 | 0.98 | 0.98 |
| 15 | 0.795 | 0.159 | 0.023 | 0.023 | 12.72 | 2.54 | 0.37 | 0.37 |
| 16 | 0.159 | 0.795 | 0.023 | 0.023 | 2.54 | 12.72 | 0.37 | 0.37 |
| 17 | 0.431 | 0.431 | 0.115 | 0.023 | 6.89 | 6.89 | 1.84 | 0.37 |
| 18 | 0.431 | 0.431 | 0.023 | 0.115 | 6.89 | 6.89 | 0.37 | 1.84 |
| 19 | 0.454 | 0.454 | 0.046 | 0.046 | 7.26 | 7.26 | 0.74 | 0.74 |
| 20 | 0.454 | 0.454 | 0.046 | 0.046 | 7.26 | 7.26 | 0.74 | 0.74 |
| 21 | 0.454 | 0.454 | 0.046 | 0.046 | 7.26 | 7.26 | 0.74 | 0.74 |

Where: $x_1$, $x_2$, $x_3$, and $x_4$ are the coded levels for whey protein, egg albumin, NaCl, and $CaCl_2$, respectively.

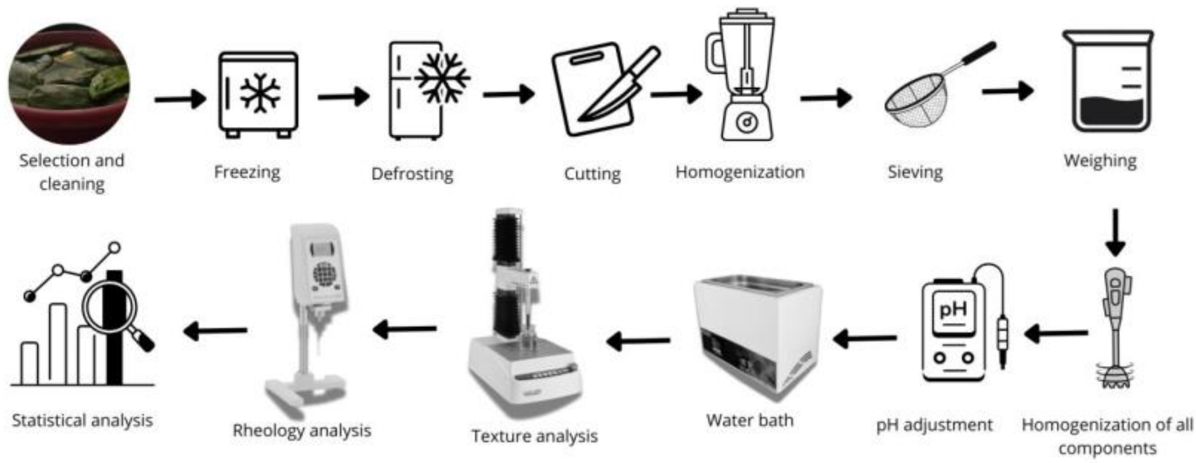
## Preparation of the gels

The cladodes of forage cactus were selected, and only healthy and intact specimens were used. The selected cladodes were sanitized using a 200 ppm sodium hypochlorite solution for 15 minutes, followed by rinsing under running water. Subsequently, the glochids were carefully removed with the aid of a knife, and the cladodes were stored in polypropylene bags under freezing conditions in a household freezer until further use.

For processing, the cladodes were thawed at 7 °C in a BF Inox 2520 cold chamber (BF Cozinhas, São Paulo, Brazil) for approximately 24 hours. They were then manually cut along the longitudinal axis and subjected to manual extraction of mucilage. The extracted mucilage was homogenized using a high-speed Lar-2 industrial blender (Metal Ferreira, São Paulo, Brazil) to ensure uniform distribution. The mucilage was subsequently sieved through a domestic sieve with an aperture of approximately 1 mm to remove larger fibers.

For gel preparation, all components were weighed using an AUY 220 analytical balance (Shimadzu, Piracicaba, Brazil). Approximately 50 g of distilled water was initially added to the dry components, and the mixture was homogenized using a high-speed TE-102 Turratec homogenizer (Tecnal, Piracicaba, Brazil) operating at 4000 rpm. The palm mucilage was then incorporated into the mixture and manually homogenized using a glass rod. The pH of the mixture was adjusted to 6.0 using saturated solutions of citric acid and sodium bicarbonate. Subsequently, the total volume of the sol was adjusted to 180 g using distilled water, followed by additional manual homogenization with a glass rod. The sol was then conditioned in a water bath at 25 °C for 1 hour and subsequently subjected to texture and viscosity evaluation using a rheometer. Figure 1 illustrates the process flowchart encompassing all stages of the experiment, from raw material selection to statistical analysis.

**Figure 1** – Experimental process flowchart.




**Instrumental texture**

The instrumental texture profile analysis was performed using a TA-XT Plus texture analyzer (Stable Micro Systems, Haslemere, England). The test employed an acrylic probe with a diameter of 35 mm and a thickness of 6 mm, along with a cylindrical container of 50 mm in diameter and approximately 50 $cm^3$ in volume. The gel samples were carefully deposited into the container up to a uniform height of 25 mm to ensure standardized experimental conditions.

The analysis conditions were as follows: pre-test, test, and post-test speeds set at 1 $mm \cdot s^{-1}$; probe height of 70 mm; trigger force of 0.0981 N; load cell capacity of 25 kg; and a compression distance corresponding to 30% of the initial sample height. Each sample was analyzed in quadruplicate. The evaluated parameters were firmness (N), consistency (N·s), cohesiveness (N), and viscosity index (N·s).

**Rheological behavior**

The rheological properties were evaluated using a RM-200 Rheomat rotational rheometer (Lamy Rheology, Champagne au Mont d'Or, France), which was employed to determine the dynamic viscosity and to characterize the rheological behavior of the samples. For each test, approximately 20 g of gel was used, with accessories MK-DIN No. 2 and tube base DIN 2 TUBE. The shear rate profile was planned to increase from 5 to 100 rpm, followed by a decreasing rate from 100 to 5 rpm, with regular increments every 5 seconds.

The Newtonian, Ostwald, Casson, and Bingham models were tested, and the Ostwald model was selected based on the highest coefficient of determination ($r = 0.9852$), indicating the best fit to the experimental data. The data obtained from the equipment included values for shear rate, shear stress, and dynamic viscosity, which were fundamental for conducting all rheological analyses. These parameters enabled a detailed characterization of the gel's behavior under different deformation conditions, ensuring the precision and reliability of the experimental results.

The dependent variables evaluated (in quadruplicate) included the area under the ascending curve, the area under the descending curve, hysteresis, and chewing viscosity, defined as the apparent viscosity (Pa·s) at a shear rate of 10 $s^{-1}$, which is considered representative for soft foods (Riquelme *et al.*, 2023). The areas under the ascending and descending curves were determined by integrating the shear stress versus apparent viscosity data using Origin 2019 software and expressed as area under the curve (AUC). Hysteresis was calculated as the difference between the areas of the ascending and descending curves and expressed in AUC.

**Statistical analysis**

The results were evaluated through Response Surface Methodology (RSM) using Statistica 7.0 software, in which regression coefficients were estimated and analysis of variance

(ANOVA) was performed. A confidence level of 90% ($p < 0.10$) and a minimum coefficient of determination of 80% were considered as criteria for model adequacy, as recommended by Rodrigues and Iemma (2014). Numerical optimization was performed using the Design Expert 6.0 software, following the methodology proposed by Derringer and Suich (1980).

