Research Article

Influence of Concentration on the Surface Tension of Milk and Whey by Vacuum Evaporation

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Abstract: The influence of the concentration by evaporation under vacuum on the surface tension of milk and whey was determined. Whey was obtained by enzymatic coagulation of the milk during the production of fresh cheese. Milk and whey were concentrated in a vacuum evaporator with single-acting plates. To correlate the total solids and the active surface components with whey's surface tension, a simple and multiple linear regression analysis were performed using the SAS 9.1.3 program. The results show that the increase in milk concentration causes a slight decrease in surface tension (40.62 to 39.21 mN/m). However, different zones of variation for the surface tension were presented. As for whey, a linear increase in the surface tension (35.8 to 43.78 mN/m) was present. The component that most influenced the surface tension are lipids; this is due to the denaturation of fat globules, which reduces the adsorption capacity at the surface.

Keywords: Concentrated milk, concentrated whey, denaturation, evaporation, surface tension

INTRODUCTION

Whey is the transparent liquid part of milk that remains from the cheese making process. It contains about 50% of the total solids of whole milk, including all the lactose (between 70% and 75%) and whey proteins (10-13%); this mainly includes lactalbumin and globulins. Also, it contains minerals, such as calcium, magnesium, manganese, phosphorus, copper, iron, zinc, sodium and potassium. Whey is commonly used in animal feed, dietary food (infant food), bread, pastries, sweets and beverages (Lapčík, *et al.*, 2015; Smith *et al.*, 2016).

To take advantage of whey, a by-product of the dairy industry and to use its technological and biotechnological characteristics, it has been used in several industrial applications. These include the use of whey as protein aggregates and gels in the preparation of edible films, in controlling the texture of products such as yogurt and surimi, as microencapsulating agents to protect ingredients sensitive to the deterioration and loss of bioactive components and the use of whey as nutraceutical nutrients and flavor (Chandrapala *et al.*, 2016; Eckert *et al.*, 2017). In addition, whey has recently been used in applications involving the separation and utilization of lactose (Cuartas-Uribe *et al.*, 2009; Gajendragadkar and Gogate, 2017) and as a coating that increases the shelf life of products, such

as Pike-Perch fillets (Shokri and Ehsani, 2017), cheese (Ramos *et al.*, 2012) and chicken eggs (Wardy *et al.*, 2010).

To use whey in the aforementioned applications, it has to undergo different separation and concentration operations (ultrafiltration, atomization and evaporation). In the design of such operations, it is necessary to know the physicochemical properties of the foods that are processed. One of these properties is the surface tension, whose role is fundamental for the stability of foams and emulsions. For spray applications (spray drying and coatings among others), in particular, the surface tension is directly related to the size of the atomized drop, the time it takes to fall from the nozzle and the drying time (Fox, 1997; Andrade et al., 2012; Drusch et al., 2012; Nishanthi et al., 2017).

When milk and whey are put through separation and concentration operations, the surface tension is affected. This is mainly due to changes in the concentration of its components (fat, proteins and salts). These changes are related to the surface activity of these components, which act as active surface agents that are absorbed in the liquid-air interface. They generally decrease the surface tension (Williams *et al.*, 2005; Xu *et al.*, 2012).

In this study, the effect of milk and whey concentration on surface tension was studied using a vacuum plate evaporator. This study is important for predicting the behavior of dairy products during processing, which ensures the quality and stability of emulsions and foams in dairy products.

MATERIALS AND METHODS

Raw material: The milk used was obtained from a collection center located in the industrial district of the municipality of Juiz de Fora (Minas Gerais, Brazil). This milk comes from Dutch cows that were reared in semi-confined systems, milked mechanically and immediately cooled (4-7°C). The transport of milk to the experiment site (Dairy Research Institute Cândido Tostes) was carried out in a refrigerated truck. As a pretreatment, the milk was standardized at 3.2% fat and pasteurized at 65°C for 30 min.

Whey was obtained by enzymatic coagulation of milk during the production of fresh cheese. The cheese was made with standardized milk with 3.3% fat and pasteurized at 65° C/30 min. The whey was mechanically separated from the formed clot and then characterized.

