Caracterization and optimization of nonfat yogurt based on texture properties: instrumental texture profile and rheological properties

Caracterização e otimização das propriedades de textura de iogurte sem gordura: perfil de textura intrumental e propriedades reológicas

Caracterización y optimización de yogur desnatado basado em propiedades de textura: perfil de textura instrumental y propiedades reológicas

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Abstract
Milk fat plays an important role in the texture of yogurt. Thus, producing fat-free yogurt is a challenge for the food industry. We investigated and modeled the effect of yogurt composition and process parameters on the texture properties obtained by analyzing the texture profile analysis (TPA) and on the rheological properties. The experiment followed the Box-Behnken (DBB) design with three factors: centrifuge rotational speed (CRS) (3000, 4250 and 5500 rpm), casein (Cas) (3.4, 4.9 and 6.4% p/v) and transglutaminase enzyme (TgE) (0, 1 and 2 U/g of protein) concentrations. A complete second-order model better explained the effect of factors on texture properties. Desirability function was selected for the optimization of responses (firmness, cohesiveness and apparent viscosity placed at a maximum level and adhesiveness, gumminess, thixotropy and loss tangent (tan δ) were placed at a minimum level). The results suggest that the addition of TgE showed less effect on texture properties. The optimized values of each factor, Cas, TgE and CRS were, respectively, 3.4%, 1 U/g of protein and 5500 rpm with general desirability (D) of 0.6531. Under these conditions, it is possible to prepare a concentrated fat-free yogurt with a higher protein content due to the concentration, obtained after centrifugation, as well as obtaining desirable texture characteristics such as greater firmness and consistency, low gumminess and adhesiveness.
Keywords: Rheology; Transglutaminase; Casein, Concentrate yogurt; Modeling**.**

Resumo
A gordura do leite desempenha um papel importante na textura do iogurte. Assim, a produção de iogurte sem gordura é um desafio para a indústria de alimentos. Nesse sentido, objetivou-se investigar e modelar o efeito da composição do iogurte e dos parâmetros do processo nas propriedades da textura obtidas por meio da análise do perfil da textura (TPA) e do estudo reológico. O experimento foi conduzido segundo Delineamento Box-Behnken (DBB) com três fatores: velocidade de rotação da centrífuga (CRS) (3000, 4250 e 5500 rpm), concentração de caseína (Cas) (3,4, 4,9 e 6,4% p/v) e de enzima transglutaminase (TgE) (0, 1 e 2 U/g de proteína). O modelo completo de segunda ordem explicou melhor o efeito dos fatores nas propriedades da textura. Para otimização simultânea das variáveis respostas,

foi utilizada a função desejabilidade (firmeza, coesão e viscosidade aparente ao ponto de máximo, enquanto a adesividade, gomosidade, tixotropia e tangente de perda (tan δ) ao ponto de mínimo). Os resultados sugerem que a adição de TgE teve menor efeito nas propriedades de textura. Os valores otimizados de cada fator Cas, TgE e CRS foram, respectivamente, 3,4%, 1 U/g de proteína e 5500 rpm com desejabilidade geral (D) de 0,6531. Nestas condições, é possível preparar um iogurte concentrado sem gordura com maior teor proteico devido à concentração obtida após centrifugação, bem como obter características de textura desejáveis, tais como maior firmeza e consistência e menor gomosidade e adesividade.
Palavras-chave: Reologia; Tranglutaminase; Caseína, Iogurte concentrado; Modelagem.

Resumen
La grasa de la leche desempeña un papel importante en la textura del yogur. Por ello, la producción de yogur sin grasa es un reto para la industria alimentaria. Investigamos y modelamos el efecto de la composición del yogur y de los parámetros del proceso sobre las propiedades de textura obtenidas mediante el análisis del perfil de textura (TPA) y reológia. El experimento siguió el diseño Box-Behnken (DBB) con tres factores: velocidad de rotación de la centrífuga (CRS) (3000, 4250 y 5500 rpm), caseína (Cas) (3.4, 4.9 y 6.4% p/v) y enzima transglutaminasa (TgE) (0, 1 y 2 U/g de proteína). Modelo completo de segundo orden explico el efecto de los factores sobre las propiedades de textura. Se seleccionó la función de deseabilidad para la optimización de las respuestas (la firmeza, la cohesividad y la viscosidad aparente se situaron en un nivel máximo y la adhesividad, la gomosidad, la tixotropía y la tangente de pérdida (tan δ) se situaron en un nivel mínimo). Los resultados sugieren que la adición de TgE mostró un menor efecto sobre las propiedades de textura. Los valores optimizados de cada factor, Cas, TgE y CRS fueron, respectivamente, 3.4%, 1 U/g de proteína y 5500 rpm con una deseabilidad general (D) de 0.6531. En estas condiciones, es posible preparar un yogur concentrado sin grasa con un mayor contenido en proteínas debido a la concentración, obtenida tras la centrifugación, así como obtener características de textura deseables como una mayor firmeza y consistencia, baja gomosidad y adhesividad.
Palabra clave: Reología; Transglutaminasa; Caseína; Concentrado de yogur; Modelado.

1. Introduction

Yogurt is one of the most popular fermented dairy products in the world and can be defined as the product resulting from the fermentative process of milk by *Streptococcus salivarius* subsp. thermophilus and *Lactobacillus delbrueckii* subsp. bulgaricus (Gharibzahedi & Chronakis, 2018). The most concentrated yoghurt is obtained through modifications of traditional manufacturing processes where it is possible to remove part of the acidic whey from the solid part or addition different components (Aprodu et al., 2012; Darnay et al., 2016).

Milk fat plays an important role in the texture of yogurt, so the production of fat-free yogurt is a challenge for the food industry. Thus, partial removal of whey by centrifugation and / or addition of casein protein (Cas) can be used to obtain fat-free yogurt with a desirable texture.

The enzyme transglutaminase (TgE) has been widely used in yogurt as an alternative to improve texture properties (Demirkaya & Ceylan, 2009; Gauche et al., 2009). This enzyme is produced by specific microorganisms and improves the desirable characteristics in the product, such as increased viscosity and consistency (Macedo & Sato, 2005). Some works relating the influence of TgE on the texture properties of yoghurts are found in literature (Darnay et al., 2016; Gauche et al., 2009; Iličić et al., 2014).

