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Title: Characterization of Bloomy Rind Cheese Recipes and the Impact of High Pressure Processing (HPP) on Cheese Quality.

Abstract approved:

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Bloomy rind cheeses, including Camembert and Brie type cheeses, are highly susceptible to contamination by environmental pathogens during their manufacture and ripening. These cheeses undergo many physiochemical changes during ripening that provides these pathogens with a suitable environment to grow. One example of this change is an increase of pH to greater than 7 during the initial stages of the cheese ripening. The risk associated with this cheese type has been well documented and people with compromised immune systems are advised against consuming these cheeses. Currently, there are no options available for bloomy rind cheese producers to add a kill step in late in the manufacture/ripening of bloomy rind cheeses. Due to the lack of kill step, cheese makers are forced to rely on stringent sanitation techniques and environmental monitoring to mitigate the risk of contamination.

There are many ways to manufacture bloomy rind cheeses, and we hypothesized that different cheese recipes would perform differently under HPP condition with the stabilized cheeses being most likely to withstand this treatment

with minimal impact on the overall quality. To be able to test this hypothesis we first manufactured five varieties of bloomy rind cheeses to investigate the composition and characteristics of the recipes as a baseline for the expected quality from these different bloomy rind cheese varieties. Next, we evaluated the effect of HPP on cheese quality for selected cheese varieties. Finally, HPP was evaluated for the overall reduction of *Listeria monocytogenes*. The overall hypothesis of this research is that HPP (high hydrostatic pressure processing) could be effectively used on bloomy rind cheeses as a post-manufacture kill step, and that the cheese making practices used will influence how the cheeses withstand HPP.

Camembert cheese varieties varied in composition and characteristics that were influenced by the cheese making practices. The most notable difference between the varieties was the paste stability. Paste stability is defined by the distance that the center of the cheese is displaced from the rind after slicing. Another notable observation is the variation in total Ca content, which is related to the amount of colloidal calcium phosphate that is associated with the protein matrix that retains the cheese structure. When determining the effect of HPP on cheese quality, the most notable discovery was the destruction of the mycelium from the surface fungi used to ripen these cheeses. HPP treatment caused significant discoloration, from white to brown/yellow, and would be unacceptable to consumers of bloomy rind cheeses. Finally, when evaluating the reduction of *L. monocytogenes* using HPP there were large reductions (> 5-log CFU/g) in the microbial load at greater than 450 MPa for 10 minutes.

Based on the evidence provided, HPP successfully reduces *L. monocytogenes* in Camembert; however, the tested treatments produced unacceptable changes in cheese appearance. Until a technology is found that can be successfully applied to bloomy rind cheeses, cheese makers must emphasize proper sanitation procedures and environmental monitoring to control the risk of contamination.

Characterization of Bloomy Rind Cheese Recipes and the Impact of High Pressure Processing (HPP) on Cheese Quality

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Chapter 1. Introduction

Overview of bloomy rind cheese

Soft cheeses are a category of cheeses that is very broad, with the specific requirement being greater than ≥67% moisture on a fat-free basis (MFFB) or ≥50% total moisture (FAO/WHO, 2011; CDC and Health Canada, 2015). These cheeses make up a sizable portion of the cheese market and are therefore an important component of the ready-to-eat food marketplace.

Bloomy rind cheeses, including Camembert and Brie, are a unique group of cheeses that belong to the soft-ripened cheese category. These cheeses are unique in that surface yeasts and molds are used during ripening and contribute a characteristic white surface bloom, creamy texture, and unique flavors (Shaw, 1981; Lessard et al., 2012). Bloomy rind cheeses originated in France (Gripon, 1997). A survey of specialty cheese makers in the US found that forty seven percent of cheese makers produce at least one surface mold-ripened cheese variety (The American Cheese Society, 2016).

Food safety risks associated with soft cheeses

Soft cheeses, including bloomy rind cheeses, make up the group with the highest food safety concern of all cheeses. Pathogens survive and grow in these cheeses due to many factors including the moisture content, pH, salt, aging time, aging conditions, and mechanism of ripening (Choi et al., 2016). Foodborne illnesses caused by *Staphylococcus aureus*, *Listeria monocytogenes, Salmonella*, and Shiga toxin-producing *E. coli* have been linked to consumption of contaminated cheese (Choi et al., 2016)

The composition and physiochemical properties of food determine if the food is capable of supporting microbial and pathogen growth. Water availability is a major limiting factor that controls pathogen growth. The safety of many foods relies on reduced water activity (Aw; <0.85) to prevent pathogen growth. Soft cheeses commonly have a moisture content of >50% with water activity of >0.95, indicating a high potential to support the growth of nearly all pathogens. The pH of soft cheeses is between 4.5 and 8; a range that also supports bacterial pathogen growth.

Refrigeration is the primary means to control the growth of pathogens in soft cheeses; however, *Listeria monocytogenes* is capable of growth in refrigerated conditions. *L. monocytogenes* causes listeriosis, which has a mortality rate of up to 30% (Pearson and Marth, 1989; Kagkli et al., 2009; Food and Drug Administration, 2012) and is occasionally detected in soft cheeses (Choi et al., 2016). This pathogen presents a serious risk to cheese producers and consumers because of its persistence in the environment and resistance to wide ranges of conditions permitting survival and grow. Resident *L. monocytogenes* strains have been found persist for years in dairy processing environments (Ferreira et al., 2014). *L. monocytogenes* can also grow in a wide pH range (4.4-9.4), high salt concentrations (10% salt in the moisture phase), and a wide temperature range (-0.4 to 45°C; National Advisory Committee on Microbiological Criteria for Foods, 2010). Therefore, *L. monocytogenes* is the primary pathogen of concern for soft cheese producers.

Other bacteria of concern for cheese producers are coliforms, including *Escherichia coli.* Coliforms are a group of bacteria that are routinely used as indicators of

sanitary quality of many foods, including cheese (Trmčić et al., 2016). Purchasers typically demand low levels of coliforms (< 10 CFU/g) in finished dairy products. If a dairy product is regulated by the Grade A Pasteurized Milk Ordinance (PMO), coliform levels cannot exceed 10 CFU/g or mL (Food and Drug Administration, 2015). There are many routes for coliform bacteria to get in to cheese, including contamination during milking and environmental contamination from processing equipment. If the cheese is made from raw milk, the likely source of contamination is from the farm, and if cheese is made from pasteurized milk the source is commonly post-pasteurization contamination (Trmčić et al., 2016). Some of the contamination sources for post-pasteurization contamination can be by unsanitary processing equipment, contaminated water, and unhygienic practices used by cheese makers.

Most soft cheeses in the United States are made from pasteurized milk, which mitigates the potential risk of pathogens from the farm; however, post-pasteurization contamination of milk and cheese is a significant risk. During the manufacture of cheese, especially in an artisan setting, there are many possible points of contamination during production and ripening (Muhterem-Uyar et al., 2015). Some of the likely contamination points include handling at salting, moving cheese in the molds, removing cheese from molds, and aging prior to final packaging. Due to the nature of soft cheese production and product composition, the risk of post-pasteurization contamination from environmental pathogens is high. Producers and consumers of soft cheeses would benefit from a microbial kill step after the product is ready for final distribution to the marketplace.

Bloomy rind cheese and unique risks

The biochemical reactions that contribute to the development of flavor and texture in bloomy rind cheeses also modify the physiochemical properties of these cheeses. At the surface of the cheese, lactic acid and lactate are consumed, increasing the pH of the cheese at the surface from its original value of 4.4-5.2 (Lawrence et al., 1987; Schlesser et al., 1992). Proteolytic enzymes associated with bloomy rind cheese catabolize the protein matrix that makes up the body of the cheese. The proteolysis is significantly higher near the surface due to proteases produced by the yeasts and molds. This leads to deamination at the surface that liberates ammonia, further increasing the pH at the surface to 7-8 (Lawrence et al., 1987). Lipolytic enzymes associated with the surface yeasts and molds break down the triglycerides in to free fatty acids and other flavor compounds. A pH gradient from the surface to the center of the cheese develops. Due to the pH gradient a mineral gradient develops as soluble calcium migrates towards the center of the cheese (Brooker, 1987; Tansman et al., 2017). As the soluble minerals are depleted in the protein matrix, the protein hydrates and the cheese texture softens (Lucey and Fox, 1993). This mechanism of ripening results in cheeses that mature shortly after manufacture (3 weeks); however, this rapid process also leads to problems with product stability and a short shelf-life (three and seven weeks post-manufacture; Galli et al., 2016).

The physiochemical changes that bloomy rind cheeses undergo present an increased risk for growth of environmental pathogens like *L. monocytogenes* (D'Amico et al., 2008). These changes during manufacture, such as pH increase, provide these pathogens with a more suitable environment to survive. Even though there are other bacteria, yeasts, and molds present, *L. monocytogenes* is a capable co-inhabitant of the cheese rind. It has been shown previously by Genigeorgis et al. (1991) and Back et al.

(1993) that *L. monocytogenes* grows on the surface of Camembert and Brie cheeses during refrigerated storage.

In addition to the unique biochemical changes that can influence the growth of pathogens, the manufacture and ripening procedures also contribute to the high risks associated with bloomy rind cheeses. After pasteurization, the milk for cheese manufacture and cheese comes in contact with many surfaces and possibly many hands over the next two weeks. The main concern for contamination in many ripened cheeses is during the process of aging. For bloomy rind cheeses, the cheese is flipped multiple times over two weeks to allow the surface yeasts and molds to grow on both sides. This provides many opportunities for contamination through contact by hand, equipment, and aerosols.

Fundamentals of high pressure processing (HPP)

High pressure processing (HPP) is a food processing technology that helps preserve foods by reducing the microbial load of pathogenic bacteria and spoilage organisms. Rather than using the traditional method of heat, this technology employs extreme pressures (100 to 1000 MPa, but typically < 600 MPa) at refrigerated or ambient processing temperatures (Yordanov and Angelova, 2010; Muntean et al., 2016; Huang et al., 2017). As consumers of specialty cheese and other foods have developed a preference for minimally processed or low temperature processed foods, the implementation of HPP technology has expanded. The number of commercial units in use worldwide has increased from less than 10 in 1990 to more than 300 in 2015 (Huang et al., 2017).

The principle of this processing technology uses extreme processing pressure (up to 600 MPa), but there are other requirements for this technology to be successful. First, the processing chamber is a high-pressure vessel that is completely filled with a processing fluid, typically water, glycol solutions, silicone oil, ethanol solutions, or castor oil. Second, the food must be sealed in a flexible container. This allows for pressure to be evenly transmitted through the food. Third, a pump is needed to apply pressure to the fluid medium (Yordanov and Angelova, 2010). Temperature is another parameter that is typically monitored or controlled because of adiabatic heating. This phenomenon is a result of the pressurized fluid heating as the chamber is compressed. When the chamber pressure is released, the temperature of the fluid/food returns to its original temperature or slightly lower (Yordanov and Angelova, 2010; Ferreira et al., 2014)

HPP and bloomy rind cheeses

The suitability of using HPP during and after cheese manufacture and ripening has been investigated for many varieties of cheese, including bloomy rind cheeses. Not only has this process been assessed for its microbial reduction efficacy, but it has also been evaluated for its effect on the ripening and functional characteristics of cheese. Many cheese characteristics have been evaluated, with some of the more notable being the effect of high pressure on ripening, proteolysis, and textural properties.

The magnitude of applied pressure affects the cheese during ripening. Ripening of cheeses involves many reactions including proteolysis, lipolysis, and glycolysis. An increased rate of ripening has been achieved using lower pressures in Cheddar, Camembert, Gouda, and goat milk cheeses (Kolakowski et al., 1998; Saldo et al., 2002;

O'Reilly et al., 2003). At lower pressures (≤ 400 MPa), there is an increased rate of proteolysis due to an increased activity of the proteolytic enzymes. This has been demonstrated using Cheddar, Camembert, Blue-veined, Hispanico, and La Serna cheese varieties (Martínez-Rodríguez et al., 2012). At higher pressures (> 400 MPa) the activity of the proteolytic enzymes vary greatly depending on pressure, time, and temperature. An increase in any of these parameters over 400 MPa generally decreases the activity of proteolytic enzymes in both milk and cheese (Huppertz et al., 2002; Martínez-Rodríguez et al., 2012). Higher pressures could lead to delayed ripening of bloomy rind cheeses due to the potential to inactivate proteases critical for texture development.

Calzada et al. (2014a) investigated how HPP at 400 MPa and 600 MPa at 2 and 3 weeks post-manufacture influenced Brie cheese, a larger wheel size cheese (compared to Camembert) in the bloomy rind category. A key finding was that pressure treatment slowed down the rate of proteolysis, a fundamental part of cheese ripening and texture development. This reduced rate of proteolysis is also apparent because of the reduced rate of pH neutralization. The pH on the untreated cheese remained significantly higher than HPP-treated cheese; this is most likely due to the additional ammonia produced during the deamination process during proteolysis. Pressure-treated cheeses were softer immediately after the HPP treatment, but firmer than the control cheeses at 120 days of aging. A similar observation was made for the elasticity of the cheeses. Pressure-treated cheeses were initially less elastic than the untreated cheeses; however, the elasticity of the pressure-treated cheeses remained stable throughout shelf-life while the elasticity of the untreated cheeses degraded over time.

Calzada et al. (2014a) also investigated the sensory and microbiological quality of HPP-treated Brie cheese. The microorganisms enumerated in this study included lactic acid bacteria and *Penicillium camemberti*. Currently, there is limited information on the resistance of *Penicillium camemberti* or *Penicillium candidum* to high pressures. It has also been demonstrated in raw milk Serra da Estrela that higher pressures (>400 MPa) reduced the total aerobic mesophilic bacteria and lactic acid bacteria (Inácio et al., 2014).

Despite the textural differences and microbiological differences with the Brie cheeses after HPP treatment, Calzada et al. (2014a) found that the sensory quality of these cheeses were not negatively impacted. They found that the flavor quality was better and the cheeses were less bitter after 60 days of ripening. With these findings there is some evidence that shelf-life could be improved for bloomy rind cheeses with the use of HPP. In this study, the cheeses were cut into slices prior to treatment. This would typically not be an acceptable way to ripen and distribute the cheeses and may have impacted some of the results they observed. Treating the full wheels as they would be distributed could impact the quality of the cheese after HPP.

HPP as a process control for pathogens in cheese

The efficacy of high pressure as a non-thermal processing technology has been evaluated for many types of foods, including different cheese varieties. Commercially, HPP is currently being used to process jellies, guacamole, meats, sauces, juices, oysters and dairy products as a post-process microbial control step while having minimal impact on product quality (Ferreira et al., 2016). It has been shown that many pathogens can be

adequately reduced by higher pressures (> 400 MPa) and longer hold times (up to 15 min).

Different varieties of cheese have been evaluated for the reduction of *L.* monocytogenes using HPP. The application of HPP on fresh goat cheese resulted in a reduction of *L. monocytogenes* greater than 5 log CFU/g using the processing parameters of 500 MPa for 5 minutes and 450 MPa for 10 minutes (Gallot-Lavallee, 1998). Queso fresco cheese, with a similar composition (protein, fat, moisture, and salt) to bloomy rind cheeses, achieved a >4.6 log CFU/g reduction when treated with 600 MPa at 5 minutes (Tomasula et al., 2014). In model washed-curd cheese, López-Pedemonte et al. (2007) demonstrated that *L. monocytogenes* could be reduced by ~ 5 log by using 500 MPa at either 5 or 20 minutes. These examples demonstrate that HPP can effectively be used in cheese to achieve a 5-log reduction as a control step for *L. monocytogenes* at higher pressures and longer holding times.

Moving forward: Investigating the effect of HPP of different bloomy rind cheeses

Based on previous evidence, we believe that HPP could be applied to bloomy rind cheeses as a post-manufacture and post-ripening kill step to reduce pathogens not controlled by pasteurization. Bloomy rind cheeses can be made using multiple manufacturing methods; however, how these methods might influence cheese quality and withstand HPP processing is unknown. This led us to decide to investigate how the manufacture protocols of bloomy rind cheeses influence cheese characteristics and their behavior following application of HPP. Based on the previous work done on bloomy rind

cheeses and the current gaps in knowledge we decided to investigate the following objectives:

- Characterize cheese making practices utilized to manufacture bloomy rind cheeses (chapter 2).
    i. To complete this objective five different recipes were used to manufacture Camembert type cheese.
    ii. The cheeses were then analyzed for composition and key quality metrics throughout shelf-life.
- Determine the effect of HPP on the quality of different bloomy rind cheeses (chapter 3).
    i. HPP was applied to three varieties of Camembert type cheese at different time points (3, 11, and 45 days post manufacture).
    ii. The cheeses were then analyzed for key quality metrics throughout shelf life.
- Evaluate the reduction of *Listeria monocytogenes* using HPP (chapter 3).
    i. HPP was applied to the cheese 11 days post manufacture after a simulated contamination event.
    ii. Reduction was evaluated using microbial enumeration within 24 hours of the treatment.

Chapter 2. Influence of cheese making recipes on the composition and characteristics of Camembert type cheese

Interpretive Summary

Camembert type cheese and related bloomy rind varieties make up a significant portion of the soft specialty cheese market. Many different manufacturing practices, including starter selection, variable pH control points, and curd handling can impact the characteristics of the cheese and the length of shelf-life. Composition and physical characteristics are significantly impacted by the cheese making practices. Cheesemakers can select and modify recipes to increase the shelf-life of Camembert type cheese.