## RESULTS AND DISCUSSION

### Instrumental texture

The texture of foods is considered an essential and adjustable aspect to promote consumer health, in addition to playing a crucial role in influencing taste and preference for a food product (Wang *et al*., 2024). Texture is directly related to food formulation and processing, with lipids, proteins, and polysaccharides playing fundamental roles in molecular interactions and product structure definition. The results of texture instrumental analysis in the experimental design assays are presented in Table 3.

**Table 3 –** Instrumental texture of gels from the Simplex Centroid Mixture Design.

| Trial | Firmness (N) | Consistency (N·s) | Cohesiveness (N) | Viscosity index (N·s) |
|---|---|---|---|---|
| 1 | 0.615± 0.02 | 0.433 ± 0.36 | 0.242 ± 0.01 | 1.825 ±0.24 |
| 2 | 0.810 ± 0.05 | 5.883 ± 0.75 | 0.323 ± 0.02 | 3.019 ± 0.39 |
| 3 | 0.611 ± 0.03 | 3.913 ± 0.51 | 0.224 ± 0.01 | 1.304 ± 0.36 |
| 4 | 0.426 ± 0.04 | 2.631 ± 0.42 | 0.158 ± 0.01 | 0.660 ± 0.12 |
| 5 | 0.430 ± 0.03 | 2.768 ± 0.27 | 0.166 ± 0.01 | 0.784 ± 0.15 |
| 6 | 0.455 ± 0.02 | 3.391 ± 0.25 | 0.180 ± 0.01 | 0.858 ± 0.11 |
| 7 | 0.634 ± 0.01 | 3.837 ± 0.35 | 0.219 ± 0.01 | 1.201 ± 0.20 |
| 8 | 0.483 ± 0.03 | 3.069 ± 0.23 | 0.171 ± 0.01 | 0.719 ± 0.14 |
| 9 | 0.629 ± 0.02 | 3.987 ± 0.24 | 0.237 ± 0.01 | 1.491 ± 0.18 |
| 10 | 0.544 ± 0.03 | 3.520 ± 0.27 | 0.195 ± 0.01 | 0.895 ± 0.08 |
| 11 | 0.402 ± 0.02 | 2.799 ± 0.39 | 0.151 ± 0.01 | 0.715 ± 0.16 |
| 12 | 0.529 ± 0.01 | 3.407 ± 0.46 | 0.197 ± 0.01 | 0.933 ± 0.17 |
| 13 | 0.525 ± 0.04 | 3.404 ± 0.55 | 0.181 ± 0.02 | 0.891 + 0.16 |
| 14 | 0.494 ± 0.04 | 3.326 ± 0.24 | 0.188 ± 0.01 | 0.932 ± 0.12 |
| 15 | 0.474 ± 0.02 | 3.133 ± 0.30 | 0.172 ± 0.01 | 0.780 ± 0.12 |
| 16 | 0.746 ± 0.03 | 4.615 ± 0.43 | 0.271 ± 0.01 | 1.969 ± 0.24 |
| 17 | 0.522 ± 0.04 | 3.080 ± 0.34 | 0.183 ± 0.01 | 0.762 ± 0.06 |
| 18 | 0.569 ± 0.02 | 3.928 ± 0.54 | 0.215 ± 0.02 | 1.290 ± 0.42 |
| 19 | 0.509 ± 0.03 | 3.080 ± 0.37 | 0.176 ± 0.00 | 0.799 ± 0.18 |
| 20 | 0.533 ± 0.02 | 3.654 ± 0.16 | 0.193 ± 0.01 | 0.894 ± 0.09 |
| 21 | 0.505 ± 0.01 | 3.209 ± 0.20 | 0.171 ± 0.01 | 0.839 ± 0.07 |

Values correspond to the arithmetic mean of 4 repetitions ± standard deviation.

Firmness

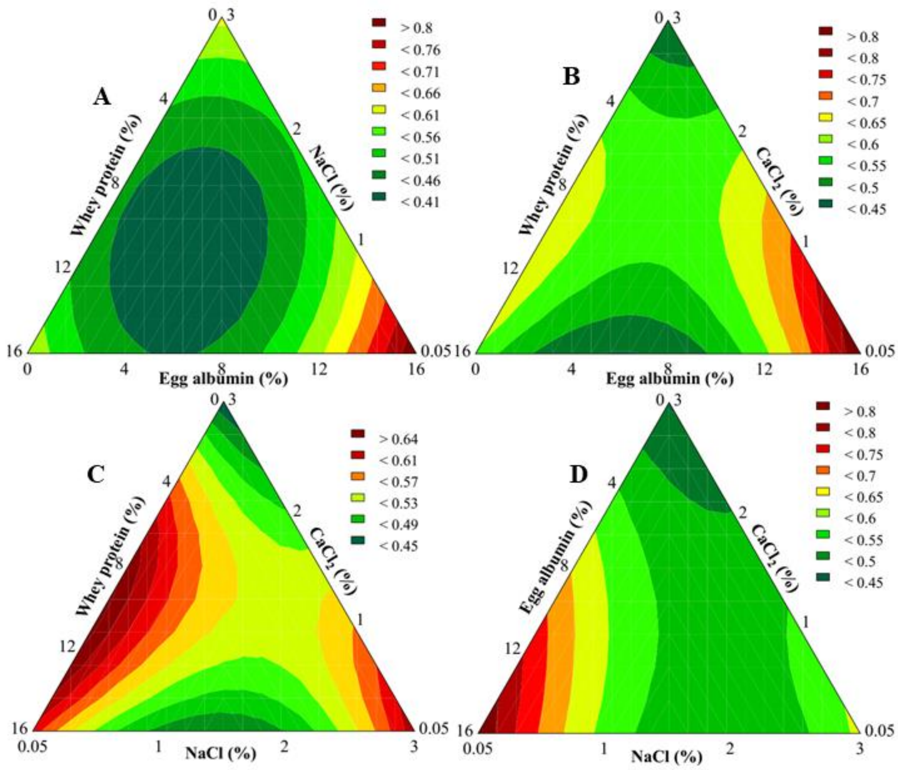
Firmness values were found to range from 0.402 to 0.810 N (Table 3), with 81.56% of the variability accounted for by the mathematical model (Equation 2), where: $x_1$, $x_2$, $x_3$, and $x_4$ represent the coded levels of whey protein, egg albumin, NaCl, and $CaCl_2$, respectively. Analysis of variance (ANOVA) resulted in an $F$-value ratio ($F_{calc}/F_{tab(7;13;0.10)}$) of 3.68 and a $p$-value of 0.001, thereby supporting the generation of contour plots (Figure 2).

$$\text{Firmness (N)} = 0.59x_1 + 0.85x_2 + 0.61x_3 + 0.44x_4 - 1.03x_1x_2 + 0.56x_1x_3 + 0.47x_1x_4 - 0.83x_2x_3 \quad \text{(Eq. 2)}$$

The mathematical model for firmness indicated that all individual factors contributed to an increase in firmness. The most pronounced effect was attributed to egg albumin ($\beta_2 = 0.846$, $p < 0.001$), a structural agent known for its ability to form stable protein networks through denaturation and thermal coagulation (Sun and Hayakawa, 2000). Whey protein ($\beta_1 = 0.591$, $p < 0.001$) also played a key role in gel structure formation, as its globular proteins contribute to the development of a more organized network, particularly when combined with calcium salts (Havea, 2006). Sodium chloride (NaCl) ($\beta_3 = 0.614$, $p < 0.001$) contributed to increased firmness, likely due to its effect on protein–protein interactions, which enhances the cohesiveness of the protein matrix (Damodaran, 2017). Calcium chloride ($CaCl_2$) ($\beta_4 = 0.444$, $p < 0.001$) assisted in promoting protein cross-linking, thereby strengthening the gel structure (Barbut, 2015).

