Cheddar Cheese Review: I Cheese Manufacture

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Abstract

Cheddar cheese is one of the most popular cheese varieties in the world. It can be categorized as hard cheese. According to its standard of identity, Cheddar cheese is required to have a minimum of 50% fat content and a maximum of 39% moisture content. Cheddar cheese is rennet coagulated and will require up to two years of aging before consumption. The production of Cheddar cheese can be divided into two stages, which are manufacture and ripening. The manufacture of Cheddar cheese consists of preparing and standardizing milk, adding starter culture with rennet, coagulating milk, cutting the coagulum into small cubes, heating and agitating the cubes, removing the whey, fusing the curd into slabs followed by milling or continuous stirring the Cheddar curd, salting, pressing, vacuum packaging, and ripening prior to consumption.

Keywords: Cheddar cheese, milk protein, rennet coagulation

Introduction

The global cheese consumption is expected to reach 21 million metric tons, which is a 20% increase, between 2008 and 2015 (PRWeb, 2008). About two-thirds of total U.S. cheese production in 2010 was from Cheddar and Mozzarella cheeses. Cheddar cheese is ranked the second behind Mozzarella with a difference of only approximately 22,000 lbs (National Agriculture Statistics Service, 2011). Although Cheddar making is a cheese relatively simple process, it is still challenging to produce Cheddar cheese with consistent quality (Lawrence et al.,

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2004). Cheddar cheese manufacture has been continuously studied for almost 150 years as the first reliable Cheddar cheese analyses were reported and published in 1877 (van Slyke, 1893). Significant improvements in the cheese manufacture have been made over a century. For instances, pasteurization process was applied to the cheese milk in the beginning of the 1900's. The use of pasteurized milk in place of raw milk for the Cheddar cheese manufacture was proven to provide more uniform and better quality Cheddar cheese, which ultimately provided more profits for the cheese manufacturers (Price, 1927).

Mechanization for the Cheddar cheese manufacture was introduced around the 1950's. The mechanized system could essentially shorten the whole cheese manufacture, which would be beneficial for the cheese manufacturers (Czulak, 1958; Olsen, 1980). The objective of this article is to give an overall review on manufacture of Cheddar cheese.

Milk quality

Cheese is a complex biological system. To manufacture Cheddar cheese with consistent quality, good quality and clean-tasting milk that has relatively low microbial count is needed (Farkye, 2004). Some aspects of milk quality maybe defined under a broad range of characteristics including microbial, chemical and enzymatic.

Microbial quality

Raw milk can contain pathogenic bacteria such as *Escherichia coli*, *Salmonella*, *Listeria*, *Campylobacter*, *Mycobacterium* and *Brucella*. Yeast growth during cheese ripening can also originate from raw milk. However, most, if not all, current Cheddar cheese production in industrial scale use pasteurized milk. Thus, pathogenic bacteria and yeast from raw milk will be of no greater threat to public health. Proteinases from psychrotrophic bacteria in milk such as *Pseudomonas fluorescens* and *P. putrefaciens* are heat stable, which are not affected by pasteurized temperature. These enzymes can hydrolyze C-terminal region of β-casein and α_{s1}-casein, resulting in bitter hydrophobic peptides that can cause bitter flavor to accumulate during cheese ripening (Sheehan, 2013).

Chemical residues

Antibiotics are the main chemical residues that can be found in milk. Normally, antibiotics are used to treat mastitis or infections of the cow mammary gland caused from bacterial infection. If present in cheese milk, antibiotic residues will cause partial or total inhibition of starter culture growth and acid production. During Cheddar cheese manufacture, if low levels of antibiotic residues are present, rate of acidification during draining and salting is reduced, which will result in longer manufacture time and may cause a higher pH in cheese. High levels of antibiotic residues can cause a complete termination

of acidification after rennet addition, which will result in an exceptionally high pH in cheese. This resultant cheese may also have an uneven texture with pasty body and unusual off-flavors (Sheehan, 2013).

Enzyme activity

Plasmin is the main indigenous proteinase in milk. If high level of plasmin is present in milk, the rate of primary proteolysis will also be elevated. This will result in longer rennet gelation time and low gel firmness with more porous and open structured rennet gel. Because plasmin is relatively high-heat stable, its contribution to primary proteolysis is more obvious in cheeses that used high cooking temperature during manufacture such as Swiss and grana-type cheeses.Comparing to Cheddar cheese, the level of plasmin activity in high-cook cheeses is higher because plasmin inhibitor is inactivated (Sheehan, 2013).

