Effect of the addition of chickpea protein on the physicochemical properties of low-fat yogurt

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Abstract:

In this work, the effect of concentration of chickpea protein and time fermentation on the properties of low-fat yogurt was evaluated and compared with two control, fat complete (FC) and reduced fat (RF). The results show that the concentration of chickpea protein affected the water-holding capacity of the yogurt but had no significant effect on acidity values; however, the treatment that presented the best WHC was Y0.5CP8h with 97.47 %. In the other hand the time of fermentation affected de acidity of the yogurts, with the fermentation time of 8 h being the one that affected the acidity. Both parameters, concentration of protein and time of fermentation, showed significant effects on viscosity of the yogurt, but the factor that had a greater effect on the viscosity was the protein concentration, although the treatment that presented de higher initial viscosity value was Y0.5CP8h. The results of the sensory properties demonstrated that there was no significant difference in overall acceptability of the different yogurt treatments. These results demonstrated that chickpea protein is a potential alternative as a source to produce functional dairy foods.

Keywords: viscosity, acidity, sensory, acceptability, chickpea, yogurt.

INTRODUCTION

Yogurt, a fermented dairy beverage highly valued for its nutritional composition, has gained popularity among consumers. The rising demand for low-fat yogurt stems from the well-documented association between fat intake and an increased risk of obesity, arteriosclerosis, hypertension, and certain cancers (Ribes *et al.*, 2020). Nevertheless, reducing yogurt's fat content can influence its sensory attributes and physical properties (Zhao *et al.*, 2018; Lamarche, 2021). To tackle this concern, diverse alternatives to fat have been explored, including whey protein gel emulsion microparticles (Li *et al.*, 2022), chia mucilage (Ribes *et al.*, 2020), and agavins (Santiago-García *et al.*, 2021). However, recent lifestyle trends favoring healthier choices have prompted the investigation of plant proteins as substitutes for animal proteins in food products (Gao *et al.*, 2015). The incorporation of high-quality plant proteins in the diet contributes to a reduced incidence of health conditions such as cancer, diabetes, and cardiovascular ailments. Plant proteins possess functional properties encompassing foam formation, gelation, thickening, and emulsification, in addition to exhibiting antioxidant, antifungal, and antibacterial activities, thereby making them valuable components in food formulations (Kumar *et al.*, 2021). The structural differences between plant and animal proteins pose challenges for direct substitution in various products, significantly affecting sensory properties (*Day et al.*, 2022).

Historically, legumes were associated with poverty, but they have now gained recognition as a noteworthy source of plant proteins. Legumes exhibit high levels of lysine, aspartic acid, and arginine, while their content of methionine, cysteine, and tryptophan is comparatively low (Bessada et al., 2019; Venkidasamy *et al.*, 2019). Nevertheless, the advantageous gelling, thickening, and emulsifying properties of legume proteins make them valuable as functional food ingredients, enabling texture modification in food products (Bessada *et al.*, 2019; Shevkani *et al.*, 2019). Chickpeas, among legumes, have emerged as a crop of significant interest due to their status as a complete protein devoid of allergenicity and phytoestrogens. Chickpea flour, with over 70% protein content, has found application in infant foods and as an isolated ingredient. Legumes, including chickpeas, possess not only diverse nutritional compositions but are also effectively categorized based on their functionality (Goldstein and Reifen, 2022). Chickpeas, abundant in protein, boast a content ranging from 19 to 29 g/100 g, coupled with vitamins and minerals of higher biological value compared to other legume proteins, which range from 75 to 85 g/100 g (Sofi *et al.*, 2020). Recent investigations have explored the effects of oat proteins (Brückner-Gühmann *et al.*, 2019), hemp protein (Dabija *et al.*, 2018), and hydrolyzed grape seed protein (Varedesara *et al.*, 2021) on the physicochemical properties of yogurt. Nevertheless, no study has investigated the addition of chickpea protein and its impact on the fundamental properties of yogurt. Hence, this study aimed to evaluate the influence of chickpea protein supplementation on the physicochemical properties of low-fat yogurt.

