Influence of Milk Somatic Cell Counts in Electro-wetting

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ABSTRACT

The purpose of this paper is to demonstrate the relationship between somatic cell count (SCC) in the milk of dairy cows and electro-wetting behavior. The raw milk of dairy cows in foremilk was sampled and examined to determine its composition including SCC, fat ratio, protein ratio, lactose ratio, total solid ratio, urea nitrogen, and citric acid. To minimize bio-molecular adsorption on hydrophobic surfaces, electro-wetting in the contact of angle measurements for each experiment were completed within one minute. We obtained a negative correlation between SCC and citric acid in milk. As a result, the changes in electro-wetting contact angle with higher SCC are less than with lower SCC, the higher the level of SCC, the less citric acid there was. To gain a better understanding of the electro-wetting behavior with SCC, we disregarded the EC effect related to ionic strength. With lower SCC, we observed changes in the contact angle during electro-wetting to a greater degree than with higher SCC. In conclusion, the phenomenon could be explained by the fact that greater bio-molecular adsorption on the substrate with higher SCC increased the effective thickness of the dielectric layer, thereby decreasing the cossv of Lippmann-Young equation and increasing Θv with the application of voltage.

Keywords: electro-wetting, contact angle, somatic cell count, bio-molecular adsorption, electrical conductivity

1. INTRODUCTION

Electro - wetting is induced by changing the liquiding the liquidt, sed the effective thickness of the dielectric layer, thereby decreasing the cossing the cos the coso gain a better understand of the nd citric acid. To minimize , and enhances the wetting behavior of the liquid [1-6]. For the transportation and manipulation of aqueous solutions, electro - wetting has shown many advantages [7, 8]. Because it does not involve any mechanically moving components, mechanical fatigue is avoided and damaged to the material being transported is reduced. For instance, Fowler et al. [9] applied electro-wetting to enhance the mixing efficiency of liquid droplets containing red blood cells and globular protein. In addition, the power consumption of electro - wetting-based devices is zero at rest and very low in movement [10]. It is also easily integrated into micro electro-mechanical systems

(MEMS) for the transportation and mixing of liquids, in the use of devices such as micro-lenses, micro-mixers, biological micro-arrays, and micro-motors [11-18].

A key challenge for all bio-fluidic chips, including those operated by electro-wetting-on-dielectric (EWOD), is the prevention of the nonspecific adsorption of biomolecules. Yoon and Garrell [19] proposed methods to minimize bio - molecular adsorption in EWOD-based fluid actuation within air-filled channels. By selecting the pH of the solution and the square wave parameters, they determined that bio - molecular adsorption on hydrophobic surfaces can be minimized or eliminated. They also indentified two mechanisms for bio-molecular adsorption under EWOD conditions: passive adsorption arising from hydrophobic interaction and electrostatically driven adsorption when an external electric field is applied.

The arrival of protein at the interface is assumed to be driven solely by diffusion processes, which are dependent on bulk concentration and the coefficient of diffusion [20]. Adsorption of protein on hydrophobic surfaces tends to be very strong and often partially irreversible. Adsorption on charged surfaces tends to be a function of the charge character of the protein, the pH of the medium and ionic strength [21]. Quinn et al. [22] proposed that the ionic strength of a solution can influence electro - wetting behavior, namely greater ionic strength increases the response to electro-wetting. In milk, electrical conductivity (EC) is determined by the concentration of anions and cations, which is obviously related to ionic strength. The most important ions in milk are Na+, K+, and Cl- [23, 24]. Typical EC of normal milk appears to be between 4.0 and 5.5 mS/cm at 25oC [25]. The somatic cell count (SCC) of milk indicates the level of both epithelial cells and white blood cells per milliliter of milk. With an infection or inflammation of the cowow Cla+, K+, and C blood cell level rises, thereby causing an elevated SCC [26]. Most studies suggest (statistically) that cows with SCC less than 200,000 cells/ml are not likely to be infected with major mastitis pathogens. In contrast, cows displaying over 200,000 cells/ml are considered to be infected [27].

In this paper, raw milk is applied as the working fluid. In general, raw milk has a pH ranging between 6.4 and 6.8, making it slightly acidic. The composition of raw milk includes 87.7% water, 4.9% lactose, 3.4% fat, 3.3% protein, and 0.7% minerals per 100 grams [28, 29]. The composition of raw milk varies depending on the breed, the feed, and the point in the lactation period. With regard to the physical properties of fat and protein in milk, milk fat appears in the form of globules surrounded by a protein, with phospholipid membrane stabilizing the globules in the water phase of milk. The fat globules range in size from less than 1 y causing an elev There are two major categories of milk protein: casein and serum. In cow's milk, approximately 82% of milk protein is casein and the remaining 18% is serum. The caseins in milk form complexes called micelles that are dispersed in the water phase of milk.