Milk protein

Originally, milk proteins were believed to be a simple homogeneous protein, but about a century or more ago, milk proteins were divided into two broad classes. The first fraction, which is about 80% of the protein in bovine milk, is precipitated at pH 4.6 (isoelectric pH) at 30ºC, and is now called casein. The second minor fraction, makes up about 20% of protein, is soluble under those conditions, and is now referred to as whey protein, serum protein, or non-casein nitrogen (Dalgleish, 1982). It is suggested that the proteins in casein micelles are bound together by two types of bonding, and it is a balance between the attractive hydrophobic interactions and electrostatic repulsion. Hydrophobic interaction is the driving force for the formation of casein micelles, while electrostatic repulsions are limiting the growth of polymers or in other words defining the degree of polymerization. The conformation of α_{s1}- and β-caseins when they are adsorbed at hydrophobic interfaces form a train-loop-train and a tail-train structure, respectively, and both caseins polymerize or self-associate, by hydrophobic interactions.

Accordingly, the self-association of caseins makes it possible for polymerization to occur. Calcium phosphate nanoclusters, or CCP, are considered to be one of the linkages between casein micelles and act as neutralizing agents of the negative charge of the phosphorserine residues. Consequently, electrostatic repulsion is reduced and the hydrophobic interaction between caseins is still dominant, resulting in more associations of proteins. Unlike the other caseins, κ-caseins can only interact hydrophobically and acts as a propagation terminator, because they do not have a phosphoserine cluster to bind calcium and also another hydrophobic point to prolong the chain (Horne, 1998). The structure of casein micelle is as shown in **Figure 1.**

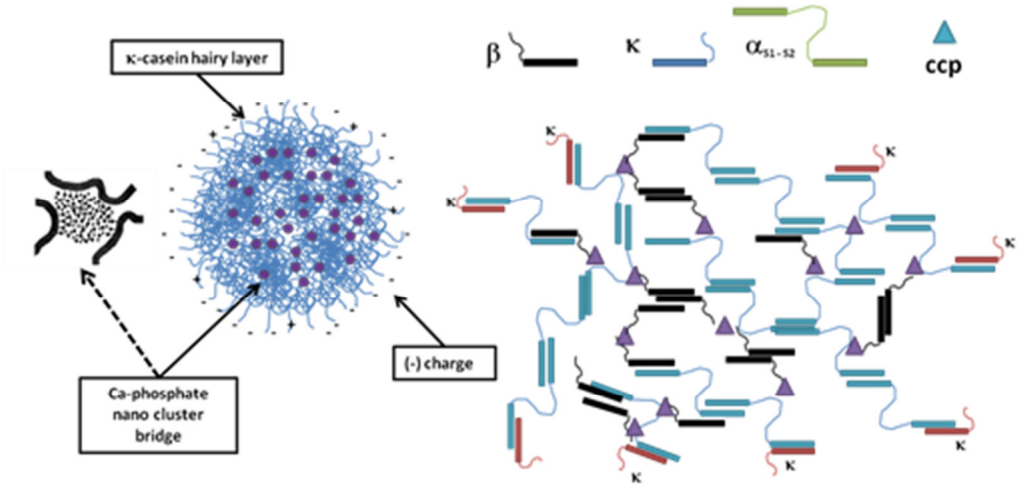

Figure 1 The dual bonding model of casein micelle structure, with α-, β-, κ-casein portrayed as indicated. Bonding appears between the hydrophobic regions, shown as rectangular bars, and by linkage of hydrophilic regions containing phosphoserine clusters to colloidal calcium phosphate (CCP) clusters. Molecules of κ-casein (K) limit further growth of the structure.
Adapted from Goff, 1995 and Horne, 1998

Importance of calcium-phosphate and κ-casein to the casein micelle

One of many important functions of the casein micelle is to solubilize calcium phosphates in milk (Farrell Jr. et al., 2006). The dry matter of bovine casein has been found to consist of about 94% protein and 6% mineral, which is colloidal calcium phosphate (CCP) (Horne, 2006). The relationship between investigated for over a century. However, this relationship has not yet been fully understood (Fox and Brodkorb, 2008). As hypothesized by De Kruif and Holt (2004), CCP could be bound and stabilized by phosphopeptide portions of α_s- and β-caseins, resulting in the formation of calcium-phosphate nanoclusters CCP and casein micelles has been vigorously or CCP (**Figure 2**). This CCP would randomly grow and precipitate without bridging with peptides. In addition, the formation of CCP is believed to generate casein micellestructure by randomly binding with phos-phorproteins until a size limited colloid is formed.

