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Effect of process variables on the physicochemical and rheological properties of yogurt

Efecto de las variables de proceso sobre las propiedades fisicoquímicas y reológicas de yogur

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ABSTRACT

In this research, the effect of the lactic starter culture, the fermentation time, the incubation temperature and the percentage of fat on the firmness and consistency of a fermented milk drink was evaluated through physicochemical characteristics of total acidity, pH, syneresis and flow behavior. The purpose was to find the best formulation that extends the shelf life of yogurt, leading to an improvement in the manufacturing process of a local dairy company in Barrancabermeja (Santander, Colombia). Quality criteria were evaluated by means of the percentage of acidity and pH of the yogurt, as well as its syneresis and rheological parameters during a storage time of 24 hours and 7 days in refrigeration. Acceptable quality parameters were obtained for the yogurt prepared with an inoculum concentration of 2%, an incubation temperature of 40°C and an incubation time of 150 minutes. In addition, better properties of firmness and consistency were found, with absence of syneresis and viscosity in the accepted range, for this dairy product.

Keywords: Acidity; Lactic culture; Syneresis; Viscosity; Yogurt.

RESUMEN

En esta investigación, se evaluó el efecto del cultivo iniciador láctico, el tiempo de fermentación, la temperatura de incubación y el porcentaje de grasa en la firmeza y consistencia de una bebida de leche fermentada, a través de las características fisicoquímicas de acidez total, pH, sinéresis y comportamiento de flujo. El propósito fue encontrar la mejor formulación que extienda la vida útil del yogur, lo que lleva a un perfeccionamiento del proceso de fabricación de una empresa local de productos lácteos en Barrancabermeja (Santander, Colombia). Se evaluaron criterios de calidad por medio del porcentaje de acidez y pH del yogurt, así como su sinéresis y parámetros reológicos, durante un tiempo de almacenamiento de 24 horas y de 7 días en refrigeración. Se obtuvieron parámetros de calidad aceptables para el yogurt, preparado con una concentración del inóculo de 2%, una temperatura de incubación de 40°C y un tiempo de incubación de 150 minutos. Además, se encontraron mejores propiedades de firmeza y de consistencia, con ausencia de sinéresis y viscosidad en el rango aceptado, para este producto lácteo.

Palabras clave: Acidez; Cultivo láctico; Sinéresis; Viscosidad; Yogur.

INTRODUCTION

Milk and dairy products have an important role in the human diet due to their many beneficial nutrients such as proteins, sugars, minerals, and water-soluble vitamins (Barkallah *et al.* 2017). Besides, dairy products, mainly fermented milk, are the most popular vehicles for supplementation and probiotics delivery in the human body due to their good compatibility, their pleasant and attractive sensory profile, and their high consumption worldwide (Heydari *et al.* 2018). Furthermore, in all its derivatives presentations that include low-fat yogurts, probiotics, smoothies, firm, ice cream, yogurt mousse (Casarotti *et al.* 2014; Ramírez & Ruiz, 2014), several health benefits such as anticarcinogenic and anti-infection effects are offered, improving immune system, cholesterol reduction, relief of lactose intolerance and/or reduction of antibiotic side effects in the consumer (Heydari *et al.* 2018). The essential microorganisms used in the production of yogurt are *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Pancar *et al.* 2016) as well as bifidobacteria which are bacterial species considered as possible probiotics (Panesar & Shinde, 2012; Kailasapathy, 2006). The latter are live microorganisms that if they are administered in adequate amounts, they provide health benefit on the host (Hill *et al.* 2014).