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2.1 Abstract

Bloomy rind cheeses, including Camembert and related varieties, can be produced using alternative processes that vary based on milk preacidification, cutting, curd handling, and ripening. Modification of these parameters creates distinct cheeses such as lactic curd, stabilized curd, and hybrids of the two. The objective of this study was to determine the influence of five Camembert type cheese recipes on the composition and characteristics during ripening. Five varieties of Camembert-type cheese were produced: i) lactic curd, ii) sweet curd, iii) washed curd, iv) solubilized curd, and v) stabilized curd. Cheeses were aged at 13°C for 10 d, during the mold growth phase, and 7°C from d 11 until 50. Key quality metrics including texture development, pH (center and surface), and color were monitored throughout shelf-life. Compositional evaluation (d 5; fat, protein, moisture, salt, and minerals) grouped cheeses into three categories: I) lactic curd, II) sweet and washed curd, and III) solubilized and stabilized curd. The lactic curd and stabilized curd were consistently the most different varieties for composition and quality metrics. Moisture content of Camembert-type varieties ranged from 53.15% to 57.99%, Ca ranged from 0.23% to 0.45%, and P ranged from 0.21% to 0.40%. All varieties followed the expected pH evolution on the rind and in the paste with the pH of the rind reaching 7 by d 10, and paste pH reaching 7 between 35 and 50 d. The displacement of the paste (distance traveled upon cutting) for the lactic curd was the greatest amongst the five varieties, reaching an average of 27 ± 1.9 mm after 50 d of ripening and 60 min of flow time. The stabilized curd on the other hand traveled the shortest distance, reaching an average of 4 ± 0.4 mm at the same time point. Browning, considered a defect in mold-ripened cheeses, was observed in all varieties, but was most substantial for lactic curd (L*

decrease from 87.19 to 68.58). Based on these quality metrics the shelf-life of these recipes were estimated with the lactic curd having the shortest, and the stabilized curd having the longest. Examining Camembert-type cheese quality metrics for these five varieties can assist cheese makers during recipe formulation and selective of cheese-making practices to achieve optimum product quality.

Keywords: mold-ripened cheese, stabilized cheese, shelf-life

2.2 Introduction

Soft, bloomy rind cheeses, predominantly Camembert and Brie type cheeses, comprise a small, but significant, segment of the total cheese market in the US. Forty-seven percent of the cheese makers within the American Cheese Society produce surface mold-ripened cheeses (The American Cheese Society, 2016). In the past decade, specialty cheese production volumes greatly increased. From 2005 to 2015, the annual production of specialty cheese in Wisconsin (the leading state of specialty cheese production) doubled from 164 to 328 million kg (Wisconsin Milk Marketing Board, 2017). Grocery sales for Camembert and Brie type cheeses increased by 4.7% from 2004 to 2005, totaling 4.9 million kg, annually (Buragas, 2006). This increase in availability and sales of specialty cheese is in part due to the increasing number of artisan cheese makers. In Oregon alone, the number of artisan specialty cheese processors has experienced significant growth from 3 in 1999 to 26 in 2013 (Bouma et al., 2014). On a national level there has also been an increase, with The American Cheese Society (2018) reporting a membership increase from 1,418 to 1,831 over the last decade (2007 to 2017).

Soft ripened cheeses were traditionally manufactured in France using basic soft cheese making practices (Shaw, 1981). Key characteristics for soft ripened cheese processes are the fermentation of cheese milk by mesophilic lactic acid bacteria (LAB) to a low pH (<4.6-5.2) with an absence of both cooking and pressing steps. This category of cheeses can be broken into two subcategories based on rind development: washed rind and bloomy rind. Camembert and Brie belong in the bloomy rind cheese category.

Camembert and related bloomy rind varieties are known for possessing a white/grey rind that is formed as Penicillium candidum, and yeasts such as Geotrichum candidum, Debaryomyces hansenii, and Kluyveromyces spp. grow on the surface of the cheese (Gripon, 1997; Leclercq-Perlat, 2011; Galli et al., 2016). Soft ripened cheeses that undergo transformation by surface molds are unique because of the many physiochemical and biochemical changes that occur over the brief period of ripening (typically 3-5 weeks). These reactions transform a chalky, crumbly, and firm cheese into one that is soft, viscous, and flowing (Lawrence et al., 1987; Sousa, 2003; Tansman et al., 2017). Cheese softening for surface-ripened varieties can be partly explained by mineral migration from the center to the surface of the cheese. This results in the swelling and hydration of the protein matrix (Lucey and Fox, 1993). The mineral migration phenomenon is primarily a result of the pH increase caused by enzymatic activity from the surface molds (Le Graet et al., 1983; Karahadian and Lindsay, 1987; Tansman et al., 2017).

The optimum time frame for consumption of Camembert type cheese is between three and seven weeks post-production (Galli et al., 2016). Therefore, getting the cheese

to market and consumers is a significant challenge for manufacturers of traditional Camembert type cheeses with large distribution networks. Camembert type cheese can be consumed outside of this optimum quality window; however, defects will be noticeable and often unpleasant. These defects including 1) flavor: being overly ammoniacal, sulfury, or bitter, 2) texture: having an overly runny paste, and 3) appearance: deterioration of rind color (browning).

Manufacturers have modified cheese making practices to lengthen the window of optimum quality and create "stabilized" bloomy rind cheeses (Lawrence et al., 1987). Stabilization of the paste is primarily accomplished by controlling the rate and level of acid development during draining for a final pH after draining ≥ 5.2 (Gripon, 1997). These modifications lead to a young cheese with a softer and more elastic texture, but one that retains physical integrity throughout ripening. Cheesemakers have modified many manufacturing steps to produce stabilized cheeses, including starter selection (i.e., LAB species and strain choice), fermentation time/temperature, target rate and degree of acidification, cut size, and curd handling practices (Lawrence et al., 1987; Gripon, 1997); however, there is limited data demonstrating the impact of these modifications on Camembert type cheese composition and quality characteristics throughout ripening. The objective of this study was to objectively describe and compare the quality characteristics and shelf-life of Camembert type cheeses produced using five common cheesemaking procedures. This information could assist artisan cheesemakers in appropriate recipe selection to achieve optimum quality and extend shelf-life as appropriate for their distribution channels.

2.3 Materials and Methods

Overall experimental design

Five different Camembert type cheese varieties (lactic curd, sweet curd, washed curd, solubilized curd, and stabilized curd) were manufactured. These five varieties capture many of the procedural differences used to manufacture bloomy rind cheeses in the industry. Differences in manufacturing procedure for the five varieties are summarized in Table 2.1. Each variety was manufactured in duplicate on two separate days using two different lots of milk. We hypothesize that modifications in the manufacturing procedure will produce Camembert type cheeses of different composition and rate of ripening as indicated by reduction in firmness and stability of the cheese paste.

Cheese making

Pasteurized whole milk (average P/F ratio of 0.97; 25 kg/batch) was supplied by a regional milk processor and transported to the Arbuthnot Dairy Center at Oregon State University (Corvallis, OR, USA) for cheese making. The milk was placed in a round cheese vat (C. van't Riet Dairy Technology B.V., Nieukoop, Netherlands) and warmed to the appropriate fermentation temperature for the recipe (Table 2.1). A thermophilic starter culture, Streptococcus thermophilus (Choozit DVI TA 50 series, Danisco, Copenhagen, Denmark) or a mesophilic starter blend (Flora Danica-DVS, Chr. Hansen Inc. Milwaukee, WI, USA), was added the milk as determined by recipe (Table 2.1). The ripening cultures (Penicillium candidum (PCA 3, Chr. Hansen Inc.), Geotrichum candidum (Choozit Geo 15 LYO, Danisco), and Kluyveromyces marxianus (LAF 4, Chr.

Hansen Inc.,)) were added to the milk at a rate of 0.40 U/100 kg, 0.15 U/100 kg, and 0.15 U/100 kg, respectively. When preparing the stabilized curd variety, an adjunct culture, Leuconostoc mesenteroides subsp. cremoris (Choozit LM 57, Danisco), was added at rate of 0.73 U/100 kg to enhance flavor development. Except for the lactic curd variety, calcium chloride (DCI Calcium Chloride, 32-33% w/v, Dairy Connections Inc., Madison, WI, USA) was added during fermentation at a concentration of 6.6 mL/100 kg. The milk was fermented until the desired set pH was achieved (Table 2.1).

For all varieties, excluding the lactic curd, rennet (see concentration in Table 2.1; DCI Star Coagulant, Dairy Connections Inc., Madison, WI, USA) was stirred gently into the fermented milk and the mixture set for 30-40 min prior to cutting with either 1-cm or 2-cm knives (Servi Doryl, Langeais, France). The cut time and size used for each cheese type is shown in Table 2.1. The cut time was determined by visual assessment of the gel firmness and flocculation time using a multiplication factor of five. After cutting, the vats were drained and the forms (Fromagex, Rimouski, Québec, Canada; 7 cm in diameter) were filled using a curd distributor (Fromagex). For the lactic curd variety, rennet was added once the desired set pH was achieved (Table 2.1). Once the lactic curd variety achieved the desired cut pH, it was ladled into the cheese molds over the course of 2-3 hours. Each mold was filled with curd every 30-45 minutes for 6 to 7 passes, until the molds were filled. Forty molds were filled for each replicate cheese make with an average cheese weight of 95 ± 10 g at salting.

Cheeses were drained at 23 ± 1°C with turning at 30 minutes, 5 hours, 10 hours and 19 hours. The cheeses were removed from the molds at either 20 or 24 hours and

salted with 2% salt (w/w) and open air dried for an additional 1-2 hours. This marked day 0 (start of ripening) for all downstream sampling points. Cheeses were transferred to an incubator (15 ± 1°C, 85% RH) for 24 hours. The relative humidity was then increased to 95% and temperature decreased to 13 ± 1°C for 10 days and cheeses were flipped daily to develop surface mold. Cheeses were then wrapped in white mold paper (Fromagex) and stored at 7 ± 1°C for up to 40 days. After wrapping, cheeses were flipped on days 14, 21, 35, and 50 post manufacture.

Composition and physiochemical analysis

Milk composition. Compositional analysis (fat, protein, lactose, and solids-not-fat (SNF)) of milk samples was conducted using a milk analyzer (Lacticheck-01, RapiRead, MA, USA) prior to culture addition on the day of cheese manufacture.

Cheese composition. Cheese samples were collected after 5 days of ripening (before development of surface molds) for macrocomponent analysis (fat, protein, moisture, minerals). Whole cheese wheels (~90 g) were homogenized by blending until the size of the cheese particles were uniform (~15 s). Fat was measured according to the Gerber Van Gulick method (ISO, 2008). Protein was estimated using a combustion analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Hanau, Germany) for total nitrogen determination according to the Dumas combustion method (Wiles et al., 1998). Crude protein was determined by multiplying total nitrogen by 6.38. Moisture content was determined using a rapid moisture and solids analyzer Computrac Max 4000XL (Arizona Instrument LLC, Candler, AZ, USA). For elemental analysis (Ca, P, Na) 10 sub-samples were taken from each wheel and dried at 35°C for 96 hours. The

samples were then ground with a mortar and pestle. The samples were wet-ashed with nitric acid (Macron Fine Chemicals, Center Valley, PA, USA) using microwave digestion (MultiwaveGO, AntonPaar USA, Ashland, VA, USA). Analysis was performed by inductively coupled plasma atomic emission spectroscopy (ICP-OES: Optima 2100 DV. PerkinElmer, Waltham, MA, USA) operated using the radial view mode. Total nitrogen and elemental analysis was conducted by the Collaborative Analytical Laboratory (Oregon State University, Corvallis, OR, USA).

Texture analysis. Texture was analyzed by penetrometery throughout ripening using a modified method previously described by Abraham et al. (2007). A texture analyzer TA XT2i (Texture Technologies Corp., Hamilton, MA, USA) was equipped with a 5 kg load cell and a 6 mm cylindrical probe. The analysis was performed on three cheeses from two replicate cheese makes on day 7, 14, 21, 35, and 50 post manufacture. The cheese was tempered to room temperature (22 ± 1.5°C) for 1 hour prior to testing. Axially penetration was accomplished at a rate of 0.4 mm/s with the penetration depth set at 75% of the total cheese height. Firmness of the cheese paste was recorded at the minimum peak and maximum peak within the paste. The "paste" firmness was determined to be the measurements observed after the initial "fracture" peak that was due to the rind breaking.

pH. The pH measurements for all in-process and ripening samples were taken using a portable pH meter equipped with a conical penetration probe designed for measuring semi-solid and solid food products (Portable Food and Dairy pH Meter, Hanna Instruments, Woonsocket, RI, USA). The pH of the rind was measured daily for the first

10 days of ripening and throughout total ripening (day 14, 21, 35, and 50). At each sampling time, three cheeses were measured in six locations. The sampling locations were arranged in a triangle pattern, equal distance apart on the top and bottom of the cheese wheel. The pH of the center paste was measured on day 7, 14, 21, 35, and 50 post manufacture. pH was measured for three replicate cheeses for duplicate cheese makes through ripening.

Color. The color of the rind was measured using a spectrophotometer (LabScan XE Spectrophotometer, Hunter Associates Laboratory Inc., Reston, VA, USA). All color measurements were performed on three separate cheeses for duplicate cheese makes of the rind throughout ripening, 14, 21, 35, 50 days post manufacture.

Paste Displacement. The stability of the cheese paste was measured quantitatively by measuring the distance the paste traveled from the cut rind of the cheese wheel. On d 35 and 50, three cheeses for each cheese make were removed from 7°C storage, unwrapped, cut in half, and left undisturbed at ambient temperature for 60 minutes. Paste displacement (mm) was measured from the edge of the cheese rind to the farthest point of displacement after 15, 30, 45, and 60 minutes.

Data Analysis. The data was analyzed using JMP Pro 13.0 (SAS Institute Inc., Cary, NC, USA). Means and standard errors were calculated for duplicate (n=2) cheese makes for composition and shelf-life characteristics. Comparison of means for significance ($P < 0.05$) was conducted using Tukey's HSD.

2.4 Results

Compositional analysis

Milk. The average composition of the milk used to manufacture Camembert type cheese over two separate days comprised of 3.57% fat, 3.48% protein, 5.05% lactose, and 9.22% SNF. The average protein-to-fat ratio was 0.97. Protein and SNF of milk differed significantly ($P < 0.05$); however, protein-to-fat ratio of the milk did not vary significantly.

Cheese composition. Complete Camembert cheese composition for each variety is shown in Table 2.2. Variation in the five cheesemaking recipes resulted in significant differences in most of the compositional components analyzed in this study. Na (dry-basis; 0.94-1.04%) was the only analyte that did not differ significantly by recipe. Based on overall compositional comparisons, the five Camembert varieties can be organized in to three separate groups. These groupings are I) lactic curd, II) sweet curd and washed curd, and III) solubilized curd and stabilized curd. These groupings shared comparable manufacturing recipes.

Compositional analysis demonstrated that the lactic curd cheese was the most different from the other varieties. The lactic curd cheese is acidified for up to 24 hours, and is ladled in large pieces rather than cut, which results in the highest moisture cheese (57.99%) with the lowest protein (15.43%), fat (21.75%), salt (1.05%), and S/M (1.81%). The calcium and phosphorus contents of the lactic curd variety were the lowest (0.0.23% and 0.21%, respectively).

There were no significant differences in composition between the sweet and washed curd varieties except for protein (15.90% and 16.62%, respectively). These

varieties are very similar in manufacture, the only exception being starter culture quantity and the washing of the curd. The washed curd was significantly lower in moisture (56.29%) and it was significantly higher in protein (16.62%) compared to lactic curd. The sweet curd was significantly higher in fat (23.66%) compared to the lactic curd. The higher pH through the process led to significantly higher ($P<0.05$) calcium content in these cheeses (sweet curd: 0.33%; washed curd: 0.35%) compared to lactic curd with (0.23%).

The solubilized and stabilized curd cheeses had no significant differences between the major compositional components. Their manufacture differed from other varieties by starter culture type, fermentation parameters, and cut size which lead to cheeses with distinct characteristics. The moisture content of these two varieties was significantly lower (53.15-53.28%) than the washed curd (56.29%) sweet curd (56.44%), and lactic curd (57.99%) varieties. The protein content (w/w and dry basis) of these two cheeses were the highest among the five varieties at 18.23-18.34% and 39.02-39.15%, respectively. The calcium content of the solubilized curd (0.36%) was comparable to the sweet curd (0.33%) and washed curd (0.35%) varieties, whereas the calcium content of the stabilized curd (0.45%) was significantly higher than all other varieties. A similar pattern was seen for the phosphorous content.

Lactic curd

Quality characteristics of lactic curd Camembert type cheese during ripening are shown in Figure 2.1. The pH of the cheese at salting (day 0) was 4.31 (Figure 2.1a). Rind pH increased quickly achieving a pH of 6.98 by day 10 and a maximum pH of 7.92

on day 35. Increases in paste pH lagged, as expected, with an increase to 6.15 by day 21. By day 50, the pH of the rind and paste harmonized at 7.65-7.70. The maximum firmness of the lactic curd paste started low at just 1.13 N on day 7 of ripening with little difference between minimum and maximum firmness (Figure 2.1b). Firmness continued to decrease until day 21 and held at a very low firmness (0.34 N) through the end of the study (day 50). During ripening there was a change in the color, primarily the L* and b* values (Figure 2.1c). The L* value started at 87.19 on day 14, and by day 50 it had decreased to 68.58. The b* value saw an increase over from 6.58 (day 14) to a maximum of 14.56 (day 35). The a* value changed only minimally from -0.43 to 1.42. Upon cutting the lactic curd wheel at the end of ripening (day 50), paste quickly and almost completely left the rind of the lactic curd cheese (Figure 2.1e). The paste of the lactic curd variety spread quickly over a large distance traveling 14 mm within 15 min after cutting (Figure 2.1d). The average distance of paste displacement for the lactic curd variety was not significantly different at day 35 and 50 at 23.5 mm and 27.3 mm, respectively, after 60 minutes of flow time. Taken together, this data indicates the rapid ripening of this cheese and demonstrates quality characteristics that explain its short shelf-life.