Figure 2 Casein-calcium/phosphate cross-linked network; the black strands represent casein network, the oval dots with the black tails represent organic phosphate, the oval dots represent inorganic phosphate, and the circle dots represent calcium ions.
Source: Metzger, 2009 (personal communication).

According to Horne and his dual-binding casein micelle structure model, CCP is considered to begin the process of casein micelle formation by acting like a bridge and neutralizing agent for the phosphoproteins, which hydrophobically interact to each other. The hydrophobic blocks of protein-protein interactions and the CCP linkage further generate the casein micelle formation. This casein micelle has a gel-like structure with embedded CCP and κ-caseins as chain terminator (Horne, 1998; Farrell Jr. et al., 2006). CCP along with hydrogen bonds, hydrophobic and electrostatic interactions are responsible for casein micelle stability. It was found that the micelles dissolve into small particles in milk solution once the CCP is removed by acidification, dialysis or Cachelator; thus this phenomenon suggests that CCP play an important role in cementing the micelles together (Fox and Brodkorb, 2008

At the concentration of protein and calcium found in bovine milk, Ca-sensitive caseins (α_{s1}-,α_{s2}- and β-caseins) are readily precipitated by calcium bound to their phosphoserine residues. However, κ-casein, which is soluble in calcium, can interact and stabilize about 10 times its mass around the core of Ca-sensitive caseins. In addition, because of the negative charge from oligo-saccharide chains at the carboxy-terminal ends of κ-caseins, they can provide steric stabilization for the casein micelles in milk. It has long been believed that κ-casein is

the only type of casein protein in the surface layer. This has been confirmed by the decrease in hydrodynamic diameter during renneting, since chymosin removes the protruding macropeptide portion of κ-casein. However, other researchers have found that N-terminal residues of all caseins are released by super-polymerized amino peptidase that cannot diffuse into the micelle. These phenomena suggest that κ-casein is not very exposed and that the surface of the micelle is not exclusively covered with κ-casein. Consequently, some of the other casein fractions are also located on the surface of the micelle (Horne, 2003; Horne, 2006; Farrell Jr. et al., 2006; Fox and Brodkorb, 2008). A study by Dalgleish et al. (2004) suggested that the casein micelle surface was more complex than just a simple hard sphere covered by a 'hairy layer'. The electron micrograph from their study also suggested that the micelles consist of seemingly casein tubules with the end protruding from the bulk structure that protects the micelles. They then hypothesized that κ-casein is probably only located at the ends of the tubes and not evenly covering the entire surface, since the amount of κ-casein in milk is not sufficient to cover the entire micelle surface on its own

Cheddar cheese manufacture

Milk is a highly perishable food; thus, cheese manufacture is a form of milk preservation. All cheeses from both acid and rennet coagulate can be roughly classified, based on their moisture content, as soft, semisoft (semi hard), hard, or very hard (**Figure 3**). (Farkye, 2004).