The traditional yogurt manufacturing process is mainly based on the microbial acidification of milk, due to lactic acid bacteria, which feed on milk lactose, generating lactic acid. Consequently, they reduce the pH of the milk to 4.5, reaching the isoelectric point of the protein (casein), which coagulates, and yogurt is obtained (Vera & Rodríguez, 2013) with its lactic flavor and typical aroma (Andrade *et al.* 2010). The acidification stage is the one that demands more time; therefore, accelerating the fermentation process would allow increasing the production capacity (Sahan *et al.* 2008; Vera & Rodríguez, 2013). However, there are other factors that influence on this such as milk composition, dry matter, heating, homogenization, type of initial culture, incubation temperature, final fermentation pH, cooling, storage time, among others (Kücükcetin, 2008). One of the most important attributes in yogurt is texture or firmness because it determines the quality and approval of the product. Thus, proper firmness without syneresis is essential to obtain a higher quality yogurt (Joon *et al.* 2017). Likewise, yogurt can have two main defects: variation in viscosity and spontaneous separation of whey that appears on the surface of yogurt (syneresis) (Hematyar *et al.* 2012); the latter situation is associated with the manufacture of industrial yogurt causing the loss of water from the yogurt gel (Pancar *et al.* 2016).

One of the most important aspects of this study is the use of lactic acid bacteria, which plays an essential role in yogurt manufacture. Therefore, if the inoculum level is increased in the presence of enough substrate, acidification occurs in less time (Vera & Rodríguez, 2013). For this investigation semi-skimmed (2.6%) and fat-free (0%) milk are used for the yogurt process considering that several studies indicate that the fat content of yogurt alters its rheological and sensory characteristics (Sandoval-Castilla *et al.* 2004). This fact is due to that in the structuring process, fatty globules interact with the protein network that acts as protein binding agents (Sandoval-Castilla *et al.* 2004). Hence, low-fat and fat-free dairy products exhibit a weak body, poor texture and therefore whey separation as the total fat and solid contents are reduced (Delikanli & Ozcan, 2014). In the same way, different levels of inoculum are established to evaluate the reduction of the fermentation time. Thus, the temperature, which is a very important factor, must be considered. The genus *Streptococcus* has an optimal growth between 42 and 45°C and *Lactobacillus* between 40 and 43°C, considering that they act in symbiosis and both stimulate each other. Investigations on the effect of different incubation temperatures (37, 40, 43 and 45°C) stated that it is possible to significantly reduce the fermentation time by increasing the temperature, taking into account that at temperatures greater than 45°C the lactic acid bacteria decrease their acidification power (Vera & Rodríguez, 2013).

The purpose of this study was to evaluate and to compare the effect of the type of milk, lactic culture, fermentation time and incubation temperature on pH, acidity, syneresis, viscosity and general acceptability in beaten yogurt under refrigerated storage conditions.

MATERIALS AND METHODS

Materials. skim milk with 0% fat and semi-skim milk with 2.6% fat were used. The stock solution used for the preparation of yogurt contained the microorganisms *L. bulgaricus*, *S. thermophilus*, and probiotic culture *L. casei* and *L. acidophilus*.

Yogurt preparation. fresh and pasteurized milk (fat percentages of 0 and 2.6) were subjected to a thermal process to reach 30°C and then, sugar (10% w/w) was added. Subsequently, the mixture was homogenized at higher temperature (36 and 40°C for each combination) and inoculated with stock culture (2 and 2.5%). At the end of the inoculation the samples were incubated for a period of 120 min and 150 min (at temperatures of 36 and 40°C). At the end of the incubation, the yogurt was stored at 4°C for 7 days. The analyses were performed at the end of the incubation, at 24h and 7 days after cooling (at 4°C) in the Laboratory of Agroindustrial Biotechnology of the Instituto Universitario de la Paz, UNIPAZ.

Physicochemical analysis. the pH values of the samples were measured using a pH meter (HACH HQ11d, Loveland, Colorado, U.S.A.) previously calibrated. The titratable acidity of yogurt was determined by taking a 10 mL sample of yogurt and holder with 0.1 N of NaOH using 1% phenolphthalein as an indicator until a weak pink color is obtained. Titratable acidity was calculated as % lactic acid (% w/v) (Horwitz, 2000) using equation 1.

$$\% \text{ Lactic Acid} = \left(\frac{V * N * 0{,}09}{m_s}\right) * 100 \qquad \text{equation 1}$$

Where V is the volume of NaOH spent, N is the normal concentration of NaOH used (0.01 N) and m_s is the mass of the yogurt sample used.

