THE IMPACT OF INTRINSIC AND EXTRINSIC FACTORS ON THE SAFETY AND QUALITY OF HARD AND SEMI-SOFT NATURAL CHEESE

by

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Abstract

This paper reviews the safe production of hard and semi-soft natural cheeses made from pasteurized milk, starter cultures, and enzymatic coagulation. Historically, raw milk has been a source of pathogenic bacteria; however, High Temperature Short Time (HTST) pasteurization has been proven to effectively control these pathogens. The US Public Health Service (USPHS) and US Food and Drug Administration (FDA) promulgate the legal operational parameters in the Pasteurized Milk Ordinance (PMO) and Code of Federal Regulations (CFR) to ensure milk is properly pasteurized and that dairy products are made in accordance with these regulatory standards.

A combination of factors in the production of these natural cheeses further inhibits microorganisms. Intrinsic factors include pH, oxidation-reduction potential, water activity, nutrient content, natural inhibitors, and physical integrity. Extrinsic factors include temperature, relative humidity, gaseous environment, cumulative stress, and storage time. These factors contribute to a multiple hurdle effect that inhibits pathogens and spoilage organisms while also providing operational parameters to ensure flavor, texture, and other quality targets.

Hard and semi-soft natural cheeses have been associated with few cases of food borne illness over the last few decades. Nevertheless, many operations in the dairy industry have voluntarily implemented food safety systems such as HACCP to ensure the continuous safe production of hard and semi-soft natural cheese.

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Acknowledgements

I would like to thank Jessica, Shiloh, and Siena for their patience and support. I would also sincerely like to thank John Boortz and Steve Yeager for their mentorship.

Dedication

This work is dedicated posthumously to my mother, Delsie Oleta Beard, for teaching me patience and perseverance.

Preface

“I have had dreams and I have had nightmares, but I have conquered my nightmares because of my dreams.” - Jonas Salk.

CHAPTER 1 - Introduction to Cheese Making

Cheese making is an ancient tradition that began as a way to improve the storage and transportation of milk by nomadic tribes. The art of cheese making was further developed in Europe by Trappist monks in the 14th Century. Regional differences in milk and cheese making techniques affected the texture and flavor of the cheese from one monastery to another (Fung 2007). The development of Cheddar cheese began in the village of Cheddar in England during the 16th century. The original process of cheddaring incorporated piling and re-piling of warm curd in a cheese vat for two hours. Jesse Williams and Robert McFadden pioneered industrial cheese making in America in the 1800s (Fung 1995). Today, numerous varieties of natural cheeses are produced commercially. Natural cheeses use bacterial cultures and enzymes, as opposed to direct acidification, to coagulate the milk into cheese curds for curing. Cheeses have standards of identity and nomenclature that have been legally defined. The US Food and Drug Administration (FDA) and Codex Alimentarius have promulgated legal nomenclature based on three characteristics: hardness, fat content, and type of curing (Nelson 1984). Natural "American-style" (e.g., Cheddar, Colby, Monterey Jack) and "Italian Style" (e.g., pasta filata Mozzarella and Provolone) cheeses have legal manufacturing specifications and are categorized as hard or semi-soft (US FDA 2007).

Although natural cheese making has undergone many technological changes over the years—for example, cheddaring may now be accomplished with large-scale cheese belts capable of stirring or matting, rather than by hand piling (Thunell and Burningham 2007)—the basic premise has remained the same. The pH of milk is lowered naturally using "starter" bacteria, and the milk is enzymatically coagulated in a process called "renneting" to yield cheese curd. The curd is then salted, compressed into form, and allowed to "ripen." The process facilitates the concentration of milk fat and protein by the syneresis of water, lactose, and minerals in the form of whey (University of Guelph 2009). This seemingly simple process incorporates a broad range of complex chemical and microbiological interactions. This paper explores the relationship of components, intrinsic parameters, extrinsic parameters, and other microbiological considerations relative to the total quality of two natural cheese categories: hard and semi-soft.

Milk Components

Milk Fats

Natural cheese quality begins with fresh raw milk. Milk is legally defined in §131.110 of the Code of Federal Regulations Title 21 (21 CFR) as "the lacteal secretion, practically free of colostrum, obtained by the milking of one or more healthy cows" (US FDA 2007, pg. 296). Milk is typically comprised of 85-88% water, 4-5% lactose, 3-5% fat, 3-4% protein, and ≤1% minerals such as calcium (Christen and Smith 2000). However, milk may vary among species of cow, and milk from the common domestic cow, *Bos taurus*, also varies greatly among breeds such as Jersey and Holstein (Coulon et al. 2009). Studies show that concentrations of milk fat (4.7-3.3%) and protein (3.8-3.0%) were greater for Jersey cows compared with Holstein cows (Andrew 2000). As shown in Table 1, ruminant milk fat (butterfat) is made up of a combination of long and short chain fatty acids.

Table 1: Typical Fatty Acids Present in Milk

Unaturated Fatty Acids	Saturated Fatty Acids
C18 - Oleic acid	C4 - Butyric acid*
C18 - Linoleic acid	C6 - Caproic acid
C18 - Linolenic acid	C8 - Caprylic acid
C18 - Arachidonic acid	C10 - Capric acid
	C12 - Lauric acid
	C14 - Myristic acid
	C16 - Palmitic acid
	C18 - Stearic acid

** butyric fatty acid is responsible for the rancid flavors when cleaved from glycerol by the enzyme lipase.*

Adapted from (Tetra Pak, 2003)

Fatty acid composition also varies among breeds. For example, butterfat from Jersey cows contain less oleic acid (cis-C18:1) and more medium-chain fatty acids than that from Holstein cows. The composition of milk from both breeds is greatly affected by a cow's environment and diet (Drackley et al. 2001). These variations contribute greatly to both cheese yield and intrinsic interactions in cheese curing. Emulsified fats also combine with proteins to

form micelles in milk. Micelle structures allow solids to stay suspended in liquid and are an important part of cheese making.

Milk Proteins

Concentrations of milk proteins will also vary among breeds of milk cows such as Jersey and Holstein (Ginger and Grigor 1999). Milk proteins not only contribute to the percentage of total solids, but they also are essential for the coagulation of milk in natural cheese making. Milk proteins contain a combination of all 20 amino acids. Differences in hydrophobic interactions, hydrophilic interactions, calcium binding, and stability (e.g., sulfide bridges) depend upon the properties of the functional R-Groups (Figures 1 & 2) of the amino acids (Smith 2008).

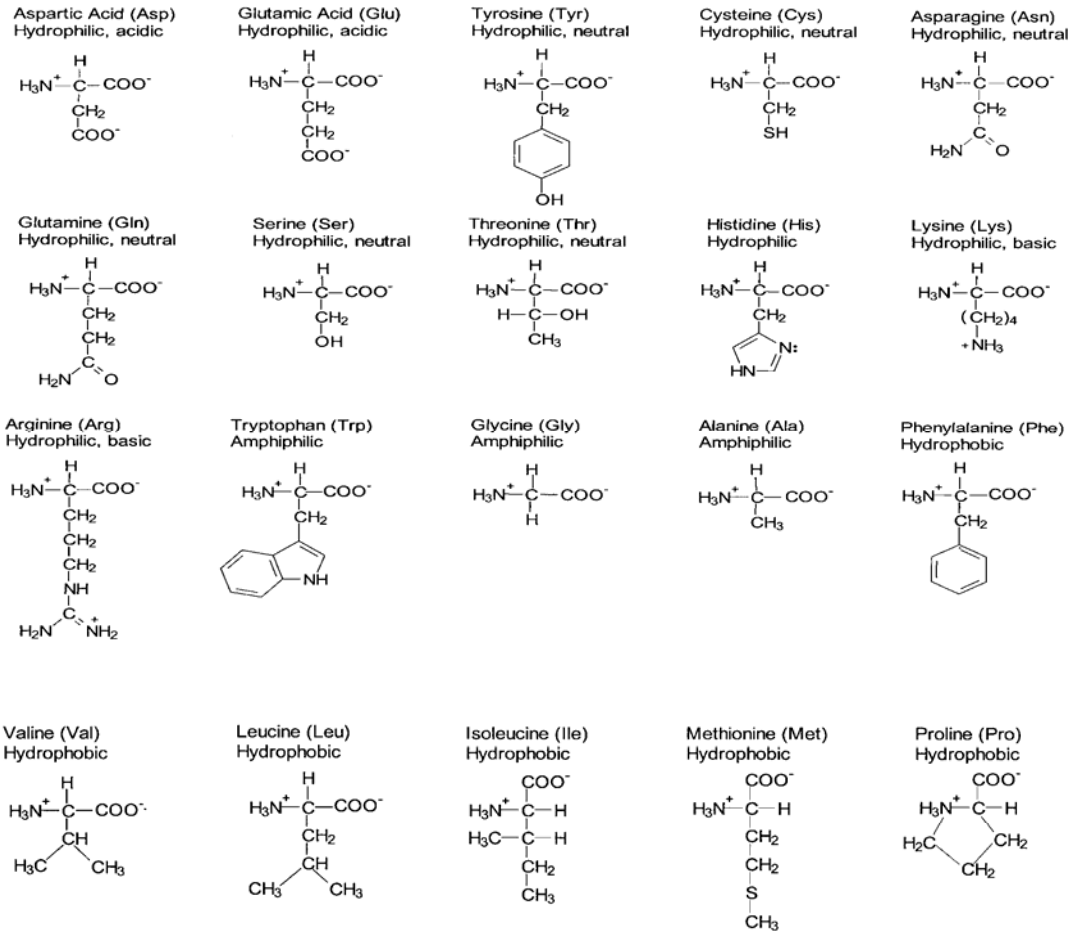

Figure 1: Hydrophilic and Hydrophobic Amino Acids

Adapted from (Smith 2008)

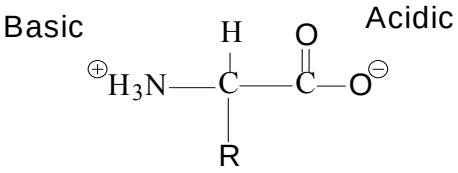

Figure 2: Basic Amino Acid Structure

(Smith 2008)

Milk proteins can be classified into two categories based on their solubility at 68°F (20°C) and pH 4.6; whey proteins remain in solution at pH 4.6 at 68°F, while caseins precipitate (Christen and Smith 2000). Whey proteins are classified as α-(alpha) lactalbumin, bovine serum albumin (BSA), β(beta)-lactoglobulin(LG) A, and β-LG B. Caseins (CN) are classified as α_{S1}(alpha S1)-CN, α_{S2}-CN, β-CN, and κ(kappa)-CN (Wedholm et al. 2006).

Table 2: Total Protein Percentages

Name	g/L	% of total protein
Total Protein	**33.0**	**100.0**
Total Caseins	**26.0**	**80.0**
alpha S1 (α_{S1}-)	10.1	31.0
alpha S2 (α_{S2}-)	2.6	8.0
beta (β-)	9.4	29.0
kappa (κ-)	3.9	12.0
Total Whey Proteins	**7.0**	**20.0**
alpha lactalbumin	1.8	5.0
beta lactoglobulin	3.3	9.3
BSA	0.4	1.2
Immunoglobulin	0.7	2.1
Proteose peptone	0.8	2.4

Adapted from (University of Guelph 2009 and CDR 2002)

Whey Proteins

Whey proteins account for approximately 20% of total proteins in milk (Smith 2008). Variations in whey protein and casein composition both affect milk clotting properties and cheese yield (Wedholm et al. 2008). Whey protein and caseins concentrations are shown in Table 2. Alpha-lactalbumin is essential for the formation of lactose in cows and humans. It is more heat stable than β-LG A and β-LG B. It does not directly play a major role in enzymatic coagulation of cheese; however, it plays an important role in the whey protein industry. Milk containing excess colostrum may contain BSA from blood, which can have adverse effects on cheese making. Similarly, high concentrations of β-LG A may have negative effects. Concentrations of β-LG B, on the other hand, were found to be significant for the cheese yield percentage. Milk containing high concentrations β-LG B and κ-CN relative to total CN has been shown to have better curd formation (Wedholm et al. 2006).