MATERIALS AND METHODS

Chickpea seeds were obtained from a local market in the city of Salvatierra, Guanajuato, Mexico. All reagents used in this study were of analytical grade.

Chickpea protein extraction

Chickpea protein was obtained from chickpea flour through alkaline extraction. To extract the protein, the chickpea flour underwent defatting using the Soxhlet method with hexane as the solvent. Subsequently, the defatted flour was subjected to drying for 24 h at 35 °C to remove any remaining hexane. Once the excess hexane was eliminated, protein extraction was carried out following the methodology proposed by Ghribi *et al*. (2015) with slight modifications. Initially, the defatted flour was mixed with distilled water at a ratio of 1:10 and stirred for 10 minutes at 800 rpm. Then, the pH of the mixture was adjusted to 9.5 using 0.5 N sodium hydroxide and stirring continued for 40 minutes at 800 rpm. The resulting alkaline extract was centrifuged at 5000 rpm for 15 minutes at 4 °C, and the supernatant was filtered using filter paper. The pH of the filtrate was subsequently adjusted to 4.5 (isoelectric point) by adding 2.0 N HCl with continuous stirring for 30 minutes. The precipitated protein was isolated through centrifugation at 5000 rpm for 20 minutes at 4 °C. The obtained isolate underwent three washes with distilled water at a ratio of 1:10, followed by centrifugation at 5000 rpm for 20 minutes at 4 °C. Finally, the sediment was dried in a drying oven at 50 °C for 42 hours.

Proximal analysis of chickpea protein

The protein content was determined using the Bradford method, the fat, moisture, and ash content were determined according to the procedures described in the AOAC (2005), methods 920.39, 925.09 and 923.03, respectively.

Zeta potential

The chickpea protein was dissolved in deionized water to prepare a 1 mg/mL dispersion using a magnetic stirrer at 300 r/min for 5 min. The mean zeta potential of the dispersion was measured by dynamic light scattering using a particle size analyzer Mastersizer 3000 (Malvern, Worcestershire, UK) at room temperature.

Protein solubility

The protein solubility was determined from pH 4.5 to pH 9.0 using the method reported by Liang and Tang (2013), with some modifications. Protein solutions in destilled water were prepared with magnetic stirring for one hour, then centrifuged at 5000 rpm at 20 °C. The supernatant was recovered, and the protein content was determined by the Bradford method (1976). Protein content at pH 12.0 or 9.0 was defined as 100% solubility.

Yogurt preparation and fermentation

A 2^2-factorial design was employed for the preparation of chickpea protein-enriched yogurt, with the factors being protein concentration (0.2% and 0.5%) and fermentation time (5 and 8 hours), the levels of the factors were chosen to evaluate the minimal effect on the properties of the treatments. Additionally, two control treatments were prepared, one complete in fat (FC) and another reduced in fat (RF) and fermented for 5 h. These controls were used in the physicochemical properties of yogurts and sensory analysis to compare them with those added with chickpea protein. Yogurt was prepared using a combination of whole milk powder and skimmed milk powder to achieve a 1.3% fat content in RF and 2.6% fat content in FC. Additionally, a sugar quantity of 60 grams per liter of milk was utilized. All solid components, including chickpea protein, were hydrated for 12 hours. Subsequently, the mixture was homogenized and pasteurized at 83 °C for 12 minutes. After cooling the milk to 45 °C, it was inoculated with a blend of lactic acid bacteria (YC-X11Yo-Flex, Chr. Hansen, Denmark). Finally, fermentation took place at 45 °C for the duration specified by the experimental design. The different yogurt treatment was coded as follows: Y0.2CP5h (yogurt added with 0.2 % chickpea protein for 5 hours), Y0.2CP8h (yogurt added with 0.2 % chickpea protein for 8 hours), Y0.5CP5h (yogurt added with 0.5 % chickpea protein for 5 hours) and Y0.5CP8h (yogurt added with 0.5 % chickpea protein for 8 hours).