Sweet curd

Quality characteristics of sweet curd Camembert type cheese during ripening are shown in Figure 2.2. This cheese, like the lactic curd, has a short ripening period and shelf-life with substantial changes occurring between 35 and 50 days as demonstrated by various quality characteristics. The pH at salting (day 0) was 4.65 (Figure 2.2a). The pH reached 7.38 by day 10 and the maximum of 7.85 by day 21. The pH of the paste

increased slowly to 5.27 by day 21 and reached a maximum of 7.53 by day 50. This cheese was quite firm with a maximum firmness of 4.29 on day 7 of ripening (Figure 2.2b). Minimum firmness of the paste was 1.91 on day 7. The difference between the maximum and minimum decreased throughout ripening, harmonizing on day 35 at < 0.80 N. The color of the sweet curd cheese rind slightly browned over time, with the L* value decreasing from 82.43 to 69.13 over ripening (Figure 2.2c). The a* and b* value exhibited minor changes, ranging from 0.24 to 1.00 and 6.35 to 8.78, respectively. Paste displacement significantly increased from day 35 to 50, averaging 11.7 mm on day 35 and 24.3 mm on day 50 after 60 minutes of flow time (Figure 2.2d and e).

Washed curd

Quality characteristics of washed curd Camembert type cheese during ripening are shown in Figure 2.3. The pH of the cheese at salting (day 0) was 4.80 (Figure 2.3a). The rind pH increased rapidly to 7.37 by day 10, increased to a maximum of 7.81 by day 21, and remained above 7.62 through 50 days of ripening. The pH of the paste was slower to increase, as expected, reaching 5.08 by day 21 and 7.60 by day 50. The maximum firmness of the washed curd paste was recorded on day 7 at 3.96 N, with the minimum firmness on the same day at 2.14 N (Figure 2.3b). By day 35, the maximum firmness decreased to 0.65 N which was congruent with the minimum firmness of 0.45 N; low firmness remained constant throughout the remaining ripening. Like the aforementioned described varieties, there was a decrease in the L* over ripening, from 84.77 to 74.07 (Figure 2.3c). The a* value ranged from 0.23 to 0.70 and the b* ranged from 6.72 to 8.22. Upon cutting the washed curd wheel, the cheese paste moved away

from the cheese wheel at a lesser extent than the sweet and lactic curd, with much of the paste remaining in the wheel (Figure 2.3e). The displacement distance after 60 minutes of flow time significantly increased over ripening, averaging 7.3 mm on day 35 and 18.7 mm on day 50 (Figure 2.3d). The difference in paste displacement indicates that washed curd cheese undergoes much of the ripening between day 35 and 50, similar to the sweet curd variety.

Solubilized curd

Quality characteristics of solubilized curd Camembert type cheese during ripening are shown in Figure 2.4. The pH at salting (day 0) started at 4.95 (Figure 2.4a), with the rind pH reaching 7.29 by day 10 and a maximum of 7.48 by day 50. The paste pH increased to 5.04 by day 21 and 7.21 by day 50. Initial firmness (day 7) ranged from 2.06 N to 3.41 N (Figure 2.4b). The maximum and minimum firmness continued to decrease until day 50, reaching 1.03 N and 0.53 N, respectively. The L* value decreased over ripening like the other varieties, from 87.57 at day 7 to 80.50 on day 50 (Figure 2.4c). The a* value exhibited minor fluctuation from -0.06 to 0.55. The b* value also remained fairly constant ranging from 6.93 to 7.56. Upon cutting the solubilized curd wheel at 50 days, the paste remained closely associated with the wheel even after 60 minutes from cutting (Figure 2.4e). The average distance of paste displacement for the solubilized curd variety was similar at day 35 and 50 at 4 mm and 6 mm, respectively, after 60 minutes of flow time (Figure 2.4d). These quality characteristics indicate the potential for a longer shelf-life for the solubilized curd relative to the lactic, sweet, and washed curd variants.

Stabilized curd

Quality characteristics of stabilized curd Camembert type cheese during ripening are shown in Figure 2.5. The pH of the cheese at salting (day 0) was the highest of all varieties at 5.24 (Figure 2.5a). The rind pH increased to 7.10 by day 10 and reached a maximum by day 50 of 7.52. The pH of the paste was slower to increase reaching 5.30 by day 21 and 7.36 by day 50. The initial maximum firmness of the stabilized curd was quite low at 2.51 N on day 7, while the minimum firmness was 1.70 on day 7 (Figure 2.5b). The firmness decreased over ripening, with the largest decrease happening between day 35 and 50. The maximum firmness on day 50 was 1.35 N while the minimum was 0.78 N. Like the other varieties, the L* value decreased over ripening from 83.75 to 77.58 (Figure 2.5c). The a* value ranged from -0.16 to 0.76. The b* value remained relatively unchanged, ranging from 5.97 to 6.96. Upon cutting the wheel, the cheese paste moved very little over the 60 minutes as shown in Figure 2.5e. The displacement distance after 60 minutes of flow time was 3 mm on day 35 and 4.3 mm on day 50 (Figure 2.5d). Considering these physical quality characteristics, the stabilized curd variety would have the longest shelf-life of the varieties evaluated in this study.

2.5 Discussion

Camembert recipe variations result in the production of cheeses that differ substantially in their composition and a variety of quality characteristics throughout ripening. In this study, we successfully produced Camembert type cheeses using five different recipes. All recipes produced cheeses that had a composition representative of Camembert type cheese early in ripening (Schlesser et al., 1992; Kulmyrzaev et al., 2005). The salting procedure or the alternative method used to calculate salt in this study

resulted in salt levels that were slightly lower than expected; however, this was consistent across all cheese varieties. Comparing the Na (dry weight basis) content to that of the commercial cheeses studied by Tansman et al. (2017) the content was quite similar.

The alternative processes (as compared to the traditional lactic curd recipe) resulted in Camembert type cheeses with varying curd type, composition, and quality. The traditional lactic curd cheese relies on acidification as the catalyst for gel formation, whereas alternative recipes limit acidification and drive enzymatic coagulation through the addition of rennet. Washed curd, solubilized curd, and stabilized curd varieties had an elevated pH compared to the other curd types which likely contributed to improved paste stability; however, only through the use of a thermophilic starter (stabilized curd variety in this study) were we able to achieve the desired pH at salting for a stabilized type cheese (of ≥ 5.2). Paste stabilization was also likely impacted by the use of a mixed starter (thermophilic/mesophilic), and curd washing to limit acid production by the starter culture.

Shelf-life of Camembert cheese is largely a function of texture deterioration. Texture stability in Camembert cheese recipes was correlated with increasing calcium content in young cheeses. Lactic curd had the lowest initial total calcium content (0.23 %) and resulted in the least stable texture throughout shelf life. At 50 days, this cheese was of unacceptable quality since the paste nearly liquefied as demonstrated by the lack of cheese remaining in the rind during the paste displacement test (Figure 2.1e). Optimum textural quality of this cheese would be <35 days of ripening. Substantial improvement in texture stability was apparent when total Ca content was increased,

leading to Ca concentrations of ≥ 0.33%. Ca concentration in the young cheeses differed as a function of pH at salting and throughout the cheese make. Higher pH at this stage and throughout the cheesemaking process increases the insoluble colloidal calcium phosphate (CCP) content and overall interactions with casein (Keller et al., 1974; Lucey et al., 2003). leading to a decrease in flowability and an overall more stable cheese paste. While all recipes with higher total Ca showed improved texture stability, the stabilized curd with the highest pH at salting (pH 5.2-5.3) and highest total Ca content (0.45%) showed little softening and minimal paste displacement (flow) by day 50. The optimum time for consumption of this cheese would be >50 days of ripening.

Ca content in cheese is influenced by pH throughout the process

The pH throughout the process controlled the final total Ca in the cheese. Across the five varieties of Camembert type cheese the degree of acidification during the make varied significantly with the pH at salting ranging from 4.3 to 5.3. Total Ca content trend for all cheeses was directly related to the pH at salting and inversely related to the total degree of acidification. It has been previously reported that three main factors affect demineralization during cheese manufacture including: milk preacidification, pH during draining, and degree of cooking (Lucey and Fox, 1993). In this study multiple methods were used to control pH and ultimately Ca content. This included pH at rennet addition, quantity of starter culture added, type of starter culture used, fermentation temperature, and washing of the curd. These process modifications limited the degree of acidification during draining, and ultimately were successful in controlling total retained Ca in the cheeses. Similar methods for controlling Ca content have previously been used for

Mozzarella, Cheddar, and Colby cheeses (Guinee et al., 2002; Upreti and Metzger, 2006; Lee et al., 2010). The cut size was also modified (1 cm cubes) for the stabilized and solubilized as described by Spinnler and Gripon (2004) when referring to the manufacturing methods for stabilized cheeses. This was done to achieve acceptable moisture content as the large cut size decreased whey expulsion during draining. This was likely due to the lesser degree of syneresis occurring at a higher pH (Lund et al., 1971; Walstra, 1993).

Cheesemakers, particularly artisans, must produce cheeses that meet customer quality expectations within the constraints of their distribution chain. For cheese makers who distribute regionally (local markets and restaurants) it may not be necessary to produce a cheese with a longer ripening time and shelf life. Generally, traditional Camembert type cheeses would be consumed shortly after manufacture. When these cheeses were developed the goal was not to distribute through long distances, but rather to bring these cheeses to a local market. However, many Camembert type cheese producers ship their cheese long distances necessitating modifications to cheese recipes to withstand longer shelf life without the risk of over-ripening and texture deterioration. There are advantages to making both traditional and stabilized Camembert type cheese depending on the destination of the cheese and the desired quality by the consumer.

This study was designed to characterize the compositional and quality characteristics of Camembert cheeses produced by differing methods currently used by artisan cheesemakers. This study was not designed to systematically evaluate the impact of individual modifications; however, these results suggest how modifications in specific

cheese making processes influence important cheese quality outcomes. It is also important to note, that all cheeses in this study were of a single diameter (7 cm). Camembert cheese diameter and height will influence the rate of ripening and related quality characteristics; nevertheless, the mechanism for bloomy rind cheese ripening and relative performance of recipes should be the same regardless of cheese size.

2.6 Conclusion

This study demonstrates significant differences in product composition as well as demonstrating the development of pH, texture/cheese body, and color development during ripening of five Camembert type cheese variants. The study provides the framework of different procedures that can be used to make Camembert type cheese and the impact these recipes will have on the quality and shelf life of the cheese. Understanding which factors control Camembert type cheese composition will assist cheese makers in controlling final product quality and allow them to manufacture cheese that will meet the desired expectations of the consumer and requirements for their distribution network.

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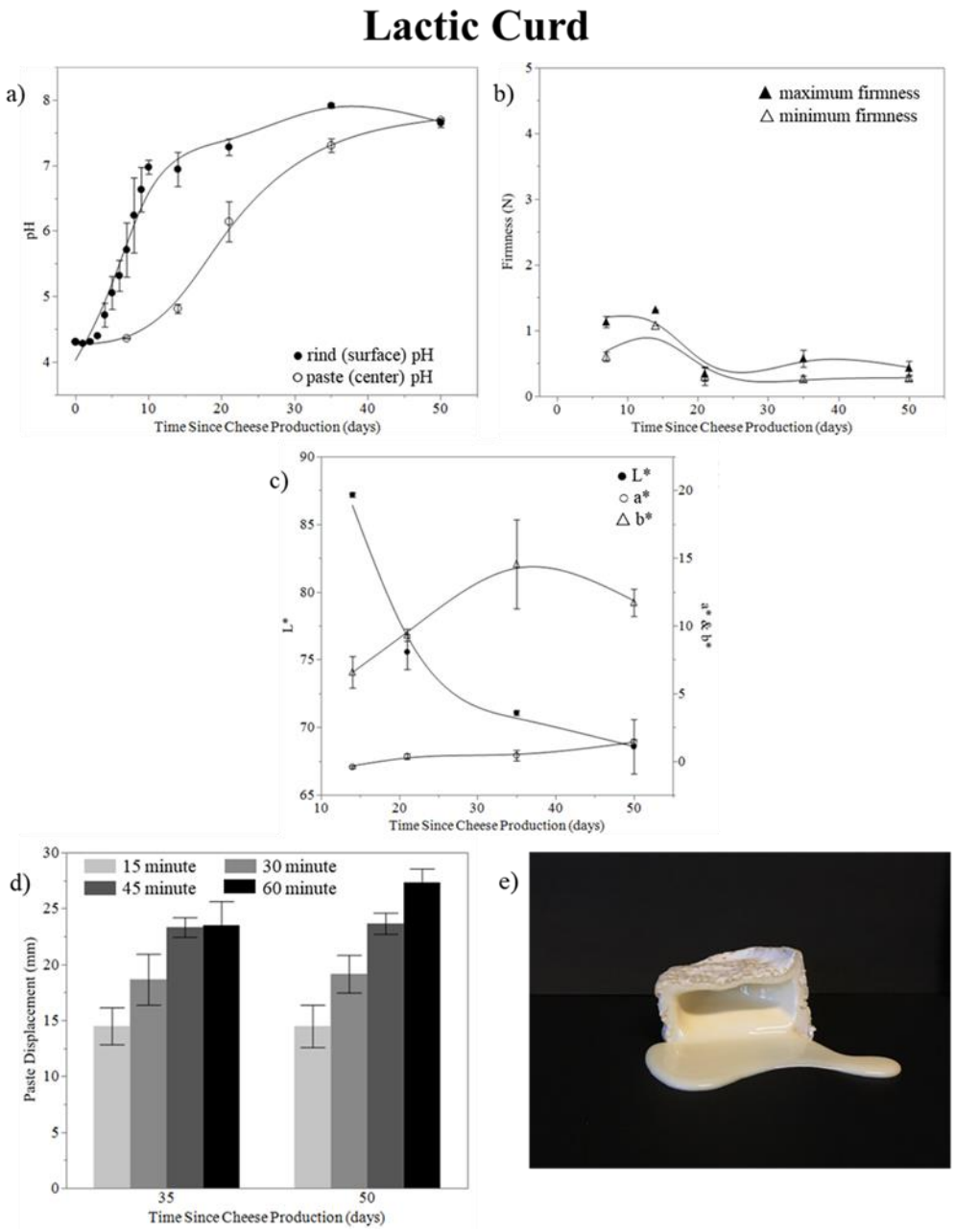

Figure 2.1. Characteristics of lactic curd Camembert variety from production throughout ripening (50 days). a) pH of the rind and paste: rind (surface) pH (●); paste (center) pH (○). b) Firmness of the paste: maximum firmness (▲); minimum firmness (ripe zone, △). c) Color of the surface represented by L* (●), a* (○), and b* (△). d) Paste displacement: 15 min (●); 30 min (●); 45 min (●); 60 min (●). e) Image of the paste displacement 50 days in to ripening at the 60 min time point. Error bars represent the standard error of the mean for duplicate cheese makes (n=2)

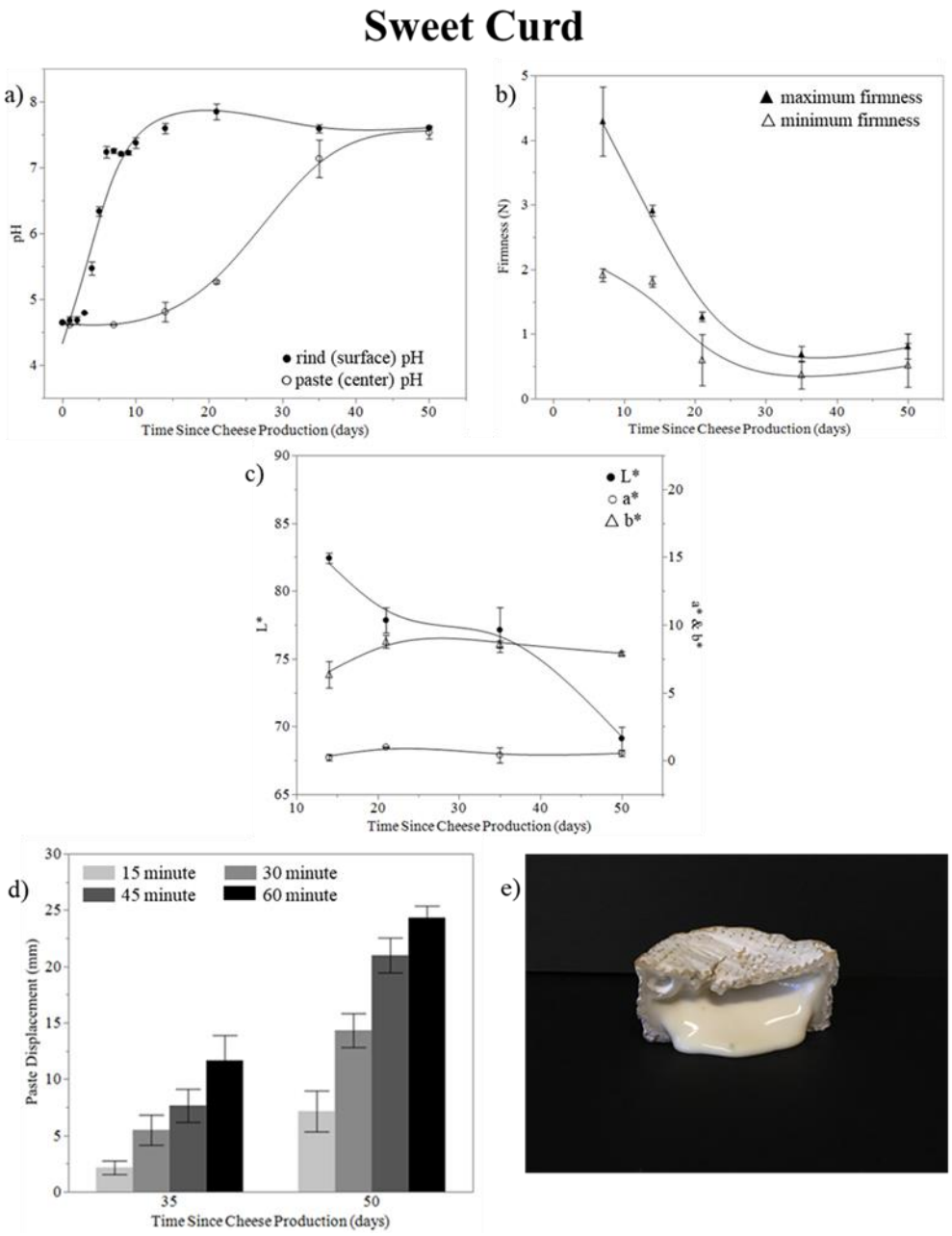

Figure 2.2. Characteristics of sweet curd Camembert variety from production throughout ripening (50 days). a) pH of the rind and paste: rind (surface) pH (●); paste (center) pH (○). b) Firmness of the paste: maximum firmness (▲); minimum firmness (ripe zone, △). c) Color of the surface represented by L* (●), a* (○), and b* (△). d) Paste displacement: 15 min (●); 30 min (●); 45 min (●); 60 min (●). e) Image of the paste displacement 50 days in to ripening at the 60 min time point. Error bars represent the standard error of the mean for duplicate cheese makes (n=2)

