Survival of *Staphylococcus aureus* During the Manufacture and Ripening of Camembert Cheese

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Abstract

Staphylococcal Food Poisoning (SFP) is the third most common cause of food poisoning internationally, caused by an enterotoxin produced by *Staphylococcus aureus. S. aureus* contamination in dairy products, including cheese, can lead to SFP. The survivability of *S. aureus* during the manufacture and ripening of Camembert cheese was the focus of this study. Camembert cheeses were manufactured using pasteurized milk inoculated with one of three *S. aureus* strains, comprising two reference strains ATCC 4163, ATCC 9144 and one dairy strain 172 RR. Each strain was tested in triplicate. The results showed that manufacturing and ripening of Camembert cheese reduced the risk of food safety associated with contamination with *S. aureus* with a 1.6 to 3.1 log reduction. The largest decrease occurred following drainage, which was particularly evident in 172 RR, and coincided with the lowest pH. The combined effect of culture blend (starter and secondary flora) activity and low pH are believed to contribute to the death of *S. aureus*.

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Abbreviations

AOC	appellation d'origine contrôlée
APHA	American Public Health Association
a_w	water activity
CFU/g	colony forming units per gram
CMP	caseinomacropeptide
g	gram
h	hour (s)
min	minute (s)
MSSA	methicillin susceptible *Staphylococcus aureus*
NSLAB	non-starter lactic acid bacteria
SC+	coagulase-positive staphylococci
SFP	Staphylococcal food poisoning

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1 Introduction

Staphylococcal Food Poisoning (SFP) as the third common cause of foodborne disease and is sometimes associated with dairy products. The possible occurrence of foodborne pathogens in cheese is an international concern, in particular with raw milk cheese. Until now, no specific study has been done in terms of identifying the survivability of *S. aureus* during the manufacture of Camembert cheese, which is the subject of this research.

1.1 Research questions

a. Is *S. aureus* able to grow in Camembert cheese during manufacture and ripening?
b. What intrinsic properties of Camembert cheese influence the survivability of *S. aureus*?

1.2 Hypothesis

S. aureus may survive during the manufacture of Camembert cheese but numbers will be reduced with the activity of lactic acid bacteria and low pH at the end of curd drainage but will increase during ripening.

1.3 Purpose of Research

Investigating the survivability of *S. aureus* during the manufacture and storage of Camembert cheese.

2 Literature Review

2.1 Staphylococcus aureus

2.1.1 Emergence of Staphylococcal Food Poisoning (SFP)

Staphylococcus aureus is the third cause of foodborne illness internationally. *S. aureus* was found responsible for about 25 % of all foodborne diseases in America 40 years ago (Boothby, Genigeorgis, & Fanelli, 1979; Genigeorgis, 1974; Minor & Marth, 1976). In the 2009 *S. aureus* was responsible for 1 % - 3 % of foodborne illnesses reported in the US in 1990s (Bean, Goulding, Lao, & Angulo, 1996; Olsen, MacKinnon, Goulding, Bean, & Slutsker, 2000). In Europe, from 1993 – 1998 *S. aureus* accounted for 5.1 % of foodborne illnesses (Tirado & Schmidt, 2001). In New Zealand, from 2007 to 2016, as shown in Figure 1, the number of outbreaks caused by foodborne *S. aureus* was between 0 – 2 each year with 3 out of 10 years showing no reported outbreaks (Pattis, Lopez, Cressey, Horn, & Roos, 2017). It is important to note that many cases of *S. aureus* food poisoning may not be reported.

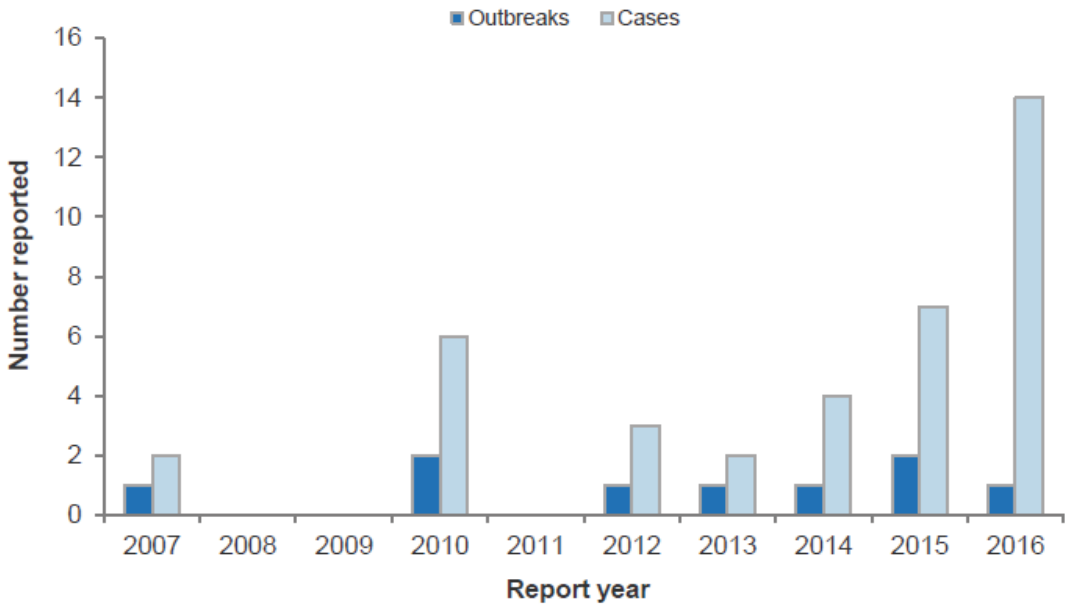


Figure 1 The number of reported foodborne *S. aureus* outbreaks out of all associated cases during 2007 and 2016 in New Zealand ((Pattis et al., 2017)

2.1.2 Growth Boundaries of *Staphylococcus aureus*

S. aureus can grow in an environment with minimal requirements. It can grow in foods at a very low water activity (approx. 0.86) and a relatively wide range of pH (4.2 to 9.3), although the precise range depends on several factors (Juneja & Sofos, 2009; Seo, Bohach, Juneja, & Sofos, 2010; Stewart et al., 2002; Valero et al., 2009). The optimal temperature for its growth is 37 °C, while the limit can be extended to over 44 °C with the presence of monosodium glutamate and NaCl (Jay, Loessner, & Golden, 2005; Juneja & Sofos, 2009; Seo et al., 2010; Stewart et al., 2002; Valero et al., 2009). It grows well in 7 %- 10 % NaCl, and can even grow in up to 25 % NaCl (El-Banna & Hurst, 1983; Juneja & Sofos, 2009; Valero et al., 2009). The growth boundaries of *S. aureus* stated in literature may have some minor differences due to the different test environment, but generally at a similar level. The conditions for *S. aureus* growth are presented in Table 1.

Table 1 Conditions for the growth of *S. aureus* and production of toxin (Stewart, 2003)

Parameter	Growth	Toxin production
Temperature (°C)	7 – 48	10 – 48
pH	4 - 10	4.5 – 9.6
Water activity	0.83 – 0.99	0.87 – 0.99

2.1.3 Survival and resistance

Survival of foodborne pathogens like *S. aureus* during the manufacture and processing of food products is important to understand the food safety risk (Seo et al., 2010). *S. aureus* does not show significant resistance to common food preservatives. However, it is resistant to some antimicrobial agents and heavy metals largely through acquiring resistance genes (Seo et al., 2010). The a_w tolerance of *S. aureus* is equivalent to 3.5 M NaCl (a_w =0.83) (Harvey & Gilmour, 1999; Townsend & Wilkinson, 1992). The osmotolerance of *S. aureus* is a food safety concern since the growth of *S. aureus* can be accelerated through the inhibition of competing bacteria (i.e. those that may be inhibited in a high salt/sugar environment). It has been shown that taurine, proline, glycine betaine and choline are osmoprotectants for *Staphylococcus aureus* (Townsend & Wilkinson, 1992) enhancing resistance to NaCl. Both low- (maximum transport rate [VmaxJ = 22 nmol/min/mg [dry weight], Km = 132 µM) and high-affinity (maximum transport rate [VmaxJ = 1.1 nmol/min/mg [dry weight], Km = 1.7 µM) systems in *S. aureus* are dependent on the activity of Na^+. The low-affinity system is stimulated by the increase of osmotic strength, while the high-affinity system is L-proline specific and stimulated by low Na^+ concentrations (Harvey & Gilmour, 1999; Townsend & Wilkinson, 1992). It is suggested that in *S. aureus*, the uptake of proline is critical in the primary response to osmotic stress (Townsend & Wilkinson, 1992).

The occurrence of *S. aureus* resistance to multiple antibiotics has stimulated the discovery of new antibiotics. Its mechanisms of antibiotic-resistant against Vancomycin, Penicillin and

Methicillin have been comprehensively studied (Control & Prevention, 2002; Lowy, 2003; Rammelkamp & Maxon, 1942; Smith et al., 1999; Velázquez-Meza, 2005; Zhao et al., 2012). Studies show that all of the staphylococcal species carry a gene that confers resistance to Methicillin and some plasmids conferring resistance to other antimicrobials are also contained in some species. There are many reasons for the antibiotic-resistance of *S. aureus* including the exchange and/or mutation of genes and the overuse of antibiotics in humans and animals (Gillaspy & Iandolo, 2009). Due to the rapid evolution of bacteria, the microorganisms that have not been destroyed by certain antibiotics may survive and adapt to resist another antibiotic.

2.1.4 Presence of *Staphylococcus aureus* in Cheese and food poisoning Outbreaks

S. aureus may be isolated from milk produced by cows with mastitis. Since traditional cheeses are produced by raw milk, the presence of *S. aureus* is a major safety issue (De Buyser, Dufour, Maire, & Lafarge, 2001). In France, it is reported that *S. aureus* is the most frequent foodborne pathogen in raw milk cheeses (De Buyser et al., 2001). The production of staphylococcal enterotoxins (SEs) by *S. aureus* was the leading cause of many food poisoning cases associated with the milk and cheese consumption in Europe (Delbes, Alomar, Chougui, Martin, & Montel, 2006). According to European standards, the control analyses were required at the time when the density of *S. aureus* reaches the "*M*-value" (5 log CFU/g). When the value is above 5 log CFU/g, the cheeses are considered to be defective and will be tested for Staphylococcal enterotoxins. This is due to the fact that when *S. aureus* reaches a set number (normally > 5 log CFU/g), SEs may be produced, even though the *S. aureus* count may decrease during the ripening period of the cheese (Delbes et al., 2006).

