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# Impact of Natural Cheese Composition on Proteolysis and its Effects on Process Cheese Functionality

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# IMPACT OF NATURAL CHEESE COMPOSITION ON PROTEOLYSIS AND ITS EFFECTS ON PROCESS CHEESE FUNCTIONALITY

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THESIS ACCEPTANCE PAGE

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I would like to dedicate this to my second father, Vern Landeen. I could not have started my journey in Dairy Science without him.

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# TABLE OF CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| $\alpha_{S1}$-CN | Alpha $S_1$ Casein |
| $\alpha_{S2}$-CN | Alpha $S_2$ Casein |
| α-LA | Alpha-lactalbumin |
| β-CN | Beta Casein |
| β-LG | Beta Lactoglobulin |
| κ-CN | Kappa Casein |
| AMF | Anhydrous Milk Fat |
| CEP | Cell-Envelope Proteinase |
| CFR | Code of Federal Regulation |
| CIC | Calculated Intact CN |
| CMP | Casein Macropeptide |
| CN | Casein |
| CO2 | Carbon Dioxide |
| FAA | Free Amino Acid |
| FDA | Food and Drug Administration |
| GDL | glucono-***δ***-lactone |
| HAV | Hot Apparent Viscosity |
| HCl | Hydrochloric Acid |
| HTST | High-Temperature Short Time |
| HVEM | Hot Viscosity At The End Of Manufacture |
| LAB | Lactic Acid Bacteria |
| lb. | Pound |

| | |
|---|---|
| LPL | Lipoprotein lipase |
| MNFS | Moisture In Nonfat Substrate |
| N | Nitrogen |
| NaCl | Sodium Chloride |
| NCN | Non-Casein Nitrogen |
| NPN | Non-Protein Nitrogen |
| NSLAB | Non-Starter Lactic Acid Bacteria |
| PC | Process Cheese |
| PCF | Process Cheese Food |
| Prt P | Lactocepin |
| PTA | Phosphotungstic Acid |
| RPM | Revolutions Per Minute |
| RVA | Rapid Visco Analyzer |
| S/M | Salt to Moisture |
| T5 | Time at 5000 cP |
| TCA | Trichloroacetic Acid |
| TN | Total Nitrogen |
| TPA | Texture Profile Analysis |
| TSC | Trisodium Citrate |
| VAM | Apparent Viscosity After Manufacture |
| WSF | Water Soluble fraction |
| Wt | Weight |

# LIST OF FIGURES

## Chapter II



## Chapter III

# LIST OF TABLES

# ABSTRACT

IMPACT OF NATURAL CHEESE COMPOSITION ON PROTEOLYSIS AND ITS EFFECTS ON PROCESS CHEESE FUNCTIONALITY

Natasha Laska Scherber

2022

Cheddar cheese is a commodity and a top-selling cheese in the United States. Enhanced flavors of cheddar cheese requires aging, which will allow proteolysis increase. Proteolysis is the most complex process that happens in cheese manufacturing and can be difficult to control because of environmental factors and compositional characteristics. The ripening process, which is the most timely and costly procedure in cheese manufacturing, can also be unpredictable when aspects, such as cheese composition, are changed. The coagulant is responsible for the first stage in proteolysis, which will produce large peptides. Small peptides are subsequentially produced during the second stage by starter bacteria and non-starter lactic acid bacteria.

Cheddar cheese was analyzed for composition and amount of proteolysis in this study. Analytical methods were used to determine composition including fat, moisture, salt, and protein. The level of proteolysis in the cheese was analyzed by the Kjeldahl fractionation method, and two different fractions were examined: soluble N percent of TN at pH 4.6, soluble N in 12% TCA as a percent of TN. Small peptides increased over time, using TCA separation, and large and small peptides increased over time, using pH 4.6 separation. Samples that only had a greater amount of fat had slower proteolysis. Over the length of the study the expected composition of Cheddar cheese had a slower

rate of proteolysis for TCA-soluble N as a % of TN, compared to Cheddar cheese that had higher low S/M, low salt and low pH. Cheese samples with a greater amount of fat and lower amount of moisture had an increased amount of TCA-soluble N as a % of TN. Samples that had a greater amount of fat, lower moisture and a pH below 5.06 or above 5.15 had the most amount of primary and secondary proteolysis.

Process cheese is affected by its ingredients and the natural cheese. A correlation from the current study was as proteolysis increased, the apparent viscosity and hardness decreased. The meltability increased when the proteolysis increased. Compositional factors of the natural cheese that contributed to the functionality of the PC were pH, S/M and ash minus salt.

# OBJECTIVE

In this study two objectives were evaluated for this study: the impact of cheese composition and storage on the ripening rate of Cheddar cheese and how the ripening rate impacts the functionality of process cheese that is made from cheddar cheese. To evaluate the first objective, cheese was gathered from three manufacturers in different locations in the United States. Each company selected 6-11 cheeses at 7-14 days for analysis. Samples were submitted with no information on processing conditions, starter culture type or dosage, coagulant type or dosage. Natural cheese samples were analyzed for composition using standard methods for composition. Composition for the samples were tested at 30 days and included: pH, Fat, Salt, Moisture, Ash, and Total Protein. At 15 day, 30 days, 60 days, 90 days and 120 days samples were tested for primary and secondary proteolysis.

The second objective was to determine how proteolysis impacts the functionality of process cheese made from cheddar cheese. For this study, process cheese was then made using a formula with Moisture 44%, Fat 30%, Protein 17%, Salt 2.5%, Sodium Citrate 3.0%, and contained 66.16-81% natural cheese. The mixture of ingredients was put in a Kitchen Aide mixer until ingredients were mixed into a homogenous paste. Rapid Visco Analyzer was used to heat the cheese and determined a hot apparent viscosity after manufacturing each sample. Once samples were melted, they were poured into a mold for further analysis. The molded, process cheese was used for a Schreiber melt test and a TPA. All samples were utilized in the process cheese formulations at 15 days, 30 days, 60 days, 120 days of ripening.

# CHAPTER I: REVIEW OF LITERATURE

## 1.Introduction- Natural Cheese

The International Dairy Federation (IDF, 1982) states that there are more than 500 types of cheese. The manufacture of cheese is dependent on milk (Guinee, Mulholland, Kelly, & Callaghan, 2007), which is composed of water, protein, fat, minerals, and trace elements (Varnam, 2001). Milk is preferred to be standardized to a certain protein-to-fat ratio and is essential for cheese composition, quality, yield and, manufacturing efficiency (Guinee, Mulholland, Kelly, & Callaghan, 2007). Milk used to make Cheddar cheese with a fat content of 3.6% and total protein of 3.2% has a protein-to-fat ratio of 1.125 and will produce a 10% manufacturing yield on average (Lucey & Kelly, 1994).

In the cheese-making process starter culture and a coagulant are added to the standardized milk which is then set, cut, cooked, drained, salted, pressed, and finally ripened (Kosikowski & Mistry, 1997). Cheese undergoes significant changes during ripening including changes to flavor, body and texture (Lawrence et al., 1986). Factors impacting ripening include cheese composition, storage conditions, starter bacteria used, type of coagulant used, non-starter bacteria, and ripening duration (Kosikowski & Mistry, 1997). The amount of moisture in the cheese plays a prominent role in the ripening rate, as water activity influences bacteria enumeration and lysis (Kosikowski & Mistry, 1997). As ripening occurs, the intact protein is hydrolyzed into peptides in a process called proteolysis (Fox et al., 2004). During ripening lipolysis and glycolysis also occur but are minor biochemical processes, relative to proteolysis (Fox et al., 2004). The coagulant

used is responsible for the first step in proteolysis and is the most critical enzyme in cheese processing (Delfour et al., 1965). The lysis of the starter bacteria will release enzymes, and Cell-enveloped proteinase (CEP, PrtP or Lactocepin) that will break down the proteins which will expose flavor complexes on the peptide chains and free amino acids (Fitzsimons et al., 1999). The non-starter lactic acid (NSLAB) bacteria will contribute to ripening via proteolytic enzymes from the NSLAB after the starter bacteria begin to die off (Fox et al., 2004). Time is also a significant factor for ripening (Fox et al., 2004). For example, a 30-day cheese will taste mild in comparison with a one-year-old cheese (Visser, 1993).

## 2. Cheese Components

### 2.1 Casein

The protein casein is composed of several different fractions: $\alpha_{S1,}$ $\alpha_{S2,}$ β-casein, and κ- casein (Heck et al., 2009). The casein micelle has the ratio in milk of 4:1:4:1 $\alpha_{S1,}$ $\alpha_{S2,}$ β-casein, and κ- casein (Heck et al., 2009). It is composed of a hydrophobic core, core-coat, internal structure, and submicelles (Fox et al., 2004), and proline residues.

Rennet precipitates the K-casein, which takes the protective shell of away from the calcium sensitive alpha s casein and beta casein (Kosikowski & Mistry, 1997). The hydrolysis of κ- casein happens at the Phe (105)-Met (106) bond. This releases the hydrophobic c-terminal of the k-casein and is also called glycomacropeptide (Fox, 2005).

Dejong (1976) found a correlation between the firmness of cheese and the quantity of intact $\alpha_{s1}$-casein. As the peptides cleave they will tie up the free ionic groups and free water (Lawrence et al., 1986). According to Lawrence et al. (1986), the casein network is substantially weakened when 20% of $\alpha_{s1}$—casein, which is a single bond, is cleaved at $Phe_{23}$-$Phe_{24}$ and will give a new peptide of $\alpha_{s1}$-I (f 24-199). As a result of the cleavage, the texture of the cheese changes in the first two weeks from a rubbery cheese to a more homogenous one. This is the first phase of texture development during ripening. The next texture changes, like a weak, pasty, short or brittle, are slower and happen over months, as opposed to days (Lawrence et al., 1986).

**2.2 Fat**

The CFR states that legal Cheddar cheese has to have a minimum milkfat being 50% by wt./solids and maximum moisture 39% by wt. (CFR Title 21. 22.2. Ch.1.B, Sec. 133.113) The cheese fat will contribute to aroma and flavor during ripening (Fox et al., 2004). The biological process of the breakdown of fats into free fatty acids is called Lipolysis (Fox et al., 2004). Fat will affect cheese texture, as it is dispersed evenly in the casein matrix in the cheese (Mistry, 2001). The fat will act as a filler in the matrix giving a creamier mouthfeel (Visser, 1991).

**2.3 Lactose**

Lactose is the sugar in milk. Lactose is a disaccharide composed of glucose and galactose. Only around 2% of the lactose in milk will be in the fresh

cheese curd, as most goes into the whey. The biological process that lactose contributes to is glycolysis, which will reduce the pH (Fox et al., 2004). The lactose in cheese curd will be fermented from lactose to lactic acid. The lactose will metabolize into L-lactic acid, D-lactate, acetate, propionate acid (Swiss Cheese), $CO_2$ (Swiss Cheese), citric acid, $NH_3$ (surface ripened cheese), and other volatile flavor components (Fox et al., 2004, Singh and Cadwallader, 2003).

**2.4 Water**

Cheddar cheese has a legal maximum of 39% moisture by weight. (CFR Title 21. 22.2. Ch.1.B, Sec. 133.113) Lawrence et al. (1986) state that cheese's texture during ripening is affected by its moisture content. The peptides that have been cleaved will then tie up the ionic groups and water from the new peptides (Lawrence et al., 1986). Cheeses that have low moisture will be harder as they age (Lawrence et al., 1986).

**2.5 Minerals, Vitamins, and Trace Elements**

**Minerals**

Minerals that can be found in cheese are salt, calcium (Ca), and phosphorus (P).

Calcium (Ca) and Phosphorus (P) are found in the milk and will be in the cheese. In an one ounce serving size, Cheddar cheese will have 15% of the daily value of calcium and 10% of the daily value of Phosphorus (Fox et al., 2004). In 100g of Cheddar cheese, there will be 40-50% of the daily P requirement, and 100% of the

daily Ca requirement (Fox,1993). Salt is added to cheddar cheese and is used to help preserve food, decrease microbial growth, control enzyme activity and to help the cheese curd release whey (Fox et al., 2004).

Vitamins

In Cheddar Cheese, the vitamins are from the milk. Vitamins that are found in cheese are Vitamin A, B1, B2, B6, B12, folic acid, and tocopherol (Fox et al., 2004). The fat soluble vitamins will be depended on the cheese fat content. Cheese will have 80-85% of Vitamin A that is from the milk (Fox et al., 2004).

Trace Elements

Milk will have trace elements, some of which will stay in the cheese curd. The elements that can be found in trace amounts in cheese are Zinc (Zn), Iron (Fe), Iodine (I), Manganese (Mn), Selenium (Se), Copper (Cu) and Aluminum (Al), (Fox et al., 2004).

Calcium

The calcium contributes to the cheese structure. The calcium that is in cheese can be associated with the casein. The associated calcium in the cheese will decrease as the cheese ages, in Cheddar it is typically 64% then goes down to 56%, and this is the cause for the structure of cheese weaking over time (Lucey et al., 2003).

Salt and Proteolysis

Salt will have an influence on cheese texture, and proteolysis. The contribution of salt will allow an increase of whey being expelled from the cheese curd. Salt will slow down the starter culture and control the acid production (Fox et al., 2004). The residual lactose and salt to moisture ratio will also affect the slowing of acid production and the rate of proteolysis (Thomas & Pearce, 1981). Proteolysis is strongly inhibited when salt is added to the curd at 20% salt for of alpha-s-1 and 10% salt for beta casein; most cheddar cheese the salt is added around 2% (Fox et al., 2004). Cheese that has a low level of salt will have more extensive proteolysis and a less firm texture (Fox,1993). Low amounts of salt in cheese will have greater chance to be bitter (McSweeney, 1997)

## 3. Natural Cheese Biochemical Reactions

The biochemical reactions that create texture, flavor and aroma are important for cheeses that are ripened. The three biochemical processes are glycolysis, lipolysis, and proteolysis. Each of the processes importance depends on the cheese variety (Fox et al., 2005).

### 3.1 Glycolysis

Glycolysis is the hydrolysis of a carbohydrate (Merriam-Webster, 2021). Cheddar cheese predominately uses the lactose, and turns it into lactic acid, but will also have other organic acids of Citric, Formic and Acidic acid (Mullin &

Emmons, 1997). Glycolysis will influence taste because it creates acid that contributes to aroma (McSweeney, 1997).

### 3.2 Lipolysis

Lipolysis is the breakdown of lipids into fatty acids and glycerol by the enzyme lipase (Random House Unabridged Dictionary, 2021). Indigenous milk lipoprotein lipase (LPL), starter lactic acid bacteria, non-starter lactic acid bacteria (NSLAB), and milk microflora are responsible for lipolysis in cheddar cheese (Hickey et al., 2007). Other than NSLAB, psychotropic bacteria and enterococci are other sources that contribute to lipolysis in cheddar cheese (Hickey et al., 2007). To consumers, any amount of free fatty acids in cheddar cheese that tastes rancid, is a negative trait (Fox, 2005).

### 3.3 Proteolysis

Proteolysis is the breakdown of proteins. Proteolysis in cheese can be described as a process in which a protein that will have an interaction with plasmin, chymosin, exogenous enzymes from NSLAB and starter bacteria (Upreti et al., 2006). The coagulation enzymes will clot the milk and initiate proteolysis (Hill, 2020). According to Rank et al. (1985), cheese ripening consists of the conversion of casein into lower-molecular-weight products.

Primary proteolysis consists of casein being broken down into large peptides (Varnam, 2001; Fox, 2005). In contrast, secondary proteolysis consists of large peptides being hydrolyzed into small peptides and free amino acids (Fox,

2005). Different cheeses types have different levels of proteolysis. Hard cheese has about 25–35% insoluble protein, or intact casein (Varnam, 2001). Soft cheese has around 80% of the proteins that have solubilized or broken down from proteolysis (Varnam, 2001).

Flavor does not come from the large peptides in cheese, or from intact casein (Boudreau, 1979). The short peptides and FAA are responsible for the flavor of cheese (Boudreau, 1979). Free amino acids directly contribute to the quality and flavor of cheese and are an indicator to how the flavor components will age in the cheese (McSweeny, 1997). The breakdown of amino acids happens via an enzymatic, non-enzymatic, or chemical pathways that can produce flavor components (Sousa et al., 2001). As previously mentioned, bitterness is a flavor that is not always perceived as positive. Some amino acids are bitter; the bitter peptides are hydrophobic or non-polar (McSweeney, 1997). The retention of chymosin, and the salt level in the cheese curd affects the degree of bitterness in the ripened cheese (Law et al., 1993).

Many different flavor components – both desirable and undesirable – derive from proteolysis. Flavor components in a ripened or aged cheese may, for example, be acidic, sulfuric, fruity, or bitter (Fox, 2005). The acidic flavor is caused by lowering the pH of cheese (Visser, 1993) A cheese that is considered acidic has a pH around 5.2 or lower (Visser, 1993). Peptidases will cleave one or two amino acids at a time, and this will enable exposure of amino acids that produce flavors (Fox, 2005). A fruity flavor in cheese is caused by exposure to aldehydes, similar to sulfur (Fox, 2005).

## 4. Ripening

### 4.1 Enzymes

There are many kinds of enzymes. Like puzzle pieces, these enzymes are designed to work in a specific area and will not work in another region. Two different types of peptidases are endopeptidases and exopeptidases. The endopeptidases will only cleave bond within a peptide, and exopeptidases will cleave bonds from the free N- terminal of the peptide (Fox et al., 2004). Milk contains indigenous enzymes, and cheesemaking involves the addition of a coagulant to the milk.

#### 4.1.1 Indigenous Milk Proteinases

Indigenous milk proteinase can be heat-labile, acidic, neutral, aminopeptidase, and heat-stable serine proteinases. Proteinase is an enzyme that will hydrolyze proteins into peptides (Fox, 2005). The main native proteinase is alkaline serine proteinase and is active at pH 7.5–8.0, and milk is at pH 6.5-7.0 (Grappin et al., 1985). This enzyme is thermo-stable and cleaves Arg-X and Lys-X in casein. It is indistinguishable from blood plasmin and travels into the milk from blood (Grappin et al., 1985).

Zymogen plasminogen is the primary source of plasmin present in milk; inactivated plasmins can be turned into activated plasmin that naturally occur in milk (Visser, 1993). Plasminogen, plasmin, and activators survive pasteurization, which will increase activity (Visser, 1993). Plasmins are responsible for cleaving, β-casein, $\gamma_3$-3 casein, and proteose peptone (Fox et al., 2005).

### 4.1.2 Cell-envelope proteinase (CEP)

Cell-envelope proteinase (CEP), a cell-wall-associated proteinase, comes from starter proteolytic enzymes (Fox, 2000). Fox states that LAB used as starter bacteria for cheese will have a range of proteolytic enzymes and substrates specific to CEP (Fox, 2000). These enzymes will then hydrolyze the casein into peptides and free amino acids (FAA).

### 4.1.3 Coagulants

Coagulants are the enzymes that start the cheesemaking process. The function of the milk clotting enzyme is to begin the coagulation of milk, which is a specific cleaving of hydrophilic casein macropeptide (CMP) away from the κ-casein (CN) located on the casein micelles (Vissier, 1993). The hydrolysis of casein micelle will allow for the cheesemaking process to continue and produce a solid product at the end of the process. Without them, there would be no cheese to be made.

Rennet is found in the fourth stomach of ruminant animals (Fox et al., 2004). The calf's rennet is how cheese was first discovered: milk was stored in a calf's stomach pouch and was taken on a journey (Fox et al., 2004). At the end of the trip, they had curdled milk – what is now called cheese (Fox et al., 2004). Rennet sources include calf, chymosin from ruminants, and pepsin from pigs and chicken, as well as vegetables (ficin, papain, bromelin), mold bacteria, and acid bacteria (Fox et al., 2004). Recombinant rennet which is commercially available, is a cloned gene that are expressed in microorganisms (Fox et al., 2004). The

microbial proteases are *Mucor pusillus, Mucor miehei,* and *Endothia parasitica* (Hill, 2020). The microbial rennet is GRAS approved.

The coagulant is the primary source of and initializes proteolysis (Fox et al., 2004). Coagulants help with flavor development and texture (Lawrence et al., 1986), and proper temperatures and optimum dosage of coagulant are needed during setting of the cheese curd (Hill, 2020).

## 4.2 Bacteria

### 4.2.1 Starter Culture

Starter bacteria are responsible for secondary proteolysis and contribute to flavor development. A major function of starter bacteria is when the bacteria cells die and undergo lysis, thereby releasing the intracellular enzymes (Fox, 2005). Non-starter single-strain bacteria contributes to flavor development in cheddar cheese, which is called an adjunct culture added for flavor (Law et al., 1976). The starter's proteinases, and residual rennet will help break down caseins (Farkye et al., 1990).

Cheese types are influenced by the starter culture and manufacturing process. Not all cheese starter bacteria will contribute to flavor development. Starter streptococci is one starter bacteria that does not contribute to flavor (Law et al., 1976). Heterofermentative lactic streptococci will produce body defects and undesirable flavors (Marth, 1974). Cheddar flavor can be obtained from *Lactobacillus casei* through proteolytic and lipolytic activities (Marth, 1974). The bacteria will free ammonia and break down amino acids. In some instances,

*Lactobacillus lactis, Lactobacillus plantarum,* and *Lactobacillus brevis* can contribute to flavor development, through lipolytic and proteolytic modes to hydrolyze amino acids and to free ammonia. However, some *Lactobacillus* strains can generate undesirable flavor in ripened cheese (Marth, 1974). Cheddar cheese can contain *Lactococcus lactis* subsp. *cremoris.* Seventy-one *Lactococcus lactis* subsp. *cremoris* strains have been identified in seven different mesophilic starter cultures (Bissonnette et al., 2000).

### 4.2.2 NSLAB

Non-starter lactic acid bacteria (NSLAB) can convert the lactose to glucose and galactose (Upreti et al., 2006). Non-starter bacteria, is usually lactobacillus, and will help with cheese flavor, and utilizing amino acids (Fox et al., 2004). NSLAB can be found in cheese milk; most are inactivated through pasteurization (Fitzsimons et al., 1999). NSLAB post pasteurization contamination can come from airborne flora living on equipment and ingredients, or it could be the thermoduric strains that survive pasteurization (Fitzsimons et al., 1999).