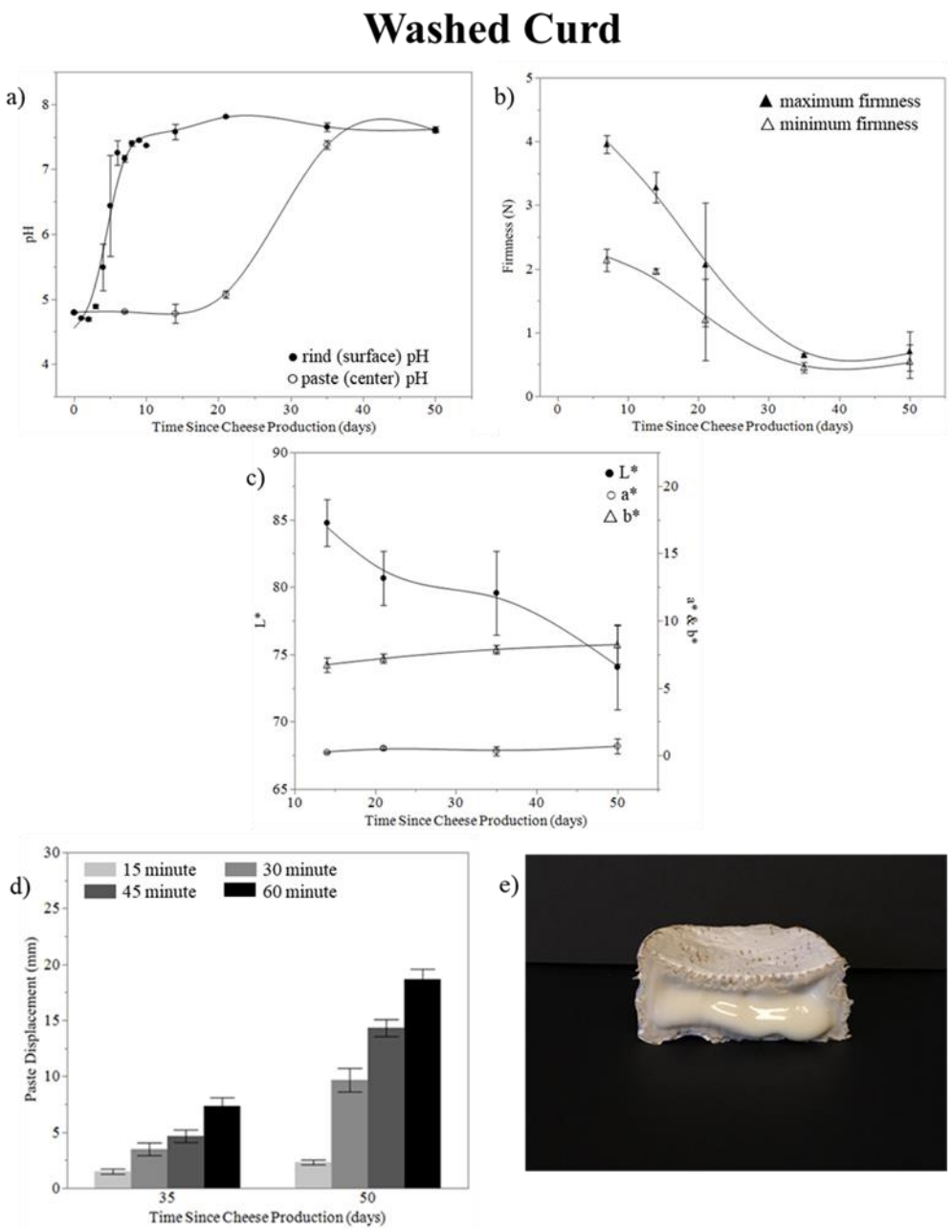

Figure 2.3. Characteristics of washed curd Camembert variety from production throughout ripening (50 days). a) pH of the rind and paste: rind (surface) pH (●); paste (center) pH (○). b) Firmness of the paste: maximum firmness (▲); minimum firmness (ripe zone, △). c) Color of the surface represented by L* (●), a* (○), and b* (△). d) Paste displacement: 15 min (●); 30 min (●); 45 min (●); 60 min (●). e) Image of the paste displacement 50 days in to ripening at the 60 min time point. Error bars represent the standard error of the mean for duplicate cheese makes (n=2)

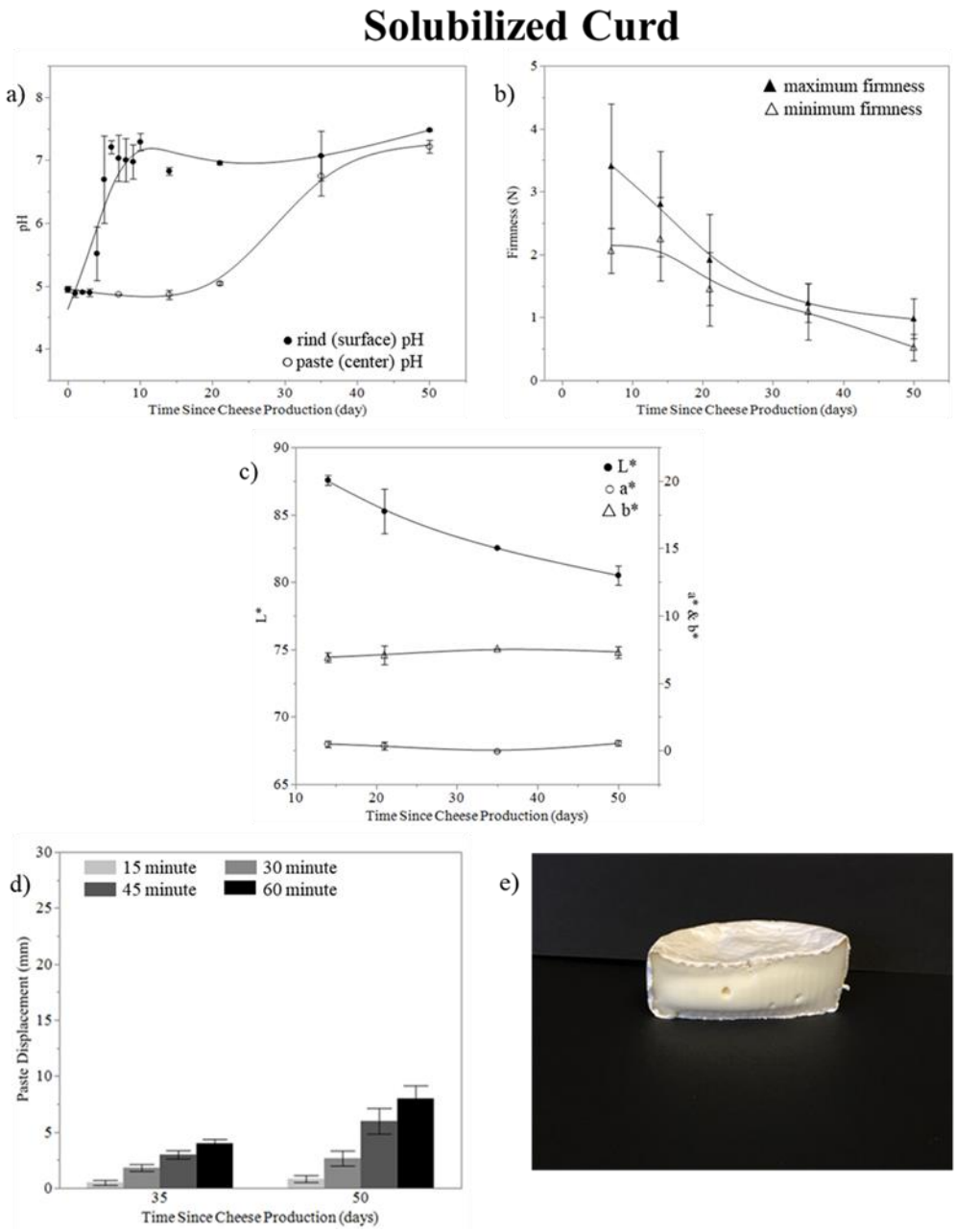

Figure 2.4. Characteristics of solubilized curd Camembert variety from production throughout ripening (50 days). a) pH of the rind and paste: rind (surface) pH (●); paste (center) pH (○). b) Firmness of the paste: maximum firmness (▲); minimum firmness (ripe zone, △). c) Color of the surface represented by L* (●), a* (○), and b* (△). d) Paste displacement: 15 min (●); 30 min (●); 45 min (●); 60 min (●). e) Image of the paste displacement 50 days in to ripening at the 60 min time point. Error bars represent the standard error of the mean for duplicate cheese makes (n=2)

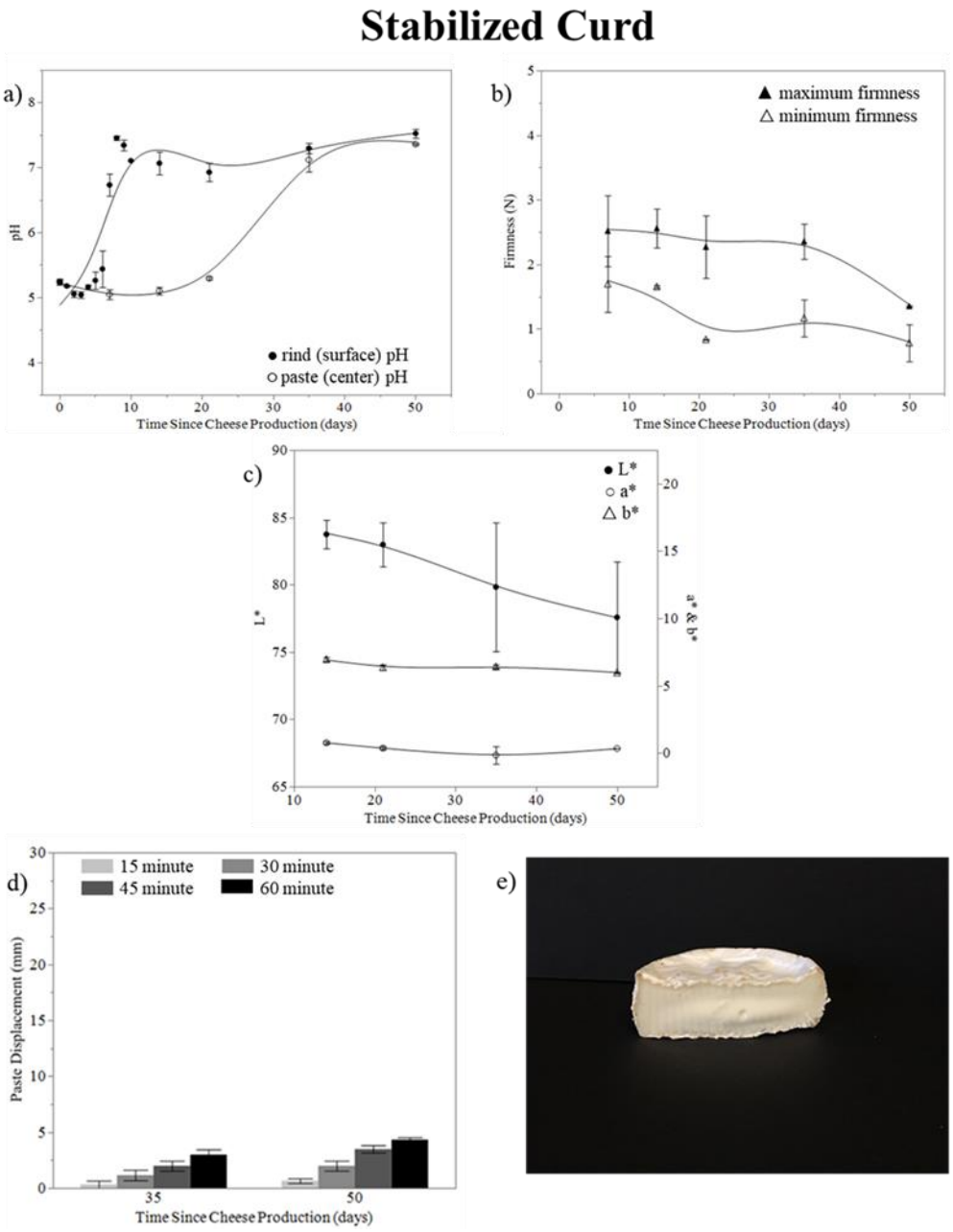

Figure 2.5. Characteristics of stabilized curd Camembert variety from production throughout ripening (50 days). a) pH of the rind and paste: rind (surface) pH (●); paste (center) pH (○). b) Firmness of the paste: maximum firmness (▲); minimum firmness (ripe zone, △). c) Color of the surface represented by L* (●), a* (○), and b* (△). d) Paste displacement: 15 min (●); 30 min (●); 45 min (●); 60 min (●). e) Image of the paste displacement 50 days in to ripening at the 60 min time point. Error bars represent the standard error of the mean for duplicate cheese makes (n=2)

Table 2.1. Modifications in cheese manufacture procedure to attain multiple variants of Camembert type cheese.

Camembert variety	Lactic curd	Sweet curd	Washed curd	Solubilized curd	Stabilized curd
$CaCl_2$ quantity (ml/100 kg)	Not added	6.6	6.6	6.6	6.6
Starter type	Mesophilic	Mesophilic	Mesophilic	Mesophilic + Thermophilic	Thermophilic + Adjunct
Starter quantity (U/100 kg)	7.6	7.6	9.7	3 + 8.8	9.7
Fermentation temperature (°C)	22	35	35	40	40
Rennet quantity* (ml/100 kg)	1.6	5.3	5.3	5.3	5.3
Set pH	6.0	6.2	6.2	6.2	6.45
Cut time (min)	***	30	30	30	40
Cut size (cm)	Ladle	2	2	1	1
Vat operations**	None	3 stirs	3 stirs	3 stirs	3 stirs
Washing	None	None	Replace 30% (v/v) whey with 35°C water	None	None
Drain pH	4.6	6.1	6.0	6.0	6.2
pH at salting	4.2-4.4	4.6-4.7	4.8-4.9	4.9-5.0	5.2 -5.3
Salting	All varieties were dry salted at a rate of 2 % (w/w)				

*Rennet was added at a 1:40 dilution with deionized water

**Vat operations or curd handling: number of stirs over the course of a 40 min holding time between cutting and hooping

***The lactic curd drain pH was achieved by letting the curd acidify and set for 24 h in the vat

Table 2.2. Composition expressed as % weight of cheese for five different varieties of Camembert type cheese sampled five days after manufacture. Values presented are the means ± standard error of two replicate cheese makes (n=2).

Component	Lactic curd	Sweet curd	Washed curd	Solubilized curd	Stabilized curd
Grouping	I	II	II	III	III
Moisture	57.99 ± 0.60^{a}	56.44 ± 0.74^{ab}	56.29 ± 0.97^{b}	53.28 ± 0.42^{c}	53.15 ± 0.69^{c}
Solid-not-fat (SNF)*	20.59 ± 0.37^{b}	19.90 ± 0.81^{b}	20.79 ± 0.88^{b}	23.96 ± 0.52^{a}	23.50 ± 1.15^{a}
Fat**	21.75 ± 0.44^{b} (50.98 ± 0.86^{bc})	23.66 ± 0.65^{a} (54.33 ± 1.25^{a})	22.92 ± 0.53^{ab} (52.46 ± 1.27^{ab})	22.77 ± 0.79^{ab} (48.71 ± 1.37^{c})	23.35 ± 0.57^{a} (49.88 ± 0.92^{bc})
Protein**	15.43 ± 0.42^{c} (36.72 ± 0.71^{b})	15.90 ± 0.26^{c} (36.51 ± 0.66^{b})	16.62 ± 0.32^{b} (37.16 ± 0.48^{b})	18.23 ± 0.19^{a} (39.02 ± 0.39^{a})	18.34 ± 0.25^{a} (39.15 ± 0.31^{a})
Salt*	1.05 ± 0.06^{b}	1.13 ± 0.11^{ab}	1.12 ± 0.14^{ab}	1.17 ± 0.12^{ab}	1.24 ± 0.06^{a}
Salt-to-moisture (S/M) *	1.81 ± 0.10^{b}	2.00 ± 0.19^{ab}	2.01 ± 0.28^{ab}	2.20 ± 0.21^{ab}	2.33 ± 0.10^{a}
Na**	0.41 ± 0.02^{b} (0.98 ± 0.06)	0.44 ± 0.04^{ab} (1.02 ± 0.09)	0.44 ± 0.05^{ab} (1.01 ± 0.11)	0.46 ± 0.04^{a} (0.99 ± 0.11)	0.48 ± 0.02^{a} (1.03 ± 0.04)
Ca**	0.23 ± 0.02^{c} (0.54 ± 0.06^{c})	0.33 ± 0.04^{b} (0.75 ± 0.08^{b})	0.35 ± 0.03^{b} (0.80 ± 0.05^{b})	0.36 ± 0.02^{b} (0.77 ± 0.05^{b})	0.45 ± 0.03^{a} (0.96 ± 0.06^{a})
P**	0.21 ± 0.01^{c} (0.51 ± 0.02^{b})	0.32 ± 0.02^{b} (0.75 ± 0.05^{a})	0.31 ± 0.03^{ab} (0.72 ± 0.06^{a})	0.34 ± 0.01^{ab} (0.73 ± 0.03^{a})	0.40 ± 0.02^{a} (0.86 ± 0.04^{a})

[a-c]Cheese composition values within a row that do not share the same superscript are significantly different ($P<0.05$)

*Calculated values including SNF ($100\ \% - \text{moisture}\ \% - \text{Fat}\ \%$), Salt ($\text{Na} \times 2.54$), S/M $\left((\text{salt}\ \% \times 100) \div \text{moisture}\ \%\right)$, % "as is" protein and minerals (Na, Ca, and P; $(\text{attribute} \times ((100 - \text{moisture}\ \%) \div 100))$), % fat "dry basis" $((\text{attribute} \div (100 - \text{moisture}\ \%)) \times 100)$

**Values in parentheses indicate component percentage on dry basis.

Chapter 3. Camembert type cheese quality implications in relation to the timing of high pressure processing (HPP) during aging

Interpretive summary

Camembert type cheese is highly susceptible to contamination by environmental pathogens, especially *Listeria monocytogenes,* during manufacture and ripening and growth occurs throughout ripening to high cell densities. High pressure processing (HPP) is a non-thermal processing technology that is currently applied to many foods (sauces, guacamole, juice, and meats) as a post-manufacture food safety control step. The current study demonstrated the efficacy of HPP to reduce *L. monocytogenes* in bloomy rind cheeses; however, HPP treatment also had a significant negative impact on cheese quality. HPP treatment of Camembert cheese led to the destruction of the characteristic surface mold producing a final product with unacceptable appearance.