The occurrence, pathogenesis and food poisoning incidences of *S. aureus* have been well studied. In 1999, two SFP outbreaks involving 378 individuals happened in Brazil due to the consumption of Minas cheese and raw milk. The first outbreak occurred in February with 50 ill individuals consumed Minas cheeses while other persons infected in the second outbreak were due to the consumption of raw milk. In outbreak one, the density of microorganisms varied from 2.4×10^3 CFU g^{-1} to 2.0×10^8 CFU g^{-1} while those involved in outbreak two

exceeded 2.0×10^8. The contamination sources in case one and case two were food handlers and cattle mastitis, respectively (do Carmo et al., 2002; Hoorfar, 2011; Stessl, Hein, Wagner, & Ehling-Schulz, 2011). Research in Italy investigating the detection of *S. aureus* in Monte Veronese was conducted in 2007. Monte Veronese is a kind of semi-hard cheese made with raw milk. The study indicated that the density of *S. aureus* in 78 % of cheese samples was higher than 10^3 CFU g^{-1}, the Italian food legislation threshold (Poli et al., 2007; Stessl et al., 2011). It was also shown that longer ripening times did not usually lead to a reduction in the pathogens (Stessl et al., 2011).

In general, the causes of SFP incidences are:

1. Foods post processing frequently contaminated by the introduction of a human strain (through people handling the food) or from animal strains introduced by the cross-contamination of processed (cooked) and raw food (Harvey & Gilmour, 1999)

2. Food contaminated with *S. aureus* and stored at temperatures that allow the proliferation of *S. aureus* may result in the production of Staphylococcal enterotoxins. Apart from the small outbreaks or individual cases that occur at home, these are associated with social occasions on a larger scale when foods are prepared and held warm before eating, which provides opportunities for *S. aureus* growth (Harvey & Gilmour, 1999).

Therefore, improving the manufacturing hygiene and reducing the initial contamination are the most effective ways to prevent food poisoning. For cheese made with pasteurised milk, the most likely source of contamination is at some point after the pasteurisation of the milk while there is still a chance that raw milk stored at higher temperatures for a long time might possibly produce staphylococcal enterotoxins before pasteurization.

2.1.5 Anti-staphylococcal Activity of Starter Lactic Acid Bacteria (LAB)

The inoculation of lactic acid bacteria (LAB) in milk at the beginning of cheese manufacture stage is an essential step for Camembert cheese manufacture. *Leuconostoc* spp., *Lactococcus lactis*, subsp. *cremoris*, and subsp. *lactis*, are commonly used in surface mould-ripened cheeses production. LAB produce several kinds of antimicrobial substances which can be

grouped by molecular mass. Substances with molecular mass <1000 Da are low molecular mass antimicrobials including organic acids, diacetyl, acetaldehyde, acetoin, carbon dioxide, hydrogen peroxide, reuterin and reutericyclin, whereas substances with >1000 Da molecular mass are bacteriocins and bacteriocin-like antimicrobials. Organic acids are the best characterised and most important antimicrobials produced by LAB. The antimicrobial effects of organic acids are achieved by the interference of crucial metabolic functions through the movement of molecules across the microbial cell membrane. Previous studies have reported the inhibitory effects towards *S. aureus* with the combination of pH and organic acids. Studies indicated that the growth of *S. aureus* is completely inhibited with acidification down to pH 4.4-4.5 using lactic acid (Charlier, Even, Gautier, & Le Loir, 2008; Haines & Harmon, 1973; Tatini, Jezeski, Olson, & Casman, 1971). Compared to a culture without acid, the milk being acidified to pH 4.6 by lactic acid showed a 2 log reduction in bacterial cells (Charlier, Cretenet, Even, & Le Loir, 2009; Minor & Marth, 1970). The similar result was detected for hydrochloric acid at pH 4, phosphoric acid at pH 4.1, citric acid at pH 4.5 and acetic acid at pH 5 (Charlier et al., 2009; Minor & Marth, 1970).

2.2 Camembert Cheeses

2.2.1 Background

Camembert, a representative mould-ripened cheese, which was originally only consumed in France, has become increasingly popular in various countries (Fox, McSweeney, Cogan, & Guinee, 2004). Traditionally, according to the appellation d'origine contrôlée (AOC), Camembert cheese could only be produced by unpasteurised raw bovine milk in order to produce natural textures and flavours (Galli, Martin, da Silva, Porto, & Spoto, 2016). Many hygiene problems, however, occurred on sale and importation in various countries leading to the increasing use of pasteurised milk (Smith, 2005). Many dairy manufacturers are only using pasteurised milk for Camembert cheese production to comply with local regulations and avoid safety problems.

White bloomy mould, the distinctive appearance on the rind of Camembert cheese, is a result of the growth of *Penicillium camemberti* and *Penicillium roqueforti* (Fox et al., 2004).

Compared to other cheeses with simple microflora, a more complex ripening is achieved by these moulds.

2.2.2 Manufacturing Process, Technology and Biochemical Changes

2.2.2.1 Principles and Fundamentals of Camembert Manufacture

Camembert is a non-scalded and non-pressed cheese under both French law and Codex standards (Alimentarius, 1973; Shaw, 1981). The whole cheese in the shape of flat square or cylinder should weigh approximately 80 g to 500 g within the height of 5 cm. The level of dry matter content may vary between 38% to 55% according to the fat content in the dry matter (Alimentarius, 1973).

Fundamentally, the manufacture of cheese is a way to extend the shelf life of milk by lowering its water activity and pH. The shelf stability correlates to the pH and water activity (Table 2). The relationship between pH and water activity of different kinds of cheese is given in Figure 2. Relatively speaking, Camembert has a low pH value (4.6 - 4.8) and high water activity value (0.98) before the ripening stage (Shaw, 1981). In cheese, the water activity can be reduced by salting and draining while the pH reduction can be achieved by the fermentation with lactic acid bacteria (Shaw, 1981). In Camembert cheese, the pH and water activity are high resulting in a short shelf-life of approximately 2 months. (Fox et al., 2004; Nooitgedagt & Hartog, 1988). The high water activity of this cheese is a potential food safety issue that may provide a suitable environment for bacteria such as *S. aureus* to grow.

Table 2 Storage categories of products based on pH and a_w (Shaw, 1981)

Category	Criteria	Storage Temperature
Easily perishable	a_w >0.95 and pH > 5.2	+ 5 °C
Perishable	a_w 0.91 – 0.95 or pH 5.0 – 5.2	+ 10 °C
Shelf-stable	a_w < 0.91 only or pH < 5.0 or a_w <0.95 and pH < 5.2	No refrigeration required

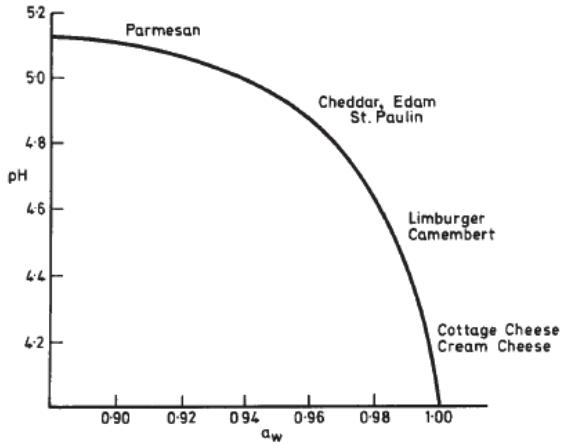


Figure 2 Relation between a_w and pH of different cheeses (Shaw, 1981)

2.2.2.2 Manufacture of Camembert cheese

The methods for Camembert manufacture have evolved since this cheese was first invented. The amount of starter culture, the temperature and time at different stages may vary with different manufacturers (Shaw, 1981). Basically, there are two kinds of core manufacturing methods, traditional and industrial (Shaw, 1981). The traditional method is usually used for homemade cheeses, and raw milk is always used for better result. In contrast, the industrial

method is for a much larger scale production, in which the pasteurized milk is considered to be the first choice for safety reason. The decrease of the amount of calcium during the heat-treating process will lead to a less firm cheese curd. Therefore, Calcium chloride is always added when the milk is heated up to around 32 °C in both methods for restoring the natural balance between protein and calcium.

2.2.2.2.1 Traditional Method

Fundamentally, the traditional process follows these steps:

a. Raw milk is essential for traditional Camembert production since the acidification process is mainly using the natural lactic acid bacteria from raw milk. Starter cultures containing mesophilic mixed strains may be utilised for a better development of the acidity rate.
b. After the milk is heated up to 30 - 32 °C in a pot or tank, Calcium chloride is added at 0.06 – 0.1 g per litre of milk for the preparation of coagulation.
c. When the lactic acid reaches 0.20 %, the rennet is added for stimulating the coagulation of milk.
d. Rennet is added at the rate of 0.15 - 0.2 ml per litre of milk once the level of acidity has reached.
e. The milk is stirred and allowed to set for 1 h to 1 h 30 min.
f. The curd is ladled by hand from the tank to cheese moulds without being cut. Cheese moulds which have lots of holes are placed onto cheese drainage mats for better drainage.
g. The cheese is flipped over every 5 hours, and whey drainage takes approximately 1 to 2 days.
h. The cheese is removed from the mould and sprinkled with dry salt over the whole cheese surface.
i. The cheese is placed in a cheese mature box and put into a fridge at a temperature of 10 -13 °C for 3 to 4 weeks.
j. The cheese is then stored in a fridge at 4 -8 °C (Shaw, 1981).

2.2.2.2.2 Industrial Method

a. In order to predict the fat in the dry matter of cheese, milk is normally standardised for fat content. Milk is pasteurised at 72 – 76°C 15-20 seconds and then inoculated with 2-3% mesophilic mixed strain starter culture and ripening culture. Calcium chloride is then added at a rate of 0.5 – 2.0 g / 10 L.
b. The milk is then filled into basins (or similar equipment) at 32 -35 °C
c. When the lactic acid reaches 0.18 – 0.20 % (about 20 min), rennet is added.
d. Rennet is added to the milk at 2.2 – 3.0 ml / 10 L.
e. After 1 to 2.5 hours ripening, the coagulum forms.
f. The curd is cut into 3 cm cubes and allowed to set for 20 to 30 min to remove the excess whey. The curd is then transferred and evenly distributed into cheese moulds.
g. For better drainage, the cheese moulds are firstly placed at 27 °C for 5 h and then be transferred to 23 °C and held for 15h.
h. These cheeses are then salted by a mechanised dry salter or a saturated brine solution to achieve a salt concentration of 1.5 – 1.8 % in the final product.
i. The cheeses are ripened at a relative humidity of 90 – 95 % and a temperature of 12 - 15 °C for 7 – 14 days.
j. The cheeses are wrapped and stored at 4 – 8 °C (Shaw, 1981).