Evaporation process for milk and whey: The milk and whey were preheated to 65-70°C before being evaporated, which was done in a single-plate vacuum evaporator (APV, Junior model). 150 L of milk and 350 L of whey were used. The volume of whey used was defined based on the tests described by Silveira *et al.* (2013). The addition of the samples to the evaporator was performed slowly until the specified volume was reached; the concentrated samples were removed from the evaporator at 10 min intervals for a total of 8 samples, which were labelled as: M1, M2, M3, M4, M5, M6, M7 and M8 for milk and W1, W2, W3, W4, W5, W6, W7 and W8 for whey.

Determination of physicochemical properties: Concentrated milk and whey samples were put through physicochemical analysis according to the Resolution 68/2006 of the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA), which are described below:

pH: The pH was determined by the direct method using a pH meter (P21, Hanna, USA) at 25°C.

Total acidity: The acidity was determined by titration with a known concentration of an alkaline solution. 20 mL of the milk sample and sodium hydroxide solution (0.1 mol/L) were measured as the titrant. The Total Acidity (TA) was expressed as a percentage of lactic acid in the sample, according to Eq. (1):

$$TA = (V \times C \times f \times 0.09) / Va \times 100$$
(1)

where,

- V = The volume of the sodium hydroxide solution (mL)
- C = The solution concentration (mol/L)

- f = The concentration correction factor for the sodium hydroxide solution
- 0.09 = The volume conversion factor for the alkaline solution used for the lactic acid
- Va = The volume of the sample (mL)

Soluble solids: The soluble solids content (°Brix) was determined with a digital refractometer (Reichert, model AR200).

Total solids: The total solids were performed using gravimetry with approximately 5 g of sample. The solids content was calculated using Eq. (2):

$$\Gamma S = M_i / M_d \times 100 \tag{2}$$

where,

TS = The total solids content of the sample

 M_i = The initial mass (g)

 M_d = The mass (g) of the dry sample

Protein content: The protein content was determined using the Kjeldahl method with a nitrogen distiller (TE-0363, Technical, Brazil).

Lipid content: The lipid content was determined by the Rose-Gottlieb method, which involves the dissolution of 10 g of sample with ethyl ether and petroleum ether. The weight of the lipid residue was expressed as a percentage of the weight of the sample.

Ash content: The ash content was determined by incineration of the organic material in a muffle furnace (Q318M, Quimis, Brazil). Approximately 5 g of sample was used. The mass of ash residue was expressed as a percentage.

Lactose content: The lactose content was determined using the chromatographic method with an ion chromatograph (850 Professional IC, Metrohm, Switzerland) equipped with an isocratic pump, dialysis system and an amperometric detector (896 Professional Detector). Initially, about 1 g of sample was weighed and diluted to 20 mL of water at 45°C. It was then transferred to a 100-mL volumetric flask, where 1 mL of a 15% (w/v) potassium ferrocyanate solution and 1 mL of a 30% (w/v) zinc acetate solution were added. The contents were stirred to precipitate the proteins and the volume of the balloon was completed with deionized water. The sample was inversely mixed and filtered on quantitative filter paper and subsequently, on a 0.45 µm membrane (Millipore, USA). A 0.1 mol/L aqueous solution of NaOH was used as the mobile phase at a flow rate of 1 mL/min. The injection volume of the sample was 10 µL, where its contents were analyzed in a Metrosep Carb 1 column (150*4 mm) at a controlled temperature (25°C). Data acquisition and

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Samples	pH	Soluble solids (°Brix)	Total acidity (% w/w)	Total solids (% w/w)
W	6.15±0.04a	6.23±0.02a	0.15±0.00a	6.45±0.11a
W1	5.19±0.05b	27.20±0.12b	0.90±0.03b	28.53±0.38b
W2	5.15±0.03b	32.50±0.34c	1.07±0.01c	31.90±1.58c
W3	5.13±0.04b	37.00±0.07d	1.21±0.00d	37.43±0.61d
W4	5.10±0.09b	41.60±0.17e	1.38±0.03e	41.03±0.12e
W5	5.09±0.06b	45.80±0.22f	1.47±0.02f	45.93±0.38f
W6	5.07±0.06b	49.50±0.15g	1.64±0.02g	47.07±0.25g
W7	5.06±0.04b	55.50±0.08h	2.00±0.02h	49.14±0.22g
W8	5.05±0.05b	59.50±0.02i	1.98±0.03h	57.73±1.73h
Samples	Lipid (% w/w)	Ash (% w/w)	Protein (% w/w)	Lactose (% w/w)
W	0.22±0.07a	0.59±0.06a	1.25±0.02a	4.95±1.43a
W1	1.63±0.10b	1.93±0.06b	3.99±0.09b	15.68±0.93b
W2	2.07±0.40c	2.07±0.08c	4.32±0.04b	18.81±1.35c
W3	2.53±0.03d	2.19±0.17d	5.10±0.11c	22.84±2.71d
W4	2.92±0.34e	2.36±0.04e	5.76±0.05d	25.76±6.36e
W5	3.03±0.02e	2.85±0.79f	6.42±0.03e	28.56±7.06f
W6	3.53±0.03f	3.65±0.26g	6.72±0.06f	31.53±6.42g
W7	4.37±0.01g	3.63±0.17g	6.44±0.21e	32.53±1.53h
W8	4.97±0.03h	4.13±0.32h	7.88±0.10g	36.85±0.04i