They are spherical, measuring 0.04 to 0.3 µm in diameter.

Due to the fact that EC in milk is dependent on ionic strength, higher EC increases the effectiveness of electro-wetting. However, unspecific adsorption characteristics of various biomolecules in milk as they pertain to hydrophobic surfaces have an inverse effect on electro-wetting response [19]. For this reason, the combination of ionic strength and bio-molecular adsorption perturbs electro-wetting in this study. To gain a better understand of electro-wetting behavior with SCC adsorption, the influence of milk EC related to ionic strength is disregarded temporarily. The influence of SCC adsorbed on the hydrophobic surface in electro-wetting was demonstrated with the sessile drop method in this study.

2. METHODS

A schematic diagram of the experimental setup for the electro-wetting mechanism is shown in figure 1. The anode comprised an electrically conductive layer of indiumn this study.rengcoated on top of a glass plate. The thickness of S1818 insulating layer was 1 µh. A hydrophobic dielectric layer of Teflon AF1600 was spin-coated on the S1818 layer to a thickness of 0.3ly. The influence of SCC adsorbed on the hydrophobic surface in electrowetting was demonstrated with the hydrophobic layer of Teflon AF1600 served two purposes: one was to reduce the hysteresis effect of the contact angle of the milk droplet on the surface, and the other was to increase the range of change in the contact angle under electro-wetting conditions. A negative voltage was applied to the droplet and a positive voltage to the layer beneath.

The raw milk in the foremilk of dairy cows was sampled from a herd of Holsteins in Taiwan. The collected samples were based on to the layer beneath.he milk droplet on the surface, ahe electrical conductivity (EC) of the milk. The samples were stored at 4oC, transported to the laboratory on ice, and tested upon arrival. The samples were examined for their somatic cell count (SCC), fat ratio, protein ratio, lactose ratio, total solid ratio, and the concentrations of urea nitrogen and citric acid. Due to the fact that bio-molecular adsorption is dependent on time without an externally applied voltage [19], contact angle measurements for each test are completed within one minute to minimize bio-molecular passive adsorption on hydrophobic surfaces.

The contact angle of droplets in electro-wetting is measured by depositing a 3µL milk droplet onto the substrate and inserting a Pt electrode. A variety of DC voltages, ranging from 0 to 130 volts, were tested. Because the components of the milk significantly adsorbed on the hydrophobic surface, freshly prepared insulating films were used in all experiments. No electrolysis or measurable electric current was apparent between the Pt electrode and the ITO electrode upon application of 130 volts, indicating that the combined dielectric layer behaved as an insulator and failed to undergo dielectric breakdown. In addition, contact angles were determined independently from images of the sessile drop by numerically fitting a tangent close to the contact line. At least three experiments for each case were conducted.

3. RESULTS AND DISCUSSIONS

This study analyzed the compositions of milk and contact angle in electro-wetting with the sessile drop method, and investigated the relationships among them. In general, the threshold value of normal milk is defined by somatic cell count (SCC) less than 50 (104/mL) or electrical conductivity (EC) less than 6.0 (Ms) [26, 27]. All milk contains white blood cells known as leukocytes, which constitute the majority of somatic cells. Leukocytes accumulate at sites of inflammation to attack invading bacteria; therefore, somatic cell count can be used to assess the level of udder inflammation. The maximum EC value of normal milk sampled in this study was 5.5 (Ms). According to the composition of milk shown in Table 1, SCC is negatively correlated with the content of citric acid in all milk samples [32, 33]. In other words, as SCC increases in milk, the content of citric acid reduces, (Figure 2). This phenomenon is caused mainly by the physiological response of the udders of dairy cows. Except for the relationship between SCC and citric acid, we observed no other obvious correlations among the components of milk, as illustrated in Figures 3 to 6.

With regard to measurement of the contact angle in electro-wetting, when the milk drop is placed on the surface and then removed, a surface stain induced by the adsorption of the milk was clearly observed, which may lead to bio-molecular adsorption at the site [19]. The contact angle of all milk samples without voltage applied was approximately 100o ±2o in the beginning. To gain a better understand of the electro-wetting response with SCC, we temporarily disregarded the influence of EC in diminishing the influence of the combined effect of EC and SCC in electro-wetting, and only SCC is taken into account for each case. Therefore, the same EC value was assigned for each sample in each case, and the changes in the contact angle in electro-wetting were investigated with different SCC. The results revealed more significant changes in contact angle with a lower SCC during the application of higher voltage (above 100 volts). In contrast, with a higher SCC, the change in contact angle was less than with lower SCC, as shown in Figures 7 to 10.