In addition to the individual effects of these ingredients, their combinations also influenced the final texture of the product. A negative interaction between whey protein and egg albumin ($\beta_{12} = -1.027$, $p < 0.001$) suggested that this combination may reduce firmness, possibly due to competition between the two proteins for binding sites, which hinders the formation of a stable structure (Foegeding *et al.*, 2006). In contrast, a positive interaction between whey protein and NaCl ($\beta_{13} = 0.563$, $p = 0.020$) indicated that the presence of salt enhances protein network organization, resulting in a firmer gel (McClements, 2020). A similar effect was observed when whey protein was combined with $CaCl_2$ ($\beta_{14} = 0.467$, $p = 0.060$), supporting the notion that calcium plays an essential role in protein matrix stabilization (Havea *et al.*, 2009). However, the combination of egg albumin and NaCl ($\beta_{23} = -0.831$, $p < 0.001$) appeared to have the opposite effect, reducing firmness. This may be explained by the partial denaturation of egg albumin at high salt concentrations, which impairs its water-holding capacity and, consequently, results in a less firm final texture (Xiong, 1997).

**Figure 2 –** Contour plots for firmness (N) as determined by the Simplex Centroid Mixture Design.




Consistency

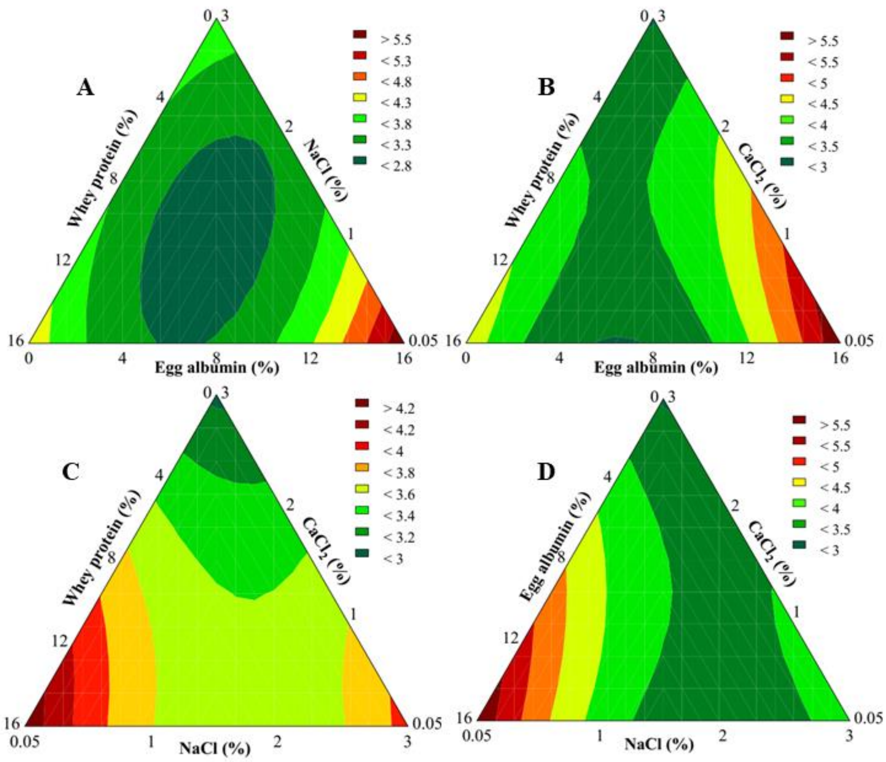
The consistency of the gels ranged from 0.433 to 5.883 N·s (Table 3). The mathematical model (Equation 3), where: $x_1$, $x_2$, $x_3$, and $x_4$ represent the coded levels of whey protein, egg albumin, NaCl, and $CaCl_2$, respectively, accounted for 84.39% of the experimental data, and the analysis of variance (ANOVA) yielded an $F$-value ratio ($F_{calc}/F_{tab(6;14;0.10)}$) of 5.64 with a $p$-value < 0.001, thereby supporting the generation of contour plots (Figure 3).

$$\text{Consistency (N·s)} = 4.35x_1 + 5.91x_2 + 3.89x_3 + 2.96x_4 - 8.38x_1x_2 - 2.66x_1x_3 - 6.45x_2x_3 \quad \text{(Eq. 3)}$$

The positive coefficients of the individual variables indicated that each ingredient contributed to increasing the consistency of the food matrix. Whey protein and egg albumin exhibit gelling properties, meaning they can form structural networks that enhance viscosity and increase the firmness of the system (Damodaran, 2017). Furthermore, the addition of salts such as NaCl and $CaCl_2$ affects protein solubility and promotes the formation of intermolecular bonds, thereby contributing to the stabilization of the gel structure (Sun and Holdsworth, 2018).

On the other hand, the negative coefficients observed for certain ingredient interactions ($\beta_{12}$, $\beta_{13}$, and $\beta_{23}$) suggested that specific combinations may lead to a decrease in system consistency. The negative interaction between whey protein and egg albumin ($\beta_{12}$), for instance, may be attributed to structural differences between these proteins, which tend to compete for binding sites, thereby hindering gel formation (Zhang *et al*., 2019). A similar phenomenon was observed in the interaction between whey protein and NaCl ($\beta_{13}$), in which the presence of salt may compromise the stability of the protein matrix, reducing its water-holding capacity and, consequently, impairing the consistency of the final product (Tang *et al*., 2020).