Caseins

Caseins comprise approximately 80% of the total proteins in milk (Smith 2008). Therefore, CN composition is a key element in natural cheese making. Caseins play an important role in the formation of micelles and subsequent enzymatic coagulation, which will be discussed later. The four basic caseins are α_{S1}-CN, α_{S2}-CN, β-CN, and κ-CN (Wedholm et al. 2006). These caseins are typically present in milk in the ratio of 4:1:4:1, respectively (Christen and Smith 2000). Their functionalities include hydrophobic and hydrophilic interactions, viscosity, heat stability, enzymatic coagulation, foaming, and emulsifying properties (Augustin and Udabage 2007). Caseins also form sub-micelles within the micelle (Tetra Pak, 2003). Alpha S1-CN is calcium sensitive, has multiple phosphorylation sites, and has hydrophobic N- and C-terminals. Alpha S2-CN, on the other hand, is the most hydrophilic. It also is highly phosphorylated and, subsequently, is the most sensitive to precipitation in the presence of calcium. Alpha S2-CN can form disulfide bonds due to the presence of Cysteine (Cys) residues as shown in Figure 2. Beta-CN is the most hydrophobic, although it has a hydrophilic N-terminal, and has the most hydrophobic Proline (Pro) residues (Figure 2).

Arguably, κ-CN plays the most important role in natural cheese making. It is the critical factor in controlling micelle stability (Figure 3) and subsequent enzymatic coagulation. Kappa-CN is different from other CNs in that it remains soluble in a range of calcium concentrations.

Like α_{S2}-CN, κ-CN is capable of forming di-sulfide bonds with it and other proteins. It is also highly glycosylated. Consequently, carbohydrates contribute to the steric stabilization of micelle. Moreover, micelle stability is dependent on the presence of κ-CN on the surface of the sub-micelle (Figure 3) where it functions as an interface between the hydrophobic caseins of the sub-micelle interior and the aqueous environment (Creamer et al. 1998). It consists of 169 amino acid residues; including 20 hydrophobic Proline (Pro) structures. Notably the protein contains a peptide bond between the sequential 105 residue from the hydrophobic Methionine (Met) and the 106 hydrophilic Phenylalanine (Phe) residues. Enzymatic cleavage at the Phe105-Met106 bond eliminates the stabilizing ability, leaving a hydrophobic portion, para-κ-CN, and a hydrophilic portion referred to as κ-glycomacropeptide (GMP) (University of Guelph 2009). However, prior to enzymatic cleavage, the pH of milk must first be adjusted. This is accomplished naturally using starter cultures.

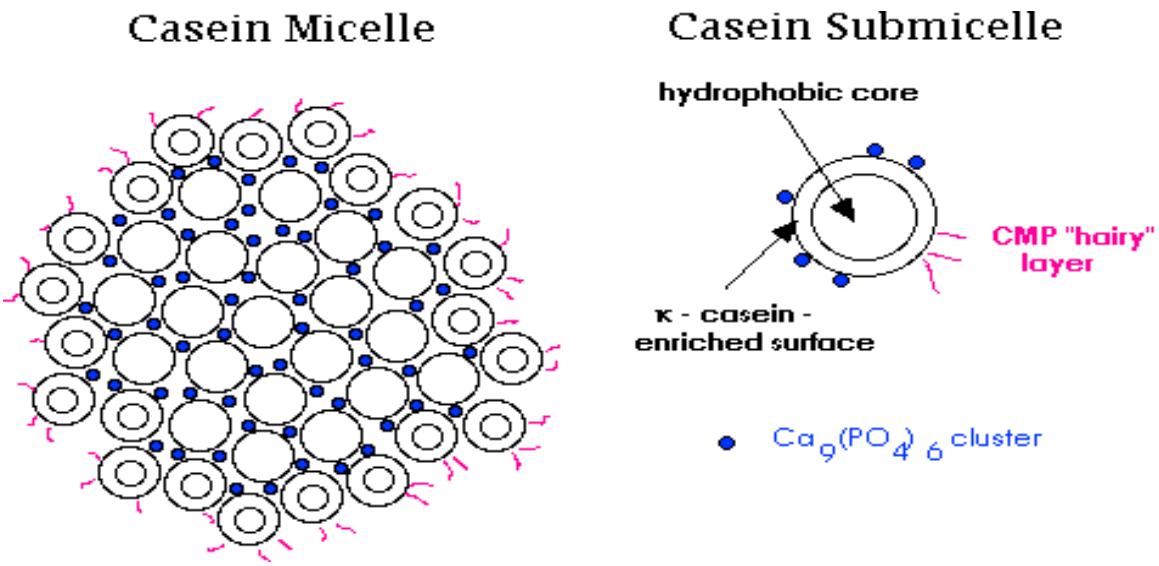

Figure 3: Casein Micelle Structure
(University of Guelph 2009)

Starter Cultures

Protein cleavage and subsequent curd formation in natural cheese making is achieved using enzymes that have optimum pH ranges. Natural cheese making uses lactic acid produced by the fermentation of lactic acid bacteria (LAB) to control the pH. Lactic acid bacteria are

Gram-positive, non-motile, and non-spore forming. A cheese maker may select starter cultures or starter culture combinations to achieve the desired functionality as shown in Table 3.

Table 3: Lactic Acid Bacteria Commonly Used in Cheese Making.

Mesophilic Cultures		
Old Name	New Name	Description
Leuconostoc citrovorum	*Leuconostoc mesenteroides* spp *cremoris*	Hetero cultures; ferment citrate; produce both CO_2 and diacetyl
Leuconostoc lactis	*Leuconostoc lactis*	May be used for cheese with small holes
Streptococcus diacetylactis	*Lactococcus lactis* ssp *lactis* biovar *diacetylactis*	Hetero culture; ferments citrate; produces both CO2 and diacetyl . Used with homofermentative lactococci for cheese with small holes
Thermophilic Cultures		
Old Name	New Name	Description
Streptococcus thermophilus	*Streptococcus salivarius* ssp *thermophilus*	Commonly used coccus/rod blend for high temperature varieties, Swiss and Italian
Lactobacillus helveticus	*Lactobacillus helveticus*	*L. helveticus g*alactose used to reduce browning in Mozzarella, and to promote proteolysis
Lactobacillus bulgaricus	*Lactobacillus delbrueckii* ssp *bulgaricus*	Commonly blended with S. salivarius ssp thermophilus for yoghurt. Alternative to *L. helveticus* in high temperature cheese
Lactobacillus lactis	*Lactobacillus delbrueckii* ssp *lactis*	Commonly blended with *S. salivarius* ssp *thermophilus* for yoghurt. Alternative to *L. helveticus* in high temperature cheese

Adapted from (University of Guelph 2009)

Starter cultures may be mesophilic, with optimum growth in medium range temperatures, or thermophilic, preferring hotter temperatures. Starter cultures are not psychrophilic or psychrotrophic in that they do not grow well in colder storage temperatures. They can grow aerobically, anaerobically, or may be micro-aerophilic in that they tolerate only low oxygen concentrations (University of Guelph 2009). They may be non-gas producing (homofermentative) or gas producing (heterofermentative), but all are also fastidious needing ample nutrients for growth. Flavor characteristics may develop as a direct result of the primary starter culture, or may be modified by a secondary "adjunct" culture. As their name implies, LAB ferment lactose as a carbohydrate source yielding lactic acid as a by-product (Fung and Goetsch 2004). Lactic acid formation "drives" the pH drop (Δ pH) in the milk necessary for curd formation. Starters may also work by microbial succession to achieve the desired functionality.

The lowering of pH is critical in controlling moisture content as acidity causes the protein matrix in the curd to undergo syneresis—that is, to contract and squeeze out moisture (University of Guelph 2009). Fermentation failure in LAB reduces starters ability to drive, and is often due to the presence of highly specific viruses called bacteriophage (McGrath et al. 2007). Bacteriophages inhibit the production of lactic acid and have an negative economical impact in commercial cheese making (Chopin et al. 2005). Starter selection, therefore, is based on many factors that affect the overall functionality as shown in Table 4.

Table 4: Considerations in Selecting a Starter Culture for Food Fermentation

Functionality	
1)	Organism must be pure.
2)	Organism must be able to grow at desired rate.
3)	Organism must be able to reproduce at desired rate.
4)	Organism must be genetically stable.
5)	Organism must produce product in desired time.
6)	Organism must be free from producing undesirable products.
7)	Organism must be able to produce uniform product.
8)	Organism must be able to maintain for long time.
9)	Organism must provide protection from other microorganisms.

Adapted from (Fung 1995)

Enzymatic Coagulation and Curd Formation

Nomenclature

Natural cheese making uses the addition of an enzyme to cleave the Phe105-Met106 peptide bond of κ-CN to leave the hydrophobic portion, para-κ-CN, and the hydrophilic portion, GMP. Traditionally, commercial cheeses were produced using the natural enzyme "rennet," which was derived from young bovine rennets (i.e., calf abomasums). This enzyme aids in the calves' digestion and nutrient absorption of milk. Rennet consists mainly of the two proteolytic enzymes chymosin (rennin) and pepsin. Chymosin is a highly specific clotting component, while pepsin is involved in other proteolytic activities with less specificity. The quantity and concentration of the enzymes in the stomach lining depends upon the age of the ruminant. Chymosin to pepsin ratios for a calf may be 9:1, respectively, where a mature animal's ratio may be 1:9 (Rogelj et al. 2001). A shortage of calf abomasums and compliance with Halahic (i.e., Kosher) food laws has led to the development of other coagulants of non-animal origin (Regenstein et al. 2003). Aspartic acid proteinases, for example, can be derived naturally from microbial (e.g., *Rhizomucor miehei*) and plant origins. Alternatively, they may be derived from genetically modified organisms (GMOs) such as the recombinant calf and lamb chymosin (rennin) produced in different microorganisms, including *Escherichia coli*, *Kluyveromyces lactis*, and *Aspergillus niger* var. *awamori*. Recombinant, highly pure chymosin from genetically modified bacteria and fungi may be advantageous as they are less expensive, more specific, more predictable, and may have Kosher certification and vegetarian approval (Rogelj et al. 2001). Customarily, natural rennin, recombinant chymosin, and microbial enzymes are all referred to generically as "rennet," which is somewhat of a misnomer and rather confusing to the layman.

Enzymatic Functionality

It is beyond the scope of this paper to comprehensively review the process by which the cheese maker would select a clotting enzyme. However, key considerations would include operational parameters, clotting ability, curd firmness, and cheese grading potential. Different milk clotting enzymes have different optimum pH and temperature ranges for activation and efficiency (Braun and Kunath 1988). Microbial enzymes derived from *Rhizomucor miehei*, for example, are less heat-sensitive than other enzymes and may be preferred for cheese operations

with higher cooking temperatures. They are also preferred by the European Union over genetically modified organisms (GMOs). However, cheese produced from these microbial coagulants (MCs) may develop off flavors that contribute to poor grades as shown in Table 5.

Table 5: Sensory Assessment on Cheeses with Different Clotting Enzymes

Properties	Possible Points	Calf Rennet	Recombinant Calf Chymosin	Microbial Coagulant	Recombinant Lamb Chymosin
Appearance	0-3	2.7	2.7	2.7	2.8
Odor	0-2	1.8	2.0	1.7	2.0
Texture	0-5	3.7	3.9	4.0	4.0
Flavor	0-10	8.0	8.6	5.5	9.0
Total	**0-20**	**16.2**	**17.2**	**13.9**	**17.8**

Adapted from (Rogelj et al. 2001)

Milk clotting activity is another quality factor associated with enzymes. Clotting activity is important to both time and yield in cheese making. International Milk Clotting Units (IMCUs) are standards for measuring milk clotting activity as described in the International Dairy Federation Standard Methods (IDFSM). Coagulant rate comparison is performed using equivalent IMCU/ml (IDF 1996). Another metric, curd firmness (a_{30}), can be quantified using IDFSM to analyze curd width (in mm) at thirty minutes after the addition of the enzyme. Studies show that a_{30} and coagulation activity could be increased by adjusting concentrations of chymosin and pepsin. For example, a_{30} could be increased with high pepsin concentrations due to its proteolytic activity. However, this could result in non-specific proteolytic activity and excessive proteolysis during cheese ripening (Rogelj et al. 2001). Controlling non-specific proteolysis allows the cheese maker to produce cheese with proper body development during ripening while reducing off-flavors such as “bitter”, “sour”, or “metallic.”