Physicochemical properties of yogurts

Fat content, acidity, pH, and total solids of yogurt samples

The fat content was determined using the Gerber method, acidity was measured using titratable acidity and expressed in Dornic degrees (°D), and the total solids content was determined by oven drying (AOAC, 2005). To measure pH, a pH meter HI 2221 was used. To measure the pH of the samples, the pH meter was first calibrated by 2 buffers 4 and 7.All analyses were performed following the yogurt preparation period.

Water holding capacity (WHC)

The water-holding capacity was assessed in yogurt samples after 7 days of preparation following the method outlined by Lalau *et al.* (2017) with slight modifications. Ten grams of each yogurt treatment were placed in 15 mL conical tubes at 4 °C ± 1 °C and subjected to centrifugation at 1250 x g for 10 minutes at 4 °C ± 1 °C. The resulting supernatant whey was meticulously decanted and weighed to determine the water-holding capacity, which was computed as the weight difference between the sample and the supernatant whey.

Yogurt viscosity

Yogurt viscosity was measured using a Brookfield RVF viscometer equipped with a number 4 needle at various speeds (10 rpm-100 rpm) to examine the relationship between viscosity and increasing speed. The measured viscosities were reported in Pa.s and the speed were converted from rpm to 1/s. The sample temperature at the time of viscosity reading was 15°C and its volume was 100 ml. This test was performed for each sample in three replications.

Sensory properties of yogurts

The sensory attributes of the yogurts were evaluated three days after preparation by 47 untrained people between 18 and 30 years old (28 men and 19 women) who regularly consume fermented dairy drinks. Randomized samples of yogurt (20 mL each) were presented to participants in plastic cups and coded for blind evaluation. Odor, flavor, creaminess, viscosity, and general acceptance were rated on a 9-point hedonic scale, with 9 indicating "extremely like" and 1 indicating "extremely dislike" (Meilgaard *et al.*, 1999).

Statistical analysis

Experimental measurements were conducted in triplicate, and the obtained data were expressed as the mean ± standard deviation. Variance analysis (ANOVA) was performed using a Tukey test with a 95% confidence level. To analyze the effect of protein concentration and fermentation time on the acidity, WHC and viscosity of the yogurts, it was used a factorial design 2^2. Data analysis was conducted using Statgraphics (Statistical Graphics Corp., Manugistics, Inc., Cambridge, MA, USA).

RESULTS AND DISCUSSION

Proximal composition of chickpea protein

The proximal composition of chickpea protein was 81.6 % of protein, 2.3 % fat, 8.87 % moisture, 3.68 % ash and 3.5 % carbohydrate, these results are slightly different from those reported by Mesfin *et al.,* (2021) who used two different chickpea varieties, Arerti and Natoli, this difference is possibly due to the variety of chickpea since in this work the kabuli variety was used.

Solubility of protein

The solubility of protein is very important for the formation of gels and foams and depends on the balance between the protein and the solvent besides to the protein-protein hydrophobic interaction (Ladjal-Ettoimi *et al.*, 2016), this interaction is a function of the surface charge, which is correlated with pH. The solubility characteristic of the protein at different pH are presented in the table 1. The lowest solubility of the protein occurred at low pH and as the pH increased, the percentage of solubility increased, with pH 9.0 presenting the highest value. Moser *et al.,* (2020) reported a similar value, they reported a solubility of 38% at pH 5.0 and 99% at pH between 8 and 10. According to Jones and Mcclements (2011) and Madadlou *et al.,* (2018), the low solubility at a pH close to the isoelectric point of the protein is due to the fact that they tend to aggregate due to the weak electrostatic repulsion that exists between them, in addition to the fact that at a pH close to the isoelectric point the water-protein interaction is minimal resulting in in a low solubility and an increase in the tendency to precipitation (Gehring *et al.*, 2011). The figure 1 shows the surface net charge of the chickpea protein at different value of pH, as the pH increases, the Z potential gradually changes until it reaches a negative maximum. On the other hand, the charges reveal that slightly below pH 4.5 corresponds to the isoelectric region of the protein where the net charge is zero and therefore there is no electrostatic repulsion and the solubility of the protein at that point is very low. However, at high pH values, the increase in the net negative charge of the protein dissociates the aggregates and solubility may increase. Finally, the Z potential profile is agreeing with the solubility profile of the protein, which indicates that the solubility of the chickpea protein is related to the net surface charge of the protein.

