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Wear Behaviors of Process Cheese With Varying Formulations and the Development of Predictive Models on Shreddability

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WEAR BEHAVIORS OF PROCESS CHEESE WITH VARYING FORMULATIONS AND THE DEVELOPMENT OF PREDICTIVE MODELS ON SHREDDABILITY

by

Jason Young

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ABSTRACT

Wear Behaviors of Process Cheese with Varying Formulations and the Development of Predictive Models on Shreddability

by

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This study investigated the effects of process cheese formulation on microstructure, material, and rheological properties of process cheese to determine the usefulness of wear behavior in predicting cheese shreddability. Experimental process cheese formulations were made with varying levels (2.0, 2.5, 3.0%) of trisodium citrate (TSC) and varying average ages (1, 22, 83, 102 d) of natural cheese (varying levels of intact casein) to create a spectrum of shreddability, material behaviors, and rheological properties for use in shreddability modeling. A modified full factorial design with 12 formulations and 3 replicates on each of the 2 central points was used to study effect of treatments.

Microstructure of process cheese samples was characterized using confocal laser scanning microscopy and transmission electron microscopy. Micrographs of process cheese indicated that size of fat globules decreased with increasing of both age of natural cheese and TSC concentration. Wear behavior for process cheese was determined using a pin-on-disk tribological attachment at sliding velocity of 50 mm/s, 5°C, and 1 N normal force. Age of natural cheese had a significant ($p < 0.01$) positive correlation with penetration depth (mm) and mass loss (g). Rheological characterizations of all samples were performed at 5°C using large and small amplitude oscillatory shear tests. With increasing of natural cheese age, G* values (at 0.01% strain) decreased significantly ($p < 0.01$), and G' (at 0.01% strain) decreased ($p < 0.01$). Texture Profile Analysis performed at 25% compression, 1 mm/s, and 5°C showed that natural cheese age had a significant ($p < 0.01$) negative correlation with gumminess and hardness. Shreddability tests were performed on the Texture Analyzer with a grating rig attachment at 5°C and 15 mm/s sliding speed with 2 kg force. Natural cheese age had a significant ($p < 0.01$) positive correlation with work to grate and negative correlation with crumbliness.

A shreddability index (SI) was developed using sieve data for the process cheese samples. Significant correlations ($p < 0.05$) were found between tribological (wear), rheological and material properties and the SI. Predictive models were created from this correlation. Of the models tested, variables from wear tests (mass loss and penetration depth) were able to account for much of the variation in shredding behavior ($R^2 = 0.74$, $p < 0.01$). It is therefore determined that wear tests are an effective tool in predictive models of process cheese shreddability.

(121 pages)

PUBLIC ABSTRACT

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Process Cheese is manufactured by grinding, mixing, and heating with agitation one or more of the same varieties of natural cheese with an emulsifying agent to create a cheese with desirable properties. After process cheese is made, it is often sliced or shredded. Some of its properties affect how well it can be sliced or shredded and can lead to loss of material due to cheese sticking to equipment or being too crumbly. The loss of material, called wear behavior can incur significant losses to cheese manufacturing operations. The purpose of this study was to produce process cheese formulations with wide range of shredding properties by changing formulation and also to develop predictive model for shreddability using cheese wear behavior data.

Experimental process cheese formulations were made with varying levels of an emulsifying agent and varying average ages of natural Cheddar cheese. The effects of these treatments on wear behavior, shredding behavior, and other important processing-related attributes were significant. We found that with increasing age, extent of proteolysis increased, and material became softer and sticky. A good correlation was found between shredding data and wear behavior and the other processing-related process cheese attributes. Various models with different combinations of variables from wear data and mechanical properties of cheese were tested statistically and the model including only wear behavior (mass loss, penetration depth) was found suitable to predict the shredding behavior of process cheese.

DEDICATION

Dedicated to my wife Kaity for her endless support, patience, love, and sacrifice.

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LIST OF ABBREVIATIONS

BIC = Bayesian’s Information Criterion

CLSM = Confocal Laser Scanning Microscopy

CP = Mallows' Cp-Statistic

DSP = Disodium Phosphate

ES = Emulsifying Salt

LAOS = Large Amplitude Oscillatory Shear

LVR = Linear Viscoelastic Region

OM = Optical Microscopy

PC = Process Cheese

PPC = Pasteurized Process Cheese

PCF = Pasteurized Process Cheese Food

PCS = Pasteurized Process Cheese Spread

PCP = Pasteurized Process Cheese Products

PRESS = Prediction Sum of Square

RMSE = Root Mean Square Error

RSM = Response Surface Methodology

RVA = Rapid Visco Analyzer

S = Strain Stiffening Factor

SI = Shreddability Index

SAOS = Small Amplitude Oscillatory Shear

SEM = Scanning Electron Microscopy

T = Strain Thickening Factor

TEM = Transmission Electron Microscopy

TPA = Texture Profile Analysis

TSC = Trisodium Citrate

VIF = Variation of Inflation Factors

INTRODUCTION

Processability of cheese (slicing, dicing, and shredding) is a major concern for cheese manufacturers (Banville et al., 2014). During processing, problems such as loss of material can arise from cheese sticking to the moving parts of machinery or crumbling during high speed operations (Fox et al., 2004a). The mass removal process, called wear phenomenon, can incur significant losses to cheese manufacturing operations. Identifying material properties (including compositional factors) that control processability of cheese (specifically shreddability) and the prediction of such properties could be of commercial significance to minimize operational losses. The focus of processability of cheese in this research is on shreddability which was defined in terms of mass loss (production of fines and sticking to equipment) and was altered by compositional factors. Notwithstanding the need for a focus on shreddability, the factors which influence shreddability have received little attention and therefore remain unclear (Banville et al., 2014). According to researchers, the absence of a suitable method for testing shreddability is responsible for the lack of information regarding the subject (Childs et al., 2007). Apostolopoulos and Marshall, (1994) developed an objective method to quantify shreddability of cheese using physical characteristics of individual shreds (shape and size and amount of fines) as measured through image analysis and tendency of shreds to stick to the equipment. Some research has been done on predicting individual components of cheese shreddability i.e. length of shreds, quantity of fines and adhesion characteristics (Banville et al., 2014), yet there are no models developed to predict wholesome shreddability of cheeses. Moreover, developed models for individual components either relied on empirical observations, lack statistically significant predictive power, and/or required many compositional and rheological descriptors to predict shredding behavior. The aim of this research was to create a robust predictive model for the cheese shreddability index obtained by using the sieve method described by Apostolopoulos and Marshall, (1994); by using wear behavior data which includes mass loss and mechanical breakdown characteristics as indicated by penetration depth similar to that is incurred during shredding operation of cheese. The use of wear tests to predict mass loss can be a better predictor because of their close relationship with the processing behaviors. Process cheese was used as a model system for this research to study mass loss and shreddability because of its ability to be formulated with wide range of shreddability characteristics ranging from brittle to viscous and sticky failure of the material. Extent of proteolysis as indicated by average age of PC formulations and the level of Tri-sodium citrate (TSC) were manipulated to obtain diverse textural attributes of PC. Process cheese was tested to determine if wear behavior (alone, or in combination with various material and rheological properties) could be used to create a predictive model for evaluating the shreddability of cheese.

HYPOTHESIS AND OBJECTIVES

Hypothesis of this Study

It is hypothesized that wear behavior of PC is a good indicator of its processability, more specifically shreddability because during shredding and slicing operations PC undergoes similar wearing patterns and mechanical breakdown processes. Hence, we propose that it is possible to develop a predictive model for PC slicing and shredding abilities by measuring its wear behavior, mechanical, and functional properties.

Objectives of this Study

1. Prepare process cheeses with different physical properties based on age of cheese used and emulsifying salt concentrations.
2. Determine the effect of cheese age and emulsifying salt level on process cheese material properties such as hardness, gumminess, work to grate, and crumbliness.
3. Determine the correlation between the shreddability rig test and the sieve shreddability index.
4. Determine correlations between wear behavior and other material properties.
5. Develop a predictive model for shreddability using wear data.

LITERATURE REVIEW

Shreddability

The ability of cheese to be sliced, shredded, or diced from a block format is a major concern for cheese manufacturers (Banville et al., 2014). Shredding of cheese in particular, is an important operation because it allows faster melting as compared with other methods of size reduction such as slicing and cubing (Ni and Guansekaran, 2004).

The term shreddability is a general term which encompasses various characteristics of shredded cheese (Banville et al., 2013). These characteristics include the ease of machinability, the geometry and integrity of cheese shreds, the propensity of shreds to mat during storage, and the production of fines during shredding (Childs et al., 2007; Banville et al., 2013). When shredding problems occur, they happen because of lack of optimal mechanical properties of the cheese (Banville et al., 2014). It is widely known that shredded cheese must meet specific functional properties such free oil release, meltability, and stretchability (Kindstedt et al., 2010; Banville et al., 2014). However, it must also meet specific physicochemical properties to have optimal shredding behavior.

Despite the obvious need for a focus on processability, many factors which influence shreddability have received little attention and thus remain unclear (Banville et al., 2014). Unavailability of more easily obtained and statistically viable method for testing shreddability is responsible for the lack of information on this topic (Childs et al., 2007). Currently, most data for shreddability is based upon empirical observations (Banville et al., 2014).

Several authors used methods to quantitate shreddability based upon visual characterization of shreds (Apostolopoulos and Marshall, 1994; Sundaram Gunasekaran and Mehmet, 2003; Banville et al., 2014). Other researchers proposed measuring adhesion to equipment, creep and recovery, tack energy, and shred distribution to evaluate cheese shred quality (Childs et al., 2007). The latter method was altered to determine the effects of various cheese making conditions on the shreddability of Mozzarella cheese (Banville et al., 2013). Most recently, a predictive shreddability model for pizza Mozzarella was created using compositional and textural descriptors (Banville et al., 2014). In this model, none of the compositional or rheological descriptors taken alone could predict shredding behaviors, but a minimum of a combination including four relevant descriptors (water soluble nitrogen as a percentage of total nitrogen, frequency dependence of complex modulus (G*), colloidal calcium, the log of G* at 1 Hz, hardness, adhesiveness, and frequency dependence of the phase angle) were needed for prediction (Banville et al., 2014). Despite of the fact that deformation regime while measuring wear behavior is closer to actual that taking place during shredding process, there is no systematic study on predicting shreddability of cheese using wear data. A significant challenge with measuring shreddabilty of a natural cheese is that it may have some structural variations such as splits , slits, cracks, which will reduce shreddability of a cheese block. It is difficult to account for these variations while developing predictive models. Also, while determining their material properties e.g., TPA, rheology, tribology, it is convenient to use samples that are devoid of these defects which is not a true representation of the actual sample. Since the structure of process cheese is more uniform than natural cheese and it can be produced with diverse material properties, we selected process cheese in this study as means to avoid these problems and provide a cheese that doesn’t change during storage time to accomplish all the testing.

Process Cheese - definition and standards

Process cheese is the generic name for three categories of emulsified cheese products, namely: pasteurized process cheese (PPC), pasteurized process cheese food (PCF) and pasteurized process cheese spread (PCS) (U.S. Food and Drug Administration, 2018). Each category is made by grinding, heating, and mixing different ages and varieties of cheese with an emulsifying agent until a homogenous mass is formed (U.S. Food and Drug Administration, 2018). An overview of the entire PC making process is outlined in Figure 1. In order to be legally classified as PC, specific levels of ingredients, moisture, fat, and pH in the final product must be met as shown in Table 1 (Fox et al., 2004; Kapoor & Metzger, 2008;).

Although PC is required to meet the limits within the standards of identity in the CFR as partially outlined in Table 1, there are variations of formulations made by manufacturers within those standards to meet consumers’ needs. These variations directly affect the functional properties of the product (Guinee, 2002). Functional properties refer to the performance of PC through all stages of preparation and consumption that contribute to the flavor and aesthetic appeal of the prepared food (Guinee, 2002). Some of these attributes include meltability, shreddability, flowability, the propensity of the cheese to mat, and others. Functional properties which help characterize these attributes and can be related to processability and are measured by rheological, textural, cooking, and sensory-related properties (Guinee, 2002). Rheological and textural attributes have been demonstrated to be most related to processing behaviors

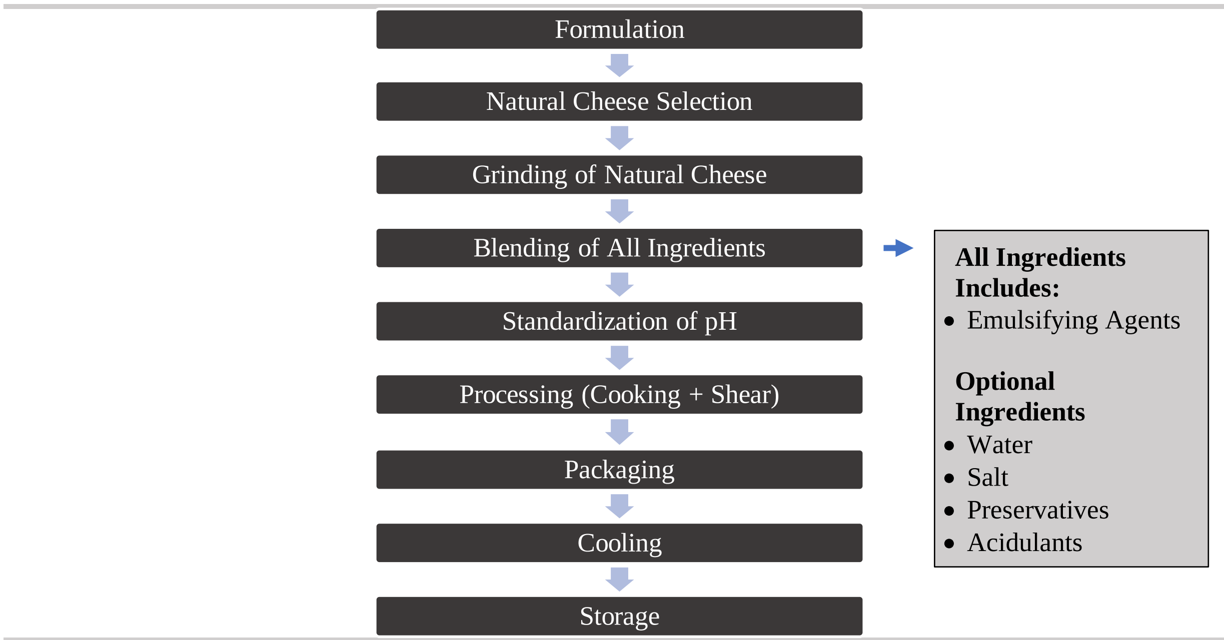


Figure 1. Schematic of the basic manufacture of process cheese

Table 1. The CFR definition of the 3 major categories of PC in the United States

Category	Major ingredients and other optional ingredients (with allowed levels)	Moisture (%w/w)	Fat (%w/w)	pH
Pasteurized Process Cheese	Natural cheese	≤ 40	≥ 30	≥ 5.3
	Emulsifying agent(s) ≤ 3% (w/w) of the final product			
	Cream, anhydrous milk fat, dehydrated cream (weight of the derived fat is ≤ 5% (w/w) of the final product)			
	Acidifying agent (vinegar, lactic acid, acetic acid, phosphoric acid) so that the pH ≥ 5.3			
	Water, salt, mold inhibitors ≤ 0.2% (w/w) or ≤ 0.3% (w/w) of the final product, coloring, spices or flavorings, enzyme-modified cheese, anti-sticking agent ≤ 0.03% (w/w) of the final product			
Pasteurized Process Cheese Food	Natural cheese ≥ 51% (w/w) of the final product	≤ 44	≥ 23	≥ 5.0
	All other ingredients and their permitted levels are the same as those in Pasteurized Process Cheese including milk, skim milk, buttermilk, and cheese whey			
Pasteurized Process Cheese Spread	Natural cheese ≥ 51% (w/w) of the final product	≤ 44	≥ 23	≥ 5.0
	All other ingredients and their permitted levels are the same as those in Pasteurized Process Food including sweetening agents, nisin (≤ 250 ppm of the final product) and food gums			

(Childs et al., 2007; Banville et al., 2014). Rheological characterizations including dynamic oscillatory shear tests, and creep and recovery tests have been used to evaluate the processing behaviors of cheese (Childs et al., 2007; Banville et al., 2014). Textural attributes have also been used to evaluate processing behaviors and include two major categories: melted texture and unmelted texture properties (Kapoor and Metzger, 2008). Both groups, along with rheological behaviors are essential in formulating process cheese with desired end-use attributes.

Melted texture properties of cheese include meltability, flowability/viscosity, and stretchability (Guinee, 2002; Kapoor and Metzger, 2008). Melted properties are considered during formulation because they contribute to the acceptability of the final product, but they are not considered when predicting and correlating processing behaviors. In PC formulation, both melted and unmelted texture properties are considered; for example, when PC is made for use in cheeseburgers. PC should have unmelted texture properties of higher firmness and cohesiveness and lower adhesiveness, while at the same time it should have melted texture properties of an average/normal melt (Kapoor and Metzger, 2008). However, it is important to consider melted texture properties; it is the unmelted properties that can be used to help determine processability of PC because unmelted texture properties are those which can be measured before processing (shredding/slicing) at the temperature and shear conditions relevant to processing conditions of PC. In this study we decided to use PC as a model system to test the hypothesis that wear data in combination with other material properties can be used to predict shreddability index obtained from sieve analysis of shreds using method suggested by Apostolopoulos and Marshall (1994). Rationale to select PC as a model system was based upon the fact that it is easier to produce cheese with varying shedding behaviors by changing the formulation, and that the structure of PC is remains mostly intact during storage.

Process Cheese Pilot-Scale Manufacturing

Pre-Processing. After formulation, ingredients are prepared by grinding or shredding natural cheese followed by standardization and blending all the ingredients together. Generally, natural cheese is ground to reduce the particle size and to break the body of the cheese in order to facilitate blending with other ingredients, and to ensure melting at an even rate as the entire mixture is processed with heat. Blending is done after grinding/shredding to ensure even distribution of ingredients and decrease processing time. It can be performed in a variety of ways, but can be done either in the cooker or in a separate processor depending on the manufacturing procedure (Hladká et al., 2014; Shirashoji et al., 2016). If standardization of pH is done, it is generally performed during/after blending of ingredients (Fox et al., 2015).

Processing and Cookers. There are many types of cookers used to make process cheese commercially. Some of these cooker types include pilot-scale cookers such as the Blentech twin-screw cooker (BTS; Blentech Corporation, Rohnert Park, CA), and the Stephan Cooker (UMM-SK25, Stephan, Hameln, Germany). Other benchtop equipment which have been used as cookers in the research include the Rapid Visco Analyzer (RVA) (Newport Scientific Pty Ltd., Australia), and the Vorwerk TM 31 (Vorwerk & Co., GmbH, Wuppertal, Germany) (Lee et al., 2003; Kapoor and Metzger, 2005; Buňka et al., 2014; Bi et al., 2016). Each cooker provides two of the most important aspects of process cheese manufacture—the application of heat and agitation (shear force). Both heat and agitation can vary, but their application at the same time is essential for creating a homogenous mass. Heat can vary between 70 to 90°C, and agitation speeds generally vary between 50-150 rpm for screw-auger agitation and 1500 to 3000 rpm for blade-type cookers (Kapoor and Metzger, 2008).

Cheese Functional and Mechanical Properties relevant to Processability

Unmelted texture properties of cheese include firmness (hardness), brittleness/fractureability, springiness/resilience, and adhesiveness/stickiness (Kapoor and Metzger, 2008). Firmness (hardness) is defined as “the ability of [cheese] (at ambient or low temperatures) to show resistance to deformation when subjected to an external force” (Kapoor and Metzger, 2008). Firmness is an important property because it has been directly correlated to cheese shreddability (Kindstedt, 1995; Childs et al., 2007; Banville et al., 2014). As firmness increased the adhesion of cheese to the processing blade during shredding decreased thereby improving shreddability (Banville et al., 2014) Brittleness is the tendency of cheese to break and fracture when subjected to external stress (Guinee, 2002; Fox et al., 2004b). Natural cheese, specifically mozzarella, that is overly firm and dry is expected to exhibit brittle/crumbly properties due to an increase in the production of fines; however, firmness relationships between cheese and shreddability were not otherwise been established (Kindstedt, 1995). Natural cheese that is too young, too firm, or dry is expected to exhibit brittle/crumbly properties (Kindstedt, 1995). A review on cheese texture from Fox et al. (2004b) reports that at higher salt-to-moisture ratios (>5%, w/w) a lower degree of casein hydration favors an overall more elastic casein matrix in natural cheese and a more elastic fracture behavior would facilitate shredding process; however, excessive ratios would lead to a firmer, shorter, and more brittle cheese therefore possibly reducing shreddability. Springiness is the ability of the cheese to recover its original shape after being subjected to external force (Kapoor and Metzger, 2008). Gupta et al. (1984) found that firmness and springiness of PC were directly correlated ($p < 0.05$, $r = 0.965$). Cohesiveness is the degree to which the mass holds together, or the “strength of the internal bonds making up the body of the product” (Fox et al., 2004b). Since cheese can exhibit pressure-sensitive adhesion during processing (shredding/slicing), cohesiveness is important because sufficient cohesiveness allows for cheese to be peeled away from the processing surface without leaving mass behind (Childs et al., 2007). Banville et al. (2014) showed that the cohesive property of natural cheese (Mozzarella) had negative correlation with and a significant impact ($p < 0.05$) on the production of fines but with poor predictive ability (explained variability < 0.36).