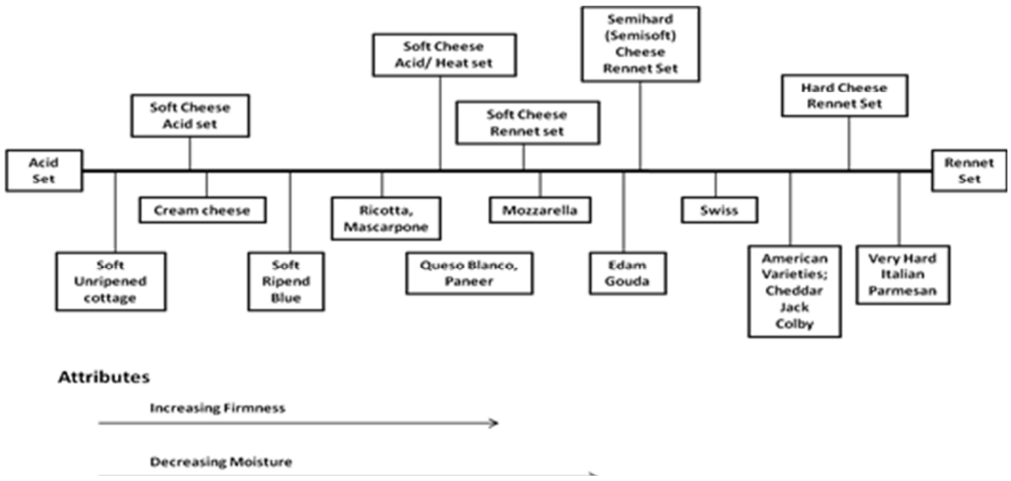

Figure 3 Classification of cheeses based on their moisture content.
Adapted from Farkye, 2004.

Cheddar cheese can be categorized as hard cheese, which will require some pressure to chew and break its structure apart. Cheddar cheese is rennet coagulated and required two months to two years of aging before consumption. Cheddar cheese that has been ripened for a longer period of time will allow its flavor, aroma and texture to be fully developed (Mc Sweeney, 2004). The production of Cheddar cheese can be divided into two stages, which are manufacture and ripening. The manufacture of Cheddar cheese (**Figure 4**) consists of preparing and standardizing milk, adding starter culture with rennet, coagulating milk, cutting the coagulum into small cubes, heating and agitating the cubes, removing the whey, fusing the curd into slabs followed by milling or continuous stirring the Cheddar curd, salting, pressing, vacuum packaging, and ripening prior to consumption. (Hill, 1995; Lawrence et al., 2004; Fox and McSweeney, 2004).

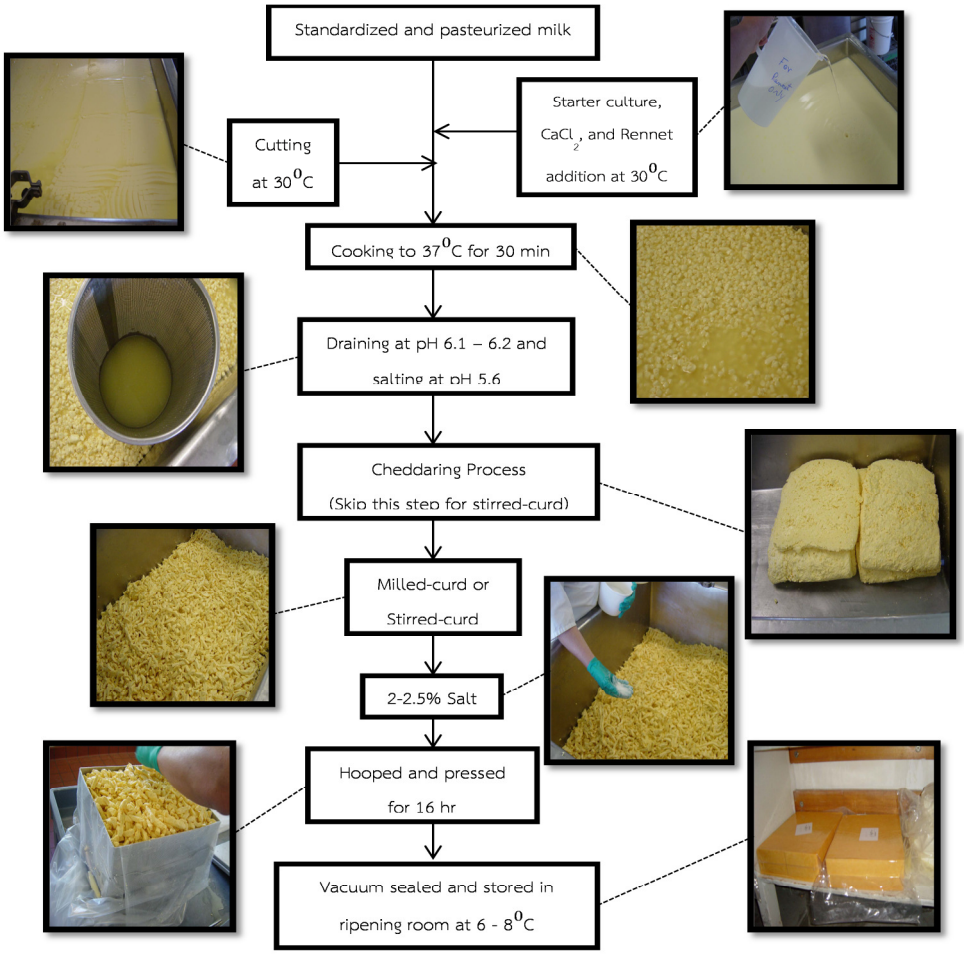

Figure 4 The manufacture diagram of Cheddar cheese.
Adapted from Avila, 2014.