Table 1. Solubility of chickpea protein at different pH

pH	Solubility (%)
4.5	2.98
6.0	33.57
7.0	56.13
7.5	62.97
9.0	92.96

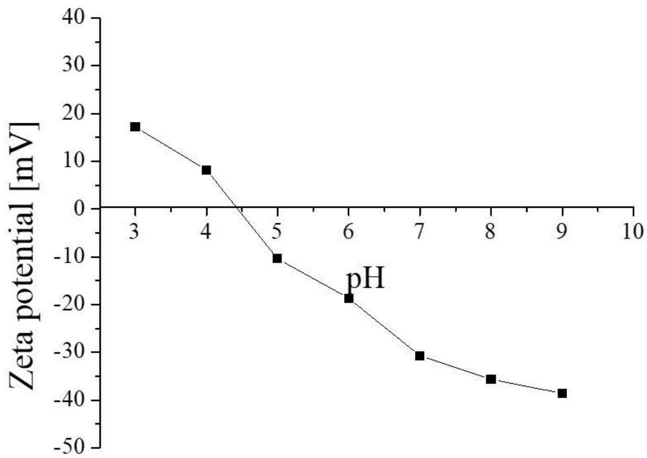

Figure 1. Zeta potential (net charge) at different pH (3.0-9.0) for chickpea protein.

Physicochemical characteristics of yogurts

The results of the physicochemical characteristics of the different yogurt treatments are presented in table 1. For all yogurt treatments, the pH was below 5.0. According to De Vuyst *et al*., 2001; Duboc and Mollet, 2001; Purwandari *et al.*, 2007, lower pH values indicate an increase in the production of lactic acid, which allows a reorganization and interconnection of the casein, resulting an increase in texture. There were some variations in the pH values, with the Y0.5CP8h treatment having the lowest value, followed by the Y0.2CP8H treatment, these values can be explained due to the duration of the fermentation time. Brückner-Gühmann et al., (2019) found low pH values (4.0) in yogurt added with oat protein fermented for 24 hours. Fermentation time also influenced acidity values, with the treatments that presented the highest acidity (°D) being those fermented for 8 hours (Y0.2CP8h and Y0.5CP8h), this because there was greater conversion of lactose to lactic acid by lactic acid bacteria.

The WHC of the yogurt is closely connected to its microstructure, which can characterize the colloid properties. Yogurts supplemented with chickpea protein retained a higher quantity of water compared with FC and RF, as shown by the greater water holding capacity value. The content of chickpea protein had an effect on WHC of yogurts (Table 1). Comparing the two level of concentration of chickpea protein present in the different treatments of yogurts, it can be noted that a small amount of chickpea protein present in the yogurt, has a less percentage of WHC compared with the treatments of yogurt that had a relative high amount of protein. The data analysis, showed a statistically significant difference between the treatments that contained a higher concentration of chickpea protein and those that contained a low concentration of chickpea protein and that without chickpea protein.

Table 2. Effect of the addition of chickpea protein at different concentration and fermentation time on the physicochemical properties of low-fat yogurt.