Effect of Natural Cheese on PC Functional and Material Properties

One ingredient in the formulation of PC that has a major impact upon the final end-properties of PC is natural cheese. Variations in pH, flavor, age, chemical composition, and intact casein content of natural cheese all influence the functional properties of PC (Caric et al., 1985; Shimp, 1985; Kapoor et al., 2007a; Kapoor and Metzger, 2008).

The pH of natural cheese has significant impact on the functional properties of PC. In a study performed by Olsen et al. (1958), PC was manufactured with natural cheddar cheese having differing final pH levels and varying ripening ages. Functional properties were measured using penetrometer (for unmelted texture) and the tube melt test (for melted texture). Even after adjusting the final pH of the PC between 5.4 to 5.5

the PC made using Cheddar cheese with a higher pH was harder and less meltable at all stages of ripening as compared to the cheese with a lower (normal) pH.

The amount of intact casein in natural cheese affects functional and material properties of PC. As natural cheese ripens the amount of intact casein decreases and pH 4.6 soluble nitrogen fraction increases due to enzymatic breakdown of caseins (proteolysis) from starter and nonstarter lactic acid bacteria and enzymes thereof (Purna et al., 2006). Several researchers studied the effect of natural cheese age (amount of intact casein) on functional and material properties of PC (Olson et al., 1958; Piska and Štětina, 2004; Purna et al., 2006). Overall, firmness of PC decreased as the amount of intact casein decreased (Olson et al., 1958; Piska and Štětina, 2004; Purna et al., 2006)

Other variables in natural cheese such as the level and state of calcium, phosphate, salt-to-moisture ratio, and amount of residual lactose impact (direct or indirect) functional and material properties of PC (Thomas et al., 1980; Kapoor et al., 2007a; Upreti and Metzger, 2007). These variables alter the physicochemical properties of natural cheese by affecting the rate and extent of casein hydrolysis, the pH and the state and quantity of casein (Kapoor et al., 2007a; Upreti and Metzger, 2007).

Microstructure of Natural Cheese. The structure of natural cheese plays a large role in influencing the final texture of process cheese and its functional and material properties (Lamichhane et al., 2018). Additionally, the microstructure of cheese (and the interaction of individual components) helps explain large- and small-scale deformation behaviors of materials. These behaviors, in turn, help define or predict mechanical behaviors such as shreddability (Banville et al., 2014). It is therefore useful to have both the mechanical behavior data and the microstructure to explain the nature and consistency of the cheese (Černíková et al., 2017)

Natural cheese structure, in general, is built on the network of interlocking casein molecules (Fox et al., 2004a). This network is formed as a result of rennet action on casein micelles and is also due to the end products of biological activity from starter cultures, namely lactic acid bacteria which solubilizes calcium and changes the physical structure of proteins (Fox et al., 2004a). Within this structure, there is a large amount of space not occupied by the proteins. This space traps milkfat globules and serum. These constituents do not form a part of the protein network but have a large influence upon its functional properties because fat and serum are of a considerable percentage (up to 69%) of the final product, and they are released while heating the cheese mass (Lucey et al., 2003; El-Bakry and Sheehan, 2014; Lamichhane et al., 2019). Fat globules affect functional properties of PC by disrupting the continuity of interconnected proteins within the casein network. Due to their large size, in comparison to casein micelles, fat globules affect the density of and distance between aggregates. Additionally, the large inhomogeneities or "weak spots" in the cheese matrix caused by fat globules strongly affect large deformation and fracture properties of the cheese. (Luyten et al., 1991).

With aging, the breakdown of protein takes place which softens the protein network overall and results in the presence of weaker spots. This weakening of the protein network changes functional properties of PC such as lowering melting points and increasing stretchability (Johnson, 2000).

Compositional Factors Affecting Functional and Material Properties of PC

There are number of compositional factors that influence functional and material properties of PC including pH (Marchesseau et al., 1997; Lee and Klostermeyer, 2001), intrinsic lipid/fat content (Hong, 1990), moisture quantity (Hong, 1989), calcium content, phosphate levels (Sood and Gains, 1979; Piska and Štětina, 2004; Kapoor et al., 2007a) and intact casein (Hladká et al., 2014). Table 2 presents a list of these major factors with their possible impact on PC functionality.

pH has large effect on functional and material properties of PC (Marchesseau et al., 1997; Lee and Klostermeyer, 2001). With an increase in the pH of PC, the material changes its character from a solid-like to more liquid-like character (Lee and Klostermeyer, 2001). Additionally, as the pH increases, the hardness, storage modulus (G'), and viscosity of PC increase (Lee and Klostermeyer, 2001). Small changes in pH affect the ionic interactions of proteins and therefore the stability of the gel-like network of PC (Marchesseau et al., 1997). One of the largest contributors to the final pH of PC is

Table 2. A list of factors influencing the functional and material properties of PC

Factors	Influence of the factor on functional or material properties of PC
pH	Increased hardness, moduli, and viscosity increased, and more viscous behavior was demonstrated with increasing pH (Lee and Klostermeyer, 2001)
Lipid content	As lipid content increased the firmness decreased, and ratio of protein-to-fat decreased (Hong, 1990)
Moisture quantity	As moisture increased the hardness decreased, ratio of protein-to-fat decreased, pH increased, and melting temperatures increased (Hong, 1989)
Calcium content	As calcium increased, pH, hardness, and viscosity increased (Sood and Gains, 1979)
Phosphate levels	As phosphate levels increased, pH, hardness, and viscosity increased (Kapoor et al., 2007a)
Intact casein	As intact casein decreased the hardness decreased and adhesiveness increased (Hladká et al., 2014)

natural cheese. The pH of natural cheese can vary between batches and affect the final pH of PC, additionally emulsifying agents can also affect the pH of the final PC product (Kapoor and Metzger, 2008). Overall, the optimal pH for processing conditions was found to be between 5.7-6.0 (Marchesseau et al., 1997).

The Influence of Emulsifying Agents on Functional and Material Properties of PC

Emulsifying agents, also known as melting salts, greatly influence the functional and material properties of PC. They sequester calcium from renneted casein, releasing individual casein fractions for making a stable emulsion matrix, and also adjust the pH of the final product (Kapoor and Metzger, 2008). Both calcium sequestration and pH adjustment create conditions in which caseins can form proper emulsifications, which directly influences functional (e.g. shreddability) and material properties (e.g. hardness) of the PC (Caric et al., 1985; Fox et al., 2004a; Mizuno and Lucey, 2007; Kapoor and Metzger, 2008). These physicochemical attributes greatly influence certain melted textural properties, such as viscosity/flow, meltability, and stretchability/extensibility of PC (Kapoor and Metzger, 2008). Melting salts also influence the unmelted textural properties of PC, including brittleness/fractureability, firmness, adhesiveness/stickiness, and springiness/resilience (Kapoor and Metzger, 2008).

During the cooking of PC, bound calcium molecules are freed from casein micelles because of chelating action of emulsifying salt. When agitation is applied, these freed polar casein molecules find their way to a fat-water phase interface, increasing the structural stability in PC. Heat and shear process cause the formation of smaller fat globules which are then dispersed evenly throughout the cheese matrix. The decrease in fat globule size directly influences functional and material properties which ultimately impact processability (sliceability and shreddability).

The Effect of Processing Parameters on Functional and Textural Properties of PC

PC is used in many forms such as slices and shreds. Textural characteristics and machinability are specific to each cheese type (and cheese form) and influence its functionality (Lucey et al., 2003; Kapoor and Metzger, 2008). For example, the textural characteristic of hardness has an impact on shreddability (Apostolopoulos and Marshall, 1994), if a cheese is too hard it may be too crumbly, therefore may exhibit poor shredding characteristics (by correlation), this crumbly cheese will also have poor functional properties (e.g. melting). Some research has emphasized the impact of processing parameters on these functional properties (Shirashoji et al., 2006, 2010; Černíková et al., 2017, 2018).

Cook temperature, cook time, and shear force applied during processing are the parameters which directly affect the functional and material properties of PC (Kapoor & Metzger, 2004; Shirashoji et al., 2006, 2006, 2010; Černíková et al., 2017;). As processing time is increased for PC made with different emulsifying agents, the meltability decreases, and the firmness of the cheese increases (Rayan, 1980). The rate at which PC is cooled after cooking also has an influence on the stickiness and firmness of PC (Piska and Štětina, 2004). PC that was cooled rapidly showed a decrease in the rigidity and an increase in stickiness (Piska and Štětina, 2004). The differences in rigidity and stickiness caused by varying cooling rates were attributed to structural variations in PC caused by the formation of an interconnected network of casein molecules (Piska and Štětina, 2004).

Measurement Techniques

Microstructural Characterization of PC. Several methods have been employed to characterize the microstructure of cheese and cheese products, these include lower resolution microscopic techniques such as optical microscopy (OM) (Černíková et al., 2010; Hladká et al., 2014), and confocal laser scanning microscopy (CLSM). Additionally, high resolution techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have also been used in the past (Rayan, 1980; Awad et al., 2002; Lee et al., 2003; Fu et al., 2018). Various preparation and fixation techniques for cheese samples were developed based upon the nature of the sample and sample-to-sample variation. In cheese, these microscopy and fixation techniques have been used to study changes in the size and distribution of fat globules (Rayan, 1980; Awad et al., 2002; Černíková et al., 2010), the structural rearrangement of the paracaseinate network (Lee et al., 2003), and the defect of undissolved emulsifying salt crystals (Kapoor and Metzger, 2008).

Cheese Rheology and Oscillatory Shear Tests. Rheology is the study of deformation and flow of material in response to stress or strain (Steffe, 1996). In general, when applied to the food industry, rheological measurements can be performed for quality assurance purposes (Steffe, 1996). These tests can assist in maintaining product consistency and reduce complications during processing.

Oscillatory shear tests are commonly used rheological techniques to characterize viscoelastic materials. The principle of oscillatory shear testing is to induce a sinusoidal shear deformation in the material being tested and measure the resulting stress. These tests may be performed on strain- or stress-controlled rheometers (Melito et al., 2013).

Oscillatory shear tests on viscoelastic materials can be separated into two categories or regimes (Figure 2): linear viscoelastic response which is measured by small amplitude oscillatory shear (SAOS) and nonlinear viscoelastic response which is measured by large amplitude oscillatory shear (LAOS) (Melito et al., 2013). SAOS tests differ from LAOS in that testing is performed within the linear viscoelastic region (LVR), whereas LAOS testing can extend beyond the LVR region (Melito et al., 2013). The majority of studies on viscoelastic material employ the use of SAOS tests (Melito et al., 2013); these tests remain in the LVR in which the stress response is proportional to the applied strain. In SAOS no permanent microstructural deformation occurs within in the material. In LAOS however, as it extends beyond the LVR the stress becomes disproportional to the strain input (usually under high strains) and permanent microstructural deformation occurs (Steffe, 1996). SAOS characterization has been used

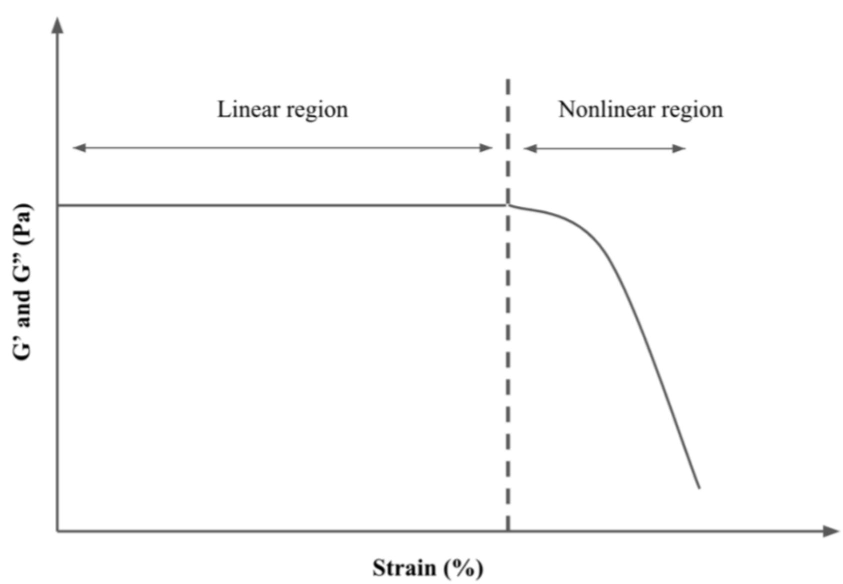


Figure 2. Illustration of the linear and nonlinear viscoelastic regions

previously to assist in predicting the shreddability of mozzarella cheese. However, SAOS tests alone are insufficient for predicting shreddability, additionally, predictive models that were created showed varying degrees of predictability using SAOS data (fines production R^2 = 0.85, adhesion to blade R^2 = 0.45) (Banville et al., 2014). The characterization of LAOS behavior however, has not been directly used in shreddability studies and could be a useful tool for cheese shredding or slicing because these processes impart strains and stresses that surpass the LVR (Steffe, 1996; Melito et al., 2013).

Texture Profile Analysis. Texture Profile Analysis (TPA) is a common test for determining the textural properties of foods. It uses a double compression test to determine these properties and has generally been used to determine how samples behave when chewed. Regardless of the end-application it gives five important textural parameters (hardness, adhesiveness, cohesiveness, brittleness, and gumminess) (Rosenthal, 2010). These parameters can provide useful information to predict processability when correlated with functional properties. TPA for PC has been used to help predict processability during shredding, slice-on-slice manufacturing, for maintaining slice identity for cold sandwich food preparation, or predicting stickability to food when used in dips (Kapoor and Metzger, 2008). Hardness, in particular, has been correlated with other rheological properties to predict the propensity of cheese to stick to equipment (Banville et al., 2014). However, TPA tests alone have been shown to be insufficient for predicting processability, additionally, predictive models that were created showed varying degrees of predictability using TPA data (long shred production R^2 = 0.67, adhesion to blade R^2 = 0.45) (Banville et al., 2014).

Cheese Wear Behavior

Because wear behavior involves the same mechanisms of mass loss as commercial shredding behaviors it is considered that wear and rheological behaviors are useful for understanding both texture attributes and processing behaviors. Wear is a part of tribology which is the study of wear and friction behaviors or lubrication between two interacting surfaces in relative motion (Tan and Joyner, 2018). Wear itself can be defined as the removal of material from a surface caused by rolling or sliding contact against a countersurface (Axén et al., 2000). This removal of material can generate a wear track with a specific size, shape, depth, or pattern on the sliding surface which indicates wear pattern (Wang et al., 2017; Zad Bagher Seighalani and Joyner, 2019). These characteristics along with generated debris can provide valuable information as to the mechanism(s) of wear and the surface wear status (Axén et al., 2000).

There are different classifications of wear including abrasive, adhesive, erosive, fretting, and surface fatigue wear (Axén et al., 2000). In relation to sliding tests, abrasive and adhesive wear classifications are most appropriate. Adhesive wear happens when two materials interact with one another and a material transfer between one surface to the other occurs, results in mass loss (Figure 3, A) (Axén et al., 2000). Abrasive wear occurs when two surfaces of differing hardness interact with each other and the movement of the harder surface on the softer material results in the loss of soft material (not due to sticking) (Figure 3, B) (Axén et al., 2000). Generally, abrasive wear can be identified by the creation of grooves which can be identified visually or measured by penetration depth, whereas adhesive wear can be identified by the presence of a film or particle on one or both of the sliding surfaces (Ozcan and Filip, 2013).

In recent years, wear behavior has appeared as a novel technique of interest in food science research and has been correlated with various sensory attributes (Nguyen et al., 2016; Laiho et al., 2017; Zhang et al., 2017). In viscoelastic materials, wear behaviors can be correlated to the processing characteristics of foods, including adhesion, cohesion, fracture behavior, deformation under force, and friction. The correlation between these properties might provide manufacturers with simple wear tests, which alone (or with other tests), can determine the quality of products during processing or can be used to predict processability of food materials (Sparkman and Joyner, 2019).

Of the several existing studies on food materials, a study on κ-carrageenan and whey protein gels was one of the wear behavior studies on the food processing aspect of research (Tan and Joyner, 2018). This study employed a twin ball-on-plate geometry with varying normal forces to determine the wear behavior of various gels. The purpose of this

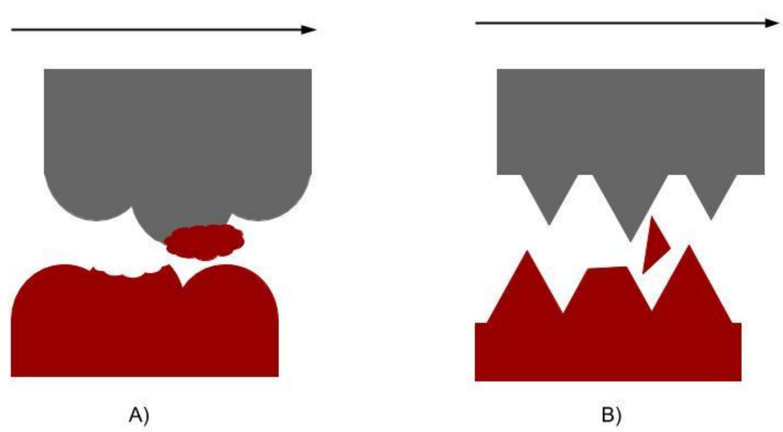


Figure 3. Different examples of wear regimes: A) adhesive wear and B) abrasive wear

study was to validate wear tests for use in soft materials such as food, and to model the processability of soft materials using mechanical and wear properties.

In 2019, the wear behavior of high-protein bars (using a twin-ball geometry) was used to determine processability (Sparkman and Joyner, 2019). The objective of the study was to create bench-level instrumental tests that could predict the processability of different formulations of protein bars (Sparkman and Joyner, 2019). In this study, both rheological and wear behaviors were used to determine the effect of varying formulations of whey protein isolate, high fructose corn syrup, and added fats on processing behaviors (Sparkman and Joyner, 2019). The results of the study demonstrated that mechanical, material, and wear behaviors were all related to processability and were controlled by variation in formulation. Furthermore, the wear test was shown to be a good indicator of processability and shows potential for benchtop testing of food processing ability (Sparkman and Joyner, 2019).

Another recent study on wear behavior provided insight into cheese rheology-wear relationships (Zad Bagher Seighalani and Joyner, 2019). The objective of the study was to determine the wear behaviors of Monterrey Jack and Cheddar cheeses and their association with rheological behaviors. The wear test was performed on a rheometer using a steel twin-ball apparatus. After testing, Monterrey Jack (when compared to Cheddar) demonstrated significantly greater small-train loss and storage moduli values and a lesser degree of nonlinear viscoelastic behavior; additionally, Monterrey jack had less penetration depth and mass loss during wear testing. Overall a negative correlation was demonstrated between penetration depth and rigidity, penetration depth and elasticity, mass loss and rigidity, and mass loss and elasticity (Zad Bagher Seighalani and Joyner, 2019).

Variations in PC composition and processing variables may result in cheese with undesirable shredding or slicing behaviors (sticking or crumbling). The literature lacks fully accurate tests which can determine this processability. We hypothesized that processing behaviors of cheese are related to wear behaviors of cheese, and that wear behaviors can be used to predict processability. Currently, there is also not enough information in the literature to test this hypothesis. Additionally, the factors and mechanisms influencing cheese wear behavior are largely unknown. Therefore, the first focus of this study was to identify factors which affect wear behavior by creating a spectrum of PC formulations. Second, determine material and rheological properties which correlate to wear behaviors and can be used to predict processing behaviors, namely shreddability. A predictive model based on wear behavior could provide the industry with a powerful new tool which requires a small amount of time (< 20 min.) and a small amount of sample (< 25 g) to predict processing ability of a cheese system.

MATERIALS AND METHODS

Overview

Model process cheeses with a range of shredding behaviors was manufactured and used for correlating wear behavior with microstructure, rheological, functional and material properties. The end goal was to create a predictive model for shreddability using these properties. The two variables used to create a range of unmelted textural properties (including shredding behaviors) were varying levels of trisodium citrate and ages of natural cheese (indicating extent of proteolysis and quantities of intact casein). Higher percentages of TSC (3%) in combination with younger cheeses (1 d) were expected to give hard crumbly cheese (abrasive wear), and lower percentages of TSC (2%) in combination with older cheeses (102 d) were expected to give more adhesive behaviors (adhesive wear) and possibly the worst shreddability. Overall, different combinations of the two variables were designed to give a range from good to poor shreddability.