The growing demand for fat-free foods due to new eating habits on the consumers creates problems related to the consistency of yoghurts, presenting an unstable and more fragile structure, which can lead to sensory rejection (Steffe, 1996; Surber, Jaros, & Rohm, 2019). However, optimization studies applied to yoghurts are rarely found (Granato et al., 2010; Popović et al., 2013; Pakseresht et al., 2017) and there are no studies related to optimization of the texture profile and rheological characteristics of yogurt supplemented with transglutaminase.

Partial whey syneresis by centrifugation and/or the addition of casein protein (Cas) and transglutaminase enzyme (TgE) can be useful to obtain a desirable textured non-fat yogurt. In this context, the present study evaluated the effect of casein addition, the concentration of TgE and centrifugation speed on the rheological properties and texture profile of yogurt

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formulations. In addition, the most relevant parameters were simultaneously optimized using the desirability function in relation to the texture profile (TPA) and the rheological properties of non-fat yogurts.

2. Material and Methods

2.1 Material

The enzyme transglutaminase (WM 100U) and the casein (96%) were provided by Ajinomoto (Sao Paulo, Brazil) and Via Farma (Sao Paulo, Brazil), respectively. Skimmed-milk powder and sugar were purchased from local market (Viçosa, MG, Brazil). The culture (YC-X11 Yo-Flex) was purchased from Chr. Hansen (Sao Paulo, Brazil).

2.2 Methods

2.2.1 Experimental design

The experiments were conducted according to the Box-Behnken (DBB) design, to evaluate the influence of the factors concentration of the casein protein (CAS) (3.4%, 4.9% and 6.4%), concentration of the transglutaminase enzyme (TgE) (0, 1U and 2U) and the centrifuge rotational speed (CRS) (3000 rpm, 4250 rpm and 5500 rpm), in the different formulations of non-fat yogurt. The formulations were evaluated in three levels coded as -1, 0 and +1, representing the values as low, medium and high, respectively. The experimental conditions were determined based on tests performed previously and according to information obtained from the literature.

Three factors were studied with 5 repetitions of the central point, resulting in a total of 17 experiments.

2.2.2 Processing of nonfat yogurt

The mixture of reconstituted skimmed-milk powder (3.4%) and sugar (10%) was subjected to a heat treatment at 83 ºC for 30 min. The other concentrations were obtained by complementing the initial protein concentration present in powdered milk with casein. After the heat treatment the mixture was cooled to approximately 40 ºC for the addition of TgE (0, 1 or 2U/g protein), and this temperature was maintained for 90 min for enzyme action. Thereafter, the enzyme was deactivated by subjecting the mixture to 80 ºC for 2 min. The mixture was then cooled to 43 ºC and 1% lactic culture containing *L. delbrueckii subsp. bulgaricus* and *S. thermophilus* was added to start the fermentation process. This process took place until the mixture reached a pH between 4.4 and 4.6 and titratable acidity 0.65% expressed in percentage of lactic acid (w/w). At the end of fermentation, the yogurt was stored under refrigeration at 8 ºC. Then, the yogurt was centrifuged (3000, 4250 or 5500 rpm) at 10 ºC for 5 min, and the volume of acidic whey released was quantified. The yogurt was then refrigerated (± 8 ° C) in polyethylene containers for further analysis.

2.2.3 Physicochemical analyses

Analyses of pH, acidity and protein were used to characterize the samples of yogurt according to the established by normative Instruction nº 30 (Brasil, 2018). The pH was determined by the potentiometric method by introducing the electrode directly into the samples. The acidity was determined by titrating the samples with 0.1M NaOH, with phenolphthalein indicator until the turning point. Protein determination was performed using the micro Kjeldahl method. The measurement of water activity was performed in AquaLab series 4TE. The volume of partial whey syneresis by centrifugation was measured in a graduated cylinder. All measurements were performed in triplicate.

2.2.4 Texture profile analysis (TPA)

Texture profile analysis (TPA) was performed according to Vidigal et al. (2012), with modifications, and the firmness (N), cohesiveness (dimensionless), gumminess (N) and adhesiveness (J) parameters of the yogurts were determined using the universal machine testing (Instron Corporation, Norwood, MA, USA). A 15 mm diameter probe was used, which was moved perpendicularly under the yogurt sample, placed in a 50 mm diameter and 40 mm high container. The analysis conditions were: 250 N load cell, compression distance of 60% of the sample height, speed of 1 $mm.s^{-1}$, with two penetration cycles. The analysis was performed in triplicate.

2.2.5 Rheological measures

Flow curves were determined by applying a shear rate of 0.05 to 200.0 s^{-1} in three curves (ascending, descending and ascending) of 180 s each, measuring the corresponding shear stress (σ). The Herschel Bulkley and Power Law rheological models were adjusted to the experimental data (Eq. 1 and 2).

$$\tau = \tau_0 + k\,\dot{\gamma}^n \qquad \text{(Eq. 1)}$$

$$\tau = k\,\dot{\gamma}^n \qquad \text{(Eq. 2)}$$

where τ is the shear stress (Pa), τ_0 is yield stress (Pa), k is the consistency coefficient ($Pa.s^n$), n is the flow behavior index (dimensionless), and $\dot{\gamma}$ is the shear rate (s^{-1}).

The hysteresis area that represents a thixotropy measure was obtained by the difference between the curves (ascending and descending). Apparent viscosity was evaluated at a shear rate of 50 s^{-1} to compare the treatments (Yilmaz et al., 2015; Laguna, Farrell, Bryant, Morina, & Sarkar, 2017).

The viscoelasticity of yogurts was studied in dynamic oscillatory tests, where the linear viscoelastic region was determined by performing a strain scan (0.01–1000%) at a constant frequency of 1 Hz. Then a frequency scan was performed from 0.01 to 10 Hz, with a constant strain of 0.5% (according to the determined linear viscoelastic range). The results obtained at the frequency of 1 Hz were presented in terms of storage module (G') and loss module (G") as a function of frequency and Tan δ = G"/G' (Sendra et al., 2010).