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3.1 Abstract

Bloomy rind cheeses, including Brie, Camembert, and related varieties, are at high risk of contamination by environmental pathogens during manufacture and ripening. This is due to the cheese ripening process and during the initial ripening and surface flora growth. Currently, no kill step is applied post-manufacture or post-ripening to control the food safety risk associated with *Listeria monocytogenes.* Currently, cheese makers must rely on sanitation and environmental monitoring to reduce this risk. High pressure processing (HPP) is a nonthermal food processing technology that can effectively reduce bacterial contaminants with minimal impact on the organoleptic properties of various foods. The objective of this study was to evaluate the application of HPP on Camembert cheese. Timing of HPP treatments (3, 11, and 45 days post-manufacture) were selected based on the growth of *Listeria monocytogenes* during Camembert cheese ripening. HPP treatment of fully ripened cheeses (45 days) resulted in destruction of the surface mold which caused a browning and yellowing of the cheese rind. The level of *P. candidum* on the cheese was significantly ($P < 0.05$) reduced and was below the detection limit for many of the cheeses tested. HPP treatment applied earlier in the ripening process (11 days) resulted in a similar degradation of cheese appearance that was not improved during continued ripening. HPP treatment shortly after production (3 days; before the surface flora developed) caused a delay in the development of the cheese rind and overall appearance and texture of the cheese. This early treatment time also resulted in free serum being expelled from the cheese, creating a firmer body. HPP application 11 days post manufacture resulted in significant reductions ($P < 0.05$) of *Listeria monocytogenes* at 450 and 550 MPa, resulting in greater than 5-log reduction. Even though HPP is

effective at reducing *Listeria monocytogenes* associated with bloomy rind cheeses, the quality deterioration must be addressed before this technology can be successfully used on this type of cheese. Cheese makers must continue to rely on sanitation and environmental monitoring to reduce the food safety risk of bloomy rind cheeses.

Keywords: bloomy rind cheese, stabilized cheese, *Listeria*, contamination time

3.2 Introduction

Bloomy rind cheeses, including Camembert and Brie, belong to the soft surface-ripened category and originated in France (Shaw, 1981). These cheeses make up a significant portion of the specialty cheese market and are now produced around the world. Many varieties of bloomy rind cheeses are produced using different recipes and cheesemaking practices; however, they all rely on surface yeasts and molds for ripening. The predominant fungi associated with bloomy rind cheeses include *Penicillium candidum*, *Geotrichum candidum*, and *Kluyveromyces marxianus* (Leclercq-Perlat, 2011) These yeasts and molds are not only important for cheese ripening but give these cheeses their distinctive white/grey appearance (Shaw, 1981; Galli et al., 2016).

Soft cheeses, including bloomy rind cheeses, are open-air ripened and therefore are at a high risk of contamination from environmental pathogens. Foodborne illnesses have been linked to cheese due to contamination by *Listeria monocytogenes,* Shiga toxin-producing *E. coli* (STEC), *Salmonella*, and *Staphylococcus aureus* (Choi et al., 2016). Between 1986 and 2008, there were 22 recalls associated with *L. monocytogenes* in soft-

ripened cheeses and 24 additional recalls for soft fresh cheeses within the United States and Canada (CDC and Health Canada, 2015). Between 1995 and 2013, there were 151 reported illnesses and 20 deaths worldwide linked to Camembert and Brie consumption (CDC and Health Canada, 2015).

The high risk of contamination for bloomy rind cheese is due to many factors including cheese composition and physiochemical changes during ripening, high frequency of handling, and lack of a food safety control after manufacture (CDC and Health Canada, 2015). During the ripening process, a unique environment is created that supports the growth of *L. monocytogenes*. The increase in pH and elevated ripening temperatures (up to 15°C) sustain or encourage growth of microorganisms (CDC and Health Canada, 2015). This is a concern because cheese making environment and ripening areas have previously been identified as likely locations for contamination by environmental *L. monocytogenes* (Muhterem-Uyar et al., 2015). The product typically remains open to the environment in aging room for up to two weeks to encourage fungal growth on the cheese surface. This prolonged environmental exposure and frequent handling increases the likelihood of *L. monocytogenes* to come in contact with the cheese. Currently, there is not an effective strategy to reduce the risk of *L. monocytogenes* in bloomy rind cheeses.

High pressure processing (HPP) is a non-thermal food processing technology that is used to improve the microbial safety and delay spoilage of fresh oysters, processed meats, guacamole, fruit juice, and fresh cheeses. For these foods, there are minimal to no noticeable quality changes (visual, texture, etc) between the “raw” product and the HPP

treated product (Yordanov and Angelova, 2010). HPP applications for microbial reduction typically range from pressures of 300 to 600 MPa with holding times of 1-10 min (Yordanov and Angelova, 2010; Martínez-Rodríguez et al., 2012).

The efficacy of HPP to reduce *L. monocytogenes* has been demonstrated for various soft cheeses including queso fresco, fresh goat cheese, and model washed-curd cheese. Tomasula et al. (2014) observed a significant destruction (4.6 log CFU/g) of *L. monocytogenes* at 600 MPa for 5 min in queso fresco cheese. In fresh goat cheese, Gallot-Lavallee (1998) achieved greater than a 5-log reduction of *L. monocytogenes* using the processing parameters of 550 MPa for 5 min and 450 MPa for 10 min. Using a model washed-curd cheese matrix, a similar result was observed with *L. monocytogenes* being reduced by ~5 log CFU/g at 500 MPa for 5 and 20 min (López-Pedemonte et al., 2007). These studies present evidence that HPP could be an effective processing strategy to reduce the risk of *L. monocytogenes* in bloomy rind cheeses.

Bloomy rind cheeses present a unique challenge in that their ripening is a dynamic combination of microbial and enzymatic activity. These reactions transform a firm and chalky curd into a complex cheese with a distinct outer rind and soft interior that flows when cut (Gripon, 1997; Tansman et al., 2017). Enzymes associated with surface yeasts and molds induce deamination reactions which increases levels of ammonia further increasing the pH at the surface of the cheese (Karahadian and Lindsay, 1987; Boutrou et al., 2006; Picque et al., 2010). A pH gradient from the surface of the cheese to the center develops and at the same time a reverse mineral gradient develops from the center to the surface (Le Graet et al., 1983; Gripon, 1997; Tansman et al., 2017). Soluble

Ca migrates to the rind of the cheese leading to increased hydration of the para-casein network (Lucey and Fox, 1993). The softening result is a combination of protein hydration, demineralization of the curd, and proteolysis. These cheeses can also be manufactured using alternative practices that result in cheeses with varying paste stability and structural integrity (Batty et al., 2018). With these two variables, the timing of HPP treatment and bloomy rind cheese variety could influence the ripening of the cheeses and overall product quality. Brie cheese has previously been investigated with 400 MPa and 600 MPa treatments at 14 and 21 days post-manufacture (Calzada et al., 2014a). In this study they also observed a reduced rate of proteolysis in the HPP treated cheeses, suggesting that the shelf-life of the cheese could be increased. They also had promising results with improved flavor quality at the end of shelf-life.

The objective of this study was to determine the effect of HPP treatment on the physical and microbiological quality of Camembert type cheese applied at different points in the aging process. Pressure treating the cheese at different stages of ripening has great implications for the success of HPP as a food safety control and was determined by growth potential of *L. monocytogenes* at various stages of ripening. Due to the lack of information available on HPP treatment of Camembert type cheese we chose to evaluate three different recipes that represented the range of physiochemical properties, ripening rate, and shelf life: i) traditional (sweet curd) Camembert, ii) stabilized Camembert, and iii) hybrid Camembert.

3.3 Materials and Methods

Overall experimental design

The growth of *L. monocytogenes* was evaluated on the surface of Camembert type cheese using the stabilized variety and multiple possible points of contamination during ripening. Three different manufacturing recipes were used to produce Camembert type cheese. The recipes included a more traditional variety (sweet curd), a stabilized variety (stabilized), and a hybrid of the two (hybrid). These three Camembert type cheese varieties were subjected to high pressure processing (HPP) at three time points post-manufacture: 3 days, 11 days, and 45 days. Times were selected to evaluate possible treatment points based on the ripening of this cheese type. To evaluate the reduction of *L. monocytogenes* only the 11 day treatment time and stabilized variety were used. Pressure treatments were performed in duplicate at each time point and the composition, physical, and microbiological quality was monitored throughout shelf-life (up to 50 days post manufacture).

Cheese making

Camembert cheese making procedures followed those described by Batty et al. (2018). Pasteurized whole milk (average P/F ratio of 0.99; 75 kg/batch) was obtained from a regional fluid milk processor and transported to the Arbuthnot Dairy Center at Oregon State University (Corvallis, OR, USA) for cheese manufacture. Milk was added to a round cheese vat (C. van't Riet Dairy Technology B.V., Nieukoop, Netherlands) and heated to the set fermentation temperature depending on the recipe (Table 3.1). Depending on recipe, a mesophilic blend (Flora Danica-DVS, Chr. Hansen Inc. Milwaukee, WI, USA) or the thermophilic starter *Streptococcus thermophilus* (Choozit DVI TA 50 series, Danisco, Copenhagen, Denmark) was added to the cheese vat along

with the ripening cultures (*Penicillium candidum* (PCA 3, Chr. Hansen Inc.; 0.40 U/100 kg), *Geotrichum candidum* (Choozit Geo 15 LYO, Danisco; 0.15 U/100 kg), and *Kluyveromyces marxianus* (LAF 4, Chr. Hansen Inc.; 0.15 U/100 kg)). The quantity of starter culture used for each cheese type is shown in Table 3.1. *Leuconostoc mesenteroides* subsp. *cremoris* (Choozit LM 57, Danisco; 0.73 U/100 kg) was also added as an adjunct culture to the stabilized variety for flavor development. Calcium chloride (DCI, 32-33% w/v, Dairy Connections Inc., Madison, WI) was also added to all recipes at this point at a rate of 6.6 mL/100 kg to promote curd formation. Fermentation continued until the targeted set pH of each recipe was achieved (Table 3.1). The milk was then transferred to soft cheese semi-cylindrical coagulation basins (Servi Doryl, Langeais, France). Coagulant (DCI Star Coagulant, Dairy Connections Inc., Madison, WI, USA) was added to the fermented milk at a rate of 5.3 mL/100 kg and left undisturbed for 25-35 minutes. Coagulum firmness was determined by visual assessment and by using the flocculation assessment with a multiplication factor of 5 for the traditional variety and a factor of 3 for the stabilized and hybrid variety (Caldwell, 2012). The coagulum was cut using either 1-cm or 2-cm knives (Table 3.1; Servi Doryl, Langeais, France), the curd/whey mixture was stirred 3 times over 30 minutes, and the whey was drained 10 minutes after the final stir. Cheese molds (n = 120/vat) (Fromagex, Rimouski, Québec, Canada; 7 cm diameter) were filled using a curd distributor (Fromagex) to an average cheese weight of 95 ± 10 g.

Cheeses were drained overnight at 21 ± 1°C with turning at 30 minutes, 5 hours, 10 hours and 19 hours. The cheese was removed from the mold at 14-20 hours (Table 3.1) and salted (2% w/w). The cheese was then open air dried for an additional 1-2 hours.

This marked day 0 (start of maturation) for downstream treatments and sample time points. Cheeses were transferred to an incubator (15 ± 1°C, 85% RH) for 24 hours. The relative humidity was then increased to 95% and temperature decreased to 13 ± 1°C for 14 days and cheeses were flipped daily to encourage uniform surface mold development. Cheeses were then wrapped in white mold paper for final maturation (Fromagex) and stored at 7 ± 1°C for up to 50 days post manufacture. Wrapped cheeses were flipped weekly.

Contamination and growth of *Listeria monocytogenes* in Camembert

L. monocytogenes cocktail preparation. All isolate stock cultures were stored at the OSU Food Safety Lab (Oregon State University) at -80° C in 40% glycerol until time of use. The bacterial strains were revived from the stock cultures by inoculating in tryptic soy broth (TSB; Neogen, Lansing, MI, USA) and incubating at 37°C for 24 hours. The strains were streaked on CHROMagar™ Listeria (DRG International, Springfield, NJ) to verify purity of the stock culture. A representative colony of each strain was selected and transferred from the media to TSB (Neogen) and incubated at 37°C for 24 hours. TSB cultures were stored at 4°C (less than 24 hours) until they were used to make a lawn culture. Individual TSB cultures were aliquoted on to tryptic soy agar (TSA; Neogen) plates to create a bacterial lawn. These plates were incubated at 37°C for 24 hours. The resulting bacterial lawn was harvested by adding whey (obtained from the cheese make) to the TSA plate. The plates were scraped using a bacterial lawn spreader and the inoculum was pipetted from the plate in to a 50 mL conical vial. The harvested cultures were then combined with the other strains of the same species in equal volumes in a 50

mL conical tube. This inoculum was then serial diluted using whey from the cheese make of the stabilized curd to a target concentration of 2 log CFU/g on the cheese.

Cheese inoculation. Four possible points of contamination were selected to assess the post-pasteurization risk for Camembert cheese when *L. monocytogenes* is in the environment. These four points include pre-salting immediately after removal from the cheese molds, post-salting (2 hours after salt application), 5 days post-manufacture (after the surface yeasts and molds begin to grow), and 10 days post-manufacture. These four time points were selected to demonstrate the different stages of cheese ripening while the cheese is open to the environment and at risk of contamination. Stabilized Camembert cheeses were artificially contaminated by spot inoculating 100 µl of a four strain *L. monocytogenes* cocktail on the surface of each side of the cheese. These strains include ScottA (serotype 4b, clinical isolate), California (serotype 4b, isolated from Mexican-style cheese associated with an outbreak in 1985), Ohio (serotype 4b, isolated from recalled cheese), and ATCC 19116 (serotype 4c, isolated from chicken in England). The microbial load was monitored through 60 days. Inoculation studies and reduction evaluation of *L. monocytogenes* were performed in duplicate (n=2) using two unique sets of prepared inocula. For the evaluation of *L. monocytogenes* reduction, the methods of culture preparation and inoculation were the same but only the post salting inoculation time was used for this treatment.

High pressure processing (HPP)

Cheeses were treated with high pressure processing (HPP) at 3, 11, and 45 days post manufacture. For the evaluation of *L. monocytogenes* reduction cheeses were

processes 11 days post manufacture. Cheese wheels were packaged in white mold paper, placed into a 3-mil plastic pouch (UltraSource, Kansas City, MO) and sealed under light vacuum. Cheeses were shipped with ice packs overnight to the Cornell HPP Validation Center (Ithaca, NY, USA) for processing in a 55-L commercial scale unit (Hiperbaric, Miami, FL, USA). Cheeses were pressurized at 550 MPa at 25°C with a hold time of 10 minutes for the quality evaluation and 350, 450, and 550 MPa at 25°C with a hold time of 10 minutes for the *L. monocytognes* reduction. Cheeses were boxed and returned to OSU via overnight shipping and returned to the ripening area upon arrival. All shipped cheese was returned to normal aging within 48 hours after initial boxing. Travel controls were shipped with the cheese (for each set) and aged normally upon return to ensure shipping didn't degrade the cheese quality. Controls used for quality analysis were aged normally (without shipping). For the *L. monocytogenes* reduction there were two sets of inoculated controls. The first one was left in typical aging conditions while the other traveled with the cheese to ensure travel didn't impact survival.

Composition, physiochemical, and microbiological analysis

Milk and cheese composition. Milk composition (fat, protein, lactose, and solids-not-fat (SNF)) was conducted using a milk analyzer (Lacticheck-01, RapiRead, MA, USA) on the day of cheese manufacture. Two samples of each cheese type were removed from ripening on day 5 for composition analysis including fat, moisture, protein, and microcomponents (Ca, Na, P). Prior to macrocomponent (fat, protein, and moisture) analysis, whole cheese wheel samples were homogenized by blending for 15 seconds until pieces were small and uniform. Moisture content was determined using a rapid

analyzer for solids and moisture (Arizona Instrument LLC, Candler, AZ, USA). Fat was analyzed by the Van Gulick method (ISO, 2008). Total nitrogen was measured using a combustion analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Hanau, Germany). Crude protein was calculated by multiplying the total nitrogen by 6.38. For micro component analysis (Ca, P, Na), 10 sub-samples were taken from each wheel and dried at 35°C for 96 hours prior to grinding with a mortar and pestle. The samples were wet-ashed using microwave digestion (MultiwaveGO, AntonPaar USA, Ashland, VA, USA) with nitric acid (Macron Fine Chemicals, Center Valley, PA). The cheese was then analyzed for micro components using inductively coupled plasma atomic emission spectroscopy (ICP-OES: Agilent 5110, Agilent technologies, Santa Clara, CA, USA).