Process Cheese Manufacture

An overview of process cheese manufacture is shown in Figure 1. Details of each step are given below.

The Formulation of PC. PC formulations were made in randomized order as per experimental design creating 12 different formulations of varying compositions (Table 3). There were 6 replications including two sets (formulas 5 and 6) of three replicates of central formulas. The variations in formulations were based upon 2 variables: intact casein levels (different ages of natural cheese) and emulsifying salt quantities. These variables were used to produce cheeses with a range of material, rheological, and textural

Table 3. Process cheese formulations including schedule of manufacture and testing

Assigned Formula Number	Parameters			Notes:
	Generic Formulation (Y, M, S)	Average Age of Natural Cheese (d)	TSC %	
10	70.0% M, 30.0% S	102	2.0	These Six formulations were made and tested within 6 wks of each other. 4a and 4b are part of the 6 replicates
8	33.3% Y, 33.3% M, 33.3% S	83	2.5	
5	70.0% Y, 30.0% M	22	2.5	
7	33.3% Y, 33.3% M, 33.3% S	83	2.0	
1	100% Y	1	2.0	
6	70.0% Y, 30.0% M	22	3.0	
2	100% Y	1	2.5	These Six formulations were made and tested within 6 wks of each other. 8a and 8b are part of the 6 replicates
9	33.3% Y, 33.3% M, 33.3% S	83	3.0	
5	70.0% Y, 30.0% M	22	2.5	
4	70.0% Y, 30.0% M	22	2.0	
6	70.0% Y, 30.0% M	22	3.0	
6	70.0% Y, 30.0% M	22	3.0	
12	70.0% M, 30.0% S	102	3.0	These Six formulations were made and tested within 6 wks of each other. 12e and 12f are part of the 6 replicates
3	100% Y	1	3.0	
5	70.0% Y, 30.0% M	22	2.5	
11	70.0% M, 30.0% S	102	2.5	
5	70.0% Y, 30.0% M	22	2.5	
6	70.0% Y, 30.0% M	22	3.0	

Y- young cheese, 1 d old; M- medium age cheese, 70 d old cheese; S- sharp cheese, 176 d old.

properties. It was expected that 2.5% TSC quantities, and formulations with 70% 1-d-old cheese would have the most ideal properties for shreddability. The average ages of natural cheese in process cheese formulations were taken as an indicator for intact casein levels. Composition of natural cheeses is given in Table 4. This was calculated using the age of three different ripened cheddar cheeses (Old Juniper, Utah State University) i.e., 1, 70, 176 d. Emulsifying salt levels of 2.0, 2.5, and 3.0% were used in this study. Targeted levels of salt and pH were 2.0 and 5.5 respectively.

Table 4. Proximate composition of natural cheddar cheese with varying ages

Natural Cheese Age (D)	Average Moisture, %	Average Fat, %	Average Salt, %
1-Day	35.08±0.11	35.50±0.00	1.84±0.06
70-D	34.49±0.88	36.50±0.00	1.59±0.04
176-D	33.02±0.32	36.50±0.50	1.70±0.05

Grinding. Natural cheese for formal PC trials was ground to less than 4 mm size pieces using the medium cutting head on a Comitrol Processor Model 3640 (Urschel Laboratories Inc., Chesterton, IN). All cheese was immediately vacuum packaged in 1-2 kg bags and frozen at -29°C to prevent further breakdown of intact casein.

Blending. Blending of all ingredients was done in a Cuisinart Model 70723 food processor (Hamilton Beach Brands INC., Glen Allen, VA) for 5 min. at 22.5°C (room temperature) with on speed setting puree/mix. During blending, deionized hot water (65 ± 5°C) was mixed with trisodium citrate, sodium chloride, and potassium sorbate. This solution was then added to mixer of ground natural cheese and processed for the allotted time. After blending was completed, the mixture was adjusted for pH and blended for an additional three min. All formulations were prepared in three-pound batches (1360.78 g).

Standardization of PC. Salt was standardized to 2.0% by considering inherent salt content in each of the 3 differently aged natural cheeses (Table 4). Salt analysis was performed using a Chloride Analyzer 926 (CORNING, Corning NY).

pH Standardization. For standardizing, the pH of complete batch an aliquot (10% of the batch size) was transferred to a mini HC2000 Black and Decker food processor (Black and Decker, New Britain, CT). The pH of the smaller quantity was standardized to a pH of 5.5 (at room temperature) by adding either 10% NaOH (Fisher Scientific, Waltham, MA) or 20% lactic acid (Mallinckrodt Baker, INC., Phillipsburg, NJ) solutions in 250-ml increments. The slurry was mixed for 30 s after each addition and repeated until the desired pH was reached. The amount of standardizing solution needed for the unstandardized slurry was scaled to standardize the remaining 90%. The smaller standardized mixture was added back to the entire mixture and everything was then processed for another 3 min. at the previously used speed setting.

Processing and Storage. After blending and standardization, ingredients were added to the Vorwerk TM 31 blender cooker (Vorwerk & Co., GmbH, Wuppertal, Germany) and processed using modified methods described by Černíková et al. (2010). Cooking was done at 85°C using a speed of 3,100 rpm. Batches were processed for 4 min. then stopped to hand mix ingredients to ensure even blending, and agitation and cooking continued for the remaining time for a total of 10 min. processing time. Immediately following processing, the cheese temperature was recorded, and the molten mass was poured into an 8.5×4.5×2.5-inch Wilton Recipe aluminum loaf pan (Wilton

Brands LLC., Naperville, ILL.) lined with cheese cloth. Each batch was sealed with Daily Chef Food Service Film (Sam's West INC., Bentonville, AR) and was immediately stored at 5°C. Samples were drawn after 1 to 3 d and vacuum sealed for storage. All samples were stored for a minimum of 14 d before tests were performed.

Process Cheese Proximate Analysis

PC proximate composition (pH, moisture, fat, protein, and pH 4.6 soluble nitrogen) were determined using standard methods for dairy products. Fat content in processed cheese samples was determined using a modified Babcock method (Richardson, 1985).

Final pH Determination. Final pH was determined using a Thermo Scientific Orion Star A211 pH meter (Thermo Scientific, Waltham, MA). Samples were prepared by mixing 20 grams of cheese with 10 g of distilled water at 80 ± 5°C in a Seward 400 BA6041 Standard Sterilized Blender Bag (Seward, Minneapolis, MN). Bags are then stomached in a Seward Stomacher 400 Circulator (Seward, Minneapolis, MN) at 260 rpm for 4 min. Sample bags were then rolled or folded down until the pH probe was submerged in the liquid. Measurements were then taken using the pH meter and values were recorded.

Salt Analysis. Final salt content in the process cheese was determined using a Chloride Analyzer 926 (CORNING, Corning NY). For this test, all samples were prepared using a Seward Stomacher 400 Circulator (Seward, Minneapolis, MN). To stomach samples, 5 grams of cheese was mixed with 98.2 grams of distilled water at 80 ± 5°C in a Seward 400 BA6041 Standard Sterilized Blender Bag (Seward, Minneapolis, MN). Samples were then processed in the stomacher at 260 rpm for 4 min. Samples were then removed, and gravity filtered using 50-ml funnels, 50-ml Erlenmeyer flasks, and 2V Qualitative 12.5 cm (8µm) Whatman filter papers (Whatman, Maidstone, UK). The chloride analyzer was then calibrated using acid buffer and 200 mg/l sodium chloride solution. A 250-ml aliquot of filtered sample was then added to the acid buffer solution to perform the test. Tests on one sample were repeated for 3 or 4 times until the chloride analyzer indicates to change the acid buffer solution. The acid buffer solution was then replaced, the machine was zeroed, and tests were continued. All data was recorded and then multiplied by 0.04 to get the percentage of salt.

Moisture Determination. Final moisture content of process cheese samples was determined using the gravimetric method in a vacuum drying oven model 5831 (National Appliance Company, Portland, OR). Approximately 2.5 ± 0.5 grams of each ground sample was weighed in aluminum dishes. The samples were transferred to an oven maintained at 78 ± 2°C. Samples were dried overnight at approximately 78 ± 2°C. The samples were removed and placed in a desiccator chamber for a minimum of 12 hours before they were re-weighed.

Intact Casein in Natural Cheese. The level of intact casein in natural cheeses was indirectly determined by measuring the proportion of pH 4.6 soluble nitrogen to the total nitrogen available in natural cheese samples. The pH 4.6 soluble nitrogen procedure measures the water-soluble products of proteolytic activity produced during cheese making and is inversely correlated to the level of intact casein found in cheese (Fenelon and Guinee, 2000). By measuring pH 4.6 soluble nitrogen an idea of the amount of intact casein can be determined by calculating the ratio of total nitrogen to pH 4.6 soluble nitrogen. Total nitrogen was determined using the cheese method on a Sprint Rapid

Protein Analyzer (CEM Corporation, Matthews, NC) and by the pH 4.6 soluble nitrogen method using a modified procedure based on that described by Kuchroo and Fox (1982).

To perform the pH 4.6 soluble nitrogen test, 60 grams of grated sample was weighed and placed in a Seward 400 BA6041 standard sterilized stomacher bag (Seward, Minneapolis, MN). 120-ml of distilled water at 50°C was added to each bag and then each bag was stomached for 5 min. at 260 rpm. After stomaching, each sample was incubated in a 55°C water bath for 1 hour. The contents were then centrifuged at 3000 RCF for 20 min. at 4°C. Each sample was then be poured through borosilicate glass fiber of 8 pore size (glass wool) (Sigma-Aldrich, St. Louis, MO) into a 150-ml beaker. The remaining supernatant was then adjusted to a pH of 4.6 using 10% HCl (Fisher Scientific, Waltham, MA). Each sample was then centrifuged again at 3000 RCF for 20 min. at 4°C. Contents of each sample were then filtered again through glass wool and diluted to fit the estimated protein ranges of the UF Permeate method on the Sprint Rapid Protein Analyzer. All dilutions were based on the approximate pH 4.6 soluble nitrogen percentages found in literature (Hou et al., 2014). Nitrogen content was then determined by the Sprint Rapid Protein Analyzer (CEM Corporation, Matthews, NC) method on 15-ml aliquots, and the end protein percentage was multiplied by the dilution factor to account for dilution.

Microstructural Characterization

Confocal Laser Scanning Microscopy. Cheese microstructures were characterized using a Carl Zeiss Confocal Laser Scanning Microscope model LSM-710 (Carl Zeiss, Oberkochen, Germany). Protein and fat were stained for ease of visualization using the methods described by (Lamichhane et al., 2019). Fat was stained using Nile

Red with an excitation of 488 nm and an emission spectrum between 500-580 nm (Sigma Life Science, St. Louis, MO). Protein was stained with Fast Green with an excitation of 633 nm and an emission spectrum between 650-700 nm (Merck KGaA, Darmstadt, Germany) (Lamichhane et al., 2019). Nile red was diluted to 1% w/v in 1-2 propanediol and mixed 3:1 with a 1% wt/vol dilution of Fast Green in distilled water. Each piece of cheese to be stained was cut from the center of each sample batch. Samples were cut into approximately 8×8 mm squares with a thickness of approximately 0.2 mm using a GEM/STAR single edge microscopy prep razor blades (Electron Microscopy Sciences, Hatfield, PA). Dye was applied to the cheese by adding 40 µl of dye mixture to a cover slip and using it to evenly spread the dye across the piece of cheese resting on a microscope slide. Then, samples were left to rest for 20 s before they are turned over. Additional dye was applied to cover the back side of the cheese. Cover slips were placed and glued by four dots of clear nail polish on each corner. All samples were kept in sealed containers in refrigerated conditions and viewed within 4 hours of preparation.

Transmission Electron Microscopy. Cheese microstructures were characterized using a JEOL-1400 Plus transmission electron microscope (JEOL USA Inc., Peabody, MA). Samples were prepared as described by Vollmer et al. (2019). Formulations 2, 4, 5, 6, 8, and 11 were cut into small cubes (≈1-2 mm) and fixed with a primary fixative (formaldehyde and glutaraldehyde each at 2.5%, 0.1 M sodium cacodylate buffer, pH 7.4) at room temperature for a minimum of 2 hours, then stored at 4°C. Further processing including secondary fixation, dehydration, and infiltration is listed in Table 5.

Following initial fixations and preparation, samples were then thick-sectioned (0.5 µm), stained with Toluidine Blue, trimmed and thin-sectioned (≈70 nm), transferred

Table 5. Processing procedure for TEM samples

Action	Agent	Duration	Time
Rinse	Sodium Cacodylate Buffer	10 min.	10 min.
Rinse	Sodium Cacodylate Buffer	10 min.	20 min.
Post-Fix	2% OsO_4 (2-ml/vial)	1 hr.	1 h 20 min.
Post-Fix	2% OsO_4 (2-ml/vial)	1 hr.	2 h 20 min.
Rinse	dH2O	10 min.	2 h 30 min.
Rinse	dH2O	10 min.	2 h 40 min.
Dehydrate	50% EtOH	20 min.	3 h
Dehydrate	70% EtOH	20 min.	3 h 20 min.
Dehydrate	95% EtOH	20 min.	3 h 40 min.
Dehydrate	95% EtOH	20 min.	4 h
Dehydrate	100% EtOH	30 min.	4 h 30 min.
Dehydrate	100% EtOH	30 min.	5 h
Dehydrate	100% EtOH	30 min.	5 h 30 min.
Dehydrate	100% Acetone	10 min.	5 h 40 min.
Dehydrate	100% Acetone	10 min.	5 h 50 min.
Dehydrate	100% Acetone	10 min.	6 h
Infiltrate	1:1 Acetone/plastic	Overnight (rotate)	O/N
Infiltrate	1:3 Acetone/plastic	2 h (rotate)	2 h
Infiltrate	1:4 Acetone/plastic	2 h (rotate)	4 h
Infiltrate	1:5 Acetone/plastic	Overnight (rotate)	O/N
Infiltrate	100% plastic	2 h (1 h rot, 1 h vac)	2 h
Infiltrate	100% plastic	2 h (1 h rot, 1 h vac)	4 h
Infiltrate	100% plastic	2 h (1 h rot, 1 h vac)	6 h
Embed & Cure		16 h (overnight)	O/N

to 3 mm copper grids, contrasted with saturated uranyl acetate and Reynold's lead citrate, and then examined at 120 kV accelerating voltage. Images were captured on the Gatan SC1000 digital CCD camera (Gatan, Inc. Pleasanton, CA) in a magnification range from 500 – 20,000x. Lower magnifications (500-2,000x) were observed for fat globule distribution and higher magnifications (20,000x) were observed for changes in the protein matrix.

Mechanical Property Measurements Overview

Cheese mechanical properties were characterized using a variety of methods. Cheese viscoelastic properties were characterized using strain and frequency sweeps. All the mechanical properties were determined at 5°C to mimic the temperature used during commercial shredding operations. Strain sweep test was conducted to see how sensitive each batch of cheese is in respect to shear force, a force that is applied during slicing or shredding. Cheese large-strain behavior was characterized using large amplitude oscillatory shear (LAOS) described by Zad Bagher Seighalani and Joyner (2019). Cheese Texture Profile Analysis (TPA) was characterized using a compression test with the Ta-XT Plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). Details of these experiments are presented as below.

Cheese Rheological Measurements

Sample Prep. Cheese was sliced to a thickness of 2.5 mm using a Beswood model 250, 10" commercial deli slicer with a chromium-plated carbon steel blade. For slicing, cheese samples were stored at 5°C for at least overnight. Cheese samples (in the form of 25 mm discs) were stamp-cut using a 25 mm inner-diameter steel cheese borer (custom-made). All spots with major defects were avoided. The 25.0 × 2.5 mm disk was immediately placed on the lower geometry of the rheometer and trim gap was set (1 N force). The sides of the sample were then coated with vegetable oil to avoid sample drying. The H-PTD 200 temperature hood (Anton Paar GmbH; Graz, Austria) was lowered and samples were given three min. before starting any test to equilibrate to the proper temperature.

Performing the Test. The measurements for rheology were performed on a rheometer model MCR302 (Anton Paar GmbH; Graz, Austria) using stainless-steel crosshatch (serrated) geometry (PP25). Before the test, there were several adjustments and pre-tests which were done to the machine. These adjustment/tests include: a quick adjust (which includes zero-gap), air check, initialization, setting the moving profile, and resetting the force.

LAOS Test. LAOS data was obtained using strain sweeps on an Anton Paar rheometer model MCR 302 (Anton Paar GmbH; Graz, Austria). The upper and lower geometries were crosshatched parallel plates (25 mm diameter). The tests were performed at 5℃. The rheometer Peltier plate was set to 5℃ and a temperature hood was used for further temperature control. The strain sweep test was performed by increasing shear strain from 0.1 to 100% at a frequency of 1 Hz. The critical strain from this test was calculated as the first point at which the complex modulus changes by more than 2.0%. LAOS data was determined by comparing strain sweep tests and varying strain percentages (0.1, 1.0, 4.0, 40.0%).

Frequency Sweep Test. Frequency sweep data was obtained using an Anton Paar rheometer model MCR 302 (Anton Paar GmbH; Graz, Austria) equipped with crosshatched parallel plates (25 mm diameter). The test was performed at 5°C. The rheometer Peltier plate was set to 5°C and a temperature hood was used for further temperature control. PC samples were prepared as mentioned above (2.5 mm thick, 25 mm diameter) and allowed to equilibrate to 5°C. The frequency sweep was performed by applying frequencies in descending order from 100 Hz to 0.01 Hz. This included 25 data points with a constant profile, and a shear strain (oscillating) value of 0.05%. Elastic (G') and viscous (G") moduli were extrapolated from this data based on Equation 1 and 2 (Steffe, 1996; Sharma, 2016; Sharma et al., 2016) where n, $k_{elastic}$ and $k_{viscous}$ are all constants, and n is the degree of frequency dependence. All measurements were taken in triplicates.

$$G' = k_{elastic}{}^{n} \quad (1)$$

$$G'' = k_{viscous}{}^{n} \quad (2)$$

Wear Testing

Cheese wear behavior was characterized using three-pin-on-disk tribological attachment (T-PID/44) on an MCR 302 rheometer (Anton Paar GmbH; Graz, Austria). Custom-made cylindrical stainless-steel pins with a hemispherical bottom were used for point-contact wear (Figure 4). Samples were prepared by slicing PC to a thickness of 5 mm. Each slice was then stamped by a stainless-steel cylindrical borer (inner diameter 69.00 mm). The Peltier plate on the rheometer was set to 5°C and the temperature control hood was used for additional temperature control. The wear test itself was done at 5°C using 50 mm/s sliding speeds and 1 N normal force (1/3 N tribological force) for 10 min. Samples were weighed before and after the wear test to calculate mass loss.

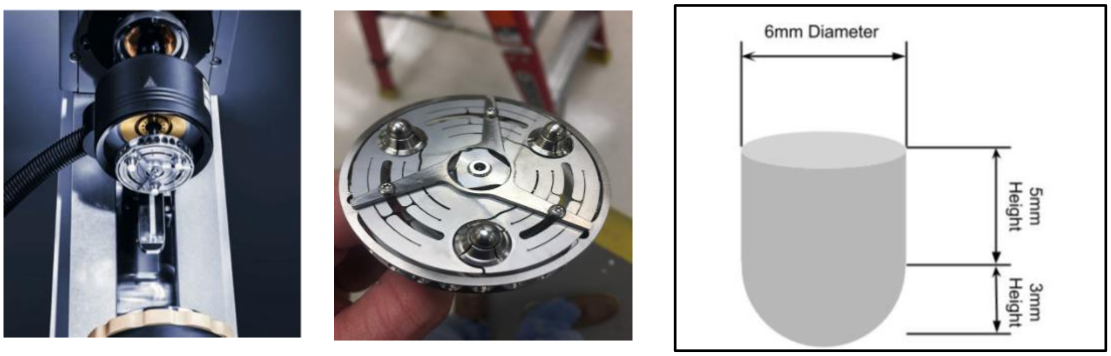


Figure 4. Pin-On-Disk Tribological Attachment T-PID/44 on MCR 302 rheometer. Cylindrical Stainless-Steel Pins with a Hemispherical Bottom were attached to the geometry.

Texture Profile Analysis

Cheese TPA was performed using a modified double compression test (Paglarini et al., 2019). It was done on a Ta-XT Plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). For TPA measurement, PC samples were cut into 12.5 mm cubes using a Prepworks potato cutter (Progressive Intl., Kent, WA) and a wire cheese slicer (RSVP Intl., Seattle, WA). Samples and equipment were both equilibrated to 5℃ and cooling packs were used to maintain temperatures during testing. A 5 kg load cell with a TA-30 (75 mm diameter) cylindrical aluminum probe was calibrated with a 2 kg load for the test. PC samples were compressed at a constant speed of 1 mm/s with a compression strain of 25%. Hardness was calculated as the maximum peak force obtained during the first compression. Each test was performed with 6 replicates.