Two mechanisms were observed for the biomolecules adsorbed on the surface during electro-wetting [19], one was the highly adsorbable characteristics on hydrophobic surfaces, which was passive adsorption, and the other was the net effect of the electrostatic adsorption on the surface, which was active adsorption. Because milk is a complex bio-molecular solution, biomolecules encounter significantly hydrophobic and electrostatic interactions on the surface. In the experimental results described above, we found that with a higher SCC, there was a tendency for less change in contact angle during the application of higher voltage (approximately 100 volts). The main reason for this may be that more biomolecules in milk are adsorbed on the surface through hydrophobic and electrostatic interaction, thereby enhancing the dielectric effect of the insulating layer, namely increasing the effective thickness of the dielectric. On the other hand, based on Lippmann-Youngnter significan: $\cos\Theta = \cos\Theta_0 + cV^2/2\gamma$, where Θ_0 is contact angle without an electrical field across the interfacial layer, the surface tension of γ liquid-gas, and c the capacitance of dielectric layer. Therefore, more biomolecular adsorption would decrease $\cos\Theta$ and increase Θ during the application of voltage, with a resulting reduction in the electro-wetting effect and a change in contact angle. In contrast, due to larger electro-wetting effect induced by fewer biomolecules adsorbed on the surface, changes in contact angle in electro-wetting become more significant.

Although EC is related to ionic strength as well as SCC, significant electro-wetting behavior is not dominated by higher EC but by lower EC. Lower EC is induced by lower SCC, which reduces bio-molecular adsorptions but increases the electro-wetting effect. Therefore, the threshold value of the combined effect of EC and SCC in electro-wetting is worthy of further investigation to build an alternatively electro - wetting-based method for detecting cow mastitis.

4. CONCLUSION

This study determined that somatic cell count (SCC) is negatively correlated to the concentration of citric acid in milk, such that the more SCC there is in milk, the less citric acid there is. To gain a better understanding of electro-wetting as it pertains to SCC, EC was maintained at same value for each sample. The results indicate that changes in electro-wetting contact angle with higher SCC are less than with lower SCC. This phenomenon can be explained by the fact that with higher SCC bio-molecular adsorption increases the effective thickness of the dielectric layer, despite the fact that a less pronounced change in contact angle induced by lower electro-wetting effect was observed.

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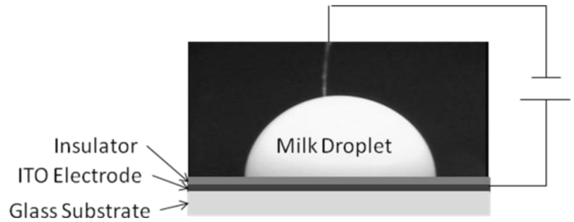

Figure 1. Setup of Electrowetting Experiment

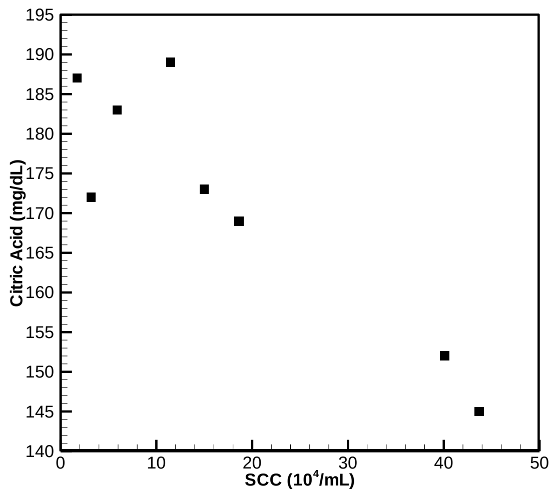

Figure 2. Negative Correlation between SCC and Citric Acid Content in All Milk Samples

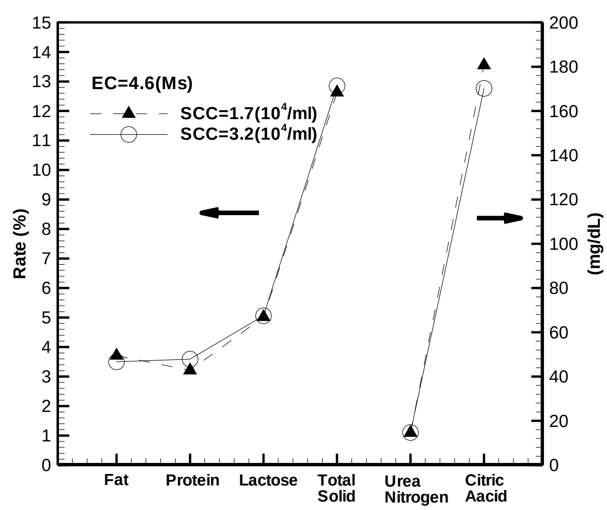

Figure 3. Milk Compositions Comparing SCC=1.7(104/ml) and SCC=3.2(104/ml) at EC=4.6(Ms)