**Figure 3 –** Contour plots for consistency (N·s) as determined by the Simplex Centroid Mixture Design.



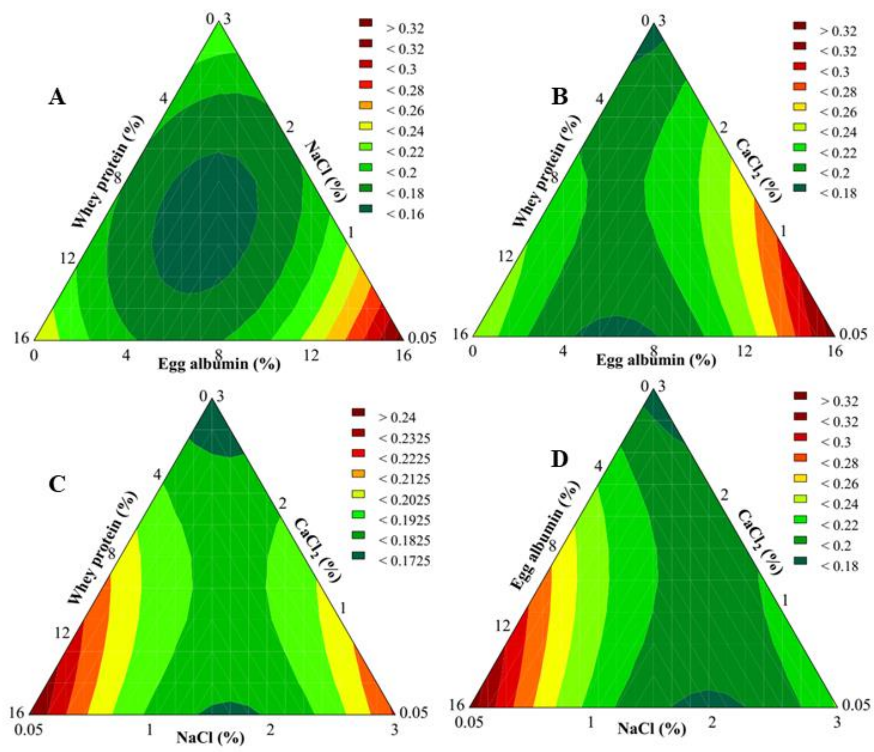

Cohesiveness

The cohesiveness values of the gels ranged from 0.242 to 0.323 N·s (N). ANOVA demonstrated that the $F_{calc}$ value was 6.40 times higher than $F_{tab(6;14;0.10)}$ ($p < 0.001$), and the mathematical model (Equation 4), where: $x_1$, $x_2$, $x_3$, and $x_4$ represent the coded levels of whey protein, egg albumin, NaCl, and $CaCl_2$, respectively, accounted for 85.99% of the experimental results, thereby allowing the construction of contour plots (Figure 4).

Proteins such as whey protein and egg albumin play a fundamental role in the formation of structural networks that enhance the product's resistance to deformation, thereby increasing the cohesiveness of the food matrix (Sun and Holdsworth, 2018). Additionally, the presence of salts such as NaCl and $CaCl_2$ influences protein–protein interactions, contributing to the stabilization of intermolecular bonds and improved moisture retention, which in turn leads to a firmer and more uniform structure (Damodaran, 2017).

$$\text{Cohesiveness (N)} = 0.24x_1 + 0.33x_2 + 0.22x_3 + 0.17x_4 - 0.43x_1x_2 - 0.21x_1x_3 - 0.37x_2x_3 \quad \text{(Eq. 4)}$$

**Figure 4 –** Contour plots for cohesiveness (N) as determined by the Simplex Centroid Mixture Design.




On the other hand, certain combinations of these ingredients may reduce the system's cohesiveness. The negative coefficients observed for the interactions between whey protein, albumin, and salts ($\beta_{12}$, $\beta_{13}$, and $\beta_{23}$) indicate that these components may compete with one another, affecting protein solubility and hindering the formation of a stable gel (Zhang *et* *al*., 2019). Specifically, the interaction between whey protein and egg albumin ($\beta_{12}$) appears to be impaired by structural differences between these proteins, which may compromise the organization of the protein network (Tang *et al*., 2020). Thus, the mathematical model demonstrates that the cohesiveness of the product is not solely dependent on individual ingredients, but also on the interactions among them. This factor is essential in the formulation of food products that require a well-defined and stable texture.

Viscosity index

The instrumental texture of the gels exhibited viscosity index values ranging from 0.660 to 3.019 N·s. The experimental results were explained in 88.98% by the mathematical model (Equation 5), where: $x_1$, $x_2$, $x_3$, and $x_4$ represent the coded levels of whey protein, egg albumin, NaCl, and $CaCl_2$, respectively, and based on the $F_{calc}/F_{tab(6;14;0.10)}$ ratio of 8.42 and $p <$ 0.001 from the ANOVA, the construction of contour plots was made possible (Figure 5).

$$\text{Viscosity index (N·s)} = 1.72x_1 + 3.00x_2 + 1.24x_3 + 0.63x_4 - 5.84x_1x_2 - 2.29x_1x_3 - 4.87x_2x_3 \quad \text{(Eq. 5)}$$

Trial 2 exhibited the highest viscosity index value, whereas trial 4 presented the lowest. Salts played a crucial role in the cohesion and viscosity of the gels, as they influenced intermolecular interactions and the organization of the gel network. Monovalent ions, such as $Na^+$ from sodium chloride, neutralized the negative charges of proteins and anionic polysaccharide groups, thereby reducing electrostatic repulsion and promoting closer molecular interactions, which enhanced the cohesion of the gel. This effect was more pronounced for the $Na^+$ cation than for the $Cl^-$ anion, due to the smaller atomic radius and higher polarity of $Na^+$, resulting in a more intense ionic strength compared to $Cl^-$ (Chu *et al*., 2019).

The positive coefficients of the individual variables ($\beta_1$ to $\beta_4$) indicated that each of these ingredients contributed to the increase in system viscosity. Both whey protein and egg albumin exhibited a strong ability to form gels and interact with water, creating a three-dimensional protein network that thickened the mixture (Zhu *et al*., 2020). Additionally, salts such as NaCl and $CaCl_2$ were found to influence protein hydration and the formation of intermolecular bonds, thereby modifying the viscosity of the food matrix (Guler, 2014). Conversely, the negative coefficients observed for some component interactions ($\beta_{12}$, $\beta_{13}$, and $\beta_{23}$) suggested an opposing effect, reducing viscosity. The combination of whey protein and albumin may result in competition for binding sites within the protein structure, impairing network cohesion and consequently decreasing viscosity (Schmidt, 2016). Similarly, the simultaneous presence of proteins and NaCl may affect solubility and molecular interactions, rendering the matrix less resistant to flow and, therefore, less viscous (Steffe, 1996). Figure 6 presents the texture profile curves obtained using the texture analyzer for trials #2, #11, and #19, corresponding to the highest and lowest firmness values and the central point, respectively.