The density was determined by a gravimetric method using glass pycnometers where the empty pycnometer, pycnometer with distilled water and the pycnometer with yogurt are weighed, and the density value is obtained with equation 2:

$$\rho_{yogurt} = \frac{\text{Weight}_{\text{pic+yogurt}} - \text{Weight}_{\text{empty pic}}}{\text{Weight}_{\text{pic+water}} - \text{Weight}_{\text{empty pic}}} \qquad \text{equation 2}$$

Total soluble solids were found using a manual refractometer (BRIXCO INSTRUMENTS, 0-90%) (AOAC, 1998).

Syneresis assay: the syneresis was determined at 120 and 150 min of incubation and at 24 h and 7 days of yogurt incubation through the method of Cárdenas *et al.* (2013): 10 g of sample were put in a centrifuge tube that was centrifuged at 5000 rpm for 20 min. The supernatant weight was used to calculate the percentage of syneresis employing equation 3:

$$\text{Syneresis} = \frac{\text{supernatant weight}}{\text{Sample weight}} * 100 \qquad \text{equation 3}$$

Flow behavior. the Brookfield DV-I digital viscosimeter (Brookfield Engineering Laboratories Inc., Massachusetts, U.S.A.) was used to determine the rheological parameters. Measurements were made at different shear rates of 50 to 100 rpm using 350 mL of the product stored at different storage times (24 h and 7 days) and incubation times (120, 150 min). The samples were placed in 400 mL glasses, and the HA / HB-2 needle was used in all systems.

Experimental design. the experimental design was established to analyze the interactions between the dependent variables and to understand the phenomenon under study. In this way, a multivariate arrangement was made 2^k = treatments (2^4) using two levels (minimum and maximum) and four factors (k) corresponding to % fat (A), % starter culture (B), incubation temperature (C), incubation time (D) (Table 1). Statistical analysis was performed using Minitab software version 17 (State College, Pennsylvania, U.S.A), using a level of significance of $P < 0.05$ for the analysis of variance (ANOVA). All physicochemical analyzes were performed in triplicate and the results were expressed as the average of the data. The entire methodological procedure was carried out under a strict sterilization protocol to avoid possible cross contamination and unwanted microbiological growths.

Table 1. Experimental design.

Factors	Levels	Variable response
A) Fat content	0 % (-)	pH of the final product - fermented milk beverage - Yogurt
	2.6 % (+)	
B) Inoculum concentration	2 % (-)	
	2.5 % (+)	
C) Incubation Temperature	36 °C (-)	
	40 °C (+)	
D) Incubation Time	120 min (-)	
	150 min (+)	

Treatment	Factor			
	A	**B**	**C**	**D**
T1	-	-	-	-
T2	+	-	-	-
T3	-	+	-	-
T4	+	+	-	-

Continuación Tabla 1

T5	-	-	+	-
T6	+	-	+	-
T7	-	+	+	-
T8	+	+	+	-
T9	-	-	-	+
T10	+	-	-	+
T11	-	+	-	+
T12	+	+	-	+
T13	-	-	+	+
T14	+	-	+	+
T15	-	+	+	+
T16	+	+	+	+

The significant effects are A:% Fat; C: T°C Incubation; AxC:% Fat Vs. T°C Incubation; CxD: T°C Incubation Vs. Incubation Time; AxBxD: % Fat Vs.% Culture Vs. T°C Incubation; AxBxCxD: % Fat Vs.% Culture Vs. T°C Incubation Vs. Incubation Time.