Coagulum

Chymosin acts upon casein in three phases: primary, secondary, and tertiary. The primary phase is independent of temperature and involves the splitting of the para κ–CN and GMP. The secondary or "clotting" phase, however, is temperature dependent. Coagulation efficiency increases approximately 1.5% for each degree raised between 68 to 100°F. However, activity begins to drop off as chymosin reaches its maximum temperature at approximately 106°F (Thunell and Burningham 2007). This phase is also calcium dependent and can only take place in the presence of Ca^{++} cations. During this phase, the proteins form together into a mass known as the "coagulum." The tertiary phase occurs once the curd is formed; the micelles are destabilized and begin to form a three-dimensional lattice (Fung 1995).

The formation of the coagulum depends upon on the quantity and type of rennet selected. Coagulation may be inhibited by residual chlorine in the cheese vat; as little as 2 ppm of residual chlorine will destroy 40% of rennet activity in 3 minutes. Typically hard water (pH > 7.0) also decreases rennet activity. Functionality may be improved, however, by the addition of extra calcium in the form of calcium chloride. The $CaCl_2$ assists in the formation of calcium bridges that will facilitate knitting and syneresis. Calcium chloride is also important for maintaining the mineral balance in milk that has just undergone pasteurization. This may also be more feasible economically as less chymosin may be used to achieve the same strength of coagulum (Thunell and Burningham 2007). Even heat distribution is critical in the cheese vat. Uneven temperatures cause localized setting differences, and may result in an increase of small, shattered curd particles in the whey known as "fines." Furthermore, overheating may result in a soft curd (University of Guelph 2009). Even after coagulation, up to six percent of the enzyme may still be active (Fung 1995).

Cutting the Coagulum

The time at which the coagulum is cut is extremely important to both quality and yield. Cutting too early when the curd is fragile or cutting too late when the curd is brittle may cause loss to fines. Cutting time may be determined using modern technology. However, many cheese makers still prefer to determine the cutting time manually using a spatula blade. The clean blade is inserted at a 45° angle below the surface layer and then raised slowly. The coagulum is ready to cut if it breaks cleanly (University of Guelph 2009). Additionally, a greenish color in the

whey will indicate the coagulum is ready (Fung 1995). Cutting should begin immediately to prevent the coagulum from matting in the vat. Modern cheese vats are equipped with blades that are sharp on one side and dull on the other. The sharp side ensures that the curd is cut cleanly, as shown in Figure 4. This reduces the curd size which, in turn, increases its surface area. Large curds may result in yield loss due to fines. Curds that are too small may result in yield loss due to excessive loss of moisture. Cutting speed may be set to a specific rate (rpm). The rpm is critical in yielding optimum fat and protein recovery. Freshly cut curd is very fragile; allowing the curd to "heal" before cooking allows the curd to better withstand agitation during cooking.

Cooking

During cooking, which occurs in the cheese vat, the curd shrinks in size. The same mechanical blades used for cutting are reversed to use the dull opposing side for agitation (Figure 4). As with cutting, the correct rpm is very important during cooking. The correct combination of heat and subsequent ΔpH facilitates the syneresis of whey: expulsion of moisture, lactose, lactic acid, soluble minerals, and whey proteins. Cooking too soon may result in a "soft set" where the curds have soft, thin fibers that collapse easily. Starting too late may result in a "hard set" where thick, rigid fibers will not properly facilitate syneresis (Thunell and Burningham 2007).

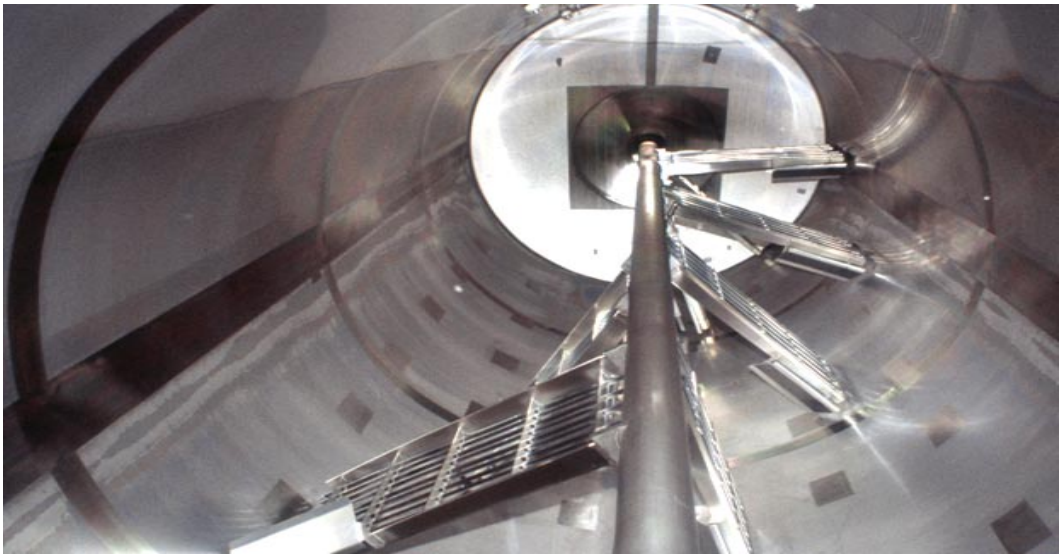

Figure 4: Internal View of Horizontal Cheese Vat
(Tetra Pak 2009)

Draining

After cooking, the whey is initially drained off as "pre-draw" prior to export to the cheese belt. After pre-draw, the cheese slurry is sent to the belt in a process known as "pump over." Draining excess whey during pre-draw greatly reduces the amount of time required to pump over the slurry of curd and whey to the cheese belt. This not only increases production efficiency, but also more control of pH in the vat. Natural cheese is typically drained in the range of pH 6.0-6.4. The pH during this step is dependent the time it takes to transfer the slurry to the belt. Draining time should, therefore, be uniform for consistency. Draining also increases curd collisions which further promotes syneresis. More draining occurs as the slurry is pumped across a drain weir at the beginning of the cheese belt. The weir allows the curds to pass onto the belt as a large volume of whey is drained. Fines may be lost at this time resulting in lower cheese yields. Draining continues as the cheese is gently stirred on the cheese belt prior to washing.

Figure 5: Internal View of Cheese Belt

(Tetra Pak 2009)

Washing and Stirring

Washing the newly formed curd consists of spraying the cheese with temperature-controlled water as it travels along the cheese belt. During this time, the whey continues to drain through openings in the belt, which reduces lactose and subsequent lactic acid content. The control of pH and rate of syneresis is determined by the temperature of the water. Hotter water promotes syneresis. Thermophillic starter strains still in the curd continue to drive better on the belt when washed with warmer water. Warm temperatures do not promote syneresis as well, but facilitate greater ΔpH when using mesophilic starter strains by returning them to their log phase of growth. In addition to the physical removal of lactose, washing with water also creates an osmotic concentration gradient. Residual lactose within the curd will travel out towards the less concentrated water (Thunell and Burningham 2007). Washing will, therefore, actually reduce the moisture content and subsequent water activity in the remaining in the curd. In fact, hot water is used in the production of Gouda to dry the curd and develop texture. The control of total moisture, available water, and pH continues during the salting phase.

Salting

Salting is a critical step in the cheese making process. The amount of salt obviously affects the flavor of the cheese; however, it is also affects biochemical processes. Salting takes place towards the end of the cheese belt. Salting agitators may be set to higher rpms as the cheese is less fragile at this stage. The amount of salt applied is highly controlled to facilitate even distribution and the desired concentration. Hardness and crumbling occurs in the finished product as the salt content increases. A high salt concentration may cause poor body; however, it may reduce bitterness and decreases excessive proteolysis during ripening. For example, degradation of α_S-CN may be reduced by higher percentages of salt. However, no associations have been made to the degradation of β-casein (Mistry and Kasperson 1998).

Salting is also used to halt fermentation by LAB, thereby stopping their pH drive. This allows the cheese maker to achieve the desired final pH in the cheese. Salting also inhibits spoilage bacteria and reduces available water (a_w) which provides intrinsic inhibition of pathogens; this will be discussed later in more detail. Therefore, pH and a_w are both affected by salt content. After salting, the curd is transferred by special solid food pumps to the top of the cheese towers for pressing.

Pressing

Modern pressing occurs in long vertical columns or "cheese towers" as shown in Figure 6. Cheese towers use a combination of vacuum and gravity to compact the curd into the desired form (e.g., blocks). Pressure in the cheese towers further promotes syneresis, and forms the cheese into a compact block. Expelling any residual whey reduces the amount of mechanical openings in the block. This facilitates proper tertiary adhesion or "knitting" and reduces oxygen penetration. Vacuum may be set to 170-175 kPa for firm Cheddar cheese (University of Guelph 2009). Once the cheese is formed into blocks, it is ready for packaging.

Figure 6: Cheese Towers
(Tetra Pak 2009)

Packaging

Cheese may be packaged in many forms: 20 lb block, 40 lb block, or 640 lb block. It may be packaged in traditional wooden crates, or it may be vacuum packed and fit into wrap-around, corrugated containers. Proper packaging facilitates proper knitting which enhances the appearance of the cheese body. Knitting is derived from tertiary casein interactions that give the cheese its body, and reduces mechanical openings. The penetration of oxygen is controlled intrinsically by knitting. Decreasing oxygen penetration helps to control microbial growth.

Packaging also provides functionality for cheese being sent to converting operations. Modern slicing and shredding equipment is more efficient when processing a uniformly shaped blocks or barrels. Hermetically-sealed packaging allows the cheese maker to extrinsically control the amount of oxygen available, thereby preventing cheese dehydration and surface oxidation. Sealed packaging reduces the chance for case hardening and hydrolytic rancidity, which develops off flavors as lipids in the form of triglycerides are be broken down into glycerol and free fatty acids (Smith 2008). The reduction of oxygen is also important in food safety. The metabolic functions of microorganisms depend upon the amount of oxygen they prefer or can tolerate. Adjusting the amount of available oxygen will be discussed later. Improperly sealed containers (i.e., "leakers") result in the loss of moisture and the penetration of oxygen. Therefore, proper packaging is critical prior to introducing the product to storage.

Storage

Properly packaged cheese is sent to the cooler or "cold box" while still warm from fabrication. Depending upon the packaging, temperature, and air movement, it may take 7-10 days for cheese to reach 40-45^{o}F in the cooler. The aging process may take 12-24 months, depending on the desired texture and flavor development. During this time lipid and protein degradation enzymes continue to break down solids. Many cheeses are selected in the first 30 days for mild flavor or mechanical functionality. However, other cheese types such as certain sharp Cheddars may take years to reach the desired flavor and texture. During this time acid formation is slow, but does continue (Thunell and Burningham 2007). It is the acid development that gives sharp Cheddar its flavor. Flavor development, however, may come at the expense of body. Some older cheeses may loose their mechanical functionality and may not slice, cube, or shred as well. Therefore, flavor and functionality determine storage time and retail shelf life. Additional or "adjunct" starter cultures may also be used to create artificial flavors such as enhanced sharpness in "young" cheese. Flavor development may also be accelerated using higher holding temperatures; however, this may shorten the shelf life and may allow for the growth of unwanted microorganisms.

