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The effect of emulsifying salts on the turbidity of a diluted milk system with varying pH and protein concentration

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ABSTRACT

Solutions of 10 commonly used emulsifying salts (ES) listed in the Code of Federal Regulations (21CFR133.179) for pasteurized process cheese were tested for their effect on the turbidity of a diluted milk system at different pH and protein concentrations to characterize the conditions that affect micellar structure. Emulsifying salt solutions were made by mixing the ES in a 1-in-20 dilution of water in skim milk ultrafiltrate (3 kDa molecular weight cut-off) to obtain ES concentrations from 0 to 248 m*M*. Skim milk was added to solutions containing nanopure water, skim milk ultrafiltrate, and a specific ES ranging in concentration from 0 to 248 m*M* and pH 5, 5.8, 6.8, 7.8, and 8.8. The turbidity of the samples was measured as the optical density at 400 nm immediately after mixing (time, t = 0), after 30 s (t = 30s), and after 30 min (t = 30min). Emulsifying salts were found to cause a decrease in the turbidity of the system, which was modeled using an exponential decay model, where C* represents a threshold salt concentration at which rapid dissociation occurs. At pH values 5.8 and 6.8, the ES caused the greatest decrease in turbidity of the diluted milk system. At pH 5, the ES had the least effect on the turbidity of the system. Sodium hexametaphosphate was found to have the strongest dissociative effect, with a C* value of 0.33 m*M* for t = 0 at pH 6.8. In contrast, the largest C* value calculated at pH 6.8 was monosodium phosphate at 278.22 m*M*. Increased time resulted in lower C* values. The model established for this study can be used to predict the dissociation of casein micelles in the presence of various types of ES.
Key words: emulsifying salt, process cheese, turbidity, micellar structure

INTRODUCTION

Although emulsifying salts (**ES**) are essential in the manufacturing of processed cheese for melting uniformity, texture, spreadability, and oiliness, the specific chemistry behind their mechanism of action is still under investigation. So far, our understanding of the effect that the 13 ES listed in the Code of Federal Regulations (**CFR**; 21CFR133.169) will have on the physical properties of cheese has relied on empirically observing the outcome of the final products (Mounsey and O'Riordan, 1999; Kaliappan and Lucey, 2011). To replace the current trial-and-error approach that governs the usage of ES, a deeper understanding of the physicochemical effects and functional attributes of ES is required. As the effects of ES become better understood, manufacturers will have an easier time adopting their usage for specific applications, such as meeting the demand for clean label products.

It is well documented both that the action of the ES is to affect the structure of casein micelles present in cheese and that the ES chelate calcium from the micelle, causing the micelles to dissociate (Holt et al., 2003; Kaliappan and Lucey, 2011). In processed cheese, these dissociated, amphiphilic proteins move to the interfaces between the fat molecules and aid with emulsification (Dickinson, 1999; Awad et al., 2002). Thus, the ES used in the manufacture of processed cheese are not emulsifiers themselves but cause emulsification in the cheese system through their dissociating influence on caseins (Carić et al., 1985; Kaliappan and Lucey, 2011). Different ES have varied effects on the calcium distribution as well as the final cheese product (McIntyre et al., 2016). The exact structure of the casein micelle is not known; however, it is commonly depicted as a spherical quaternary protein structure with more hydrophilic κ-caseins surrounding an inner layer of more hydrophobic α-and β-caseins. Also present inside the casein micelle is colloidal calcium phosphate, an ionic complex that serves as the "glue" that helps hold the individual casein proteins together in a micelle along with hydrophobic interactions (Holt, 2016).

Individual ES are known to produce different effects in the final cheese product (Kaliappan and Lucey, 2011). Tetrasodium diphosphate (**TSDP**) has been found to have properties unique from other phosphate salts that are affected by a critical ES concentration in the system (Shirashoji et al., 2016). In the case of phosphates, the

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distribution of charges on phosphate and casein molecules and the pK_a values all contribute to the potential for ES-protein interaction. These ES might play a role similar to salts in the Hofmeister series that work at the protein–water interface to change the conformation of the protein due to interaction with the water (Stankey et al., 2011). Some salts in the Hofmeister series (perchlorate, nitrate, and bromide) have been found to be misplaced in terms of the dissociative effects that they have on β-casein (Sawyer and Puckridge, 1973). The authors concluded that individual proteins are likely to vary in their susceptibility to dissociation by chaotropic salts; they also concluded that temperature, pH, and the protein concentration of the system will determine the amount of salt needed to cause dissociation.