The ripening process of Cheddar cheese will not be process of Cheddar cheese will not be discussed in this paper.

Rennet coagulation of milk

Rennet is a general term for proteinase used to coagulate milk. Milk coagulants from several sources such as vegetable, animal, bacteria and fungi have been used in cheese making. Rennet, a natural coagulant extracted from the fourth stomach of the calf was the main choice for the early cheese makers.

Traditionally, rennet extracted from young calf stomachs are used to make Cheddar cheese. This rennet contains 88–94% chymosin, providing approximately 90% of total milk clotting activity, and 6–12% pepsin, providing approximately 10% of milk clotting activity. Bovine chymosin is an aspartyl proteinase containing approximately 320 amino acid residues. Its physiological role is to coagulate milk in the young mammal stomach, resulting in an increase in the digestion efficiency. (Horne and Banks, 2004; McSweeney, 2007).

The cheese production has increased, while the supply of calf rennet has decreased; thus this led to the use of alternative products, which are other types of aspartyl proteinases. Rennet substitutes should have high milk clotting activity compared to general proteolytic activity and specific activity on κ-casein. Rennet substitutes include bovine and porcine pepsins, microbial aspartyl proteinases, and fermentation-produced chymosin cloned from microorganisms. Rennet extracted from older bovine contains about 6–10% chymosin and 90–94% pepsin. Bovine pepsin is quite effective once blended with chymosin. Porcine pepsin is unstable at pH more than 6; thus it is usually used along with calf rennet.

Some yeasts and molds such as *Rhizomu cormeihei*, *R. corpusillus*, and *Cryphonectria parasitica* can naturally produce proteinase. Fermentation-produced chymosin or recombinant chymosin is produced by fermentation of identical calf protein chymosin obtained from cloning calf chymosin into host micro-organisms (*Kluyveromyces lactis, Aspergillus niger,* and *Escherichia coli*). These rennets have shown excellent results in cheese processing, but their use is still subjected to regulation (McSweeney, 2007).

The coagulation reaction can be divided into two phases, as shown in Figure 5. The primary phase: the protolithic enzymes (chymosin, pepsin or microbial proteinases) cleave κ-caseins at a specific bond. The secondary phase: casein micelles start to aggregate. These coagulation phases are in fact overlapping, since casein micelles may begin to aggregate before the κ-casein hydrolysis is completed. As previously mentioned, κ-caseins are glycosylated with hydrophilic short sugar chain in the carboxy-terminal ends called glycomacropeptide (GMP). This GMP consists of residues 106-169, and hydrophobic para-κ-casein (residues 1-

105). In order for the bovine casein micelles to aggregate, κ-casein has to be hydrolyzed at the Phe_{105}–Met_{106} bond between para-κ-casein and GMP.Chymosin is considered suitable for cheese manufacture, because it is specifically active in hydrolysis of the Phe_{105}–Met_{106} bond of κ-casein. Thus, during milk coagulation, κ-casein is hydrolyzed at the Phe_{105}–Met_{106} bond, and the hydrophilic GMP is released into the serum phase, while para-κ-casein remains bound to the casein network. The ongoing loss of GMP results in the decrease in the micelles zeta potential from -20 mV to about -10 mV, and destabilization of the micelles.

Once κ-caseins have been sufficiently hydrolyzed; casein micelles will aggregate (Dalgleish, 1993; Horne and Banks 2004). In addition, the micellar calcium and phosphate are dissolved into the serum phase at the lower pH (Denmark and Walstra, 2004).