2.3 Statistical analysis

2.3.1 Regression model

The effect of the factors Cas, TgE and CRS on the texture profile and rheological properties was evaluated by regression analysis, at a significance level of 5% of probability. A complete second-order model was selected as to explain the effect of factors on textural properties and rheological properties. The lack of adjustment, the regression coefficients and the determination coefficients were considered when choosing the model. Statistical analyses performed using Statistica software, version 8, at the significance level of 0.05.

The results of the texture profile analysis were also submitted to Principal Component Analysis (ACP), in which the data were organized in a treatment matrix (in rows) and the texture properties (in columns).

Pearson's correlation analysis was performed to investigate the relationship between the physicochemical parameters in relation to the texture profile and the rheological properties.

2.4 Desirability

The desirability function (Derringer & Suich, 1980) was used to perform simultaneous optimization of the significant response variables. The response variables estimated for each analysis performed in the present work were transformed into a desirable value (di) by the desirability function shown in Eq 3.

$$di = \begin{cases} 0 & \hat{y} \leq y_{i\,min} \\ \left[\frac{\hat{y}i - ymin}{yimax - yimin}\right] & y_{i\,min} < \hat{y} < y_{imax} \\ 1 & \hat{y} \geq y_{imax} \end{cases} \qquad \text{(Eq.3)}$$

Where:

- yi_{min} corresponds to the minimum value;
- yi_{max} corresponds to the maximum;
- ŷi corresponds to the acceptable value.

The values of individual desires were combined using Eq. 4, generating the global desirability (D).

$$D = (d1 \times d2 \times \cdots \times dk)^{1/k} \qquad \text{(Eq. 4)}$$

Overall desirability (D) is the geometric mean of the individual desirable values. Thus, the higher the D value the greater the desirability of the response, i.e., the maximum D value corresponds to the optimized solution.

The properties obtained by the texture profile analysis (firmness, cohesiveness, adhesiveness and gumminess) and rheological properties (apparent viscosity, thixotropy and Tan δ) were optimized.

3. Results and Discussion

3.1 Physicochemical analysis

The characterization of the yogurt samples in relation to pH, acidity, water activity (a_w), protein content and volume of partial whey syneresis by centrifugation is presented in table 1. No significant differences were found between the formulations ($p > 0.05$) for pH, acidity, and a_w. However, for final protein and volume partial whey syneresis, there was a significant difference between treatments ($p < 0.05$).

The acidity values are within the values established by the technical regulation of product identity and quality (0.6 a 1.5 g/ 100g de acid lactic) (Brasil, 2007), and the pH also presented values consistent with the range suggested in the literature (4,4 - 4,6) (Bong & Moraru, 2014; Pimentel et al., 2017; Şanli et al., 2011). Water activity is an important parameter related to product stability; the higher the free water content, the more susceptible the samples will be, which means, subject to enzymatic and / or microbiological modifications (Barros et al., 2020). Values close to those found in this work for this parameter, were reported by other authors, where the range obtained by them is between 0.9850 and 0.9900 (Karnopp et al., 2017). The final protein content of yogurts ranged from 2.7 to 7.5%. In a study by Tamime et. al., 2014, the authors concluded that the Greek name should not be applied to products that have a protein concentration below 5%, with the range found being

4.5 to 8% protein. Codex Alimentarius (2011) establishes a minimum value of 5.6% of protein for concentrated fermented milk. Thus, only formulations 7, 11 and 12 could be categorized as consistent yogurt.

The volume of acidic whey removed from the solid part was positively correlated with the protein content in the product (0.584, $p<0.05$), regardless of the concentration of added protein. Thus, the greater the volume removed in the centrifugation step, the greater the amount of protein in the yogurt**.**

Table 1. Values of pH, acidity, a_w, protein and volume of partial whey syneresis by centrifugation for non-fat yogurts.

Formulation	**Cas**	**TgE**	**CRS**	**pH**	**Acidity (g/100g de lactic acid)**	a_w	**Final protein (%)**	**Volume of partial whey syneresis (mL)**
1	-1 (3.4)	-1 (0)	0 (4250)	4.6	0.80	0.9727	5.2	405
2	1 (6.4)	-1 (0)	0 (4250)	4.4	1.21	0.9784	3.1	296
3	-1 (3.4)	1 (2)	0 (4250)	4.6	0.82	0.9803	2.7	308
4	1 (6.4)	1 (2)	0 (4250)	4.5	0.68	0.9857	4.9	400
5	-1 (3.4)	0 (1)	-1 (3000)	4.5	0.73	0.9819	3.4	192
6	1 (6.4)	0 (1)	-1 (3000)	4.5	0.76	0.9786	3.8	278
7	-1 (3.4)	0 (1)	1 (5500)	4.6	0.80	0.9797	6.2	440
8	1 (6.4)	0 (1)	1 (5500)	4.6	0.79	0.9754	4.0	235
9	0 (4.9)	-1 (0)	-1 (3000)	4.6	0.84	0.9790	3.6	189
10	0 (4.9)	1 (2)	-1 (3000)	4.5	0.66	0.9818	3.3	180
11	0 (4.9)	-1 (0)	1 (5500)	4.5	0.94	0.9785	7.3	535
12	0 (4.9)	1 (2)	1 (5500)	4.5	0.79	0.9792	7.5	270
13	0 (4.9)	0 (1)	0 (4250)	4.5	0.74	0.9786	3.7	221

Source: Authors.

3.2 Texture profile evaluation

Texture is widely used to assess the quality of yoghurt, being a very important parameter for its acceptance (Sah et al., 2016). The results obtained for the different formulations in relation to firmness, cohesiveness, gumminess and adhesiveness are shown in Table 2.

All factors studied had a significant influence ($p<0.05$) on texture properties. The adjusted equations for each response variable and the determination coefficient are described in Table 3. As they are considered complex equations, response surface graphs (Figure 1) and the main component analysis were used to assist in the discussion of the results.

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Table 2. Texture properties obtained by TPA of non-fat yogurts.

