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Effect of the milk-whey relation over physicochemical and rheological properties on a fermented milky drink

INGENIERÍA DE ALIMENTOS

Efecto de la relación leche-lactosuero sobre las propiedades fisicoquímicas y reológicas en una bebida láctea fermentada

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Abstract

The effect of the milk-whey relation over the physicochemical properties (pH, total acidity, soluble solids and syneresis) and rheology (deformation rate, apparent viscosity, flow behavior index and consistency index) of a fermented dairy drink was studied. A completely randomized experimental design of two factors: percentage of whey (0, 5, 10 and 15% w/w) and type of milk (whole and skim) was used. The significance was determined using an analysis of variance (ANOVA). All properties analyzed except pH had statistically significant differences ($P<0.05$). The use of whole milk in comparison to skim milk generated an increase in soluble solids and viscosity of the beverage but caused a decrease in acidity and syneresis. The growth in whey concentration increased syneresis and apparent viscosity but decreased soluble solids and consistency index. The changes in properties are related to the contribution of fat from milk and the contribution of calcium and phosphate from whey, affecting both the interaction between the casein micelles and the water retention capacity, which changes the composition and fluidity of the beverage.

Keywords: *Casein, fermented milk, lactic acid, pH, syneresis, viscosity.*

Resumen

Se estudió el efecto de la relación leche-lactosuero sobre las propiedades fisicoquímicas (pH, acidez total, sólidos solubles y sinéresis) y reológicas (velocidad de deformación, viscosidad aparente, índice de comportamiento al flujo e índice de consistencia) de una bebida láctea fermentada. Se utilizó un diseño experimental completamente al azar de dos factores: porcentaje de lactosuero (0, 5, 10 y 15% p/p) y tipo de leche (entera y descremada). La significancia se determinó mediante un análisis de varianza (ANOVA). Todas las propiedades analizadas excepto el pH presentaron diferencias estadísticamente significativas ($P<0,05$). El uso de la leche entera en comparación con la leche descremada generó un incremento en los sólidos solubles y en la viscosidad de la bebida, pero causó una disminución en la acidez y la sinéresis. El incremento en la concentración de lactosuero aumentó la sinéresis y la viscosidad aparente pero disminuyó los sólidos solubles e índice de consistencia. Los cambios en las propiedades se relacionan con el aporte de grasa que realiza el tipo de leche y la contribución de calcio y fosfato que efectúa la adición de lactosuero, afectando tanto la interacción entre las micelas de caseína como la capacidad de retención de agua, que resulta en cambios en la composición y fluidez de la bebida.

Palabras Clave**:** *Ácido láctico, caseína, leche fermentada, pH, sinéresis, viscosidad.*

1. Introduction

In recent decades the dairy industry and various research groups have been seeking different strategies to give added value to the whey, by-product in the manufacture of cheese (1-3). Due to the high volume of whey production, about 180-190 million metric tons per year, traditionally much of the whey has been discharged into water sources such as rivers and seas and in other cases has been used on agricultural land as fertilizer (4-7). However, as it is well known, the whey is one of the currents of food sector that major environmental problems generates when is poured both in water and on land due to its high biological oxygen demand (BOD) (> 35000 ppm) and high chemical oxygen demand (COD) (> 60000 ppm) (8-10).

The whey chemical composition is directly related to the method used in cheese production. Water is the main component with a 90-92%, while the remaining 8-10% is composed mainly of lactose and proteins with a 60-80% and 10-20%, respectively. There are other compounds in lower concentrations such as minerals, vitamins, fats, lactic acid and trace elements (11, 12, 6). The production of fermented dairy drinks composed of milk and whey has been growing significantly worldwide due to the simplicity of its process and above all, good acceptance from the customers (13-15).

Diverse papers concerning fermented milk drinks have been performed around the world. Janiaski et al. (15) evaluated the sensory profiles of yogurt and fermented dairy drinks, finding that there were no substantial changes in the sensory attributes between the two types of beverages, with and without whey. Moreover, Castro et al. (14) determined the relationship between the content of whey in fermented milk drink and rheological properties, determining that the increase in whey content increased the fragility of the gel structure, probably as a result of the substitution of casein for whey proteins. Gomes et al. (16) evaluated the physicochemical and sensory properties of a fermented milk drink made from the milk of a different nature, goat or cow. The drinks prepared with a mix of milk and whey of goat and cow had lower values of pH and lactose than goat´s origin drinks, but higher than those presented in beverages prepared with milk and whey of cow. Despite differences in physicochemical properties, all beverages had similar scores on all sensory attributes evaluated.