**Figure 5 –** Contour plots for the viscosity index (N·s) according to the Simplex-Centroid Mixture Design.

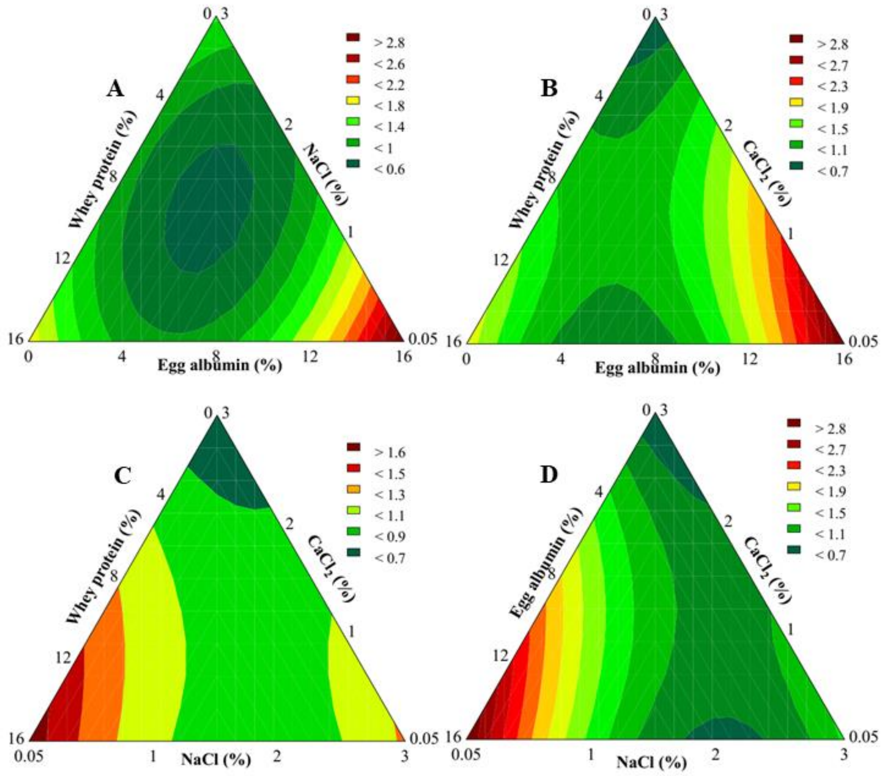
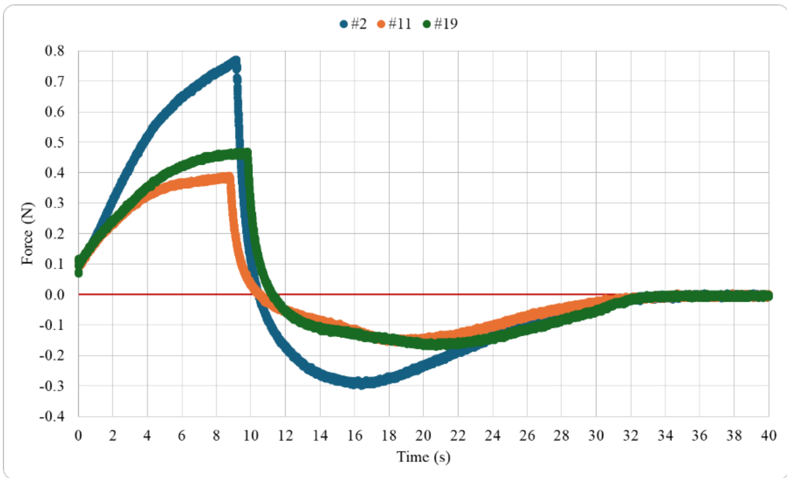



**Figure 6 –** Texture profiles of trials #2 (highest firmness), #11 (lowest firmness), and #19 (central point).




The overall behavior suggests that egg albumin was the independent variable that led to the highest texture values of the gels. Furthermore, the use of the same concentration (in coded levels) of whey protein, egg albumin, and NaCl did not meet the target condition in the study, a situation represented by the central point.

## Rheological behavior

The analysis performed using the viscometer enabled the acquisition of upward and downward flow curves, from which the hysteresis area was calculated, providing valuable insights into the rheological behavior of the formulations. Table 4 presents the mean values of the ascending and descending areas, hysteresis, and chewing viscosity.

**Table 4 –** Apparent viscosity and rheological behavior of the gels from the Simplex-Centroid Mixture Design.

| Trial | Area of the ascending curve (AUC) | Area of the descending curve (AUC) | Hysteresis (#AUC) | Chewing viscosity 10 $s^{-1}$ (Pa.s) |
|---|---|---|---|---|
| 1 | 7808 ± 1499 | 4939 ± 968 | 2870 | 4.72 ± 0.86 |
| 2 | 10336 ± 381 | 10249 ± 292 | 88 | 7.55 ± 0.17 |
| 3 | 6508 ± 599 | 6005 ± 548 | 504 | 4.56 ± 0.25 |
| 4 | 4789 ± 121 | 4605 ± 118 | 184 | 3.50 ± 0.07 |
| 5 | 4500 ± 99 | 4244 ± 115 | 256 | 3.30 ± 0.10 |
| 6 | 6058 ± 308 | 5810 ± 296 | 248 | 4.43 ± 0.23 |
| 7 | 5402 ± 906 | 4055 ± 382 | 1347 | 3.39 ± 0.45 |
| 8 | 4903 ± 198 | 4681 ± 153 | 222 | 3.62 ± 0.05 |
| 9 | 6223 ± 609 | 5774 ± 600 | 450 | 4.77 ± 0.48 |
| 10 | 5658 ± 587 | 5260 ± 598 | 398 | 4.28 ± 0.41 |
| 11 | 5800 ± 536 | 5653 ± 229 | 148 | 4.10 ± 0.14 |
| 12 | 4442 ± 555 | 4007 ± 374 | 434 | 3.02 ± 0.23 |

| | | | | |
|---|---|---|---|---|
| 13 | 5251 ± 328 | 4895 ± 326 | 356 | 3.89 ± 0.31 |
| 14 | 5597 ± 333 | 5337 ± 377 | 259 | 4.19 ± 0.14 |
| 15 | 5170 ± 213 | 4900 ± 249 | 270 | 3.81 ± 0.08 |
| 16 | 7356 ± 636 | 6835 ± 506 | 521 | 5.34 ± 0.38 |
| 17 | 5023 ± 326 | 4743 ± 360 | 280 | 3.77 ± 0.28 |
| 18 | 5794 ± 521 | 5376 ± 418 | 418 | 4.19 ± 0.22 |
| 19 | 5536 ± 271 | 5270 ± 152 | 266 | 4.13 ± 0.15 |
| 20 | 5703 ± 328 | 5372 ± 256 | 331 | 4.19 ± 0.23 |
| 21 | 5455 ± 124 | 5162 ± 90 | 294 | 3.84 ± 0.12 |

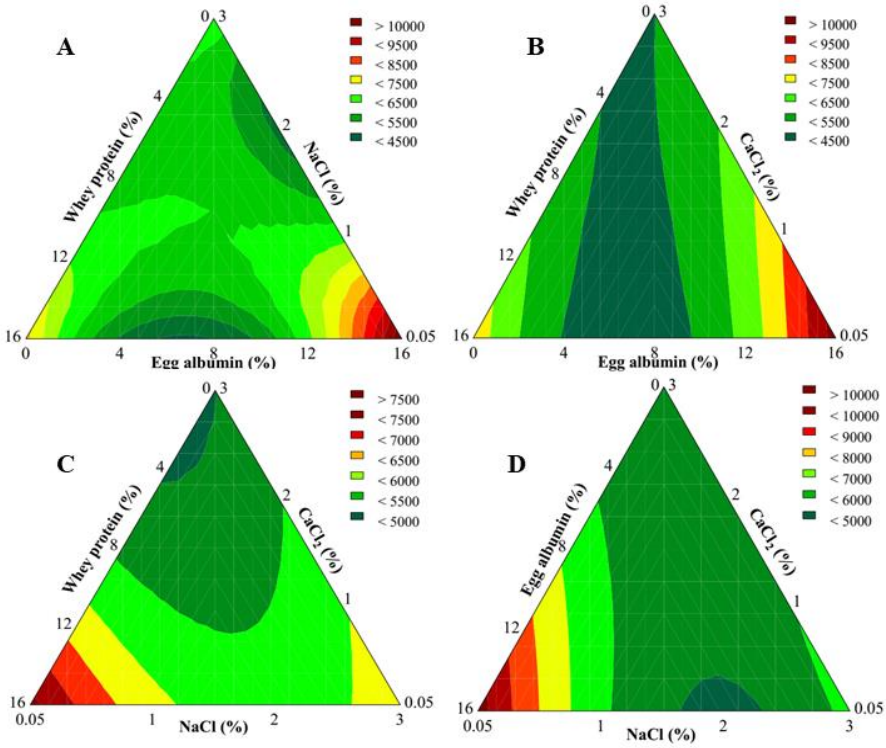
Values correspond to the arithmetic mean of 4 repetitions ± standard deviation. #Values calculated by difference (Ascending curve area – Descending curve area). AUC = Area Under Curve.