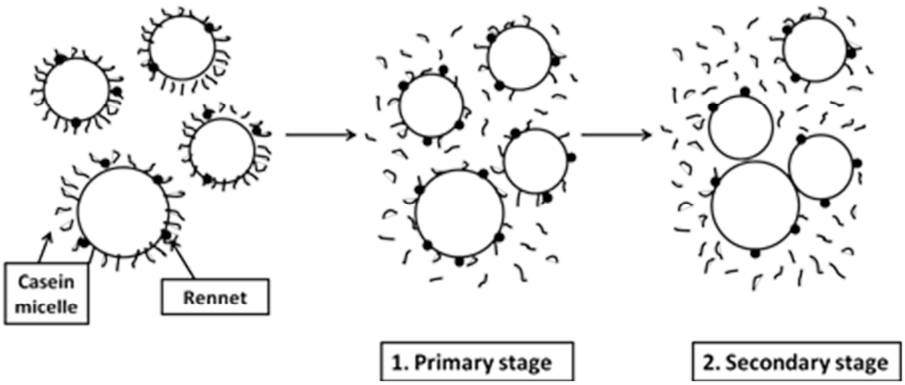

Figure 5 Two stages of rennet coagulation. Primary stage–rennet enzyme cuts off κ-casein fragments, thus removing the net negative charge from the micelle surface. Secondary stage–casein micelles aggregate and form a gel network.
Adapted from Dalgleish, 1993.

Milk coagulation properties

Milk coagulation properties (MCP) are important factors for cheese processing, cheese yield, and cheese quality. The most common approach to study MCP is to monitor the viscosity of milk samples after rennet addition that have been maintained at a fixed temperature. The formgraph is an apparatus that allows simultaneous evaluation of MCP from several milk samples. Formagraph-based MCP measurement is based on movement of small-loop stainless-steel pendulums immersed in linearly oscillating samples of coagulating milk. Forces are applied to the pendulums because of the gel formation in the moving milk samples. These forces are recorded,

and a typical formagraph is produced as shown in Figure 6. From the formagraph, three parameters that are considered to be useful in order to monitor MCP include: 1) rennet coagulation time (RCT, r, min), which measures the interval from zero (enzyme or rennet addition time) to the baseline begins to widen, 2) the time to curd firmness of 20 mm (k_{20}, min), which measures the interval from the start of gel formation to the time that oscillation width becomes 20 mm, and 3) the curd firmness 30 min after rennet addition (a_{30}, mm), which corresponds to the width of the formagraph 30 min after enzyme addition (Bittante, 2011).

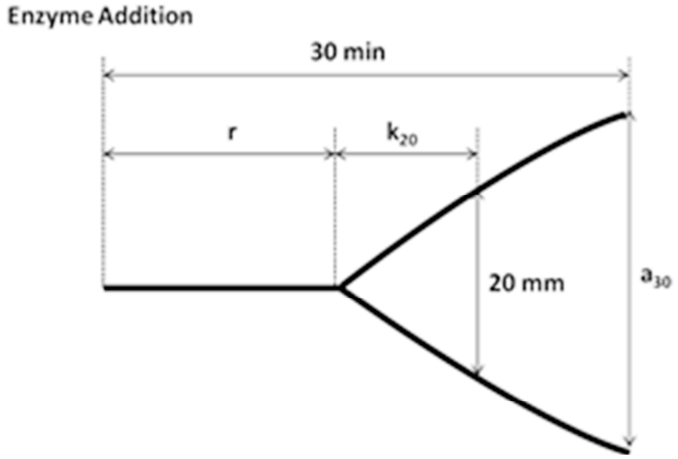

Figure 6 Diagram of rennet coagulation time (r = RCT, min) and time to curd firmness of 20 mm (k_{20}, min), and curd firmness 30 min after enzyme addition (a_{30}, mm) as a function of time as recorded with the Formagraph. Adapted from Bittante, 2011.

Many indigenous factors have great impact on MCP such as ruminant species, breeds, and genetic variants of milk protein especially κ-casein. **Figure 7** depicts direct and indirect genetic effects on MCP. Milk from different ruminant species has different curd firmness pattern. For example, milk from smaller ruminants such as goat and sheep coagulates earlier than cow milk. This means that RCT from the formagraph is longer, and, thus, k_{20} and a_{30} might not be measurable. For bovine breeds, milk from Holstein-Friesian and some Scandinavian breeds is considered to be non-coagulating milk (De Marchi et al., 2013), which is milk that has longer RCT, lower a_{30}, and immeasurable k_{20} than does milk from Brown Swiss, Simmental and other local alpine breeds (Bittante et al., 2012). Genetic variation of κ-casein in the degree of glycosylation (GD)

has direct effect on MCP. It has been recently reported that GD of milk from Simmental breed ranged from 22 to 76%, and milk with higher GD had shorter RCT than milk with lower GD. Thus, the association between RCT and κ-casein content mainly owes the glycosylated fraction of the protein (Bonfatti et al., 2014).