CHAPTER 2 - Intrinsic and Extrinsic Parameters

Raw milk has long been recognized as a potential source of pathogenic bacteria such as *Mycobacterium tuberculosis*, *Streptococcus pyogenes, Corynebacterium diptheriae,* and *Shigella dysenteriae* (Evans et al. 1970). However, many dairy products use a combination of intrinsic and extrinsic factors to inhibit pathogens. The combination of intrinsic and extrinsic factors used to inhibit microbial growth is known as the "multiple hurdle" concept (US FDA 2004). Intrinsic factors include pH, oxidation-reduction potential, moisture content and availability, nutrient content, natural inhibitors, and physical integrity. Extrinsic factors include temperature, relative humidity, gaseous environment, cumulative stress, and storage time (Fung and Goetsch 2004). Multiple hurdles are often a result of that product being manufactured to meet its legal definition. Cheese standards for American manufacture are listed in §133 of 21 CFR.

Both the FDA and Codex define cheeses by their hardness, fat content, and type of curing (Nelson 1984). Hard cheeses like Cheddar, for example, must be $\leq$ 39% moisture and $\geq$ 50% milkfat as FDB (fat measured on dry basis). Semi-soft cheeses must legally contain 39 $\leq$ 50% moisture and $\geq$ 50% FDB per 21 CFR 133.187. Monterey Jack, for example, is a semi-soft cheese and is allowed up to 44% moisture although the FDB is the same as Cheddar. Other pasta filata types, such as Provolone and Mozzarella, may be allowed 45% and 60% moisture, respectively. Although Mozzarella is allowed up to 60% moisture in 21 CFR 133.155, it may no longer be considered as semi-soft if the moisture content is greater than 50%. Furthermore, the FDA has promulgated in 21 CFR that all of these cheese styles must be made from pasteurized milk or "cured at a temperature of not less than 35^{o}F for not less than 60 days" (US FDA 2007). Studies have examined the role of intrinsic antimicrobial properties in conjunction with other factors to control pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* in dairy foods (Arques et al. 2008). Hard and semi-soft cheeses are rarely linked to cases of food borne illness, and are exempt from legislation applying to other Ready to Eat (RTE) products in the FDA Food Code (US FDA/ CFSAN 2009a). Current food safety standards appear adequate for the safe production of these cheeses (Holsinger et al. 1997), but intrinsic and extrinsic factors should be understood in order to advance the development of food safety systems.

Intrinsic Factors

Intrinsic factors are those characteristics that are inherent to the food. They occur naturally within the food itself, and are often a result of that product being manufactured to meet other quality standards. Intrinsic factors include pH, oxidation-reduction potential, moisture content and availability, nutrient content, natural inhibitors, and physical integrity (Fung and Goetsch 2004).

Hydrogen Potential (pH)

Naturally increasing the acidity of cheese through starter fermentation has been used as a preservation method for many years. The pH is a function of the hydrogen ion concentration in the food and is defined as the negative logarithm (base 10) of the hydrogen ion concentration: pH = -log10 [H+] (Campbell and Reece 2005). The pH scale is inversely proportional to the amount of H+ present—as the pH declines, the H+ concentration increases. The pH scale is a base 10 logarithmic scale that ranges from 0-14 (7 is neutral). The hydrogen potential may be measured using hydrogen, quinhydrone, antimony, silver halide, or calomel (mercurous chloride) electrodes (Dean and Lange 1973). In Cheddar cheese, for example, a cheese maker typically targets a finished product pH of 5.1-5.3 for maximum quality as shown in Table 6. Quality parameters also make good food safety targets for controlling spoilage organisms and pathogens.

Table 6: Impact of Final pH on Cheddar Cheese Texture

Taste	pH	Characteristic
	5.5	
"plastic"	5.4	Springy
	5.3	
	5.2	Cheddary
	5.1	
"mealy"	5.0	Short
"non - cohesive"	4.9	
	4.8	

Adapted from (Thunell and Burningham 2007)

Different microorganisms have different optimum, minimum, and maximum pH ranges for growth in foods (ICMSF 1980). Table 7 lists the approximate pH ranges for many of the pathogens associated with many foods. Although the pH in natural cheese is not low enough to exclusively control the survival of all pathogens, it is below the optimum for growth of many related to food manufacturing. Retarded bacterial growth due to pH may be used in conjunction with other parameters in the food to inhibit growth. These interactions may be intrinsic or extrinsic combinations. Pathogens may be inhibited by the interaction of a combination of factors such as pH, a_w, salt, temperature, redox potential, and preservatives (US FDA 2004). Therefore, pH is critical to both quality and food safety in natural cheese fabrication.

Table 7: Approximate pH values permitting growth of selected pathogens in many foods
Adapted from (ICMSF, 1980)

Microorganism	Minimum pH	Optimum pH	Maximum pH
Clostridium perfringens	5.5-5.8	7.2	8.0-9.0
Vibrio vulnificus	5.0	7.8	10.2
Bacillus cereus	4.9	6.0-7.0	8.8
Campylobacter spp.	4.9	6.5-7.5	9.0
Shigella spp.	4.9	NA	9.3
Vibrio parahaemolyticus	4.8	7.8-8.6	11.0
Clostridium botulinum toxin	4.6	NA	8.5
Clostridium botulinum growth	4.6		8.5
Staphylococcus aureus growth	4.0	6.0-7.0	10.0
Staphylococcus aureus toxin	4.5	7.0-8.0	9.6
Enterohemorrhagic *Escherichia coli* (EHEC)	4.4	6.0-7.0	9.0
Listeria monocytogenes	4.4	7.0	9.4
Salmonella spp.	4.2	7.0-7.5	9.5
Yersinia enterocolitica	4.2	7.2	9.6

Adapted from (ICMSF, 1980)

Buffering capacity is another characteristic that must be considered when using acidity as a control mechanism. The buffering capacity of a food is its ability to resist changes in pH by

minimizing, accepting, or donating hydrogen ions from the solution (Campbell and Reece 2005). Milk is typically more buffered than foods such as vegetables because of its components (e.g., fats and proteins). However, the buffering capacity of milk may be overcome by the ΔpH that occurs naturally due to the drive of the starter culture.

Titratable acidity (TA) is also a useful tool for a cheese maker. Titratable acidity is the quantity of standard alkali (usually 0.1 N NaOH) required to neutralize an acidic solution (ICMSF 1980). It measures the amount of hydrogen ions released from undissociated acid during titration. Titratable acidity is very useful when measuring highly buffered or highly acidic foods (US FDA 2004). Therefore, it is often a good indicator when analyzing milk. Weak acids (e.g., organic acids) are usually undissociated and, therefore, do not directly contribute to pH. Titratable acidity yields a measure of the total acid concentration, while pH does not, for these types of foods. The two, however, may be used together to determine quality parameters.

Fresh milk with 6.6-6.8 pH will typically have a TA of less than 0.18% (Jeon and Schmidt 2007). High TA values are typically inversely proportional to low pH values, and are often used together to assess quality deviations, such as when milk has been allowed to remain warm too long. This is very useful as temperature-abused milk may be determined by low pH or high TA values. Temperature-abused milk is of concern to the cheese maker as it may have off flavors due to hydrolytic rancidity. It may also result in poor cheese texture and has a greater potential for pathogenic growth.

In general, pathogen growth is reduced in aged cheese at pH levels below 5.5 although this is a full log above pH 4.6, which is acknowledged as the minimum pH for the growth of *Clostridium botulinum*. However, pH 5.5 is considered a safe target for hard and semi-soft cheese types because it is used in conjunction with other factors, such as oxygen-reduction potential (Eh), within the multiple-hurdle concept.

Oxidation-reduction Potential (Eh)

Oxidation-reduction or "redox" potential is another intrinsic factor that contributes to the multiple hurdle effect in certain cheeses. Redox is the measurement by which a substance will gain or lose electrons. Redox potential (Eh) is be measured in millivolts (mV), and is the ratio

of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of the substance (US FDA 2004).

Microorganisms may be grouped based on their Eh range for growth. Aerobes such as *Aeromonas hydrophila* have an Eh range +500 to +300 mV while facultative aerobes such as *Escherichia coli* O157:H7 have a range of +300 to -100 mV. True anaerobes (e.g., *Clostridium botulinum*) and microaerophiles have typical ranges of +100 to -250 mV. Cheddar cheese has a typical Eh range of +300 to -100 mV (US FDA 2004) which makes it more ideal for the growth of facultative aerobes rather than true aerobes or true anaerobes. The relationship of Eh to growth can be enhanced by the presence of other factors such as salt content to increase the effectiveness of the multiple hurdle technology.

Another important factor is the poising (i.e., buffering) capacity of the food. Poising is the resistance changes in Eh. The poising capacity of the food will be affected by oxidizing and reducing constituents in the food. More studies need to be done to determine the poising capacity of specific cheese types.

Oxygen tension must also be considered as a function of Eh. It is the ability for O_2 to penetrate beyond the surface and into the matrix of the food. Oxygen tension is often used in the food industry as a benchmark for potential bacterial growth. Ground beef, for example, supports more growth than steak. This is partially due to increased surface area and subsequent surface contact facilitating cross-contamination. However, it is also due to the low O_2 penetration in solid tissue (Fung and Goetsch 2004). Hard and semi-soft cheeses have similar properties. The tertiary knitting of the proteins forms a solid matrix that is less likely to be penetrated by oxygen. A solid matrix free of mechanical openings is a desirable quality in cheese manufacturing, and is another example of a quality parameter that also improves the safety of the food. As discussed previously, knitting also promotes syneresis and reduces the amount of moisture in the cheese. Moisture content and subsequent water activity (a_w) are important factors multiple hurdle technology.

Moisture Content and Availability (a_w)

All microorganisms need water in an available form to grow. As discussed previously, standards of identity legally define the amount of moisture in hard and semi-soft cheeses. However, total moisture content is not the only concern in cheese making. The amount of

available water measured as water activity (a_w) is also critical to controlling the growth of microorganisms. The a_w of food describes the degree to which water is free or bound to other components. Bound water reduces its availability to participate in biochemical reactions necessary for the metabolic functions of microorganisms. Water activity is the vapor pressure of substrate (cheese) divided by the vapor pressure of solution (water) as defined by defined in Raoult's Law: $a_w = P_s / P_o$ (Fung and Goetsch 2004). Water activity is based on a scale from 0.00-1.00, where pure water is 1.00. All microorganisms have an optimum, minimum, and maximum a_w as shown in Table 8.

Table 8: Approximate a_w Values for Growth of Selected Food Pathogens.

Microorganism	Minimum	Optimum	Maximum
Campylobacter spp.	0.98	0.99	
Clostridium botulinum type E	0.97		
Shigella spp.	0.97		
Yersinia enterocolitica	0.97		
Vibrio vulnificus	0.96	0.98	0.99
Enterohemorrhagic *Escherichia coli*	0.95	0.99	
Salmonella spp.	0.94	0.99	0.99
Vibrio parahaemolyticus	0.94	0.98	0.99
Bacillus cereus	0.93		
Clostridium botulinum types A & B	0.93		
Clostridium perfringens	0.93	0.95	0.97
Listeria monocytogenes	0.92		
Staphylococcus aureus growth	0.83	0.98	0.99
Staphylococcus aureus toxin	0.88	0.98	0.99

(ICMSF 1996)

Although a_w is a function of water content, the two may not always be directly proportional. Fresh meats and vegetables have both high moisture content (>50%) and water activity levels between 0.97-0.99 a_w (ICMSF 1996). These levels approach optimum growth levels for many of the pathogens listed in Table 8. Fresh meats and vegetables are also more

frequently associated with outbreaks of food borne illness (CDC 2009). However, many foods such as syrup, ham, and honey use sugar or salt to bind available water. These foods, when manufactured properly, are less likely to support microbial growth. Likewise, hard cheeses have a typical water activity of 0.95 a_w (US FDA 2004) as water is bound to salt and other components.

Optimal water activity ranges also vary among Gram-negative and Gram-positive bacteria. Gram-negative bacteria (e.g., coliform group and *Salmonella enteritidis*) are generally more sensitive to low a_w than Gram-positive bacteria (e.g. *Bacillus* spp. and *Clostridium* spp.). *Staphylococcus aureus* (Gram +), for example, can grow at water activities of 0.85 a_w, and may still form toxins at 0.90 a_w (Stevenson and Bernard 1999). Hard and semi-soft natural cheeses do not reduce the amount of available water for a_w to be used as an independent control of pathogens such as *S. aureus*. However, water activity may be used in conjunction with other factors to promote quality and control microbial growth.