RESULTS AND DISCUSSION

Physicochemical analysis. Figure 1 and 2 show the variation in pH and acidity from the end of the incubation period and during the storage period (24 h and 7 days), for each fat content of the milk.

The behavior of pH and acidity of the yogurt measured 24 h after stored at 4°C (Figure 1) does not follow a clear tendency, which determines that there must be a different factor than those studied which directly affects this variation in pH.

On the other hand, the acidity maintains an increasing behavior except for the case of skim milk with an incubation time of 120 min, due to the lower conversion of lactose, possibly time was not enough for the proper fermentation process. In comparison with the data obtained just at the end of the incubation, there is no definite

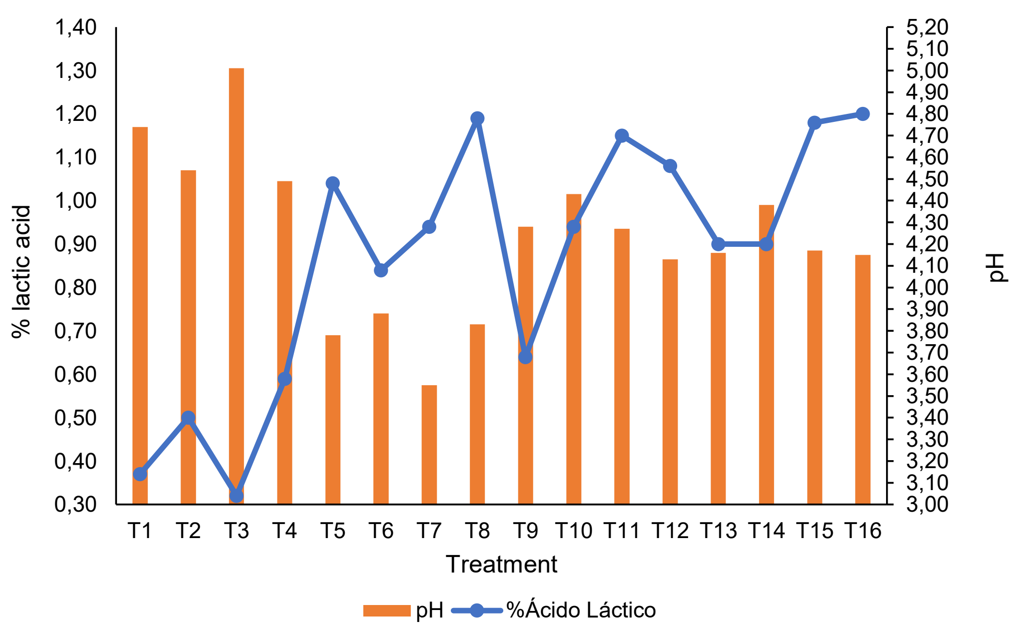

Figure 1. pH (bars) and acidity (red lines) of yogurt treatments measured after 24 h of incubation (n=3).