Table 1: Composition of whey and whey concentrate

Different letters in the same column indicate significant differences (p < 0.05).

processing were performed using the MagIC Net 3.0 program (Metrohm). An analytical curve with known concentrations of lactose was prepared previously.

Surface tension: Surface tension measurements were performed on the samples using the Wilhelmy's plate method with a tensiometer (DCAT 11EC, Data Physics, Germany) and a platinum plate (19.9*10.0*0.2 mm). The experiments were run in triplicate at a temperature of $29\pm1^{\circ}$ C. The balance of the tensiometer was calibrated internally by the SCAT program.

RESULTS AND DISCUSSION

Physical-chemical characterization of whey: Table 1 shows the composition and physical properties of the whole whey samples and their respective concentrates. For each property, the concentrated samples showed significant differences from the whole whey sample and even among themselves, at a 95% confidence level, which indicates that concentration by evaporation has a significant influence on the content of Whey's organic components.

For whey, all components (protein, lactose, lipids, ash and lactic acid) had a direct relationship with the increase of the total solid and soluble solids content (°Brix) and the characteristic behavior of concentration through evaporation. During the concentration process, the pH presented a behavior inversely proportional to the variation of the solids, which can be attributed to the release of protons by primary and secondary phosphates that occurs when calcium phosphate becomes supersaturated. As the water content decreases, the association of the ionic groups of the proteins, which decreases the pH. A change in concentration, expressed as Q (concentration factor), causes a decrease in pH; it decreased by 0.5 units for a

Γable 2: Surface tension of milk and the concentrated milk samples				
Samples	TS (% w/w)	$\sigma (mN \cdot m^{-1})$		
М	10.72±0.01ª	40.62±0.01°		
M1	11.07 ± 0.01^{b}	38.67 ± 0.87^{b}		
M2	$11.41\pm0.18^{\circ}$	41.12±0.03°		
M3	15.77 ± 0.02^{d}	42.35±0.42 ^d		
M4	20.70±0.02°	36.87±0.22ª		
M5	25.43 ± 0.02^{f}	36.48±0.24ª		
M6	28.43±0.01 ^g	42.66 ± 0.30^{d}		
M7	35.33 ± 0.01^{h}	40.41±0.27°		
M8	39.41 ± 0.02^{i}	39.21±0.25 ^b		

Different letters in the same column indicate significant d	ifferences
(p<0.05).	

Q of 3 (Walstra *et al.*, 2006), which is comparable to the behavior of sample S1, which had a decrease of 0.96 units in pH for a Q of 4.

Surface tension of milk and concentrated milk: Table 2 shows the surface tension of milk and concentrated milk samples at different processing times. The analysis of variance shows that the concentration of milk, obtained through evaporation, had a significant effect on the surface tension.

The mean surface tension for milk was 40.62 ± 0.01 mN/m, while, for concentrated milk, it was between 36.48 and 42.66 mN/m. These values agree with those reported by Kristensen et al. (1997) for milk with 3.5% fat at 25°C (41.89 mN/m). However, they are inferior to the values reported by other authors: Williams et al. (2005) reported values of 42 to 48 mN.m⁻¹ and Fox and MacSweeney (1998) reported values of 40 to 60 mN/m. These differences are from measurements that were carried out under different conditions (temperature and sample handling), which could cause free fatty acid release by lipolysis. It should be noted that the increase of free fatty acids or rancidity decreases the surface tension of milk. Another factor that may have influenced is the lactation period of the animals when the milk was obtained. Because several authors have shown that, according to the time of year, the



Fig. 1: Surface tension of the concentrated milk at different total solids content

concentration of free fatty acids in milk varies and that this variation has an effect that is inversely proportional to the variation of the surface tension (Jenness *et al.*, 1974; Chazal and Chilliard, 1985).