Sample	pH	Acidity [°D]	Fat content after fermentation [%]	WHC [%]	Total solids [%]
FC	4.78±0.05[a]	79.2±1.90[a]	2.6±0.0[a]	85.18±0.13[a]	18.80±0.00[a]
RF	4.72±0.03[a]	77.6±2.02[a]	1.3±0.0[b]	81.09±0.24[b]	16.00±0.00[b]
Y0.2CP5h	4.82±0.07[a]	77.5±2.12[a]	1.3±0.0[b]	95.50±0.14[c]	16.20±0.01[c]
Y0.2CP8h	4.55±0.03[b]	84.0±1.41[ab]	1.3±0.0[b]	96.15±0.07[c]	16.20±0.00[c]
Y0.5CP5h	4.66±0.00[ab]	78.5±2.12[ab]	1.3±0.0[b]	97.25±0.07[d]	16.51±0.03[d]
Y0.5CP8h	4.53±0.04[b]	84.5±0.71[b]	1.3±0.0[b]	97.5.0±0.28[d]	16.50±0.01[d]

Different superscripts in the same column are significantly different (*p<0.05*) by Tukey's test.

Values are presented as mean ± standard deviations.

Protein Concentration Effect and Fermentation Time

Effect on Acidity

Figure 2 shows the effect of chickpea protein concentration and fermentation time on the acidity values of yogurt treatments. It can be observed that the factor that had the greatest effect on acidity is the fermentation time, showing a linear effect, that is, as the fermentation time increases, the acidity of the yogurts increases. This can be explained by the fact that during the fermentation time of milk with lactic acid bacteria, lactose is converted into lactic acid, allowing acidification of the product. Soukoulis *et al.* (2007) found that as fermentation time increases, the pH of yogurt decreases. The analysis of variance shows an R^2 value of 0.87, which is statistically acceptable at a 95% confidence level. On the other hand, it can be observed that the concentration had no significant effect on acidity values. The first-order regression equation of acidity as a function of concentration and fermentation time, Equation (1), was formulated with regression coefficients significant at a 95% confidence level:

Acidity = 65.4444 + 6.11111*concentration + 2.27778*fermentation time – 0.0555556* concentration*fermentation time

Eq. (1)

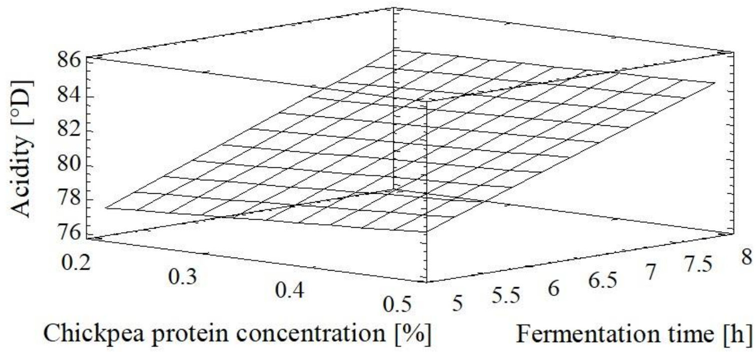

Figure 2. Response surface graph for the effect of chickpea protein concentration and fermentation time on the acidity of low-fat yogurt.

Effect on Water Holding Capacity (WHC)

Figure 3 illustrates the effect of chickpea protein concentration and fermentation time on the water holding capacity (WHC) of the yogurts. Water holding capacity is a physical property related to product ability to retain water within its three-dimensional structure (Levy *et al.,* 2021; Canon *et al.,* 2022). The response surface for WHC of the yogurt samples demonstrates the effect of chickpea protein concentration on this property. The observed effect is directly proportional to the concentration, as increasing the protein concentration leads to an increase in water holding capacity. The behavior exhibited by the treatments with higher concentrations of chickpea protein is related with greater stability of the three-dimensional network due to an increase in matrix rearrangement. Brückner-Gühmann *et al.* (2019) reported that the addition of oat protein to yogurt resulted in increased water holding capacity. This property is crucial in yogurt as it affects the quality of the product during storage. High WHC values are closely related to syneresis, and therefore, it could be an advantage for improving the shelf life of yogurts. The analysis of variance shows an R^2 value of 0.97, which is statistically acceptable at 95% confidence level. The first-order regression equation for WHC as a function of concentration and fermentation time, Equation (2), was formulated with regression coefficients significant at 95% confidence level:

WHC = 92.8056 + 8.05556*concentration + 0.305556*fermentation time – 0.444444*concentration*fermentation time

Eq. (2)

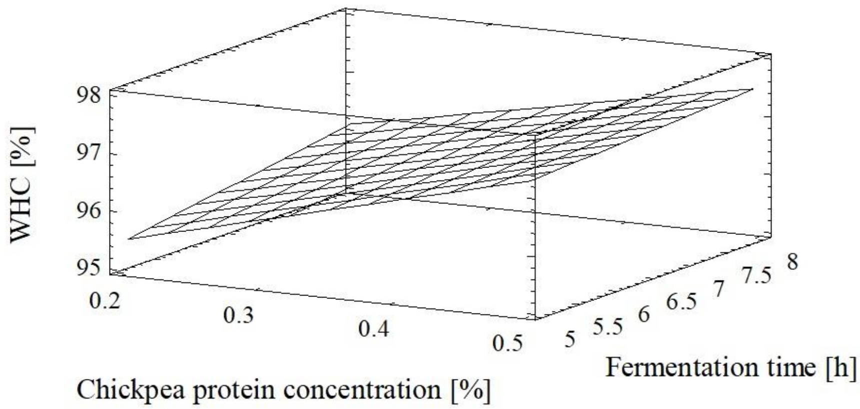

Figure 3. Response surface graph for the effect of chickpea protein concentration and fermentation time on the WHC of low-fat yogurt.

Viscosity behavior of yogurts

The viscosity behavior of the yogurts is shown in Figure 4 as a function of shear rate. It can be observed that all yogurts exhibited shear-thinning behavior, where viscosity decreased as shear rate increased. However, as the shear rate continued to increase, all samples displayed Newtonian behavior. Additionally, in Figure 3, the yogurt with 0.5% chickpea protein fermented for 8 hours (Y0.5CP8h) had a relatively high viscosity, followed by Y0.5CP5h, while the Y0.2CP5h treatment presented the lowest initial viscosity value. Yogurts added with 0.5% chickpea protein exhibited pronounced shear-thinning behavior at low shear rates values, which could be related to a high degree of molecular unfolding with respect to the shear rate. The apparent viscosities of the yogurts showed a maximum at low shear rate, corresponds the shear stress value known as the yield stress. At this value all yogurts start to flow, decreasing viscosity as the applied shear rate increases. This behavior is typical of a pseudoplastic material. Veredesara *et al.* (2021), found that by adding hydrolyzed protein to yogurt the viscosity increases. On the other hand, Dabija *et al.* (2018) also found that yogurt with protein of hemp increases its viscosity.