2.2.2.3 Enzyme-induced Milk Coagulation

The coagulation of milk is a major step converting liquid cheese milk into solid cheese. Basically, milk coagulation results from the physical and chemical changes of casein micelles. The coagulum formation can be induced by two different methods which are enzymatic-induced coagulation and acid-induced coagulation (Eck, Gillis, & Davies, 2000). Acid-induced cheeses are mainly fresh cheeses produced by whey, cream or milk which can be consumed once immediately following manufacture (e.g. Cottage cheese, Cream cheese, Ricotta cheese). The coagulation of these fresh cheeses occurs when the pH drops to the isoelectric point of casein (pH 4.6). Heat treatment may also be introduced to some specific cheeses (e.g. Ricotta) which leads to a high degree of whey protein denaturation (Guinee, Pudja, & Farkye, 1993). The coagulation of Camembert cheese is different from acid curd cheese in that it is primarily induced at a higher level of pH (pH 6.4-6.6) by rennet which is currently the most

widely used coagulating enzyme(Eck et al., 2000). The acidification by lactic acid will also contribute to the coagulation (Shaw, 1981). Lactic acid bacteria do have an inhibitory effect on the growth of *S. aureus* (Haines & Harmon, 1973; Charlier, Cretenet, Even, & Le Loir, 2009) and the expression of virulence genes (Charlier et al., 2009).

2.2.2.4 Coagulation Mechanism of Rennet

Rennet consists of pepsin and chymosin. Milk coagulation, induced by rennet, results from the aggregation of casein micelles. The action of rennet can be divided into three stages: κ-casein hydrolysis, casein micelle aggregation and reticulation (Eck et al., 2000; Fox et al., 2004; Visser, 1977).

At the first stage, caseinomacropeptide (CMP) and κ-para-casein formation take place after κ-casein hydrolysis. During this period, CMP is released due to the decrease of electrostatic repulsions, which leads to the destabilisation of this colloidal system. The second stage of coagulation starts when approximately 80% of κ-casein in milk is hydrolysed, at which point the destabilised casein micelles begin to aggregate. When the whey is separated from cheese curds by syneresis, the third stage takes place, at which these casein micelles start to reticulate (Eck et al., 2000).

2.2.2.5 Drainage of Coagulum

The drainage of coagulum is the elimination of the whey. The whey can be easily separated by gravity since the cheese curds are highly permeable (McSweeney, 2007). The removal of the whey causes the gel contraction and hardening producing a more concentrated cheese curd. This separation of the continuous phase is known as syneresis (Eck et al., 2000; Fox et al., 2004; Guinee & Fox, 1987). The fundamental mechanism of syneresis that takes place in Camembert cheeses is closely related to two properties of the cheese curd, which are (1) the capacity of the protein network (made by casein micelles) (2) the evacuation ability of the gel from the whey.

The volume of cheese keeps decreasing until the whey is fully expelled. According to previous research, the density of *S. aureus* may increase during coagulation and drainage due to the physical entrapment of cells and the microbial growth (Delbes et al., 2006; Meyrand et al., 1998). During draining, however, the acidification continues until the pH is between 4.4 and

4.8, may be low enough to cause the death of *S. aureus* combined with the activity of lactic acid. Thus, it can be predicted that the density of *S. aureus* may increase as the curds formed, but with the decrease of the cheese pH, *S. aureus* numbers may decrease until the end of drainage.

2.2.2.6 Salt in Cheese

Camembert cheeses are salted simply by rubbing the dry salt to the surface. A concentrated brine layer is created by the counterflow of water, the diffusion of which from surface to core then occurs (Guinee & Fox, 2004).

On surface mould-ripened cheeses, salting has a selective effect (Gripon, 1999). The growth of *Geotracum candidum* will be limited when cheeses are over salted, while the implantation of *P. camemberti* will not be affected in high salt conditions. On the contrary, the growth of *P. camemberti* will be hindered when the salt content is too low, whereas *G. candidum* grows too much (Gripon, 1999). Overall, the concentration of NaCl in Camembert cheese plays an important role in the surface mould growth, pH changes, as well as proteolysis (Guinee & Fox, 2004). *S. aureus* is able to grow in up to 25% NaCl (Jablonski & Bohach, 1997). The salt content in a regular Camembert cheese is usually lower than 2%, which is much lower than the growth boundary for *S. aureus*. The presence of NaCl in cheese will not influence *S. aureus* growth.

2.2.3 Biochemistry of Camembert Ripening

Ripening is an essential part of the cheese manufacturing process for many varieties of cheeses. Ripening period may vary among different cheeses ranging from 14 days to several years (Fox, Law, McSweeney, & Wallace, 1993; McSweeney, 2004; Smit, van Hylckama Vlieg, Smit, Ayad, & Engels, 2002). Various biochemical changes and reactions occur at the ripening stage that lead to the distinctive textures and flavours (Collins, McSweeney, & Wilkinson, 2003b; Fox et al., 1993; Lawrence, Creamer, & Gilles, 1987; McSweeney, 2004; McSweeney & Sousa, 2000; Smit et al., 2002; Upadhyay, McSweeney, Magboul, & Fox, 2004). Generally, these biochemical reactions can be divided into two phases: primary and secondary events. The primary events include proteolysis, lipolysis and glycolysis (metabolism of lactose and lactate) while the secondary event is mainly the metabolism of amino acids and free fatty acids (Fox et al., 1993; Fox et al., 2004; Guinee & Fox, 1987). The basic flavour and the textures

of the cheese are mainly determined by the primary reactions and modified by the secondary events (Fox et al., 1993; Lawrence et al., 1987; McSweeney, 2004).

2.2.3.1 Metabolism of Residual Lactose

Lactose metabolism induced by specific starter cultures is an important feature of fermented cheese. During the manufacture of Camembert, most of the lactose is expelled with the whey while a small amount of the lactose remains in the cheese (McSweeney, 2004). In order to avoid the development of undesirable secondary microflora, it is essential to complete the lactose fermentation. During the early ripening stage, the residual lactose is metabolised rapidly to L-lactate through the action of starter culture and affected by the levels of salt and temperature (Fox et al., 1993; McSweeney, 2004; Parente & Cogan, 2004; Turner & Thomas, 1980). Lactose that has not been metabolised by the starter may be fermented by non-starter lactic acid bacteria (NSLAB). With a high population of NSLAB, a large amount of D-lactate is produced by fermenting the residual lactose through NSLAB (McSweeney, 2004). NSLAB inhibit *S. aureus* growth, but there is no evidence of D-lactate influencing *S. aureus*.

2.2.3.2 Metabolism of Lactate

The metabolism of lactate is an essential part of ripening in Camembert cheeses. At the early period of ripening, secondary microorganisms grow on the rind of cheeses. Yeasts and *G. candidum* colonise the surface at first, followed by *Penicillium camemberti*. The lactate is rapidly metabolized by *P. camemberti* and *G. candidum* and oxidatively converted to O_2 and CO_2 (Abraham, Cachon, Colas, Feron, & De Coninck, 2007; Fox et al., 1993; McSweeney, 2004; McSweeney & Fox, 2004; Parente & Cogan, 2004; Spinnler & Gripon, 2004). This reaction de-acidifies the surface and develops a pH gradient from the core to the rind with the diffusion of lactate. When the cheese is mature, ammonia is produced at the rind of Camembert by proteolysis and disperses to the core of cheese. As the pH of the cheese surface increases, calcium phosphate migrates from core to rind and precipitates at the surface as a layer. The increased pH and the reduction of $Ca_3(PO_4)_2$ leads to a very soft interior (Abraham et al., 2007; Eck et al., 2000; Fox et al., 1993; Guinee & Fox, 1987; McSweeney, 2004; McSweeney & Fox, 2004; McSweeney & Sousa, 2000; Parente & Cogan, 2004; Spinnler & Gripon, 2004). These changes are presented in Figure 3.

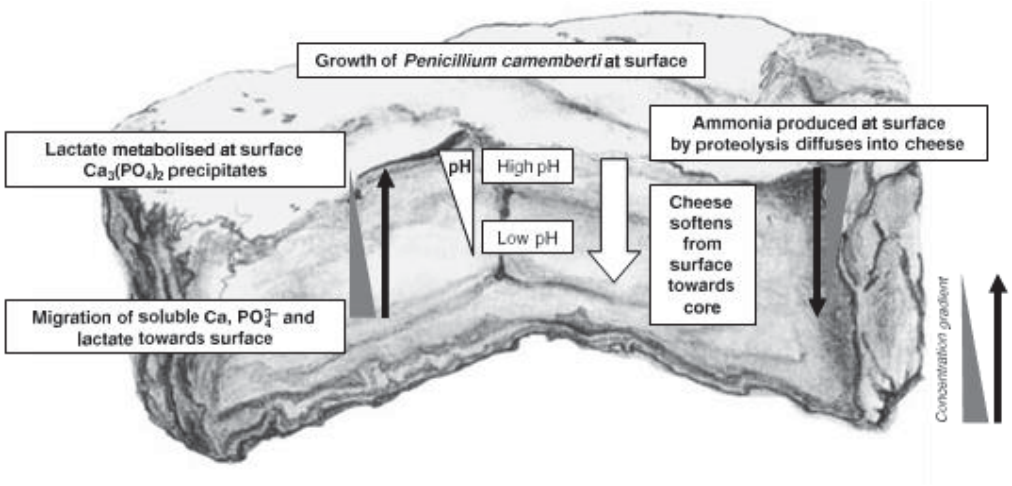


Figure 3 Changes caused by the growth of *P. camemberti* during the ripening of Camembert cheese (McSweeney, 2004)

During the metabolism of lactose and lactate, the cheese is deacidified. The pH of cheese increases due to the consumption of lactate (McSweeney, 2007). The increasing pH may gradually slow down the death rate of *S. aureus* or even help with its growth followed by a stable survival of bacteria.

2.2.3.3 Lipolysis

Normally, lipids may experience oxidative or hydrolytic degradation in foods. In cheese, however, the low oxidation/reduction potential results in a limited degradation of lipids (Collins, McSweeney, & Wilkinson, 2003a; Fox et al., 1993; McSweeney, 2004; McSweeney & Sousa, 2000). Compared to other kinds of cheeses, the extent of lipolysis in mould-ripened cheese like Camembert is much greater. The lipolysis level of triglycerides in Camembert is about 5 – 10 %, whereas the extent of lipolysis is lower than 2 % in other cheeses like Cheddar and Gouda (Fox et al., 2004; Guinee & Fox, 1987).