Formulation	Cas	TgE	CRS	Firmness (N)	Cohesiveness (N)	Gumminess (N)	Adhesiveness (J)
F1	-1 (3.4)	-1 (0)	0 (4250)	0.0549	0.51169	0.03014	-0.00060
F2	1 (6.4)	-1 (0)	0 (4250)	0.0251	0.07351	0.00422	-0.00040
F3	-1 (3.4)	1 (2)	0 (4250)	0.0305	0.25414	0.01445	-0.00035
F4	1 (6.4)	1 (2)	0 (4250)	0.0336	0.21233	0.01216	-0.00051
F5	-1 (3.4)	0 (1)	-1(3000)	0.0170	0.59651	0.03467	-0.00038
F6	1 (6.4)	0 (1)	-1(3000)	0.0259	0.09473	0.00562	-0.00045
F7	-1 (3.4)	0 (1)	1 (5500)	0.0649	0.68589	0.03901	-0.00086
F8	1 (6.4)	0 (1)	1(5500)	0.0238	0.10041	0.00582	-0.00026
F9	0 (4.9)	-1 (0)	-1 (3000)	0.0157	0.55177	0.03271	-0.00031
F10	0 (4.9)	1 (2)	-1 (3000)	0.0160	0.60779	0.03492	-0.00029
F11	0 (4.9)	-1 (0)	1 (5500)	0.0884	0.95527	0.05450	-0.00096
F12	0 (4.9)	1 (2)	1 (5500)	0.0282	0.13933	0.00803	-0.00038
F13*	0 (4.9)	0 (1)	0 (4250)	0.0263	0.48531	0.02809	-0.00031
σ				0.0020	0.03690	0.0021	0.000033

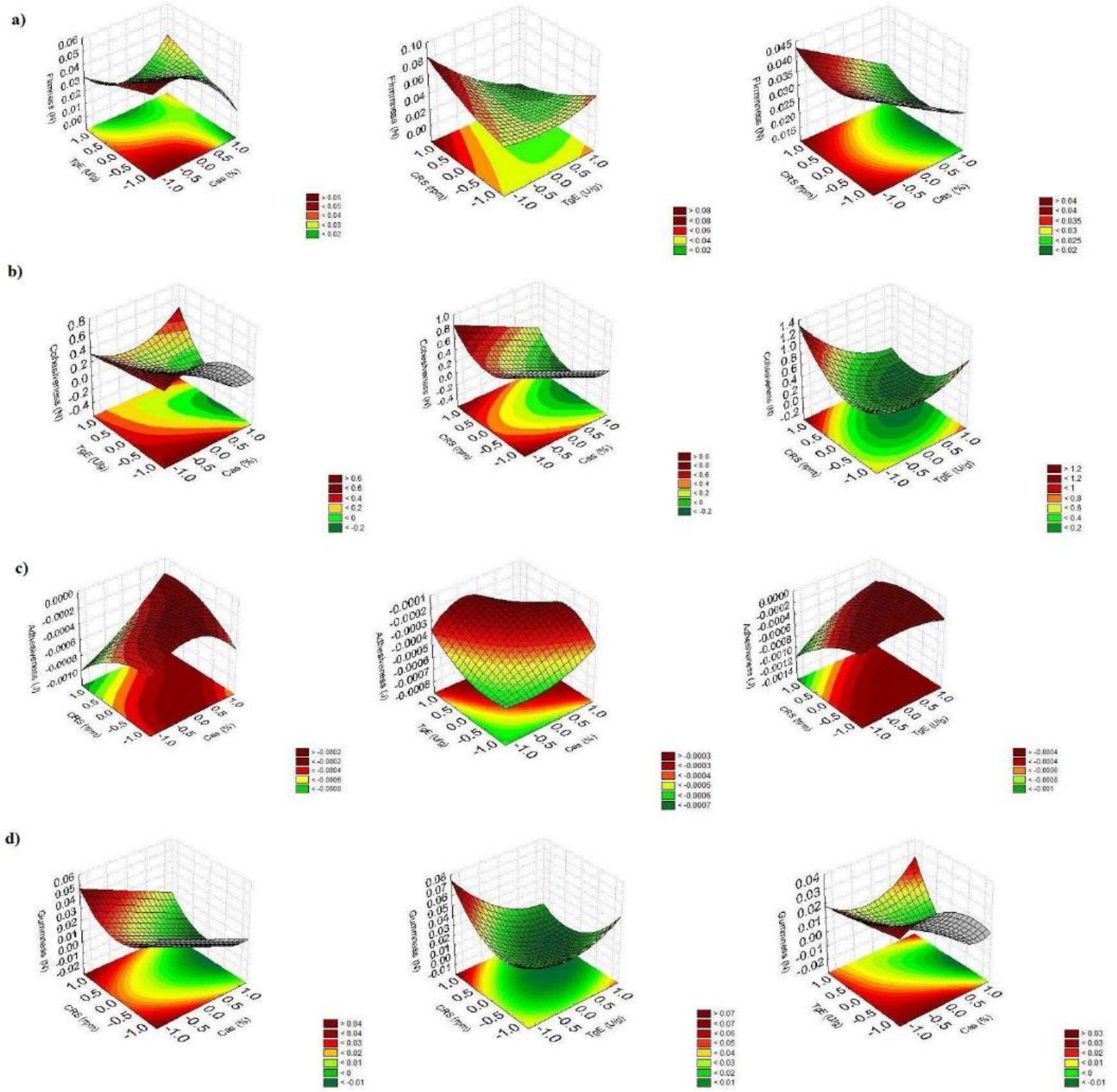
Source: The authors.

Table 3. Equations adjusted for the properties obtained in the Texture Profile Analysis (TPA) and rheology of yogurts as a function of the different concentrations of protein (Cas), enzyme (TgE) and centrifuge rotation speed (CRS).