Area of the ascending curve

The values of the ascending area ranged from 4.442 to 10.336 (AUC), as shown in Table 4. The mathematical model presented in Equation 6 accounted for 92.70% of the experimental results, with the $F_{calc}$ value being 6.91 times higher than $F_{tab(9;11;0.10)}$, and a significance level of $p < 0.001$, according to the ANOVA, where: $x_1$, $x_2$, $x_3$, and $x_4$ represent the coded levels of whey protein, egg albumin, NaCl, and $CaCl_2$, respectively. These results enabled the generation of contour plots (Figure 7), thus allowing a general visualization of the influence of the independent variables on the rheological behavior of the gels as the shear rate increased.

$$\text{Ascending area (AUC)} = 7658x_1 + 10475x_2 + 6381x_3 + 4987x_4 - 18268x_1x_2 - 4832x_1x_3 - 3731x_1x_4 - 13113x_2x_3 - 4107x_2x_4 + 50038x_1x_2x_3 \quad \text{(Eq. 6)}$$

**Figure 7 –** Curvas de contorno para a área ascendente (area under curve) de acordo com o Planejamento de Mistura Simplex Centroide.




The addition of salts, such as NaCl and $CaCl_2$, can modulate the expansion of the gel network. Studies conducted by Zhang *et al*. (2021) have shown that intermediate ion concentrations favor a larger upward area, promoting a more porous and elastic structure. Increases in egg albumin or whey protein directly influence gel network expansion, enhancing both elasticity and mechanical strength (Tavakolipour and Labbafi, 2022).

On the other hand, the negative coefficients observed in the interactions between certain ingredients ($\beta_{12}$, $\beta_{13}$, $\beta_{14}$, $\beta_{23}$, and $\beta_{24}$) indicate that the combination of these components has an opposing effect, reducing the upward area. This may occur due to competition between proteins for binding sites, ultimately compromising structural stability and diminishing the system's capacity to withstand mechanical forces (Zhu *et al*., 2020). However, the ternary interaction among whey protein, egg albumin, and NaCl ($\beta_{123}$) presents a highly positive coefficient, suggesting a significant synergistic effect. This finding indicates that, under specific conditions, the combination of these ingredients may promote the formation of a stronger and more structured protein network, thereby contributing to the maximization of the upward area (Steffe, 1996).

Area of the descending curve

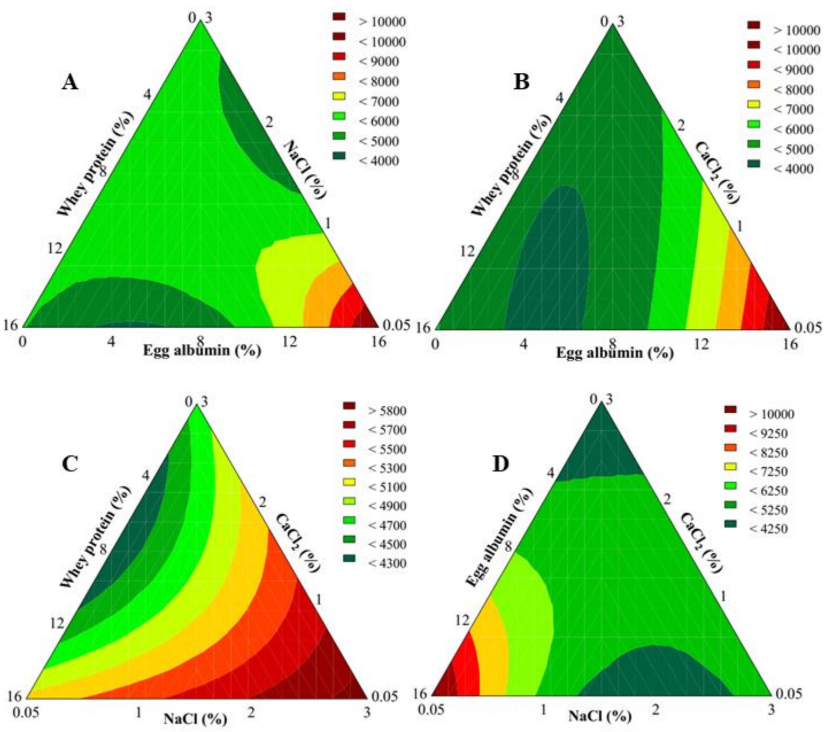
The descending curve ranged from 4007 to 10249 (AUC) for the developed gels.

ANOVA revealed a $F_{calc}/F_{tab(9;11;0.10)}$ ratio of 12.76 with $p < 0.001$, and the mathematical model (Equation 7) was able to explain 95.95% of the experimental results, where: $x_1$, $x_2$, $x_3$, and $x_4$ represent the coded levels of whey protein, egg albumin, NaCl, and $CaCl_2$, respectively, thereby enabling the generation of the contour plots shown in Figure 8.

High levels of egg albumin and whey protein promote greater compaction of the gel network, thereby increasing its mechanical resistance and, consequently, its downward area (Tavakolipour and Labbafi, 2022). The presence of NaCl can influence protein charges, leading to structural reinforcement that enhances gel cohesion (Martinez *et al.*, 2023). The negative coefficients of the binary interactions ($\beta_{12}$, $\beta_{14}$, $\beta_{23}$, and $\beta_{24}$) indicate that the combination of these ingredients exerts an antagonistic effect, resulting in a reduction of the downward area. This effect may be associated with competition among proteins for intermolecular binding sites, which compromises the structural cohesion of the protein network and weakens its stability (Schmidt, 2016).

$$\text{Ascending area (AUC)} = 5074x_1 + 10341x_2 + 5888x_3 + 4702x_4 - 13443x_1x_2 - 2696x_1x_4 - 14081x_2x_3 - 6325x_2x_4 + 45129x_1x_2x_3 + 20927x_2x_3x_4 \quad \text{(Eq. 7)}$$

**Figure 8 –** Contour plots for the descending area (area under the curve) according to the Simplex-Centroid Mixture Design.