## 5. Analysis of Proteolysis

Proteolysis is the hydrolysis of peptides or proteins into soluble and simpler products (Merriam-Webster, 2021). Proteolysis can be measured by two techniques: crude fractionation and instrumental (Fox et al., 2004). The instrumental methods include chromatographic, electrophoretic, spectrometric, and sequencing methods (Fox et al.,

2004). Crude fractionation uses chemicals that will allow for selective precipitation with salts, acids, alcohols and filtration (Fox et al., 2004).

**6. Acceleration of Ripening**

Accelerated ripening has been studied with the aim to reduce costs. Many studies are working toward finding methods to shorten the time cheese needs to ripen without impacting the flavor and texture. Some of the accelerated ripening approaches have included increased ripening temperatures, enzyme-modified cheese, attenuated starters, application of exogenous enzymes, adjunct starter, and genetically engineered starters cultures (Fox et al, 2000). The reason for the accelerated ripening is the storage costs, which account for 6–8% of the cost of cheese in the UK (Law et al.,1993). Accelerated ripening can cause an unbalance in the flavor and increase the off-flavors because the chemical reactions within the cheese are not occurring equally and in a balanced way, since the temperature is elevated (Fox, 2000).

**7. Process Cheese**

Process cheese has existed since the early 20$^{th}$ century (Meyer, 1973) and was founded on the principle of increasing the shelf life of natural cheese. It was invented to find a way to sell cheese that was imperfect and hard to sell to the consumer (Zehren & Nusbaum, 1992). In 1911, process cheese was first created using Swiss cheese and sodium citrate. Walter Gerber and Fritz from Switzerland and working with Stettler of Gerber and Co. decided to combine melted Swiss cheese and the emulsifying salt, sodium citrate (Zehren & Nusbaum, 1992). Emulsifying salt will break the bond of calcium from

the paracasein network, which solubilizes the proteins, creating a modified casein network that forms a gel during cooling (Garimella et al., 2006). The process cheese makes a smooth and homogeneous product. J.L. Kraft developed his own method for process cheese, years after it was first created in Europe. (Zehren & Nusbaum, 1992).

The United States Code of Federal Regulations (CFR) uses the term "process cheese" to describe various categories that differ in maximum moisture content, minimum fat content, and minimum final pH (FDA, 2019). The CFR also specifies the number of ingredients used and the quantity of the ingredients (Kapoor and Metzger, 2008). Process cheese describes three different types of cheese: pasteurized process cheese spread, pasteurized process cheese food (PCF), and pasteurized process cheese (PC; FDA, 2019; Kapoor et al., 2004). The main ingredient in the manufacture of process cheese is natural cheese (Kapoor et al., 2007). Process cheese is made by mixing and blending natural cheese that varies in age with emulsifying salts and dairy and non-dairy ingredients (Zehren & Nusbaum, 1992). Heating and mixing are used to make a homogeneous product with an extended shelf life (Zehren & Nusbaum, 1992).

Process cheese has many functions like being used in restaurants for food dishes, in the grocery store for consumers to purchase, and ingredients for further food processing. Process cheese is significant because it has added shelf life stability that natural cheese does not have. Process cheese can be found in sauces, blocks, shreds, or slices and is an ingredient in many products. The functional properties for the end use of the process cheese are unique for each application (Kapoor et al., 2004).

The characteristics of the natural cheese used as an ingredient in process cheese influences process cheese characteristics (Kapoor et al., 2007). Garimella et al. (2006)

noted that process cheese's functional properties are affected by the ingredients used in the formulation, precisely the type of natural cheese, age of the natural cheese factors, amount of natural cheese and the type of emulsifying salts. Additionally, processing conditions, such as cooking temperatures, cooking time, and mixing speed used during manufacture, affect the final process cheese properties (Garimella et al., 2006). Kapoor et al. (2007) found that some natural cheese characteristics (Ca, P, and salt-to-moisture ratio [S/M]) have an important effect on process cheese's final chemical and functional properties. Zehren and Nusbaum (2000), Guinee (2002), and Lucey et al. (2003) similarly found that functional characteristics of natural cheeses used in process cheese manufacturing, such as meltability and textural properties, are significant factors that may alter the quality of the final product (Kapoor et al., 2004).

To address these inconsistencies in formulation and processing parameters, Kapoor et al. (2004) offered an analysis method through small-scale manufacturing that could be used to understand the functionality of process cheese by evaluating the influence of formulation parameters and processing conditions (Kapoor et al., 2004). An analysis system for process cheese through small-scale manufacturing would allow for an expedited and low-cost assessment of the influences of formulation and processing conditions on product components (Kapoor et al., 2004). The conclusions of Garimella et al. (2006) described the flow properties of process cheese immediately after manufacture, in addition to unmelted textural properties and flow properties of process cheese during melting and post melt, which supported the conclusions to Kapoor et al., 2004 study.

Olson et al. (1958) conducted research on the significance of natural cheese pH for process cheese properties. They manufactured Cheddar cheese using a modified

manufacturing protocol to generate two Cheddar cheese treatments that included different final pH levels. Then, the two Cheddar cheeses were used to create process cheeses at 10, 30, 60, 90, and 150 days of ripening. Using penetrometry, these were evaluated for unmelted texture and incorporated meltability via the tube melt test. It was concluded that even after adjusting the PC pH to 5.4 and 5.5 the PC that used the natural cheese with higher pH were less meltable and harder compared to the samples that had natural cheese with a normal pH (Olsen et al., 1958).

According to Acharaya and Mistry (2005), natural cheeses created from concentrated milk influence process cheese's functional and chemical properties (Kapoor et al., 2007). Archaraya and Misty indicated that natural cheese Ca and P, residual lactose, and S/M significantly affected the cheese's chemical properties, including the total P content, the pH, total Ca content, and the Calculated intact Casein (CIC) of the resulting processed cheese Thus, it is imperative to balance the moisture, fat, salt, and total protein of a PC and control the final Ca, P, pH, and intact CN to manufacture a process cheese with targeted functional effects (Kapoor et al., 2007). Biwas et al. (2015) conducted a study that used natural cheese characteristics, such as Ca and P content, lactose content, and S/M content and produced process cheese. These characteristics had a notable impact on the functionality of process cheese. The researchers concluded that process cheeses produced with different natural Cheddar cheeses, including high and low Ca, P, and S/M content, affected the cheese hardness and melting characteristics. Additionally, cheeses manufactured using natural Cheddar cheese with low Ca, P, and S/M had less hardness and more apparent viscosity than the samples with a high S/M, Ca, and P (Biswas et al., 2015).

Biswas claims that the study results can allow for the cheese and food science industry to manufacture process cheeses with modified Cheddar cheesemaking protocols to create optimal cheese with desired functional qualities that have been honed to precise standards. Biswas notes that it is possible to create process cheeses with lower hardness and higher melting characteristics by preparing the natural Cheddar cheese using a lower set and drain pH and lowering the salting amount (Biswas et al., 2015). Likewise, the firmer process cheese (with higher hardness) is created by preparing the natural Cheddar cheese with a higher set and drain pH and higher salting amount (Biswas et al., 2015).

## 8. Conclusion

An aged cheese has more flavor than a young cheese, and also takes longer to ripen (Fox et al., 2004). High-quality aged cheese is in high demand and is more complex due to the extended ripening in comparison to a mild cheese. There are many aspects to cheese like the age, composition and other factors that will affects the overall ripening process. Targeting specific factors before placing cheese in the cooler for an extended period could save a company money by providing a predictable cheese in a shorter time. Moreover, flavor change is primarily affected by proteolysis. Proteins also allow for a specific body, such as hard versus soft cheese. The major protein affected by the cheesemaking process is casein. Whey is drained off and does not affect overall protein. Furthermore, starter bacteria added to cheese and the natural flora in milk create flavor in a cheese. Some coagulants are added to cheese that will allow the breakdown of proteins to start.

Cheese manufacturing is a relatively brief process, but proteolysis and ripening take time. Ripening time can be anywhere from five days to many years. Indeed, ripening of

cheese is the most time-consuming process in cheesemaking. Controlling the ripening process will reduce the ripening time without repercussions like bitterness and other adverse side effects from accelerated ripening.

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# CHAPTER II: CHARACTERISTICS OF NATURAL CHEESE

## 1. Introduction

Cheesemaking originated from food preservation, of another way to use milk before it spoils. The preservation of cheese to ensure a longer shelf life and shelf stability came from adding salt, anaerobic conditions, dehydration, antimicrobial factors, and acid (Singh and Cadwallader, 2003). Natural Cheddar cheese was analyzed to understand the factors that affect proteolysis over time. Each cheese was sent to SDSU from three different manufacturers for analysis of composition and proteolysis over time. Each cheese that was sent to SDSU had unknown differences that the manufactures did not disclose. The samples were analyzed to observe the differences in the natural cheese composition and how proteolysis was affected, then proteolysis was analyzed to observe the effects on process cheese functionality. For all samples it is expected that samples will increase in proteolysis over time, and the functionality decreases over time.

## 2. Natural Cheese Composition

In the current study 27 different samples of cheese were analyzed. The study's composition factors included pH, fat, salt, moisture, S/M, ash, ash minus salt, and total protein. All samples were analyzed on day 30 for composition. The expected composition of Cheddar cheese is 37% moisture, 25% protein, 33% fat, ash 4%, pH 5.10, salt 1.80% and salt to moisture (S/M) 4.86 (Ayala-Bribiescaet et al. 2016). Companies 1 and 2 had legal Cheddar cheese as stated in the CFR, with minimum

milkfat being 50% by wt./solids and maximum moisture 39% by wt. (CFR Title 21. 22.2. Ch.1.B, Sec. 133.113). Company 3 had a legal reduced-fat Cheddar cheese as stated in CFR; reduced fat must have 25% less fat than Cheddar cheese (CFR Title 21.2, Part 101 D Sec. 101.62).

The pH of Cheddar cheese changes during ripening due to the fermentation of lactose to organic acids, which causes the pH to lower. Cheese will buffer, which causes the pH to resist change. The decrease in pH results from acids dissociating the $H^+$ ions (Upreti and Metzger, 2007). The phosphate in the paracasein network is entrapped, which results in the buffering (Upreti and Metzger, 2007). Studies have identified an overlap in different chemical species' contributions to pH buffering in cheese (Upreti et al., 2006a). The pH of cheese will influence all aspects of cheese quality: appearance, texture, and flavor (Upreti and Metzger, 2007).

### 3. Proteolysis

Cheese proteolysis is influenced by the protein-calcium-phosphate interactions (protein), chymosin, and plasmin, as well as the exogenous enzymes from starter bacteria and NSLAB (Upreti et al., 2006). The proteolytic enzymes with different specific substrates are found in LAB when used as a starter culture in making cheese (Fox et al., 2004). The LAB will hydrolyze casein, into peptides and FAA (Fox et al., 2004). The starter culture enzyme that facilitates proteolysis is proteinase (Fox et al., 2004). This is associated with the cell wall and is known as a CEP (Fox, 2000). Cell-envelope proteinases will make small peptides in cheese ripening, resulting from primary proteolysis (Fox, 2000). This primary proteolysis is from the plasmin in the

milk and the chymosin added to the cheese milk (Fox et al., 2004). The small peptides will be further broken down by the peptidases into amino acids (Fox et al., 2004).

Dejong (1976) identified a correlation between firmness of cheese and quantity of intact $\alpha_{s1}$-casein, the more intact $\alpha_{s1}$-casein, the firmer cheese will be. According to Lawrence et al. (1986), the casein network is substantially weakened "when the coagulant hydrolyzes only a single bond in about 20% of the α s1--casein at the $Phe_{23}$-$Phe_{24}$ position to give the peptide $\alpha_{s1}$-I (f 24-199)" (Lawrence et al., 1986). Cheese texture is drastically affected by cleavage done by the coagulant. In the first two weeks, cheese can be rubbery, and it will become more homogeneous after 14 days (Lawrence et al., 1986). The coagulant contributes to the first phase of cheese texture, and this happens in the first two weeks. After the first two weeks, ripening slows down, and texture and flavor result after months rather than a week or days (Lawrence et al., 1986).

The amount of free moisture in the cheese will affect the amount of hydrolysis that can occur. Available water will be tied up as peptides will cleave and utilize available water, meaning as cheese ages it becomes harder (Lawrence et al., 1986). Mature cheese's flavor and texture can be linked to proteolysis over time, helping to create flavor and create a firmer cheese (Law et al., 1993). The pattern or degree of proteolysis can be analyzed using chemical methods to indicate the cheese's quality and maturity (Law et al., 1993).

### 3.1 **Proteolysis Testing**

One method to index the proteolysis is by quantifying, separating, and finally characterizing the compounds—specifically nitrogenous—during cheese ripening. The instrumental technique uses chromatographic, electrophoretic, spectrometric, sequencing, HPLC chromatography or Polyacrylamide Gel Electrophoresis (PAGE) (O'Shea et al., 1996).

Another method that can be used for proteolysis testing is extracting and collecting the water-soluble fraction (WSF) and completing a Kjeldahl nitrogen test (Rank et al., 1985). When analyzing proteolysis, the first step is to separate the fractions or proteolysis products from the intact casein (Fox et al., 2004). This can be done using 4.6 pH buffers or water (Fox et al., 2004). Individual peptides can be isolated using methods that fractionate the peptidases into homogenous sub-fractions. Those techniques can be combined or single to give different degrees of separation (Fox et al., 2004). The WSF method is excellent for correlating ripening with cheese flavor because most nitrogenous compounds are soluble (O'Shea et al., 1996). Reduced fat cheeses measured by pH 4.6-soluble nitrogen were lower in primary proteolysis (Fenelon and Guinee, 2000).

## 4. Materials and Methods

### 4.1 Cheese Samples

The cheese was gathered from three manufacturers in different locations in the United States. Each company selected 6–11 cheeses for analysis and sent in samples to SDSU at 7-14 days of age with no information on processing conditions,

starter culture type or dosage, coagulant type or dosage, or ripening conditions. Once the natural cheese samples were obtained, they were stored in a cooler at 44°F.

Each sample was received in a 40 lb block, cut into 5 lb slabs, and vacuum sealed. The samples were placed back in their cardboard box for storage. Compositional analysis was performed at day 30. The slabs were then taken out of the cooler at the designated time points for proteolysis testing and process cheese analysis: 15 days, 30 days, 60 days, 90 days, and 120 days. Once the samples reached the specified time points, they were shredded and placed in the freezer until analysis.

## 4.2 Total Nitrogen

### 4.2.1 Sample Preparation

Cheese samples were shredded and measured for total nitrogen at 15, 30, 60, 90, and 120 days.

### 4.2.2 Kjeldahl Analysis

Total nitrogen was analyzed by the Kjeldahl Method. The cheese was weighed to 0.7000 g into a micro Kjeldahl flask. A catalyst tablet and 1 mL of 35% hydrogen peroxide were added along with 13 mL of sulfuric acid. The Foss heating block was set at 420 °C, and the samples run for 2 hours. Once the tubes cooled, each sample go through an automated Foss distillation unit, where 40% NaOH, water, and steam were added to the

Foss micro Kjeldahl flasks. An Erlenmeyer flask with Tashiro's indicator and 30 mL of 4% boric acid solution, collected the distillate. After the sample had been distilled, it was titrated with 0.1N $H_2SO4$ until the solution achieved a color change. (Wehr & Frank, 2012)

## 4.3 Non Casein Nitrogen (NCN)

### 4.3.1 Sharps Solution Preparation

The 136.2 Na acetate was weighed into a 250 mL beaker and quantitatively transferred into a 1 L volumetric flask with 200 mL of distilled water. A stir bar and stir plate were used for the 1 L volumetric flask. Then, 47.0 g NaCl and 11.78 g $CaCl_2$ were weighed consecutively into two different 50 mL beakers. Both 50 mL beakers were transferred quantitatively with 100 mL of distilled deionized water into the 1 L volumetric flask. Next, 57.5 mL of glacial acetic acid was measured into a 100 mL graduated cylinder and then added to the 1 L volumetric flask. Then, 300 mL of distilled deionized water was added to the flask. Once the salts were in the solution, the temperature was adjusted to 20 °C, and the solution was brought to the graduated line using distilled deionized water. The solution was then poured into a glass-stoppered reagent bottle and stored at room temperature. (Metzger, Barbano, Kindstedt, & Guo, 2001; Rudan, Barbano, Joseph Yun, & Kindstedt, 1999)

### 4.3.2 Sharp's Working Solution

The 250 mL of Sharp's stock solution was poured into a 250 mL graduated cylinder. The solution was transferred into a 1 L volumetric flask. Approximately 700 mL of deionized water was added to the solution in several intervals using a 250mL graduated cylinder. The solution was stirred and adjusted to 20 °C and was brought to the graduated line using deionized water. The solution was verified to be pH 4.6 and was stoppered and stored at room temperature. (Metzger, Barbano, Kindstedt, & Guo, 2001; Rudan, Barbano, Joseph Yun, & Kindstedt, 1999).

### 4.3.3 Sample Preparation

A 0.7000 g sample of cheese was weighed into a centrifuge tube and recorded to the fourth decimal place. Sharp's working solution was added to a centrifuge tube, and 20 mL was weighed to the fourth decimal place. The samples were blended with an Omni-mixer at 12 RPM for one minute so that the cheese was broken into a fine suspension in the liquid. The samples were then centrifuged for five minutes using the IEC HT Centrifuge with an RPM setting of 42. The extract was filtered with a Whatman #2 filter paper. A second extraction was completed by adding 20 mL of Sharp's working solution into the centrifuge tube and subsequently weighed. Samples were blended using an Omni-mixer at 12 RPM for one minute. The cheese was broken into a fine suspension in the liquid. The samples were centrifuged for five minutes and filtered through the same

filter paper from the first extraction. The filtrate was then collected and placed in a container for Kjeldahl analysis. The Kjeldahl analysis used 20 mL of filtrate. (Metzger, Barbano, Kindstedt, & Guo, 2001; Rudan, Barbano, Joseph Yun, & Kindstedt, 1999).

Percent Nitrogen Equation

%N = (1.4007) x (mL HCL sample - mL HCl blank) x (N of HCl) Sample wt. of extraction solution x (wt. of cheese + solution) wt. of cheese.

## 4.4 Non Protein Nitrogen (NPN)

### 4.4.1 TCA (24% w/v) Solution Preparation

Four hundred eighty grams of trichloroacetic acid (TCA) was weighed into a 500 mL glass container and quantitatively transferred to a 2000 mL volumetric flask. The flask was filled with deionized water to the graduated line. The solution was poured into a glass-stoppered reagent bottle at room temperature. (Metzger, Barbano, Kindstedt, & Guo, 2001; Rudan, Barbano, Joseph Yun, & Kindstedt, 1999).

### 4.4.2 12% TCA Solution Preparation

Five hundred mL of 24% TCA solution was poured into a graduated cylinder and transferred to a 1 L volumetric flask. The solution was filled to the graduated line, then stoppered and stored in a reagent

bottle at room temperature (Metzger, Barbano, Kindstedt, & Guo, 2001) (Rudan, Barbano, Joseph Yun, & Kindstedt, 1999).

### 4.4.3 Sample Preparation

The centrifuge tube had 1.4000 g of cheese added and weighted to the fourth decimal place. Twenty milliliters of 12% TCA solution were added to the centrifuge tube and weighed to the fourth decimal place. The samples were blended with an Omni-mixer at 12 RPM for one minute, breaking the cheese into a fine suspension in the liquid. The samples were then centrifuged for five minutes using the IEC HT Centrifuge with an RPM setting of 42. The extract was filtered with a Whatman #2 filter paper. A second extraction was completed by adding 20 mL of 12% TCA solution into the centrifuge tube and was subsequently weighed. The samples were then blended into a fine suspension using an Omni-mixer at 12 RPM for 1 minute. The samples were centrifuged for five minutes and filtered through the same filter paper from the first extraction. The filtrate was collected and placed in a container for Kjeldahl analysis. Twenty milliliters of the filtrate were used for Kjeldahl analysis. (Metzger, Barbano, Kindstedt, & Guo, 2001; Rudan, Barbano, Joseph Yun, & Kindstedt, 1999).

Percent Nitrogen Equation

%N = (1.4007) x (mL HCL sample - mL HCl blank) x (N of HCl) Sample wt. of extraction solution x (wt. of cheese + solution) wt. of cheese.