Recognizing the advances developed in the studies conducted so far, it is still uncertain the effect of the variation of the concentration of whey on fermented milk drinks made from milk with different fat concentrations. For this reason, the objective of this study is to evaluate the possible changes in the physicochemical and rheological properties in a fermented milk drink attributable to the variation of whey concentration (0, 5, 10 and 15% p/p) and the kind of milk, whole or skim, with fat content of 3 and 0.5% w/v, respectively.

2. Materials y methods

Whey powder (Lácteos la Cristina SA, with a moisture content of 4% w/w, 11% w/w proteins, 2% w/w fat, 75% w/w carbohydrate, 0.038% w/w , phosphorus 0.035% w/w, calcium 0.044% w/w, potassium 0.137% w/w and ash 7.8% w/w), two types of commercial milk (Lechesan SA), whole and skim with a fat concentration of 3±0.2% w/v and 0.5±0.2% w/v respectively, and a commercial lactic acid culture (YO-MIX 883 LYO 50 DCU, DuPont Danisco France SAS) composed of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus salivarius* subsp. *Thermophilus* were used. The fat content of the milk was verified in triplicate using the Gerber method (17).

The fermented dairy beverage was prepared according to the procedure described in technical standard NTC 805. First, 5, 10 and 15 g of a whey solution (6% w/w) were mixed with 95, 90 and 85 g of milk respectively, at 45 °C until homogenizing. Subsequently, the mixture was inoculated with 3 g of lactic acid culture and maintained for 4 h at 45 °C and 120 rpm, using an orbital shaker incubator (LABNET, model I5311-DS). After incubation, the fermented dairy beverage was first cooled naturally to room temperature (25 °C) and then to 15 °C using a refrigerator. Finally, the product was stored at 4 °C.

2.1. Soluble solids

Soluble solids were measured in terms of °Brix at 20 °C by the refractive method according to AOAC 932.12/90, using a portable refractometer (Zhifong, model FG-109).

2.2. pH and titratable acidity

The pH was determined at 20 ºC according to the standard procedure AOAC 981.12/90 with a potentiometer Hanna HI8314N. Total titratable acidity was performed by titration with 0.1N NaOH and expressed as the lactic acid percentage (Eq. 1) according to standard procedure (AOAC 937.05).

$$\%\ Lactic\ acid = 0.09\frac{V_{NaOH} * C_{NaOH}}{m_{Sample}} * 100 \quad (1)$$

Where V_{NaOH} and C_{NaOH} are normal volume and concentration of NaOH, respectively, and m_{sample} is the mass of the sample of the fermented milk drink.

2.3. Syneresis

The syneresis was calculated according to the procedure described by Aichinger et al. (18). Samples of the dairy beverage were stirred for 1 min on a vortex stirrer (VX-200, Labnet), and then centrifuged for 20 min at 5000 rpm and 12 °C. The syneresis percentage (% S) was calculated by the ratio between the mass of the supernatant obtained and the mass of the centrifuged sample, as shown in Eq. 2.

$$\%\,S = \frac{Mass\ of\ Supernatant}{Sample\ Mass} * 100 \quad (2)$$

2.4. Rheological properties

To determine the rheological properties it was used a rotational viscometer Brookfield model DV-E (Brookfield Engineering Laboratories) and the methodology proposed by Mitschka (19), where the readings of percentage deflection (% ∝) (Eq. 3) and speed needle rotation (N) (Eq. 4), are converted to functions of viscosity.

$$\tau_i = K_{\propto\tau} * \propto_i \quad (3)$$

Where % is the deflection percentage on the dial torque, τ_i is the shear stress in Pascals (Pa) and is a conversion factor needle, which is obtained from tabulated values (19).

$$\acute{\gamma} = K_{N\acute{\gamma}}(n_{ap})N_i \quad (4)$$

Where $K_{N\gamma}$ is the conversion factor of the readings of speeds rotation of the needle N_i (rpm) at a rate of deformation (γ) (s^{-1}); which varies according to the number of the needle and the apparent index of behavior flow (n_{ap}).