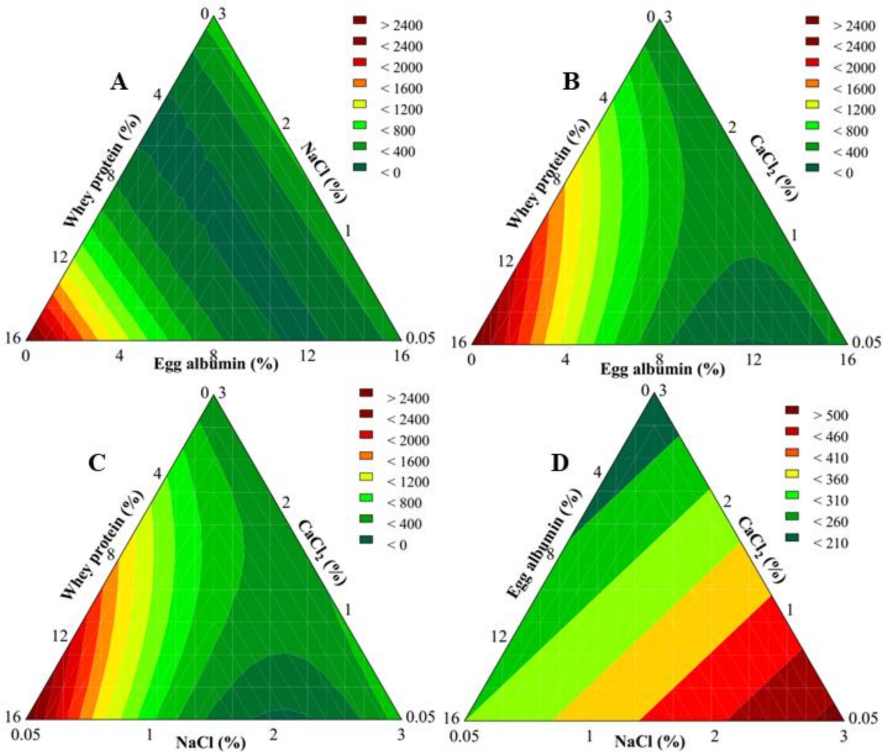

On the other hand, the positive coefficients of the ternary interactions ($\beta_{123}$ and $\beta_{234}$) suggest a synergistic effect, that is, the simultaneous presence of these components enhances the downward area. This behavior may be explained by the formation of a more cohesive protein structure when proteins and salts interact positively, thereby favoring system stability (Steffe, 1996). Thus, the analysis of the mathematical model demonstrates that the downward area is influenced not only by the individual effects of the ingredients but also by their interactions. This factor is essential for the development of food formulations with the desired textural properties.

Hysteresis

The hysteresis data, reported as the difference between the areas under the ascending and descending curves, ranged from 88 to 2870 (AUC). Trial #2 exhibited the smallest difference (corresponding to the highest concentration of egg albumin), whereas Trial #1 showed the greatest hysteresis (associated with the highest concentration of whey protein). The mathematical model (Equation 8) where: $x_1$, $x_2$, $x_3$, and $x_4$ represent the coded levels of whey protein, egg albumin, NaCl, and $CaCl_2$, respectively, accounted for 88.05% of the experimental data, with the ANOVA indicating a $F_{calc}/F_{tab(5;15;0.10)}$ ratio of 9.74 and $p < 0.001$. Accordingly, contour plots were constructed (Figure 9) to visualize the influence of the independent variables on hysteresis.

$$\text{Hysteresis (AUC)} = 2574x_1 + 300x_2 + 518x_3 + 209x_4 - 4740x_1x_2 - 5605x_1x_3 \quad \text{(Eq. 8)}$$

**Figure 9 –** Contour plots for hysteresis (area under the curve) according to the Simplex-Centroid Mixture Design.

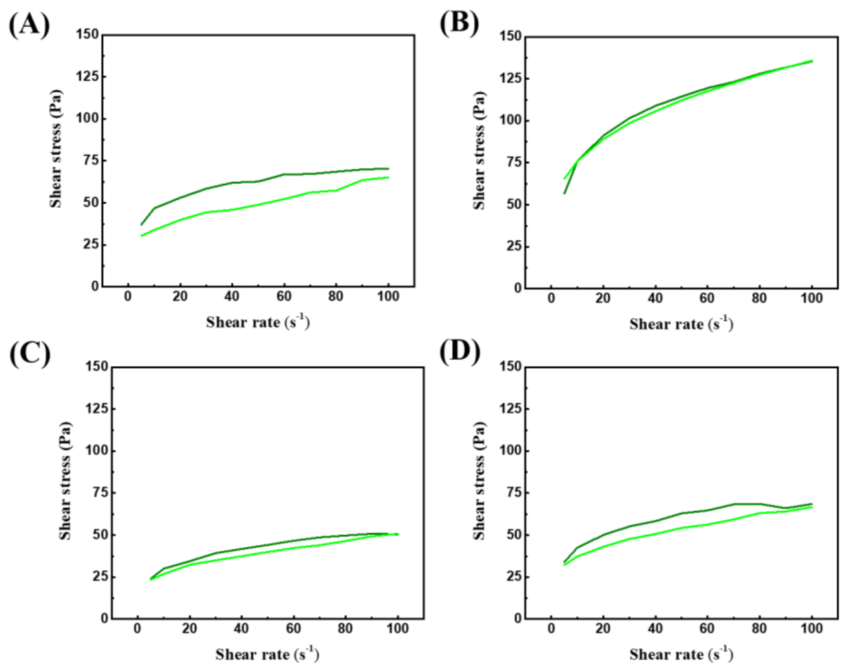



Whey protein ($\beta_1 = 2574$; $p < 0.001$) exhibited the greatest impact, suggesting that its addition significantly contributes to the retention of elastic energy within the material. This observation is consistent with studies demonstrating that whey protein forms highly structured gel networks, thereby enhancing the mechanical strength and viscoelasticity of food systems (Havea, 2006). NaCl may influence the protein network structure through ionic interactions, altering water-holding capacity and material plasticity (Damodaran, 2017).

Negative interaction coefficients indicate that certain ingredient combinations may reduce hysteresis. For instance, the interaction between whey protein and egg albumin ($\beta_{12} = -4740$; $p < 0.001$) suggests that, when used together, these ingredients diminish the system's elastic capacity. This effect may be attributed to competition between proteins for water and structural space, impairing the formation of well-organized protein networks (Foegeding *et al.*, 2006). Another negative effect was observed for the interaction between whey protein and NaCl ($\beta_{13} = -5605$; $p < 0.001$), indicating that salt may hinder whey protein's ability to form elastic networks. This outcome could result from NaCl-induced alterations in the electrical charges of proteins, affecting their aggregation and structuring behavior (McClements, 2020).

Figure 10 illustrates the hysteresis behavior according to selected formulations from the experimental design. The graphs illustrate the relationship between shear rate ($s^{-1}$) and shear stress (Pa) for different gel formulations. In Figure 10(A), it is observed that isolated whey exhibits a weaker response to shear stress, suggesting a less cohesive structure with a large hysteresis area. In contrast, Figure 10(B) shows that the presence of egg albumin results in a gel with little or no hysteresis; however, egg albumin renders the system highly resistant to shear.

**Figure 10 –** Visualization of the ascending (—) and descending (—) curves for the assessment of the hysteresis behavior in trials #1 (A) with the highest hysteresis, #2 (B) with the lowest hysteresis, #12 (C) with intermediate hysteresis, and #19 (D) corresponding to the central point of the Simplex-Centroid Mixture Design.