## 4.5 Salt

### 4.5.1 Sample Preparation

Five grams of finely ground cheese was weighed to the fourth decimal place into a clean and dry blender cup. The moisture of the cheese was determined, and the predetermined moisture was used to identify the amount of water to add to the cheese sample. (Corning 926 Automated Chloride Salt Titrator)

Amount of Water in 5 g of Cheese

(Moisture of cheese) x (g of cheese) = (Mass of water in 5 g of cheese)

Determined Amount of Water in Cheese

(Mass of water to add to cheese) = (100) - (Mass of water in 5 g of cheese)

The determined amount of water was added to the cheese in the blender cup. The mixture was blended using an Osterizer® blender on LIQUIFY for 30 seconds. The cup was removed from the blender and set down for 5–10 minutes until the solids settled to the cup's bottom. The fatty layer from the cup was then removed, and the solution was filtered

into a funnel using a #4 Whatman filter paper. The filtrate seeped into a 250 mL Erlenmeyer flask. (Corning 926 Automated Chloride Salt Titrator)

### 4.5.2 Analysis

The chloride analyzer was powered on, and five minutes passed to allow the machine to warm up. Corning® acid buffer was added to the graduated mark on the 14 mL sample beaker. The sample was then conditioned by pressing the CONDITION button, and 250 µL of Corning® standard solution (200 mg/L) was pipetted into the 14 mL beaker. The TITRATE button was then pressed, and the reading was in 100 mg/L and could be ±3 mg/L. Once the solution was titrated, 250 µL of cheese solution was pipetted, the TITRATE button was pressed, and the reading was recorded. The sample was completed in duplicates. When the CHANGE REAGENTS light turned on, two more samples could be completed. The beaker was then rinsed and dried. The beaker was filled with Corning® acid buffer with chloride to the graduated line. The sample was then conditioned by pressing the CONDITION button, and 250 µL of Corning® standard solution (200 mg/L) was pipetted into the 14 mL beaker. The TITRATE button was then pressed, and the reading was 100 mg/L and could be ±3 mg/L. Sample titrations of the cheese solutions continued until the last sample was analyzed. The electrodes were rinsed with DI water and blotted with a Kimwipe®. (Corning 926 Automated Chloride Salt Titrator)

Percent Salt Equation

Percent of salt in sample = digital reading in mg% x 4

## 4.6 Fat

### 4.6.1 Preparation

Mojonnier fat extraction flasks and aluminum weighing dishes were cleaned and dried. The aluminum weighing dishes were weighed to the fourth decimal place and stored in a desiccator until needed. All the surfaces where the aluminum weighing dishes would sit were cleaned so no extraneous material adhered to the surface. The corks were soaked in water for 1–2 hours before use. (Wehr & Frank, 2012)

### 4.6.2 Analysis

One gram of shredded cheese was weighed into the extraction flask and recorded to the fourth decimal place, and 9 mL of water at 60 °C was added to the flask with the cheese. The stopper was placed, and the flask was shaken until the cheese was mainly dissolved. Three milliliters of ammonium hydroxide were added, stoppered, and shaken until all the cheese was dissolved. Three drops of phenolphthalein indicator were added to the flask; 13 mL of ethyl alcohol was added, stoppered, and shaken for one minute; 25 mL of ethyl ether was added, stoppered, and shaken for one minute; and 25 mL petroleum ether was added, stoppered, and shaken for one minute. The flasks were centrifuged at 600 RPM for 30

seconds to allow for the separation of aqueous and ether phases. The ether layer was then decanted into a pre-weighed aluminum dish on a hotplate. Once the ether layer in the dishes had evaporated, the dishes were placed in the desiccator. A second extraction was required using the same method but with 5 mL of ethyl alcohol, 15 mL of ethyl ether, and 15 mL of petroleum ether. The flask was centrifuged under the same conditions and decanted into the aluminum dishes. Then, a third extraction was necessary using 5 mL of ethyl alcohol, 15 mL of ethyl ether, and 15 mL of petroleum ether. The flasks were centrifuged, decanted into the aluminum dishes, and evaporated. Once the aluminum dishes' ether solutions finished evaporating, the dishes were placed in a vacuum oven at 70 °C under 50.8 cm of vacuum for 7 minutes. The dishes were then removed and cooled. Finally, the cooled dishes were weighed and recorded to the fourth decimal place. (Wehr & Frank, 2012)

Percent Fat Equation

Fat (% weight) = {(weight of dish with extracted fat) - (weight of empty dish)} x 100 weight of cheese sample

**4.7 Moisture**

Aluminum dishes were labeled and placed in the oven at 100 °C for two hours, then cooled in a desiccator for 20 minutes. Two grams of cheese was added to the aluminum dishes and weighed and recorded to the fourth decimal place.

The aluminum dishes were placed in the oven at 100 °C for 24 hours and then placed in a desiccator to cool for 20 minutes. The final weights were then recorded and calculated. (Wehr & Frank, 2012)

Total Solids Equation

Total Solid (%) = (weight of dried sample / weight of original cheese) x 100

Total Moisture Equation

Total Moisture (%) = 100 - Total Solids (%)

## 4.8 Ash

### 4.8.1 Prepare Crucibles

The crucibles were fired until red and then cooled. They were submerged and placed in aqua regia for several hours, then removed and rinsed with water. The crucibles were then placed in the oven at 100 °C for drying. Once dried, they were placed in the muffler furnace until they were a dull red. Finally, we cooled them by placing them in a desiccator until use. (Wehr & Frank, 2012)

### 4.8.2 Analyze

Ten grams of cheese was weighed into the prepared and pre-weighed crucibles. The crucibles were recorded to the fourth decimal place. Crucibles were placed into an oven at 100 °C until the sample dried. The samples were then placed on a heating plate in a fume hood to slowly

carbonize. The crucibles were placed into the muffler furnace at 550 °C overnight, then were removed from the furnace, cooled in a desiccator, and weighed to the fourth decimal place. (Wehr & Frank, 2012)

Percent of Ash Equation

Ash (%) = (weight of residue x 100) / (weight of sample)

### 4.9 pH

The readings were taken using Corning pH/ion meter 340, (Corning, Inc., Corning, NY). Calibration of the machine was performed first, and new buffer solutions of pH 4, pH 7, and pH 10 were used. The probe was rinsed with deionized water and wiped with a KimWipe® between samples and buffers. For each cheese sample, a rugged non-glass body conical tip and silicon ISFET sensor was placed into the cheese with the temperature probe. The reading was displayed and recorded. The probe was rinsed and dried between each sample. (Wehr & Frank, 2012)

## 5. Results and Discussion

### 5.1 Table 1. Natural Cheese Composition Company 1

The composition of the 11 samples from Company 1 had the following ranges as shown in table 1: pH 5.01–5.22, fat 30.22–37.79, salt 1.50–1.83, moisture 32.89–39.32, S/M 4.01–5.47, ash minus salt 1.88–2.15, ash 3.51–3.87,

and total protein 22.26–24.43. Each sample came from a different 40 lb. block sent to South Dakota State University (SDSU) for evaluation.

We observed that if the sample had greater moisture, it then had a lower amount of fat. Ash and salt were directly correlated. In addition, Sample 8 had a low fat content and would not be considered a legal full-fat Cheddar cheese. Sample 9 had moisture that exceeded the moisture maximum of legal Cheddar cheese.

**5.2 Table 2. Natural Cheese Composition Company 2**

The 10 samples delivered to SDSU from Company 2 had the following ranges as shown in table 2: pH 5.0–5.20, fat 34.93–39.09, salt 0.86–1.73, moisture 34.38–36.68, S/M 2.24–5.10, salt-free ash 1.98–2.32, ash 3.0–3.98, and total protein 22.43–25.44.

The analysis of composition from Company 2 revealed low salt on Sample 1, at 0.86%. Ash and salt directly correlate in cheese. Notably, low salt will cause low S/M ratio and ash. The low salt of Sample 1 had a higher moisture content at 36.68%.

**5.3 Table 3. Natural Cheese Composition Company 3**

The six samples for Company 3 had the following ranges as shown in table 3: pH 4.9, fat 20.95–22.02, salt 1.40, moisture 43.20–45.67 S/M 3.04–3.69, salt-free ash 1.97–2.19, ash 3.37–3.74, and total protein 22.58–27.21.

The samples from this company were of legal reduced-fat Cheddar cheese. The compositions were all highly similar. Sample 4 had the lowest fat and highest ash, Sample 3 had the lowest moisture and highest protein, Sample 5 had the highest fat, and Sample 1 had the lowest protein and a lower salt content.

The samples from Company 1 and 2 were cheddar cheese and company 3's product was legal reduced fat. Company 1 and 2 had similar pH ranges for the samples. Company 3 all samples had a pH of 4.9. Company 2 had a numerically higher fat range 34.93% - 39.09% vs Company 1 having a wider fat range (30.22% - 37.73%). Company 3 had a small range for fat 20.95% – 22.02%. Company 1 had a numerically higher salt level 1.50% -1.83%, and company 2 had a numerically wider range of salt levels 0.86% - 1.78%. Company 3 salt range of 1.39% - 1.65%. Company 1 had a higher amount of moisture and a range of 32.89% - 39.32%. Company 2 had a moisture range of 34.38% - 36.68% was lower in moisture compared to company 1. Company 3 had moistures for a reduced fat cheese and a range of 54.33% – 56.80%. Company 2 had the lowest sample of S/M being 2.24. Company 1 had a higher ratios of S/M (4.01 – 5.47). Company 3 had a range of S/M of 2.55- 2.98. Company 1 had a lower amount of protein for the samples compared to company 2. Company 1 had a range of protein 22.26% - 24.75%. Company 2 had a higher amount of protein that ranged from 23.46% - 25.14%. Company 3 had a protein range of 19.73% - 23.01%.

**5.4 Figure 1. Company 1 pH 4.6 Soluble N**

As shown in Figure 1, the pH 4.6-soluble N of all samples increased over the 120-day experiment. Sample 4 had the lowest amount of pH 4.6-soluble N throughout the experiment. On day 15, Sample 4 had a pH 4.6-soluble N of 2.28%, whereas sample 8 had 3.51%. At day 30 the pH 4.6-soluble N was highest in Sample 10 (4.69%), and the lowest in Sample 4 (2.41%). At day 60 Sample 1 had the highest level of pH 4.6 Soluble N (5.88%) and the lowest in Sample 4 (3.6%). At day 90 Sample 4 had the lowest pH 4.6-soluble N (4.5%) and Sample 1 was highest level (6.75%). At day 120 Sample 4 (5.24%) had the lowest level of 4.6 soluble N and the highest in Sample 2 (7.39%).

Sample 10 had a lower pH of 5.02 at day 30, which would have facilitated proteolysis (Upreti and Metzger 2007). The cheese's buffering capacity had already decreased, which means the LAB and NSLAB had been active in breaking down proteins (Upreti and Metzger 2007). Cheese pH will change throughout cheese ripening since its buffering capacity will shift with an increased organic acid, mainly lactic acid (Upreti and Metzger 2007).

Sample 4 had the lowest amount of proteolysis over 120 days. This was caused by its lower moisture content of 32.89%, and a greater salt: moisture ratio of (5.47). Sample 1 and 8, had a greater rate of proteolysis, and was similar to an expected composition. Typical Cheddar cheese has moisture of 37%, protein 25%, fat 33%, ash 4%, pH 5.10, salt 1.80% and salt to moisture (S/M) 4.86 (Ayala-Bribiesca et al., 2016). Having an expected composition could have been the reason for the high amount of proteolysis observed.

**5.5 Figure 2. Company 2 pH 4.6 Soluble N**

In Company 2 Figure 2 pH 4.6-soluble N, all samples increased over 120 days. On day 30, Sample 5 had the highest pH 4.6-soluble N at 3.59%, and Sample 7 had the lowest at 2.58%. On day 60, Samples 1 and 6 had the lowest proteolysis for company 2 3.56% and 3.44%. Samples 5 and 10 had the highest 4.6 pH-soluble N at 4.64% and 4.68%, respectively. On day 90, the samples were all similar and ranged from 4.3% to 5.0%. On day 120, Samples 8 and 4 had the lowest pH 4.6-soluble N at 5.62% and 5.64%, and Samples 9 and 10 had the highest amount of pH 4.6-soluble N at 7.29 and 7.23%, respectively.

Sample 5 had lowest moisture compared to the rest of the sample set at 34.78% and a higher fat at 35.38%, and a lower salt at 1.64%. Sample 7 had an expected composition, which may be related to the slower start to the proteolysis. Sample 1 had a low salt level at 0.86%, a lower protein level at 23.46%, and a low S/M at 2.24. Sample 6 had lower moisture, lower salt, lower S/M and greater fat. The moisture was 35.43%, salt was 1.63%, S/M was 4.60, and fat was 35.83%. Sample 5 had lower moisture at 34.78% and higher fat at 35.38%. Sample 10 was an expected composition of Cheddar cheese. As previously mentioned, typical Cheddar cheese has moisture of 37%, protein 25%, fat 33%, ash 4%, pH 5.10, salt 1.80% and salt to moisture (S/M) 4.86 (Ayala-Bribiesca et al., 2016). Samples 4 and 8 had a higher S/M of 5.10, and 5.03. Sample 9 had a lower moisture of 34.51%, lower salt at 1.58%, lower S/M at 4.58, and the highest fat at 39.09%.

**5.6 Figure 3. Company 3 pH 4.6-Soluble N**

In all of Company 3's samples, pH 4.6-soluble N increased over the 120 days. On day 60, Sample 2 had the lowest at 4.39%, and Sample 4 had the largest amount of pH 4.6-soluble N at 5.21%. On day 90, the samples were similar; the lowest amount of pH 4.6-soluble N was in Sample 6 at 5.42% and the highest was in Sample 3 at 5.93%. On day 120, Sample 1 had the highest amount of pH 4.6-soluble N at 6.21%, and Sample 5 had the lowest amount at 5.68%

Samples 1 had the lowest amount of total protein among the sample set, which could have caused the increase in primary proteolysis at 60 days. Sample 5 had the highest amount of fat (22.02%). All the samples were a legal reduced-fat Cheddar cheese. A legal reduced-fat Cheddar cheese, according to the CFR, must have 25% less fat than Cheddar cheese (CFR Title 21.2, Part 101 D Sec. 101.62).

Company 1, 2 and 3 all samples started out similar for pH 4.6-Soluble N, and the starting range was 2.22% - 3.52%. Company 3 did not have 30-day samples available. Company 1 samples had a higher rate of proteolysis at 30 days compared to company 2. Company 1 and 3 had a higher range of proteolysis for pH 4.6-Soluble N at 60 days, 3.60% - 5.88%. Company 2 has a proteolysis range at 60 days 3.44% - 4.68%. Company 1, 2 and 3 had a rate of 4.6 Soluble N at 90 days at 4.37% - 6.09%. Company 3 had the lowest amount of pH 4.6-Soluble N at 120 days (5.68% - 6.21%) and company 1 and 2 had similar ranges (5.24% - 7.33%).

**5.7 Figure 4. Company 1 pH 4.6-Soluble N as a % of TN**

In all of Company 1's samples, pH 4.6-soluble N as a % of TN increased over 120 days. Sample 4 had the lowest pH 4.6-soluble N as a % of TN throughout the study's entirety. Sample 2's pH 4.6-soluble N as a % of TN was also low on day 30 at 12.75%. Sample 3 was lower on day 60 at 16.24% and on day 90 at 19.61%. At 120 days, Sample 4 was the lowest at 21.45%, and Samples 5, 6, and 7 were also low in pH 4.6-soluble N as a % of TN at 24.81%, 24.13%, and 23.19%. On day 15, the samples' range was 9.33% in Sample 4 to 15.35% in Sample 8. Day 30's range was 9.86% in Sample 4 and 20.10% in Sample 10. Sample 1 on day 60 was 26.21%. Day 90 Sample 1 was 30.09%. Sample 1 day 120 was 32.68%.

Sample 4 had the lowest amount of proteolysis and the lowest moisture of the sample set. Cheese with low moisture content throughout ripening becomes harder the longer the cheese ages (Lawrence et al., 1986). The amount of water or moisture available in cheese becomes unavailable as ripening occurs due to the proteins' hydrolysis. The new peptides will tie up any of the available water molecules (Lawrence et al., 1986). Since there is less water available, it can slow the proteolysis rate.

Sample 2 had the highest amount of fat for company 1 (37.79%), and a lower S/M (4.60). Sample 3 had the lowest amount of total protein (22.29%). Sample 5 had a lower pH of 5.01, a higher fat (36.30%), lower moisture (34.41%), lower salt (1.53%) and lower S/M (4.45). Sample 6 had a higher pH at

5.22, higher fat at 36.44%, and lower moisture at 34.03%. Sample 7 had a higher fat (37.73%), lower moisture (32.91%), and a higher S/M (5.17).

**5.8 Figure 5. Company 2 pH 4.6-Soluble N as a % of TN**

In Company 2, pH 4.6-soluble N as a % of TN increased over the 120 days. On day 30, Sample 5 has the highest pH 4.6-soluble N as a % of TN at 15.11%. On day 60, Samples 5 and 10 had the highest amount of pH 4.6-soluble N as a % of TN at 19.53% and 18.72%. On day 90, Sample 3 had the lowest pH 4.6-soluble N as a % of TN at 16.90%. On day 120, Samples 7, 9, and 10 had the highest pH 4.6-soluble N as a % of TN. Samples 3 and 4 had the lowest amount of pH 4.6-soluble N as a % of TN.

Sample 5 had lower moisture (34.78%), higher fat (35.38%), and lower salt (1.64%) Sample 5 had lower moisture compared to the rest of company 2 samples (34.78%), higher fat (35.38%), and lower salt (1.64%). Sample 10 was what one would expect for Cheddar cheese; typical Cheddar cheese has a moisture content of 37%, protein 25%, fat 33%, ash 4%, pH 5.10, salt 1.80% and salt to moisture (S/M) 4.86 (Ayala-Bribiesca et al., 2016). Sample 3 had a lower pH 5.01, a lower fat content of 34.93%, lower moisture (4.85%), lower salt (1.56%) and lower S/M (4.48). Samples 3 and 4 both had similar moisture levels, with Sample 3 at 34.85% and Sample 4 at 34.89%. Sample 3 had a lower pH of 5.01, and Sample 4 had a higher pH of 5.20. These factors could have resulted in less proteolysis. Samples 7 and 10 had expected compositions for Cheddar cheese. Sample 9 had a higher fat content at 39.09%, lower moisture at 34.51%,

low salt (1.58%) and low S/M (4.58). Sample 1 had the lowest salt and S/M in the sample set at 0.86%, and 2.24, which would have been the cause for the higher amount of pH 4.6-soluble N as a % of TN. Sample 1 had a low level of salt, which is prone to bitterness (McSweeney, 1997). Salt will slow down or stop starter proteinases by aggregating the large peptides that are not bitter (Sousa et al., 2001).

**5.9 Figure 6. Company 3. pH 4.6-Soluble N as a % of TN**

All samples from Company 3 exhibited increases in pH 4.6-soluble N as a % of TN. On day 15, Sample 2 had the lowest pH 4.6-soluble N as a % of TN at 10.41%. Sample 1, on day 15, had the highest amount at 11.04%. On day 60, Samples 3 and 6 had the lowest pH 4.6-soluble N as a % of TN at 17.31%, and 16.88%. On day 60, Samples 1 had the highest pH 4.6-soluble N as a % of TN at 19.92%. On day 90, Sample 6 had the lowest pH 4.6-soluble N as a % of TN at 20.20%. Day 90 Sample 2 had the highest pH 4.6-soluble N as a % of TN at 24.22%. On day 120, Samples 5 and 6 had the lowest pH 4.6-soluble N as a % of TN at 21.37% and 21.39%. The sample with the highest pH 4.6-soluble N as a % of TN was Sample 1 at day 120.

Sample 5 and 6 had the lowest pH 4.6-soluble N as a % of TN throughout the experiment, as well as the higher S/M of company 3 samples. Sample 1 was higher in pH 4.6-soluble N as a % of TN at days 15, 60, and 120. Sample 1 had a low salt level at 1.4% and a low total protein at 23.65%. All the samples were of legal reduced-fat Cheddar cheese. A legal reduced-fat Cheddar cheese, according

to the CFR, must have 25% less fat than Cheddar cheese (CFR Title 21.2, Part 101 D Sec. 101.62).

Company 3 starts at a higher level of pH 4.6-soluable N as a % of TN at 15 days 9.51% - 11.04%. Company 1 has a wider range of 15-day pH 4.6-soluble N as a % of TN, (9.33% - 15.35%). Company 2 has a range of 9.03% - 11.70% for 15-day. Company 3 does not have a 30-day sample for pH 4.6-soluble N as a % of TN. Company 1 had a very large range and a higher rate of proteolysis for pH 4.6-soluble N as a % of TN, (9.86% - 20.10%). Company 2 at 30 days was 10.26% - 15.11%. Company 2 at day 60 had the lowest amount of pH 4.6-soluble N as a % of TN, (14.23% - 19.53%). Company 1 and 3 had similar rates of proteolysis for pH 4.6-soluble N as a % of TN at day 60 with a range of 14.74% - 24.52%. Company 1 had the highest rate of proteolysis at day 90 with a range of 18.42% - 30.09% pH 4.6-soluble N as a % of TN. Company 2 had the lowest amount of proteolysis at day 90 having a range of 16.90% - 20.63%. Having lower S/M as displayed in Table 1 Company 1 Composition, would have a higher level of proteolysis which is the same findings as Upreti et al's study (2006c). At day 120 Company 1 had the highest level of pH 4.6-soluble N as a % of TN. Company 2, and 3 were similar in proteolysis with a range of 21.37% - 32.68% of pH 4.6-soluble N as a % of TN.

**5.10 Figure 7. Company 1 TCA-Soluble N**

Company 1's samples all increased over 120 days for TCA-soluble N. On day 15, Sample 2 had the lowest TCA-soluble N at 1.43%. Samples 5 and 7 had

the highest TCA-soluble N at 1.75% and 1.77%. Day 30 Sample 2 had the lowest amount of TCA-soluble N at 1.52%. Samples 5, 6, and 7 had the highest amount of TCA-soluble N at 2.39%, 2.36%, and 2.37%. On day 60, Sample 2 had the lowest TCA-soluble N at 1.75%. The highest amount of TCA-soluble N was in Sample 5 on day 60, at 3.11%. Day 90 had the lowest TCA-soluble N with Sample 3 at 2.53%. The highest amount of TCA-soluble N was observed in Sample 5 on day 90 at 3.98%. Sample 3, day 120 had the lowest amount of TCA-soluble N at 2.75%. The highest amount of TCA-soluble N was on day 120 at 4.29%.

Sample 2 had the greatest amount of fat (37.79%) for company 1 samples and had a lower S/M (4.60). Typical Cheddar cheese has 33% fat (Ayala-Bribiesca et al., 2016). Sample 5 had a lower pH at 5.01, higher fat at 36.30%, and lower moisture at 34.41%. Sample 7 had the high amount of fat (37.73%), low moisture (32.91%), and a high S/M (5.17). To measure primary proteolysis, pH 4.6-soluble N can be used, and it is not influenced by the amount of fat in a sample (Fenelon and Guinee, 2000). There was a notable difference in samples with an increased level of fat and increased proteolysis level in the current study. Sample 6 had a higher pH at 5.22, a higher fat at 36.44%, and lower moisture. Sample 3 had expected composition of Cheddar cheese; typical Cheddar cheese has a moisture content of 37%, protein 25%, fat 33%, ash 4%, pH 5.10, salt 1.80% and salt to moisture (S/M) 4.86 (Ayala-Bribiesca et al., 2016).

**5.11 Figure 8. Company 2 TCA-Soluble N**

Company 2's samples all increased over 120 days for TCA-soluble N. Throughout the entire study, Sample 1 had the lowest amount of TCA-soluble N, with 0.91% on day 15. The highest amount of TCA-soluble N was on day 15 with 1.63% for Sample 9. Day 30 had the lowest amount of TCA-soluble N with Sample 1 at 1.40%, and the highest amount was Sample 5 at 2.04%. Day 60 Sample 1 was at 1.64%, which was the lowest TCA-soluble N. The highest amount was 2.67% from Sample 5. On day 90, the lowest amount of TCA-soluble N was 2.49%. The highest amount came from Sample 2 at 3.05%. On day 120, the lowest amount of TCA-soluble N came from Sample 1 at 2.70%. The highest amount came from Sample 8 at 3.85%.