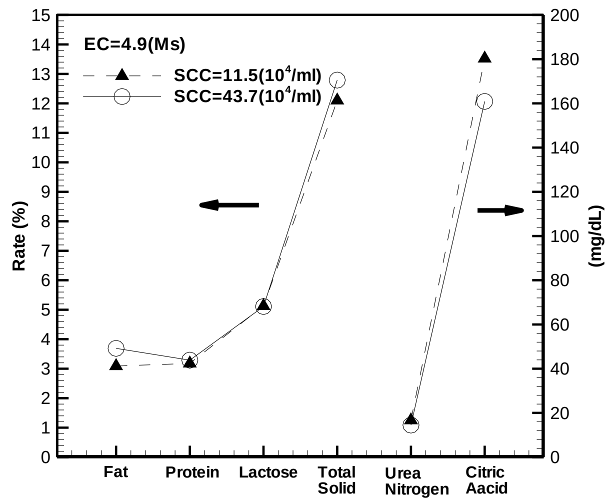

Figure 4. Milk Composition Comparing SCC=11.5(104/ml) and SCC=43.7(104/ml) at EC=4.9(Ms)

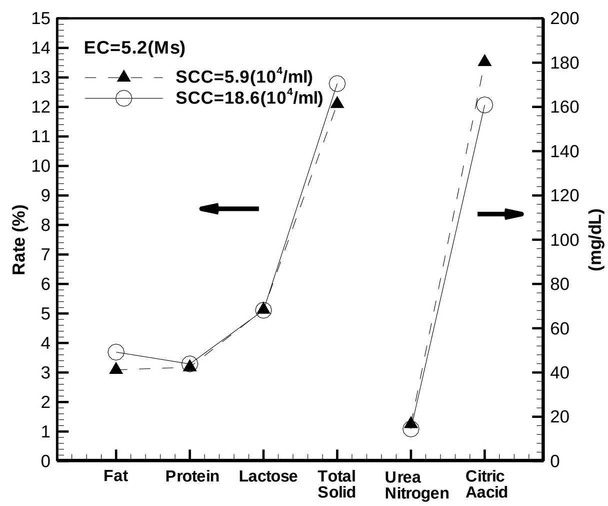

Figure 5. Milk Composition Comparing SCC=5.2(104/ml) and SCC=18.6(104/ml) at EC=5.2(Ms)

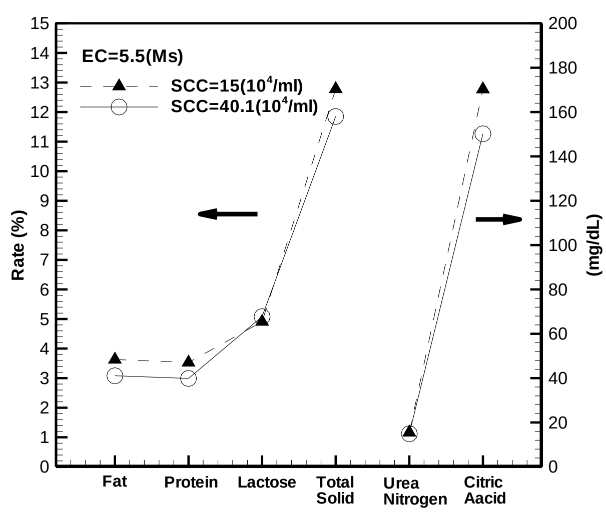

Figure 6. Milk Composition Comparing SCC=15(104/ml) and SCC=40.1(104/ml) at EC=5.5(Ms)

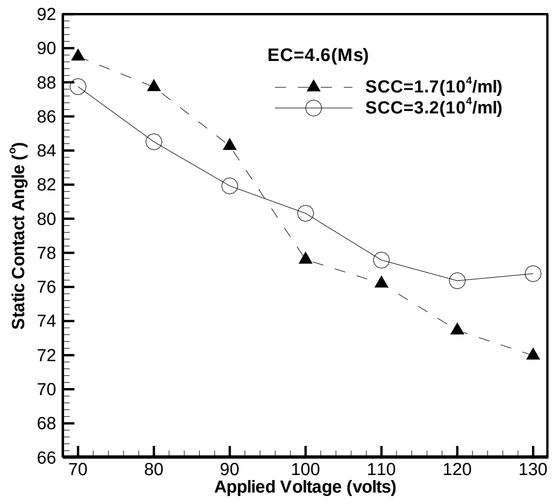

Figure 7. Electro-wetting Contact Angle Comparing SCC=1.7(104/ml) and SCC=3.2(104/ml) at EC=4.6(Ms)