Response variable	Equation	R^2
Firmness	0.0327 - 0.0074 CAS - 0.0150 TgE + 0.0212 CRS + 0.0082 CAS*TgE + 0.0110 CAS^2*TgE - 0,0125 CAS*CRS + 0.0098 CAS^2*CRS- 0.0151 TgE*CRS	0.9515
Gummness	0.0232 - 0.0198 CAS - 0.0100 TgE + 0.0104 TgE^2 + 0.0164 CRS^2 + 0.0118 CAS*TgE +0.0085 CAS*TgE^2 + 0.0091 CAS^2*TgE – 0.0122 TgE*CRS	0.9911
Adhesiveness	-0.0003 + 0.000132 CAS – 0.000080 CAS^2 + 0.000150 TgE – 0.000077 TgE^2 – 0.000185 CRS – 0.000100 CRS^2 – 0.000090 CAS*TgE – 0.000123CAS*TgE^2 – 0.000115 CAS^2*TgE + 0.000168 CAS*CRS+ 0.000113 CAS^2*CRS + 0.000140 TgE*CRS	0.9935
Cohesiveness	0.0911 - 0.2718 CAS - 0.1900 TgE + 0.1793 TgE^2 + 0.2857 CRS^2 + 0.0991 CAS*TgE + 0.1518 CAS*TgE^2 + 0.1603 CAS^2*TgE – 0.2180 TgE*CRS	0.9909
Apparent Viscosity	0.4965 – 0.1555 CAS + 0.0671 CAS^2 – 0.3465 TgE + 0.2143 TgE^2 + 0.5395 CRS + 0.1506 CRS^2 + 0.2265 CAS*TgE - 0.2370 CAS*TgE^2 + 0.0835 CAS^2*TgE - 0.3435 CAS*CRS – 0.3668 CAS^2*CRS - 0.3625 TgE*CRS	0.9989
Thixotropy	1913.89 - 13.88 CAS - 413.96 CAS^2 - 2306.79 TgE + 1275.51 TgE^2 +2394.02 CRS + 1021.73 CRS^2 + 786.37 CAS*TgE – 870.71 CAS*TgE^2 + 1255.78 CAS^2*TgE – 712.55 CAS*CRS – 2048.00 CAS^2*CRS - 2322.37 TgE*CRS	0.9970
Tan δ	0.4460 + 0.0488 TgE + 0.1504 CRS^2 + 0.1780 CAS*CRS - 0.0770 TgE*CRS - 0.1005 TgE^2*CRS^2	0.7924

Source: Authors.

Figure 1. Response surface of properties: (a) firmness, (b) cohesiveness, (c) adhesiveness, and (d) gumminess in relation to the factors Cas, TgE and CRS.



Source: Authors.

The rotation speed of the centrifugation step had the greatest influence on firmness, since a greater amount of acidic whey removed resulted in a firmer structure, with a higher protein content. This relationship has already been confirmed through the positive correlation between protein content and greater volume of centrifuged acidic whey (see 3.1). The increase in protein concentration causes an increase in water retention capacity of yogurt, changing its texture, resulting in increased firmness (Morr & Ha, 1993).

However, the addition of proteins and enzymes had a negative effect on the firmness, which means that low concentrations of these variables resulted in an increase in the firmness of yogurts. Milanović et al., (2009) and Iličić et al. (2014) reported similar results that indicated that the use of low concentrations of transglutaminase enzymes promoted greater firmness due to the formation of covalent bonds between the glutamine and lysine residues of the most stable proteins (Kuraishi et al., 1996; Martins et al., 2014).

Gumminess and cohesiveness were also influenced by the high speed of the centrifuge, with higher values of these variables being obtained at higher speeds. However, when assessing the relationship between protein and enzyme addition, an increase in cohesiveness was obtained when low concentrations were used. This result implies that a high force is required to disintegrate the sample, which is not desirable for yogurt.

However, regardless of the concentration of casein protein added (Cas factor), what influenced the texture was the final protein concentration of the yogurt. Thus, it is suggested that the enzyme transglutaminase formed intermolecular protein interactions and increased the consistency of the product. In another study conducted by Raak et al., (2016), the authors attribute the gel's lower stiffness to excessive crosslinking.

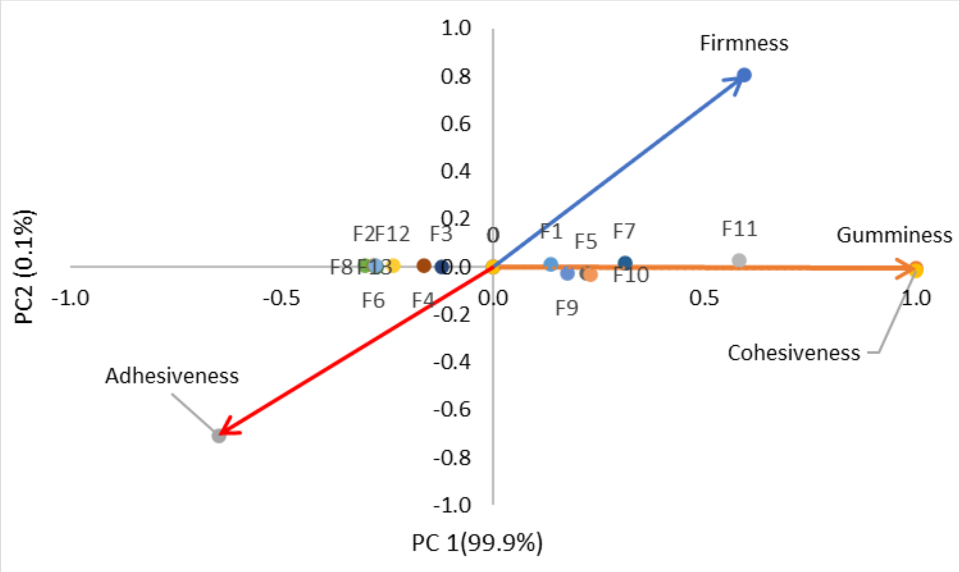
The lower concentrations of enzyme promoted an increase in the firmness and cohesiveness properties due to the formation of a more stable and uniform protein structure as a function of the bonds formed. According to Rigo et al. (2016) e Kuraishi et al., (1996), when excessive enzyme loads are used there is a loss of stability, compromising the structure of the formed gel.

Adhesiveness, in turn, was positively influenced by all factors, wherein higher concentrations of enzyme, protein and higher rotation speeds caused great adherence of the material to the probe. Domagała, Sady, Grega, & Najgebauer-Lejko (2007), when evaluating yogurts treated with TgE, found that the enzyme promoted greater adhesion to the product. This adhesion can be explained by the formation of additional bonds of protein produced by the TgE enzyme, making the force required to remove the product greater (Gauche et al., 2009; Zhang et al., 2012).