Sample 1 had the lowest pH of company 2 at 5.00, the lowest salt and S/M of the samples in the study at 0.86% and 2.24. Sample 9 had the highest fat of company 2 (39.09%), lower moisture (34.51%), lower salt (1.58%) and lower S/M (4.58). Sample 5 had lower moisture (34.78%), higher fat (35.38%), and lower salt (1.64%). Sample 2 had a lower pH of 5.01, higher fat (35.25%), lower salt (1.66%), lower S/M (4.68), and slightly lower moisture than expected (35.45%). Sample 8 had a higher fat (37.09%), higher S/M (5.03) and lower moisture (34.38%).

**5.12 Figure 9. Company 3 TCA-Soluble N**

Company 3's samples all increased throughout the experiment. Day 15 had the lowest TCA-soluble N from Sample 1, at 1.19%. Sample 4 had the

highest amount of TCA-soluble N at 1.67%. On day 60, Sample 3 had the lowest TCA-soluble N as is at 1.94%. On day 60, the highest amount of TCA-soluble N was Sample 2 at 2.23%. On day 90, the lowest amount of TCA-soluble N came from Samples 1 and 4, at 2.41% and 2.4%, respectively. The highest amount of TCA-soluble N came from Sample 5 at 2.95%. The lowest amount of TCA-soluble N on day 120 came from Samples 2 and 4 at 2.71% and 2.79%. The highest amount of TCA-soluble N on day 120 was Sample 5 at 3.42%.

Sample 1 had a total protein of 23.65%, which was the low of company 3, and a lower S/M (3.10). This could have been why it had the lowest starting TCA-soluble N. Sample 1 also had a lower salt of 1.40%. Sample 5 had a higher fat at 22.02%. The sample set composition was highly similar and consistent between the samples.

All 3 companies were similar of a starting range of under 2% proteolysis at day 15 for TCA-soluble N. Day 30 for Company 3 was not analyzed as samples were unavailable. Company 1 and 2 had similar day 30 TCA- soluble N with a range of 1.40%- 2.39%. Company 1 had the highest amount of TCA-soluble N at day 60, 90 and 120 having the highest sample be 3.11%, 3.98%, and 4.29%.

Company 1 and 3 had similar starting TCA-soluble N as % of TN for day 15, 6.03% - 7.70%. Company 2 had lower starting amount of TCA-soluble N as % of TN for day 15 at 3.88% - 5.51%. Day 30, company 1 had the highest proteolysis of TCA-soluble N as a % of TN with 6.53% - 10.06%. Company 3 30-day samples were unavailable, and company 2 had lower proteolysis at 6.99% - 11.24%. At day 60 all companies had similar rates of proteolysis for TCA-soluble

N as a % of TN with a range of 6.99% - 12.57%. Day 90 and 120 Company 1 had the highest amount of proteolysis for TCA-soluble N as a % of TN 11.35% - 16.08%, and 12.34% - 17.56%. Company 1 had a lower S/M ratio and also had a higher amount of proteolysis, this was analyzed in Upreti et al's study (2006c). Company 2 and 3 were similar for day 90 and 120.

**5.13 Figure 10. Company 1 TCA-Soluble N as a % of TN**

Company 1 TCA-soluble N as a % of TN had all samples increase over time. Day 15 Sample 2 had the lowest amount of TCA-soluble N as a % of TN at 6.14%. The highest amount was from Samples 7 and 10 at 7.38% and 7.33%. Day 30 Sample 2 had the lowest amount of TCA-soluble N as a % of TN at 6.53%, and the highest amount came from Sample 6 at 10.06%. Day 60 Sample 2 had the lowest TCA-soluble N as a % of TN at 7.51%, and Sample 5 had the highest amount at 12.57%. Day 90 Sample 3 had the lowest amount of TCA-soluble N as a % of TN at 11.35%, and the highest amount came from Sample 5 at 16.08%. On day 120, the lowest TCA-soluble N as a % of TN came from Sample 3 at 12.34%. The highest TCA-soluble N as a % of TN at day 120 was from Sample 4 at 17.56%.

Sample 2 had the highest amount of fat in company 1 samples (37.79%), lower salt (1.66%), and lower S/M (4.60). Sample 7 had a high amount of fat for company 1 (37.73%), low moisture (32.91%), and a high S/M (5.17). Fenelon and Guinee concluded, "Reduction in fat content of Cheddar cheese, in the range of 32.5–6.3 g 100 $g^{-1}$, resulted in lower levels of primary proteolysis, as measured by

the levels of pH 4.6-soluble N as % total N (Banks et al., 1989)" (Fenelon and Guinee, 2000).

Sample 10 had a lower pH of 5.02 and had the highest salt for the sample set at 1.83%. Sample 6 had the highest pH of company 1 samples at 5.22, and low moisture (34.03%). Sample 3 had what is to be expected of Cheddar cheese. Typical Cheddar cheese has moisture of 37%, protein 25%, fat 33%, ash 4%, pH 5.10, salt 1.80% and salt to moisture (S/M) 4.86 (Ayala-Bribiescaet et al., 2016). Sample 5 had a lower pH at 5.01, a higher fat at 36.30%, and lower moisture at 34.41%.

**5.14 Figure 11. Company 2 TCA-Soluble N as a % of TN**

All samples increased over the experiment for Company 2. On day 15, Sample 1 had the lowest TCA-soluble N as a % of TN at 3.88%. The highest amount at day 15 was 6.56% for Sample 9. The lowest amount of TCA-Soluble N % of TN for day 30 came from Sample 1 at 5.97%. The highest amount of TCA-soluble N as a % of TN was from Sample 5 at 8.59%. Day 60 Sample 1 had the lowest amount of TCA-soluble N as a % of TN at 6.99%. The highest amount of TCA-soluble N as a % of TN was from Sample 5 at 11.24%. On day 90, the lowest TCA-soluble N came from Sample 3 at 10.61% and 10.14%. The highest TCA-soluble N as a % of TN came from Samples 2 and 5 at 12.70% and 12.71%. On day 120, Sample 3 was the lowest at 10.97%, and the highest amount of TCA-soluble N as a % of TN was from Sample 8 at 16.04%

Sample 1 had a low pH of 5.0, a low salt of 0.86%, a low S/M of 2.24, and the lowest total protein. Sample 5 had lower moisture at 34.78% and higher fat at 35.38%. Sample 3 had a low pH of 5.01 and a lower moisture of 34.85%. Sample 2 had a lower pH of 5.01, a higher fat at 35.25%, and lower moisture at 35.45%. Sample 8 on day 120 had higher proteolysis. This sample had a high fat (37.09%), lower moisture (34.38%), and higher S/M (5.03).

**5.15 Figure 12. Company 3 TCA-Soluble N as a % of TN**

Company 3 TCA-soluble N as a % of TN had all samples increase over time. Sample 1 had the lowest amount of TCA-soluble N as a % of TN at 5.03%, and the highest amount was 6.47% from Sample 2. On day 60, Samples 3 and 6 were the lowest TCA-soluble N as a % of TN at 7.13% and 6.93%. The highest amount of TCA-soluble N as a % of TN was Sample 2 at 9.88%. Day 90 Sample 4 had the lowest amount of TCA-soluble N as a % of TN at 8.88%, and the highest was Sample 5 at 11.10%. Day 120 had the lowest TCA-soluble N as a % of TN from Sample 4 at 10.32%. The highest amount came from Samples 1 and 5 at 12.94% and 12.87%.

At days 90 and 120, Sample 5 had higher fat for company 3 samples (22.02%). Sample 2 had a total protein of 22.58%, which was the lowest of company 3, lowest salt (1.39%) and lowest S/M (3.04). Low total protein has a correlation to the lowest starting TCA soluble N % of TN. Sample 1 also had a lower salt of 1.40%. Sample 5 had a higher fat at 22.02%. The samples for company 3's composition were all very similar and consistent between the

samples. Legal reduced-fat Cheddar cheese, as stated in CFR, must have 25% less fat than Cheddar cheese (CFR Title 21.2, Part 101 D Sec. 101.62).

## 6. Discussion for Natural Cheese Proteolysis

The comparison of pH 4.6 N revealed that all samples increased in proteolysis over time. The cheese samples for company 1 and 2 were of legal Cheddar cheese according to the CFR, with a minimum milk fat of 50% by wt./solids and maximum moisture of 39% by wt. (CFR Title 21. 22.2. Ch.1.B, Sec. 133.113). Company 3 had the composition of reduced-fat Cheddar cheese and could not be compared with the full-fat Cheddar cheese of Companies 1 and 2. A legal reduced-fat Cheddar cheese, as stated in CFR, must have 25% less fat than Cheddar cheese (CFR Title 21.2, Part 101 D Sec. 101.62). Company 3's samples were all highly similar in composition. In pH 4.6 nitrogen, the lowest amount of proteolysis occurred in samples with the highest amount of fat in the sample set. The samples with the lowest amount of total protein also had the lowest amount of proteolysis over time.

### Proteolysis of pH 4.6 Soluble N

Among all of the samples from Companies 1 and 2, moisture seemed to be a critical factor, either slowing down or speeding up proteolysis. Lawrence asserts that cheese will get harden as time passes (Lawrence, et al., 1986). This is because the available moisture will be tied up as more peptides are cleaved due to the ionic groups taking the available water molecules (Lawrence et al., 1986). Indeed,

samples with lower moisture had slower proteolysis. Moreover, samples with a higher S/M had slower rates of proteolysis. In contrast, if the samples had low moisture but high total protein and high fat, they had a faster rate of proteolysis.

The expected composition of Cheddar cheese had a faster rate of proteolysis for pH 4.6 protein. The expected composition can start slow but can increase in the rate proteolysis over time. Typical Cheddar cheese has a moisture content of 37%, protein 25%, fat 33%, ash 4%, pH 5.10, salt 1.80% and salt to moisture (S/M) 4.86 (Ayala-Bribiesca et al., 2016). A low total protein will cause an increased level of pH 4.6 protein. Having a lower salt and low S/M content can cause an increase in proteolysis due to the buffering capacity of the cheese (Upreti and Metzger, 2007).

**Proteolysis of TCA Nitrogen**

All of the samples in TCA nitrogen exhibited an increase in proteolysis over 120 days. TCA nitrogen for Company 3 could not be compared to Company 1 and 2 because Company 3 was a legal reduced-fat Cheddar cheese. Company 3 had lower rates of proteolysis in samples with lower total protein, and samples with higher fat had increased rates of proteolysis for TCA nitrogen.

Companies 1 and 2 had slower proteolysis for samples that were only high fat. The expected composition of Cheddar cheese also had a slower rate of proteolysis over time for TCA nitrogen. Another contributing factor for slow proteolysis was a sample with high salt, high S/M, and high pH. Indeed, the level of salt and S/M will affect the pH, residual lactose, and proteolysis (Kapoor et al., 2007).

Composition factors that have an increased rate of TCA nitrogen were high fat, low moisture, and a low S/M ratio. The small-chained peptides were most affected by the moisture, fat, and pH. Fenelon and Guinee (2000) found increased proteolysis if they had increased protein, pH, and moisture contents. They also discovered that proteolysis decreased in samples with lower MNFS contents, lower chymosin-activity-to-protein ratios, and lower lactate-to-protein levels. Lower fat also decreased proteolysis (Fenelon and Guinee, 2000).

**Proteolysis of pH 4.6 Soluble N% of TN**

The pH 4.6-soluble N as a % of TN revealed that all the samples increased over the 120-day experiment. Samples from Companies 1 and 2 were of legal Cheddar cheese and could be compared with each other, while Company 3's samples were of legal reduced-fat Cheddar cheese.

A compositional factor of for a low level of proteolysis of pH 4.6-soluble N as a % of TN was low salt and moisture, this was Sample 2 Company 2. Sample 1 had an increase in proteolysis of pH 4.6-soluble N as a % of TN, and this sample was low in salt, S/M, and total protein. Sample 1 also had an increase in proteolysis for pH 4.6-soluble N. It can be concluded that low salt can increase proteolysis since increased salt will inhibit proteolysis (Visser, 1993). Samples with high S/M had slower rates of proteolysis.

Samples that had increased rates of pH 4.6-soluble N as a % of TN were highly similar to the results of pH 4.6 nitrogen. One factor from the composition

that increased the rate of proteolysis was low salt. If a sample had a low-fat range, this decreased the proteolysis rate (Fenelon and Guinee, 2000).

The expected composition of Cheddar cheese has a moisture content of 37%, protein 25%, fat 33%, ash 4%, pH 5.10, salt 1.80% and salt to moisture (S/M) 4.86 (Ayala-Bribiesca et al., 2016). High fat, low moisture, and low pH led to increased rates of proteolysis. The high pH affected the rate of proteolysis. In Farkye et al.'s (1990) study, the starter bacteria had the largest impact on proteolysis to cleave the peptides into small peptides and then into FAA. The researchers also stated that the GDL cheese had a lower level of chymosin activity and a higher pH than the cheese made with starter bacteria (Farkye et al., 1990).

**Proteolysis of TCA N% of TN**

TCA-soluble N as a % of TN increased in all samples over the 120-day experiment. In TCA-soluble N as a % of TN, the full-fat Cheddar cheese of Companies 1 and 2 could not be compared with Company 3's legal reduced-fat Cheddar cheese. Company 3 had lower rates of proteolysis with samples with lower total protein. Higher fat led to an increased rate of proteolysis for TCA-soluble N as a % of TN in Company 3.

Companies 1 and 2 had slower proteolysis for samples that were only high in fat. The expected composition of Cheddar cheese had a slower rate of proteolysis over time for TCA-soluble N as a % of TN. Another contributing factor for faster proteolysis was a sample with low salt, low S/M, and a low pH. Indeed, the amount of salt and the S/M affect cheese pH, residual lactose, and the hydrolysis

of proteins (Kapoor et al., 2007). The last sample had a low proteolysis rate, a low pH, and low moisture.

Samples that had composition factors of higher fat, low S/M, and lower moisture were some of the few that would increase the rate of TCA-soluble N as a % of TN. The small-chained peptides were most affected by the moisture, fat, and S/M.

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**TABLES**

**Table 1. Natural Cheese Composition, Company 1**

| Item | pH | Fat | Salt | Moisture | Salt: Moisture | Ash minus salt | Ash | Total Protein |
|---|---|---|---|---|---|---|---|---|
| Sample 1 | 5.09 | 34.82 | 1.50 | 37.45 | 4.01 | 2.01 | 3.51 | 22.43 |
| Sample 2 | 5.13 | 37.79 | 1.66 | 36.12 | 4.60 | 1.88 | 3.54 | 23.29 |
| Sample 3 | 5.13 | 34.9 | 1.75 | 35.79 | 4.89 | 2.00 | 3.75 | 22.29 |
| Sample 4 | 5.13 | 35.91 | 1.80 | 32.89 | 5.47 | 2.06 | 3.86 | 24.43 |
| Sample 5 | 5.01 | 36.30 | 1.53 | 34.41 | 4.45 | 2.06 | 3.59 | 24.75 |
| Sample 6 | 5.22 | 36.44 | 1.62 | 34.03 | 4.76 | 2.15 | 3.77 | 23.46 |
| Sample 7 | 5.06 | 37.73 | 1.70 | 32.91 | 5.17 | 1.94 | 3.64 | 23.98 |
| Sample 8 | 5.08 | 30.22 | 1.62 | 38.52 | 4.21 | 2.10 | 3.72 | 22.87 |
| Sample 9 | 5.05 | 31.51 | 1.71 | 39.32 | 4.35 | 2.10 | 3.81 | 22.26 |
| Sample 10 | 5.02 | 33.21 | 1.83 | 38.34 | 4.77 | 2.04 | 3.87 | 23.33 |
| Sample 11 | 5.08 | 33.14 | 1.62 | 37.06 | 4.37 | 2.02 | 3.64 | 24.08 |

**Table 2. Natural Cheese Composition Company 2**

| Item | pH | Fat | Salt | Moisture | Salt: Moisture | Ash minus salt | Ash | Total Protein |
|---|---|---|---|---|---|---|---|---|
| Sample 1 | 5.00 | 35.06 | 0.86 | 36.68 | 2.24 | 2.14 | 3.00 | 23.46 |
| Sample 2 | 5.01 | 35.25 | 1.66 | 35.45 | 4.68 | 2.32 | 3.98 | 24.01 |
| Sample 3 | 5.01 | 34.93 | 1.56 | 34.85 | 4.48 | 2.29 | 3.85 | 25.44 |
| Sample 4 | 5.20 | 35.73 | 1.78 | 34.89 | 5.10 | 1.99 | 3.77 | 24.50 |
| Sample 5 | 5.10 | 35.38 | 1.64 | 34.78 | 4.72 | 2.03 | 3.67 | 23.76 |
| Sample 6 | 5.16 | 35.83 | 1.63 | 35.43 | 4.60 | 2.12 | 3.75 | 24.13 |
| Sample 7 | 5.10 | 35.83 | 1.72 | 36.46 | 4.72 | 2.10 | 3.82 | 25.14 |
| Sample 8 | 5.18 | 37.09 | 1.73 | 34.38 | 5.03 | 2.08 | 3.81 | 24.00 |
| Sample 9 | 5.15 | 39.09 | 1.58 | 34.51 | 4.58 | 2.26 | 3.84 | 24.83 |
| Sample 10 | 5.11 | 36.35 | 1.69 | 35.18 | 4.80 | 1.98 | 3.67 | 25.00 |

**Table 3. Natural Cheese Composition, Company 3**

| Item | pH | Fat | Salt | Moisture | Salt: Moisture | Ash minus Salt | Ash | Total Protein |
|---|---|---|---|---|---|---|---|---|
| Sample 1 | 4.9 | 21.80 | 1.4 | 45.09 | 3.10 | 1.97 | 3.37 | 23.65 |
| Sample 2 | 4.9 | 21.76 | 1.39 | 45.67 | 3.04 | 1.99 | 3.38 | 22.58 |
| Sample 3 | 4.9 | 21.41 | 1.52 | 43.20 | 3.52 | 2.19 | 3.71 | 27.21 |
| Sample 4 | 4.9 | 20.95 | 1.65 | 44.70 | 3.69 | 2.09 | 3.74 | 27.03 |
| Sample 5 | 4.9 | 22.02 | 1.53 | 45.46 | 3.37 | 1.97 | 3.50 | 26.58 |
| Sample 6 | 4.9 | 21.69 | 1.53 | 45.19 | 3.39 | 1.97 | 3.50 | 26.83 |