Shredding/Grating Analysis

Shredding analysis was done on a Ta-XT Plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) with a Cheese Grating Rig (Stable Micro Systems, Surrey, UK). Analysis was done using equipment and samples chilled to 5°C and maintained as close to that temperature as possible during testing. Samples were cut to a size of 80x60x40 mm to fit in the block holder. A 2.0 kg weight was used to apply constant force on the block holder during the test. All tests were done in triplicates with eight grating repetitions. Only the latter five repetitions from each test were used for comparing the effect of treatments on work to grate and crumbliness (parameters obtained from the grating rig test data).

Grating rig data was collected by measuring force (g · s) over time (s) after applying a 2 kg weight to the sled containing a 40x60x80 mm PC sample at 5°C. Work to grate (g · s) was calculated as the area under the curve between force (g) and time (s). Crumbliness was calculated as the number of peaks (n) that are created as the cheese passes through the grooves during grating.

Sieve Analysis

Development of the shreddability index (SI) was performed by a sieve analysis of shreds using the methods described by Apostolopoulos & Marshall (1994) and Banville et al., (2013). Blocks of cheese were cut to approximately 80x60x35 mm and stored in a fridge at 5°C. After temperature equilibration, blocks were individually weighed, shredded, and analyzed for shred characteristics. Blocks were shredded on a Cuisinart Model 70723 food processor (Hamilton Beach Brands INC., Glen Allen, VA) fitted with a circular shredding blade with staggered holes. A 2.5 kg weight was attached to the plunger for application of constant normal force. After shredding, the shreds were added to the top of an RX-86 sieve-shaker (W.S. Tyler, Mentor, OH) with stacking sieves. All samples were shaken for 60 s at 280 oscillations per min. The cheese fractions that were retained by the mesh sieves with 6.3-, 4.0-, and 2.0-mm openings were classified as long, medium, and small shreds, respectively. Cheese shred particles smaller than 2.0 mm were classified as fines. Each size of the retained shreds (> 6.3, > 4.0, > 2.0, and < 2.0 mm) were weighed and reported as a proportion of the total weight of the shredded cheese (Banville et al., 2013). To calculate adhesion, the weight of all shreds and fines (cheese shreds < 2.0 mm) were subtracted from the initial weight of the block (Apostolopoulos & Marshall, 1994; Banville et al., 2013). The production of fines, distribution of the shreds, and adhesion to the processing equipment were used as indicators of shreddability (Banville et al., 2013).

Experimental Design and Statistical Analysis

The relationship between the treatment factors i.e. age of natural cheese (d) and emulsifying salt (ES) concentration and the responses in cheese wear behavior and textural characteristics were explored by response surface methodology (RSM). A 3x4 full factorial design with three replicates of two central points to estimate pure error due to randomness and strengthen statistical analysis was used (Table 6). The final model for each response was chosen based on RSM results and comparison between the full RSM model and the selected model with reduced F test. All selected models did not statistically differ from the full RSM models. Selected models were also tested for lack of fit tests and all proved no lack of fit.

Table 6. Response Surface Model Coded Statistical Design

		Coded Variable for Average Age of Natural Cheese	Coded Variable for Emulsifying Salt Percent	Average Age of Natural Cheese	Emulsifying Salt Content
Formula	**Std Runs**	**X1 (a)**	**X2 (b)**	**X1 (c)**	**X2 (d)**
F1	1	-1	-1	1.0	2.0
F2	2	-1	0	1.0	2.5
F3	3	-1	1	1.0	3.0
F4	4	0	-1	21.7	2.0
F5	5	0	0	21.7	2.5
F6	6	0	1	21.7	3.0
F7	7	1	-1	82.3	2.0
F8	8	1	0	82.3	2.5
F9	9	1	1	82.3	3.0
F10	10	2	-1	101.8	2.0
F11	11	2	0	101.8	2.5
F12	12	2	1	101.8	3.0
F5 R1	13	0	0	21.7	2.5
F5 R2	14	0	0	21.7	2.5
F5 R3	15	0	0	21.7	2.5
F6 R1	6	0	1	21.7	3.0
F6 R2	6	0	1	21.7	3.0
F6 R3	6	0	1	21.7	3.0

Model Development

Predictive modeling of shreddability was performed on SAS analytical software (SAS Institute Inc., Cary, NC) using regression analysis. First, we developed a SI using the sieve data i.e. length, fine fragments and adhesion (Childs et al., 2007; Apostolopoulos & Marshall, 1994; Banville et al., 2013). Then, we developed predictive models to predict this SI using most appropriate 8 variables from wear behavior, grating behavior, and rheological properties as shown in Table 7.

The justification for the use of these 8 variables in predictive model is discussed in this paragraph. Penetration depth, mass loss, and wear rate were recorded as the main responses of each wear test and were used in the model because they provide valuable information related to cheese processability. G* was recorded as the main response of the LAOS/SAOS test and was used because it gives the viscoelastic behavior of cheese at specific strains. And strain response is hypothesized to be useful in determination of processability of foods. Gumminess was recorded as one response of the texture profile analysis because it is directly correlated to both hardness and cohesiveness and has previously been correlated to the prediction of fines during processing (Kapoor and Metzger, 2008).

Table 7. Variables from selected tests used in predictive modeling

Test	**Wear**	**LAOS**	**Frequency Sweep**	**Grating Rig**	**TPA**
Variables	Penetration depth Mass loss Rate of wear	G*	G’	Work to grate Crumbliness	Gumminess

The SI was developed based on the average length of shreds, fine fragments, and adhesion to equipment using factor analysis. The index score was calculated and used as a response variable that would be predicted by the eight variables in the predictive model. From the prediction of the SI score the best predictive model was selected by stepwise method with 3-fold cross validation. Variation of inflation factors (VIF) for predictors were calculated and all VIF values below three which showed no multicollinearity of concern were used in the selected predictive model.

Final multiple regression analyses were performed using PROC RSREG, PROC REG, PROC FACTOR, PROC GLMSELECT in SAS/STAT 15.1 (SAS Institute Inc., Cary, NC). All final models were checked for normal and homogeneous error variance assumptions by model diagnostics. The assumptions were adequately held. Statistical significance is specified at 0.05 level throughout the analyses.

RESULTS AND DISCUSSION

Composition

Table 8 shows the proximate composition of all 18 formulations including moisture, fat, total protein, and salt. PC samples were prepared in this study by varying proportion of young, medium and old cheddar cheeses and emulsifying salt (2-3% TSC) and by keeping moisture, fat, and salt levels constant. It is hypothesized that the slight variation between formulations did not have a significant impact on the mechanical properties of process cheese as discussed previously in this thesis.

The total nitrogen and pH 4.6 soluble nitrogen of natural cheese samples are shown in Table 9. It is well known that as the age of natural cheese increases the amount of proteolysis increases, and therefore the amount of intact casein decreases (Hladká et al., 2014). Natural cheese age (d) and pH 4.6 soluble nitrogen (as % of total N) demonstrated a significant ($p < 0.0001$) positive linear correlation (Figure 5). As age (days) increased the pH 4.6 nitrogen (%) also increased. Therefore, natural cheese age (d) would have a negative linear correlation with intact casein. Intact casein would decrease as age increased from 1-102 d. In this thesis, intact casein of natural cheese used in PC is referred to by the measurement of d of aging.

Process Cheese Microstructure

With confocal laser scanning microscopy CLSM results were as expected (Luyten et al., 1991; Lamichhane et al., 2019), with an increasing proportion of aged cheese in the PC formulation, it was found that fat particle size decreased and dispersion of the same

Table 8. Proximate composition of all 18 PC formulations. Values are the mean ± SD of three subsamples. The results are expressed as a % (wt/wt) of cheese sample

Formula	Moisture, %	Fat, %	Total Protein, %	Salt, %	pH	pH 4.6 Soluble Nitrogen (% of Total)
1	43.1±0.1	30.9±0.0	20.20±0.01	2.09±0.03	5.50±0.01	1.25±0.00
2	42.3±0.5	29.9±0.0	20.00±0.01	1.92±0.11	5.49±0.01	1.25±0.00
3	42.0±0.2	30.4±0.0	19.83±0.01	2.21±0.14	5.50±0.01	1.25±0.00
4	41.7±0.3	29.4±0.0	20.00±0.01	2.06±0.18	5.50±0.01	2.85±0.01
5	43.3±0.3	29.1±0.0	19.80±0.01	2.43±0.24	5.50±0.01	2.85±0.01
6	41.6±0.3	29.8±0.0	19.63±0.01	2.12±0.04	5.49±0.01	2.85±0.01
7	42.4±0.2	29.8±0.0	19.87±0.01	2.14±0.05	5.51±0.01	6.99±0.04
8	42.5±0.2	29.2±0.0	19.53±0.01	2.18±0.05	5.50±0.01	6.99±0.04
9	43.0±0.4	30.3±0.0	19.48±0.01	2.02±0.04	5.50±0.01	6.99±0.04
10	42.5±0.2	28.8±0.0	19.62±0.01	2.24±0.05	5.49±0.01	11.17±0.08
11	43.7±0.1	30.6±0.0	19.43±0.01	2.07±0.05	5.47±0.02	11.17±0.08
12	43.7±0.2	29.5±0.0	19.26±0.01	2.05±0.07	5.50±0.01	11.17±0.08
F5 R1	42.6±0.3	29.5±0.0	19.63±0.01	2.11±0.07	5.50±0.01	2.85±0.01
F5 R2	42.9±0.4	29.5±0.0	19.63±0.01	2.24±0.05	5.49±0.01	2.85±0.01
F5 R3	42.8±0.2	29.5±0.0	19.80±0.01	2.20±0.05	5.52±0.02	2.85±0.01
F6 R1	42.9±0.0	29.2±0.0	19.63±0.01	2.19±0.07	5.50±0.01	2.85±0.01
F6 R2	41.6±0.3	29.2±0.0	19.83±0.01	2.19±0.10	5.50±0.01	2.85±0.01
F6 R3	42.8±0.1	29.8±0.0	19.63±0.01	2.07±0.06	5.52±0.01	2.85±0.01

$F5\ Rx^1$ Represents formula 5, replicate x, F6 Rx^2 represents formula 6, replicate x

Table 9. pH 4.6 soluble nitrogen content of natural cheeses used in PC formulation and protein contents.

Age of Natural Cheese, D	Average Total Nitrogen, %	Average pH 4.6 Soluble Nitrogen, %	Calculated Average Total Protein, %	Calculated Average of pH 4.6 Soluble Protein as % of Total Protein
1	3.83±0.01	0.05±0.00	24.43±0.06	*1.25±0.00
70	3.63±0.01	0.24±0.01	23.18±0.06	*6.58±0.16
176	3.65±0.01	0.38±0.11	23.26±0.06	*13.14±0.62

*All values are statistically significant ($p < 0.0001$)

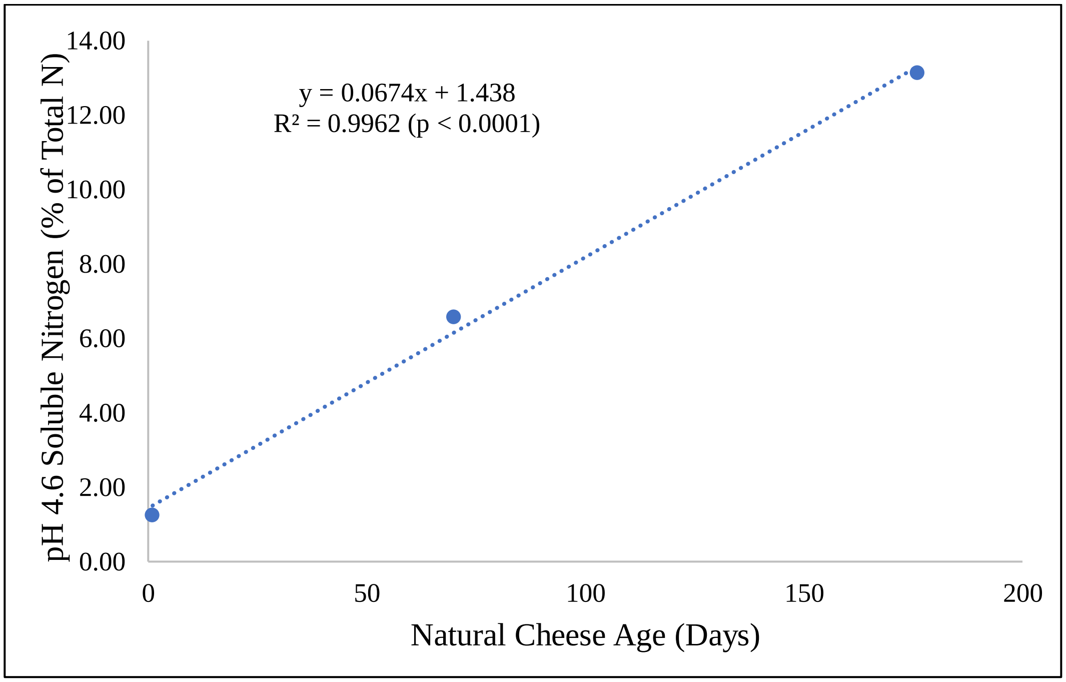


Figure 5. pH 4.6 soluble nitrogen (% of total nitrogen) as dependent on natural cheese age (d). Higher values of pH 4.6 soluble nitrogen indicate increased proteolysis and decreased amounts of intact casein.

was more prominent (Figure 6). However, observational length scale of CLSM images was not sufficient to visualize changes in the protein phase and also the interface in between fat and protein component.

TEM images of the PC samples with varying levels of TSC (Figure 7) and varying average ages of natural cheese (Figure 8) are depicted below. Visual observation of the TEM images show that as TSC levels in PC increases, the fat globule size decreases. At higher resolutions (20000 x) there were differences due TSC levels in the size of protein structures and the way they interacted with fat globules. However, exact causes for these changes are unknown and require further future scientific investigation as to find the quantifying changes in protein molecules. Impact of age of natural cheese in

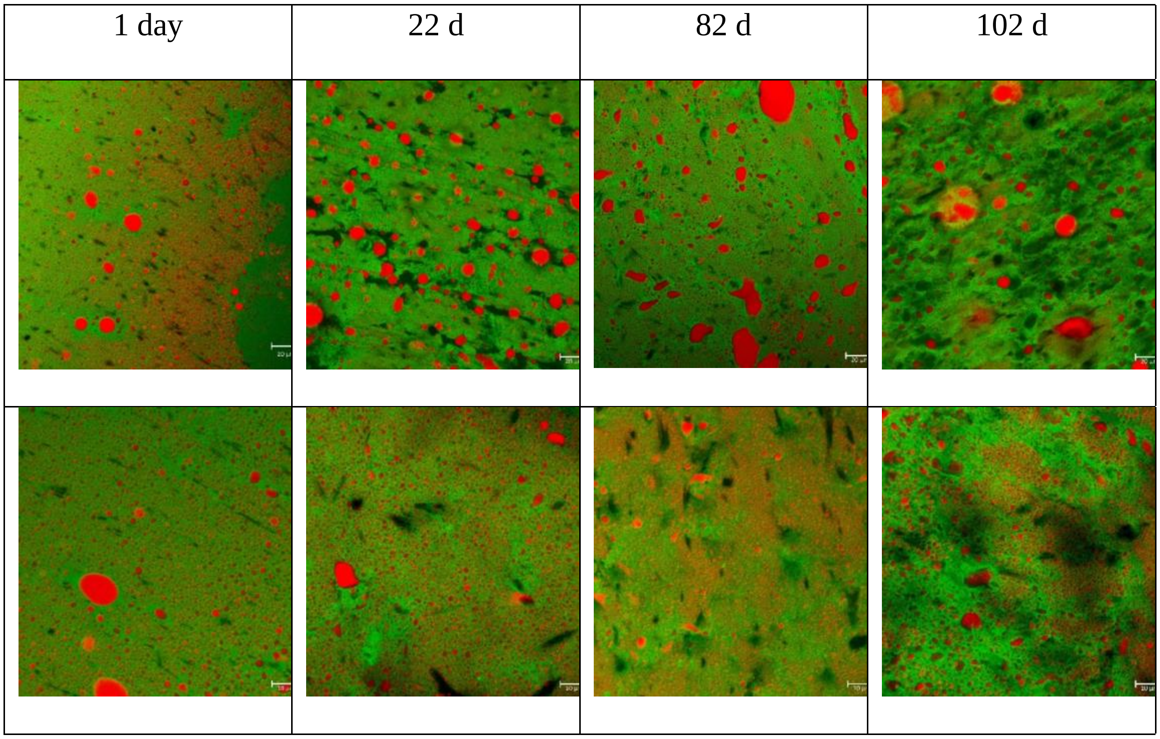


Figure 6. Microstructures from CLSM on PC samples formulated with 2.5% trisodium citrate and varying ages of natural cheese (1.0-102 d), with fat globules (red) and protein phase (green) visible. Top row images were taken at 20x, the scale bar represents 20 μm. Bottom row images are 40x magnification, scale bar represents 10 μm.

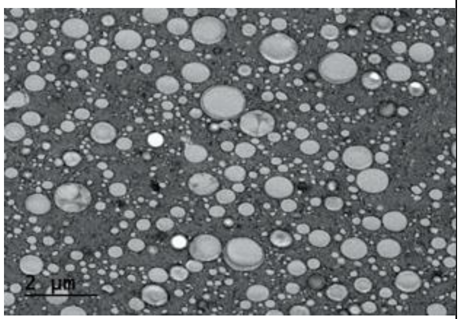
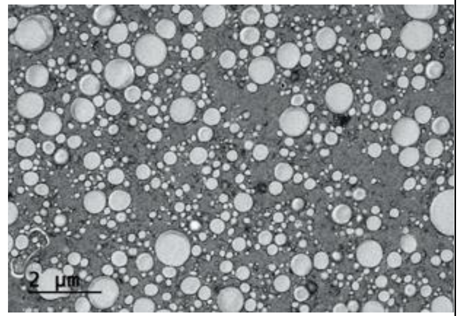
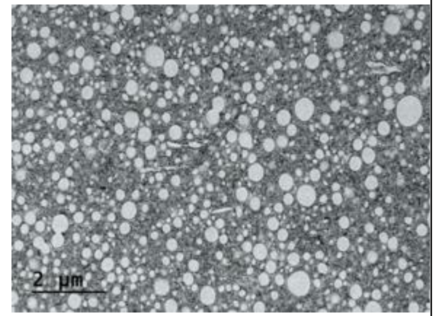
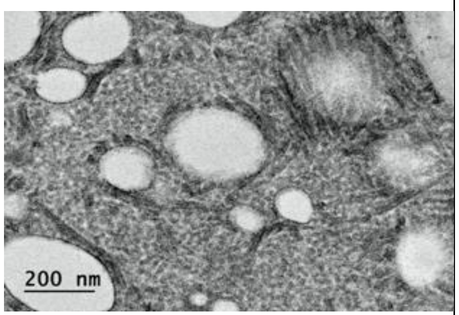
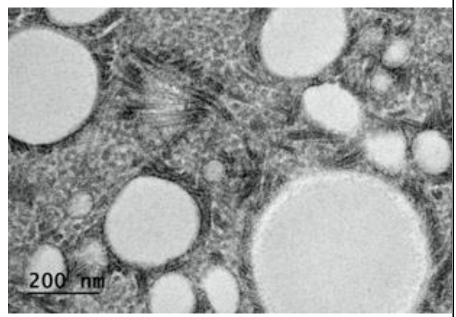
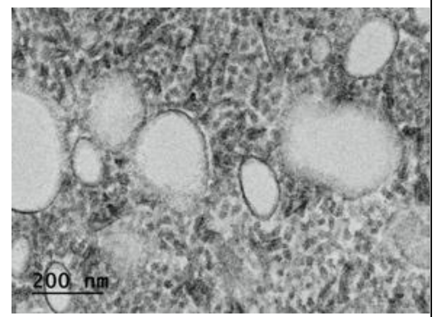
Mag. (x)	2.0% ES (F4)	2.5% ES (F5)	3.0% ES (F6)
2,000			
20,000			

Figure 7. Microstructures from TEM imaging on PC samples formulated with 22-d average natural cheese age and varying levels of trisodium citrate (2-3%). Fat globules (light gray circles) and protein phase (dark gray background) are visible. Top row images were taken at 2000x, the scale bar represents 2 ***μ***m. Bottom row images are 20,000x magnification, scale bar represents 200 nm. (TEM images provided by Dr. Almut Volmer, Utah State University, Logan, UT).