TPA results were also evaluated by multivariate analysis (Figure 2) to better visualize the differences between the formulations in relation to texture properties. The first principal component (PC1) explained 99.9% of the data variation, being, therefore, sufficient to discriminate the treatments. Considering cluster analysis, the spatial separation of formulations is formed by two distinct groups: group 1 (F1, F5, F7 F9, F10, and F11), and group 2 (F2, F3, F4 F6, F13, F8 and F12). Group 2 formulations present greater adhesiveness (negatively correlated with PC1) and lower firmness, cohesiveness and gumminess properties that were positively correlated with the PC1 ($p < 0.10$). In general, formulations with lower concentrations of enzyme (TgE) (F1, F5, F7, F9 and F11) belonging to Group 1, indicate that the addition of enzymes should be done with caution in order to favor the consistent texture of the non-fat yogurt.

The final protein content and volume of partial whey syneresis by centrifugation were positively correlated with firmness ($r = 0.721$, $r = 0.820$, respectively, $p<0.05$). Thus, a higher concentration of protein favors the interaction between them, resulting in a firmer and more consistent product (Agarwal et al., 2015; Körzendörfer et al., 2019; Shleikin et al., 2016). The study of the correlation of physicochemical characteristics with texture measures for yogurt was investigated by other authors (Güler & Park, 2011; Amani et al., 2017; Laiho et al., 2017), with good correlations being obtained.

Figure 2. PCA (Principal Component Analysis) of textural properties for non-fat yogurts**.**



Source: Authors.

3.3 Rheological behavior

The rheological behavior was evaluated by means of rotational and oscillatory tests, where it is possible to evaluate the behavior and structure of the formulations according to the applied conditions.

3.3.1 Rotational tests

The investigation of the flow behavior of fluids showed that, the Power Law and Herschel Bulkley models fitted to the experimental data, and the values of initial stress (τ_0), consistency index (k) and flow behavior index were also obtained (n), in addition to the apparent viscosity at the deformation rate of 50 s^{-1} (η_{50}) and the thixotropy of yogurts (Table 4).

Table 4. Properties obtained from the rheological models, apparent viscosity and thixotropy of non-fat yogurt formulations with different concentrations of protein (Cas), transglutaminase enzyme (TgE) and centrifuge rotation speed (CRS).

Formulation	Power Law			Hershel-Bulkley				η_{ap50}	Thixotropy
	K(Pa.s)	n	R^2	τ_0 (Pa)	K (Pa.s)	n	R^2	(Pa.s)	(Pa.s^{-1})
F1	12.53	0.33	0.9911	-30.30	35.07	0.20	0.9962	0.93	5497.40
F2	3.42	0.47	0.9939	5.53	1.43	0.61	0.9982	0.42	2155.49
F3	8.88	0.34	0.9979	2.14	7.60	0.37	0.9980	0.68	1822.65
F4	1.86	0.58	0.9895	6.09	0.49	0.81	0.9990	0.35	1626.20
F5	4.16	0.38	0.9973	3.25	2.48	0.46	0.9988	0.35	1476.97
F6	9.04	0.36	0.9988	3.41	7.10	0.39	0.9992	0.73	2874.31
F7	20.44	0.31	0.9890	-71.30	78.09	0.15	0.9970	1.39	3594.10
F8	1.35	0.65	0.9763	6.72	0.71	0.74	0.9988	0.39	2141.25
F9	1.86	0.54	0.9918	4.67	0.61	0.73	0.9984	0.31	1801.53
F10	2.70	0.48	0.9930	4.96	0.98	0.64	0.9987	0.34	1832.69
F11	29.01	0.32	0.9734	-291.80	285.00	0.08	0.9932	2.11	11234.30
F12	6.79	0.42	0.9939	9.37	2.83	0.56	0.9982	0.69	1975.99
F13	4.24	0.46	0.9925	8.20	1.34	0.65	0.9988	0.50	1913.89

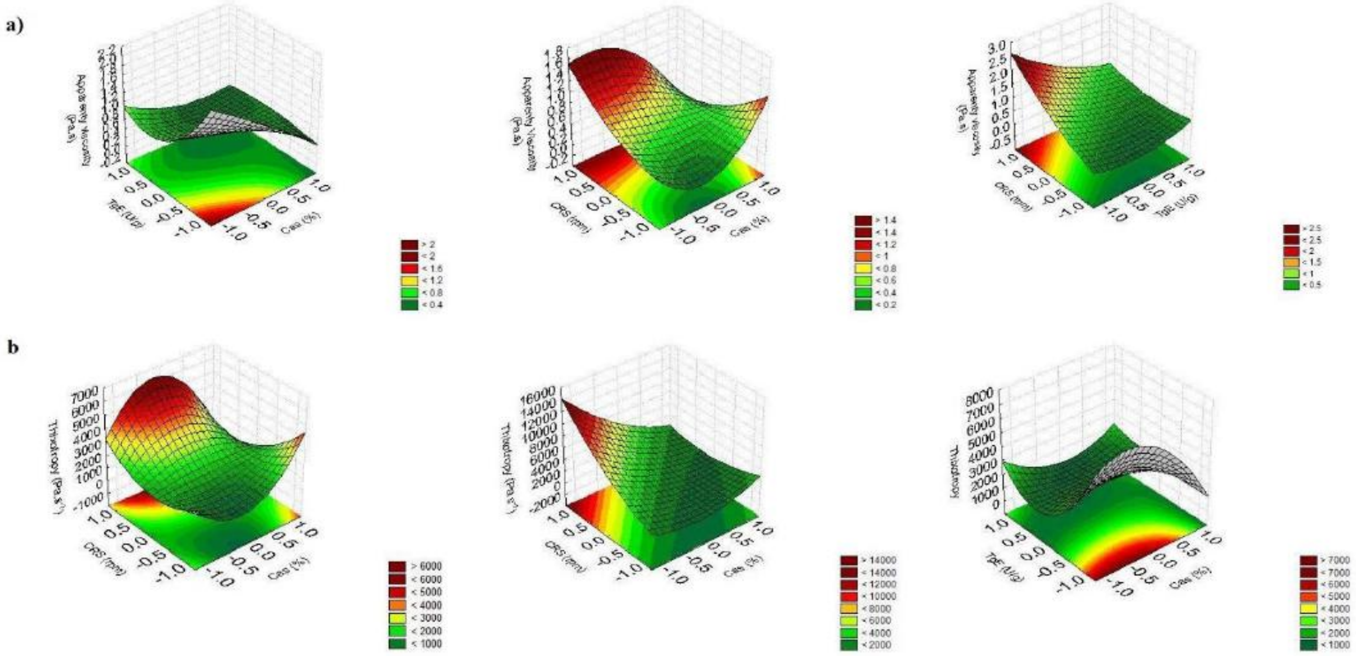
Source: Authors.