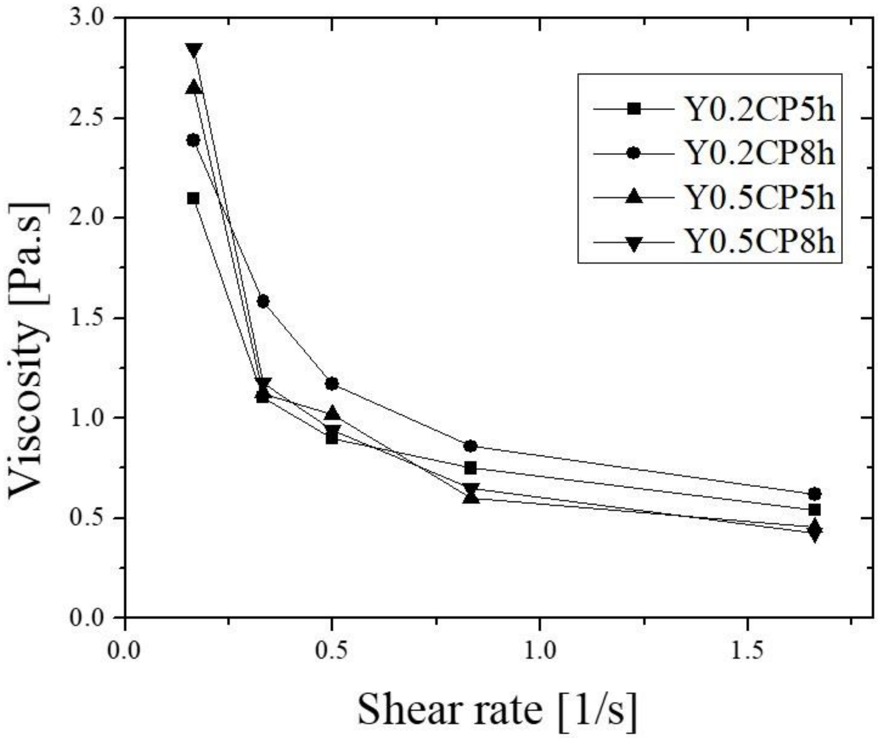

Figure 4. Shear rate dependence of viscosity of yogurts.

Effect of concentration and fermentation time on viscosity.

Figure 5 shows the response surface for viscosity. The analysis of variance presented an R^2 value of 0.93, which is statistically acceptable at 95% confidence level. The linear model for viscosity can be expressed by Equation (3), which was formulated with regression coefficients significant at 95% confidence level:

Viscosity = 1.58722 + 1.43889*concentration + 0.0672222* fermentation time – 0.0111111*concentration*fermentation time
Eq. (3)

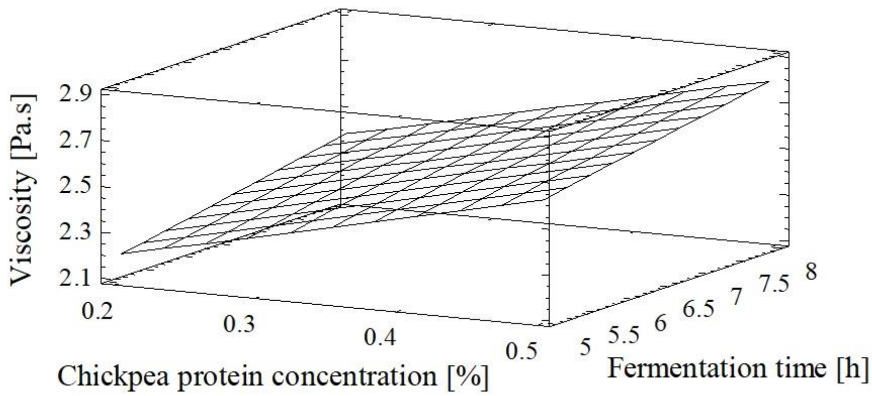

Figure 5. Response surface graph for the effect of chickpea protein concentration and fermentation time on the viscosity of low-fat yogurt.

Although both parameters showed statistically significant effects on viscosity with P=0.0022 and P=0.0317 for protein concentration and fermentation time, respectively, it can be observed that the factor that had a greater effect on the viscosity of the treatments was protein concentration. It is evident that as the protein concentration increases, viscosity also increases. Varedesara et al. (2021) reported that increasing the concentration of hydrolyzed grape seed protein in yogurts resulted in an increase in apparent viscosity, possibly due to an increase in dry matter and the binding of protein with free water.

Yogurt sensory properties

Sensory properties are one of the main factors influencing the acceptability or rejection of many products. Four yogurt treatments were prepared for sensory evaluation. Figure 6 shows the behavior of the sensory factors evaluated. It can be observed that there was no statistically significant difference in overall acceptability among all treatments ($P \geq 0.05$) with chickpea protein; however, it can be noted that there was a great difference in general acceptance between the controls and those yogurts added with chickpea protein, this difference, not only general acceptance but also the other sensory attributes between the controls and the treatments with chickpea protein is due to the chickpea flavor that the protein imparts to the yogurts. Despite that, among the yogurts that were added with chickpea protein, numerically, Y0.2CP5h had the highest value, followed by the Y0.5CP8h treatment. Regarding taste, all formulations had similar values: Y0.2CP5h (4.0), Y0.2CP8h (3.5), Y0.5CP5h (3.4), and Y0.5CP8h (3.8). The statistical analysis for this parameter reported that there was no statistically significant difference with a 95% confidence level. Mudgil *et al.* (2018), found that enhancement of yogurt texture is related to high values of sensory evaluation, for example, taste and overall acceptability.