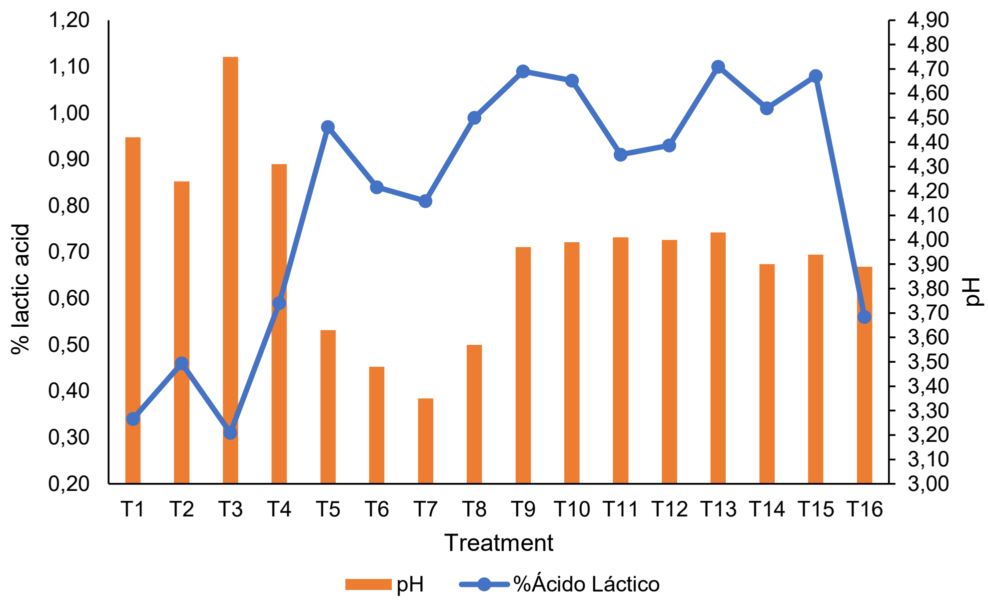

Figure 2. pH (bars) and acidity (red lines) of yogurt treatments measured after 7 days in storage (n=3).

trend that allows concluding some effect between the storage time and the pH of the yogurt. The treatments T5 to T8, in which the pH increases 24 h after the end of the incubation, and the treatments T9 to T16, where the pH decreases, stand out from the rest.

The tendency to decrease the pH can be attributed to both the activity of beneficial and harmful microorganisms. The latter can lead to a decrease in pH due to the consumption of sugar and the production of organic acids. When the sugar resources are finished, microorganisms consume proteins from the environment, and this will increase the pH as investigated by Shahbandari *et al.* (2016). Also, in general acidity shows an increasing tendency in all treatments, 24 h after finished the incubation process.

Figure 2 shows the samples behavior after 7 days of storage under refrigeration conditions. The reduction in pH and the increase in the total acidity of yogurts during storage could possibly be explained due to the increased consumption of residual lactose by lactic acid bacteria (Curti *et al.* 2017).

Other studies reported that the pH of yogurt decreased during storage under refrigeration conditions between 3.8 and 4.5 (Olson & Aryana, 2008). The reduction in the pH of yogurt may be due to the degradation of lactose in lactic acid (Hassan & Amjad, 2010). Considering the experiments carried out, the best working variables were selected: temperature (40°C), incubation time (150 min) and inoculum concentration (2%), with a refrigeration storage period of 7 days.

In the present investigation the variables that most affect the response variable (pH) are the incubation time (D) and the incubation temperature (C); especially the combination of incubation temperature with incubation time. The best treatment, to maintain a pH up to 4.5 after 24 h and 7 days of incubation, would be an incubation temperature of 40°C and incubation time of 150 min.

Although the variables of % fat and % inoculum do not significantly affect the response variables as shown by the Pareto chart (Figure 3), the normal graph and the p values (which for these two variables are greater than $\alpha = 0.05$ ($p>0.05$)), a % fat of 0% and an inoculum concentration of 2% are recommended.

Syneresis behavior: syneresis is an appreciable defect briefly, due to the appearance of two phases, whey, and water. Among the main causes of this behavior are low milk quality, very high incubation temperature, low acidity, enzymes that clot the protein and low viscosity. In this study, the consistency and stability of yogurt were analyzed through the calculation of syneresis. On treatments T1, T2, T3, T9, T10, T11, and T12 there was no syneresis in any of the samples. The results of syneresis converge as in the physicochemical analysis, in which the optimal working factors are 2% of the stock culture and incubation temperature 40°C.

Figure 4 shows the results for the other treatments where the highest point of syneresis was obtained in the T13 treatment corresponding to 0% fat milk, 2% stock culture, incubation temperature 40°C and incubation time 150 min. At incubation temperature of 40°C, a trend is revealed, at a shorter incubation time, the quality of yogurt would be better after 7 days in storage.