Nutrient Content

As previously discussed, dairy products are rich in nutrients such as fats, carbohydrates, protein, and minerals. These nutrients are critical for the metabolic function of eukaryotes (e.g., humans) and prokaryotes (e.g., bacteria). Microorganisms require certain basic nutrients for growth and maintenance of metabolic functions. They differ, however, in their nutritional requirements. Gram-positive bacteria, for example, are more fastidious in their nutritional requirements as they cannot naturally synthesize certain nutrients and must consume them through their "diet." *Staphylococcus aureus* requires amino acids, thiamine, and nicotinic acid for growth. Gram-negative bacteria are often less fastidious, but may also require certain nutrients for metabolic functions. *Salmonella enteritidis*, for example, may be limited by the availability of iron bound by the protein, bovine serum albumin (BSA). The ability to utilize the nutrients available promotes the growth of one organism over another. The large numbers of live starter bacteria compete with pathogens for available nutrients (Bauman and Dolby 2003).

Natural Inhibitors

Competitive Inhibition and Antagonism

Competitive inhibition is an intrinsically natural phenomenon whereby groups of organisms compete for nutrients within the same environment. Typically, the species that starts with largest starting population will have an advantage over smaller or less established populations. The bacteria may also be antagonistic towards each other in that they generate a product that actively inhibits the growth of another organism. Some types of starter bacteria used in fermentation contain antimicrobial substances such as bacteriocins, antibiotics, and other related inhibitors.

Bacteriocins

Bacteriocins are proteinaceous toxins that can kill competitive microorganisms (Bauman and Dolby 2003). These antimicrobial peptides are produced naturally by bacteria, and have a relatively narrow range of activity that targets closely related organisms. Bacteriocin specificity may be due to historical ecological competition between these organisms. Subtilosin A, for example, is a bacteriocin produced by *Bacillus subtilis* that has activity against *Listeria monocytogenes* (Shelburne et al. 2007). The most commonly characterized bacteriocins are those produced by LAB. Nisin, for example, is a well known bacteriocin that is produced by certain strains of *Lactococcus lactis.* Nisin is approved as a processing aid for food applications in over 50 countries around the world (Jay 2000, p 269-72). The nisin polypeptide is effective against most Gram-positive bacteria (e.g., *Clostridium*), but is ineffective against Gram-negative bacteria and most fungi (US FDA 2004).

Antimicrobials

Some animal-based foods such as milk may also contain natural antimicrobial constituents. Bovine milk may contain lactoferrin, conglutinin, lysozyme, and lactoperoxidase system. Bovine lactoferrin is produced on an industrial scale from cheese whey or skim milk. Lactoferrin has been used in a wide variety of products, and was first added to infant formula in 1986 (Thunell and Burningham 2007). Conglutinin is another natural antimicrobial agent present in the bovine immune system. Its concentration, however, has been shown to vary

among breeds (Nyman et al. 2008). Lysozyme is a small protein that can hydrolyze the cell wall of bacteria. Lactoperoxidase is a key component of the antimicrobial lactoperoxidase system: lactoperoxidase, thiocyanate, and hydrogen peroxide (US FDA 2004). Spoilage organisms and opportunistic pathogens such as Gram-negative psychrotrophs (e.g., *Pseudomonas aeruginosa*) have a high sensitivity to the lactoperoxidase system. Additionally, reducing sugars and amino acid complexes typically associated with Maillard reactions may also have some antimicrobial activity (Chuyen 1998).

Physical integrity

Many foods have natural physical barriers that prevent contamination of pathogenic and spoilage microorganisms. Fruit skins, nut shells, vegetable skins, egg shells, and animal hides are examples of natural barriers. Cow's milk is also naturally protected by the udder; however, unsanitary conditions may allow for cross-contamination resulting in mastitis, or inflammation of the mammary gland. Therefore, the FDA has established legal sanitary regulations for production, transportation, and processing of milk and milk products as promulgated in the Grade "A" Pasteurized Milk Ordinance (USPHS and FDA 2007). These regulations are primarily enforced by registered sanitarians employed by the Department of State Health Services. Guidelines include legal requirements for bacteria and somatic cell counts allowed in raw milk. Control of cross-contamination promotes the natural integrity of the milk created by the physical barrier of the udder, and relies on a combination of intrinsic and extrinsic factors that help maintain quality and food safety.

Hard and semi-soft cheeses also have a natural physical integrity created by syneresis and the subsequent knitting of the fats and proteins. The solid matrix created during ripening decreases both permeability and surface area to make it less penetrable by moisture or oxygen. Cheese is more resistant to hydrolytic rancidity and the growth of certain microorganisms due to natural barriers. The benefits of intrinsic factors, such as natural barriers, may be enhanced by using them in conjunction with extrinsic factors.

Extrinsic Parameters

Extrinsic parameters are those conditions that are easily manipulated to control the growth and proliferation of microorganisms (Fung and Goetsch 2004). Extrinsic parameters include temperature, relative humidity, gaseous environment, and time of storage. These factors often correspond with desired overall quality in cheese making. They also may be used as critical limits for food safety systems such as HACCP. Critical limits are scientifically based parameters that have a minimum or maximum value that can be controlled to prevent, eliminate, or reduce the occurrence of a food safety hazard to an acceptable level (IDFA 2007). HACCP is a proactive approach to food safety that relies on critical limits to control intrinsic and extrinsic factors such as temperature, pH, and a_w.

Temperature

Temperature, in combination with other factors, is a major requirement for microbial growth. Bacteria grow by binary fission as one cell grows to twice its normal size and divides into two daughter cells at a logarithmic rate (Bauman et al. 2005). In logarithmic growth, a single organism can produce 10^6 (1 million) cells in 21 replications, 10^9 (1 billion) cells in 31 replications, *et cetera* (Fung and Goetsch 2004). The growth rate (Table 9) of an organism is classically defined as an Arrhenius relationship:

Table 9: Arrhenius Growth Rate

$G = -\mu / 2.303\ RT$
G = log growth rate constant
μ = temperature characteristic (constant for a particular microbe)
R = gas constant
T = temperature (°K)

Adapted from (Fung and Goetsch 2004)

Under ideal conditions, the generation time for *E. coli* is 20 minutes (Bauman and Dolby 2003). Colder temperatures, however, may slow microbial metabolic activity as enzymatic activity and fluidity of the cytoplasmic membrane is decreased. High temperatures may denature and inactivate structural components and heat-sensitive enzymes. Microorganisms may be

grouped into overlapping groups based on optimal and non-optimal growth ranges: psychrophilic, psychrotrophic, mesophilic, thermophilic, and thermoduric (Figure 7).

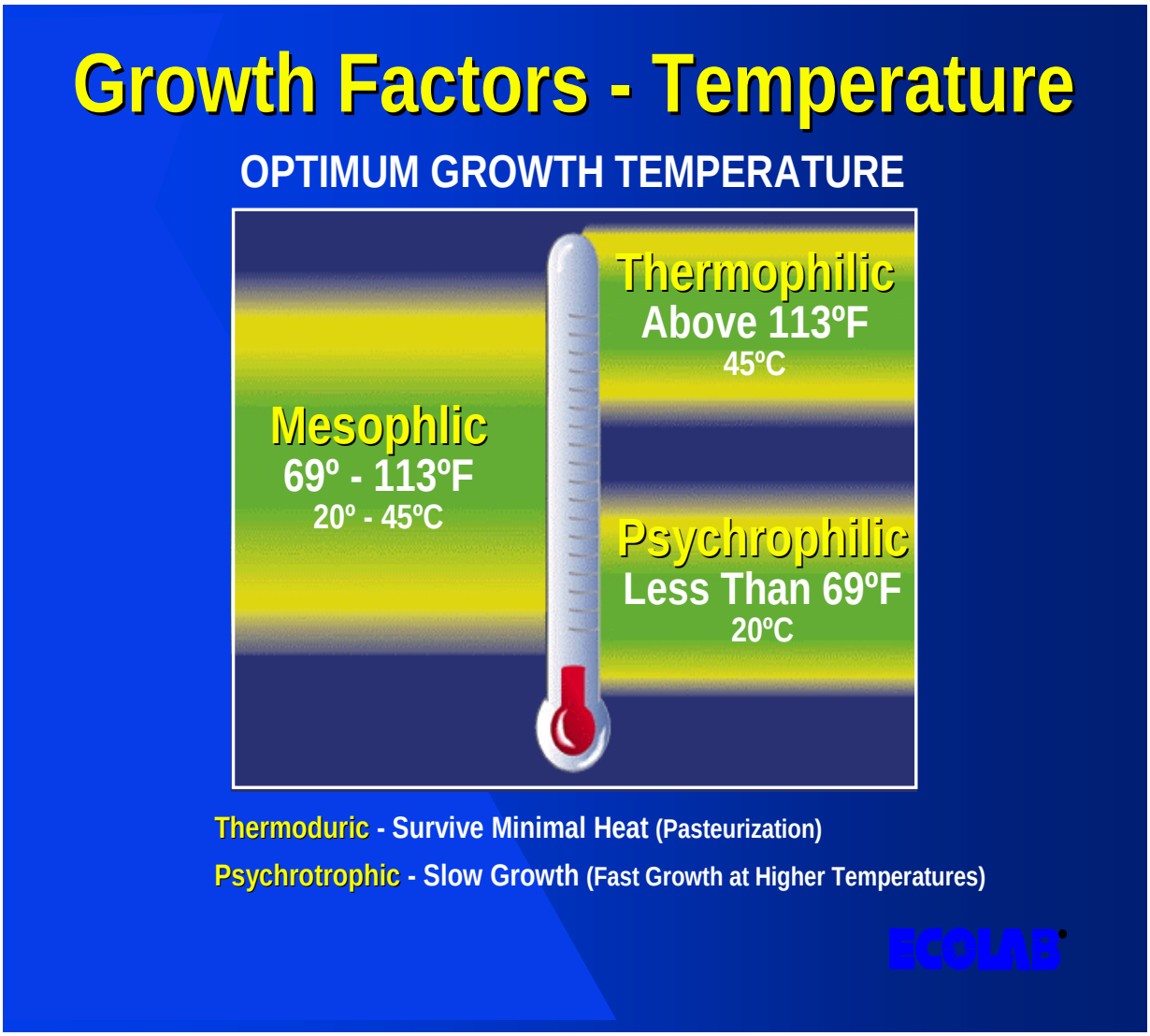

Figure 7: Growth Factors - Temperature

Reproduction courtesy of (Ecolab 2007).

A list of optimal growth temperatures for selected pathogenic organisms in food are listed in Table 10.

Table 10: Approximate Growth Temperatures °C (°F) of Selected Food Pathogens.

Organism	Minimum	Optimum	Maximum
Bacillus cereus	5 (41)	28-40 (82-104)	55 (131)
Campylobacter spp.	32 (90)	42 - 45 (108-113)	45 (113)
Clostridium botulinum A & B	10-12 (50-54)	30 - 40 (86-104)	50 (122)
Clostridium botulinum E	3-3.3 (37-38)	25 - 37 (77-99)	45 (113)
Clostridium perfringens	12 (54)	43-47 (109-117)	50 (122)
Enterotoxigenic *E. coli*	7 (45)	35-40 (95-104)	46 (115)
Listeria monocytogenes	0 (32)	30-37 (86-99)	45 (113)
Salmonella spp.	5 (41)	35-37 (95-99)	45 - 47 (113-117)
Staphylococcus aureus	7 (45)	35-40 (95-104)	48 (118)
Staphylococcus aureus toxin	10 (50)	40-45 (104-113)	45 (113)
Shigella spp.	7 (45)	37 (99)	45 - 47 (113-117)
Yersinia enterocolitica	-1 (30)	28-30 (82-86)	42 (108)

Adapted from (ICMSF 1996 in United States FDA/ CFSAN 2004)

Psychrophilic Microorganisms

True psychrophilic organisms have an optimal growth temperature of ≤ 15°C (59°F) and may continue to grow at temperatures below freezing. They may be found living in snow fields, cold water, and even ice. Organisms in this group include bacteria, algae, and fungi that do not tolerate temperatures above 20°C (68°F). They can cause operational problems if they establish growth in water cooling systems or other equipment. Psychrophilic microorganisms are not generally pathogens of concern due to the low temperatures required for their metabolic functions.