Microbiological analysis. Cheese samples were removed from ripening on days 7, 21, 35, and 50 for enumeration of microbial populations. For the reduction of *L. monocytogenes* samples were enumerated within 24 hours of the treatment. Cheese samples (25 g) were transferred to a sterile Whirl-Pak bag (Nasco, Salida, CA) and combined with a 1:1 ratio of 0.1% peptone water. The samples were stomached (EASYMIX™, bioMérieux, Marcy-l'Étoile, France) for 30 seconds until homogenous. Cheese homogenate was serial diluted in 0.1% peptone water and 0.1 mL aliquots were spread-plated on appropriate growth media. Oxytetracycline-Glucose Yeast Extract Agar (Neogen; 25°C for 3-5 days) was used for the enumeration of yeasts and molds. *L. monocytogenes* was enumerated using CHROMagar™ Listeria (DRG International; 37°C for 48-72 hours).

Texture analysis. Cheese firmness was measured on days 7, 14, 21, 35, and 50 post manufacture using methods previously described (Abraham et al., 2007 and Batty et al. 2018). The texture analyzer TA XT2i (Texture Technologies Corp., Hamilton, MA, USA) equipped with a 5 kg load cell and a 6 mm cylindrical probe was used for penetration measurements. The cheese was held for one hour at room temperature (23°C) prior to texture analysis. During the test, the penetration speed was 0.4 mm/s with a total penetration depth of 75% of the cheese height. Firmness of the cheese paste was recorded at the minimum peak and maximum peak within the paste. The paste measurements were the low and high points for firmness after the initial rind fracture peak. Texture analysis was performed on two cheeses of each variety at each time point.

pH. The pH was measured throughout the cheese make and ripening period using a portable meter (Portable Food and Dairy pH Meter, Hanna Instruments, Woonsocket, RI) equipped with a conical penetration probe designed for semi-solid foods. Cheese rind pH measurements were performed in triplicate for each side. For the paste, one pH measurement was taken from the center of the cheese. Two cheeses per treatment were analyzed on days 7, 14, 21, 35 and 50 post-manufacture.

Color. The color of the cheese paste and rind were measured using a spectrophotometer (LabScan XE Spectrophotometer, Hunter Associates Laboratory Inc., Reston, VA, USA). Color measurements of paste and rind were measured in triplicate for two cheeses/treatment on days 7,14, 21, 35, and 50 post-manufacture.

Data Analysis. All data analysis for this experiment was completed using JMP 13.0 (SAS Institute Inc., Cary, NC, USA). The means, standard deviations (cheese

composition), and standard errors (all other quality metrics) were calculated. The standard error was calculated for two replicate pressure treatments (n = 2). Mean comparisons were made by computing a one-way analysis of variance (ANOVA) and differences were further compared using the Tukey-Kramer HSD test.

3.4 Results and Discussion

Cheese composition

The traditional, stabilized, and hybrid Camembert type cheese varieties differed significantly in moisture, protein, Ca, and P contents at 5 days post manufacture ($p < 0.05$; Table 3.2). The composition results obtained are expected given the differences in the recipes that were used to produce these cheeses. These compositional differences are attributable to differences in formulations and cheesemaking practices of Camembert type cheese (Batty et al. 2018). Lower moisture of the stabilized curd was most likely due to the smaller cut size used because less distance is needed to be covered for the whey to expel from the curd, resulting in more whey expulsion (Walstra, 1993; Guinee and O ’callaghan, 2010). The higher level of protein for the stabilized curd was likely due to the displacement of components with the different moisture contents as the protein content on a dry basis was not significantly different. All three of these cheeses had a Ca content that was directly related to the pH. The stabilized variety had the highest pH and Ca content and the traditional (sweet curd) variety had the lowest pH at all control points (cutting, draining, and salting) leading to lowest levels of total Ca. Differences in Ca content are most likely due to the different pHs throughout the process as previously stated by Fox and Lucey (1993). pH as a control of the total Ca and P or the insoluble Ca

has been successfully used in many studies on cheddar, mozzarella, and Camembert type cheeses (Guinee et al., 2002; Upreti and Metzger, 2006; Batty et al., 2018). There were no significant differences in salt content between the cheese varieties in the current study.

Listeria monocytogenes growth after surface contamination

L. monocytogenes was capable of growing on the surface of stabilized Camembert type cheese at all inoculation points. From the initial level of contamination (2-3 log CFU/g), there was a slight decrease in the concentration by day 5 (Figure 3.1). Dry salting of the cheese surface likely contributed to the initial reduction of *L. monocytogenes*. Continued ripening supported exponential growth of *L. monocytogenes* on the surface of the cheese, regardless of the timing of inoculation. By 25 days post-manufacture, *L. monocytogenes* reached levels in excess of 7.4 ± 0.2 CFU/g. This high level presents a great risk to consumers and producers of Camembert type cheese.

L. monocytogenes contamination occurring later in the ripening period (11 days post manufacture) was also capable of growing on Camembert type cheese; however, the growth rate was substantially reduced as compared to contamination that occurred during production. The decreased growth rate is likely due to the cooler ripening temperature during this production stage. By the time of consumption (day 60 post-manufacture), *L. monocytogenes* levels had increased to 5.6 ± 0.5 CFU/g. These results are comparable to previous studies of *L. monocytogenes* growth during aging and refrigerated storage of bloomy rind cheeses including both Camembert and Brie (D'Amico et al., 2008; Kapetanakou et al., 2017). With all contamination events resulting in high concentrations of *L. monocytogenes* (>5.6 log CFU/g) by the end of shelf life, it would be ideal to apply

HPP as close to distribution as possible. Three timepoints in the ripening period were selected to investigate the suitability of HPP to maintain product quality and mitigate *L. monocytogenes* contamination in bloomy rind cheeses: i) 45 days post-manufacture (mid shelf-life and typical consumption time), ii) 11 days post-manufacture (after the surface yeasts and molds developed and when the cheeses are wrapped), and iii) 3 days post-manufacture (prior to surface yeast and mold development).

Reduction of *Listeria monocytogenes* using HPP

L. monocytogenes was significantly reduced ($P < 0.05$) using pressures at 350, 450, and 550 MPa compared to both controls (aging and travel). With both the 450 and 550 MPa treatments a greater than 5-log reduction was observed (Figure 3.2). These results are similar to previous studies that also achieved large reductions of *L. monocytogenes* in queso fresco, goat cheese, and washed-curd model cheese (Gallot-Lavallee, 1998; López-Pedemonte et al., 2007; Tomasula et al., 2014). Even though there is great reduction potential using HPP, there was still between 1-2 log CFU/g of *L. monocytogenes* present on the cheese. This is concerning because the level achieved on the surface of the cheese was achieved with a low-level contamination just after manufacture. Any *L. monocytogenes* remaining presents the risk of growth by viable cells that could lead to foodborne illness. It is important to note that the travel to the processing center didn't impact the survival of *L. monocytogenes*.

HPP Treatment at Day 45

The 45-day HPP treatment is representative of a treatment time that would minimize the food safety risk immediately prior to distribution of bloomy rind cheeses,

especially for the longer shelf life stabilized variety. This would be the optimum treatment time from a food safety stance because the risk for recontamination is extremely low. The cheese would also be at the ideal point for consumption and the risk would be the highest based on the *L. monocytogenes* growth study.

Cheese appearance. By day 50 post-manufacture, the white mycelium of the surface fungi covers majority of the cheese wheel with an appearance that would satisfy typical consumer expectations. The appearance of the cheese is negatively impacted by HPP treatment (Figure 3.3b, d, and f), particularly shape deformation of the cheese and discoloration of the rind. Deformation of the cheese wheel is more severe in the traditional and hybrid varieties (Figure 3.3b and d). The rind of the hybrid variety fractured (Figure 3.3d) which caused the paste to escape the wheel. This observed deformation was less apparent for the stabilized variety. The deformation may have been due to the higher moisture content and lower Ca and P content of the hybrid and traditional varieties. It has been well demonstrated that cheeses of higher moisture tend to be less firm, allowing them to be more susceptible to physical modification. The level of total Ca can be indicative of the softness and flowability of Camembert type cheese (Batty et al., 2018). The surface color of the cheese is significantly affected by the HPP treatment at day 45. The L* value (measuring lightness to darkness) was significantly ($P < 0.05$) reduced for all of the varieties with the 45 d treatment (Figure 3.4). The average reduction ranged from 13.3 to 42.4. This decrease in L* indicates browning of the rind that is apparent in Figure 3.3 (b, d, and f). The a* value had a significant increase for all varieties comparing the control and the 45 d treatment (Figure 3.4). It had an average increase ranging from 2.39 to 6.69. This observed increase in the a* value indicates that it

is becoming more red in color, also contributing to the brown appearance. The b* value, similar to the a* value, had significant increase for all varieties at the 45 day treatment time. The average increase ranged from 14.91 to 16.53, indicating the rind is becoming more yellow. The color change induced by HPP was also demonstrated by (Calzada et al., 2014b) when the treatment was applied to Brie cheese wedges 14 and 21 days post manufacture, but it was downplayed to not be as important of a finding even though the color difference would be completely unacceptable to consumers. Using both 400 and 600 MPa they found that there was a decrease in lightness and an increase in red and yellow colors on the rind of the cheese throughout storage. The differences in color they observed were not as large as what we found and may be a result of how their cheeses were treated. Typically, bloomy rind cheeses would not be cut in to wedges at this point of ripening and provides a potentially different result. Comparing to other varieties of mold-ripened cheese, Voigt et al. (2010) found that the L* and a* values of blue cheese were significantly impacted by HPP treatment.

Microbiology. The observed color change for the 45 day treatment is due to the destruction of the mycelium produced by *Penicillium candidum* which was confirmed by significant reductions in yeast and mold count ($P < 0.05$). The stabilized variety had the greatest resistance of the three varieties, decreasing from an average of 6.8 to 1.2 log CFU/g. The control group for the traditional and hybrid varieties had levels of 5.5 and 6.5 log CFU/g, respectively, and were reduced to below our limit of detection (1 CFU/g) after the HPP treatment. All counts are from 50 days post-manufacture. Interestingly, the yeasts and molds (all ripening organisms) had a significant ($P < 0.05$) reduction with 4.2 log CFU/g present after treatment compared to 7.4 log CFU/g on the control cheeses.

This reduction of *P. camemberti* has previously been documented for Brie type cheese subjected to 400 and 600 MPa of pressure. Calzada et al (2014) found that 600 MPa treatments reduced mold counts by more than 6 log CFU/g to below their limit of detection, similar to the result we observed.

Texture and pH. The HPP treatment at 45 days had no significant impact on the firmness or pH of any of the cheese varieties. The average pH of the rind and the paste for all treated and untreated cheeses were between pH 7 and pH 8. After 45 days of ripening, the pH of the cheeses had reached equilibrium that was unchanged by HPP treatment. The firmness at 50 days for all treatments and controls was between 0.41 and 1.14 N. Nearly all of the cheeses treated with HPP at 45 days had a less firm paste compared to their untreated controls. The average decrease in firmness for the traditional variety was 0.45 N, a 0.21 N decrease for the hybrid variety, and 0.19 N decrease for the stabilized variety. However, the differences between the treatment and control of each cheese variety were not significant ($P > 0.05$). All cheeses were very soft at this stage of ripening. For the traditional (sweet curd) variety, this time point will be near the end of shelf life, while for the stabilized variety this would be near the beginning of its ideal consumption time (Batty et al., 2018).

HPP Treatment at Day 11

Due to the unacceptable quality of HPP treated cheese at day 45, HPP treatment at day 11 was evaluated. HPP treatment at day 11 would be suitable because the surface fungi would have developed and the cheeses would be wrapped for final aging. After this

wrapping is complete, the risk for contamination by *L. monocytogenes* and other environmental contaminants is significantly reduced.

Cheese appearance. Visual changes of cheeses with and without HPP treatment at 11 days are shown in Figure 3.5a. Similar to the HPP treatment at 45 days, the color of the cheese was significantly impacted due to this destruction of the surface fungal mycelium. Despite continued aging, the fungus was unable to recover to repopulate the rind of any of the Camembert varieties indicating complete destruction of the mycelium and large reductions of the fungal population by HPP treatment (Figure 3.5b-d). The b* significantly increased ($P < 0.05$) due to HPP treatment (Figure 3.6). The b* value of the HPP treated cheeses also increased throughout ripening, indicating that the rind is becoming more yellow over time. The L* value decreased for the 11 day treatment compared to the control, but for most sample times and varieties this decrease not significant ($P > 0.05$). There was no apparent change in the a* value due to HPP treatment. With these changes in the b* and L* it is very apparent the appearance of the cheese was impacted by the HPP treatment and continued aging is unsuccessful at improving color quality (Figure 3.4). In addition to the obvious visual changes, the HPP treated cheeses also had an obvious sour odor.

Microbiology. Comparable to the 45 day treatment the yeast and mold counts as well as the *P. candidum* counts were significantly lower ($P < 0.05$) than that of the control, with the degree of reduction ranging from 1.7 to 2.7 log CFU/g and 2.8 to 5.0 log CFU/g at the 21 day sample point, respectively. Surprisingly, there was not a nearly complete destruction of the *P. candidum* like with the 45 day treatment, but it still was

significant and impacted the overall appearance of the cheese. Even though there was a recovery of the *P. candidum* by the end of ripening, it was not apparent because the mycelium failed to develop. The lesser degree of reduction observed could have been because the first sample point after HPP treatment was 10 days post treatment which likely allowed for the regrowth of the fungi.

Texture and pH. HPP treatment at day 11 caused a significant increase ($P < 0.05$) in the firmness of the hybrid and traditional varieties of Camembert cheese (Figure 3.7), whereas HPP treatment at this timepoint had no impact on the firmness of the stabilized variety. This increase in firmness was possibly due to free serum (up to 5 mL) being pressed from the cheese during the HPP treatment. Sandra et al., (2004) also observed an increase in firmness initially after the HPP treatment of queso fresco at 400 MPa for 20 minutes. After continued aging of the cheeses (day 50), there were no significant differences ($P > 0.05$) in firmness between HPP-treated and untreated cheeses for any of the varieties.

The pH of the surface initially decreased to the pH level of the paste (center of the cheese). This was possibly due to the migration of lactic acid in the serum to the cheese surface during the treatment. The development of pH on the surface of the cheese was significantly ($P < 0.05$) impacted by the pressure treatment. The average surface pH of the control for the three varieties was 8.13 ± 0.13 and the treated cheeses had an average of 6.26 ± 0.34 on day 50 post manufacture. This limited increase in the surface pH was also apparent with the paste pH during ripening, as there was a lagged increase as well. HPP treatment also led to a slight increase in paste pH initially; however, the increase

was not statistically significant ($P > 0.05$). The lower pH at the surface compared to the control cheeses was most plausibly due to the reduction of the surface fungi, as they are responsible for the pH increase during ripening due to proteolysis and lactate consumption (Schlesser et al., 1992; Spinnler, 2017). This observation of delayed pH increase was consistent with previous findings using HPP to treat Brie cheese (Calzada et al., 2014a).

HPP Treatment at Day 3

The previously two described treatment times were unsuccessful in retaining the cheese quality, especially due to the color degradation associated with fungal destruction. HPP treatment prior to mycelium development could reduce the likelihood of these color changes; however, HPP at this time point is not ideal as the cheeses would likely have additional environmental exposure during open-air ripening and further handling.

Cheese appearance. Initially, the HPP treatment on day 3 had minimal impact on the cheese appearance with only slight deformation of the cheese wheel (Figure 3.8a). With continued ripening, it was clear that HPP treatment on day 3 led to a reduced rate of surface fungal development. It is apparent that the surface fungi develop on the cheese by day 50, especially the traditional and hybrid varieties (Figure 3.8b and c). This is also apparent with the *P. candidum* counts observed for the sweet curd variety (Figure 3.10). There was a general trend for a decrease in the L* value but the most different was the b* value. For all varieties there was a significant ($P < 0.05$) increase in the b* value when compared to the control, with this increase ranging from 7.90 to 11.04. This indicates a yellowing of the cheese that is very aparent in the pictures.

Microbiology. The surface fungi, specifically *P. candidum*, were significantly ($P < 0.05$) effected by the HPP treatment on the day 7 sample point with a lower quantity being recovered (Figure 3.10 - traditional variety). The other varieties were comparable in mold counts. This delayed development of the surface fungi was also noticed throughout the cheese ripening, but the levels of *P. candidum* reached similar levels to the control between day 21 and 35. This level of *P. candidum* was not as apparent visually on the cheese, as the development of the mycelium was still lacking (Figure 3.8b-d).

Texture and pH**.** The firmness of the cheese was significantly increased ($P < 0.05$) for all varieties, with the average increase ranging from 3.07 to 8.29 N. This initial increase was likely due to the cheese being pressed and free serum being released. There was enough free serum in the vacuum packages after HPP that a measurable amount could be attained. Many of these samples had upwards of 5 mL of free serum in the package after treatment. This higher firmness continued throughout shelf life, but it did decrease over time.

The development of pH on the cheese surface was significantly ($P < 0.05$) impacted by the HPP treatment on day 3 (Figure 3.9). This reduced rate of pH at the surface was due to the reduced growth development of the surface fungi previously described. The surface fungi are primarily responsible for the typical pH development at the surface. This reduced rate of pH development could slow the rate of cheese ripening and texture development, but this was not observed in this study.