The syneresis values may increase as the pH decreases because the yogurt continues to produce lactic acid during storage (Rebollar, 2017), it should be mentioned that the pH of treatment 13 ranges by 4.2 and may affect the stability of the structural network of the

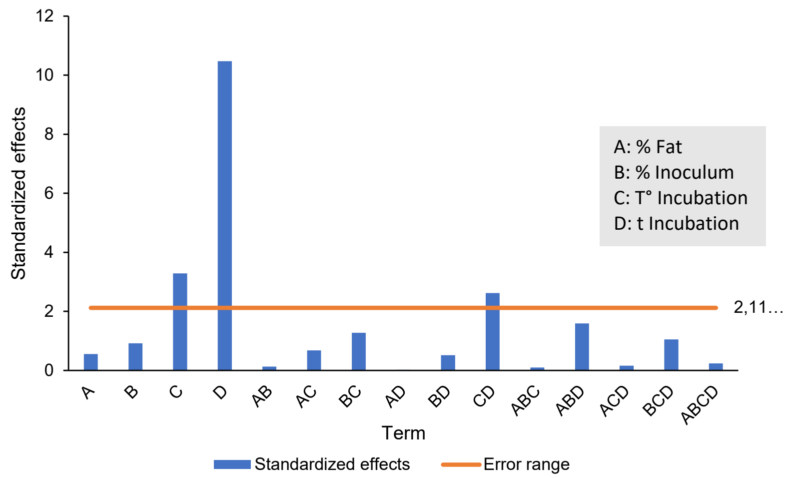

Figure 3. Pareto Chart of the Standardized Effects. (response is pH; α=0,05).

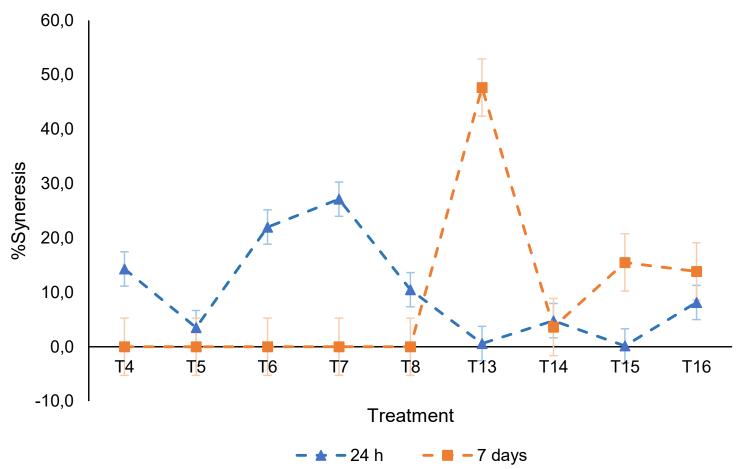

Figure 4. Syneresis of yogurt with 24 h and 7 days of storage.D.

yogurt. According to Lucey & Singh (1997), casein aggregation occurs at the isoelectric point (pH = 4.6) because of the repulsion between micelles decreases; at this point, the contraction of the structural network results in the whey release. According to the results, the isoelectric point of casein studied in this work is below the pH range of fermented beverages, this explains the syneresis values found in figure 3.

Figure 5 shows the apparent viscosity of the samples of yogurt stored at different times. A higher incubation time (150 min) of the yogurt led to an increase of the apparent viscosity at high rotation speed (graphs a and c), while a pseudoplastic behavior is observed in graphs b and d for treatments at 120 min incubation time.

Viscosity values of yogurt samples are affected by several factors: composition, starter cultures, heat treatment (Velez-Ruiz *et al.* 2012; Mohan *et al.* 2020). Likewise, as the total solids increase (e.g. fat content), the viscosity and firmness of the yogurt increase (Tarrega *et al.* 2016).