The fat transformation in cheese results from the hydrolysis of triglycerides during ripening. According to previous research, the lipolysis rate of total fatty acids is between 6 and 10 % Camembert manufactured with raw milk. Short chain fatty acids, which significantly contribute to the flavour of cheese, are abundant in the milk fat triglycerides (Fox et al., 1993; Fox et al., 2004; McSweeney, 2004; Molimard & Spinnler, 1996). In Camembert, however, due

to the higher concentration of oleic acid, the proportion of short-chain fatty acids, is only about 5 % of free fatty acids (Fox et al., 2004; Guinee & Fox, 1987). Additionally, a previous study indicated that oleic acid is able to inhibit the growth of *S. aureus* (Dilika, Bremner, & Meyer, 2000). However, the oleic acid in that study was isolated from *Helichrysum pedunculatum* and applied in traditional medicine, whereas the conditions are different in Camembert cheese.

Lipolysis is not only important for the contribution of flavour, the ripening of Camembert, but also the production of volatile flavour compounds (Fox et al., 1993; McSweeney, 2004; McSweeney & Sousa, 2000). Generally, the lipolytic agents originate from the microflora, the coagulant and the milk. In this case, the secondary flora, particularly *Penicillium spp.*, mainly contribute to the Lipolysis of Camembert. It has been found that only one extracellular lipase is produced by *P. camemberti*, which is optimally active at 35 °C and pH 9 on tributyrin agar (Fox et al., 1993; Fox et al., 2004; Guinee & Fox, 1987; McSweeney, 2004).

2.2.3.4 Proteolysis

For most kinds of cheeses, proteolysis is the most important and complex event during ripening. During proteolysis, water-insoluble caseins are sequentially hydrolysed into small peptides. For a Camembert made by raw milk, the soluble Nitrogen in the rind of cheese accounts for approximately 35 % of the total while that is only 25 % within the cheese. Many peptides are contained in the soluble nitrogen fraction. According to the profile obtained by Do Ngoc *et al.* in 1971, the amino acids show a preferential release in traditional Camembert cheeses during proteolysis, which is different from the whole casein hydrolysate (Fox et al., 2004; Guinee & Fox, 1987; Ngoc, Lenoir, & Choisy, 1971). More specifically, phenylalanine, leucine and alanine are in high amounts while serine and arginine are in lower proportions. Proteolysis, as well as the degradation of β–casein in mould–ripened cheeses, is greater and more extensive compared to that of other varieties (Fox et al., 1993; Fox et al., 2004; Guinee & Fox, 1987). Both phenylalanine and leucine are essential for the growth of *S. aureus.* The high amount of these amino acids released during the manufacture of Camembert cheese may encourage the growth of bacteria such as *S. aureus* (Mah, Fung, & Morse, 1967). In this process, proteolytic agents break down protein network into free amino acids and peptides. The proteolytic agents (peptidases and proteinases) in Camembert cheese originate from

several sources which include rennet, milk, Lactic Acid Bacteria and Secondary inoculum (*Penicillium ssp.*) (Fox, 1989; Fox et al., 1993; McSweeney, 2004).

2.2.3.4.1 Action and Effect of rennet

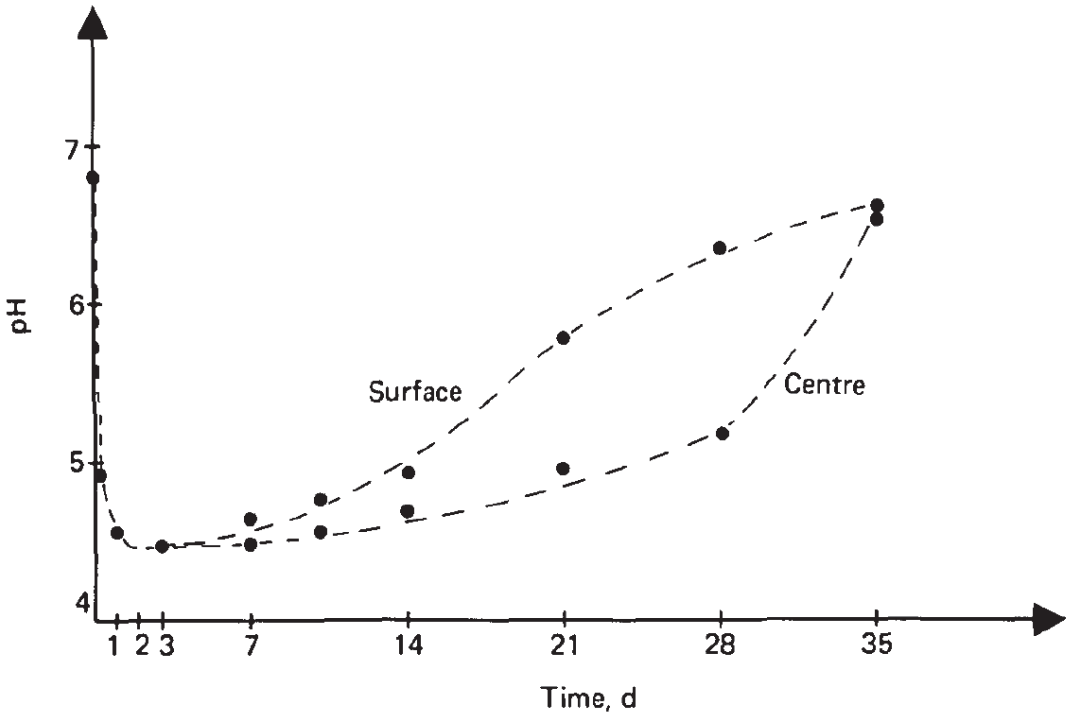


Figure 4 Changes in pH during the ripening of Camembert (P. F. Fox et al., 2004)

The hydrolysis of α_{s1}-casein and the α_{s1}-I peptide production results from the action of rennet. Six hours after draining, α_{s1}-I peptide can be detected through electrophoresis and the amount increases during ripening. According to previous research, the optimal pH for casein breakdown by rennet action is at pH 5.0 (Noomen, 1978). As shown in Figure 3, the pH on the surface of Camembert cheese is higher than 5.0 after 14 days and may reach up to 7.0 after 5 weeks. It is proposed that when the pH of cheese increases, the action of rennet decreases. Additionally, previous studies show that the hydrolysis of β-casein and the production of β-I has not been detected by electrophoresis during the ripening of Camembert cheese (which is different from the *in vitro* experiments) (Fox et al., 2004; Guinee & Fox, 1987; Trieu-Cuot & Gripon, 1982).

2.2.3.4.2 Action and Effect of Milk Proteinases

Compared to the action of rennet, the milk proteinases are less active in most cheeses during the ripening process (Visser, 1977). In Camembert, however, at the end of ripening, an increased activity of plasmin was demonstrated by the increase of γ-caseins. This phenomenon has been explained by Noomen that the pH of the Camembert surface at the end of ripening is around 7.0 which is close to the optimal pH of plasmin (pH 8.0) (Noomen, 1978). Therefore, compared to other semi-hard cheeses with lower pH (pH 5.0-5.2), plasmin is more active in Camembert at the end of ripening. However, whether the activity of plasmin contributes to the survival of *S. aureus* is unknown.

2.2.3.4.3 Action and Effect of *Penicillium*

The *P. camemberti* can synthesize several enzymes including an acid carboxypeptidase, an aspartate peptidase and a metalloproteinase. The optimum pH of aspartate proteinases of *Penicillium camemberti* is between pH 3.5 and 5.5. Compared to β- and κ-casein, α_{s1}-casein can be better hydrolysed by *P. camemberti* enzymes. The action of aspartate proteinases on β-casein breaks down 3 bonds (Lys_{29}-Ile_{30}, Lys_{99}-Glu_{100} and Lys_{97}-Val_{98}) more quickly than others. The metalloproteinase synthesised by *P. camemberti* is stable with a pH range from 4.5 - 8.5, which perfectly suits the pH of Camembert (Guinee & Fox, 1987; Spinnler & Gripon, 2004). The electrophoretograms obtained after the ripening of aseptic control curds, in which the metalloproteinase of *P. camemberti* develops alone, were very similar to that of regular Camembert, indicating the dominant role of this proteinase in cheese (Fox et al., 2004; Guinee & Fox, 1987; Lenoir & Auberger, 1982; Spinnler & Gripon, 2004; Trieu-Cuot & Gripon, 1982).

The changes of proteolytic activity in Camembert cheeses during ripening have been investigated. Studies show that the activity at the core of cheese stays low while that at the rind of cheese increases rapidly after 6 - 7 days ripening. At this time, both metalloproteinase and aspartate proteinase are synthesised and grow dramatically on the surface until 14 days following a slow decrease, which is presented in Figure 4 (Lenoir, 1984; Lenoir & Auberger, 1982). Following the electrophoretic changes of β_{mp1}- and β_{ap1}-peptidases, the effect of metalloproteinase and aspartate proteinase *in situ* were tested. After 2 weeks of ripening, β_{mp1}-peptidase decreased indicating the degradation of β_{mp1} or the decrease of the action of metalloproteinase. β_{ap1}-peptidase occurred and regularly intensified after the development

of *P. camemberti*, suggesting that the action of aspartate proteinase continues during ripening (Fox et al., 2004; Gripon, 1999; Lenoir, 1970; Noomen, 1983; Spinnler & Gripon, 2004). Further, the proteolytic activity stays low at the inner part of cheese compared to that at the surface, indicating the limited migration of *P. camemberti* in Camembert cheeses.

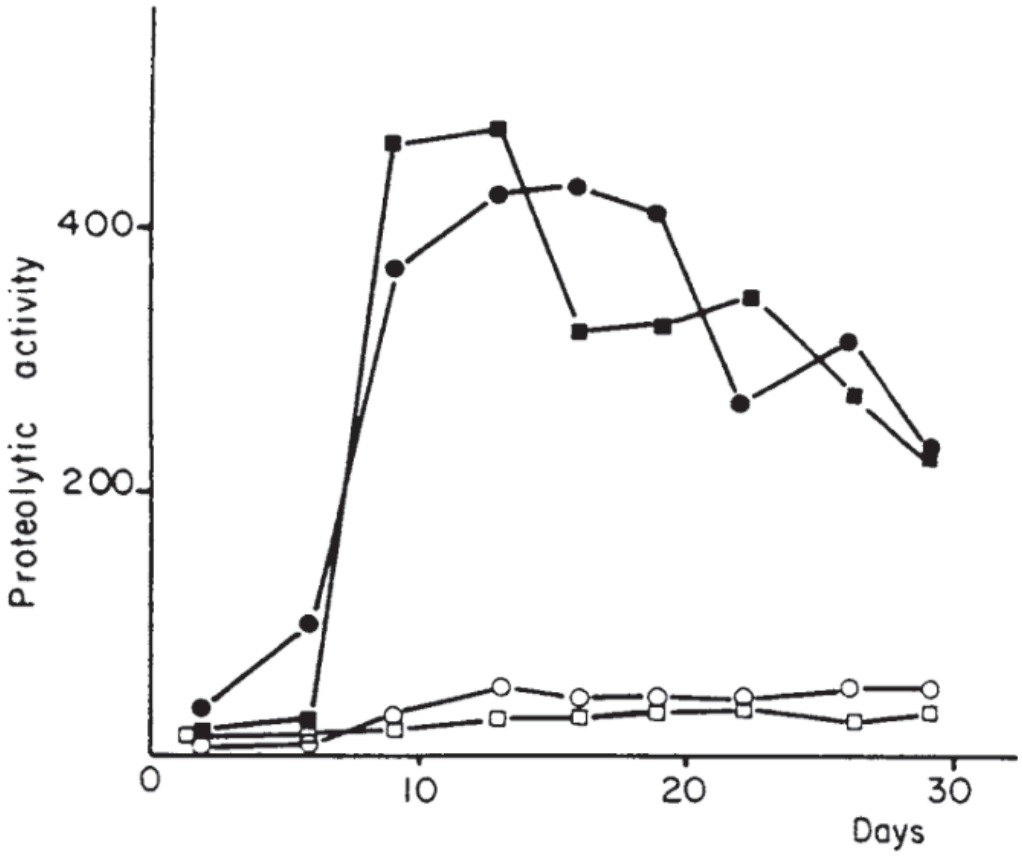


Figure 5 Changes in the activities of the aspartate proteinases and the metalloproteinase during the ripening of Camembert (Guinee & Fox, 1987; Spinnler & Gripon, 2004).