With the values found for τ_i and γ is found graphically the initial shear stress (τ_0). Converting the Herschel-Bukley equation (Eq. 5) in a linear equation, Eq. 6 was obtained.

$$\tau = K * \acute{\gamma}^n + \tau_0 \quad (5)$$

$$ln(\tau - \tau_0) = ln(K) + n ln(\acute{\gamma}) \quad (6)$$

Plotting in a graph $ln(\tau\text{-}\tau_0)$ *vs* $ln(\gamma)$ the consistency index (K) and flow behavior index (n) was determined.

2.5. Statistical analysis

A completely randomized experimental design of two factors: percentage of whey (0, 5, 10 and 15% w/w) and type of milk (whole and skimmed) was used. All experiments were replicated 6 times. Analysis of variance (ANOVA) was performed with a significance defined as P<0.05, using the statistical package IBM SPSS Statistics 21.

3. Results and discussion

Figure 1 shows the behavior of the soluble solids of fermented milk beverages prepared with different mass ratios of a mixture of whey and whole or skim milk. It is noted that the increase in the fat content increases the content of soluble solids, regardless the whey concentration. The increase in whey concentration did not cause significant changes in the soluble solids of beverages with whole milk but caused a decrease in the soluble solids of beverages with skim milk.

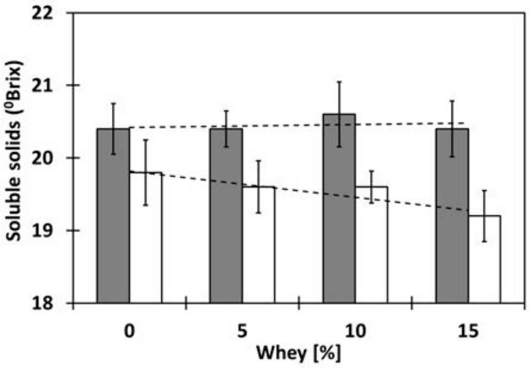

Figure 1. *Effect of whey concentration in the soluble solids of a fermented drink prepared with whole milk (filled column) and skim milk (empty column). The results are the average of six replicates and error bars represent the standard deviation. The dashed line shows the linear trend.*

Figure 2 presents the results of both the pH and acidity behavior of the different fermented milk drinks. It is observed that the pH of the beverage is not modified when the type of milk is changed (P>0.05), but change with the whey addition (P<0.05), getting a maximum value in 5% w/w of whey. Additionally Figure 2 shows that the acidity of beverages with skim milk is higher than with whole milk (P<0.05), and it presents minimum values in the beverages with 5 and 10% w/w of whey for whole and skimmed milk, respectively.

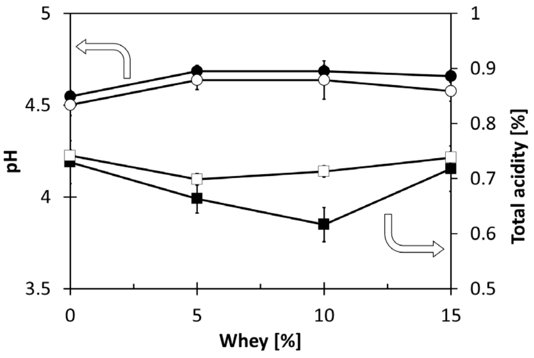

Figure 2. *Effect of the whey concentration on pH (circles) and total acidity (square) of a fermented drink prepared whole milk (filled symbols) and skim milk (empty symbols). The results are the average of six replicates and bars represent the standard deviation.*

The results of the behavior of the syneresis of fermented milk beverages are presented in Figure 3. It is observed that the beverage prepared with whole milk has less syneresis than with skim milk and that the addition of whey generates a significant increase in the values of syneresis (P<0.05), especially in beverages with whole milk. When comparing Figures 1 and 3, it can be observed that with increasing whey concentration, soluble solids decrease mainly when skim milk is used, while syneresis increases significantly in beverages prepared with whole milk.

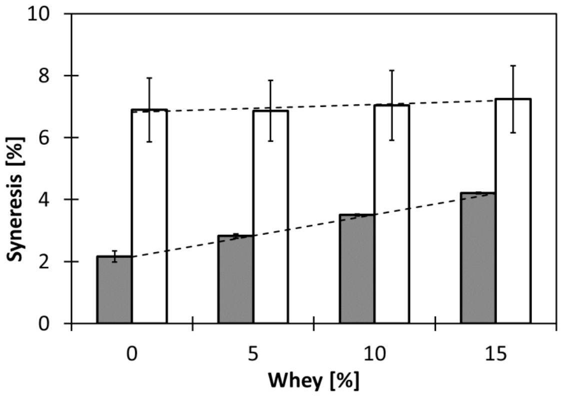

Figure 3. *Effect of whey concentration in the syneresis behavior of a fermented drink with whole milk (full column) and skim milk (empty column). The results are the average of six replicates and the error bars represent the standard deviation. The dashed line shows the linear trend.*