Psychrotrophic Microorganisms

Psychrotrophic organisms may survive in temperatures near freezing although their optimum temperatures may be mesophilic. They typically have optimal growth temperatures in the mesophilic range, and survive in temperatures approaching the thermophilic ranges. They can cause spoilage or food borne illnesses as they grow relatively rapidly at commercial

refrigeration temperatures (Speck 1984). Spoilage organisms include bacteria (e.g., *Pseudomonas* spp.) and fungi (e.g., *Geotrichum*, *Botrytis*, and *Penicillium*) that may cause unwanted flavors, odors, and slime. Pseudomonads, for example, are noted for their ability to catabolize proteins and lipids at 4°C (39°F) (Bauman et al. 2005). Other organisms, however, that survive in these temperatures may be pathogenic and of great concern to food safety.

Listeria monocytogenes, for example, is a pathogenic psychrotrophic bacteria with an optimal growth range of 30-37°C (86 - 99°F), and experiences death at 45°C (113°F), as shown in Table 10. This Gram-positive, motile, bacillus-shaped organism is non-spore forming. Infections by this organism are referred to as listeriosis. Diseases caused by this organism include septicemia, meningitis, encephalitis, and intrauterine or cervical infections in pregnant women. Complications for expectant mothers may result in spontaneous abortion or stillbirth. The infective dose of *L. monocytogenes* is believed to vary with the strain and susceptibility of the victim, and fewer than 1,000 organisms may cause listeriosis through raw or improperly pasteurized milk (US FDA/ CFSAN 2009b). As a psychrotrophic organism, it may grow at temperatures at or below freezing. It has a high affinity for stainless steel, and grows well in drains and cooling units. Therefore, it is of great concern in dairy HACCP systems (IDFA 2007).

Mesophilic Microorganisms

Many microorganisms in the mesophilic group are pathogenic to humans, as their optimal temperature ranges are close to 37°C (99°F) for metabolic functions. Organisms in this group include the bacteria family Enterobacteriaceae (en'ter-o-bak-ter-e-a'-se-ee), also known as enteric bacteria as they are typically of fecal origin. Enterobacteriaceae include members of the coliform group, such as *Escherichia*, *Klebsiella*, *Serratia*, *Enterobacter*, and Citrobacter, as well as non-coliforms like *Proteus*, and *Salmonella* (Bauman et al. 2005). Using Enterobacteriaceae as an indicator population to assess cleanliness may be accomplished using rapid methods such as media on film. The presence or absence of Enterobacteriaceae is used in the European Union to determine operational cleanliness in food manufacturing (Fung 2007). The United States, however, traditionally relies on the coliform group as an indicator of food safety (Wehr and Frank 2004). This may not always be appropriate, however, as serotypes of *Salmonella enterica* (e.g., *S. typhi* and *S. typhimurium*) are not classified as coliforms due classifications related to

metabolic functions, such as the ability to form H_2S. Using the presence or absence of coliforms as an indicator of food safety may give a false sense of security.

Escherichia coli

Escherichia coli biotype I (generic) is also used frequently in the US as an indicator organism. Petrifilm™ distinguishes *E. coli* from other species based a color change associated with the presence of the β-glucoronidase enzyme; however, some pathogenic strains of *E. coli* do not contain the β-glucoronidase enzyme necessary for a positive reaction (Benesh 2007). *Escherichia coli* are the most abundant bacteria found in the GI tract of humans and animals. The non-virulent strains have many practical applications, such as the competitive inhibition of pathogenic bacteria. Virulent strains of *E. coli*, however, are pathogenic to humans. Enterohemorrhagic (EHEC), Vero-toxigenic (VTEC), and Shiga-toxigenic (STEC) are classifications of *E. coli* containing virulence factors associated with increased pathogenicity. First associated with human illness in 1982, they cause an estimated 70,000 cases and 60 deaths annually in the United States (Bauman 2004). The presence of virulence factors associated with EHEC may be detected genetically using multiplex Polymerase Chain Reaction (mPCR) using genetic targets to determine the presence of genes that produce O antigens, effacement and attachment proteins, and Shiga-like toxins (Paton and Paton 1998). The most notable strain of EHEC is *E. coli* O157:H7 as it produces powerful verotoxins that were named for after research using Vero (African green monkey) kidney cells (Konowalchuk, Speirs, and Starvic 1977). Further research found that these verotoxins closely resembled toxins isolated from *Shigella dystenteriae*, and these specific verotoxins are often called Shiga toxins. Conventional enzymatic methods (e.g., ELISA) of food analysis are often too slow or lack the specificity required to be effective in screening for VTEC or STEC to be effectively used in the dairy industry.

Thermophilic Microorganisms

True thermophilic organisms can tolerate temperatures well over 45°C (113°F), and their cellular components do not function at lower temperatures. Thermophiles include microorganisms such as *Thermus aquaticus* that live in the sulfur-rich, acidic hot springs in Yellowstone National Park (Bauman et al. 2005). These organisms are not normally found in milk, and are not usually of food safety concern. Heat-tolerant enzymes from *Thermus aquaticus*

(Taq), however, are used as primer catalysts in mPCR as used in the detection of EHEC and other pathogenic organisms (Horton et al. 2002). *Bacillus stearothermophilus* is a sporeforming, thermophilic facultative aerobe that can cause "flat sour spoilage" of low acid canned foods (Speck 1984), but is not considered pathogenic to humans. Hermetically-sealed ampoules of *Bacillus stearothermophilus* are used frequently in the dairy laboratory to ensure that autoclaves are working properly (Wehr and Frank 2004).

Thermoduric Microorganisms

Thermoduric microorganisms are capable of surviving temperatures above their normal growth range and so are of concern to the dairy industry as they can survive milk pasteurization temperatures. Thermophilic bacteria are often mesophilic, and survival during heating or other stress is often due to the formation of endospores. Bacteria capable of forming endospores include the aerobic genus *Bacillus* and anaerobic species of *Clostridium* and *Desulfotomaculum* (Donnelly and Busta 1981). Endospores are dormant, tough, non-vegetative structures. They ensure the survival of the parent bacterium through periods of environmental stress from desiccation, certain antibiotics, UV radiation, sanitizers, and extreme temperatures. They are ubiquitous as they are commonly found in soil and water. All endospores have a similar basic structure consisting of an outer keratin-like coat, a cortex, and an inner core, but their morphology depends upon the genus of the parent bacterium, as shown in Figure 8.

Figure 8: Bacterial Spore Structure

Reproduction courtesy of (Ecolab 2007).

Anaerobic sporeformers include the genus *Clostridium* (Gram+) and the genus *Desulfotomaculum* (Gram-). *Desulfotomaculum* species are often involved in sulfur odor spoilage; however, *Clostridium* species are more likely to be pathogenic. *C. perfringens*, for example, is a pathogenic mesophile with a growth range of 20-50°C (68-122°F). *C. perfringens* is often associated with foods improperly held at temperatures outside the "safe zone" 40-140°F (4-60°C) (Stevenson and Bernard 1999). *C. botulinum* produces botulinum toxin, which is one of the most deadly natural toxins known and is responsible for the disease known as botulism.

Spores do not undergo mitosis directly; a single endospore, once reactivated, forms a single vegetative cell, and subsequently experiences outgrowth. It is imperative that thermal processing operations provide adequate destruction of vegetative cells. Thermal destruction time (D-Value) is calculated as the time to destroy 90% (1 log) of the vegetative cells of a particular microorganism at a specific temperature (Fung and Goetsch 2004). High Temperature Short Time (HTST) pasteurization, for example, was designed to achieve a 5-log reduction, killing 99.999% of the number of viable mesophilic pathogens in milk (IDFA 2008). HTST pasteurization is also capable of destroying other heat-resistant pathogenic bacteria (e.g., *Mycobacterium tuberculosis* and *Coxiella burnetii*) and fungi. To verify thermal processes, the dairy lab differentiates between Standard Plate Count (SPC) and Lab Pasteurization Count (LPC) populations. A post-HTST milk sample with low SPC indicates that most mesophilic microorganisms have been eliminated, while a low LPC indicate that thermoduric microorganisms have also been effectively destroyed. Operationally, HTST dairy facilities must be constructed using 3-A approved design, and must be maintained in accordance with the Grade "A" Pasteurized Milk Ordinance (USPHS and FDA 2007).

Relative Humidity

Relative humidity is the ratio of the of water concentration in the air at a certain temperature to the maximum concentration at the same temperature (Fung 2007). Relative humidity is very important to traditional cheese curing processes. Today's modern packaging utilizes cheese bags that control permeability and gas exchange, though some cheeses are still ripened using customary techniques. Ragusano cheese, for example, is brine-salted pasta filata cheese that is historically aged in caves at 14-16°C (57-61°F) with about 80-90% relative humidity (Licitra et al. 2000). Studies by Licitra examined block-to-block variation of Ragusano

cheeses aged naturally in caves for 12 months in the Hyblean plain region of the Province of Ragusa in Sicily. The blocks averaged 14 kg (31 lb) before salting with component means of 45.35% moisture, 25.3% protein, and 25.4% fat, and had a pH of 5.25. After ripening for 12 months at 80-90% humidity, the cheeses contained 33.6% moisture, 29.2% protein, 30.0% fat, and 4.4% salt and had a pH of 5.54. Soluble nitrogen content and free fatty acid (FFA) content increased with age indicating the breakdown of proteins and fats, respectively. Proteolytic activity was most notable in the block interior which also had higher moisture and lower salt-in-moisture content than the block exterior. The exterior had experienced "case-hardening" resulting in textural variation, and the standard deviation among blocks had increased significantly. This study illustrates block variation that occurs using traditional methodology and demonstrates the difficulty in manufacturing cheese without packaging as an extrinsic control for atmospheric influences. The Ragusano cheese in this study was held with two variables constant: temperature and relative humidity. Theoretically, changes in either of these variables would have led to even more inconsistency in quality and subsequent functionality. Large commercial cheese manufacturers may utilize packaging materials or storage rooms that extrinsically control relative humidity and gas content.

Gaseous Environment

Gases at ambient and sub-ambient pressures in foods are used to control microorganisms by two mechanisms: direct toxic effect and indirect inhibition. Carbon dioxide (CO_2), ozone (O_3), and oxygen (O_2) may be directly toxic to certain microorganisms; however, the toxicity depends upon the chemical and physical properties of the gas and the matrix of the food (US FDA/ CFSAN 2004). Ozone and oxygen generate oxidizing radicals that may be used to control certain aerobes, and obligate aerobes may be controlled by the use of CO_2. The direct toxic effect is not used frequently in natural cheese manufacturing, whereas indirect inhibition is used regularly as packaging alters the microbial ecology by creating an anaerobic environment. As discussed earlier, bacteria have optimum oxidation-reduction potentials (Eh). Aerobes and facultative aerobes may be inhibited by the mV negativity in the hermitically-sealed environment allowing pathogens and spoilage organisms to succumb to competitive inhibition. As spoilage organisms and unwanted pathogens compete for nutrients within the same environment,

anaerobic LAB have an advantage based on population size, pH, Eh, and other factors. Intrinsic and extrinsic factors continue to control pathogenic growth over time.

Storage Time

Time of storage during cheese ripening involves the controlled breakdown of proteins, lipids and carbohydrates. Ripening times depend upon the flavors, textures, and aesthetic properties desired of the cheese. Texture is important for functionality in cubing, slicing, and shredding operations, and these operations work best with cheese that has aged 30-90 days. At this aging time flavor is very mild; however, sharp Cheddars may take years to develop the desired flavor and body (University of Guelph 2009).