Other factors that affect MCP include drug residues in milk and coagulation temperature. In order to prevent the occurrence of disease in milk production, drug treatment might be used. Thus, milk might contain residual drugs, which can pose problem for the cheese manufacture. It has been reported that milk samples containing trimethoprim had longer RCT, lower a_{30}, and immeasurable k_{20}, while the presence of sulfamides did not alter MCP (Dreassi et al., 2007). Lastly, coagulation temperature poses direct impact on cheese microstructure, which consequently influences cheese and flavor. Ong et al. (2011) studied effect of coagulation temperature on the microstructure and composition of full fat Cheddar cheese. They reported that the microstructure of milk gel coagulated at 27°C consisted of a fine interconnected protein network, whereas gel coagulated at 36°C consisted of a coarse, irregular and more discontinuous protein network. At a higher coagulation temperature, hydrophobic and ionic interactions increased, which caused rearrangement of casein micelles and an increasing the size casein aggregates and the size of protein strands, as shown in **Figure** 8.

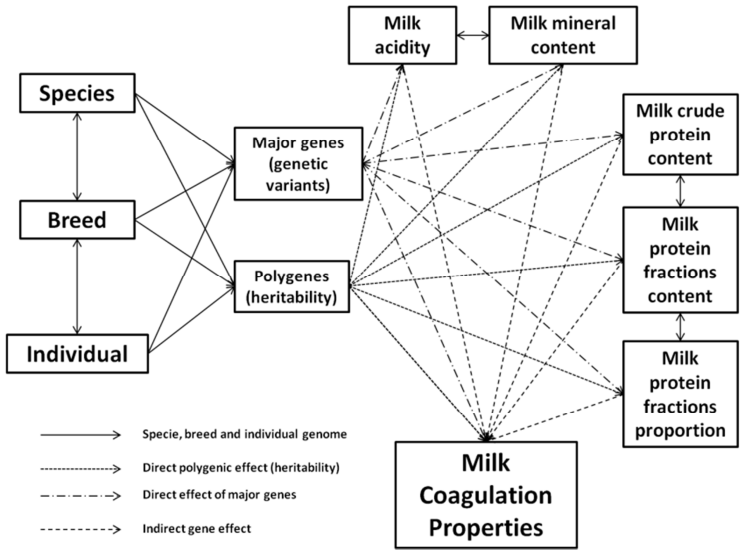

Figure 7 Direct and indirect genetic effects on milk coagulation properties.
Adapted from Bittante et al., 2012.

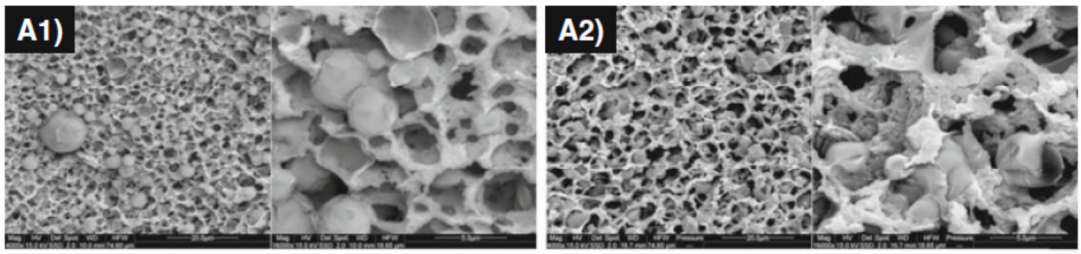

Figure 8 Cryo SEM micrographs of rennet milk gel coagulated at (A1) 27°C and (A2) 36°C.
Adapted from Ong et al., 2011.