The growth of *P. camemberti* leads to a dramatic increase in pH, especially on the surface of cheese (McSweeney, 2007). The increase in pH from 5 to 7.5 provides a suitable environment for *S. aureus* growth. Previous studies, however, indicated that the presence of *S. aureus* results in a significant increase in plasmin and proteolytic activity, which will negatively affect the quality and properties of the cheese (Larsen et al., 2010) and these changes may also influence *S. aureus* growth.

2.2.3.4.4 Metabolism of Amino Acids

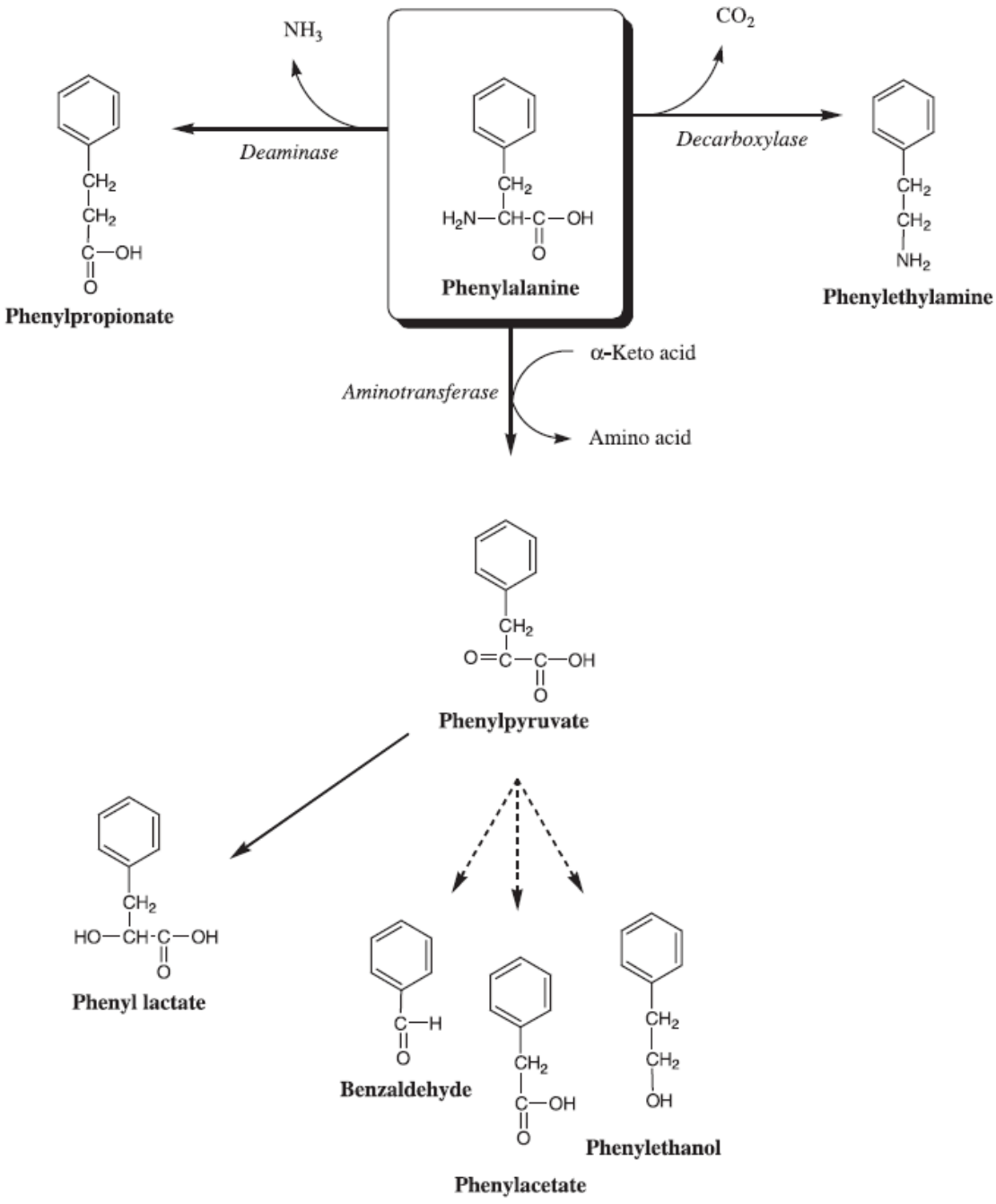


Figure 6 Catabolism of phenylalanine (McSweeney, 2004)

The metabolism of amino acids produces flavour compounds during ripening. Being considered as a precursor for catabolic reactions, the main function of proteolysis in the production of flavour compounds is the release of free amino acids. The pathway for the catabolism of phenylalanine (an aromatic amino acid) is presented in Figure 6. This is similar to the catabolism of other aromatic amino acids like tyrosine and tryptophan. Phenylalanine, tyrosine and tryptophan are catabolised by aminotransferase producing phenylpyruvate, *p*-hydroxyphenyl pyruvate and indole-3-pyruvate respectively (Curtin & McSweeney, 2004; Lee & Desmazeaud, 1985; McSweeney, 2004). As shown in Figure 7, the branched-chain amino acids valine, isoleucine and leucine, are initially degraded to α-ketoacid by aminotransferase, and then the α-ketoacid is degraded producing other compounds. This has been studied in lactococci, analysing the capability of aminotransferases transaminating branched-chain amino acids (Engels, 1997).

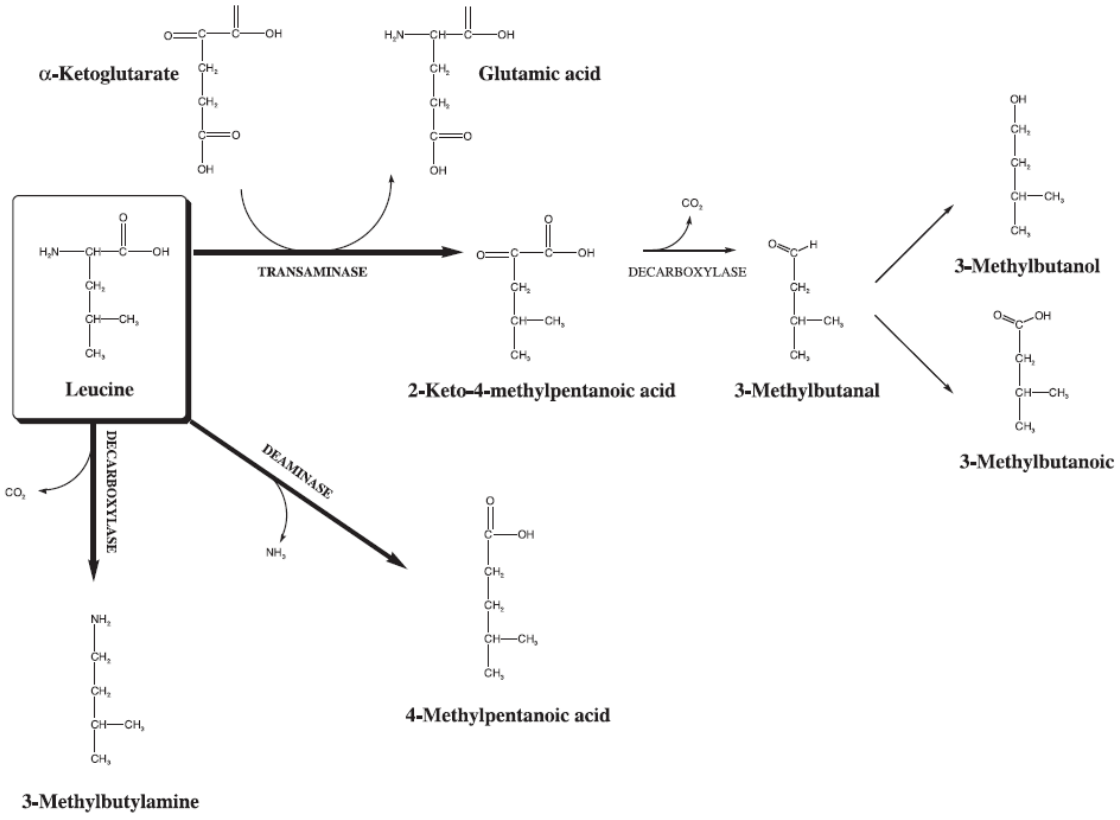


Figure 7 Pathways for the catabolism of leucine and the formation of volatile flavour compounds (McSweeney, 2004).

Deamination reactions are also pathways for the degradation of amino acids. In Camembert cheeses, producing ammonia through deamination can not only help increase the pH but also contribute to the flavour of cheeses during ripening (McSweeney, 2004; McSweeney & Sousa, 2000). However, since the deamination reactions may cause some side effects like producing unpleasant aromas, decarboxylation is correspondingly important for Camembert. The low pH optimum for decarboxylases and the ability of these decarboxylases to produce non-volatile amines through decarboxylation can help in the balance of pH and flavours in cheese (McSweeney, 2004). The metabolism of amino acids plays an important role in developing the flavour compounds of Camembert cheeses, its effect on *S. aureus* growth, however, has not been particularly investigated. The increased pH contributed by the deamination reactions may help provides a good condition for *S. aureus* growth.