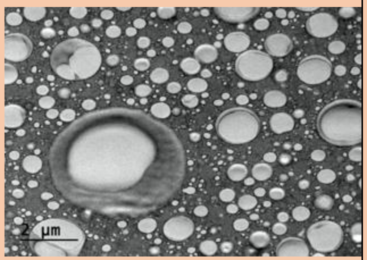
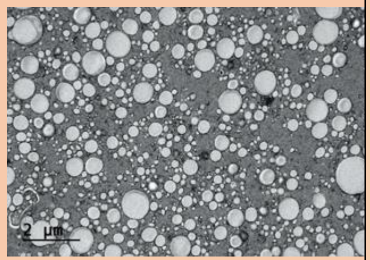
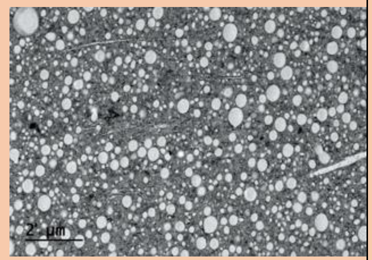
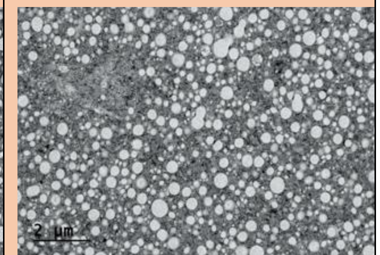
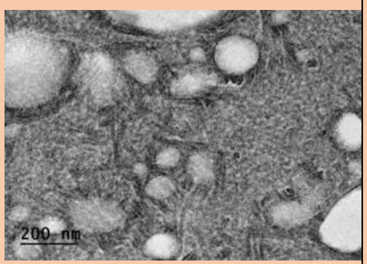
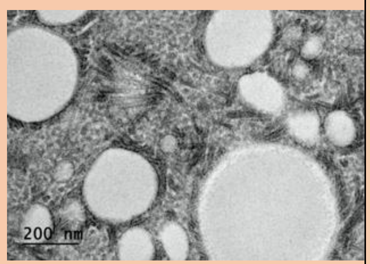
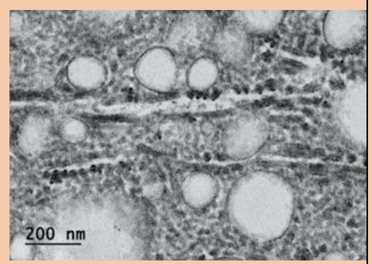
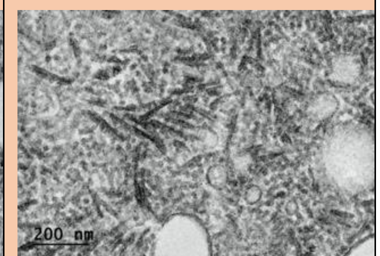
Mag. (x)	1-D (F2)	22-D (F5)	82-D (F8)	102-D (F11)
2,000				
20,000				

Figure 8. Microstructures from TEM imaging on PC samples formulated with 2.5% trisodium citrate and varying ages of natural cheese (1 to 102 d). Fat globules (light gray circles) and protein phase (dark gray background) are visible. Top row images were taken at 2000x, the scale bar represents 2 μm. Bottom row images are 20,000x magnification, the scale bar represents 200 nm. (TEM images provided by Dr. Almut Volmer, Utah State University, Logan, UT).

PC formulation is also visible in the micrograph shown in Figure 8. At low (2000 x) magnification, as the average natural age increased the size of fat globules decreased and it was more dispersed into the protein network (Figure 8). A further analysis is recommended to quantify the fat particle size using an image analysis software. These changes in fat globule size are the result of an increasing degree of proteolysis of natural cheese used in PC formulation (Luyten et al., 1991; Lamichhane et al., 2019). These changes have also been shown to reduce the viscosity of the mixture during cooking and agitation, and as a result, enhance the dispersion of fat phase into protein matrix (Luyten et al., 1991; Kapoor and Metzger, 2005; Lamichhane et al., 2019). At higher magnification (20000x), the impact of age or extent of proteolysis is clearly visible on the protein matrix with the presence of electron dense, rod or tubular shaped structures around fat globules. Constituent material of these structures is rather unknown as these could be related to fragmented protein particles, or aggregated structure of emulsifying salt or calcium lactate crystals. This requires further investigation to identify these structures and quantify them.

Functional and Rheological Properties: Wear Behavior. Wear behavior in PC samples was determined by measuring penetration depth as described in the materials and methods section. Other parameters obtained from these experiments were the mass removal (referred mass loss in this thesis) and rate of wear (mm penetration/min) upon applying tribological force. As expected, penetration depth increased as sliding distance increased for all 18 samples, indicating continuous wearing of PC (Figure 9).

The wear behavior parameters (depth of penetration and mass loss) were obtained for PC over 30 m sliding distance, however, penetration depth became inconsistent

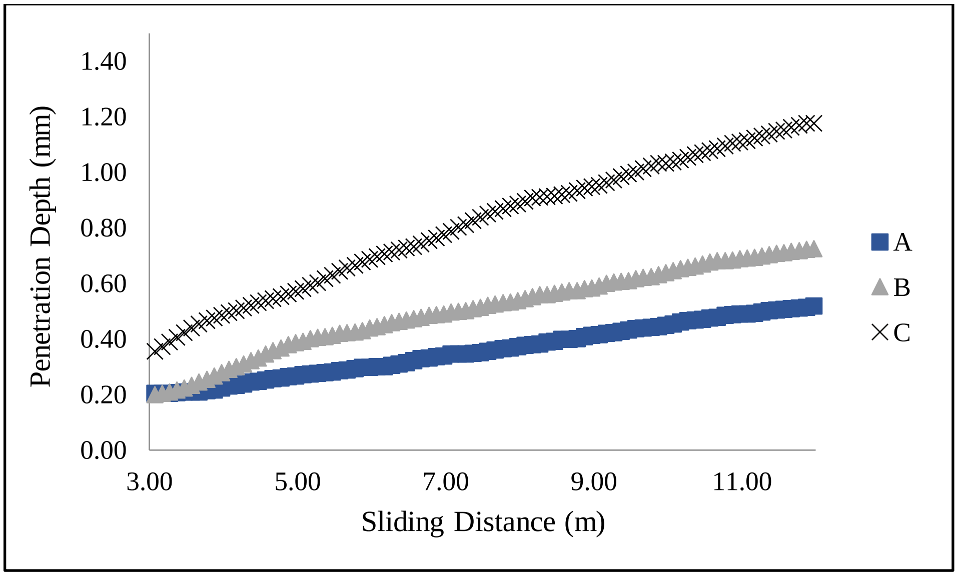


Figure 9. Penetration depth (mm) as dependent on sliding distance (12 m) for PC formulations with 2.0% TSC and varying average ages of natural cheese A) *1-day B) *22 d C) *82 d D) 102 d not shown due to presence of large artifacts during testing. *Significantly different ($p < 0.01$)

for PC samples with higher proportion of old aged cheese due to highly adhesive nature of the material and very soft body. After 12 mm of sliding distance, the data was inconsistent, we therefore, decided to report penetration depth at sliding distance of 12 m.

Impact of age of natural cheese in PC formulation was significant ($p < 0.01$) on mass loss and penetration depth (Table 10). A positive correlation was found between average age of natural cheese in the PC formulation and mass loss (Figure 10), and average age of natural cheese and penetration depth (Figure 9). These results were expected because of proteolytic changes that occur in natural cheese as it ages. No significant correlation ($p > 0.05$) was found for the TSC (%) treatment on mass loss (Figure 10).

The results of this experiment, as shown in Figure 5 and Figure 7, agreed with data reported in the literature that the extent of proteolysis in natural cheese increases with aging of cheese (Olson et al., 1958; Piska and Štětina, 2004; Purna et al., 2006). The results of our mechanical tests also agreed with reports in the literature that the effect of increased proteolysis of natural cheese used in PC formulation affects the mechanical strength (see Texture Profile Analysis) and elasticity of the cheese matrix (see Effect of Age and ES on Large Amplitude Oscillatory Shear (LAOS) Rheology) (Olson et al., 1958; Piska and Štětina, 2004; Purna et al., 2006).

Table 10. Estimated coefficients[1] and their p values (in parenthesis) of final regression models using age and emulsifying salts (ES) (coded) on the eight tested variables.

	Coded Factors in Final Models				
	Age	**ES**	**Age*Age**	**ES*ES**	**Age*ES**
Tribology/Wear Test:					
Mass loss	1.04 (<0.0001)	-	-	-	-
Penetration depth	0.38 (< 0.0001)	-	-0.15 (0.006)	-	-
Wear rate	0.55 (<0.0001)	-	-0.32 (0.02)	-	-
Grating/Shredding test:					
Work to grate	0.09 (<0.0001)	0.07 (<0.0001)	-0.07 (0.01)	-	-
Crumbliness	-10.45 (<0.0001)	7.74 (0.0002)	-	5.61 (0.02)	-5.66 (0.008)
LAOS:					
G*	-3.21 (<0.0001)	-1.70 (0.0009)	2.11 (0.02)	-	-
Textural/Compression test:					
Hardness	-0.12 (0.0001)	-	-	-	-
Gumminess	-0.10 (0.0001)	-	-	-	-

1. Coefficients are for coded age and ES. Coding is applied as:
   Coded age = (actual age – 41.65)/40.65
   Coded ES = (actual ES – 2.5)/0.5

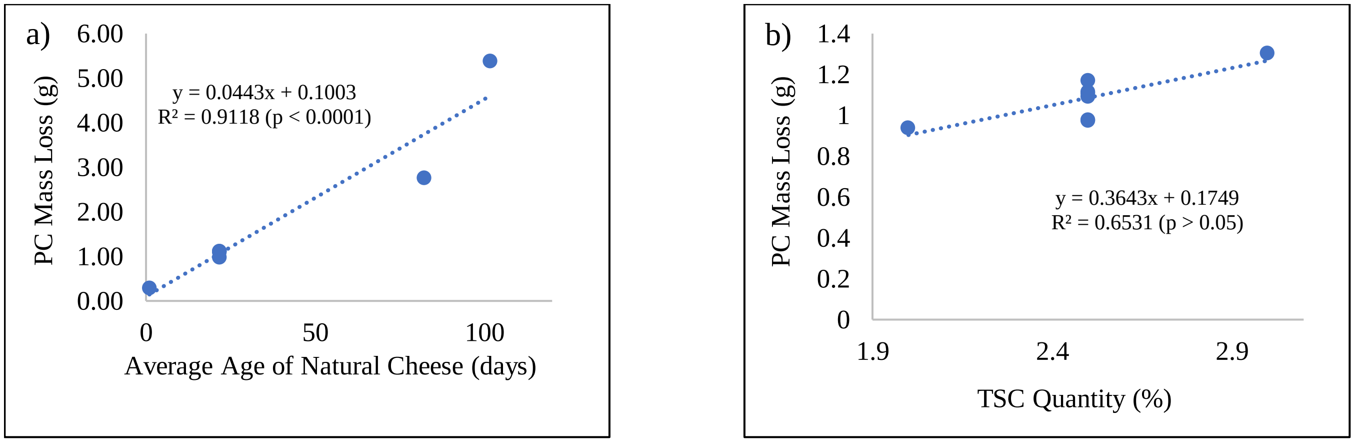


Figure 10. The effect of average natural cheese age on mass loss (g / 22 g) at 2.5% TSC during the wear test (a). *The effect of ES (%) on mass loss (g / 22 g) with natural cheese having an average age of 22 d (b). *Not statistically significant $p > 0.05$)

As the age of natural cheese increases the strength and elasticity of the cheese decreases because of breakdown of intact casein and weakening of bonds between protein and other molecules (Olson et al., 1958; Piska and Štětina, 2004; Purna et al., 2006), our results also agreed with these findings (see Frequency Dependence of Viscoelastic Properties). This breakdown of intact casein can also affect the material properties of PC impacting the wear behavior (including penetration depth and mass loss) of PC (Figure 10; Figure 11). In this study, a higher proportion of aged cheeses (indicated as average age of cheese formulation) in the PC formulation (i.e., increased proteolysis and decreased amount of the intact casein) resulted in softer material with decreased strength and elasticity (see Frequency Dependence of Viscoelastic Properties). A higher proportion of aged cheeses (measured in d) correlated well with an increase in both mass loss (g) (Figure 10) and penetration depth (mm) (Figure 11).

The effect of TSC on wear behavior (penetration depth and mass loss) was not statistically significant (Figure 10; Table 10). Effect of emulsifying salt was not visible at the measurement temperature of 5℃, because of the presence of large proportion of solidified fat at such a low temperature. Solid fat below 20℃ (G'~292 kPa) contributes more to structural strength and rheology of PC as compared to the protein phase (G'~164 kPa) (Yang et al., 2011; Sharma et al. 2018). Therefore, the solidified fat at 5℃ is expected to reinforce the cheese matrix and would easily mask effect of TSC on the material and functional properties of PC.

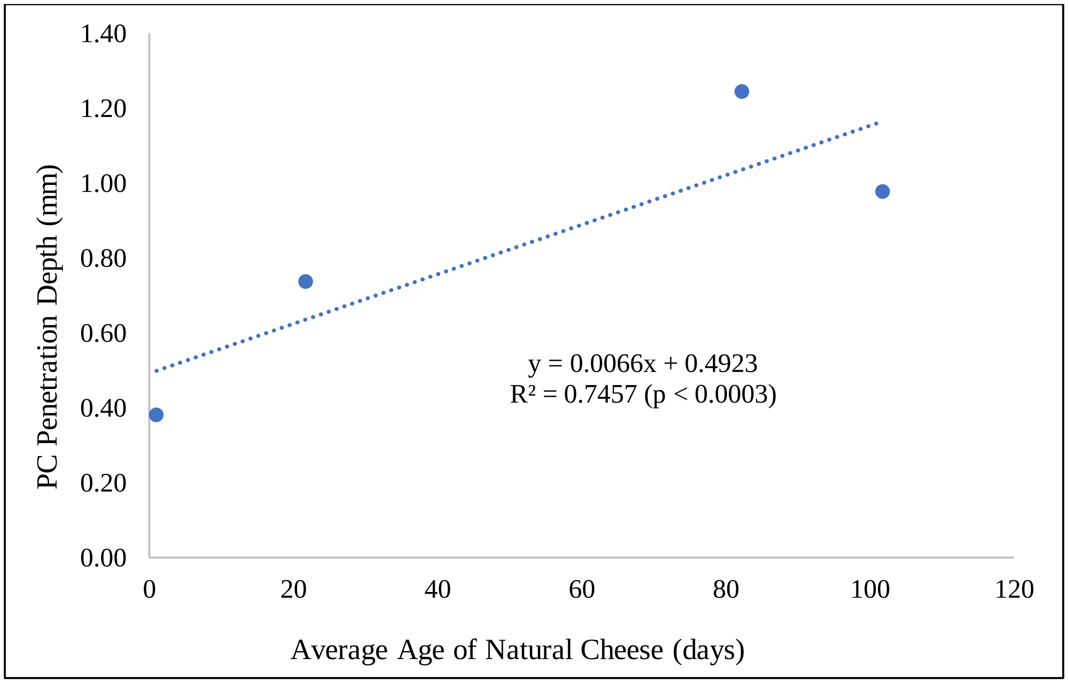


Figure 11. The linear effect of average natural cheese age on penetration depth (mm) at 2.5 % TSC during the wear test

Wear behavior for PC formulations prepared with cheeses of different average ages and levels of emulsifying salt demonstrated both adhesive and abrasive wear depending upon treatment conditions. PC made with 1-day and 22-day-old cheese demonstrated abrasive wear as shown by presence of fragmented fine particles on the wear track (Figure 12 A, B). These formulations showed very little sticking to the upper PID tribometer geometry during testing. The wear track of these cheeses also illustrated more of a crumbling behavior by the small particles left on either side of the wear track. PC made with 82 and 102-d-old cheese demonstrated adhesive wear as indicated by sticking (adhesive layer) to the probe (Figure 12 C, D). These formulations exhibited a high degree of adhesion to the upper PID tribometer geometry during testing (Figure 13).

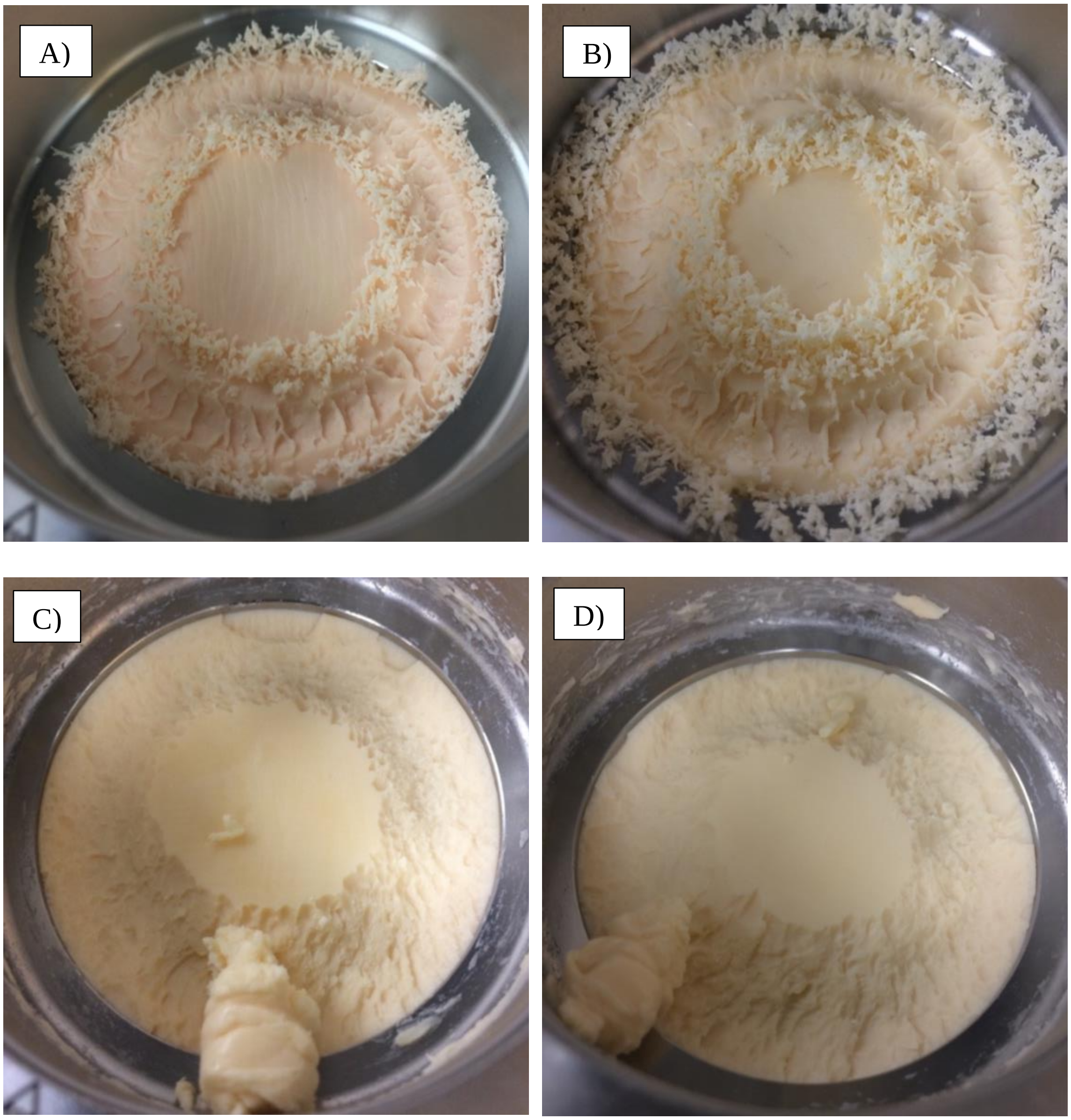


Figure 12. The wear track of PC made with A) 1-day, B) 22-day, C) 82-day, and D) 102-day average natural cheese age at 2.5% TSC.