FIGURES

**Figure 1. Company 1 pH 4.6 Soluble N**

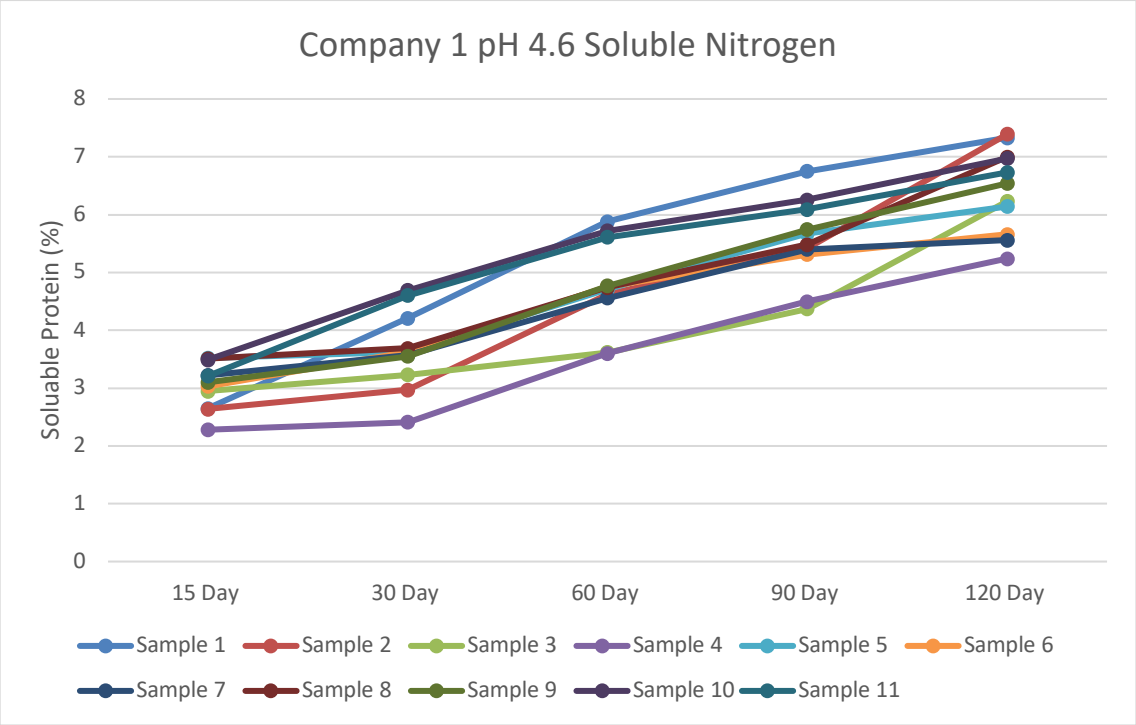


**Figure 2. Company 2 pH 4.6 Soluble** N

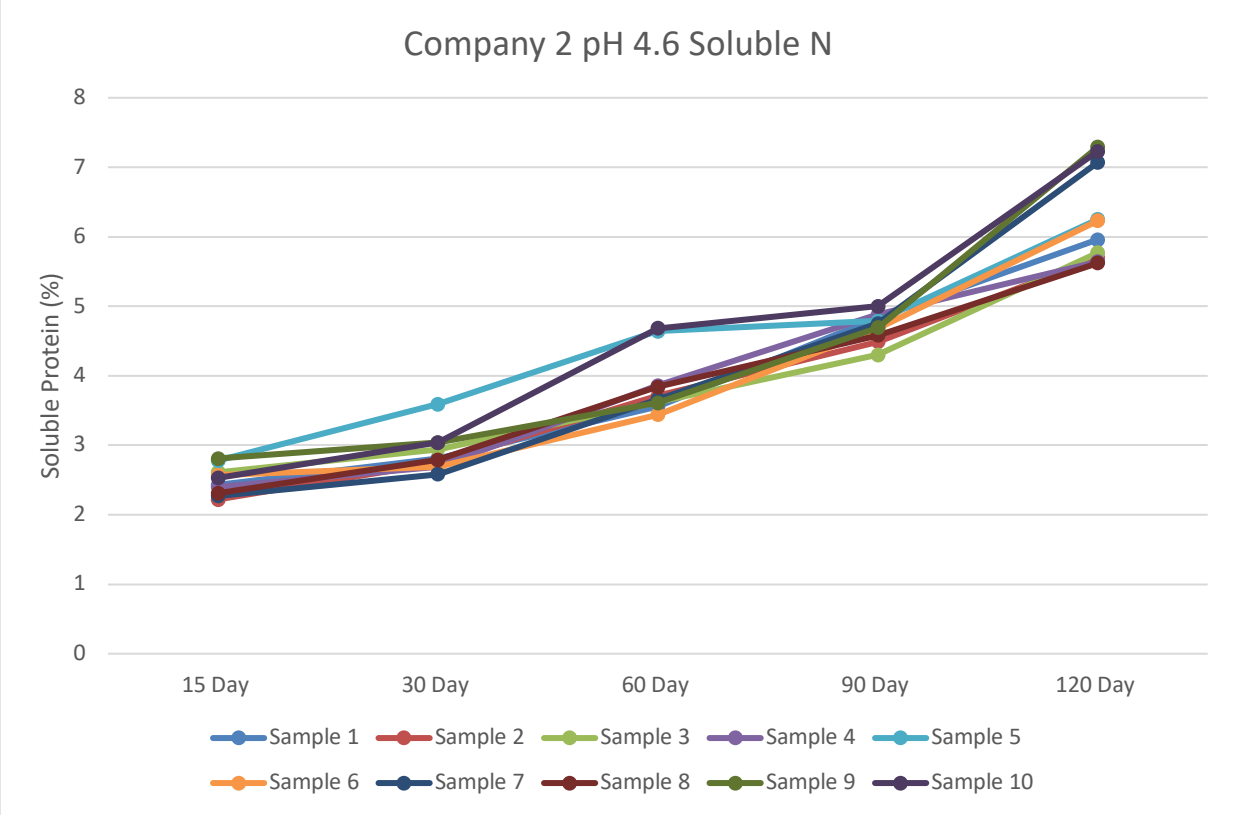


**Figure 3. Company 3 pH 4.6 Soluble N**

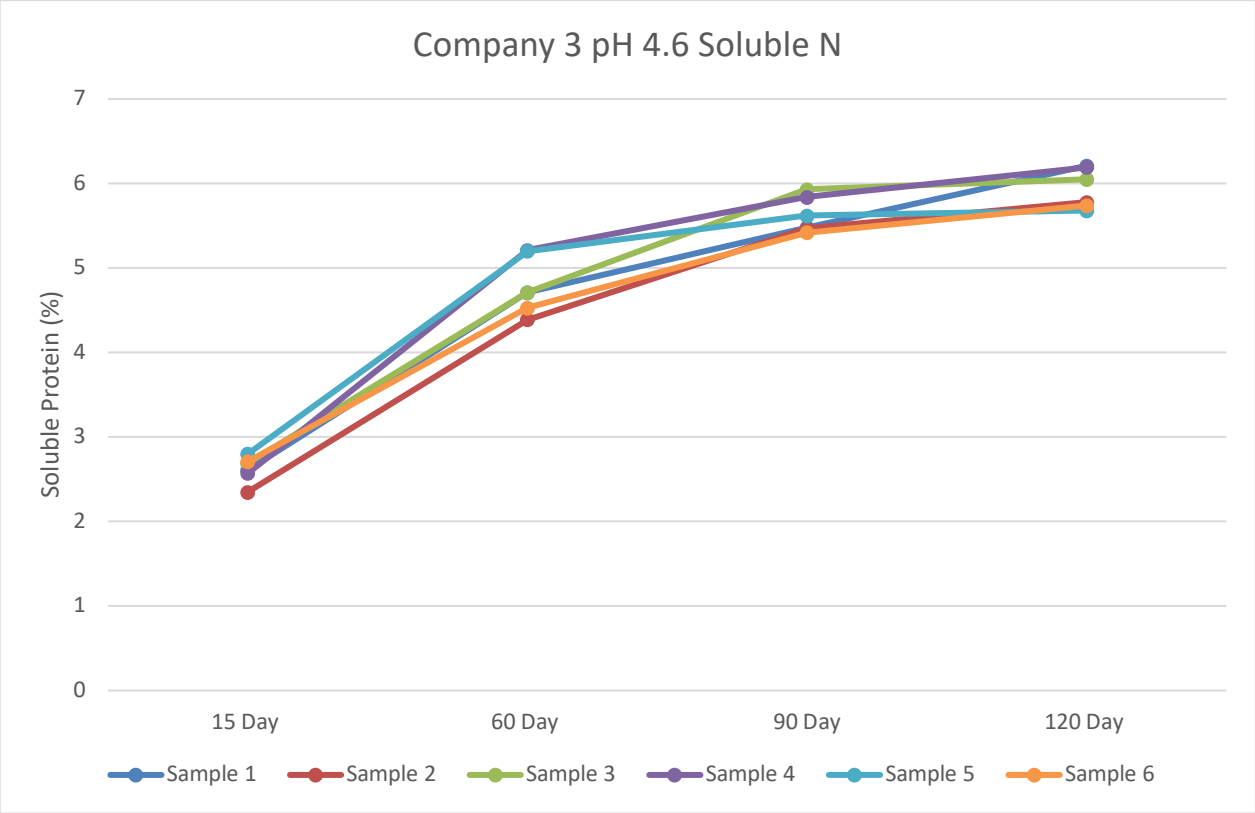


**Figure 4. Company 1 pH 4.6 Soluble N as a % of TN**

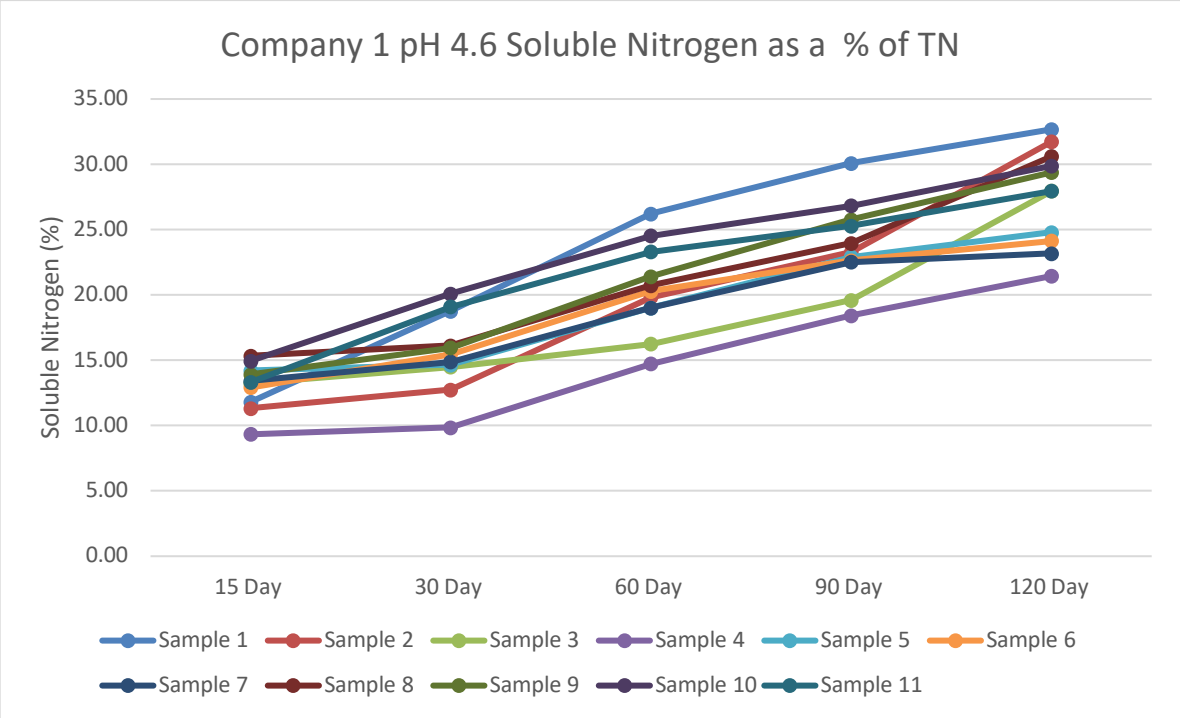


**Figure 5. Company 2 pH 4.6 Soluble N as a % of TN**

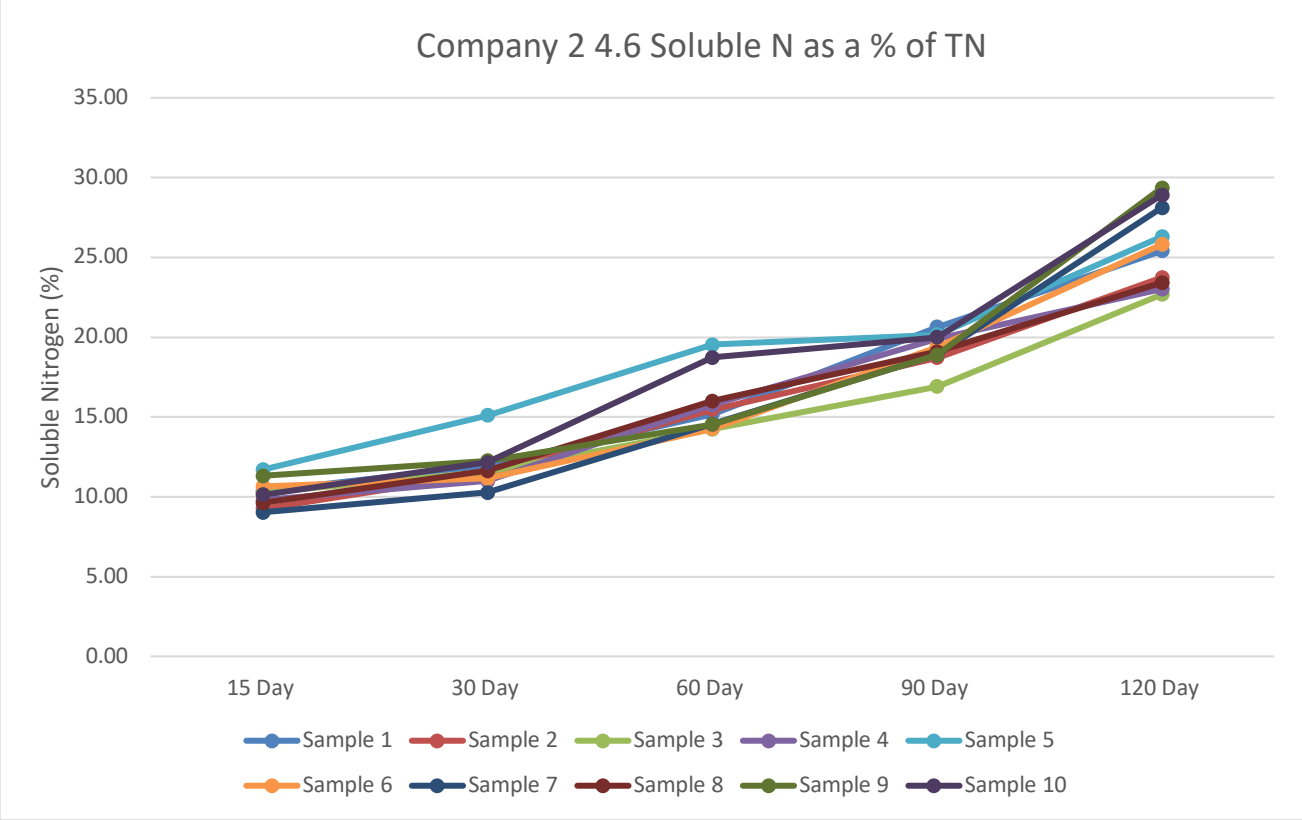


**Figure 6. Company 3 pH 4.6 Soluble N as a % of TN**

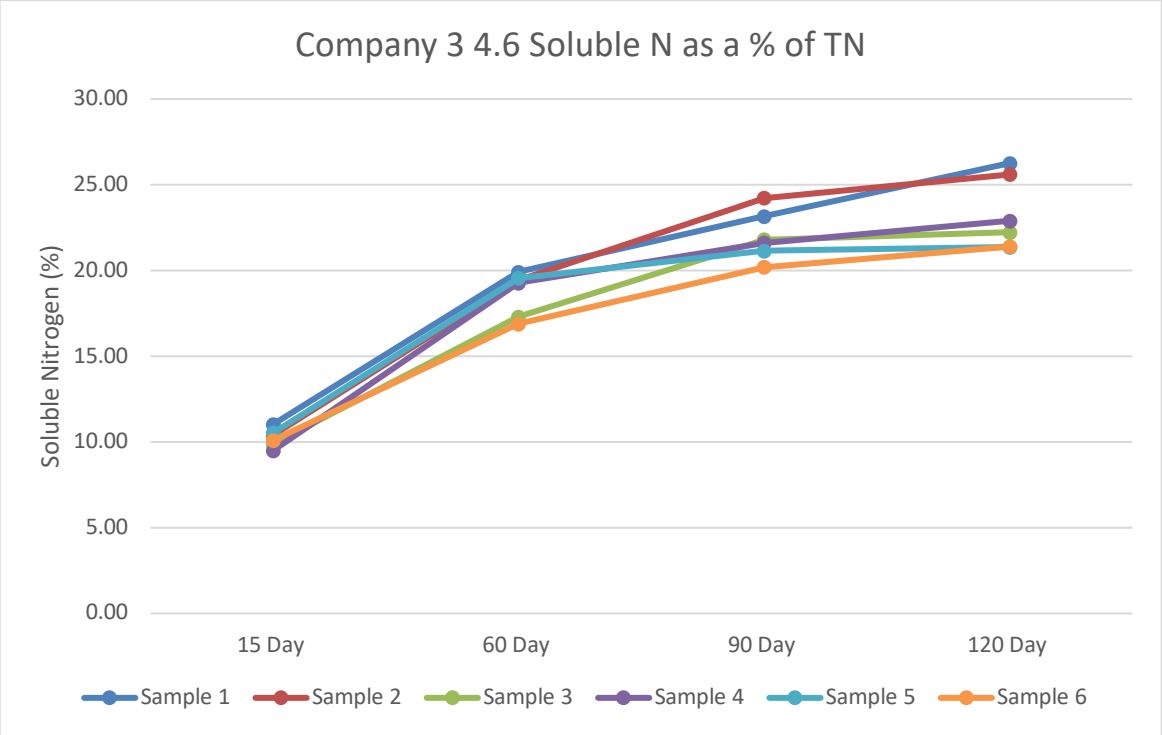


**Figure 7. Company 1 TCA Soluble N**

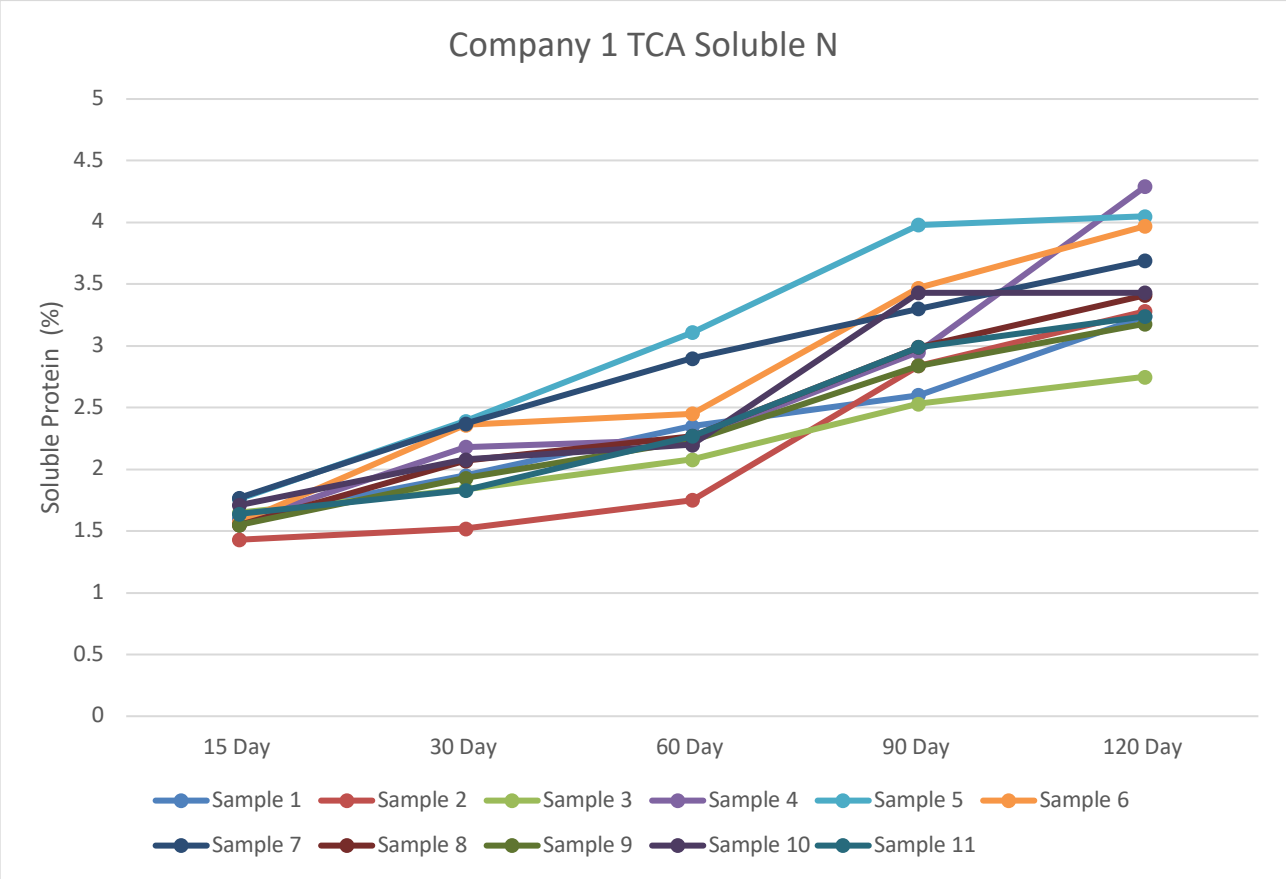


**Figure 8. Company 2 TCA Soluble N**

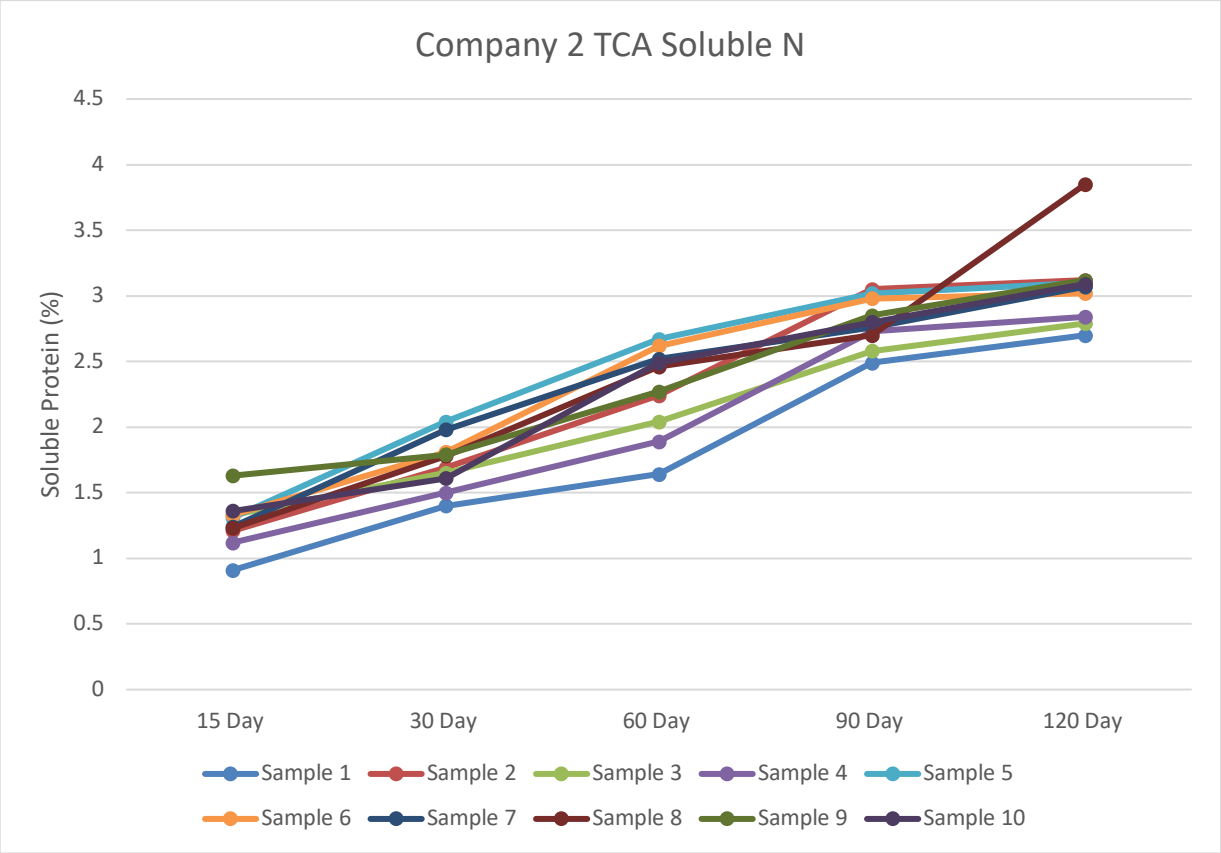


**Figure 9. Company 3 TCA Soluble N**

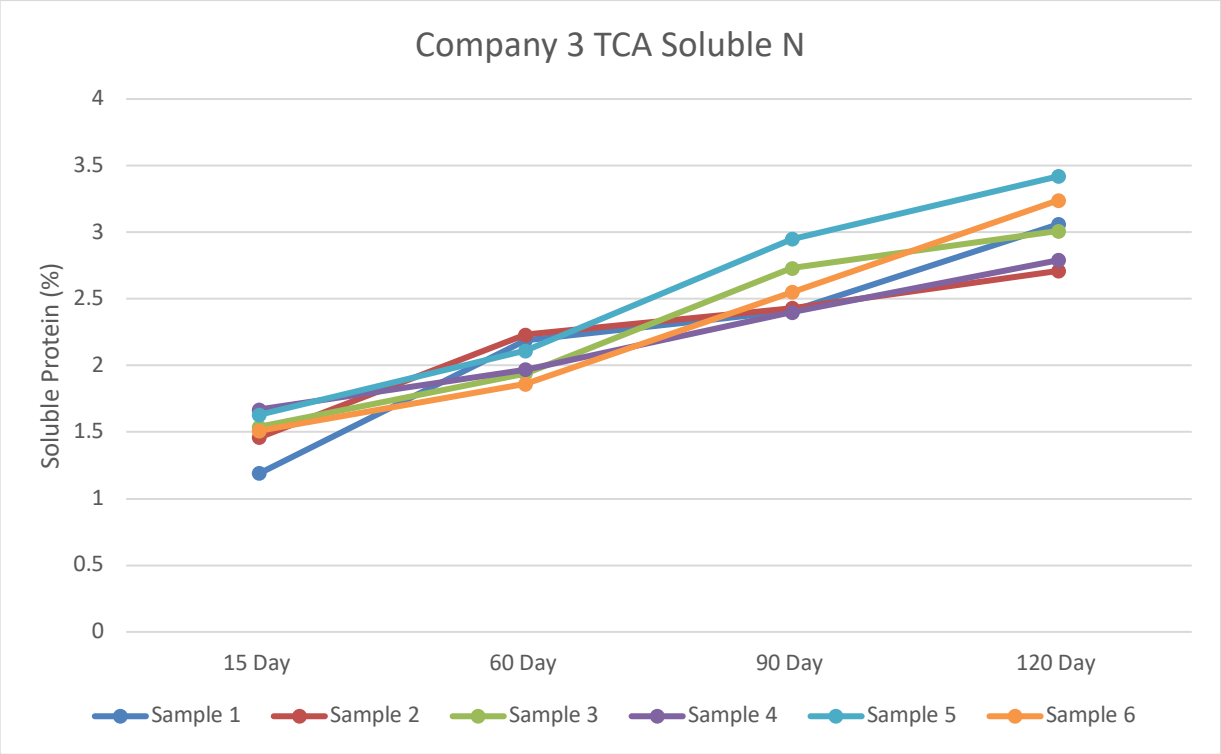


**Figure 10. Company 1 TCA Soluble N as a % of TN**

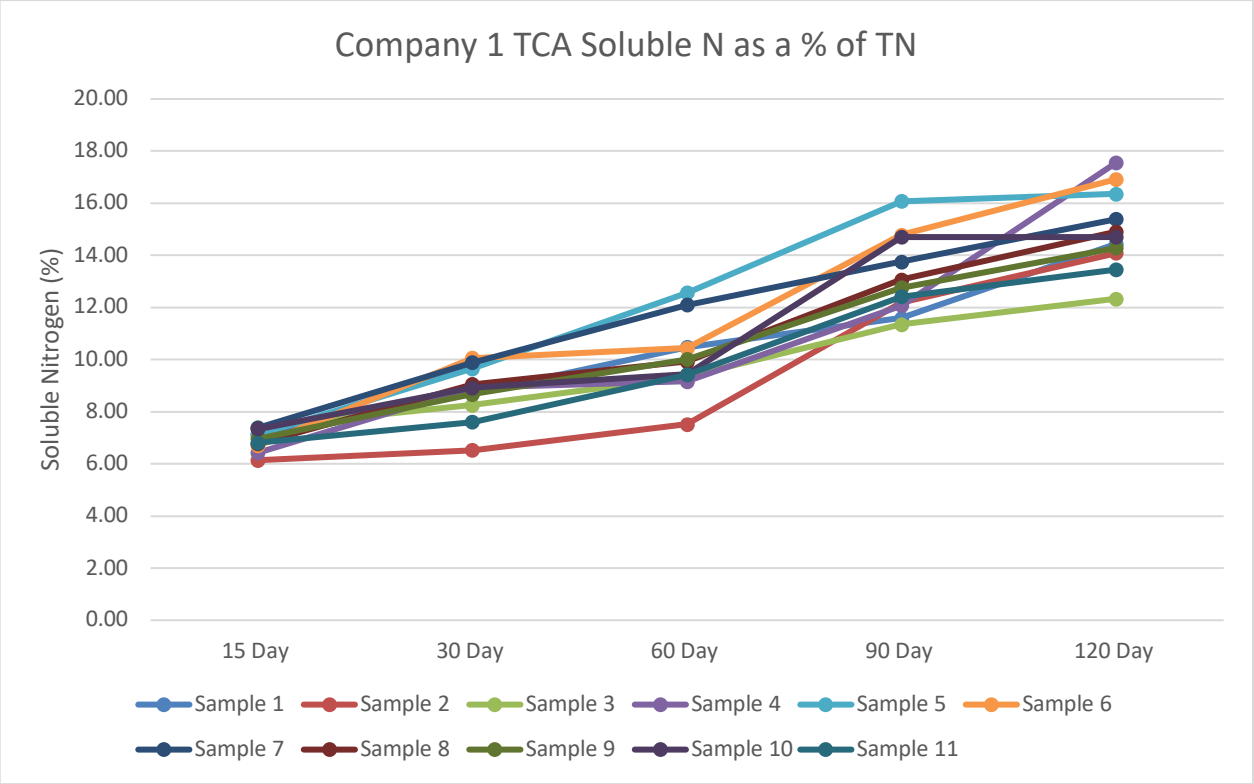


**Figure 11. Company 2 TCA Soluble N % of TN**

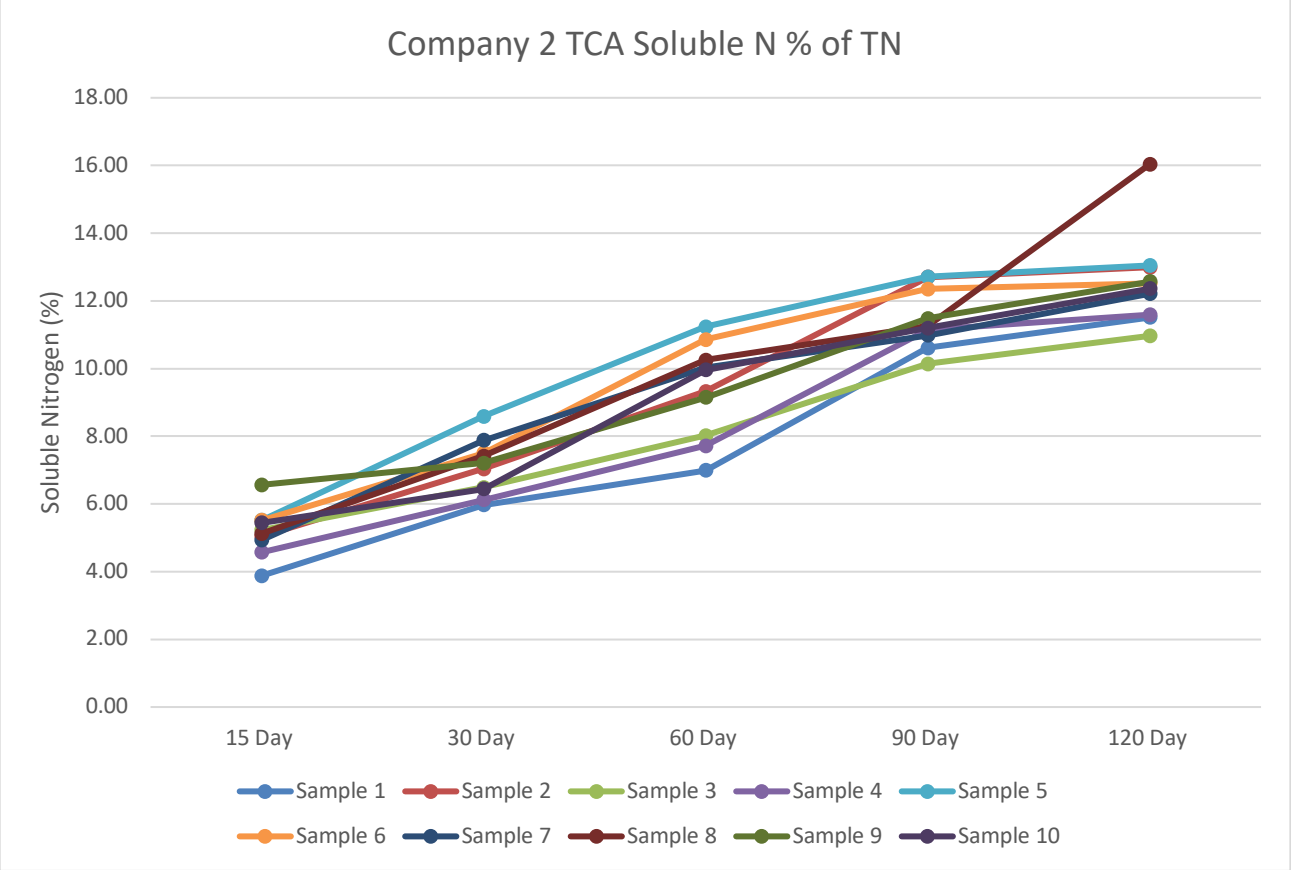
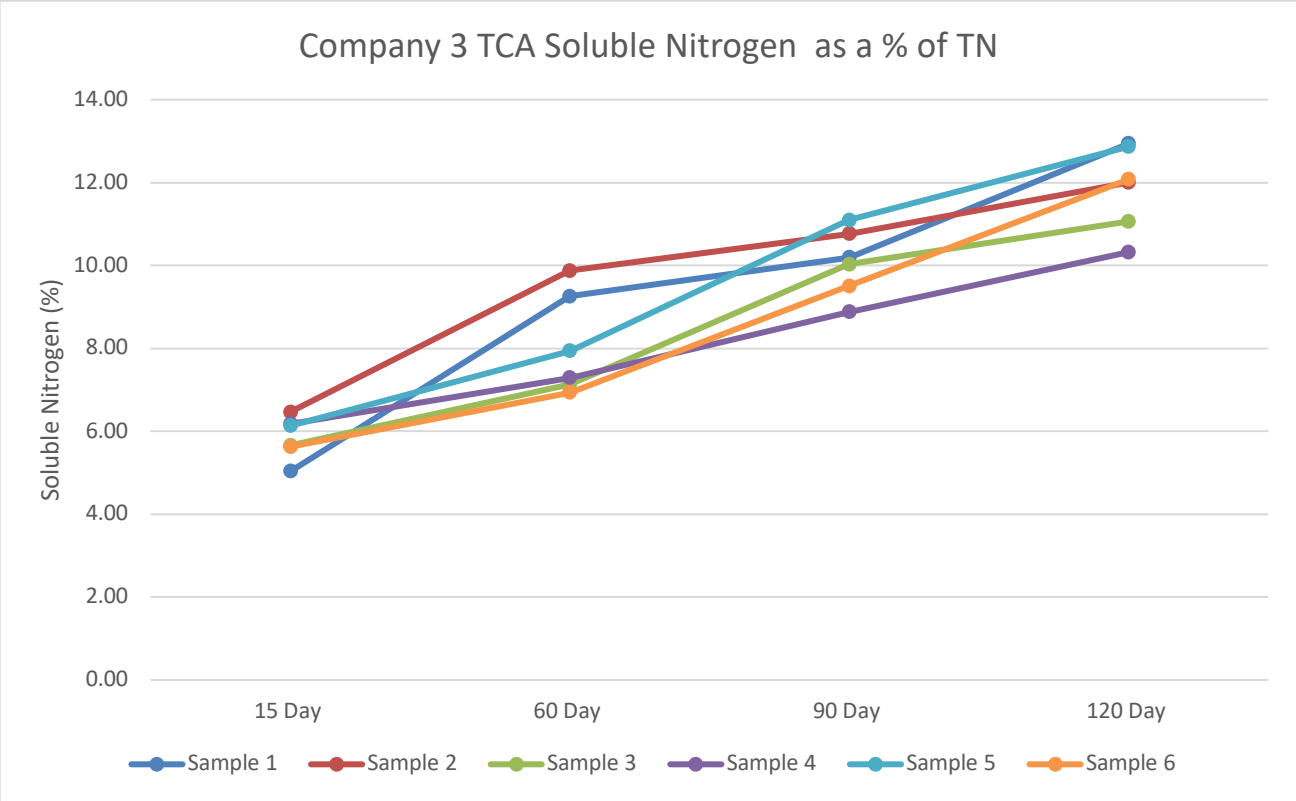


**Figure 12. Company 3 TCA Soluble N as a % of TN**



# CHAPTER III: THE EFFECTS OF NATURAL CHEESE COMPOSITION AND PROTEOLYSIS ONPROCESS CHEESE

## 1. Introduction

Process cheese was first produced in the 20th century. It was created to extend the shelf life of natural cheese and made room for cheese that has not been made to specifications deemed by the recipes for its final destination (Kosikowski & Mistry, 1997). Process cheese was first invented in Switzerland by Walter Gerber and Fritze Stettler. Swiss cheese and the emulsifying salt sodium citrate were combined together in 1912 to create the first form of process cheese (Kosikowski & Mistry, 1997). J.L Kraft then created process cheese to preserve natural cheese through heating and continuous mixing (Kosikowski & Mistry, 1997). Natural cheese is different from process cheese as process cheese acts as a stable, oil-in-water emulsion (Kosikowski & Mistry, 1997). The use of emulsifying salts, specifically, polyphosphates, citrates and sodium phosphates aide in the manufacture of process cheese by improving the combination of ingredients that would otherwise be unmixable. The emulsifying salts isolate the calcium phosphates in natural cheese. This will then solubilize the protein so that the water, fat, and protein create a homogenous mass (Kosikowski & Mistry, 1997; Caric et al., 1985).

### 1.1 Definition

Process cheese has two primary ingredients, natural cheese which will vary in age, and salts, including emulsifying salts. Natural cheese for process cheese will need to be selected based on the intact casein which can correlate with age (Garimella et al., 2006). An example of selection of cheese would be 10% aged, 35% medium

and 55% young natural cheese (Kosikowski & Mistry, 1997). Emulsifying salts are needed to separate the calcium from the paracasein network, solubilizing the protein and producing a smooth and homogeneous product (Garimella et al., 2006).

Process cheese is a dairy product that is different from natural cheese in melting and extended shelf-life (Caris et al., 1985; Kosikowski & Mistry, 1997). It is created by combining natural cheese with varying ages and degrees of maturity, unlike natural cheese, which is made directly from milk. Additionally, according to Guinee et al. (2004), in addition to blending different natural cheeses, the process of cheese manufacturing must include emulsifying salts, dairy ingredients, and non-dairy ingredients. Upon heating and continually mixing these ingredients, the combined mixture forms a homogenous product with an extended shelf life (Guinee et al., 2004). According to the CFR, the legal definition of process cheese in the United States is generic and can describe three different types: pasteurized process cheese, pasteurized process cheese food, and pasteurized process cheese spread (FDA, 2019). The CFR distinguishes the categories based on minimum fat content requirements, minimum final pH, and maximum moisture content. The ingredients that are to be used, as well as the quality and number of ingredients, are also dictated by the CFR (21CFR 133.169 to 133.180; FDA, 2019).

### 1.2 Manufacture

The manufacture of process cheese is depicted in Figure 1. The figure illustrates the two key steps. Step one is to select the ingredients and choose the formulation to be used depending on the category of process cheese that will be made

along with what functionality properties that are needed. Step two involves processing and storage. Processing consists of cooking and mixing, and storage relates to the packaging, cooling, and storing of the product (Kapoor and Metzger, 2008). The CFR specifies minimum requirements for cooking and a particular amount of time to be cooked (FDA 2019; 21CFR 133.169 to 133.180).

### 1.3 Natural Cheese's Casein

Natural cheese is the primary ingredient in process cheese as well as one of the most influential. The micellular casein that comprises 80% of milk also has a significant impact on the process cheese (Visser, 1993). The casein micellar is comprised of K-CN, αs1-, αs2-, and β-casein in the micelle (Heck et al., 2009). The size of the casein micelle is 15 to 20 nm in diameter (Fox et al., 2004). The casein micelle is a hydrophilic core with a core coat, an internal structure and the submicelles (Fox et al., 2004). Coagulant will hydrolyses the Phe (105)-Met (106) bond, which will break away from the alpha s casein and beta casein that is calcium sensitive (Kosikowski & Mistry, 1997). According to Shimp (1985), natural cheese contains under-emulsified fat; the fat phase and water phase are sustained by a water-insoluble calcium–paracaseinate phosphate network (Kapoor and Metzger, 2008).

### 1.4 Process Cheese's Casein

Casein in process cheese is essential because it will experience an interruption in its calcium phosphate structures with added mixing and heating. The mixing and heating will help to hydrate and partly disperse the calcium–paracaseinate phosphate

structure. This calcium-paracaseinate structure, which is mainly dispersed, will then interact with the fat through hydrophobic interactions. During cooling, the partially dispersed caseinate matrix will floc and the new material will create a uniform gel that is closely knit together (Zhong and Daubert 2004).