2.3 Summary and Hypothesis

Through our understanding of the growth requirements of *S. aureus*, and our knowledge of the manufacturing process and ripening biochemistry of Camembert cheese, the effect on *S. aureus* can be roughly predicted. The hypothesis is that during the initial stage of manufacture, the counts of *S. aureus* will increase as the cheese curd forms due to the physical entrapment and temperature of 32 °C, ideally suited to the growth of *S. aureus. S. aureus* will be expelled with the whey, and the bacterial numbers will decrease due to the low pH (<4.8) produced by lactic acid bacteria. During the early stages of ripening, the counts will continue to decrease due to the residual effects of the pH and lactic acid. During the later stages of ripening as the pH increases, the rate of decrease in *S. aureus* will reduce, but the storage temperature of 4 °C will prevent the growth of *S. aureus*.

3 Materials and Methods

3.1 Manufacture of Camembert Cheeses

3.1.1 Materials and Equipment

Camembert cheeses were manufactured using Mad Millie Speciality cheese-making kit containing the following components: Steriliser, Calcium Chloride, Culture Blend for White Mould Ripened Cheeses, Vegetarian Rennet Tablets, Artisan's Cheese Salt, Cheese Moulds, Cheese Cloths, Cheese Mats, Maturing Boxes, Silver Cheese Wraps 240mm x 240 mm. Other tools were used including long blade knife, large ladle spoon and pipette.

The milk used was Meadow Fresh brand (Goodman Fielder Ltd, Longburn) silver top full cream pasteurised milk purchased from a local Countdown supermarket. The blend of starter culture and secondary flora was bought from the Mad Millie official website (73002 White Mould Culture Blend 5 Sachet Pack). Both starter and ripening culture are included in each sachet. The starter culture includes the following bacteria: *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis biovar diacetylactis*, and *Leuconostoc*, whereas the secondary flora used here is *Penicillium candidum*.

Three *Staphylococcus aureus* strains were used in this project: two reference strains *S. aureus* ATCC 4163 and *S. aureus* ATCC 9144 (retrieved from the Massey microbiology lab culture collection) and one dairy farm strain was isolated from a cow with mastitis (from a Manawatu farm) which was named 172RR. The tag in the cow's ear was 172 and RR stands for the right rear teat.

3.1.2 Experimental design

Three different strains were inoculated into the Camembert cheeses separately. At least three trials were made for each strain. For better understanding whether the addition of Culture Blend is a factor influencing the fate of *S. aureus* in cheese, comparison groups without the addition of starter culture and secondary flora were made for each strain. Further control groups with or without culture blend were done in buffered peptone water with pH control.

3.1.3 Sterilisation

Iodophor was used as a steriliser to thoroughly sterilise all utensils that may be utilized in the Camembert cheese manufacture. Iodophor (4 ml) was poured into 2 L of water, and the equipment was soaked at room temperature for about 10 min.

3.1.4 Cheese Curd Preparation and *S. aureus* Inoculation

Pasteurised milk (2L) was poured into a pot and heated up to 32 °C in a water bath, at which point 1 ml of Calcium chloride was added. The milk was stirred for 30 s then 1 ml of *S. aureus* and 0.3 g of white mould culture blend added while stirring for another 30 s. The mixture was left at 32 °C for 1 hour. A half tablet of rennet in 5 ml of distilled water was added to the milk. The temperature was maintained at 32°C then left for the milk curd to form (about 2 hours).

3.1.5 Cutting, Moulding and Draining the Curds

A long blade knife was used to cut the curd into 2 cm cubes. The curds were stirred occasionally using a draining spoon for 15 min. Oversized cubes were cut into 2 cm squares while stirring, then left for another 15 min in a water bath at 32 °C. The curds were taken out with a spoon and evenly distributed into 2 sterilised cheese moulds. The moulds were placed on a sterilised cheese mat in a mature box (provided with the cheese kit) to drain the whey away from curds. The cheeses were turned over every hour for 5 hours using cheese cloth to make sure the cheese was evenly drained. The cheese was left to drain overnight at room temperature (20 °C).

Figure 8 Cutting and ladle the cheese curds before drainage

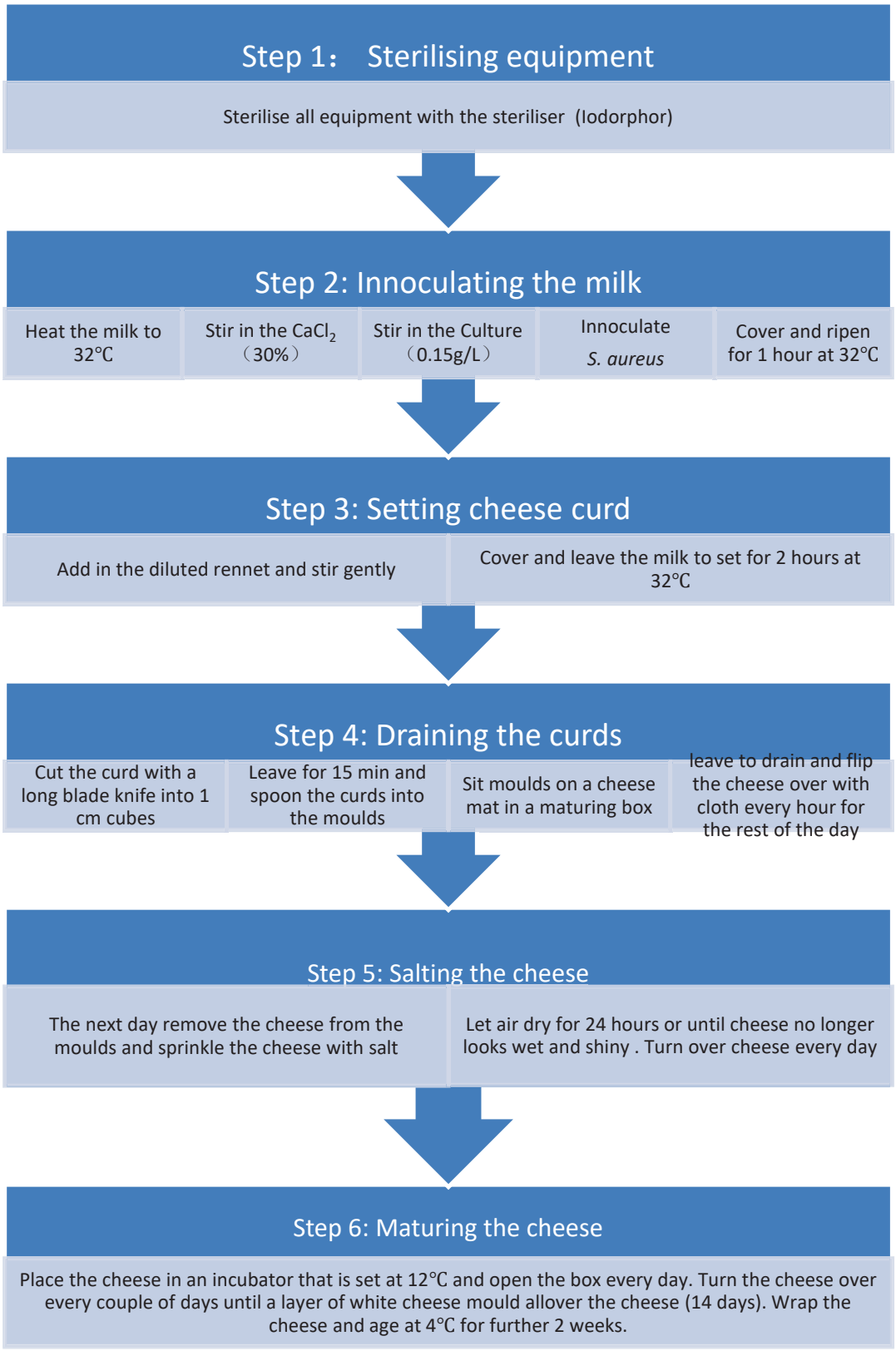
3.1.6 Salting the cheese

The cheeses were turned over twice during the following day and the cheese removed from the mould in the evening. Cheese salt (2g) (provided with the kit) was sprinkled onto the surface of the cheese, then put into the mature box (provided with the cheese kit) into the fumehood for 24 h to help dry the surface of cheeses and then the cheese was flipped over once during this period to ensure even drying of the surface.

3.1.7 Maturing the Cheeses

The cheese box was placed in a cooled incubator (myTEMP^{TM} Mini Digital Incubator) set at 12 °C. To ensure the mould is well developed, the box was opened every day to allow the air exchange. The cheese was flipped and moved every day to help the moulds grow more evenly. A thick layer of mould developed on the surface after 14 days. The cheese was then removed from the mature box and wrapped with white cheese wrap. The cheese was then put in a 4 °C refrigerator for another 2 weeks for further ripening so that the interior of cheeses develops a soft texture.

3.1.8 Flow Chart of Cheese Manufacture



3.2 Bacterial Enumeration

3.2.1 Experimental Design

The numbers of *S. aureus* were examined at the following stages: (1) pasteurised milk right before the addition of rennet (1 h); (2) the curd at the beginning of drainage (3 hours); (3) whey after cutting the curd; (4) cheese at the end of moulding (2 days); (5) cheese at the 3rd, 5th, 7th, 14th and 30th day of ripening. *S. aureus* counts in all pasteurised milk samples were tested before use to ensure no contamination.

The surface and interior of cheeses were examined separately for the enumeration of bacteria. Sampling techniques were based on (Wehr, 2004). The surface part of cheese was sampled by scraping a thin piece with a depth of 5 mm beneath the mould. The interior part was sampled by cutting a small cube with a depth between 5 mm to 15 mm. Due to the limit of incubator space, the number of cheeses was limited, so the size of samples that could be taken was limited to 1 g.

3.2.2 Plate Count Method

The plate count method of the American Public Health Association (APHA) was used for bacterial enumeration. This approach is suitable for food with an expectation of over 100 cells/g (Lancette & Bennett, 2001).

3.2.3 Procedure

To prepare samples for microbial counting, 1 ml of liquid sample (whey or milk before adding rennet) or 1 g of solid sample (cheese curds, cheese) was added to 9 ml sterile 0.1% peptone. The diluted sample was poured into a Stomacher bag of a peristaltic blender and blended for 60 seconds or until it was almost entirely dissolved. Serial 10 fold dilutions of the sample were made. Each of three appropriate sample dilutions (0.1 ml), based on the estimated counts of bacteria, were spread onto Baird-Parker Agar with a bent glass rod to spread the inoculum over the surface of agar plate. When the estimated counts were too low to count, 1 ml of sample dilution was inoculated for the test. The inoculated agar plates were placed in a 37 °C

warm room for 48 h. *S. aureus* on Baird-Parker plates are small, black or grey colonies with an opaque halo. Plates with colonies between 20 ~ 200 were selected for counting.