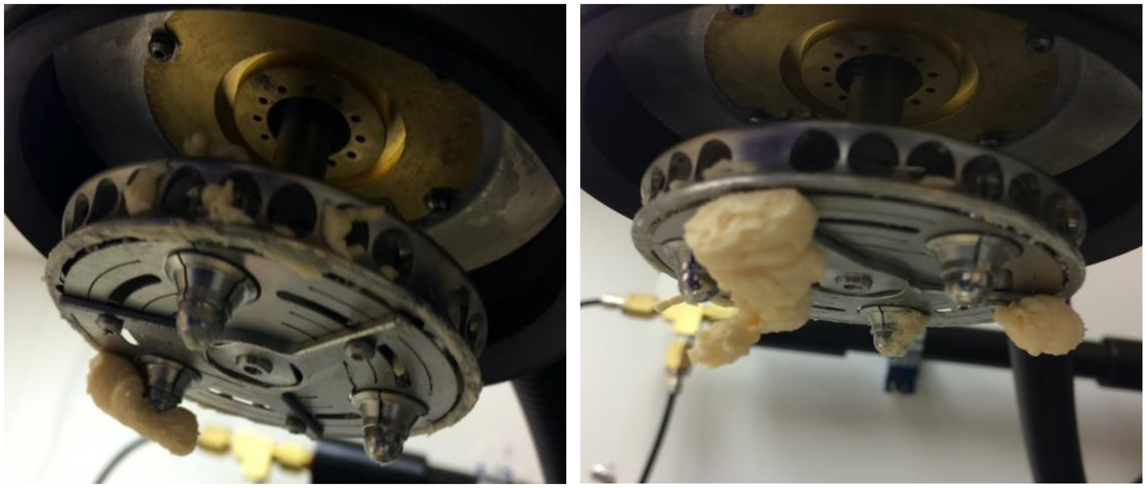


Figure 13. The PID Tribological geometry with adhesion artifacts of PC made with 82-day (left) and 102-day (right) average natural cheese age and 2.5% TSC.

The wear track of these cheeses illustrated more of an adhesive type of wear behavior with cheese particles clumping together and also sticking to the probe. The clumping effect of the adhesive behavior caused a larger wear track. The stickiness (adhesive behavior) observed in PC formulations increased from the higher intact casein formulations (1 and 22 d) to the lower formulations (82 and 102 d) and was attributed to the lower amount of intact casein content as reflected by higher amounts of pH 4.6 soluble nitrogen (Table 8). Both cases, the production of fines on the sides of the wear track and the formation of cheese lumps and adherence to a metal surface cause mass loss, however, cheese adhesive behavior appears to cause higher mass losses (> 2 g) as compared to abrasive behavior (< 1 g). Both of these wear behaviors should correspond to the shreddability index which is developed in this study using the production of fines, adhesion behaviors, and average shred length. It is proposed that adhesive wear corresponds more with mass loss due to adhesion during shredding an abrasive behavior appears to be more associated with the mass loss due to production of fines, however, further analysis is needed to support conclusions.

Frequency Dependence of Viscoelastic Properties. Frequency sweeps on PC samples were performed by applying frequencies in descending order form 100 Hz to 0.01 Hz at 5℃ using 0.05% strain amplitude (Figure 14). As frequency decreased for all 18 samples, both G' and G" decreased at the almost similar rates for all samples (Figure 14). Through all frequencies (0.01-100 Hz), storage modulus (G') remained higher than the loss modulus (G") with n < 0.2 (from Eq 1) and no crossover point (Figure 14), thus indicating all 18 cheese formulations behaved like viscoelastic solids with low frequency dependence.

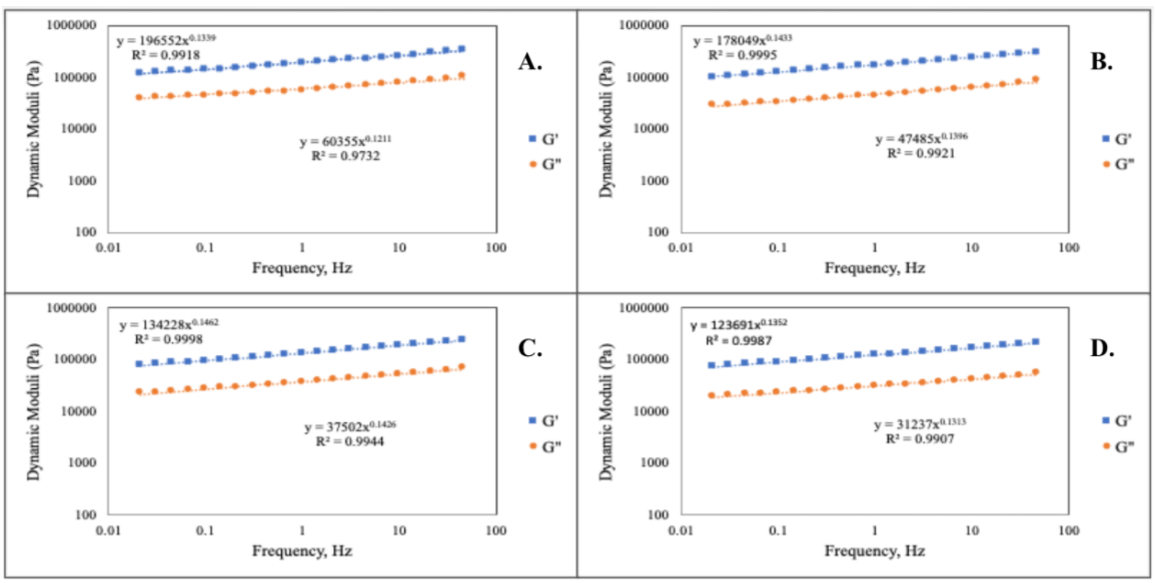


Figure 14. Varying average age of natural cheese at 2.5% TSC. A. 1-day natural cheese B. 22-day natural cheese C. 82-day natural cheese D. 102-day natural cheese

Impact of average natural cheese age in the PC formulation was significant ($p < 0.01$) on G' (Table 10). For all samples, the G' increased over all frequencies (0.01-100 Hz) (Figure 15). At 1 Hz, the storage modulus (G') decreased as age increased (Figure 16). As G' values reflect the strength of the PC network (Mezger 2014), it is suggested that the strength of the PC network decreased as average cheese age of the PC formulation increased from 1-102 d.

The effect of TSC levels on G' at 5°C was not as significant ($p > 0.05$) as the effect of age (Table 15, Appendix B). This is attributed to the nature of milkfat at 5°C. PC is commercially shredded or sliced between 5-7°C. Frequency sweeps tests were performed at 5°C for all 18 formulations to imitate these conditions.

It has been demonstrated that the temperature of milkfat has a major impact on the rheological properties of cheese, especially at lower temperatures (Zhou and Mulvaney, 1998; Yang et al., 2011; Sharma et al., 2018). Using fat-filled gel models the calculated G' of fat particles was shown to be greater than the G' of their protein matrix at 20, 15, and 10°C (Yang et al., 2011) thus emphasizing the reinforcing effect of fat on cheese G' (Yang et al., 2011). Additionally, cheese rheological properties were overcome by the solid fat phase at 15 and 10°C and demonstrated no significant change in G' with aging (Yang et al., 2011), however, our results showed significant ($p < 0.0001$) change with age (Table 16, Appendix B). Additionally, when testing TSC-treated mozzarella cheeses with varying fat contents at 20°C (which had similar solid viscoelastic behavior and low frequency dependence (from Eq. 1, $n < 0.18$) the full-fat cheeses had a higher storage moduli than the nonfat cheeses (Sharma et al., 2018). The storage modulus of the milkfat was notably higher than that of the cheese matrix (292 kPa > 164 kPa)

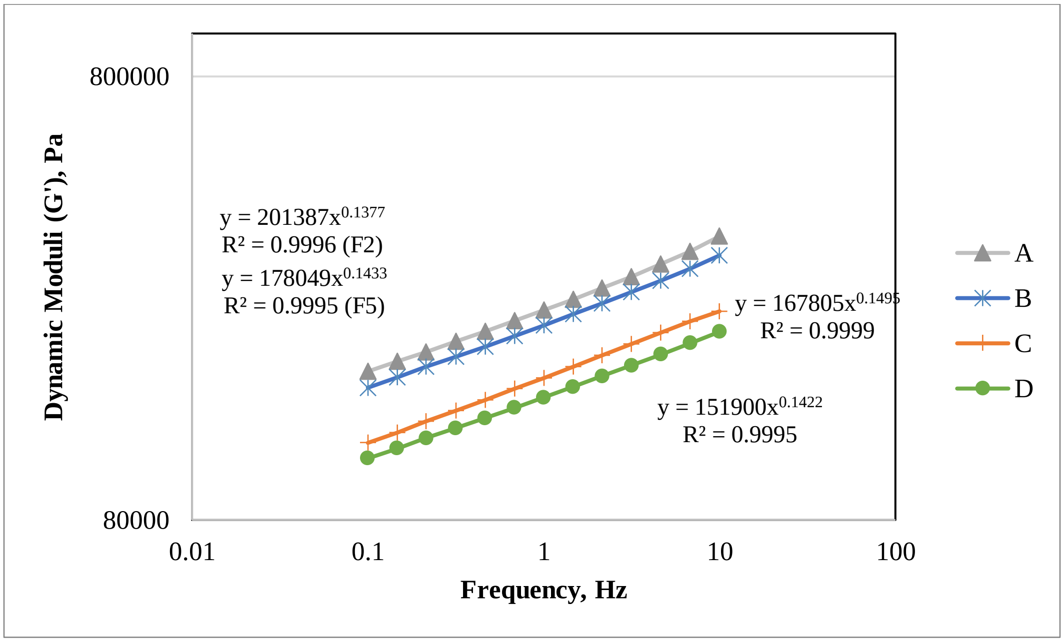


Figure 15. G' values of PC at 2.0% TSC with varying ages of natural cheese. A. 1-day B. 22 d C. 82 d D. 102 d

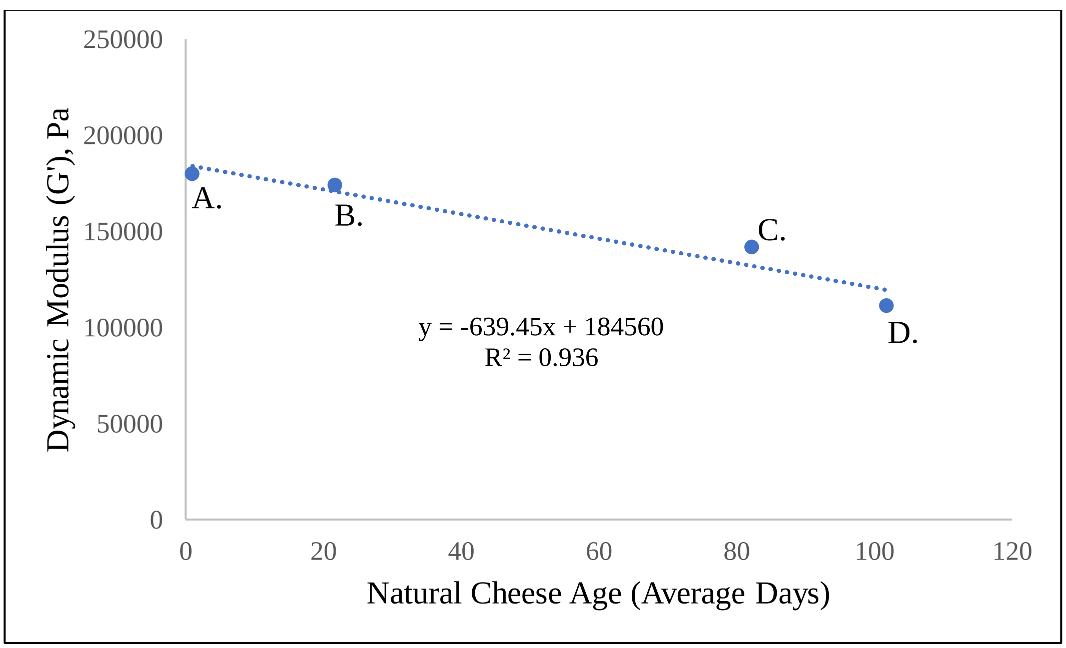


Figure 16. G' values at 1 Hz of PC with varying ages of natural cheese. A. 1-day (d) B. 22 d C. 82 d D. 102 d

(Sharma et al., 2018). The difference in G' values was attributed to the contribution of solid fat and supports the idea that milkfat reinforces the cheese matrix (Zhou and Mulvaney, 1998; Sharma et al., 2018). From these results, it can be concluded that because the rheological properties of cheese matrices were dominated by the effect of solid fat as low temperature (Yang et al., 2011) therefore, the effect of TSC levels was also overshadowed by the effect of solid fat.

Effect of Age and ES on Large Amplitude Oscillatory Shear (LAOS) Rheology. Because non-linear viscoelastic behavior corresponds to a permanent deformation which is also the case while performing shredding operations on cheese blocks, therefore non-linear viscoelastic behavior of PC samples was determined by LAOS tests. These tests were performed using strain sweeps (0.1 to 100%) at 1 Hz and 5℃. Impact of average age of natural cheese in PC formulations was significant ($p < 0.01$) for G* values at 0.025% strain (Table 10). There was a negative correlation between average natural cheese age and G* (Figure 17).

Impact of TSC in PC formulations was significant ($p < 0.01$) on G* values at 0.025% strain (Table 10), however, these values were not included in modeling due to the lack of significance (Figure 18). Additionally, the results do not corroborate with the findings in the literature that demonstrate the increase in the strength of the cheese matrix as emulsifying salt levels increase (Kapoor and Metzger, 2008). The effect of TSC concentration and the combined effect of both average age of natural cheese and TSC quantity on G* is demonstrated in Figure 18.

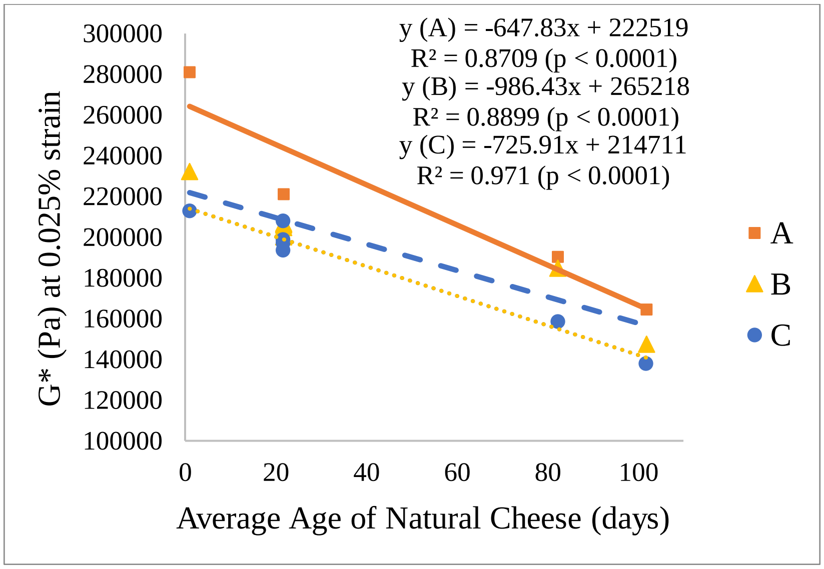
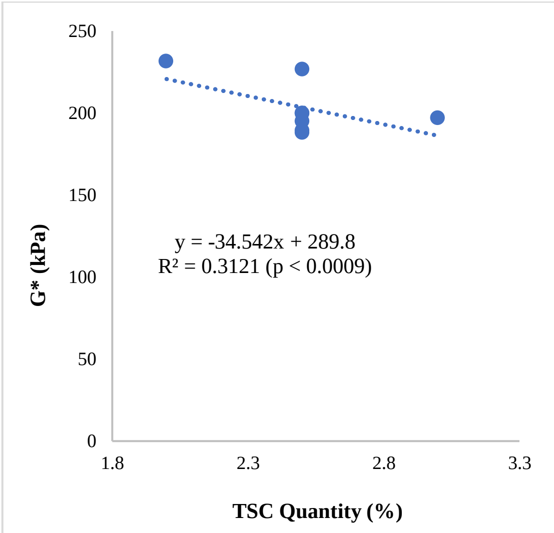


Figure 17. The effect of average natural cheese age on G* at 0.025% strain with varying levels of TSC A) 2.0% B) 2.5% C) 3.0%. Significance for data was derived from a two-way ANOVA test with a 95% confidence interval.



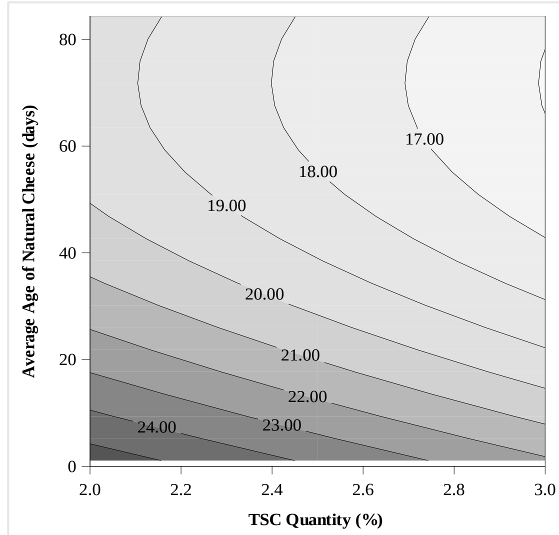


Figure 18. The effect of trisodium citrate (TSC) levels with PC having 22-day-old average natural cheese mixture on G* (kPa) at 0.025% strain (left). The effect of TSC (2.0, 2.5, 3.0%) and natural cheese age (1, 22, and 83 d) on G* as shown by a RSM contour plot.

In non-linear rheology, Lissajous plots are a graphical representation of the strain response (%) to changing stress (Pa). In PC samples used in this thesis, it demonstrates the viscoelastic behavior over increasing stress (Pa) from linear to nonlinear viscoelastic regimes. Pure elastic materials on a stress-strain plot should appear as a straight line. Purely viscous materials on similar plot should appear as a perfect circle. For viscoelastic materials, the more a plot closely resembles a line or a circle the more elastic or viscous behavior a sample is said to have. PC is a viscoelastic material and therefore exhibits a degree of both viscous and elastic behaviors, this appears on a Lissajous plot as a shape in between a line and a circle (Figure 19). As a material changes over increasing stress (deformation), the ratios of viscous/elastic behavior change and therefore the shape of the

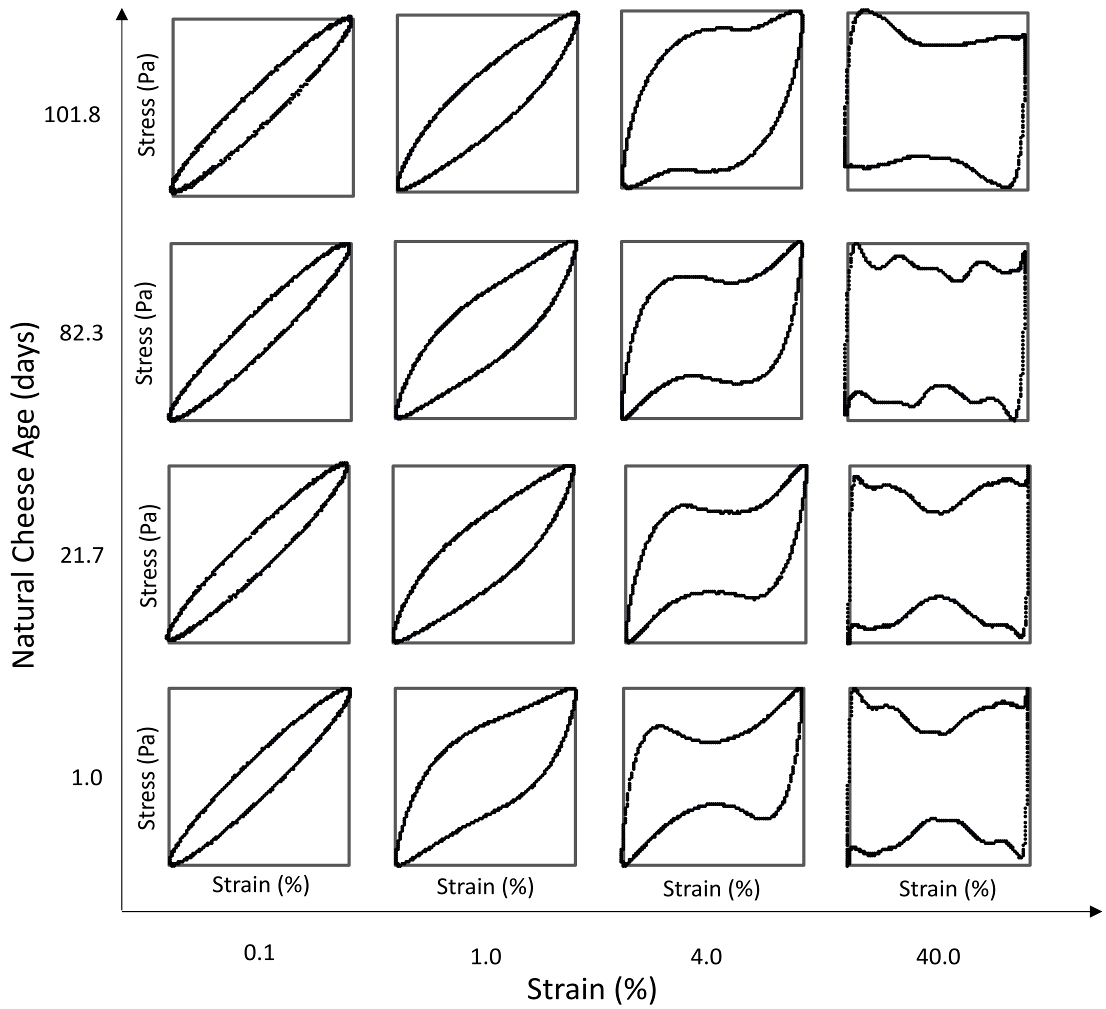


Figure 19. Lissajous plot for cheeses containing 2.5% TSC and varying ages of natural cheese (1, 22, 82, 102, d).

plot changes. Strain stiffening factor (S), or shear thickening factor (T) can also be calculated from a Lissajous plot (Giménez-Ribes et al., 2020).