### 1.5 Formulation

As previously mentioned, natural cheese is a key ingredient in process cheese. The natural cheese type, and age are important for the quality of process cheese. The intact casein in the cheese will correlate with the age (Garimella Purna et al., 2006). Young cheese will /have more intact casein, not hydrolyzed casein (Berger et al., 2002). The casein will hydrolyze due to proteolysis that occurs over time during the ripening of the natural cheese (Fox, 2005). Proteolysis of casein is from enzymes, starter culture, residual starter, or NSLAB, that will break down the protein over time (Varnam, 2001).

Emulsifying salts will allow natural cheese to be heated and mixed into a homogenous mass (Kosikowski & Mistry, 1997), which would not be possible without the salts. Emulsifying salts in process cheese with help keep the pH stabilized which is critical for the body, texture and spoilage (Kosikowski & Mistry, 1997). They will also isolate the calcium in the natural cheese paracasein network, and soluablize the protein for fat, protein and water to create a homogenous mass (Garimella Purna et al., 2006).

## 2. Materials and Methods

Natural Cheddar cheese was sent to SDSU to be analyzed for composition and proteolysis over time. The composition was analyzed for pH, fat, salt, moisture, salt-to-moisture ratio (S/M), ash minus salt, ash, and total protein. Proteolysis was analyzed on days 15, 30, 60, 90, and 120. Samples were studied for compositional effect on pH 4.6-soluble N, 4.6-soluble N as a % of TN, TCA-soluble N, and TCA-soluble N as a % of TN. All of the cheeses were then placed into the Owl Software TechWizard. The formula was standardized to 44% moisture, 30% fat, 17% protein, 2.5% salt, and 3.0% sodium citrate for company 1 and 2, Legal cheddar cheeses. Company 3, legal reduced fat cheese, samples were standardized to 45.5% moisture, 18% protein, 22% fat, 2.5% salt, and 3% sodium citrate. Each formulation used one of the 27 cheese variables with butter/AMF, deproteinized whey, sodium citrate, water, and salt, as shown in Table 3. Example Recipe Formula for Company 1 Sample 2 Legal Cheddar Cheese, and Table 4. Example Recipe Formula for Company 3 Sample 6 Legal Reduced Fat Cheddar Cheese.

The 27 samples were made into 300g batches. Then the formulations were mixed with a KitchenAid blender until it was a homogenous paste. Company 1 and 2 used cheeses aged at 15, 30, 60 and 120 days, totaling 21 samples. Company 3 used cheeses aged at 15, 60, and 120 days, 30-day sample was unavailable. All 27 samples used only 1 cheese formula. The samples were then placed into the Rapid Visco Analyzer (RVA) for manufacture, and the melted cheese was poured into molds for further analysis. The molded samples were then analyzed through texture profile analysis (TPA) and Schreiber melt. In this study, PCF was made based on CFR Title

21 Ch 1 Section 133.173 for Companies 1 and 2 and for the reduced-fat cheese of Company 3.

## 2.1 RVA

### 2.1.1 Sample Preparation

TechWizard was used to input each cheese and formulate each sample for Company 1 and 2 to 17% protein, 2.5% salt, 44% moisture, 30% fat, and 3% sodium citrate. This is displayed in a breakdown of ingredients in Table 3. Example Recipe Formula for Company 1 Sample 2 Legal Cheddar Cheese. Company 3, legal reduced fat cheese, samples we reformulated to 45.5% moisture, 18% protein, 22% fat, 2.5% salt, and 3% sodium citrate. An example formula for company 3 is in Table 4. Example Recipe Formula for Company 3 Sample 6 Legal Reduced Fat Cheddar Cheese. Once the ingredients were weighed, a KitchenAid mixer was used to turn it into a homogeneous paste. Twenty-five grams of cheese paste were then weighed into a clean, washed, and dried aluminum canister, and 500 µL of water was pipetted into each canister. Finally, the canisters were placed into the water bath at 35 °C for 15 minutes.

### 2.1.2 Analysis

The Perten RVA 4500 was powered on to allow the machine to warm up. The faucet was attached, and the computer system turned on. The TCW3 computer program was then opened on the computer. On the RUN drop-down menu, SELECT CONFIGURATION TO RUN was clicked, and the RVA file

was chosen. For the first 2 minutes of the analysis, the speed was 1000 RPM and the temperature 95 °C. For minutes 2 to 3, the speed was set at 160 RPM so the apparent viscosity could be measured.

## 2.2 TPA

### 2.2.1 Sample Preparation

Using a 10 mm diameter cylinder mold, melted process cheese was poured from the RVA canisters into 5 molds. Once the samples had been refrigerated at 5 °C for 24 hours, they were tempered at room temperature for 30 minutes and cut to 20 mm in height. The sample was placed under the probe.

### 2.2.2 Analysis

The computer and the Texture Analysis Software Exponent were started. Username and password were filled in. On the next screen, TA Settings and Library were selected. From the sequence library, the seventh CYCLE UNTIL COUNT setting was clicked. The settings were ensured to be correct (Test Mode: Compression, Pre-test Speed: 1.00mm/sec, Test Speed: 2.00 mm/sec, Post Test Speed: 2.00mm/sec, Target Mode: Strain, Strain: 10%, Count: 2, Trigger Type: Force, Trigger Force: 0.0050 kg). The machine was then calibrated by clicking on the TA drop-down menu, and the height was calibrated and set at 20 mm. To run a test, the drop-down menu Run a Test was selected, and the file ID and where to save the documents

were specified. Pick the TPA macro and TA method. Finally, the image and data were exported, and the data were saved in an excel spreadsheet.

### 2.3 Schreiber Melt

Using a 30 mm diameter mold, melted process cheese was used for the molds. The process cheese was then cut into seven mm thick slices. The cheese slices were weighed, and the five slices that were closest in weight were chosen, with a coefficient of variance of weights within 3%. The glass petri dishes were labeled, and the cheese was placed in the center of the dish. The oven rack was removed from the second shelf and the temperature set at 100 °C. The oven was preheated for at least 30 minutes. The plates were left at room temperature for 30 minutes. Following this, the rack with the plates were quickly placed in the oven. The cheeses were left to melt for seven minutes and to cool for 30 minutes. The cheese was measured for the length of flow.