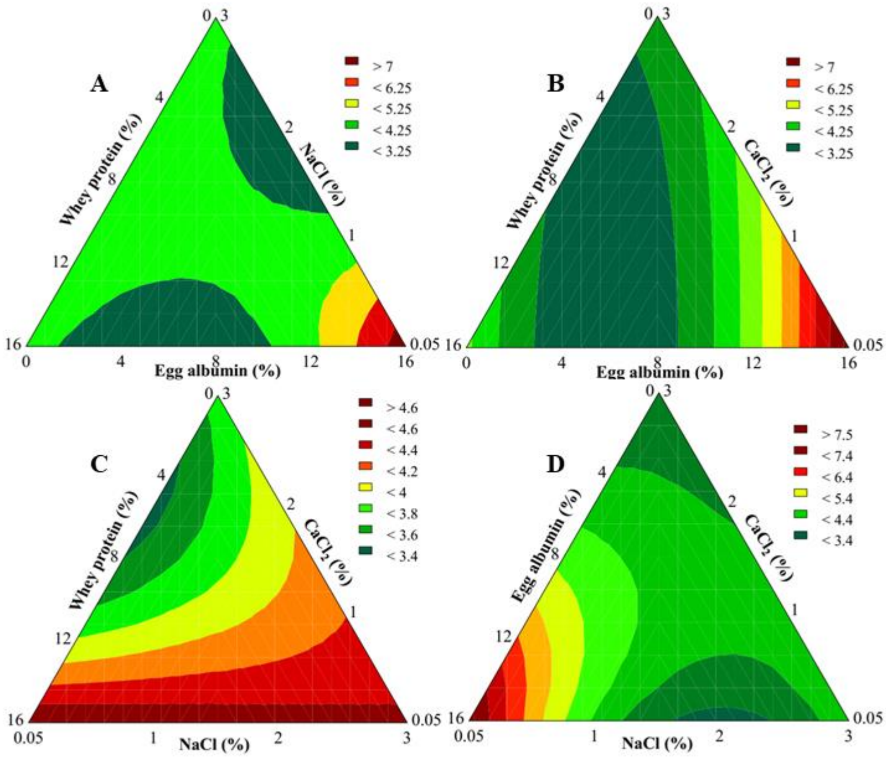

When whey and albumin were combined in equal proportions and the NaCl concentration was increased to 0.98 g, the ascending and descending curves became closer, indicating reduced hysteresis and low shear resistance. As the $CaCl_2$ amount was kept constant in assays #1, #2, and #12 (0.05 g) and elevated to 0.74 g in assay #19, its main effect can be observed in Figure 10(D), where an increase in shear stress and a reduction in hysteresis is evident.

Chewing viscosity

The chewing viscosity (10 $s^{-1}$) for the gels ranged from 3.02 to 7.55 Pa·s. The mathematical model (Equation 9) was able to predict 94.67% of the experimental data, and the ANOVA showed an $F_{calc}/F_{tab(9;11;0.10)}$ ratio of 9.57 ($p < 0.001$), where: $x_1$, $x_2$, $x_3$, and $x_4$ represent the coded levels of whey protein, egg albumin, NaCl, and $CaCl_2$, respectively, allowing the generation of contour plots (Figure 11).

$$\text{Chewing viscosity (Pa·s, 10 s}^{-1}) = 4{,}61x_1 + 7{,}68x_2 + 4{,}44x_3 + 3{,}70x_4 - 11{,}41x_1x_2 - 3{,}02x_1x_4 - 9{,}88x_2x_3 - 3{,}43x_2x_4 + 26{,}36x_1x_2x_3 + 15{,}66x_2x_3x_4 \quad \text{(Eq. 9)}$$

**Figure 11** – Contour plots for chewing viscosity (Pa·s, 10 $s^{-1}$) according to the Simplex-Centroid Mixture Design.




The positive coefficients of the individual variables ($\beta_1$ to $\beta_4$) indicate that each of these ingredients contributes to an increase in system viscosity. The presence of whey protein and egg albumin, both rich in proteins, favors the formation of a three-dimensional network, thereby densifying the matrix and increasing its viscosity (Zhu *et al*., 2020). Furthermore, the addition of salts such as NaCl and $CaCl_2$ may alter protein interactions, promoting either aggregation or solubilization, depending on the concentration applied (Guler, 2014).

Conversely, the negative coefficients of the binary interactions ($\beta_{12}$, $\beta_{14}$, $\beta_{23}$, and $\beta_{24}$) suggest that the combination of these ingredients may reduce viscosity. This effect may be attributed to competition among biopolymers for water molecules or to structural changes that hinder the formation of a cohesive protein network (Morris, 2009).

In contrast, the ternary interactions $\beta_{123}$ and $\beta_{234}$ exhibit highly positive coefficients, indicating a significant synergistic effect on viscosity. This behavior suggests that the simultaneous presence of these components strengthens the protein network, enhancing water retention and resulting in a thicker and more structured food matrix (Schmidt, 2016).

**Numerical optimization and validation of the mathematical model**

The optimal point (Table 5) was defined based on the mathematical models obtained through the analysis of the response surfaces from the Simplex Centroid Mixture Design experiments. The parameters for whey protein, egg albumin, NaCl, and $CaCl_2$ were adjusted within the studied range, with an importance factor of 3. For the statistically significant dependent variables, for which predictive mathematical models were obtained ($R^2 > 0.80$; $F_{calc}/F_{tab} > F_{tab}$; and $p < 0.10$), the parameters were maximized or minimized with an assigned importance of 5.

The optimal experimental point was defined as 14.26% egg albumin, 1.74% NaCl, and 0.03% $CaCl_2$, with a desirability value of 95.81%. The optimized formulation was processed in true quadruplicate, and the statistically significant dependent variables ($p < 0.10$) were evaluated to verify the validity of the mathematical models. Except for cohesiveness, which exhibited a relative deviation of less than 10%, the other mathematical models were not able to predict the results, as they presented relative deviations greater than 10%.

**Table 5 –** Numerical optimization for the determination of the optimal point of the Simplex

Centroid Mixture Design: Experimental vs. predicted values and relative deviations for model validation.

| Parameters | Goal | Importance | Solution | | |
|---|---|---|---|---|---|
| | | | Coded value | Real value | |
| **Independent variables** | | | | | |
| Whey protein | In range | 3 | 0.00 | 0.00 | |
| Egg albumin | In range | 3 | 0.98 | 14.26 | |
| NaCl | In range | 3 | 0.02 | 1.74 | |
| $CaCl_2$ | In range | 3 | 0.00 | 0.03 | |
| **Parameters** | **Goal** | **Importance** | **Predicted value** | **Experimental value** | **Relative deviation (%)** |
| **Dependent variables** | | | | | |
| Ascending area (AUC) | Maximize | 5 | 10094 | 8532 ± 456 | 19.54 |
| Descending area (AUC) | Maximize | 5 | 9932 | 8308 ± 412 | 18.31 |
| Hysteresis (AUC) | Minimize | 5 | 305 | 224 | 35.99 |
| Chewing viscosity (Pa·s, 10 $s^{-1}$) | Maximize | 5 | 7.39 | 6.57 ± 0.34 | 12.48 |
| Firmness (N) | Maximize | 5 | 0.82 | 0.73 ± 0.03 | 12.74 |
| Consistency (N·s) | Maximize | 5 | 5.72 | 5.09 ± 0.23 | 12.46 |
| Cohesiveness (N) | Maximize | 5 | 0.32 | 0.30 ± 0.03 | 7.00 |
| Viscosity index (N·s) | Maximize | 5 | 2.85 | 2.53 ± 0.35 | 12.69 |
| Desirability 95.81 % | | | | | |

## CONCLUSION

It was concluded that, through the Simplex-Centroid Mixture Design, the combination of egg albumin (0.98%), NaCl (0.02%) and $CaCl_2$ (0.00%) in coded levels, yielded the best result for gel structuring, proving to be more efficient in protein–salt interactions. The interactions between ingredients, such as whey protein and egg albumin, demonstrate how understanding the functional properties of food components can lead to innovations in food formulations, prioritizing both sensory quality and product functionality. Therefore, the findings not only contribute to the academic field but also have direct implications for the food industry, fostering significant advances that meet market demands and consumer needs.

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