Lissajous plots for both varying levels of emulsifying salts and natural cheese ages showed distortion from an elliptical shape with increased strain (Figure 19), this behavior indicates nonlinear viscoelastic behavior at high strain. Each variation among 18 formulas (whether by varying emulsifying salt content or average natural cheese age) showed different patterns in the shape of Lissajous plots at each strain. These differences indicate that strain variations impact nonlinear viscoelastic behavior of cheese. The results from these visual observations agree with the quantitative LAOS data demonstrating that as shear strain increased past 4.0% for all 18 PC formulations nonlinear viscoelastic behavior (phase angle > $45°$) started to appear (Table 11). At 4.0% strain, the shape (Figure 19) of the Lissajous plots changed with respect to the average age of natural cheese. As age increased, the samples displayed more viscous behaviors indicating increased plastic deformation. From this data, it is evident that PC formulations with higher proportion of aged cheeses will most likely exhibit higher degree of viscous behavior (stickiness) at larger strains.

Nonlinear viscoelastic behavior recorded from large amplitude oscillatory shear tests is further elaborated in terms of strain stiffening or strain softening ratios. These ratios are derived from a protocol described by Ewoldt et al. (2008) and Melito et al. (2012). Large-strain elastic modulus (G_L') is the secant modulus measured at maximum strain; and minimum-strain elastic modulus (G_M') is the tangent modulus measured at zero strain. A ratio of G'_L / G'_M > 1.10 demonstrates strain stiffening behavior while G'_L / G'_M

Table 11. Viscoelastic parameters for PC made with 22-day average age of natural cheese and varying levels of TSC. Values are mean ± SD after three measurements, replications.

Process Cheese Rheological Parameters Beyond 0.025% Strain and Frequency of 1 Hz at 5°C				
Age of Natural Cheese (d)	**Sodium Citrate Quantity (%)**	**Strain (%)**	G'_L / G'_M	**Phase Angle (degrees)**
22	**2**	**0.1**	0.007±0.004	15.99±0.30
		1	0.627±0.149	37.46±2.65
		4	0.963±0.062	52.34±3.86
		40	0.928±0.059	71.56±7.45
	2.5	**0.1**	0.029±0.014	16.24±0.29
		1	0.308±0.034	32.34±2.94
		4	0.875±0.280	46.88±1.60
		40	0.885±0.122	70.05±1.75
	3	**0.1**	0.015±0.004	15.85±0.60
		1	0.435±0.188	30.31±5.96
		4	1.058±0.010	44.43±2.64
		40	0.843±0.145	70.00±5.27

< 0.90 denotes strain softening behavior (Melito et al., 2013; Zad Bagher Seighalani and Joyner, 2019).

As shown in the Table 11, PC samples of 22 d average age (typical sample) showed minimal strain stiffening and major strain softening behavior. However, it was observed that as G'_L / G'_M increased as strain increased for 0.1, 1.0, and 4.0% strain, this behavior indicates increased strain stiffening and decreasing strain softening behavior (Table 11). The increasing phase angle with increasing strain shows that PC across all 18 formulations in this experiment exhibited more viscous behavior (phase angle > 45°) as strain increased (Table 11). At strains beyond 1%, viscous behavior was dominant.

For viscoelastic materials such as PC, fluid behavior is a sign of permanent deformation. Internal structure of PC at high strains cannot stretch elastically to compensate for the increasing strain similar to that of natural cheeses (Zad Bagher Seighalani and Joyner, 2019). PC microstructure consists of an interlocking casein protein network with trapped milkfat globules and serum (Fox et al., 2004a).

As applied strain is increased, the protein network is stretched, and the adjacent protein chains/structures start rubbing each other, therefore, strain hardening behavior is exhibited. (Sharma et al., 2018; Joyner Melito et al., 2018; Zad Bagher Seighalani and Joyner, 2019). With varying amount of aged cheese, the amount of intact casein present in the formulation changes, and therefore different protein networks were formed. These different networks exhibited differences in mechanical and strain stiffening behaviors, particularly at 1.0% strain (Table 17, Appendix B). At this strain, it was evident that as average natural cheese age increased from 1-102 d, the stiffening behavior decreased (Table 17, Appendix B).

Complex shear modulus (G*) is a reflection of the complete viscoelastic behavior of a material as it takes into account both the storage modulus (G') and loss modulus (G"). As cheese age increased from 1-102 d, G* at low strain (0.025%) decreased (Table 18, Appendix B). Because it is elastically dominant at 0.025% strain (phase angle < 45°), G* at this strain is therefore more reflective of its elastic portion (G'). The comparison of G* values within the elastic dominant portion (LVR) is one way to compare the strength of some material structures. All 12 formulations and replicates displayed a decrease in G* as average natural cheese age increased from 1-102 d indicating a weakening of the cheese structure (Table 18, Appendix B).

Texture Profile Analysis. Texture profile analysis was performed with a two-bite compression test at 25% compression and 1 mm/s crosshead speed at 5°C. Parameters obtained included hardness, adhesiveness, resilience, cohesion, springiness, gumminess and chewiness of PC samples. The impact of age of natural cheese in PC formulation was significant ($p < 0.01$) on hardness and gumminess (Table 10). A negative correlation was found between average natural cheese age in PC formulation and hardness (Table 19, Appendix B) or gumminess (Table 20, Appendix B). This was because of the proteolytic changes that occur in natural cheese as it ages as discussed previously in this thesis (Olson et al., 1958; Piska and Štětina, 2004; Purna et al., 2006).

The effect of TSC on TPA parameters i.e. hardness and gumminess was not statistically significant ($p > 0.05$) (Table 10). This was because of the presence of solid milkfat at 5°C as mentioned previously in this thesis. Another possible reason is that the design used in this study (without 3 replicates of factorial design) didn't allow an interaction effect to be observed clearly.

Shreddability Rig Data. The grating rig attachment on the TPA provides two variables necessary for determining shreddability: work to grate, and crumbliness. Impact of average natural cheese age and TSC levels was significant ($p < 0.01$) on both work to grate and crumbliness (Table 10). The effect of both age of natural cheese (0-82 d) and TSC concentration (2-3%) on work to grate is shown in the contour plot below (Figure 20). This graph demonstrates the increasing effects of TSC concentration on work to grate, this could be attributed to the fact if TSC concentration were to increase past 3% the effects on work to grate might be greater than the effects of natural cheese age.

As average age of natural cheese increased (1-102 d), work to grate (g · s) increased (Table 21, Appendix B) indicating less ease of grating and crumbliness decreased (Table 22, Appendix B) showing increasing tendency of cheese to stick to the grating surface. This could be attributed to the fact that as the extent of proteolysis (natural cheese age) increases, the degree of viscous behavior (and therefore stickiness, which increases work to grate) increases due to a weakened cheese matrix.

The effect of TSC concentration (2-3%) and the combined effect of both natural cheese age (0-82 d) and TSC concentration on crumbliness are shown in Figure 21. The contour plot shows the inverse relationship of natural cheese age and TSC concentration on crumbliness after 2.5 % level of ES. Similarly, after certain age, effect of TSC is more visible on crumbliness of PC.

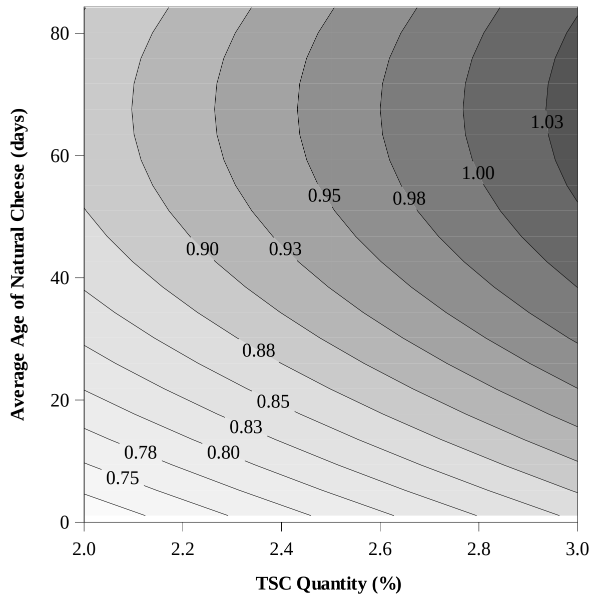


Figure 20. A contour plot of work to grate ($\boldsymbol{kg \cdot s}$) as affected by average natural cheese age (d) and TSC concentration (%).

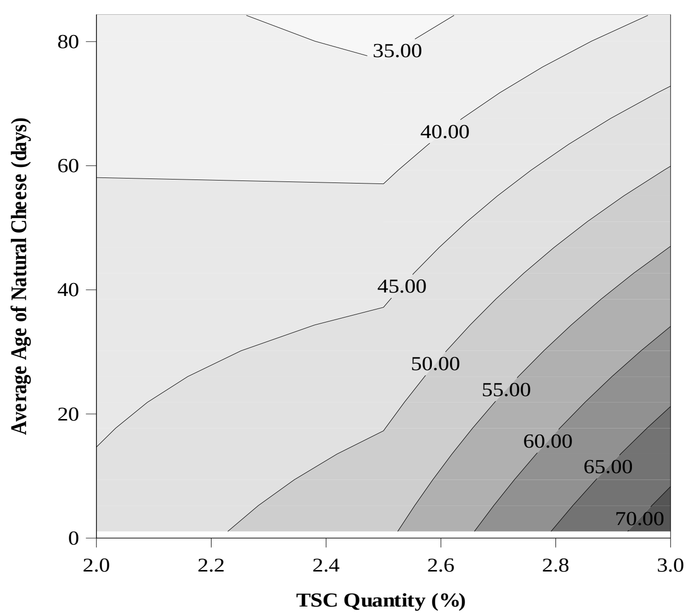


Figure 21. Crumbliness as affected by average natural cheese age (d) and TSC concentration (%)**Development of Shreddability Index and Correlation with Material Propertie**s

In this study our aim was to test whether wear behavior and other material characteristics, can be used to predict shreddability of PC. We, therefore, first developed a shreddability index using sieve data for possible correlation with these material properties.

Shreddability index analysis was performed by measuring cheese average shred length (mm), quantity of fines (length < 2.0 mm) (g) and adhesion (g) to equipment after applying a 2.5 kg weight to 175 $\pm$ 0.8 g cheese blocks during shredding (Table 14, Appendix B). The shreddability index based on the work from Apostolopoulos and Marshall (1994) was developed from these variables as shown in the Equation 3, where SI is shreddability index, A is length, B is adhesion, C is fines. The coefficients of these variables were obtained from the multiple regression statistical analysis. As expected, the effect of length was positively correlated with S ($p < 0.05$) and fines were negatively related to S ($p < 0.05$) (Figure 22). It was unexpected, however, that adhesion did not contribute negatively to the shreddability score as we originally hypothesized. Theoretically, the more adhesion there is to processing equipment, the worse the shreddability is in real practice (due to an increase in mass loss). The index developed by us has an inverse relationship with mass loss, meaning that higher SI values indicate poor shreddability. Nevertheless, our findings from this work were in agreement with the results from Banville et al. (2013) where length of shreds and adhesion exhibited positive relationships. This relationship is unknown and will need to be investigated in future work.

$$SI = 1.07\ A + 0.04\ B + -0.66\ C \qquad (3)$$

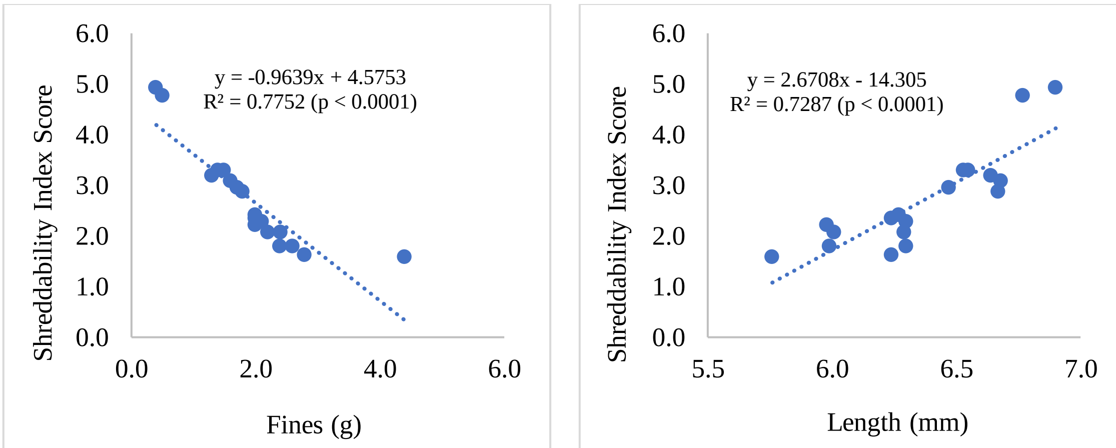


Figure 22. Shreddability index score as related to shredding fines (left) and length (right)

The shreddability index (SI) was then correlated with various material properties in order to choose best candidate for use in the predictive model. Pearson correlation matrix for all batches is shown in Table 13. The shreddability index had significant ($p < 0.01$) positive correlations with mass loss, work to grate, and a significant ($p < 0.01$) negative relationship with complex modulus G*. From TPA data we decided to use gumminess as a variable to be tested for its applicability in the predictive model for shreddability, because it takes into account both hardness and cohesiveness.

We believed that material or rheological descriptors alone could not predict the shreddability of cheese, therefore, we chose more than wear test and rheological variables for prediction. Three significant ($p < 0.01$) descriptors (Table 12) were chosen to predict the shreddability of cheese including mass loss (g) from wear data, work to grate ($g \cdot s$) from shreddability rig data, and G* (at 0.025% strain) from viscoelastic behavior.

Penetration Depth (mm) and rate of wear (mm/min.) from the wear test, and gumminess (N) from the texture profile analysis were also tested in the predictive model to provide

Table 12. Comparison of candidate models and selected model to predict shreddability index score

	R^2	**Adjusted R^2**	**p -value**	**^Cp**	**^PRESS**	**^RMSE**	**^BIC**	***Collinearity**
Full: Mass loss, *Penetration depth, *Rate of wear, *Work to grate, *G*, Gumminess	0.82	0.71	< 0.002	7	13.44	0.52	-8.54	Yes
M1: Mass loss, *Work to grate, *G*, Gumminess	0.61	0.48	< 0.01	5	18.97	0.69	-3.43	Yes
M2: Mass loss, *Penetration depth, *Rate of wear	0.78	0.74	< 0.001	4	6.43	0.50	-17.71	Yes
M3: Mass loss, Penetration depth	0.74	0.70	< 0.001	3	7.55	0.53	-17.13	No

^ Mallows' Cp-statistic (Cp), prediction sum of square (PRESS), root mean square error (RMSE), Bayesian's Information Criterion (BIC).

additional predictive models to which we could compare the accuracy of prediction to ensure the creation of the most accurate predictive model.

Predictive Model for Shreddability

Predictive models for shreddability were made on SAS/STAT 15.1 (SAS Institute Inc., Cary, NC) using regression analysis. The relationships between the treatment factors of natural cheese age (d), ES concentration (%) and the eight material (shown in Table 13) and selected rheological factors were explored by RSM. General trends of those relationships can be seen in the scatter plots in Figure 23 and also in Table 13. From each response, the final models were chosen based on RSM results and the comparison between the selected models with reduced F-test and the full RSM model. All selected models did not differ from the full RSM models and each selected model was tested for lack of fit and none demonstrated lack of fit (Table 12). Additionally, final analyses were performed using PROC RSREG, PROC REG, PROC FACTOR, PROC GLMSELECT in SAS/STAT 15.1. All final models were checked for normal and homogeneous error variance assumptions by model diagnostics. The assumptions were adequately held. Statistical significance is specified at 0.05 level throughout the analyses.

In comparison of all candidate models, the full model accounts for six model parameters (wear data, shreddability rig, viscoelastic behavior, and texture profile analysis) and explains the most variability ($R^2 = 0.82$) when predicting the shreddability index score. Other models M2 and M3 successively used a smaller number of variables, indicating stepwise regression models. The top three models demonstrate collinearity which is not a desirable property of a robust model. Additionally, for these three models

Table 13. Correlation matrix (Pearson correlation coefficients) from material and rheological tests

	Shreddability index	Mass Loss (g)	Penetration Depth at 12.04 m (mm)	Storage Modulus (G') at 1Hz	Work to Grate ($g \cdot s$)	Crumbliness (n peaks)	Complex Modulus (G*) (0.025% strain)	Hardness (g force)	Gumminess (N)
Shreddability index	1								
Mass Loss (g)	.686**	1							
Penetration Depth at 12.04m	.195	.676**	1						
Storage Modulus at 1Hz	.263	-.895**	-.728**	1					
Work to Grate ($g \cdot s$)	.642**	.687**	.608**	-.582*	1				
Crumbliness	-.367	-.683**	-.507*	.772**	-.301	1			
Complex Modulus (0.025% Strain)	-.706**	-.809**	-.652**	.720**	-.959**	.461	1		
Hardness (g force)	-.386	-.864**	-.733**	.768**	-.616**	.758**	.708**	1	
Gumminess (N)	-.400	-.863**	-.730**	.771**	-.643**	.753**	.732**	.997**	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

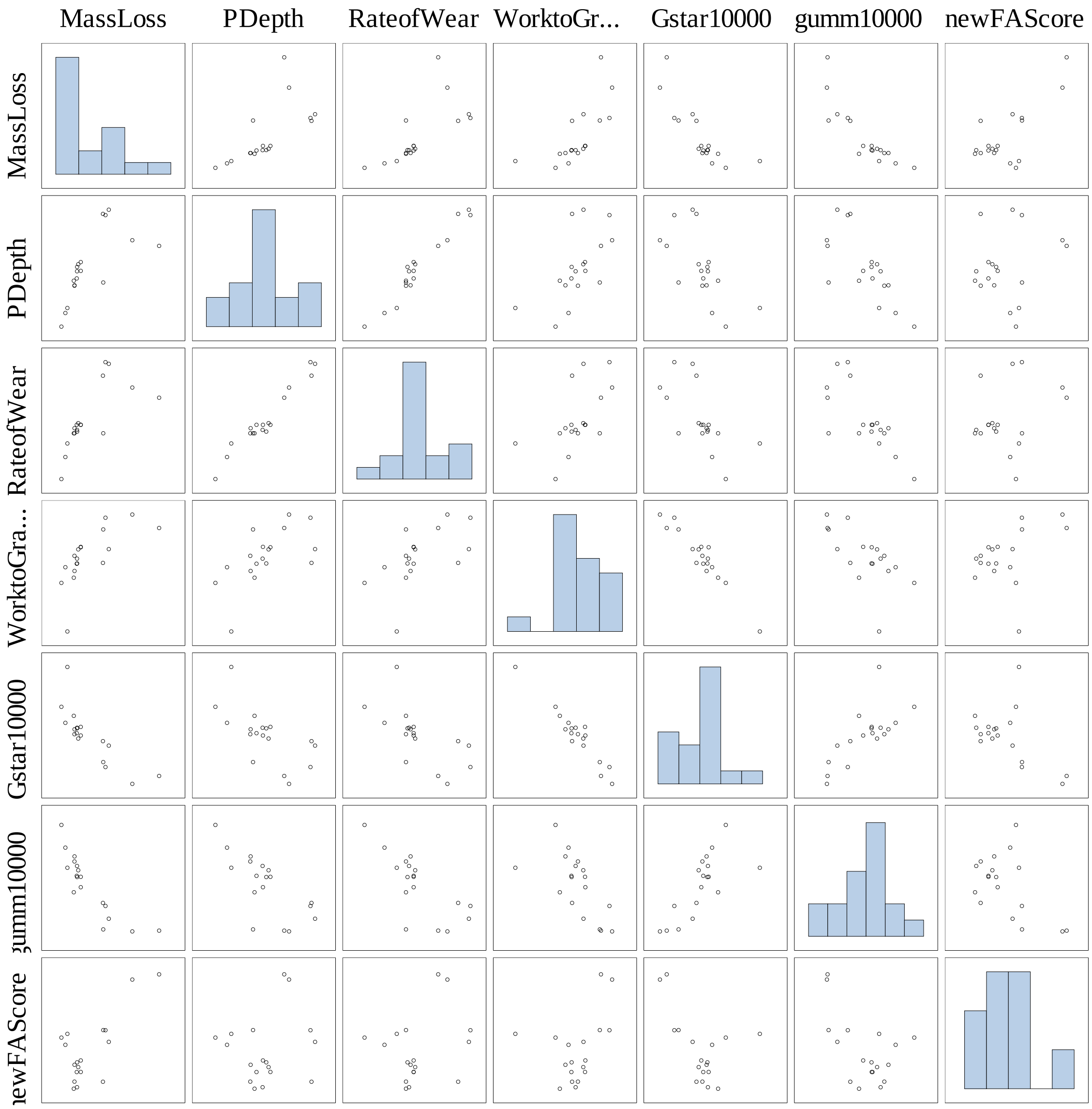


Figure 23. Scatter plots indicating the correlations between the predictors of interest

dropping all variables besides wear behavior led to small decrease in coefficient of determination (0.82-0.78). However, the M3 model appears to be robust and adequate for prediction, being able to account for 74% of the variation in modeling when trying to predict the shreddability index score. The benefit this model gives to this study is the reasonable degree of prediction ability ($R^2 = 0.74$) that a single test, specifically the wear test, can provide when trying to predict shreddability of model cheeses without any collinear effect. However, when compared to actual shredding data in a two-way ANOVA the results demonstrated that they were statistically different ($p > 0.05$).