In general, yogurts can be characterized as pseudoplastic fluids, i.e., there is a reduction in apparent viscosity with the increase in deformation rate, demonstrated by n<1 (Yu et al., 2016). The Herschel-Bulkley model can be used to represent the flow behavior of yogurt formulations with R^2> 0.99, which indicates excellent fit of the model to the experimental data.

However, formulations 1, 7 and 11 showed negative initial stress values, with no physical significance for such values. Thus, the rheological behavior of these formulations can be represented by the Power Law model, which is also used to characterize the flow of yogurts (Yilmaz et al., 2015).

The apparent viscosity obtained at the deformation rate of 50 s^{-1} and thixotropy were significantly influenced by all factors studied ($p < 0.05$). In Table 3 is shown the adjusted equations, and in Figure 3 is presented the response surface graphs.

Figure 3. Response surface of rheological properties: (a) Apparent viscosity and (b) Thixotropy in relation to the factors Cas, TgE and CRS.



Source: Authors.

Higher apparent viscosity values were achieved when a low concentration of enzymes is used, favoring the formation of cross-linkages between them, which generates a more stable structure. The rotation speed also had a strong influence on apparent viscosity because there is a higher concentration after removal of part of the acidic whey from the solid part. The final protein content of the product was also positively correlated with the consistency index (k) (r = 0.639) and apparent viscosity (η_{ap}) (r = 0.691) and negatively correlated with the behavior index (r = - 0.500) (p< 0.05). The apparent viscosity values found were similar to those obtained in a study performed by other authors when establishing the correlation for this rheological parameter in yoghurts, ranging from 0.13 to 0.76 (Shleikin et al., 2016; Abou-Soliman et al., 2017).

Thixotropy is another measure used in rheological characterization and is related to the reduction of viscosity as a function of time. Its occurrence is the result of the breaking of weak bonds between proteins that reflects in the degree of structure disruption due to the shear applied. Therefore, the higher the thixotropy, the lower the structural strength of the network formed during shear (Tabilo-Munizaga & Barbosa-Cánovas, 2005; Gomes & Penna, 2009; Iličić et al., 2014). Formulations 11, 1 and 7 showed higher values of thixotropy (greater area of hysteresis). The presence of the bonds formed between enzyme and proteins, as well as bonds formed between the proteins themselves and the greater acidic whey volume removed in the centrifugation can explain this result in formulations 1, 7 and 11 (Gomes & Penna, 2009; Aprodu et al., 2011). The significant positive correlation between the volume of acidic whey removed and the final protein content with thixotropy (r=0.660 e r=0.626, respectively, p<0.05), reinforces that the increase in thixotropy is related to the increase in these two variables. Thus, there may be an increase in the breakdown of weak interactions when the product is submitted to stress, like storage time, compromising its structure.

3.3.2 Oscillatory tests

The viscoelastic properties of fluids can be characterized using the storage module (G') and the loss module (G"). The G' is related to the viscous characteristic of the material and the G" to its elastic nature. Another property that can be used is the loss tangent (Tan δ = G"/ G'), which measures the relationship between the viscous and elastic components of the viscoelastic material (Ramirez-Santiago et al., 2010; Rosa et al., 2018) (Table 5).

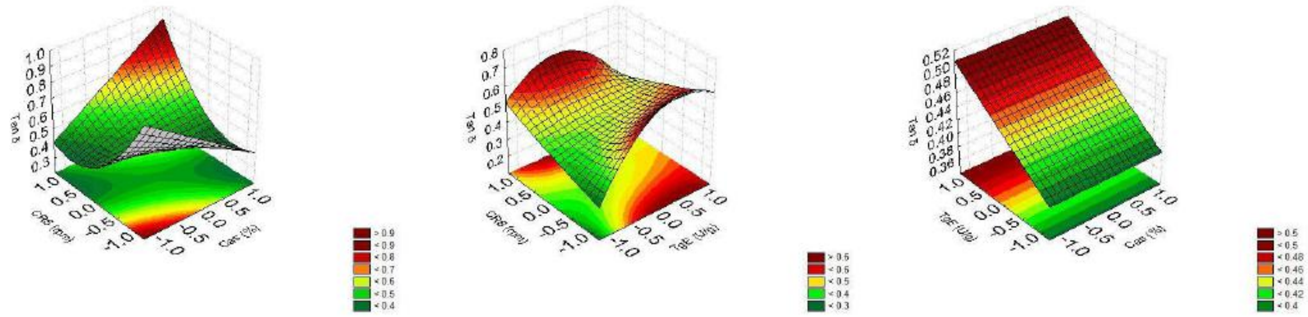
Table 5. Viscoelasticity properties of non-fat yogurts.

Formulation	G'	G"	Tan δ
F1	1969.32	690.73	0.3507
F2	303.98	107.39	0.3533
F3	1741.11	687.20	0.3947
F4	301.54	118.38	0.3926
F5	522.62	424.42	0.8121
F6	1628.18	724.91	0.4452
F7	2924.32	1144.87	0.3915
F8	1043.72	768.88	0.7367
F9	285.11	109.40	0.3837
F10	247.27	170.87	0.6910
F11	5621.96	2555.54	0.4546
F12	813.73	369.48	0.4541
F13*	2801.11	1432.16	0.4989
σ	1751.00	925.76	0.0347

Source: Authors.