3.2.4 Physico-chemical Analysis

3.2.4.1 pH

The pH values of the cheeses were determined using a pH meter (SevenCompact™ pH/Ion S220). A small piece of cheese (approximately 1g) was weighed into a small beaker and mixed with deionised water (about 9 ml). The surface and interior parts of cheese were tested separately, and the sampling technique is described in Section 3.2.1 (Experimental design of Bacteria Enumeration). Each sample was tested three times to obtain an average result.

3.2.4.2 Water Activity

A water activity meter (Aqualab) was used for testing the water activity of different parts of the cheese. Different parts cheeses were taken and evenly spread on the small plastic plate of the water activity meter. The plate was placed into the meter and the reading recorded. Eash sample was tested three times to obtain an average.

3.2.4.3 Salt Content Determination

Salt content was determined using the Volhard's method. Camembert cheese (3g) was weighed into a 250 ml conical flask.Distilled water (100 ml) was added along with 20 ml of concentrated nitric acid, 50 ml of 0.1 mol L^{-1} silver nitrate solution into a conical flask.In a fumehood, some boiling chips were added and the solution heated until boiling (Due to the smelly reaction, this operation has to be done in a fumehood).Potassium permanganate solution (5 ml 5%) was added to the boiling solution and more were added when the purple colour disappeared. The solution was kept boiling, and potassium permanganate added until the cheese sample was fully digested (no cheese particles on the surface). The solution was allowed to stand until it was cooled to room temperature (20 °C). The solution was then filtered, and the residue in the filter was washed with distilled water.The filtrate was placed in a volumetric flask and made up to 500 ml.

3.2.4.3.1 Titration

The cheese solution (filtrate) was poured into a conical flask, and one drop of saturated ferric ammonium sulphate was added as an indicator.The cheese solution was titrated with ammonium thiocyanate solution until a light red colour appeared.The titration was repeated twice and the average volume calculated.

3.2.4.3.2 Calculation

The average volume of ammonium thiocyanate used during the titration was converted into moles.In order to determine the total moles of unreacted silver nitrate in the volumetric flask (500 ml), the following equation was used to calculate the moles of silver nitrate used for 100 ml cheese extract: $Ag^+(aq) + SCN^-(aq) \rightarrow AgSCN(s)$. The total moles of untreated silver nitrate in 500 ml volumetric flask was calculated by multiplying the figure by 5.The moles of silver nitrate in the 50 ml of solution that was added during the sample preparation was calculated.The total moles of silver nitrate that reacted with the salt from the cheese was determined by subtracting the moles of unreacted silver nitrate (the excess) from the total moles of silver nitrate added to the cheese.The equation for the reaction between the silver ions and the chloride ions was used to calculate the moles of sodium chloride in the sample of cheese: $Ag^+(aq) + Cl^-(aq) \rightarrow AgCl(s)$. The concentration of sodium chloride in the cheese was expressed as grams of salt per 100 g cheese (% salt).

3.2.4.4 Control Groups

In order to investigate whether the addition of the Culture Blend is a factor influencing the fate of *S. aureus* in cheese, control cheeses were prepared without the addition of starter culture and secondary flora. These control cheeses were coagulated by the activity of rennet only. The methods of sampling, bacterial counts and physical parameter tests were the same as for the regular groups.

3.2.4.5 Statistical Analysis

The mean value of results from all three trials on a single strain was calculated for inter-strain comparison. To compare the results of different strains throughout the whole period of manufacture, T-tests were used.

4 Results

4.1 Survival of *S. aureus* During the Manufacture and Ripening Period of Camembert Cheese

Survival of *S. aureus* strains ATCC 4163, ATCC 9144 and 172RR, the changes of pH and water activity during cheese manufacture were measured during the manufacture and ripening of the cheese. Strain ATCC 4163 was used initially in preliminary experiments to become familiar with the cheese and the experimental methods. For detailed analysis in the main experiment, the three strains were compared in triplicate (Full data is presented in the appendices). In order to investigate and compare the differences in the results of three different strains, a T-test was used to compare the mean values of each strain.

4.1.1 Early Stages of Cheese Manufacture

To compare the changes of physical parameters and bacterial count at different manufacture stages, bar charts were used. Three stages are presented, which are: (1) milk right before the addition of rennet; (2) cheese curd at the start of drainage; (3) whey after cutting the curd. The bacterial counts of pasteurized milk before manufacture were not presented in these figures since none of them was contaminated before the experiment.

4.1.1.1 Survival of *S. aureus*

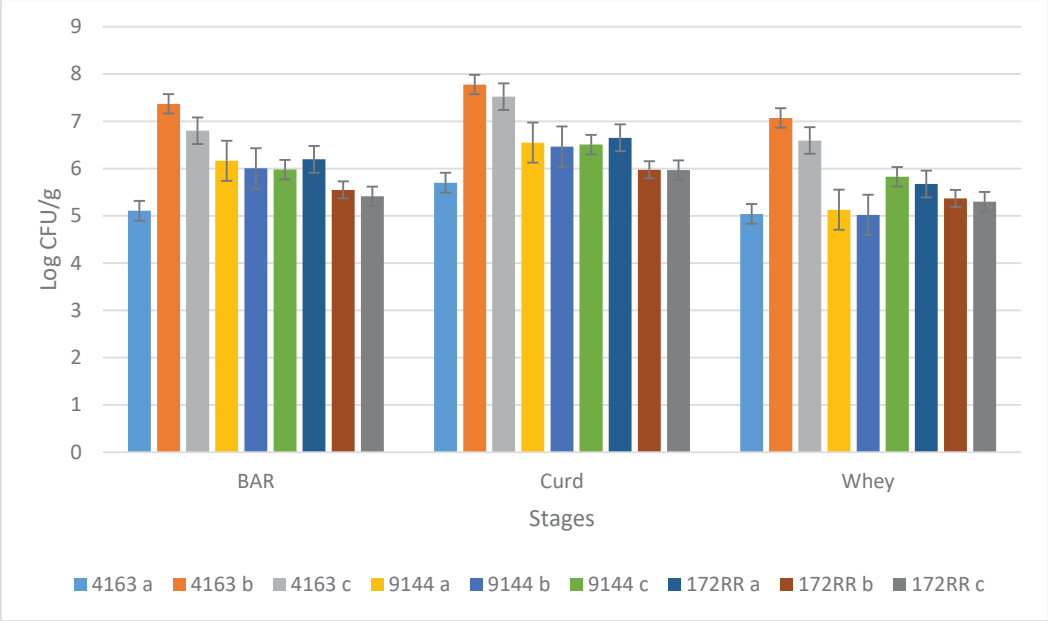


Figure 9 Survival of *S. aureus* at the beginning stages of Camembert cheese manufacture

(Coloured bar represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in each single trial.) BAR*: Cheese milk right before the rennet was added

As expected, none of the retail milk samples used in this experiment was contaminated with *S. aureus*. *S. aureus* was inoculated at the start of manufacture along with the addition of the cheese culture blend (starter culture). The density of *S. aureus* colonies at the beginning stages of cheese manufacture is recorded in Figure 9. Approximately 0.5 to 1 log increase in the count was observed during the coagulation of the cheese, at which point the majority of the bacteria were expelled through the whey with counts at approximately the same level as the cheese milk with around a 0.1 to 0.5 log reduction. No significant difference was shown by T-test between different strains ($p>0.05$).

4.1.1.2 Physical Parameters

4.1.1.2.1 pH

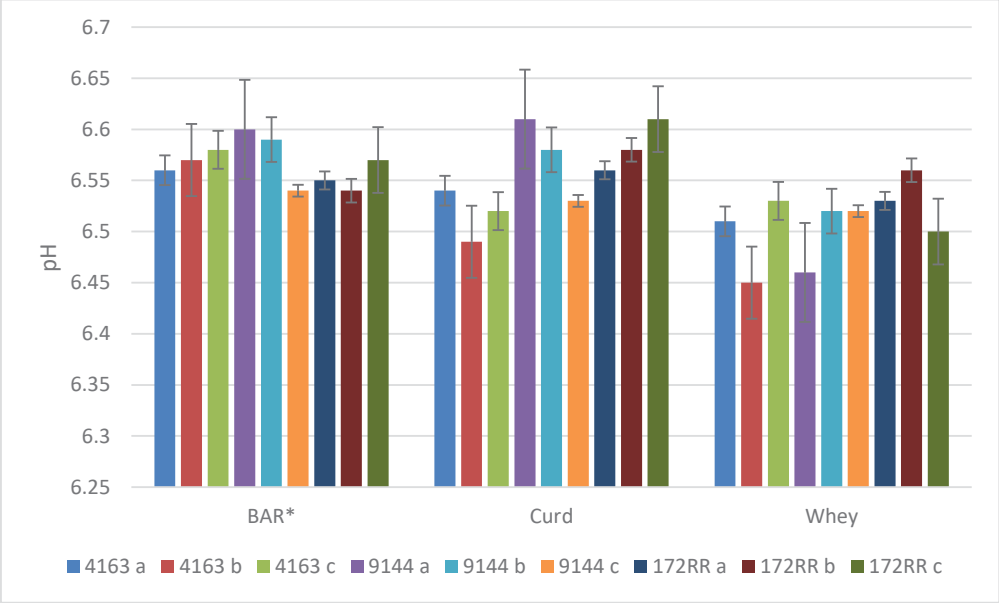


Figure 10 Changes of pH at the early stages of Camembert cheese manufacture

(Coloured bar represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in each single trial.) BAR*: Cheese milk right before the rennet was added

4.1.1.2.2 Water Activity

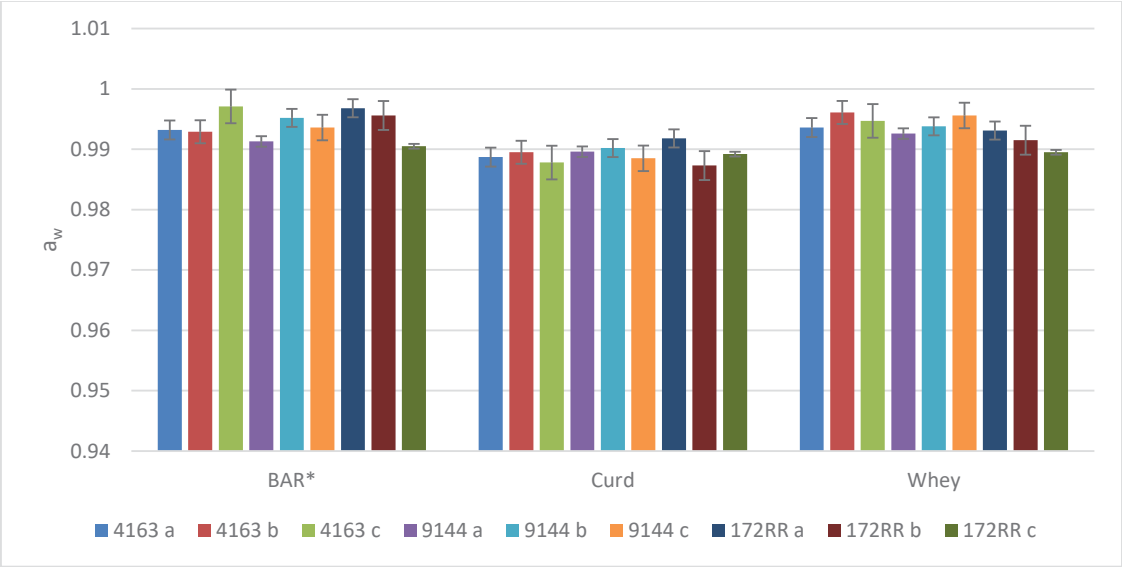


Figure 11 Changes of a_w at the beginning stages of Camembert cheese manufacture

(Coloured bar represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in each single trial.) BAR*: Cheese milk right before the rennet was added