3.5 Conclusion

HPP treatment (550 MPa, 10 minutes, and 25°C) of Camembert type cheese leads to unacceptable deterioration in product quality regardless of timing of treatment during the aging process. The primary defect in HPP-treated cheese is the destruction of the mycelium on the surface of the cheese, leading to an undesirable appearance. Until an alternative processing treatment can be identified to reduce *L. monocytogenes* in bloomy rind cheeses, pasteurization in combination with sanitation and environmental monitoring remain the only strategies to control the risk of *L. monocytogenes* associated with these products.

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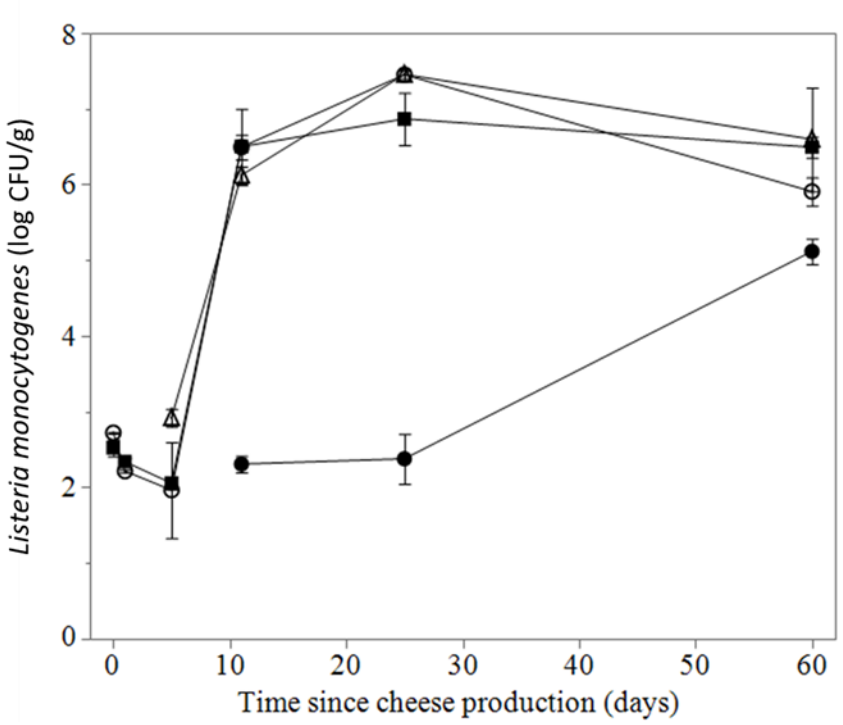


Figure 3.1**.** Microbial risk assessment of *Listeria monocytogenes* growth during the ripening and storage of stabilized Camembert cheese with different points of contamination. a) Pre-salting contamination (day 0; ○). b) Post-salting contamination (day 0; ■). c) Contamination after the first surface yeasts grow (day 5; △). d) Contamination at the end of open air storage before the cheese is wrapped (day 11; ●). Result does not appear in graph until the day of contamination.

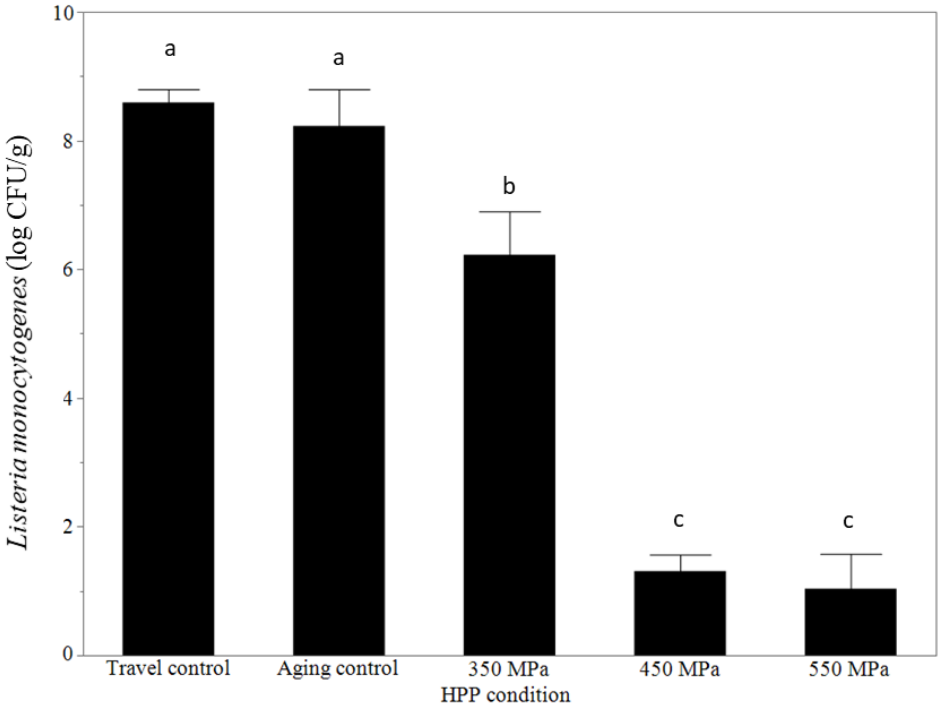
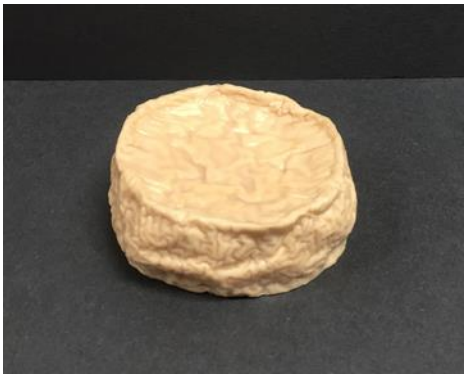


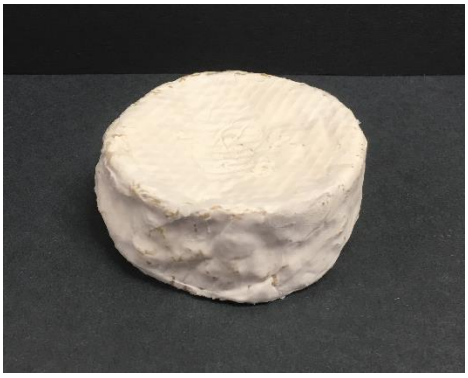
Figure 3.2 Reduction of *Listeria monocytogenes* after HPP treatment at 350, 450, and 550 MPa. Significant differences of the means are indicated by letters above the error bars ($P < 0.05$). Error bars represent the standard error of the mean for duplicate treatments (n=2).

a) Traditional control (day 50)

b) Traditional HPP treated (day 50)



c) Hybrid control (day 50)



d) Hybrid HPP treated (day 50)

e) Stabilized control (day 50)

f) Stabilized HPP treated (day 50)

Figure 3.3. Cheese appearance from the 45 day HPP treatment. Pictures were taken 50 days post manufacture. a) appearance of the traditional (sweet curd) variety untreated control. b) appearance of the traditional (sweet curd) variety 45 day treatment. c) appearance of the hybrid variety untreated control. d) appearance of the hybrid variety 45 day treatment. e) appearance of the stabilized variety untreated control. f) appearance of the stabilized variety 45 day treatment.

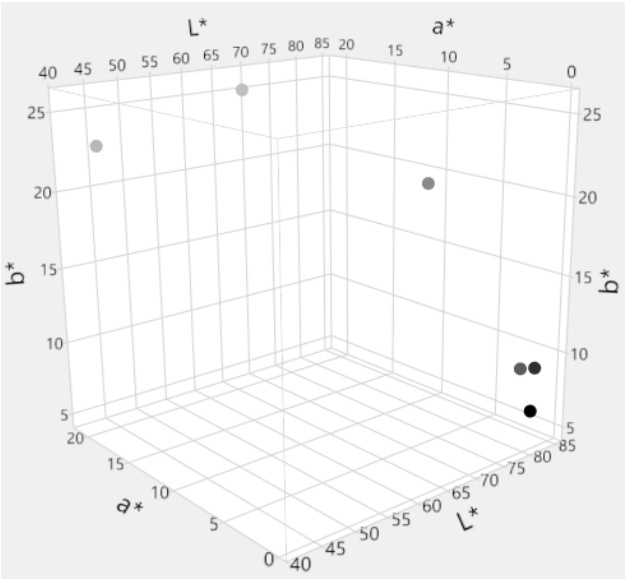


Figure 3.4. Color spectrum (L*, a*, and b*) comparing the mean values for the HPP treated and control cheese: traditional control (●), hybrid control (●), stabilized control (●), traditional HPP treated (●), hybrid HPP treated (●), stabilized.

a) Traditional variety control (left), travel control (middle), HPP treated (right; day 11)

b) Traditional 11 day HPP treated (day 50)

c) Hybrid 11 day HPP treated (day 50)

d) Stabilized 11 day HPP treated (day 50)

Figure 3.5. Cheese appearance from the 11 day treatment. Picture “a” was taken immediately after return of 11 day treatment cheeses and pictures “b” through “d” were taken 50 days post manufacture. a) appearance of the traditional (sweet curd) variety within 24 hours of treatment. From left to right: aging control, travel control, HPP treated. b) appearance of the traditional (sweet curd) variety 11 day treatment. c) appearance of the hybrid variety 11 day treatment. d) appearance of the stabilized variety 11 day treatment.

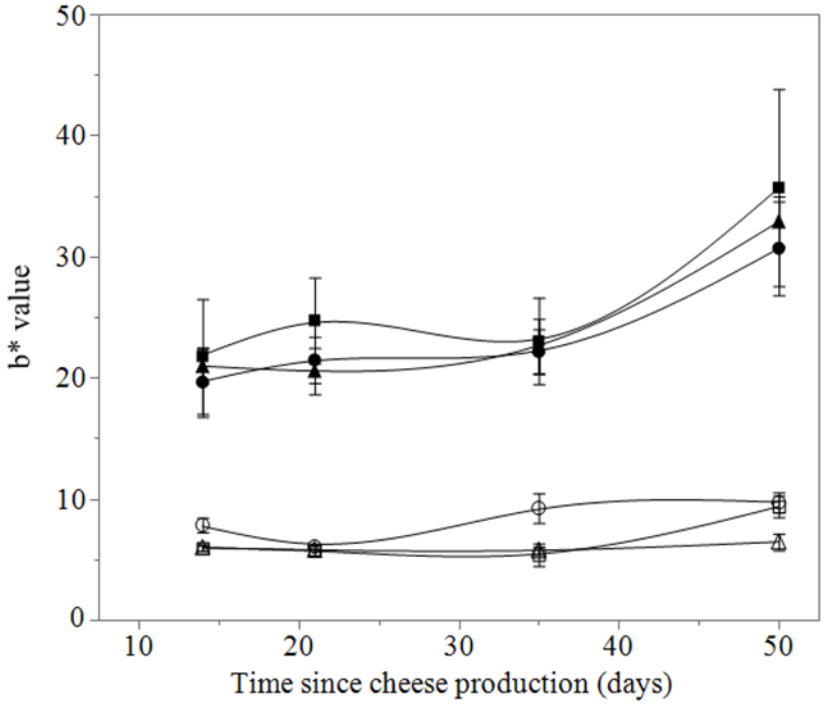


Figure 3.6. b* value of the 11 day treatment through shelf-life. a) Sweet curd (traditional) variety control (△). b) Sweet curd (traditional) variety HPP treated (▲). c) Hybrid variety control (□). d) Hybrid variety HPP treated (■). e) Stabilized curd variety control (○). f) Stabilized curd variety HPP treated (●). Error bars represent the standard error of the mean for duplicate treatments (n=2).

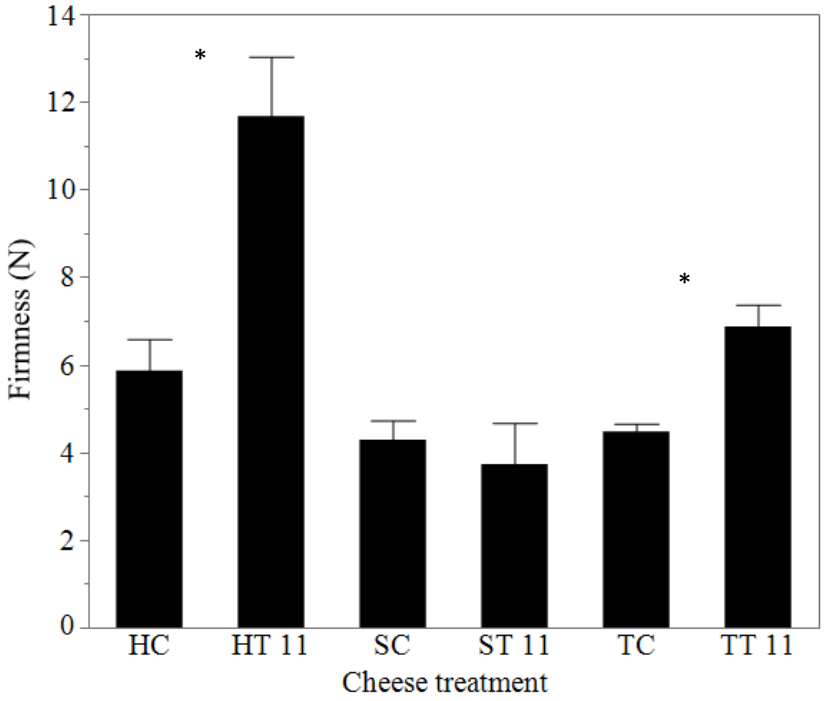


Figure 3.7. Firmness of the cheese from the 11 day treatment on the day 14 sample point. "*" indicates the treatment is significantly different from the control ($P < 0.05$). Error bars represent the standard error of the mean for duplicate treatments (n=2).

a) Traditional variety control (left), travel control (middle), HPP treated (right; day 3)

b) Traditional 3 day HPP treated (day 50)

c) Hybrid 3 day HPP treated (day 50)

d) Stabilized 3 day HPP treated (day 50)

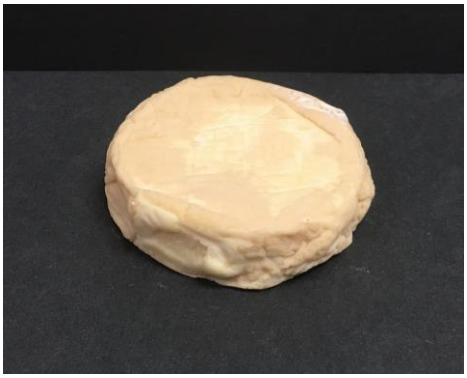


Figure 3.8. Cheese appearance from the 3 day treatment. Pictures are from immediately after HPP treatment for image a and the 50 day sample point for images b-d. a) appearance of the traditional (sweet curd) variety within 24 hhours of treatment. From left to right: aging control, travel control, HPP treated. b) Appearance of the sweet curd (traditional varity). c) Appearance of the hybrid variety. d) Appearance of the stabilized curd variety.

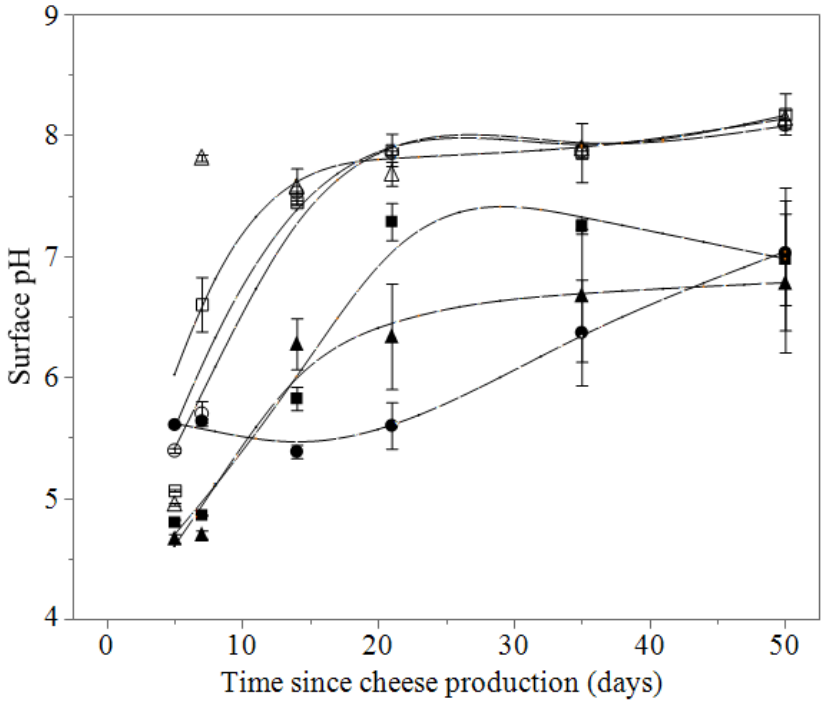


Figure 3.9. Surface pH development for the 3 day treated cheeses through 50 days or ripening. a) Sweet curd (traditional) variety control (△). b) Sweet curd (traditional) variety HPP treated (▲). c) Hybrid variety control (□). d) Hybrid variety HPP treated (■). e) Stabilized curd variety control (○). f) Stabilized curd variety HPP treated (●). Error bars represent the standard error of the mean for duplicate treatments (n=2).