Factors studied also had a significant influence ($p<0.05$) on the Tan δ, with the adjusted equation shown in Table 3 and the corresponding response surface graphs is shown in Figure 4.

Figure 4. Response surface of the Tan δ property in relation to the Cas, TgE and CRS factors.



Source: Authors.

The yogurt formulations showed G' values greater than G", indicating a predominance of elastic properties over viscous properties at the frequency of 1Hz. The values of Tan δ <1 are also indicative of this predominance, and when these values are low, they indicate a behavior more similar to that of a solid (Yeung, Lee & Chang, 2019). Similar results were reported by Lobato-Calleros, Ramírez-Santiago, Vernon-Carter, & Alvarez-Ramirez (2014) and Sendra et al. (2010) when assessing the rheological characteristics of whipped yogurt. In addition, Tan δ showed dependence on the applied frequency for all formulations, whose value decreased with increasing frequency. This result was similar to the results found by Pereira et al. (2016) when evaluating the sensory properties of probiotic petit suisse. The authors observed that the interaction between protein and rotation speed influenced the most on the sensory properties, leading to lower Tan δ values when these two variables increased.

3.4 Optimization of rheological measures and texture profile

Performing a univariate analysis of the investigated texture properties is difficult due to the influence of several factors. Thus, application of the desirability function, which simultaneously optimizes the dependent variables, is an interesting option for defining the best combination in order to find the desirable answer. To optimize rheological measurements and the texture profile, apparent viscosity, firmness and cohesiveness were maximized, and thixotropy, Tan δ, adhesiveness and gumminess were minimized. The maximization or minimization of these variables depends on how they will influence the response and the intended objective. Therefore, the desirability surface graphics are represented in Figure 5.

In Figure 5 we can observe that in the studied range the general desirability was 0.65307. This result was based on the best relationship between the parameters chosen when using 3.4% protein, 1 U of enzyme and rotation speed of 5500 rpm; in which the parameters obtained represent one of the formulations studied, where the final protein content reached was 6.2%. Thus, upon satisfying all the restrictions applied, the proposed objective of achieving the best relationship between the enzyme factor, protein and centrifugation speed for optimization was achieved.

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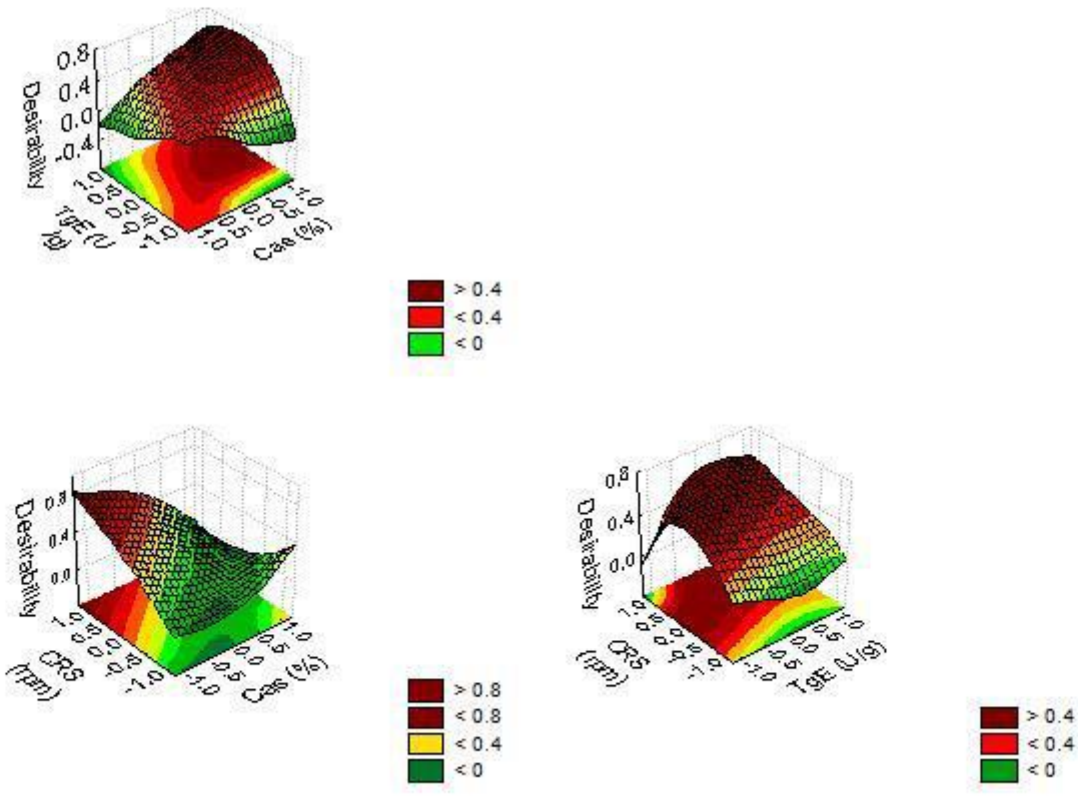
Figure 5. Desirability response surfaces as a function of Cas, TgE and CRS, for optimization of the thixotropic, apparent viscosity, Tan δ, firmness, adhesiveness, cohesiveness and gumminess variables.



Source: Authors.

4. Conclusion

The texture of skimmed yogurt was affected by the speed of rotation of the centrifuge and the composition of the product, with the increase in viscosity in some formulations and improved texture. Therefore, the use of this step in the processing of yogurt can be an alternative to obtain a more concentrated product, using a lower concentration of proteins, with a firmer texture and a high nutritional value.

The CRS influenced both the texture profile and the rheological properties since the removal of the acidic whey from the solid part led to the concentration of the other components. This result can be confirmed by the significant correlation between the final protein content of yogurts and volume of partial whey syneresis by centrifugation. The final protein content had a great impact on the texture properties (firmness and rheological measurements), resulting in a firmer product, with higher levels of consistency and apparent viscosity, characteristics that are interesting for yogurt.

From the established optimum condition containing 3.4% protein, 1U of enzyme goes rotational speed of 5500 it is possible to obtain a product with greater stability, which presented greater firmness, gum, viscosity, elasticity and less adhesion and thixotropy (properties that negatively affect yogurt texture) being a potentially more nutritious product with better acceptance.

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