Protein Degradation (Proteolysis)

Proteolysis of cheese proteins provides flavor and body, but may also yield unwanted textures and flavors if not properly controlled. Non-specific proteases (e.g., pepsin) degrade proteins into smaller peptides that may release bitter flavors. Excessive proteolysis may result in cheese with a shorter body (see Figure 6) which is less rubbery, less elastic, but more meltable. The degree of proteolysis depends upon the cheese type; Cheddar, for example, is more dependent on the breakdown of proteins than lipids.

Fat Degradation (Lipolysis)

Milk contains a full and diverse selection of fatty acids (Table 1) that provide fresh dairy flavors with very low levels of free fatty acids (FFAs). Butyric acid found in butterfat, for example, is a rich dairy flavor compound that is the only natural fat rich in short chain fatty acids (University of Guelph 2009). Exposure to oxygen, metals, or sunlight during ripening, however, can lead to off-flavors and odors as a result of FFAs released during oxidation (Smith 2008). Free fatty acids may also be released due to the esterase "lipase" enzyme cleaving them from the glycerol molecule during ripening. Free fatty acids are also more accessible for bacterial catabolism. Packaging punctures or poor seals (i.e., leakers) in cheese packaging may cause food safety and quality concerns, and should be addressed immediately.

Lactose Degradation (Glycolysis)

Residual lactose remains available to be converted to lactic acid by starter LAB. If unchecked, LAB will continue to drive during ripening until the pH of the cheese is undesirable

as shown in Table 6. Lactic acid production can be controlled by the proper application of salt to slow fermentation by LAB. Residual lactose is also available to be converted to calcium lactate by non-starter lactic acid bacteria. Calcium lactate crystals appear on the surface of ripened Cheddar cheese, and are commonly mistaken for mold. Non-starter lactic acid bacteria (NSLAB) include *Lactobacillus* spp., *Pediococcus* spp., and *Leuconostoc* spp., and may be found in biofilms that form in pitted areas of stainless steel (Somers et al. 2001). Calcium lactate is the result of racemization of lactate that converts L(+) lactic acid to the less soluble D(−) isomer, and is a function of the ratio of total lactate (lactic acid, lactose, and galactose) to moisture.

Accelerated Ripening

Normal and accelerated ripening are functions of time and temperature. Although little data is available for cheese shelf-life, we can estimate the optimum for cheese quality using the concept of enzymatic Q10. Enzymatic Q10 is the rate of increased activity based on each 10°C increase in temperature (Fung 2007). Cheddar cheese Q10 may be calculated where: Q10 = t st (06°C)/ t st+10 (16°C) = 3 (Aramouni 2008). Using this model, it would normally take 12 months at 6°C to reach optimal ripening for grading purposes; however, it would take only 4.5 months at 16°C. This would greatly reduce the ripening time, but would also reduce the shelf-life and functionality of the product due to the degradation of proteins and lipids at a rate 3 times faster at 16°C.

Cumulative Stress

Cumulative stress is placed on microorganisms during fabrication and storage and includes the effects of pH shock, pH adaptation, starvation, cold stress, heat stress, physical damage, desiccation, chemical stress, and microbial antagonism. The effect on pathogenic and non-pathogenic microorganisms of induced stress factors created by multiple hurdles have been evaluated. Leenanon and Drake investigated the stress related to heat and cold shock on *Escherichia coli* O157:H7 (ATCC 43895) and nonpathogenic *E. coli* (ATCC 25922) (Leenanon and Drake 2001). Physical stress could be amplified by the manipulation of multiple factors such as starvation, acid stress, and temperature shock. Temperature shock occurs during HTST pasteurization in dairy processing. According to the PMO, grade "A" milk is to be kept at <45°F (7°C) throughout processing (USPHS and FDA 2007). Upon introduction to the dairy heat plate exchange system, it is sent through a regeneration section to warm the milk prior to being

introduced into the heating section where it is rapidly heated to a minimum of 161°F (72°C) for a minimum of 15 seconds. During this time the milk travels across a series of plate heat exchangers. The concept of heat transfer in liquids traveling in opposing directions across a metal plate was developed in 1878 by a German named Albert Dracke (Tetra Pak 2009). The plates used in this system are thin to allow heat transfer and are also corrugated to create turbulence. The cold milk is heated as it passes through a series of channels created by pressing the plates tightly together as hot water passes along the opposite side as shown in Figure 10. Milk is then held in holding tube that ensures all of the milk achieves the proper temperature.

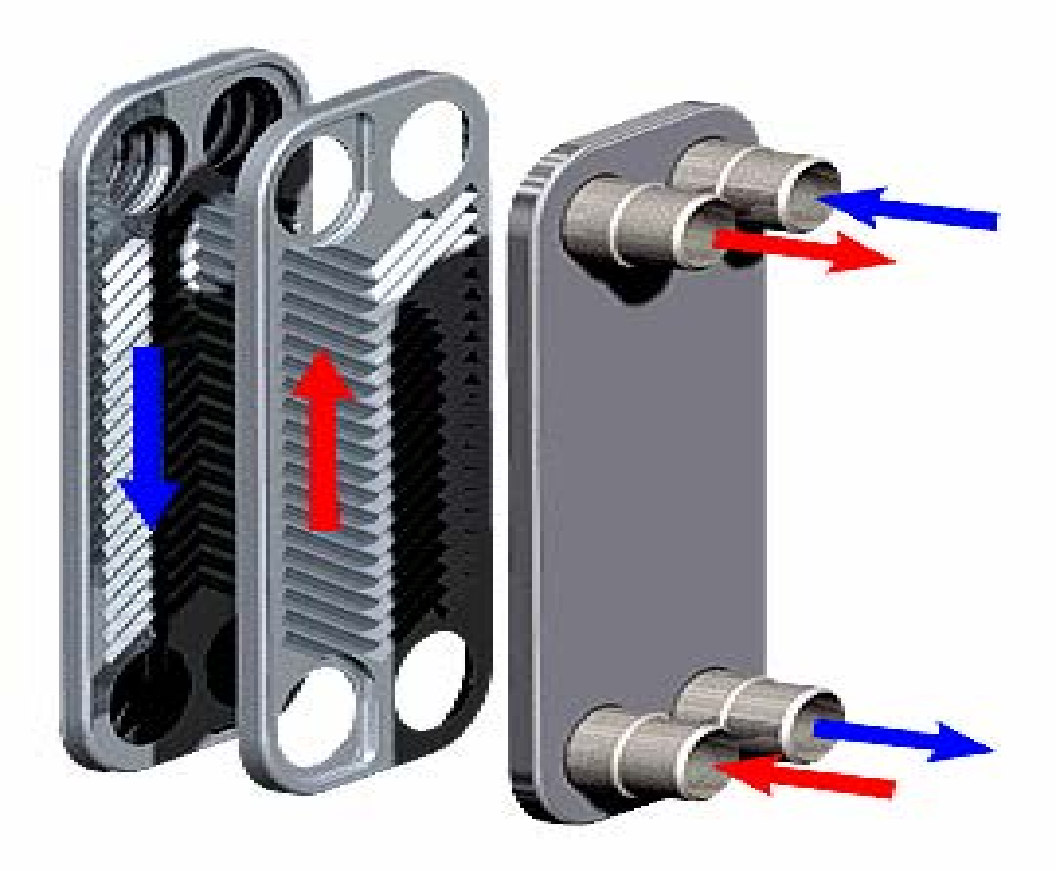

Figure 9: Plate Heat Exchanger Diagram
(AIMRH 2009)

CHAPTER 3 - Food Safety

Food safety systems, such as Hazard Analysis and Critical Control Points (HACCP), are necessary for food processors to continuously improve their food safety programs. In 2007, the United States Centers for Disease Control and Prevention (CDC) FoodNet reported a total of 17,883 laboratory-confirmed cases of infection caused by foodborne pathogens (US CDC 2009). This report indicated a recent increase in outbreaks of foodborne-disease associated with peanut butter (which had been considered low risk due to low a_w) and fresh vegetables. There have also been several cases of foodborne disease associated with milk and dairy products in the last two decades. In 2000, the CDC released a surveillance summary that detailed cases of foodborne disease from 1993 to 1997 (Table 12). During this time 86,971 cases of foodborne disease were reported, of which 1,507 cases were associated with milk and dairy products although only 34 cases (0.04%) associated with all cheese types during the same time frame (US CDC 2000).

Table 111: CDC Surveillance Summaries 1993 - 1997

Year	Milk	Cheese	Ice Cream	Other Dairy	Total Dairy	Total Cases	Dairy %	Cheese %
1993	28	20	32	41	121	17,477	0.69%	0.11%
1994	105	5	919	0	1,029	17,478	5.89%	0.03%
1995	3	9	60	0	72	17,479	0.41%	0.05%
1996	48	0	183	31	262	22,607	1.16%	0.00%
1997	23	0	0	0	23	11,940	0.19%	0.00%
1993-2007	**207**	**34**	**1,194**	**72**	**1,507**	**86,981**	**1.73%**	**0.04%**

Adapted from (US CDC 2000)

There is little statistical data available for illnesses linked to hard and semi-soft cheese types; however, there have been a few documented outbreaks. On August 3, 1976, ongoing *Salmonella* surveillance in Colorado led to the recognition of an epidemic of *Salmonella heidelberg* infections that eventually included 339 isolates. The majority of cases occurred in the summer and emerged in two widely separated cities—Denver and Pueblo. Epidemiological

investigation associated the outbreak with imported Cheddar cheese eaten at Mexican-style restaurants, and *Salmonella heidelberg* was isolated from seven production lots of the incriminated cheese (Fontaine et al. 1980).

In 1998, the Wisconsin Department of Health and Family Services initially reported 12 cases of *E. coli* O157:H7 infection among west-central Wisconsin residents who became ill during June 8 - 12 (US CDC 2007). During the subsequent investigation, the Wisconsin Department of Agriculture, Trade, and Consumer Protection visited a local dairy plant to collect cheese and environmental samples, to review employee food handling and hygienic practices, and to assess potential sources of contamination from raw milk. All product and environmental samples (e.g., vat surfaces and floor drains) from the dairy plant were screened for alkaline phosphatase (AP) activity. AP is present in all raw milk, but is deactivated at 72^{o}C (161^{o}F). The presence of AP is subsequently used as has to identify evidence of unpasteurized milk (Wehr and Frank 2004). Laboratory identification eventually linked the dairy to 55 individual cases of men, women, and children who experienced a variety of symptoms including bloody diarrhea; ultimately 43 pulsed-field gel electrophoresis laboratory-confirmed cases were associated with the consumption of fresh cheese curds. Samples taken during the investigation were also positive for AP. It was discovered in the subsequent investigation that the dairy plant had produced pasteurized Cheddar and Colby cheese products that were packaged as 40-lb blocks, daisies (rounds of cheese), and fresh cheese curds since 1977. The dairy plant also produced unpasteurized Cheddar cheese daisies every June as part of "Dairy Month". The CFR states that certain cheese products can be produced and sold legally as long as the cheese is held at >35^{o} F (1.7 ^{o}C) for at least 60 days before it is sold (US FDA 2007). Curds are consumed fresh, however, and must be made with pasteurized milk. It was determined that at least one 1,500-pound cheese vat was used to make fresh cheese curds after being used to make "raw milk" Cheddar cheese. The fresh curds, which were incorrectly labeled as pasteurized cheddar cheese curds, had been cross-contaminated with the pathogen and distributed and sold in six Wisconsin counties. These investigations illustrate the hazards associated with dairy due to the potential pathogens present in raw (i.e., unpasteurized) milk. Cheese manufacturers that produce hard and semi-soft natural cheeses rely on intrinsic and extrinsic factors to reduce the likelihood of pathogens and prevent foodborne illnesses. Dairy processors, however, may wish to take a proactive approach to ensuring continuous food safety: HACCP.