## 3. Results and Discussions

### 3.1 Table 1. Formula for Process cheese food for Cheddar Cheese Samples

Table 1 presents the formulas that were used for Companies 1 and 2. The moisture was calculated to 44%, fat 30%, protein 17%, salt 2.5%, and sodium citrate 3.0%. SDSU received 27 cheese samples from three different United States companies from three different states. Each of the cheeses was used as the main ingredient for process cheese. The ingredients used for the process cheese formulas

of Companies 1 and 2 were unsalted butter, AMF, deproteinized whey, water, salt, and sodium citrate. The formulas were made using Owl Software's TechWizard.

### 3.2 Table 2. Formula for Process cheese-like food for Reduced Fat Cheddar Cheese Sample

Table 2 illustrates the formula used for Company 3 that was made with reduced-fat Cheddar cheese. Company 3 used a different formula for process cheese because its reduced-fat cheese did not conform with the study's ingredient constraints. The reduced-fat samples used 2.5% salt, 22% fat, 18% protein, 45.5% moisture, and 3% sodium citrate. The samples came from a cheese company that sent it to SDSU in 40 lb blocks. The samples were previously studied for composition and proteolysis and were analyzed for the effects proteolysis and composition had on the process cheese. The major ingredients used in the process cheese were the reduced-fat cheese samples; the formula used unsalted butter/AMF, sodium citrate, salt, water, and deproteinized whey. The formulas were made using Owl Software's TechWizard.

### 3.3 Table 3. Example Recipe for Process Cheese Food for Company 1 Sample 2 Legal Cheddar Cheese

Table 3 is an example of a formula for Company 1 Sample 2. Each legal cheddar cheese composition was input into Owl Software's TechWizard. All the cheeses had different composition that was analyzed at 30 days for pH, fat, salt, moisture, S/M, ash and total protein. The formulas were made from unsalted butter, sodium citrate, salt,

water, each cheese variable, whey deproteinized, butter oil. Cheese had the highest addition in the formula. Water was also an ingredient that was added at an increased rate, this is because the formula was targeted for a 44% moisture. All formulas had the same amount of sodium citrate added. All the formulas were slightly different based on the composition of Company 1 and 2, and had targets of 2.5% salt, 30% fat, 17% protein, and 44% moisture.

### 3.4 Table 4. Example Recipe of Process Cheese Spread for Company 3 Sample 6 Legal Reduced Fat Cheddar

Table 4 is an example formula for Company 3 sample 6. Company 3 reduced fat cheese had a different composition than a full fat cheese. All 6 of Company 3's cheese composition was input into Owl Software's TechWizard. Each of the cheese variable were analyzed at 30 days for pH, fat, salt, moisture, S/M, ash and total protein. All the cheese formulas used the same ingredients as Company 1 and 2, unsalted butter, sodium citrate, salt, water, cheese sample, whey deproteinized, and butter oil. The formula fit the specifications for a process cheese spread (21CFR133.179). Formulas were competed to target 2.5% salt, 22% Fat, 18% Protein, 45.5% moisture, and 3% sodium citrate.

### 3.5 Figure 1. Company 1 RVA Apparent Viscosity

Figure 1 displays apparent viscosity, also known as viscosity. A normal trend would be that all samples would trend down as time goes on. Sample 2, Sample 6, Sample 8, Sample 9, Sample 10, and Sample 11 did not display that. The samples

would have created an expected model that the apparent viscosity increases, or the sample becomes less viscous, over time if 15 days would have been more viscous. This could have been due to the curd not being broken down and the mixture not being mixed well enough. Sample 1 and Sample 6 had the lowest amount of apparent viscosity at day 120. Sample 3 had a greater apparent viscosity at day 15. Sample 1, Sample 3, and Sample 10 had similar days 30 and 60 apparent viscosity. Sample 7 were similar apparent viscosity at 60 and 120 days. Sample 6 and Sample 11 had a high day 30. Sample 3, Sample 4, and Sample 5 had high apparent viscosity at day 120.

Sample 1 had a high pH 4.6-soluble N as a % of TN, and Sample 6 had a high TCA-soluble N as a % of TN. The more broken down the peptides were, the more flow Samples 1 and 6 had. Sample 1, Sample 3, and Sample 10 were similar from day 30 to day 60 because there was less breakdown in those 30 days. Lawrence et al. (1986) claims the texture of the cheese alters considerably in the first two weeks, when the cheese is rubbery in texture, and then will become smoother and more homogenous as a result of the cleavage. This is the first phase in the development of texture during cheese ripening. The texture changes occurring after this phase happen at a more gradual pace over months rather than days (Lawrence et al., 1986). Sample 6 and Sample 11 had the same ash content and similar protein content in the natural cheese. Sample 3, Sample 4, and Sample 5 had similar ash minus salt, which could have contributed to the high apparent viscosity at day 120.

### 3.6 Figure 2. Company 2 RVA Apparent Viscosity

Figure 2 displays the apparent viscosity of the company's 2 cheese samples. All the samples had expected downward trends over time except Sample 4, where day 15 had more apparent viscosity than day 30. This could have been from the cheese being young and unable to work into the mixture when blended in the KitchenAid mixer. Sample 5 had the highest day 15 and day 30 of the sample set. Samples 8 and 10 had the lowest amount of apparent viscosity. Sample 1, Sample 3, Sample 6, and Sample 9 had similar day 30 and 60 apparent viscosity. Sample 2, Sample 4, and Sample 8 had similar day 60 and day 120.

Sample 5 had the highest day 15 and day 30, and the natural cheese had low moisture and was low in ash minus salt. Sample 5 also had the highest amount of TCA-soluble N s of all the samples in the sample set at day 30. Samples 8 and 10 had low ash minus salt, lower moisture, and higher fat. Sample 8 also had the highest amount of TCA-soluble N as a % of TN, and Sample 10 had the highest pH 4.6-soluble N as a % of TN. The samples similar at day 30 and 60 were similar in pH 4.6-soluble N as a % of TN. Sample 2, Sample 4, and Sample 8 were similar in pH 4.6-soluble N as a % of TN at day 60 and day 120.

### 3.7 Figure 3. Company 3 RVA Apparent Viscosity

Figure 3 depicts the apparent viscosity of Company 3’s cheese samples. All the samples had an expected trend in which they became less viscous over time. Company 3’s samples did not have a day 15 or 30, as day 15 and day 30 cheese samples were not available to be taken from the block. Sample 4 had the highest amount of apparent

viscosity at day 60. Sample 4, Sample 5, and Sample 6 had the lowest apparent viscosity at day 120. Sample 5 had similar day 60 and day 90 apparent viscosity results. Sample 4 had the highest amount of pH 4.6-soluble N as a % of TN. Sample 4, Sample 5, and Sample 6 had similar pH 4.6-soluble N as a % of TN at day 120. Sample 5 had similar amounts of pH 4.6-soluble N as a % of TN between the time points.

### 3.8 Figure 4. Company 1 TPA Hardness

According to Figure 4, some samples from Company 1 exhibited an expected decreasing trend of TPA hardness over time. Sample 2, Sample 6, Sample 8, Sample 9, Sample 10, and Sample 11 had less force at day 15 than at day 30. This could be because the casein had not yet broken down or because the sample did not sufficiently mix in the KitchenAid mixer. Samples 1 and 6 had very low force at day 120. Sample 1, Sample 3, and Sample 7 had high amounts of hardness at day 15. Sample 6 and Sample 11 had high amounts of hardness at day 30.

Samples 1 and 6 had low hardness at 120 days, Sample 1 had the highest amount of pH 4.6-soluble N as a % of TN, and Sample 6 had a high amount of TCA-soluble N as a % of TN. Sample 1 had an expected cheese composition, Moisture 37%, protein 25%, fat 33%, ash 4%, pH 5.10, salt 1.80%, and S/M4.86 (Ayala-Bribiesca et al.,2016). Sample 6 had the highest pH of the sample set in natural cheese, as well as higher fat, lower moisture and total protein. Texture profile analysis is a common technique to measure unmelted texture in process cheeses (Kapoor and Metzger, 2008).

### 3.9 Figure 5. Company 2 TPA Hardness

Figure 5 displays the expected results for TPA hardness, with all the samples decreasing in hardness over time. Sample 5 had a high force at day 15 and day 30. Sample 2, Sample 9, and Sample 10 had high hardness at day 15. Sample 5, Sample 7, and Sample 10 had very low hardness at day 120. The natural cheese pH was very similar across Samples 5, 7, and 10 and could have contributed to the low force at day 120.

### 3.10 Figure 6. Company 3 TPA Hardness

Figure 6 illustrates the expected results for TPA hardness, with all the samples decreasing in hardness over time. Samples 3 and 4 were higher than the other samples at days 30 and 60. Samples 4 and 5 had lower hardness at day 120. Day 15 was not available due to not having sample 15 natural cheese. Samples 4 and 5 had low 120-day hardness. Sample 4 had a high pH 4.6-soluble N as a % of TN, and Sample 5 had a higher TCA-soluble N as a % of TN.

### 3.11 Figure 7. Company 1 Schreiber Melt Test

In Figure 7, all samples increased over the time points, which was to be expected, because as cheese ages the intact casein decreases because of proteolysis (Fenelon and Guinee, 2000). Vakaleris (1962) study described that as cheese aged and proteolysis increased the samples melting properties increased. Sample 3 had the lowest melt on day 15. Sample 10 had the smallest amount between days 15, 30, and 60. Sample 1 had the highest amount of melt at day 120. Sample 3 day 15 had a large

TCA-soluble N as a % of TN. Sample 1 day 120 had the highest melt and the largest pH 4.6-soluble N as a % of TN. Templeton and Sommer (1930), Olson et al. (1958), Vakaleris et al. (1962), Piska and Stetina (2003), and Garimella Purna et al. (2006) all found that the unmelted texture of the resulting process cheese decreased when increasing the age of natural cheese used in process cheese manufacturing (Kapoor et al., 2007). Moreover, Olson et al. (1958), Vakaleris et al. (1962), and Garimella Purna et al. (2006) noted an increase in the meltability of the resulting process cheese (Kapoor et al., 2007).

### 3.12 Figure 8. Company 2 Schreiber Melt Test

As illustrated in Figure 8, all samples for Company 2 increased over time for the melt test. Sample 1 had similar days 60 and 120. Samples 5 and 10 had the highest amount of melt at day 120. Sample 10 was similar at days 30 and 60. Sample 1's natural cheese had a low salt, low ash, lower pH and low S/M. The natural cheese of Sample 5 and Sample 10 had similar pH, similar S/M, and similar salt minus ash.

### 3.13 Figure 9. Company 3 Schreiber Melt Test

As depicted in Figure 9, all samples of Company 3 increased over time, which was expected, as the intact casein decreases over time due to proteolysis. Sample 4 had the lowest melt at days 60 and 90 compared to all others in the sample group. Sample 5 had the highest melt among the samples at day 90. Sample 1, Sample 2, Sample 3, and Sample 6 had similar 60- and 90-day results; they were different when compared against other samples in the set but similar to each other. Day 60 and 90 day had the

highest pH 4.6-soluble N as a % of TN for Sample 4. Finally, Sample 5 had the highest amount of TCA-soluble N as a % of TN, while Sample 1, Sample 2, Sample 3, and Sample 6 had similar natural cheese compositions.

## 4. Discussion

Process cheese is made by heating a stirred blend of a variety of natural cheeses and other ingredients and using emulsifying salts (Kalab et al., 1991). In the present study, 27 different cheese blocks were tested using a formula for Companies 1 and 2 and a second formula for company 3. The cheeses for Companies 1 and 2 were made into process cheese at 15 days, 30 days, 60 days, and 120 days. Those for Company 3 were made into process cheese at 60 days, 90 days, and 120 days. Fewer time points were taken for Company 3 because the cheese was unavailable.

### Apparent Viscosity

In the current study the samples that had a higher apparent viscosity had a lower amount of TCA proteolysis, higher pH, higher S/M. Kapoor et al. (2007) found that a higher S/M would have a higher apparent viscosity. Lower apparent viscosity had a lower S/M, and low pH 4.6 soluble N as a % of TN, and Kapoor et al. (2007) found similar findings. Natural cheese age affected the apparent viscosity, as the cheese ages the apparent viscosity decreases, and Garmella et al. (2006) found similar findings. The apparent viscosity decreases and firmness increases as the cheese ages (Garimella et al., 2006). Overall company 3 Process Cheese Spread (PCS) was the lowest viscosity after manufacture (VAM), and Company 1 was lower in VAM than

Company 2. Company 1 had lower pH's overall, and lower Ash minus salt results which could contribute to having the lowest VAM of PCF.

**Hardness**

In the study samples with lower S/M, increased rate of pH 4.6 soluble N as a % of TN, and lower ash minus salt had the least amount of hardness. The samples that were the hardest had the highest amount of S/M. Hardness represents the unmelted texture properties and firmness. In the study from Kapoor et al (2007), the texture profile analysis (TPA) had similar findings that as the cheese age increases the hardness decreases. In the study from Garimella et al (2006) intact (unhydrolyzed) casein decreases over time which will have a lower hardness. In the current study that was completed this could also be seen to be true.

**Meltability**

The current study correlates with the study from Kapoor et al. (2007). The meltability increased over time for all the samples. The samples that had the highest meltability had increased pH 4.6 soluble N as a % of TN proteolysis, a higher TCA soluble N as a % of TN proteolysis, and a low S/M. The samples that had the lowest amount of melt had the highest S/M. Kapoor et al. (2007) found similar findings with a higher S/M, higher pH has less meltability, and lower S/M, higher proteolysis will have more meltability. Process Cheese apparent viscosity and meltability correlate, and hardness is typically the opposite of those functional properties.

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## 6. TABLES

**Table 1. Formula for Process Cheese Food for Cheddar Cheese Samples**

| Components | Percentage in each Formula |
|---|---|
| Moisture | 44% |
| Fat | 30% |
| Protein | 17% |
| Salt | 2.5% |
| Sodium Citrate | 3.0% |

**Table 2. Formula for Process Cheese Spread for Reduced Fat Cheddar Cheese Sample**

| Components | Percentage in each Formula |
|---|---|
| Moisture | 45.5% |
| Fat | 22% |
| Protein | 18% |
| Salt | 2.5% |
| Sodium Citrate | 3.0% |

**Table 3. Example Recipe Formula for Company 1 Sample 2 Legal Cheddar Cheese**

| Ingredient | Percent of Ingredient (wt/wt) |
|---|---|
| Butter (unsalted), % | 3.35 |
| Sodium Citrate, % | 3.00 |
| Salt, % | 1.31 |
| Water, % | 16.89 |
| Sample 2 Cheese, % | 72.18 |
| Whey Deproteinized, % | 3.27 |
| Butter Oil, % | 0.00 |

**Table 4. Example Recipe Formula for Company 3 Sample 6 Legal Reduced Fat Cheddar Cheese**

| Ingredient | Percent of Ingredient (wt/wt) |
|---|---|
| Butter (unsalted), % | 6.93 |
| Sodium Citrate, % | 3.00 |
| Salt, % | 1.36 |
| Water, % | 10.82 |
| Sample 6 Cheese, % | 74.35 |
| Whey Deproteinized, % | 3.53 |
| Butter Oil, % | 0.00 |