Cheddaring and salting steps

Traditionally, Cheddar cheese utilizes the cheddaring process, where the curd is allowed to fuse into slabs, which are turned, piled, and re-piled at regular intervals for 1 to 2 hr until reaching the desired pH. This process causes the curd granules to fuse together under gravity, which leads to a close-knit and fibrous cheese structure. The cheddared curds are followed by milling, which involves mechanically cutting curds into small pieces. The milling process facilitates uniform salt distribution into the curds and promotes whey drainage from the curds. However, because of the development of hooping and pressing of salted granular curd under vacuum, the stirred-curd method has become more commonly accepted. In this method, drained curds are continuously stirred until reaching the desired pH. Although the constant agitating does not allow knitting of curds, the use of 'block former' hooping and vacuum pressing system yields Cheddar cheese with a close-texture characteristic. Thus, the stirred-curd method facilitating by hooping and vacuum pressing eliminate the need for cheddaring and milling. The stirred-curd method requires shorter time than the traditional method; thus this is the method of choice in highly mechanized cheese plants (Lawrence et al., 2004; Serrano et al., 2004; Rehman et al., 2008).

Salt plays an important role in the quality of Cheddar cheese, which include 1) controlling the growth of lactic acid bacteria and undesirable bacteria such as coliforms, staphylococci and clostridia, 2) controlling the final pH of Cheddar cheese as a result of starter culture activity retardation, and 3) controlling overall flavor and texture of the cheese. The main method of salting Cheddar cheese curd is directly mixing dry salt crystals into milled or stirred curd pieces after whey removal. After dry salt is distributed over the surface of cheese curd, some salt dissolves and slowly move inwards the curd. This causes a counter flow of whey from the curd to

the surface and dissolves the remaining salt crystals, creating a supersaturated brine solution around each curd particle. The rate of the salt uptake by the cheese curds highly depends on the initial moisture in the curd and the amount of the salt added. An increase in salting level results in an increase in the rate of both salt absorption by the cheese curd and whey drainage from the cheese curd (Guinee and Fox, 2004; Lawrence et al., 2004).

Cheddar cheese composition

According to USDA standard of identity, Cheddar cheese is required to have the minimum of 50% milk fat and the maximum of 39% moisture content (Office of the Federal Register, 2006). Typically, Cheddar cheese composition contains approximately 37% moisture, 25% protein, 33% fat, 1% carbohydrate, and 4% ash (Canadian Dairy Commission, 2011).

Since Cheddar cheese can be eaten fresh or aged for up to 2 years, some chemical composition of Cheddar cheese might change over time. In order to produce a commercial first-grade Cheddar cheese, a diagram **(Figure 9)** with suggested ranges of moisture in the non-fat substance (MNFS), salt-to-moisture ratio (S/M), fat-in-dry matter (FDM), and pH has been widely used. This useful diagram can be a method for deciding which cheese should be further ripened and which should be sold more quickly (Lawrence et al., 2004).

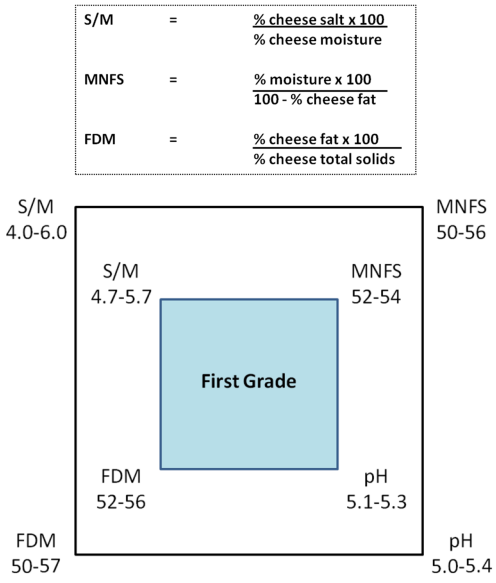

Figure 9 Suggested range of salt-to-moisture ratio (S/M), moisture in the non-fat substance (MNFS), fat-in-dry matter (FDM), and pH for first grade and second grade Cheddar cheese. Analyses 14 days after cheese manufacture.

Adapted from Lawrence et al., 2004.

Conclusions

Cheddar cheese is a complex and dynamic food system. Although there has been quite extensive research about Cheddar cheese for several decades, with an increase in the global cheese consumption, more research on this subject is needed in order to meet continually changes in consumer demands.

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