Figure 4 shows the behavior of the apparent viscosity versus the rate of deformation of the fermented milk drinks as a function of the milk type and the whey concentration. It is observed that the increase in whey concentration decreases apparent viscosity (P<0.05), although these values are considerably higher in beverages with whole milk. The effect of milk type on the behavior of syneresis and soluble solids seems to be inversely related to the effect on viscosity.

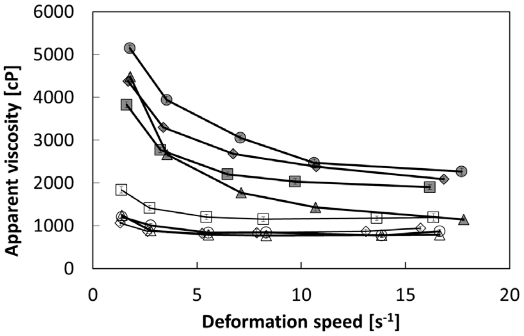

Figure 4. *Behavior of the apparent viscosity versus deformation speed of a fermented milk drink with two types of milk (filled symbols: whole and empty symbols: skim) and whey (triangles: 0%, squares: 5 %, diamonds 10% and circles: 15%).*

Table 1 shows the results of the flow behavior index (n) and the consistency index (K) of the fermented milk drinks. It is observed that using skim milk the values of n are higher than when whole milk was used, this behavior was observed indistinctly the concentration of whey in the drink. However, for both whole and skim milk, the increase in whey results in an increment in the rate of flow behavior. On the other hand, the consistency index (K) behaves opposite the flow behavior index (n). For drinks made with whole milk the values of K are higher than when skimmed milk was used, and when the percentage of whey in the beverage of both whole and skimmed milk was increased, K values decreased.

Table 1. *Effect of whey concentration and milk type on flow behavior index (n) and the consistency index (K) of fermented milk drink.*

Type of milk	Whey [%]	Rheological properties	
		n	*K* [Pas]
Skim	0	1.13 ± 0.02	0.514 ± 0.005
	5	1.14 ± 0.02	0.552 ± 0.004
	10	1.18 ± 0.01	0.575 ± 0.012
	15	1.23 ± 0.02	0.391 ± 0.008
Whole	0	0.75 ± 0.01	3.347 ± 0.009
	5	0.77 ± 0.01	2.811 ± 0.012
	10	0.81 ± 0.02	2.406 ± 0.01
	15	0.78 ± 0.01	2.887 ± 0.007

The effect of the type of milk on the studied variables may be related to its fat content whereas the influence of the addition of whey may be due to its content of calcium, phosphates and proteins. Fat and whey are substances that can alter the balance of the structure of the fermented milk drink because interact electrostatically and mechanically with the colloidal calcium phosphate (CCP), present in the casein proteins, altering the molecular arrangement of the tridimensional network of drink (20-23, 15). For the discussion of the physicochemical and rheological properties of the fermented dairy beverage, the effect of the milk type was studied initially, analyzing especially the difference in fat content, and then the particular effect of the whey concentration on the beverages.

3.1. Effect of milk type

The variation in the type of milk, and therefore of the fat concentration in the fermented milk drinks, generated significant changes in all the physicochemical and rheological properties measured ($P < 0.05$), except for pH ($P > 0.05$). Because an increase in soluble solids in milk beverages was observed when whole milk was used, it is possible to directly associate fat content with soluble solids as studied by Palmquist et al. (24). Soluble solids are closely associated with the content of dissolved salts such as calcium phosphate from the casein micelles (25, 21, 26, 27), and sugars such as lactose (28, 3), which is converted into lactic acid by bacterial action (29-32). According to Innocente et al. (32), increasing the fat concentration in milk decreases solubility and mass transfer by changes in rheological conditions, which causes a decrease in the bacterial activity associated with the transformation of lactose and this is reflected in a greater amount of soluble solids.