## 7. FIGURES

**Figure 1. Company 1 RVA Apparent Viscosity**

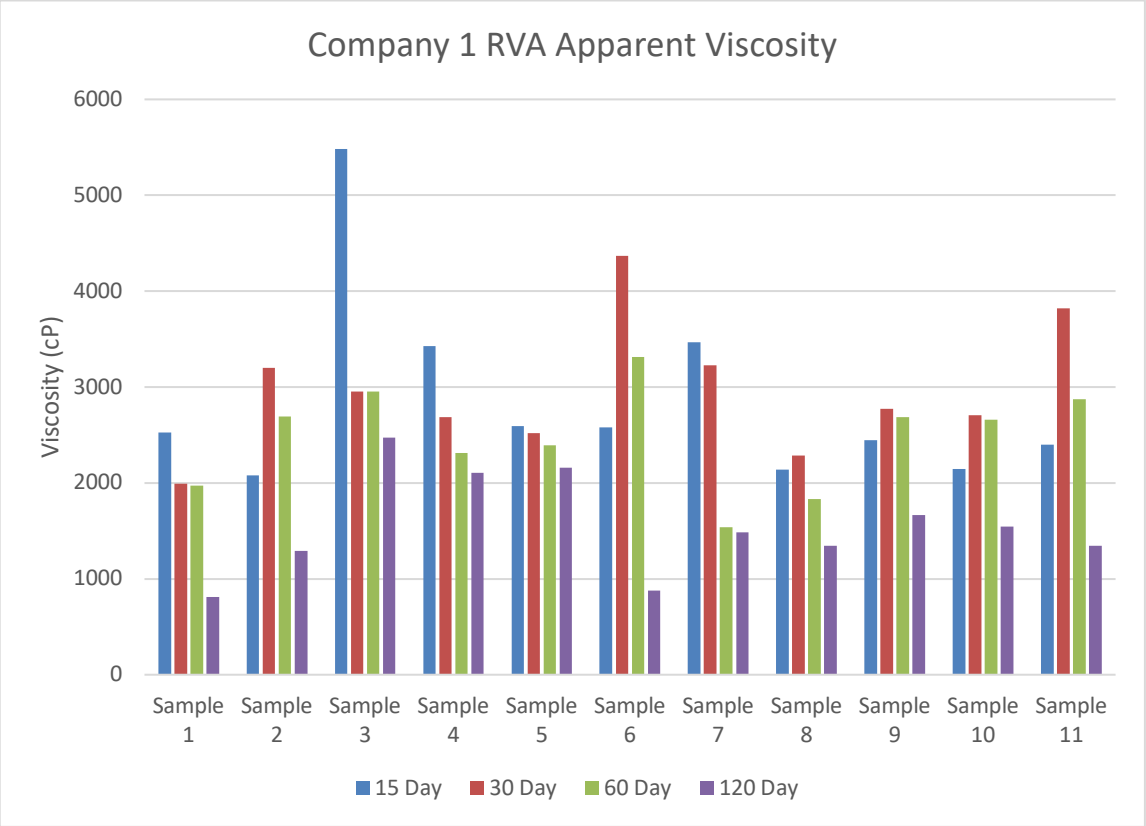


**Figure 2. Company 2 RVA Apparent Viscosity**

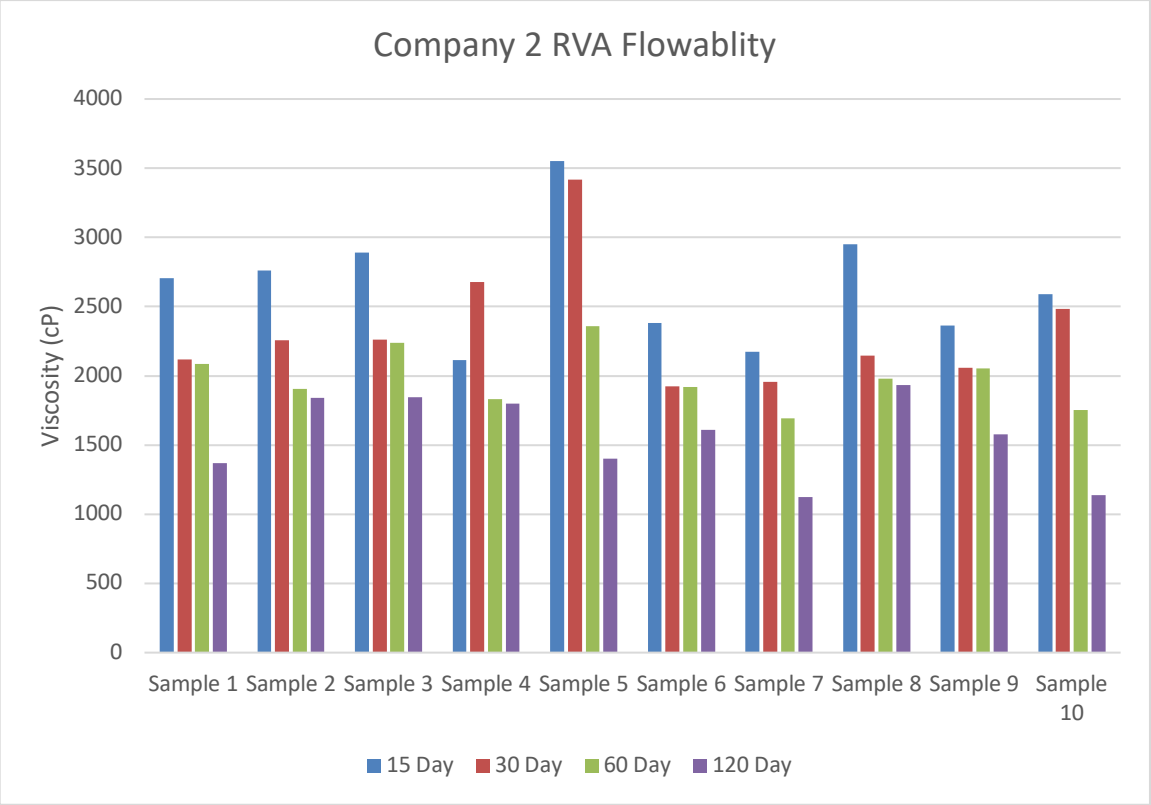


**Figure 3. Company 3 RVA Apparent Viscosity**

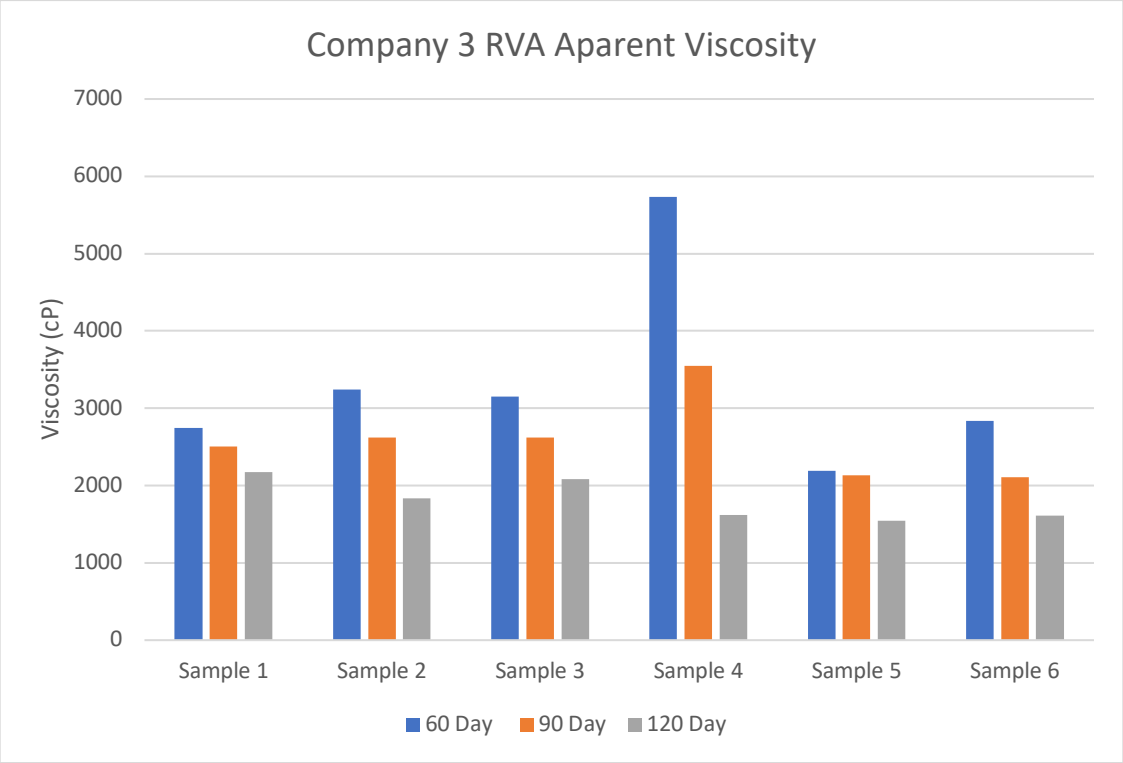


**Figure 4. Company 1 TPA Hardness**

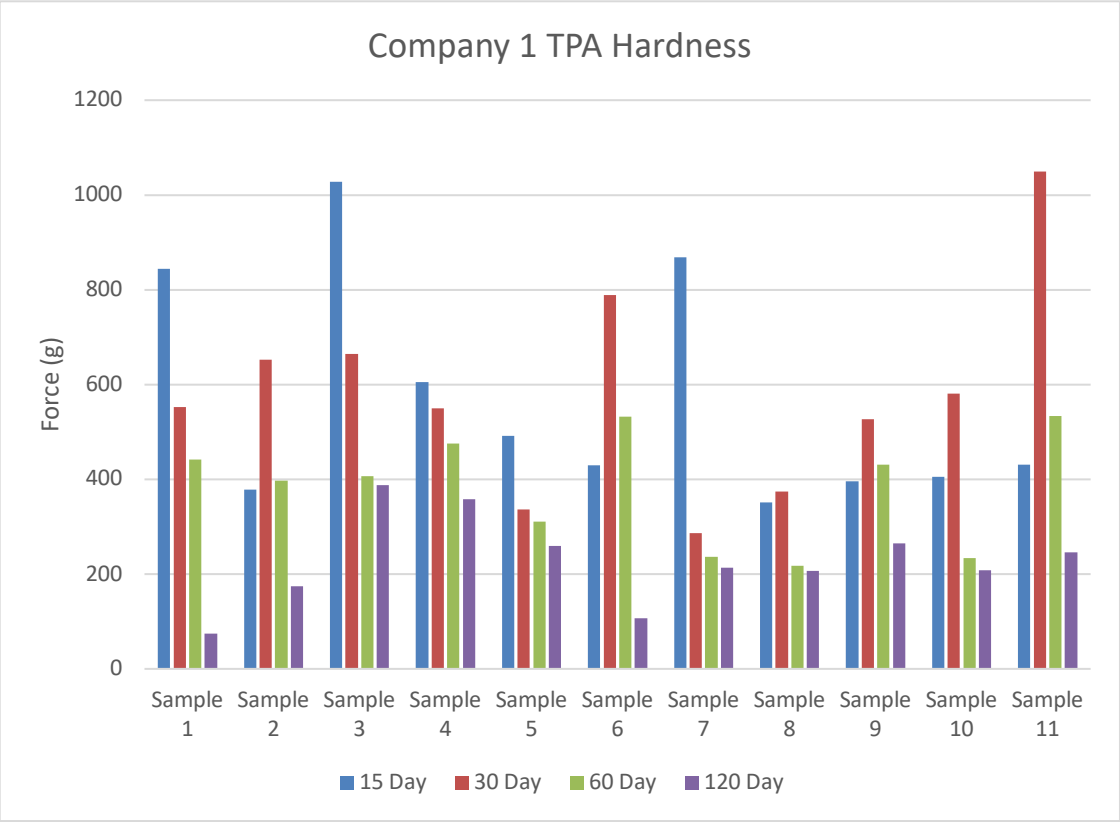


**Figure 5. Company 2 TPA Hardness**

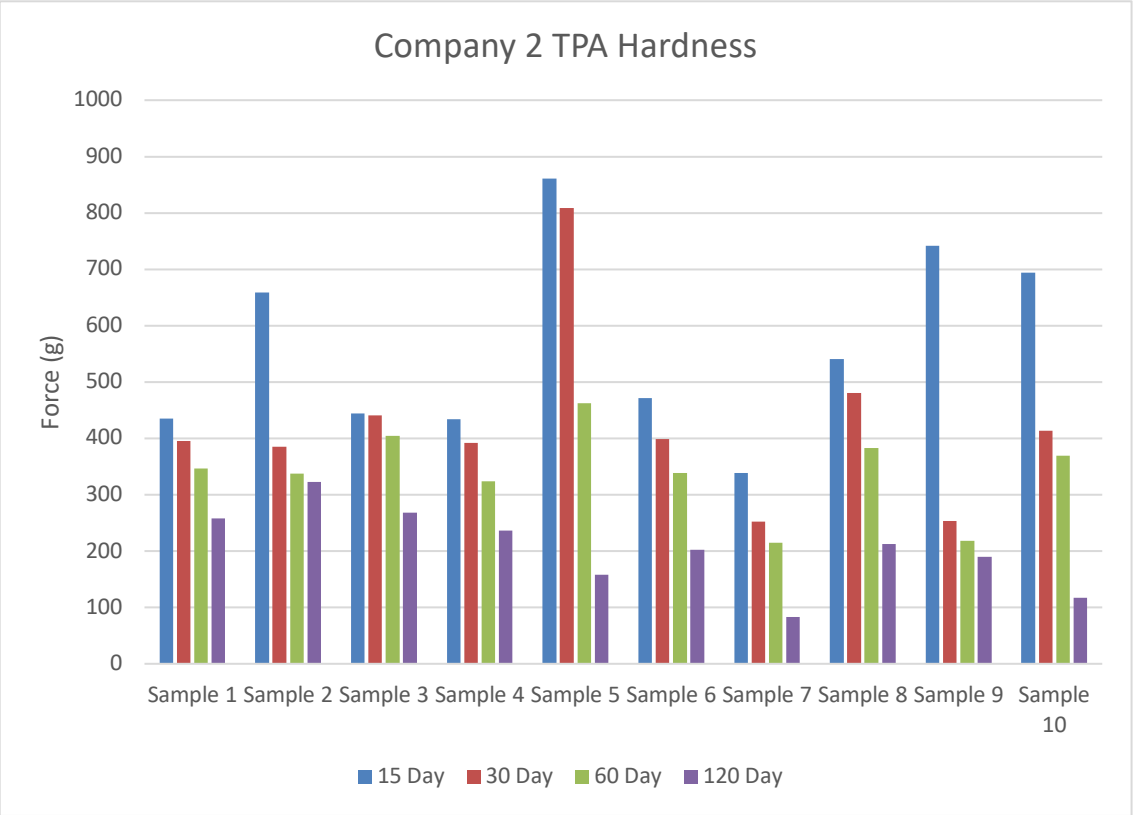


**Figure 6. Company 3 TPA Hardness**

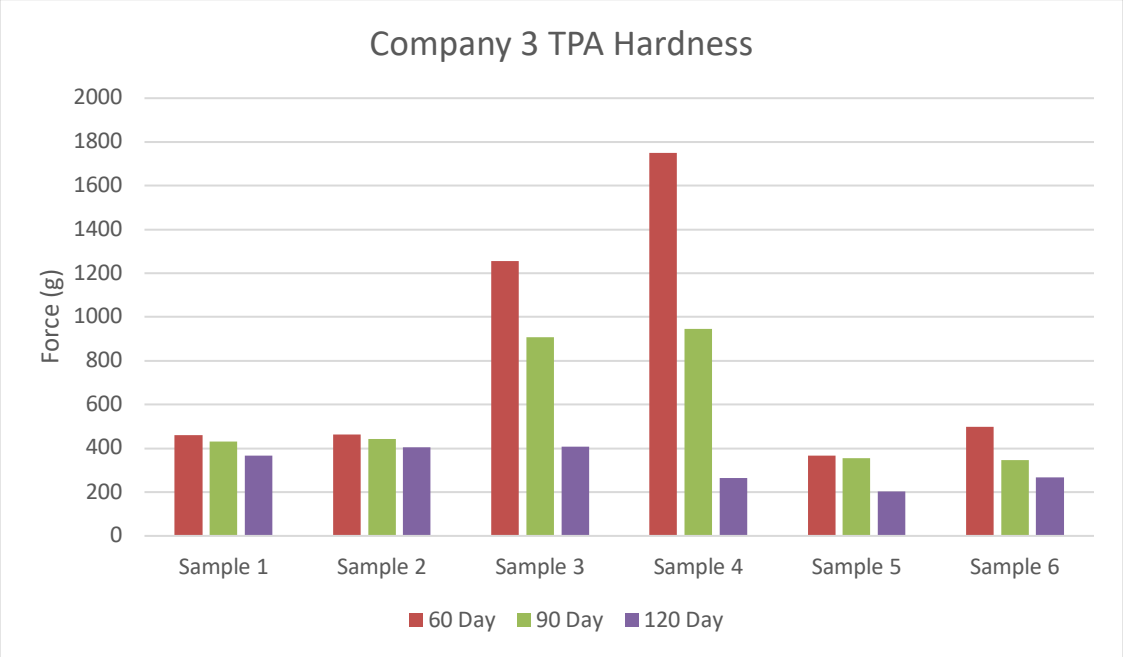


**Figure 7. Company 1 Schreiber Melt**

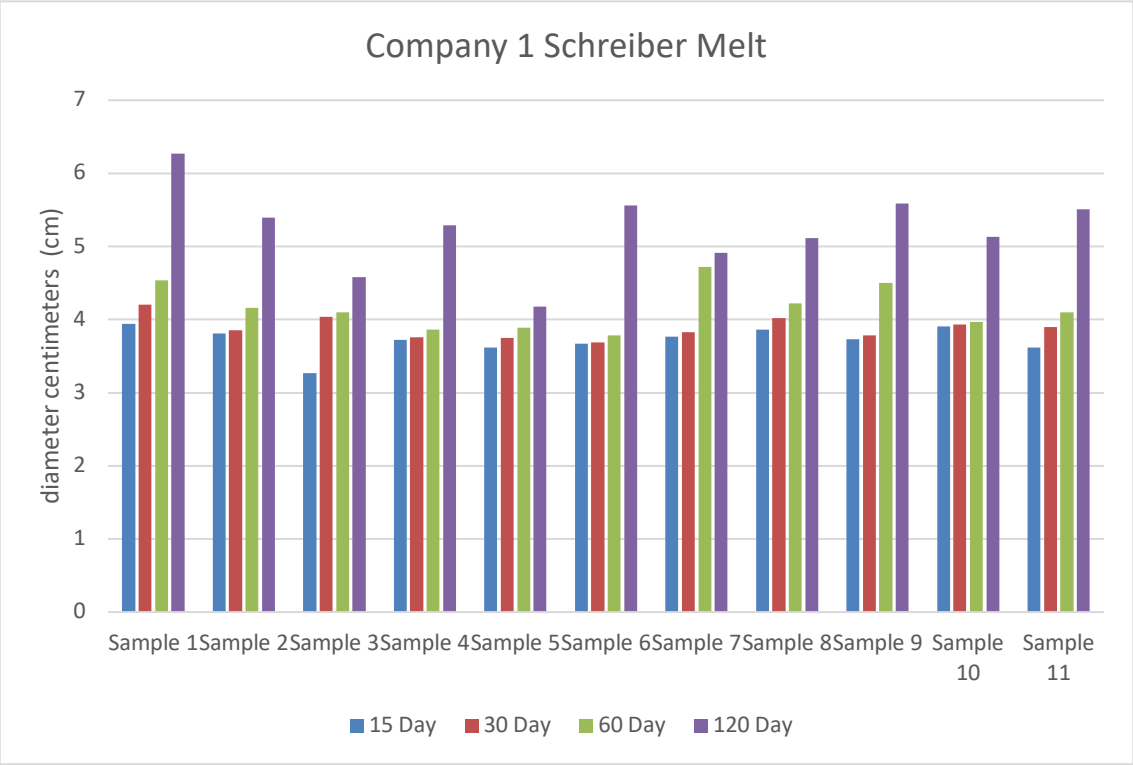


**Figure 8. Company 2 Schreiber Melt**

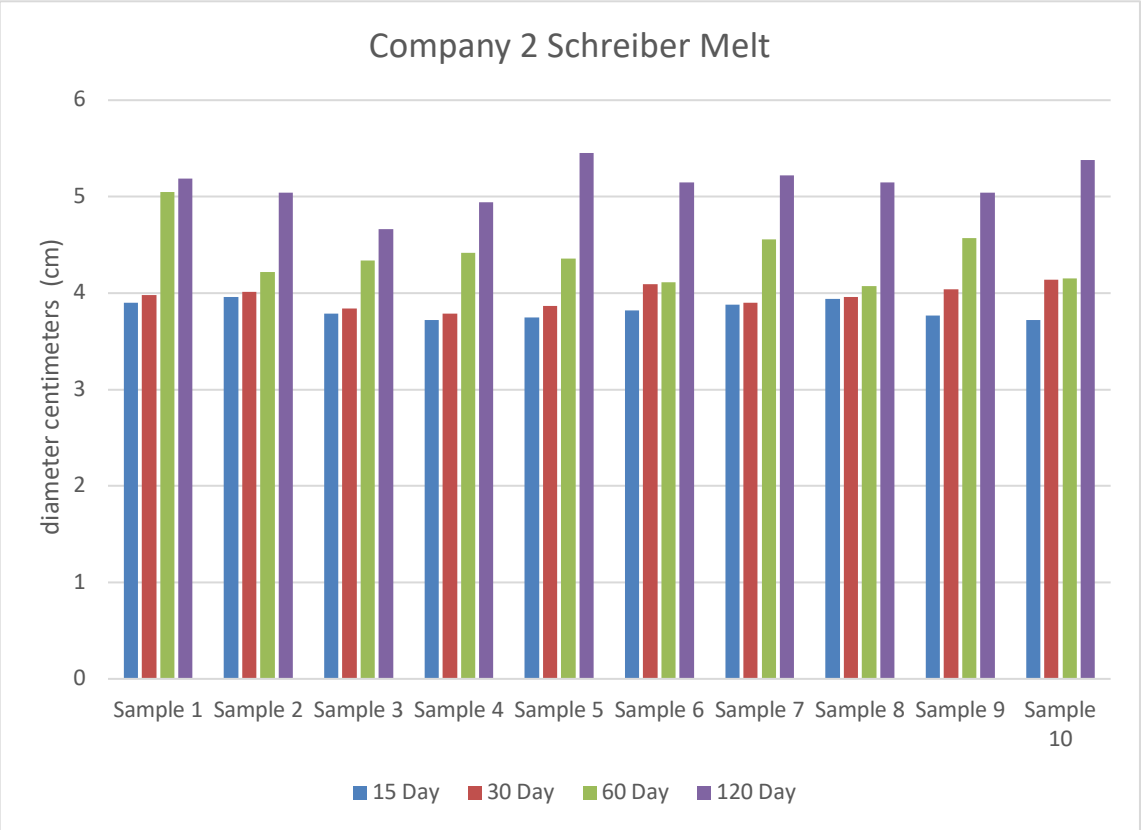


**Figure 9. Company 3 Schreiber Melt**

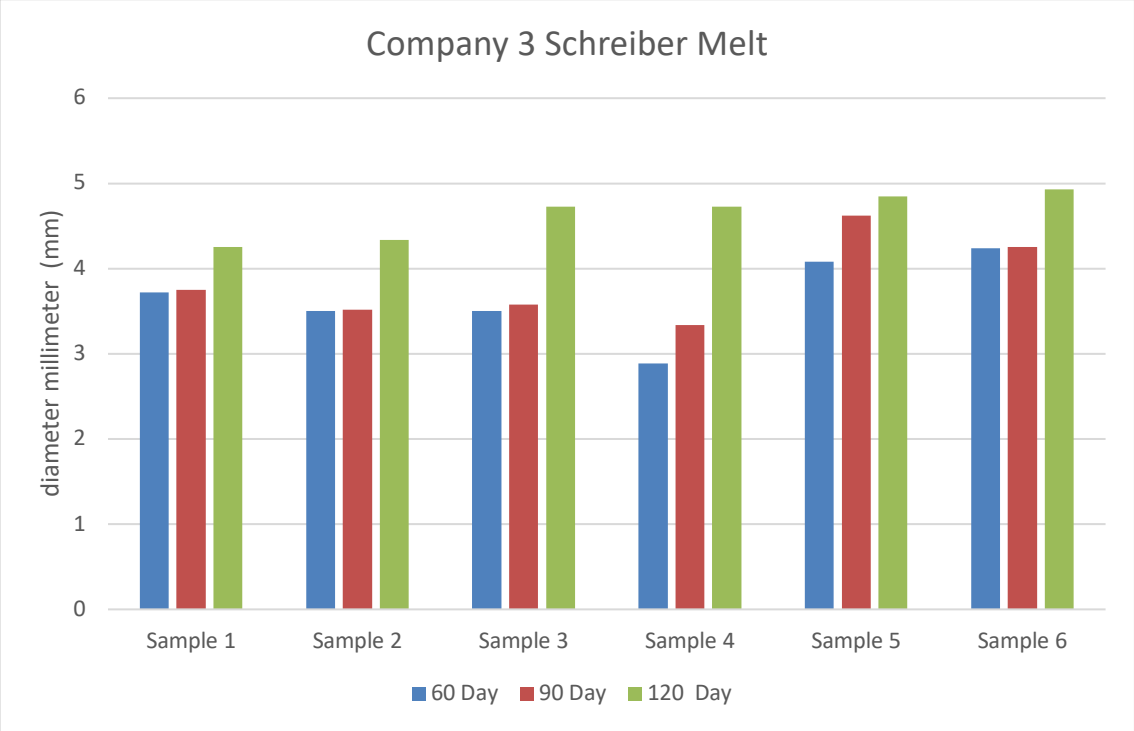
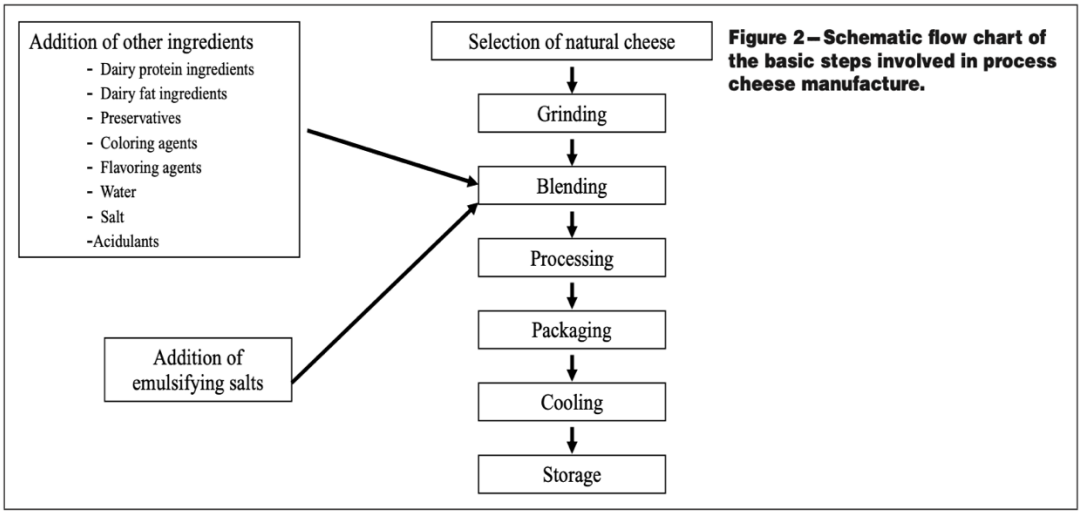



**Figure 10. Figure 2 Schematic flow chart of the basic steps involved in process cheese manufacture (Kapoor and Metzger, 2008).**



# CHAPTER IV. OVERALL CONCLUSIONS AND FUTURE WORK

The cheese that SDSU received was unknown cheese that makes coagulants and starter cultures. The natural Cheddar cheese proteolysis of pH 4.6-soluble N, pH 4.6-soluble N as a % of TN, TCA-soluble N and TCA-soluble N as a % of TN was affected by moisture, fat, salt, and the S/M ratio. The samples that had an expected composition for natural cheese also had high rates of proteolysis. In all cheese samples, as the cheese aged, the samples increased in proteolysis. The samples increasing in proteolysis over time was to be expected.

Future research should investigate more varieties of cheese factors, such as a broader range of defects, a more extensive pH range, larger moisture range, larger salt range, and a more extensive fat range. In future work, Ca, P, and lactose should be studied with the natural cheese composition and the TCA-soluble N as a % of TN and pH 4.6-soluble N % of TN. I also recommend a different study focusing on reduced-fat Cheddar cheese with wide pH, moisture, salt, and fat ranges. In the reduced-fat fat study, I also suggest including analyses on lactose, Ca, and P.

Process cheese was affected by the pH of the natural cheese, the cheese's age, the amount of proteolysis, and the ash minus salt. The intact casein was a factor that changed with age. In the study that we performed, the functionality of PC decreases over time. In the future, select ingredients that would enable having one recipe for pasteurized process cheese, a wider range of defects of the natural cheese to analyze effects of composition, and analyze Ca, P and lactose for process cheese.