Figure 1 shows the effect of the milk concentration on the surface tension. It has several zones, which are characterized by increases and decreases in surface tension. However, the overall effect is a small decrease in the surface tension, e.g., from 40.62 mN/m, in whole milk, to 39.21 mN/m in the most concentrated milk sample ($39.41\pm0.02\%$). Tomczyńska-Mleko *et al.* (2014) found a similar behavior for solutions of isolated proteins with concentrations between 6-11% and reported that there were no significant global changes; however, when studying a lower range (0-10 mg/L), the surface tension decreased exponentially from 67 to 50 mN/m.

Milk is a very complex liquid and therefore, there may be various dynamics in the active surface components while it is being concentrated. In the first zone of Fig. 1, the surface tension first decreases when it has 7.6% of total solids and then it increases when it contains 17.7% of total solids. This behavior can be explained by the release of primary and secondary phosphates from calcium phosphate during the concentration of milk, which is naturally close to its saturation point. Any change in concentration and temperature leads to its gradual insolubilization in the form of colloidal salt, which results in a decrease in intermolecular interactions and, in turn, a decrease in the surface tension. This effect occurs until the free phosphate is completely associated with the phospholactam submicelles, which presents a new equilibrium of molecular interactions and increases the surface tension. It is possible that a subsequent decrease in the surface tension corresponds to the second zone (16-28% ST) because the submicelles of casein begin to agglomerate once they are mineralized in the presence of ionic calcium and phosphate, which forms casein

micelles by reducing their electric charge. These micelles are of a considerably larger size and they have a lower degree of hydration, which decreases their molecular interactions (González-Tello *et al.*, 2009; Walstra, 2013).

The third zone is characterized by a decrease in the surface tension from a solids content of about 30%. This behavior can be explained by the supersaturation of α -lactose as insoluble crystals, which are imperceptible at low concentrations where nucleation begins. It should be noted that the formation of the alpha isomer in this sugar is favored by the experimental conditions of this study (vacuum and temperatures below 98°C) and by increasing the components, such as salts and organic acids, the molecular interactions are decreased (Gerigon *et al.*, 2013). Although lactose is not a major active surface component in milk, it can have significant influence once it is in the supersaturated state (Fox and Paul, 1998; Schuck, 2011).

Surface tension of whey: Table 3 shows the surface tension of whey and whey concentrate at each concentration level. The analysis of variance shows that the whey concentration using evaporation had a significant effect on the surface tension.

Table 3: Surface tension of the samples of whey and whey

conce	cintate	
Muestra	TS (% w/w)	$\sigma (mN \cdot m^{-1})$
W	6.45±0.11ª	35.82±0.19ª
W1	28.53 ± 0.38^{b}	36.80±0.25 ^b
W2	31.90±1.59°	38.50±0.33°
W3	37.43 ± 0.60^{d}	39.85±0.15 ^d
W4	41.03±0.12°	41.45±0.24°
W5	45.93 ± 0.38^{f}	39.13±0.13°
W6	47.07 ± 0.25^{g}	39.13±0.12°
W7	49.14 ± 0.22^{g}	44.30 ± 0.28^{f}
W8	$57.73{\pm}1.47^{h}$	43.78 ± 0.13^{f}



Fig. 2: Surface tension of whey concentrate at different total solids content

Different letters in the same column indicate significant differences (p<0.05).

The mean value of whey's surface tension was 35.82 ± 1.267 mN/m, while the value for whey concentrate was between 36.8 and 44.3 mN/m. These values agree with those reported by Roehl and Jelen (1988) for unconcentrated whey (41.7 mN/m), but they differ from those reported by other authors: 48 mN/m (Fox and MacSweeney, 1998) and 56.6 MN/m (Kessler, 2002). These differences agree with the findings for milk, where values were also found that were lower than those reported in the literature.

Some authors have studied the variability of the surface tension and reported that it may be due to factors such as the techniques used in the determination, the composition, the temperature history, the storage time and the manipulation of samples, such as with agitation (Williams *et al.*, 2005). Mukherjee *et al.* (2005) reported that the surface tension of different milk types was lower when using the Wilhelmy's plate method compared to the capillary ascent method. Kristensen *et al.* (1997) reported that the dynamic method of the drop number gives higher values than the static method using the Du Nouy ring, because of the diffusion times of the surfactants at the interface.