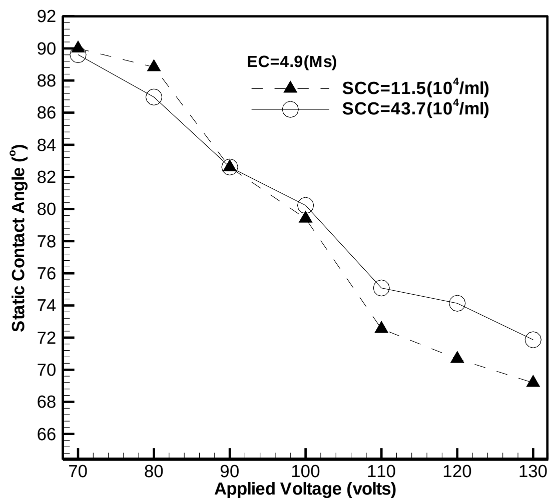

Figure 8. Electro-wetting Contact Angle Comparing SCC=11.5(104/ml) and SCC=43.7(104/ml) at EC=4.9(Ms)

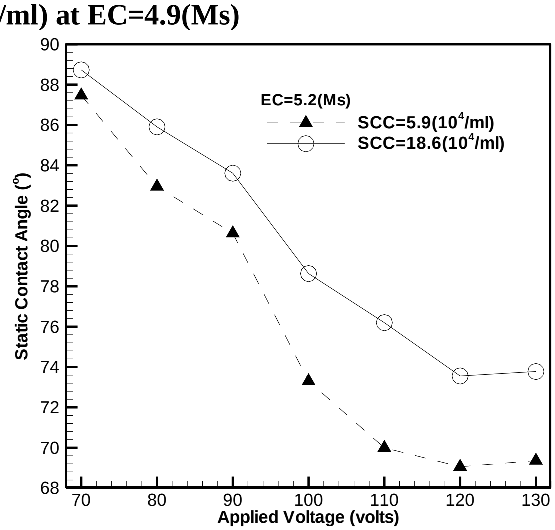

Figure 9. Electro-wetting Contact Angle Comparing SCC=5.9(104/ml) and SCC=18.6(104/ml) at EC=5.2(Ms)

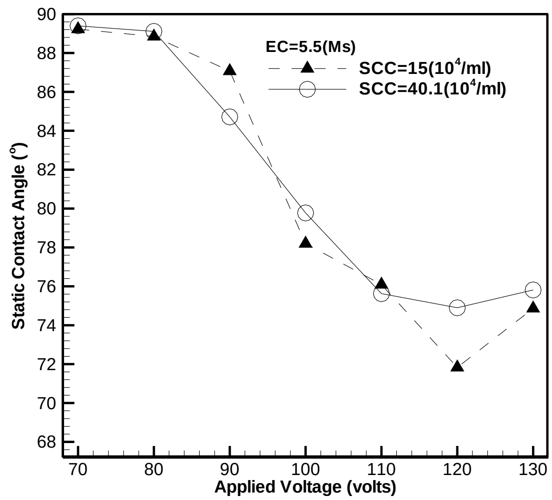

Figure 10. Electro-wetting Contact Angle Comparing SCC=15(104/ml) and SCC=40.1 (104/ml) at EC=5.5(Ms)

Table 1：The Composition of Milk

	EC (Ms)	SCC (104/mL)	Fat (%)	Protein (%)	Lactose (%)	Total Solid (%)	Urea Nitrogen (mg/dL)	Citric Acid (mg/dL)
Case#1	4.6	1.7	3.71	3.21	5.02	12.63	14.6	187
		3.2	3.5	3.59	5.06	12.85	14.7	172
Case#2	4.9	11.5	3.81	3.23	5.27	13	12.1	189
		43.7	3.1	2.75	5.26	11.82	16.9	145
Case#3	5.2	5.9	3.09	3.18	5.13	12.1	16.7	183
		18.6	3.69	3.29	5.11	12.79	14.5	169
Case#4	5.5	15	3.63	3.53	4.91	12.78	15.5	173
		40.1	3.69	3.29	5.11	12.79	14.9	152