Semi-skimmed milk (2.6%) and fat-free (0%) for the yogurt process showed variations in pH and acidity as well as in its texture and firmness structure. This may be due to various studies indicating that the fat content of yogurt alters its rheological and sensory characteristics as in the structuring process, fatty globules interact with the protein network that acts as protein-binding agents. Therefore, low-fat and fat-free dairy products exhibit a weak body,

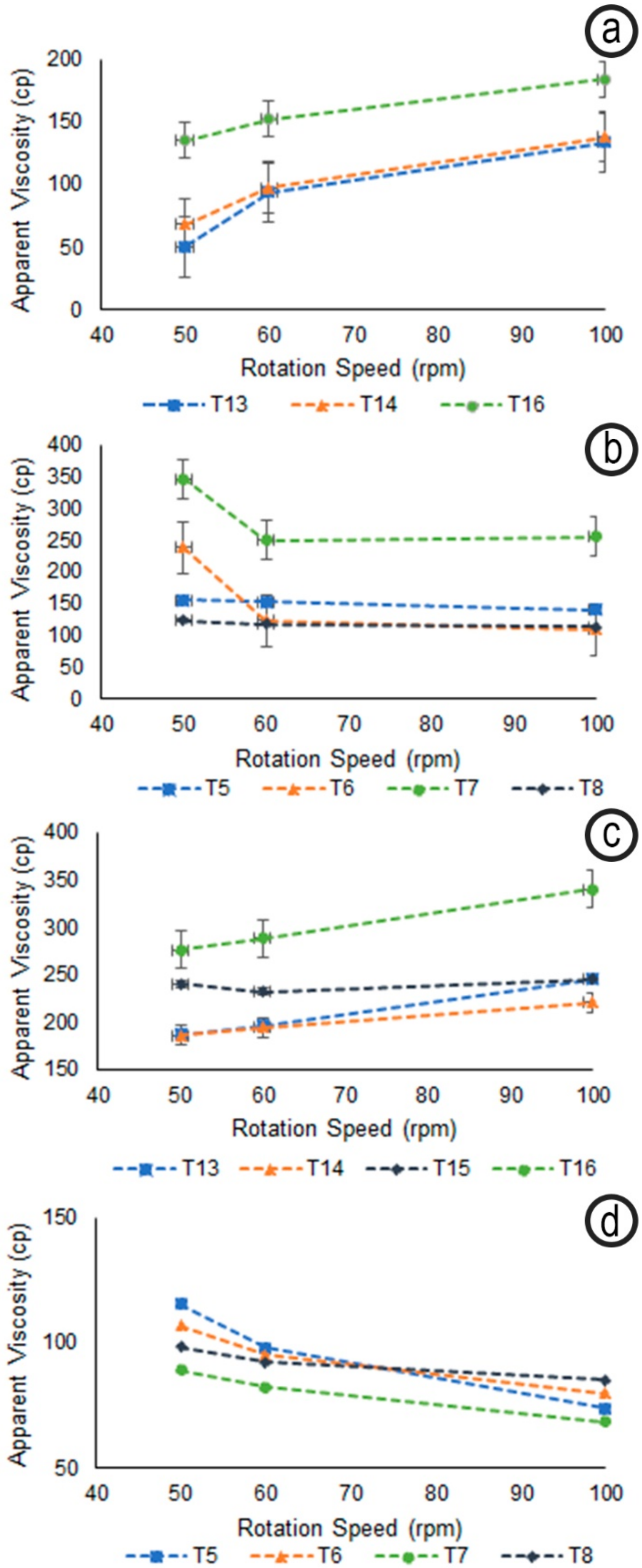

Figure 5. Viscosity behavior at different rotation speeds for yogurt stored for 24h (a and b) and 7 days (c and d).

poor texture and, therefore, whey separation as the total fat and solid contents are reduced.

The variables for the preparation of yogurt were: 40°C incubation temperature, 150 min incubation time and 2% stock culture, as they did not show syneresis; pH and acidity values were found in the acceptable ranges and the best properties of consistency and firmness were obtained, after 7 days in storage at 4°C.

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