Hazard Analysis Critical Control Points (HACCP)

HACCP in Dairy

The HACCP system concept was originally developed in the 1960s by the Pillsbury Company, NASA, and the US Army Natick Laboratories to ensure the safe production of foods for the space program. It is based on a preventative, systematic approach to food safety, and has been endorsed by the National Advisory Committee on Microbial Criteria for Foods (NACMCF) and the international Codex Alimentarius Committee on Food Hygiene (Codex) since 1992 and 1997, respectively (Stevenson and Bernard 1999). Although HACCP is not legally required in the dairy industry, this effective and highly structured system has been used in the dairy industry to supplement 21 CFR and the PMO. The National Conference on Interstate Milk Shippers (NCIMS) piloted a program from 1999 – 2003 to evaluate the effectiveness of a HACCP system. In 2003, the NCIMS HACCP pilot program was adopted by Grade "A" dairy plants as a voluntary alternative to state regulatory enforcement of the PMO (IDFA 2007). The NCIMS HACCP pilot program encompasses the terminology (Table 13) and guidelines established by the NACMCF and Codex HACCP systems.

Table 12: NCIMS HACCP Terminology

Term	Definition
Adulterated	A food that bears or contains any poisonous or deleterious substance that may render it injurious to health.
Control	To manage the conditions to maintain compliance with established criteria and the state where correct procedures are being followed.
Critical Point	Any step at which biological, chemical, or physical factors can be controlled.
Critical Control Point	Any point at which control can be applied and a food safety hazard can be prevented, eliminated, or reduced to acceptable levels.
Critical Limit	A maximum and/ or minimum value to which a hazard must be controlled at a CCP.
Deviation	A failure to meet a Critical Limit at a Critical Control Point.
HACCP Program	The written HACCP Plan and prerequisite programs such as GMPs.
HACCP Team	Personnel responsible for developing the HACCP Program.
Hazard	A biological, chemical, or physical agent that is of sufficient severity or is likely to cause injury or illness in the absence of its control.

Adapted from (IDFA 2007)

Prerequisite Programs

An overall dairy HACCP program will include the HACCP Plan as well as prerequisite programs. Prerequisite programs are the foundation of a HACCP system, and include current Good Manufacturing Practices (cGMPs) as outlined in 21 CFR 110, and are required per Appendix K of the PMO. Current Good Manufacturing Practices are outlined in §110 to include general provisions, building and facilities, equipment, production and process controls, and defect action limits. Key components of cGMP programs include personnel practices, production related practices, housekeeping, equipment and utensils, building and grounds, maintenance, visitors and contractors, training, record keeping, and self-assessment (IDFA 2008). The NCIMS HACCP program further requires the program to address the safety of water, condition and cleanliness of contact surfaces, prevention of cross-contamination, maintenance of hand washing and toilet facilities, protection of food from adulteration, proper labeling and storage of toxic compounds, control of employee health conditions, and the exclusion of pests. Prerequisite programs should designate personnel, frequency, and materials required to maintain these systems in written Standard Operating Procedures (SOPs) and Sanitation Standard Operating Procedures (SSOPs).

HACCP Plan

The HACCP Plan is the portion of the overall HACCP Program that addresses specific food safety concerns. The manufacturer must perform five preliminary tasks as shown in Table 14 prior to developing the HACCP Plan. Dairy processors may have HACCP Plans that are unique to their operation; however, each plan will be developed using seven basic principles outlined by the FDA, NACMCF, Codex, and NCIMS, as shown in Table 15.

Table 13: Preliminary Tasks

Step	Action
1	Assemble the HACCP Team.
2	Describe the food and its distribution.
3	Describe the intended use and consumers of the food.
4	Develop a flow diagram which describes the process.
5	Verify the flow diagram.

Adapted from (Stevenson and Bernard 1999)

Table 14: Seven Principles of HACCP

Step	Action
1	Conduct hazard analysis and identify control measures.
2	Identify Critical Control Points (CCP)
3	Establish Critical Limits (CL)
4	Monitor each CCP
5	Establish corrective action taken each time a CL deviation occurs.
6	Establish Verification procedures.
7	Establish a record-keeping system.

Adapted from (IDFA 2008)

The first preliminary task in developing a HACCP Plan is to assemble the HACCP Team that consists of a HACCP Coordinator, who has formal training in HACCP, and a multidisciplinary team. The team should include personnel with a variety of skills and expertise including quality assurance, microbiology, production, maintenance, engineering, etc. The next step is to describe the food and its distribution in order to fully understand the nature of the product and how it will enter into commerce. The team should then describe the intended use and consumers of the food as foods that require further processing may have specific concerns as do those that are sold as ready to eat, and they should note whether the intended consumers may be children, elderly, or immuno-compromised individuals who may be more susceptible to certain food safety risks. The HACCP Team must then develop a flow diagram which describes the process in which the food will be manufactured and distributed. The flow diagram will outline all steps from the receipt of raw materials through the processing, storage, and shipping operations, and the last step will be to verify the flow diagram by performing an on-site inspection to ensure its accuracy (Stevenson and Bernard 1999). Once these preliminary tasks have been completed by the HACCP Team, they may begin to apply the seven basic principles.

The first principle step in developing a HACCP Plan is to conduct a hazard analysis and identify control measures. The hazard analysis is the process of collecting and evaluating information on hazards that are associated with the food being manufactured or processed. It should take the type of food into consideration to address all potential hazards that may be associated with the food using the flow diagram. During this step, any physical, chemical, or biological concern should be addressed and designated as a critical point (CP). Once CPs have been identified, the team will establish critical control points (CCPs). A CCP is any step where

control can be applied to prevent a food safety hazard or reduce the hazard to an acceptable level. It is important to note, however, that a CP (e.g., milk receiving) might not be designated as a CCP if there is a subsequent step (e.g., pasteurization) that will control the hazard. Federally regulated operations, such as β-lactam antibiotic testing and HTST pasteurization, shall always be addressed as CCPs; however, the manufacturer may also choose to designate metal detection, a_w, pH, or AP testing as CCPs. The HACCP team may then establish critical limits (CLs) for those steps where a lack of control will likely result in the development of a potential food safety hazard. The CLs are parameters that define whether the CCP is in control or if a deviation has occurred, and are based on operational parameters or established regulator limits. Operational parameters in the production of cheese may include setting pH target values to control the growth of pathogens in those instances where there is no established regulatory limit. Other CLs such as HTST temperature and flow rate are clearly defined in the PMO as well as 21 CFR to ensure that every particle of milk has been properly pasteurized. The screening of raw milk for the presence of β-lactam antibiotics should also be conducted using equipment with limits of detection outlined in Appendix N of the PMO. The HACCP Team should then establish monitoring procedures to determine whether the CLs are being met. Monitoring is a key element in determining if operations are being conducted in a manner sufficient to control the identified hazards, and should be performed by trained operational or quality assurance personnel at a designated frequency. The failure of a CCP to operate within CLs is identified as a deviation, and monitoring personnel should take immediate action to ensure that any product affected will not enter into commerce. The HACCP Team should establish a pre-determined set of corrective actions to be taken for each deviation that addresses the root cause of the deviation and preventative measures to reduce future occurrences. The team should then establish verification procedures, other than monitoring, to determine the validity of the HACCP Plan. Verification includes the validation and re-validation of procedures by reviewing technical data, customer feedback, and current food safety events to ensure that the plan is effective. The team should also establish record-keeping and documentation procedures that include the summary of the hazard analysis (Table 16), the HACCP Plan, support documentation, and daily operational records. Record-keeping assures that all documentation is complete, and provides the dairy establishment with the evidence necessary to verify that the product was produced in accordance with the HACCP Plan (Stevenson and Bernard 1999).

Table 15: Cheddar Cheese HACCP Summary Example

HACCP Summary			Monitoring						
CCP	Hazard	Critical Limits	Process Step	Method	Freq	Personnel	Corrective Action	Verifi-cation	Record Keeping
CCP-1C Antibiotic Screen	Beta Lactam Antibiotics	>5 ppb Penicillin G	Raw Milk Receiving	Charm Rosa SL3 or equivalent	Each tanker truck of raw milk Startup and once per shift	Production Supervisor Trained QA Technician	Positive screen will result in product on "Hold" until confirmation. State Sanitarian must be notified.	Review at end of each Shift Start up and once per shift	Training Records, Continuous printout and Screen Log, Calibration Records, State Confirm. Log.
CCP-1B HTST Past-eurization	Pathogens	>161F >15 sec	Milk to HCV	Temp and Flow Meter	Continuous Monitoring Startup and once per shift	Production Supervisor Trained QA Technician	Flow divert to raw balance tank. Failure to divert will result in product on "Hold" until phosphatase analysis by approved lab.	Review at end of each Shift Start up and once per shift	Training Records, Continuous HTST Log, Calibration Records, Corrective Action Log, State Timing and Sealing records
CCP-1P Detection of Metal	Metal	7.0 Stainless Steel, 6.0 Non-ferrous, 5.0 Ferrous	Cheese prior to Boxing	Continuous Metal Detector	Continuous Monitoring Startup and once per shift	Production Supervisor Trained QA Technician	Detection of metal of failure to reject wand due to sensitivity will result in product on "Hold" back to last acceptable verification.	Review at end of each Shift Start up and once per shift	Training Records, Continuous Monitoring Log, Calibration Records, Corrective Action Log

Adapted from (Aramouni 2008)

As mentioned previously, many food safety parameters may also have quality implications. The laboratory screening of raw milk for Beta lactam antibiotic residue, for example, is listed in Table 15 as a CCP-1C. The Beta lactam group consists of common antibiotics that are routinely used to treat dairy cattle. They are considered a chemical hazard as some consumers are allergic to the presence of these drug residues, and raw milk coming into the cheese plant must be screened for their presence per Appendix N of the PMO (USPHS/ FDA 2007). If these antibiotics entered the milk stream into the cheese plant, they would continue to affect both cheese quality and food safety as they would destroy the cell walls of the starter

cultures; consequently, there would be no Δ pH. The finished cheese would have a high pH (> 5.5) that would give it a "plastic" texture (as shown in Figure 6) and make it more susceptible to the growth of pathogens or spoilage organisms.

HTST pasteurization, listed in Table 15 as CCP-1B, is another food safety parameter with operational criteria outlined in both the PMO and 21 CFR 133.3. As discussed previously, HTST pasteurization is designed to ensure the proper heating of "every particle of milk or product, in properly designed and operated equipment" (USPHS/ FDA 2007, pg. 7). The legal pasteurization of raw milk to ≥ 161°F (72°C) for ≥ 15 seconds is critical for the reduction of mesophilic pathogens (e.g., EHEC and *Salmonella* spp.); however, it is also an important factor in cheese quality. Improperly pasteurized milk would allow heterotrophic (i.e., gas forming) bacteria (e.g., coliforms) to cause "early blowing and a disagreeable taste" during cheese grading (Tetra Pak 2003, pg. 306). Exceeding the legal operational parameters for time and temperature, however, would denature the caseins and whey proteins. The resulting yield loss in cheese and whey protein operations as well as potential sensory concerns should always be considered before using a heat treatment that is more intense than the legal limit.

CHAPTER 4 - Conclusion

This paper has reviewed the safe production of hard and semi-soft cheeses made naturally using pasteurized milk, enzymatic coagulation, and starter cultures manufactured in accordance with the Pasteurized Milk Ordinance and Title 21 of the Code of Federal Regulations. Natural cheese making uses a combination of intrinsic and extrinsic factors to create multiple hurdles that reduce the likelihood of pathogens and spoilage organisms in the final product while providing quality benefits. Milk components, milk flora, starter cultures, enzymes, and salt also give cheese its flavor and texture while creating natural antimicrobial properties that provide natural protection against unwanted microorganisms. Thermal processing, cooking, packaging, cooling, and aging all impact quality while further providing measures of control to ensure the safety of the finished product. These parameters also provide critical control points and critical limits that may be used voluntarily by the dairy industry in proactive food safety systems such as HACCP to reduce the likelihood of foodborne disease. Consequently, these factors do not work independently, but interact as food safety, sensory, and performance parameters during the production of hard and semi-soft cheese types.

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