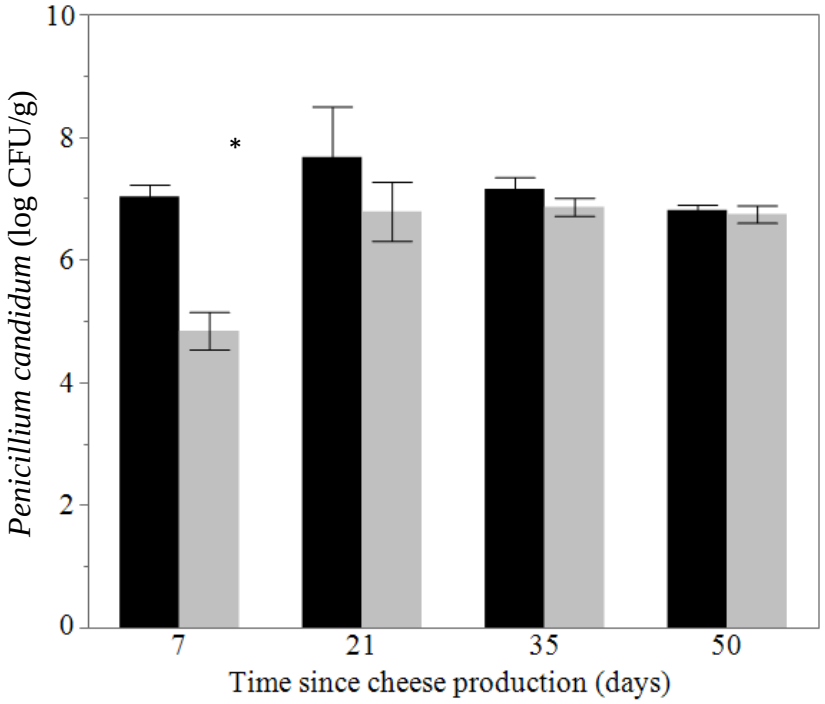


Figure 3.10. Level of *Penicillium candidum* from the 3 days treatment through 50 days for the sweet curd (traditional) variety. Control is represented by the black bars and the treatment is represented by the grey bars. Error bars represent the standard error of the mean for duplicate treatments (n=2). Significant differences ($P < 0.05$) of the mean are represented by "*" above the bars with differences.

Table 3.1. Cheese making controls for each recipe of Camembert type cheese.

Camembert variety	Sweet curd	Hybrid	Stabilized curd
Starter type	Mesophilic	Mesophilic	Thermophilic
Starter quantity (U/100 kg)	7.6	7.6	9.7
Fermentation temperature (°C)	35	35	40
Set pH	6.2	6.5	6.45
Cut size (cm)	2	2	1
Drain pH	6.1	6.4	6.2
Drain time (h)	20	14	20
pH at salting	4.6-4.7	5.1-5.2	5.2 -5.3
pH day 1	4.5-4.6	4.9-5.0	5.2-5.3

Table 3.2. Composition expressed as % weight of cheese for three different varieties of Camembert type cheese sampled five days after manufacture. Values presented are the means ± standard deviation.

Component	Sweet curd	Hybrid	Stabilized curd
Moisture	57.17 ± 0.59[a]	58.13 ± 0.40[a]	53.67 ± 0.61[b]
Fat**	23.35 ± 0.21 (54.53 ± 1.25)	23.13 ± 0.25 (55.23 ± 1.12)	23.90 ± 0.35 (51.59 ± 1.54)
Protein**	15.63 ± 0.34[b] (36.44 ± 0.29)	15.52 ± 0.33[b] (37.05 ± 0.43)	17.29 ± 0.39[a] (37.31 ± 0.36)
Salt*	1.52 ± 0.13	1.65 ± 0.17	1.63 ± 0.16
Salt-to-moisture (S/M) *	2.67 ± 0.25	2.85 ± 0.32	3.06 ± 0.37
Na**	0.60 ± 0.05 (1.41 ± 0.10)	0.65± 0.07 (1.56 ± 0.15)	0.64 ± 0.06 (1.39 ± 0.12)
Ca**	0.35 ± 0.02[b] (0.78 ± 0.04[b])	0.39 ± 0.03[ab] (0.94 ± 0.06[ab])	0.47 ± 0.07[a] (1.01 ± 0.16[a])
P**	0.27 ± 0.03[b] (0.62 ± 0.06[b])	0.28 ± 0.02[ab] (0.67 ± 0.06[ab])	0.36 ± 0.02[a] (0.86 ± 0.04[a])

[a-c]Cheese composition values within a row that do not share the same superscript are significantly different ($P<0.05$)

*Calculated values including Salt ($\text{Na} \times 2.54$), S/M $\left((\text{salt \%} \times 100) \div \text{moisture \%}\right)$, % "as is" protein and minerals (Na, Ca, and P; $(\text{attribute} \times (\text{moisture \%} \div 100))$), % fat "dry basis" $((\text{attribute} \div (100 - \text{moisture \%})) \times 100)$

**Values in parentheses indicate component percentage on dry basis.

Chapter 4. Conclusions

Bloomy rind cheese can be made using different formulations and cheese making practices to produce cheese of varying shelf life. The shelf life of these cheeses are typically indicated by the degradation of texture from a semi-solid paste to a free flowing liquid. At different points in shelf life, 35 and 50 days post manufacture, the cheese exhibited different degrees of stability and rate of softening. From our study, we found that the degree of softening and flow distance of the cheese was inversely related to the total Ca content of the cheese and the pH of the cheese after manufacture. This indicates that pH can be an effective tool at controlling shelf life by controlling the rate of softening of these cheeses.

The risk of contamination time was evaluated. Regardless of contamination time, *L. monocytogenes* can grow on the surface of bloomy rind cheeses to high levels. *L. monocytogenes* was even able to grow and persist through 60 days of aging, providing evidence that Camembert type cheese ripening is not effective at controlling pathogens post-manufacture as previously thought. Due to the nature of contamination and *L. monocytogenes* growth, this poses a risk for consumers of bloomy rind cheeses who are susceptible to listeriosis unless an alternative solution can be found.

The alternative solution we tested to control post-manufacture and ripening contamination was HPP. It is an emerging food safety technology that is successfully applied to many food products, but may not be appropriate for bloomy rind cheeses. Three different HPP treatment times that were tested resulted in cheese of unacceptable appearance. We hypothesized that this technology could successfully be applied to

bloomy rind cheeses to be used as a post-manufacture and ripening kill step to control environmental pathogens like *L. monocytogenes*. *L. monocytogenes* was successfully reduced by the HPP treatment; however, the resulting appearance of the cheese would be unacceptable to consumers.

With this technology resulting in cheese of unacceptable quality, cheese makers will have to continue to rely on regular and rigorous sanitation practices. This helps to keep the cheese processing and aging environment clean and reduces the risk of contamination by post-pasteurization and environmental contaminants such as *L. monocytogenes*. Regular environmental monitoring, the verification used to ensure proper sanitation, can help mitigate the food safety risk and help cheese makers identify areas on concern in their facility.

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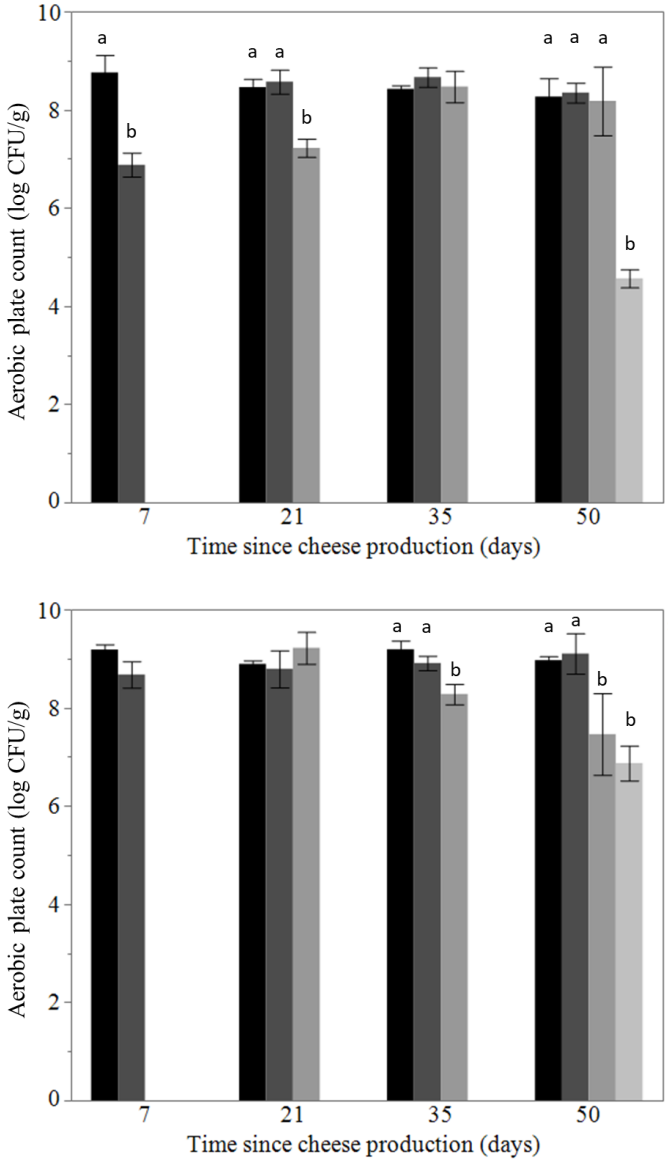
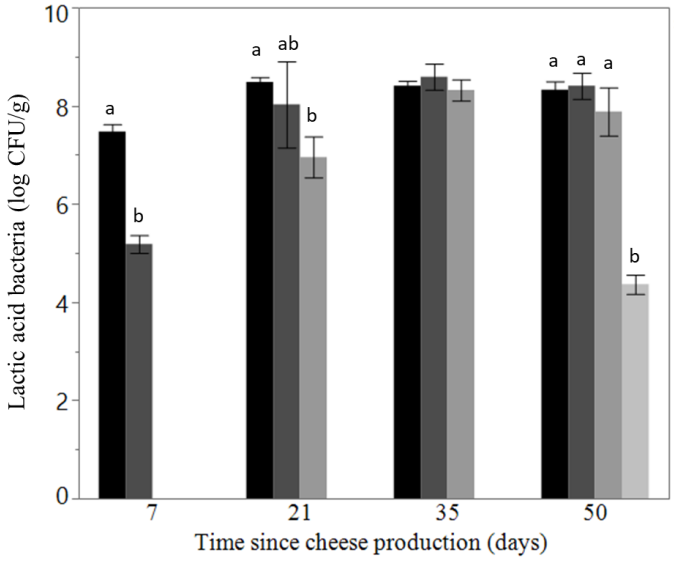
Appendices

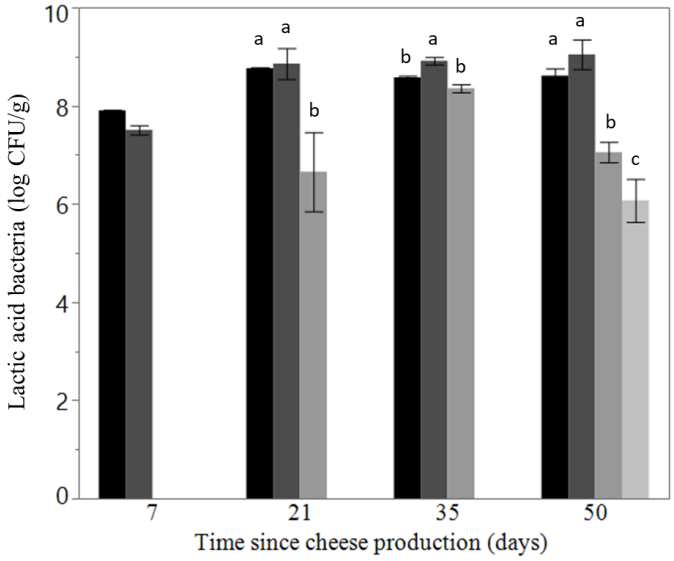
Appendix A: The Effect of HPP on Aerobic Plate Count.



This figure shows the effect of HPP (550 MPa, 10 minutes, and 25°) on the aerobic mesophilic bacteria for the traditional variety (top) and the stabilized variety (bottom). Color differences indicate treatment times: control (■), 3 day treatment (■), 11 day treatment (■), and 45 day treatment (■) Significant differences ($P < 0.05$) within a sample day are indicated by different letters above the bar graph. Error bars represent standard error of the mean (n=2).

Appendix B: The Effect of HPP on Lactic Acid Bacteria.

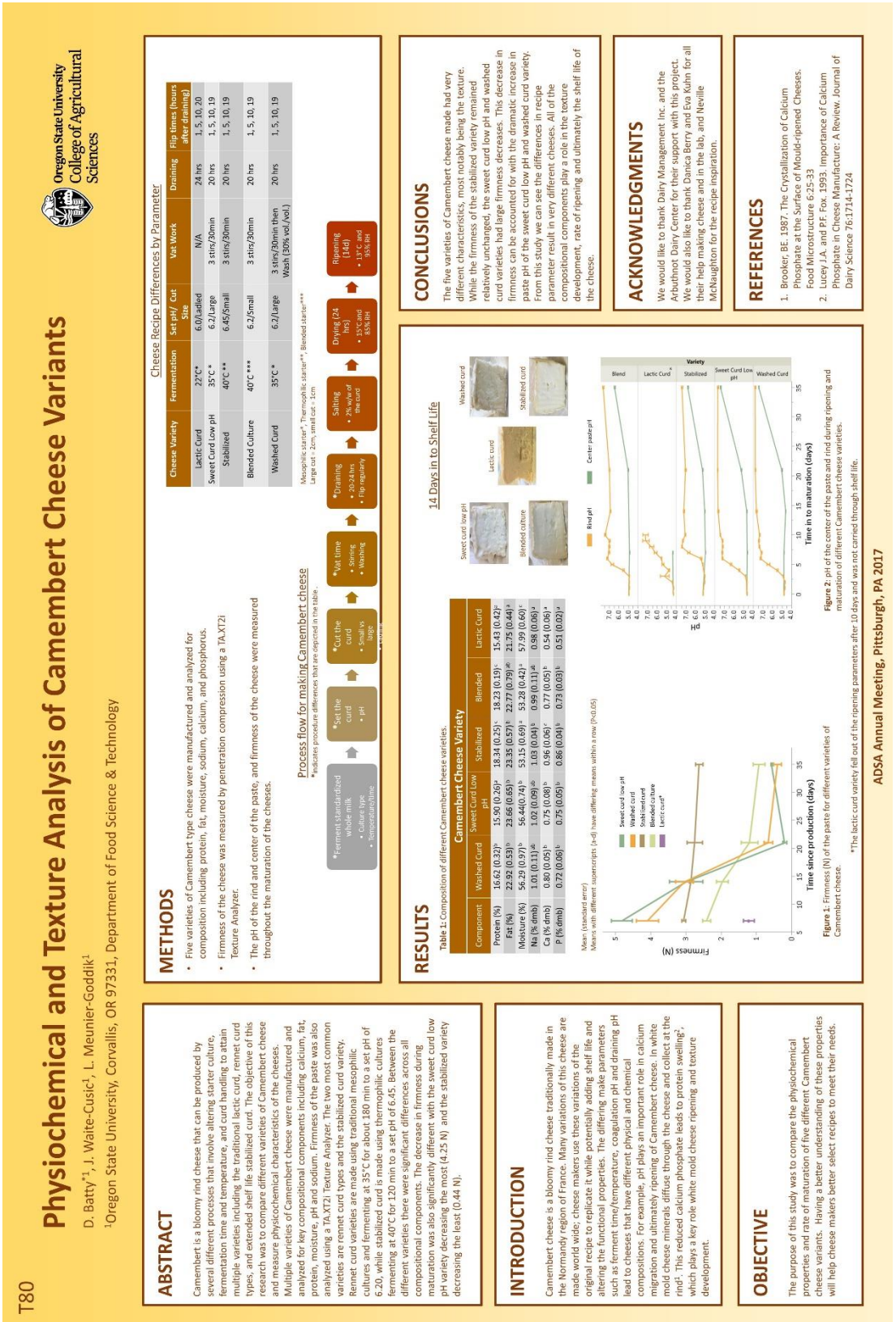




This figure shows the effect of HPP (550 MPa, 10 minutes, and 25°) on the lactic acid bacteria for the traditional variety (top) and the stabilized variety (bottom). Color differences indicate treatment times: control (■), 3 day treatment (■), 11 day treatment (■), and 45 day treatment (■) Significant differences ($P < 0.05$) within a sample day are indicated by different letters above the bar graph. Error bars represent standard error of the mean (n=2).

Appendix C: Taste of Research 2017 infographic poster.

Appendix D: American Dairy Science Association meeting 2017 poster.



Appendix E: Abstracts accepted for upcoming presentation

Batty, D., Emch, A., Meunier-Goddik, L., Waite-Cusic, J. The effect of high hydrostatic pressure on the microbiological quality and shelf life of Camembert type cheese. Poster presentation at 2018 ADSA annual meeting, June 2018, Knoxville, TN

Batty, D., Berry, D., Meunier-Goddik, L., Waite-Cusic, J. The effect of high hydrostatic pressure on the texture, appearance, and shelf life of Camembert type cheese. Poster presentation at 2018 ADSA annual meeting, June 2018, Knoxville, TN

Batty, D., Waite-Cusic, J., Meunier-Goddik, L. Method development to quantify paste stability for surface mold-ripened cheeses. Poster presentation at 2018 ADSA annual meeting, June 2018, Knoxville, TN

Batty, D., Meunier-Goddik, L, Waite-Cusic, J. Growth of Listeria Monocytogenes on the Surface of Camembert Cheese Is Influenced by Timing of Contamination. Poster presentation at 2018 IAFP annual meeting, July 2018, Salt Lake City, UT

Appendix F: Oral presentations

Batty, D. Reducing the risk of *Listeria monocytogenes* and *E. coli* in bloomy rind cheeses. Oral presentation at 2017 Oregon Dairy Industries Annual Conference, April 11th, 2017, Salem, OR

Batty, D. High Pressure Processing Camembert Cheese: Product Optimization and Pathogen Reduction. Oral presentation at 2017 Taste of Research, June 9th, 2017, Philomath, OR

Batty, D. High pressure processing Camembert cheese variants. Oral presentation at 2017 BUILD dairy annual meeting, July 19th, 2017, Moscow, ID

Batty, D. The effect of high hydrostatic pressure on the quality of Camembert type cheese. Oral presentation at 2018 Oregon Dairy Industries Annual Conference, April 10th, 2018, Salem, OR

Batty, D. Update: high pressure processing of bloomy rind cheeses. Oral presentation at 2018 BUILD dairy annual meeting, May 30th, 2018, Corvallis, OR