On the other hand, Ramírez-Sucre & Vélez-Ruiz (33) presented results of syneresis with the same tendency as those reported in the present study, an increase of the fat from 0.2 to 3.2% w/v decreased the syneresis by about 32%. Several authors have reported that one of the possible causes of reduced syneresis is associated with the presence of fat in the three-dimensional network structure forming the drink (34, 33). A low content of fat, such as skim milk, structural proteins network comprised mostly of casein is compact due to the formation of large numbers of crosslinks and low water retention (26, 34, 35). Crosslinks occur mainly by the interaction of CCP present on the surface of the casein micelle. In this matrix state, it may release whey and indeed an increase in syneresis occurs (36, 37). Moreover, by increasing the fat content, for example by using whole milk, a change occurs in the order of the tridimensional network. In this case, fat globules are deposited within the structure promoting a decrease in the crosslinks which reduces the contraction of the matrix and increases water retention capacity (28, 34). In this state, the network has the ability to retain the whey and low syneresis effect, as seen in Figure 3.

The increment in the fat content increases the viscosity values as shown in Figure 4. This effect is mainly associated with the physicochemical properties that were influenced by the increase in fat. Castro et al. (14) and Sodini et al. (38) report that the increment in soluble solids content and low syneresis are related to the growth in viscosity. According to Jumah et al. (39), high levels of syneresis affect gel formation and decrease its consistency. This is shown in Table 1 where skim milk drinks, with lower fat content, showed higher syneresis and lower consistency index (K). According to Ramírez-Sucre & Vélez-Ruiz (33) the decrease in the rigidity of the casein network and increased water retention promotes better flow behavior.

3.2. Effect of whey content

The pH and total acidity showed a maximum and minimum value between 5 and 10% w/w of whey, respectively. Modler et al. (40) reported results of pH and acidity of fermented milk drink similar to those presented in this research. These authors found that there were variations in the pH between 4.24 and 4.32 and in the acidity between 0.96 and 1.19 using whey protein at concentrations of 0.5 and 1.5% w/w. Both, the results presented in Figure 2 and the results of the work of Modler et al. (40), showed that the behavior of the pH and the acidity depending on the concentration of whey. The pH presented a maximum while the acidity had a minimum. According to Gomes et al. (16), the acidity of fermented milk drink is mainly associated to the lactic acid formed in the transformation of lactose from milk and whey by microbiological action during the incubation stage of the milk drink. According to several authors (21, 34, 37, 41) changes in pH and acidity can affect the stability of the structural network of the drink which is reflected in the values of syneresis, because it has implications on the surface charge of casein micelles. According to Lucey & Singh (42) casein aggregation occurs at the isoelectric point (pH=4.6) because it decreases the repulsion between micelles; at this point the contraction of the structural network resulting in the release of whey occurs, as seen in the results of syneresis. According to the results presented in Figure 2, the isoelectric point of casein is within the range of pH (4.5 and 4.7) of fermented drinks studied in this work, which explains the values of syneresis found in Figure 3.

As the concentration of whey increments, there is an increase in pH followed by a decline. This behavior may be due to the influence of several factors: the effect of whey in the production of lactic acid modifying both acidity and pH, and the growth in the content of whey increases the amount of Ca^{+2} in the drink, which promotes protonation of the phosphate species present on the surface of the micelle, causing an increase in pH. This protonation increases the strength of the network crosslinks of proteins making them more rigid. In this sense, this increase in Ca^{+2} concentration has an influence on syneresis, as seen in Figure 3. According to Park & Haenlein (36), a low pH and a high acidity cause the releases of whey, increasing solubilizing of CCP micelles which it promotes a loss of soluble calcium in the whey.

4. Conclusion

The type of milk and the addition of whey affect the physicochemical and rheological properties of a fermented milk drink, taking into account a significance less than 0.05. It was found that increasing the percentage of fat also increases soluble solids, pH, and viscosity, but at the same time decreases syneresis and acidity. However, there were no significant variations in pH (P>0.05). On the other hand, the addition of whey increased the acidity and syneresis especially in drinks with higher fat content. The different physicochemical properties are affected by the interaction between the fat globules of milk and the calcium present in the whey, with phosphates present on the surface of casein micelles. This interaction causes changes in the ordering of the three-dimensional network formed by micelles. By increasing fat, the rigidity of casein network is decreased, increasing its ability to retain water and reducing the release of whey; this considerably improves the stability of the fermented milk drink. Conversely, increasing the whey content rise the calcium content of the beverage and therefore the interaction of the casein micelles is promoted, resulting in a stronger network with low water retention capacity and high instability.

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