Emulsifying salts mentioned in the CFR can be classified into the following groups: monophosphates, condensed phosphates, glassy (long-chain) phosphates, citrates, and tartrates. A general trend in the calcium chelating ability of some of the ES has been established as monophosphate < citrate < diphosphate < hexametaphosphate (Van Wazer and Callis, 1958). Several roles have been established for the ES. The first is their calcium-binding ability, which has a very significant effect on the properties of the cheese because it is thought that an ideal ES consists of the combination of a monovalent/divalent cation with a polyvalent ion (Guinee et al., 2004). The ability of a salt, specifically an ES, to bind Ca^{2+} depends on the valence, type of ion species formed, pH, ionic strength, and temperature (Kaliappan and Lucey, 2011). The direct binding of phosphate ES, especially sodium hexametaphosphate (**SHMP**), to positively charged amino acid residues and the indirect binding via calcium bridges have also been suggested as potential action mechanisms (Mizuno and Lucey, 2005; de Kort, 2012).

Turbidity measurements have been established as a way to indirectly examine the aggregation and dissociation of casein micelles (Parker and Dalgleish, 1977; Orlien et al., 2010; Kaliappan and Lucey, 2011). Because casein micelles are primarily responsible for the white color, or turbidity, in skim milk or casein powder solutions, a decrease in turbidity is due to a dissociation of the casein micelles into smaller structures. This method has been used to confirm that the turbidity of a solution containing casein micelles decreases as the ES dissociate the micelles (Pitkowski et al., 2008; Orlien et al., 2010; Anema and Klostermeyer, 1997). Turbidity measurements have also been used to show that increasing pressure causes a decrease in micellar aggregation at varying temperatures and pH levels (de Kort, 2012).

The aim of this paper was to examine and model the effect that ES have on the structural integrity of casein micelles by measuring their turbidity in a diluted skim milk system at various pH levels over time. This wide range of salts, concentrations, and pH levels examined over time have been chosen to generate a body of data to directly compare the different effects between ES.

MATERIALS AND METHODS

Materials

Monosodium phosphate [**MSP**, molecular weight (**MW**): 119.98 Da; CAS no. 7558-80-7], trisodium phosphate (**TSP**, MW: 163.96 Da; CAS no. 7601-54-9), sodium acid diphosphate (**SAD**; MW: 221.94 Da; CAS no. 7758-16-9, listed in the CFR as sodium acid pyrophosphate), sodium tartrate (**ST**, MW: 230.08 Da; CAS no. 6106-24-7), disodium phosphate (**DSP**, MW: 141.96 Da; CAS no. 7558-79-4), calcium citrate (**CC**, MW: 570.49 Da; CAS no. 5785-44-4), and sodium potassium tartrate (**SPT**, MW: 282.22 Da; CAS no. 6381-59-5) were obtained from Sigma-Aldrich (St. Louis, MO). Dipotassium phosphate (**DPP**, MW: 174.18 Da; CAS no.7758-11-4) and sodium citrate (**SC**, MW: 294.1 Da; CAS no. 6132-04-3) were obtained from VWR (Radnor, PA). Sodium hexametaphosphate (MW: 611.77 Da; CAS no. 10124-56-8) and TSDP (MW: 265.9 Da; CAS no. 7722-88-5, listed in the CFR as tetrasodium pyrophosphate) were obtained from Alfa Aesar (Ward Hill, MA). Potassium citrate (**PC**, MW: 306.396 Da; CAS no. 6100-05-6) and sodium aluminum phosphate (**SAP**, MW: 221.94 Da; CAS no. 10305-76-7) were obtained from Spectrum Chemical MFG Corp. (New Brunswick, NJ). Pasteurized, homogenized skim milk was obtained from the Berkey Creamery at the Pennsylvania State University (University Park).

Sample Preparation

Stock solutions of each ES were prepared at 250 mM by adding the appropriate weight of ES to a 1-in-20 dilution of protein-free serum (**PFS**) in water. Protein-free serum was obtained using a 3-kDa tangential flow regenerated cellulose filtration filter (Prep/Scale Spiral Cartridge, Millipore, Darmstadt, Germany). The pH of the stock ES solutions was adjusted using small quantities of NaOH or HCl as appropriate to pH 5, 5.8, 6.8, 7.8, and 8.8. These stock solutions were further diluted using pH-adjusted, 1-in-20 PFS-in-water solutions to create ES concentrations of 0, 0.1, 1, 5, 10, 15, 25, 50, 100, and 248 mM. Milk samples for pH 5, 5.8, 6.8, and 7.8 were adjusted using small amounts of 1 M HCl or NaOH as appropriate at least 2 h before experimentation and were again measured and adjusted as necessary. In a separate experiment, MSP and DPP were