$$SI = 3.22 + 0.83M - 2.41P \quad (4)$$

The wear behavior model is shown in Equation 4, where SI is the shreddability index score, M is the mass loss, and P is the penetration depth. Figure 24 shows correlation between actual and predicted shreddability index for the 18 PC samples

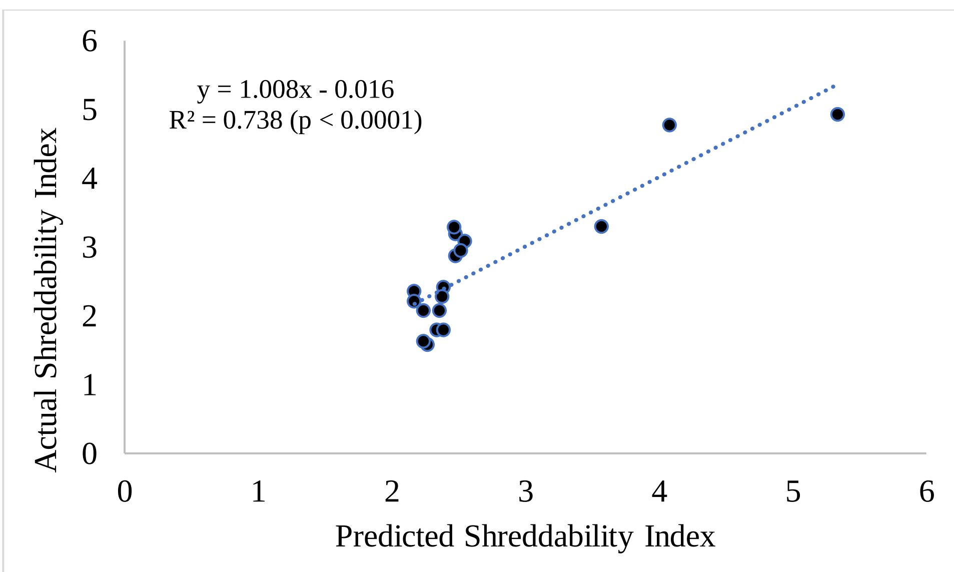


Figure 24. Correlation between predicted and actual shreddability index for 18 PC samples.

produced in this study. The results indicate reasonable agreement between actual and predicted values, however, further analysis is needed to improve correlation, as we believe that formulations that were too soft did not have consistent results for wear behavior. Furthermore, it is to be noted that our shreddability index represents an inverse relationship with mass loss. It would be worthwhile to revisit the data, particularly for the samples that had most distinctive differences in wear behavior, shreddability rig data and shreddability index. It will be useful for developing a better shreddability score. Since most of SI data points clustered and it would be better to identify variables that produce more differences in SI.

CONCLUSIONS

We successfully produced PC samples with a shreddability spectrum by changing the amount of aged cheese and varying emulsifying agent content. Material properties and structure of PC changes significantly with average age of PC formulation. Also, an increased emulsification occurred with older cheese, and with TSC above 2.5%. As observed in CLSM and TEM micrographs of PC size of fat globules decreased with increasing of both age of natural cheese and TSC concentration. Penetration depth and mass loss increased linearly ($p < 0.05$) as natural cheese age increased, indicating strong influence of extent of proteolysis. With a larger proportion of aged cheese in PC formulation, hardness (N) and gumminess (N) both decreased ($p < 0.05$), suggesting softer PC matrix due to decreased intact casein levels. With age of natural cheese, work needed to grate a block of PC increased, and crumbliness decreased, indicating loss of shredding good properties. Effect of TSC on these properties was not significant, as solid fat at 5°C, largely dominated the material characteristics. The findings of this study provide useful information to cheese manufacturers for optimizing formulation and processing conditions to attain desirable shredding properties with minimal material loss.

It was found that with increasing average age of natural cheese, PC samples became softer and more viscous as indicated by a decrease ($p < 0.05$) in G^* values (at 0.01% strain), gumminess, and hardness; leading to increase in penetration depth and mass loss. Age of natural cheese had a positive impact ($p < 0.05$) on the work to grate (extent of difficulty in grating) and negative impact on crumbliness. A good correlation found between SI and tribological, rheological, and material properties.

A shreddability index (SI) was developed from shred characteristics obtained after passing them through a mechanical sieve. Mechanical sieve data in terms of length of the shreds, quantity of fines and adhesion to the surface was used for developing the SI. Various multiple regression models were used on selective variables for testing their ability to predict the SI. A model having mass loss, penetration depth, rate of wear, work to grate, G*, and gumminess, as predicting variables, was found the best fit (R^2 = 0.82). However, the model with wear data only, was also able to predict of the shredding behavior with acceptable confidence (R^2 = 0.74). These results suggest potential use of wear data for predicting shreddability of cheese and for minimizing material losses at commercial scale operations.

FUTURE IDEAS

Future steps to prepare these models for use in the industry would include specification of prediction to not only include overall SI but to predict specific shreddability attributes (adhesion, production of fines, etc.), and adjustment of the current model for use with other cheese varieties, and validation of those models in commercial operations. Other variables to consider in these models might be the propensity of shreds to remain free flowing (and not mat together), shape and integrity of shreds, sensory analysis, proximate composition, and processing conditions. Lastly, accuracy of processing prediction will need to be evaluated.

Now that the ability of wear behavior to assist in the prediction of cheese shredding behavior has been investigated, it might also be helpful for the industry to investigate the ability of wear behavior to aid in the prediction of slicing operations of PC and other cheese varieties. It is hypothesized that these processing operations (shredding and slicing) are influenced by the same material and rheological behaviors (adhesion, friction, and fracture) and should therefore have similar processing behaviors, thus similar processing problems.

Future work should also include a more direct measurement and correlation between the fines produced during the wear test and fine production during shredding. Considering the fact that wear behavior and processing operations (namely friction, fracture, and adhesion behaviors) follow same mechanisms and therefore should be directly correlated.

Other research areas where this research could extend would be to investigate the effect of the milkfat on processing behaviors of cheese at lower temperatures. Currently, there is little research on the effect of the fat temperature, size, and distribution on processing behaviors under processing conditions (e.g. lower temperatures). This research could help the industry understand how to improve quality and reduce waste from processing.

Overall, this research gives insight into the wear behavior of cheese and provides the industry with another tool to improve shreddability prediction modeling. Extending this research would help to eliminate waste from commercial shredding operations and improve the quality of shredded cheese products. Future work with wear behavior can also be extended to other processing behaviors of cheese and other food products to improve quality and eliminate waste.

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APPENDIX A

Thermomix Process Cheese Make Sheet Mix Preparation and Cooking

1. Place all vacuum sealed cheese in the fridge the day before and ensure temperature has equilibrated to 5°C
2. Obtain all ingredients, formulation, and necessary equipment
    1. Equipment needed:

Thermomix (TMX) blender-cooker	TMX jars	TMX jar lids plus lid cap	loaf pans (8-1/2 x 4-1/2 x 2-1/2 inches)
plastic cheesecloth	rubber scraper	8-10” strong plastic heat-resistant spoon	medium bowl for weighing cheese
two small nonmetal bowls and transfer pipettes for weighing water	small plastic cups & spatulas or spoons for weighing	labeling tape and permanent markers	sturdy metal (only) thermometer
hot pads	heat-resistant plastic wrap	dish soap for cleaning	stiff bristle brush

3. Line a bread loaf pan with plastic cheesecloth in a cross pattern, one strip is lengthwise, and the other is widthwise, ensure that there is enough length to be able to fold over the top of the cheese after pouring
4. Insert the TMX jar into the machine
5. Using the medium bowl, weigh the natural cheese (remember to set aside 250 grams from each type of natural cheese for proximate analysis).
6. Add the weighed cheese to the TMX jar by breaking it up in pieces smaller than 1” in diameter (if needed)

7. Using the small plastic cups and spatulas/spoons, weigh the emulsifying salt(s), sodium chloride, and potassium sorbate and combine all into the small bowl
8. Weigh the deionized water and warm it in the 1000Watt microwave (at max power) for 1 min., the temperature should be approximately 65°C
9. If there are any residual ingredients in the small cups, use the warmed water to rinse them out, combine all water with salts and sorbate in the small bowl and stir until fully dissolved

Blending

10. Add the liquid mixture to the cheese in the TMX jar, add lid and cap, and begin blending all ingredients for 2.5 min. at 37.8°C and 1,100 RPM (speed setting 4)
11. After 2.5 min., invert the contents by hand (move the top mixture to the bottom and bottom to top)
12. Continue blending at 37.8°C and 3100 rpm (speed setting 6) for 2.5 more min. After blending is completed, the mixture will again be mixed by hand before beginning to cook

Processing/Cooking

13. Set the TMX time to 11 min. and cook temperature to 185°F (85°C)
14. Set stirring speed to 6 and begin cooking and agitation
15. Process for 4 min. then stop to hand mix ingredients to ensure even blending
16. Continue cooking until emulsification is complete, note the time

    Note: The mixture will continue to be agitated and cooked for 1 min. after the target emulsification has been reached. Target emulsification occurs after the cheese has melted, free fat is released, and then free fat is incorporated back into the matrix so that it is no longer visible. Furthermore, during cooking, there is a short time in which the cheese texture visibly changes, and an audible tax is put upon the motor. This time, along with the temperature are noted down below.

17. After cooking is complete, record the temperature and time
18. After cooking is finished, quickly scoop the cooked cheese into the loaf pan and fold over the flaps of the cheesecloth to cover the cheese
19. Using high heat foodservice film (Daily Chef 18 x 3000 FT) completely seal the pan - wrapping about 3 times.
20. Immediately place cheese in the walk-in fridge set at 4°C

*Formulation #*________________ *Date* __________________

Formulated weight & ingredient name - actual weight of ingredient

- ________ barrel (young) cheddar cheese - actual ________________
- ________ 3 mo. aged cheddar cheese - actual ________________
- ________ 6 mo. aged cheddar cheese - actual ________________
- ________ Deionized water - actual ________________
- ________ Trisodium citrate (%) - actual ________________
- ________ Potassium Sorbate - actual ________________
- ________ Salt (Sodium Chloride) - actual ________________

Cook Time and Temperature

Time began cooking ______________ Time began emulsifying ______________
Temp. at emulsification ______________ Temp. at finish ______________
Time finished agitating ______________ Time finished pouring ______________

Cooling of process cheese

Batch 1 cooling time:
Time put in fridge ______________ Fridge Temp ______________

APPENDIX B

Table 14. Shreddability score chart

Run	Age (d)	TSC (%)	Length (mm)	Adhesion (g)	Fines (g)	Score (S)
F1(B4)	1.00	2.00	6.64	4.70	1.30	3.19
F2(B8)	1.00	2.50	6.68	7.39	1.60	3.08
F3(B7)	1.00	3.00	6.67	7.08	1.79	2.87
F4(B1)	21.70	2.00	5.76	5.20	4.40	1.58
F5(B2)	21.70	2.50	6.29	7.79	2.40	2.07
F6(B9)	21.70	3.00	6.27	7.10	2.00	2.41
F7(B4)	82.30	2.00	6.30	6.21	2.60	1.79
F8(B6)	82.30	2.50	6.47	9.34	1.71	2.95
F9(B12)	82.30	3.00	6.53	9.15	1.39	3.29
F10(B3)	101.80	2.00	6.55	10.66	1.49	3.30
F11(B11)	101.80	2.50	6.90	16.39	0.40	4.93
F12(B5)	101.80	3.00	6.77	16.85	0.50	4.77
F5_R1	21.70	2.50	6.30	6.29	2.10	2.28
F5_R2	21.70	2.50	6.24	6.49	2.00	2.35
F5_R3	21.70	2.50	6.24	7.28	2.79	1.63
F6_R1	21.70	3.00	6.01	7.49	2.20	2.07
F6_F2	21.70	3.00	5.99	6.08	2.39	1.79
F6_R3	21.70	3.00	5.98	6.90	2.00	2.21

Table 15. Two-way ANOVA with 95% confidence interval for the effect of emulsifying salts on G' at 0.025% strain for all 12 process cheese formulations.

	df	SS	MS	F	Significance F
Regression	1	513777778	513777778	0.638	0.428
Residual	52	4.1868E+10	805148148		
Total	53	4.2381E+10			

Table 16. Two-way ANOVA with 95% confidence interval for the effect of natural cheese age (intact casein content) on G' at 0.025% strain for all 12 process cheese formulations.

	df	SS	MS	F	Significance F
Regression	1	2.2025E+10	2.2025E+10	56.2605338	7.88308E-10
Residual	52	2.0357E+10	391476746		
Total	53	4.2381E+10			

Table 17. Analysis of variance for rheological parameters of strain stiffening ratios at 1% strain between varying average natural cheese ages (d) and TSC (%).

Trisodium Citrate Concentration (%)	Average Natural Cheese Age (d)	Average Strain Stiffening Ratio (S)	ANOVA					
	1.0	0.63±.15		*DF*	*Sum of Squares*	*Mean Square*	*F-value*	*Significance F*
2.0	21.7	0.63±.15	Regression	1	0.24342	0.24342	18.4998	0.00155864
	82.3	0.43±.09	Residual	10	0.13158	0.01316		
	101.8	0.28±.07	Total	11	0.374999			
2.5	1.0	0.58±.12						
	21.7	0.31±.03	Regression	1	0.168375	0.16838	15.3755	0.00286097
	82.3	0.28±.01	Residual	10	0.109509	0.01095		
	101.8	0.20±.06	Total	11	0.277884			
3.0	1.0	0.49±.28						
	21.7	0.43±.19	Regression	1	0.030857	0.03086	1.00267	0.34027974
	82.3	0.48±.05	Residual	10	0.30775	0.03078		
	101.8	0.29±.14	Total	11	0.338607			

Table 18. Analysis of Variance on the effect of average natural cheese age on G* at 0.025% strain with varying levels of TSC A) 2.0% B) 2.5% C) 3.0%.

Trisodium Citrate Concentration (%)	Average Natural Cheese Age (d)	G* at 0.025% strain (kPa)	ANOVA					
	1	281.0±12.7		*DF*	*Sum of Squares*	*Mean Square*	*F-value*	*Significance F*
2	21.7	221.2±8.3	Regression	1	20190878.7	20190878.7	58.8	<0.0001
	82.3	190.2±14.4	Residual	10	3431662.3	343166.2		
	101.8	164.5±5.2	Total	11	23622541.1			
2.5	1	232.2±12.6						
	21.7	199.9±16.4	Regression	1	9696838.3	9696838.3	39.7	<0.0001
	82.3	184.7±7.3	Residual	10	2443603.1	244360.3		
	101.8	147.5 ±5.0	Total	11	12140441.3			
3	1	212.6±1.8						
	21.7	197.1±7.5	Regression	1	10120887.5	10120887.5	50.1	<0.0001
	82.3	158.4±3.2	Residual	10	2019553.8	201955.4		
	101.8	137.8±4.6	Total	11	12140441.3			

Table 19. Analysis of variance for the effect of average age of natural cheese (d) on PC hardness (g force) with varying levels of TSC A) 2.0% B) 2.5% C) 3.0%.

Trisodium Citrate Concentration (%)	Average Natural Cheese Age (d)	Average Hardness (g force)	ANOVA					
	1	4082.8±342.4		*DF*	*Sum of Squares*	*Mean Square*	*F-value*	*Significance F*
2	21.7	3109.6±164.6	Regression	1	12588455.7	12588455.7	80.4	<0.0001
	82.3	2694.5±97.9	Residual	18	2817726.7	156540.4		
	101.8	1714.0±287.7	Total	19	15406182.3			
2.5	1	5903.4±236.0						
	21.7	4052.6±257.4	Regression	1	56959388.6	56959388.6	286.8	<0.0001
	82.3	1978.3±69.7	Residual	18	3575382.6	198632.4		
	101.8	1589.6±137.7	Total	19	60534771.2			
3	1	5220.9±199.0						
	21.7	3568.3±238.8	Regression	1	32438772.3	32438772.3	135.9	<0.0001
	82.3	2724.8±216.4	Residual	18	4297962.0	238775.7		
	101.8	1527.5±76.6	Total	19	36736734.3			

Table 20. Analysis of variance for the effect of average age of natural cheese (d) on PC gumminess (N) with varying levels of TSC A) 2.0% B) 2.5% C) 3.0%.

Trisodium Citrate Concentration (%)	Average Natural Cheese Age (d)	Average Gumminess (g force)	ANOVA	*DF*	*Sum of Squares*	*Mean Square*	*F-value*	*Significance F*
	1	3344.6±269.7						
2	21.7	2506.1±106.2	Regression	1	9947738.7	9947738.7	87.7	<0.0001
	82.3	2141.6±140.8	Residual	18	2040674.8	113370.8		
	101.8	1235.8±171.4	Total	19	11988413.5			
	1	4809.5±154.1						
2.5	21.7	3068.2±166.4	Regression	1	37279212.4	37279212.4	203.1	<0.0001
	82.3	1601.1±55.3	Residual	18	3303210.2	183511.7		
	101.8	1193.6±135.9	Total	19	40582422.7			
	1	4033.2±93.7						
3	21.7	2679.0±114.4	Regression	1	19453022.5	19453022.5	133.5	<0.0001
	82.3	2037.7±164.4	Residual	18	2622934.1	145718.6		
	101.8	1166.8±69.0	Total	19	22075956.6			

Table 21. ANOVA for work to grate ($g \cdot s$) as affected by average natural cheese age (d) between A) 2.0% TSC B) 2.5% TSC C) 3.0% TSC

Trisodium Citrate Concentration (%)	Average Natural Cheese Age (d)	Average Work to Grate ($g \cdot s$)	ANOVA					
	1	6344.5±270.3		*DF*	*Sum of Squares*	*Mean Square*	*F-value*	*Significance F*
2	21.7	8277.1±308.3	Regression	1	29273546.1	29273546.1	60.4	<0.0001
	82.3	8809.5±166.4	Residual	18	8725441.8	484746.8		
	101.8	10012.9±741.6	Total	19	37998987.9			
2.5	1	8090.1±506.6						
	21.7	8779.8±110.1	Regression	1	9624381.8	9624381.8	66.8	<0.0001
	82.3	9304.7±364.8	Residual	18	2592540.0	144030.0		
	101.8	10064.4±206.3	Total	19	12216921.8			
3	1	8654.0±176.3						
	21.7	9386.0±172.4	Regression	1	11727703.0	11727703.0	220.8	<0.0001
	82.3	10433.3±235.3	Residual	18	956132.6	53118.5		
	101.8	10547.9±91.1	Total	19	12683835.6			

Table 22. ANOVA of crumbliness as affected by average natural cheese age (d) between 2.0, 2.5, and 3.0% TSC.

Trisodium Citrate Concentration (%)	Average Natural Cheese Age (d)	Average Crumbliness (n peaks)	ANOVA					
	1	47.6±4.7		*DF*	*Sum of Squares*	*Mean Square*	*F-value*	*Significance F*
2	21.7	44.6±5.4	Regression	1	1358.9	1358.9	57.1	<0.0001
	82.3	36.2±4.2	Residual	18	428.1	23.8		
	101.8	25.4±2.5	Total	19	1787.0			
2.5	1	57.8±6.6						
	21.7	52.8±3.1	Regression	1	2519.8	2519.8	149.6	<0.0001
	82.3	37.4±3.6	Residual	18	303.2	16.8		
	101.8	30.0±2.4	Total	19	2823.0			
3	1	71.0±8.0						
	21.7	65.2±4.4	Regression	1	4821.6	4821.6	182.9	<0.0001
	82.3	39.2±4.4	Residual	18	474.6	26.4		
	101.8	35.4±2.4	Total	19	5296.2			