Figure 2 shows the variation of the surface tension of the whey concentrate samples as a function of the Total Solids content (TS). It is observed that there is a linear increase in the surface tension as the TS increases. The linear model Eq. (3) presents a coefficient of determination (\mathbb{R}^2) equal to 0.67, which means that the model explains only 67% of the variation of the surface tension as a function of TS:

$$\sigma = 33.80 + 0.16 * TS$$
(3)

As the total solids did not adequately explain the variation of the surface tension in whey concentrated through evaporation, it was analyzed according to its major components (protein, lipids, lactose and ash). It was observed that the lipid content (p = 0.00062), protein (p = 0.031) and ash (p = 0.006) had significant effects on the surface tension. Protein matter and ash had inverse influences, i.e., the surface tension decreased with the elevation of this content. This is because these components are important surface active agents in dairy products, which are absorbed in the air/water interface and form a monomolecular layer on the surface. The decrease in the surface tension with increasing protein concentration has been reported for proteins isolated from whey (Xu et al., 2012; Tomczyńska-Mleko et al., 2014) and peas (González-Tello et al., 2009); however, this effect is lower than that produced for pea proteins because globular protein peptides with larger molecular weight slowly diffuse to the interface.

The surface tension was directly affected by the lipid concentration, i.e., the increase in lipid content increased the value of the surface tension. For protein emulsions isolated from whey, the addition of fat increases the surface tension because more protein is needed to stabilize the interface of the fat droplets (Xu et al., 2013). On the other hand, it was possible to alter the fat globules in the whey samples, which could have occurred due to manipulation and the temperature history that samples were subjected to: cooling (\sim 12°C), pre-heating (\sim 35°C), concentration (\sim 45°C), cooling (~5°C) and finally, heating for surface tension measurements (~30°C). According to Williams et al. (2005), the denaturation of proteins on the surface of fat globules can be present, which increases the surface tension because they lose the capacity to adsorb on their surface. In thermal denaturation, proteins are added on the surface of fat globules because of the disulfide bonds between them. Although denaturation is delayed at high lactose concentrations, it may still occur, which would allow aggregation and would increase the surface tension. This mechanism was dominated by concentrating on whey samples and therefore, the surface tension increased linearly as the total solids content was increased as observed in Fig. 2.

For 50% of the total solids, the surface tension was stable. This can be explained by the supersaturation of crystals of alpha and beta-lactose, which begin to appear rapidly when the total solids are above 45-55% during the concentration of whey (Lapčík et al., 2015), the which decreases molecular interactions. Additionally, the concentration of components, such as salts and organic acids, favors the nucleation of alphalactose crystals. These dynamics counteract the effect of increased surface tension by protein denaturation in fat globules (Gerigon et al., 2013; Smart and Smith, 1992).

Considering the coefficients of significant effectslipid, protein and ash content-the data can be represented by a linear model. It should be noted that the coefficient for the lactose content variable was not significant (p = 0.159), which indicates that a change in this variable does not significantly affect the surface tension of the concentrated whey. The linear model Eq. (4), whose contents included lipids (L), proteins (P) and ash (A), presented an R^2 of 0.98 and an average error of 0.306. This equation is useful for predicting changes in the surface tension caused by changes in the concentration of whey constituents when they are subjected to evaporation processes:

$$\sigma = 38.26 - 1.52 * P + 5.73 * L - 3.44 * A \tag{4}$$

CONCLUSION

The surface tension in milk is affected during evaporation by the concentration of protein, ash, lactose and lipids, which act as active surface components. However, it does not show a marked behavior when the concentration of the milk passes from 10.7 to 40% of the TS and the overall effect is a decrease in the surface tension from 40.62 to 39.21 mN/m.

The surface tension of whey is affected during evaporation by the concentration of protein, ash and lipids, which act as active surface components. The effect is a linear increase in the surface tension, which decreases from 35.82 to 43.78 mN/m as the total solids vary from 6.5 to 58%. Other components, such as lactose, have no significant influence on this property.

Lipids have the greatest influence on the surface tension. This is due to the denaturation of fat globules, which reduces the adsorption capacity at the surface. Denaturation is proportional to its concentration and is caused by manipulation and the heat treatments. The surface tension of the whey can be represented by a linear model taking into account the content of proteins, lipids and ash.

CONFLICT OF INTEREST

The authors of this study do not have conflict of interest to report.

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