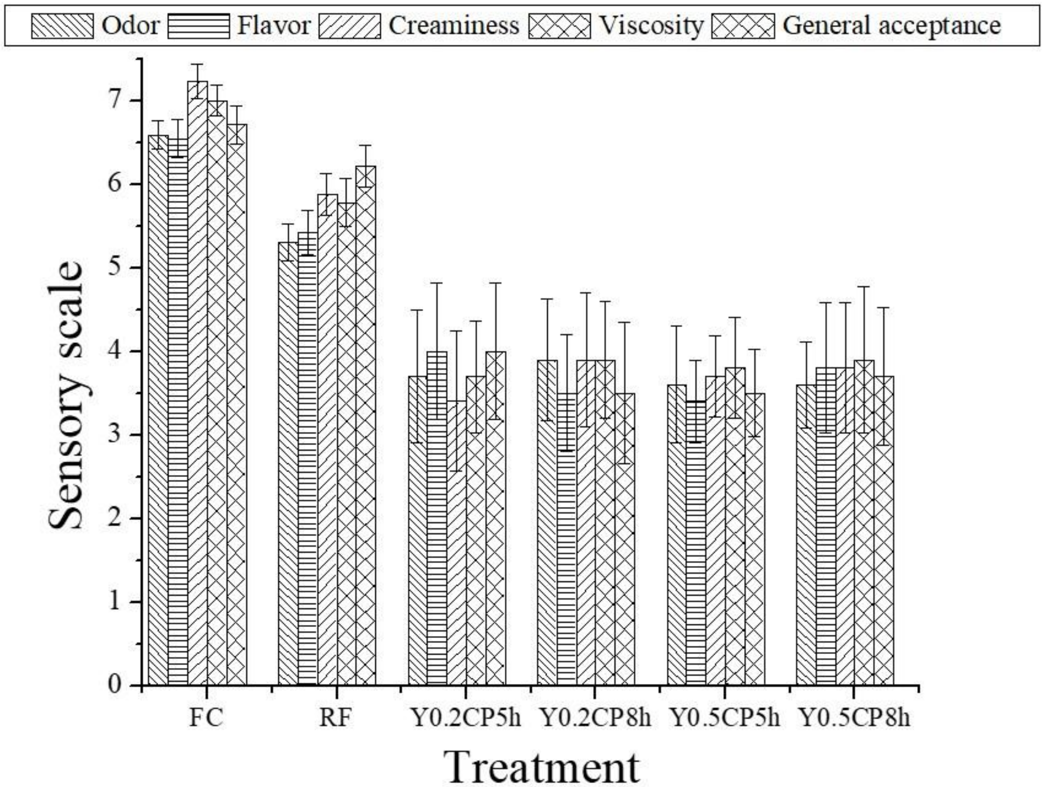

Figure 6. Sensory evaluation in the different treatments of yogurts.

CONCLUSION

The results of this work demonstrated that the addition of chickpea protein to low fat yogurt affects its properties. Increasing the concentration of chickpea protein, the water holding capacity increase, this effect of the concentration also can see in the initial viscosity of the yogurts. On the other hand, the time fermentation affected the acidity and pH and had a good participation in affecting the other properties like viscosity and water holding capacity. The sensory properties of the yogurts demonstrated that the level of concentration of chickpea protein in the samples and time of fermentation no presented difference between them in the effect that have in the yogurts properties. Both factors had effect on the properties of the low-fat yogurt. Based on all the analyses conducted, it can be said that the best formulation was Y0.5CP8h, but it is necessary to investigate the addition of a higher percentage of protein to low-fat yogurts and evaluate its effect on its physicochemical and sensory properties. The use of chickpea protein in dairy products will be an alternative to produce fermented milk products like yogurt.

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