Results of pH and water activity at the early stages of Camembert cheese manufacture are presented in Figure 10 and 11. The pH of cheese milk remained steady when forming the curds, whereas the pH of whey was lower than both cheese milk and cheese curd. The water activity of cheese milk before adding the rennet was between 0.990 and 0.997 which was almost at the same level as the whey, while the a_w of cheese curd was 0.987 and 0.992. No significant difference was shown by T-test between different strains, in terms of both pH and water activity values ($p>0.05$).

4.1.2 Survival of *S. aureus* in different part of the cheese

To compare the changes in physical parameters and bacterial count at different manufacture stages as well as the ripening period, line graphs were used for comparisons. Since the time

unit during the initial manufacture stage was ‘hour’, while that during the ripening time was ‘day’, in order to clearly state the change of data and make it more understandable, the test results were recorded in three separate graphs. Graph one stands for the whole period of Camembert production (From milk to the end day of ripening) whereas graph two and three stand for the manufacture stage and ripening stage respectively. In graph one, the corresponding time points are: (1) the origin (0 h) is corresponding to the time before adding rennet; (2) 2 h is curd at the start of drainage; (3) 50 h represents the end of moulding; (4) 122 h, 172 h, 218 h and 386 h are the 3rd day, 5th day, 7th day and 14th day of ripening at 12 °C (1 - 14 ripening days), respectively; (5) 770 h represents the 30th day of ripening at 4 °C (14 - 30 ripening days).The corresponding time point in graph two is as follows: (1) 0 h is the time before adding rennet; (2) 2 h is curd at the start of drainage; (3) 50 h (2 days) represents the end of moulding. In graph three, the numbers represent ripening days: (1) 3, 5,7, 14, are the 3rd, 5th, 7th and 14th days of ripening at 12 °C (1 - 14 ripening days), respectively; (2) 30 represents the 30th day of ripening at 4 °C (14 - 30 ripening days).

4.1.2.1 Mould Rind of Camembert Cheese

4.1.2.1.1 Survival of *S. aureus*

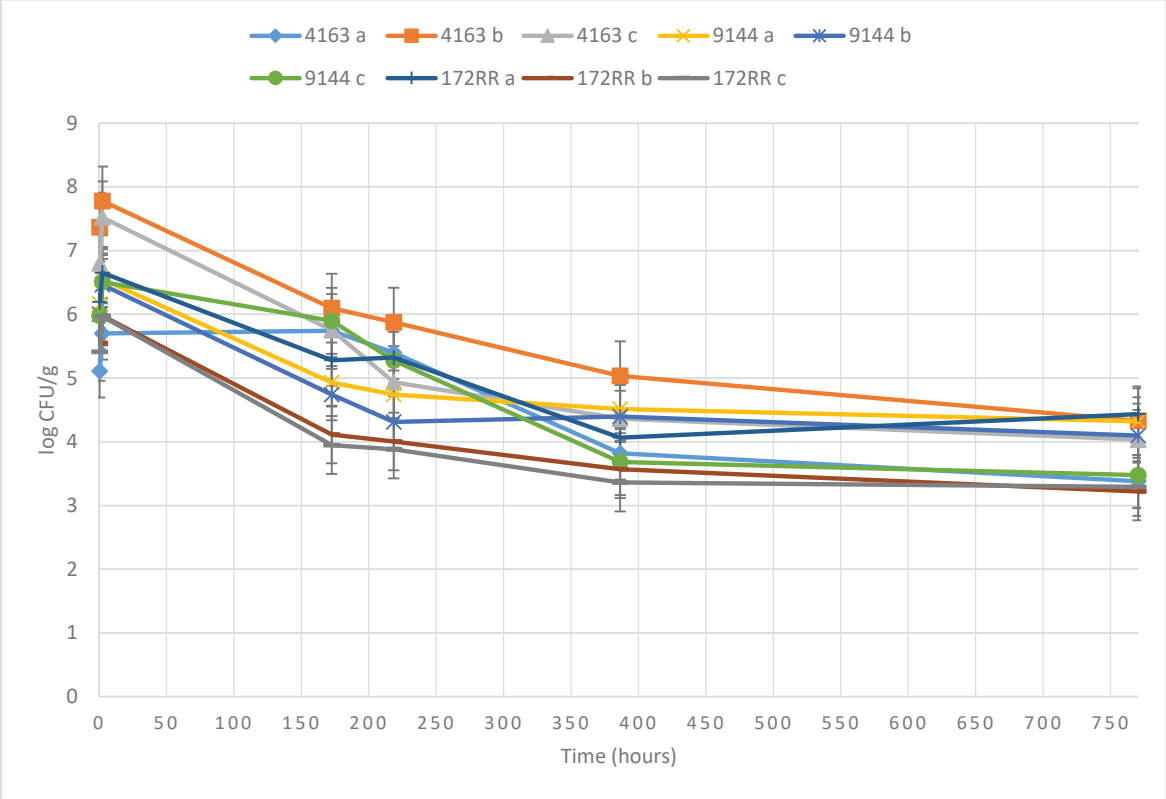


Figure 12 Survival of *S. aureus* at the mould rind of Camembert cheese throughout manufacture and ripening stages

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in each single trial.)

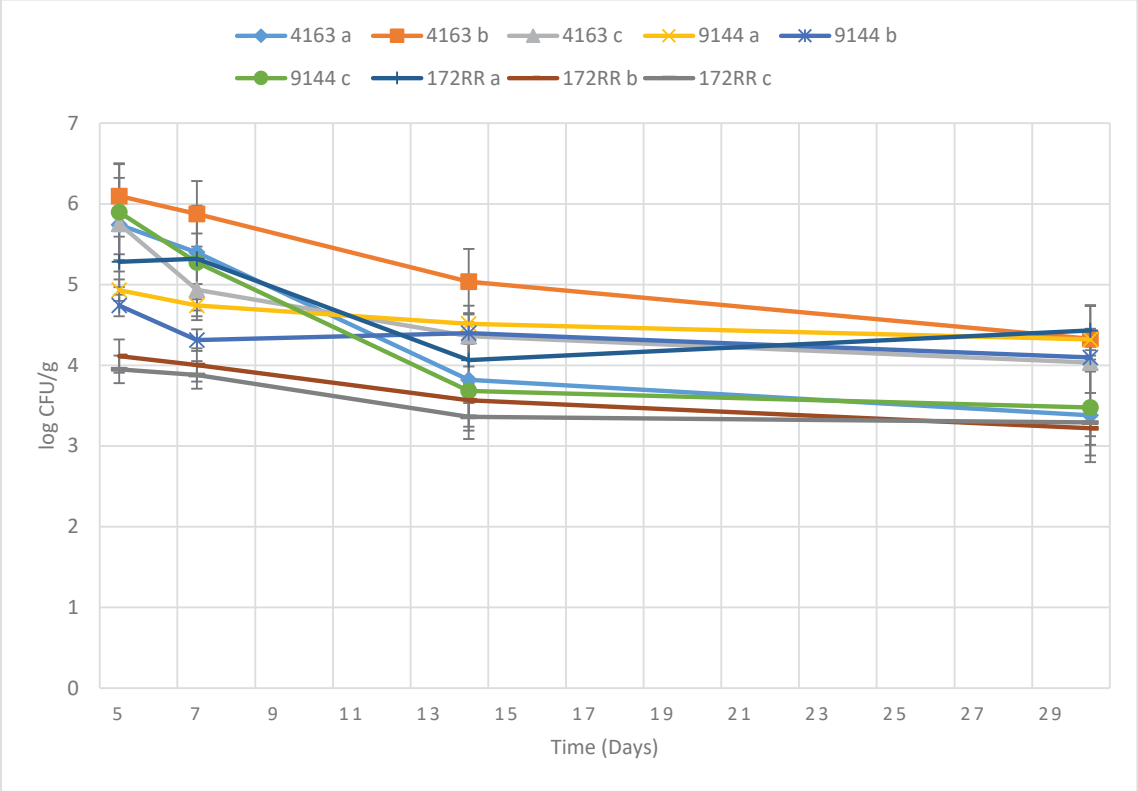


Figure 13 Survival of *S. aureus* in the rind of Camembert during the ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

Since the mould rind parts of cheeses were not fully grown until the 5th day of ripening, the pH, water activity, as well as the survival of *S. aureus* in the mould rind of Camembert cheese, were examined at the 5th, 7th, 14th and 30th days of ripening. The temperature in the first 3 hours was 32 °C, followed by 12 °C from 5 days to 14 days ripening and 4 °C from 14 days to 30 days. The number of *S. aureus* in the rind of Camembert cheese decreased during the manufacture and ripening of the cheese with the rate of decrease slowing after approximately 14 days. The reduction in *S. aureus* across all cheese samples and all trials was between 1.9 logs (9144 a and b) and 3.0 logs (4163 b). The maximum count of *S. aureus* in most samples was during the formation of the curd with one exception, 4163 (a), which reached a peak on the 5th day of ripening. Only the count of 172RR (a) increased from the 5th to the 7th ripening days while the counts in the other samples decreased. From the 7th to the 14th days, the

growth in the *S. aureus* occurred for 9144 (b) whereas a 1.5 log reduction occurred for 4163 (a), 9144 (c) and 172 RR (a), a 1 log reduction for 4163 (b) and a 0.5 log reduction for both 172 RR (b) and (c). From day 14 to day 30, only 172 RR (a) showed a 0.5 log increase while a 0 - 0.7 log reduction appeared for the other samples. There was no significant difference between the results for each cheese ($p>0.05$).

4.1.2.1.2 Physical Parameters

4.1.2.1.2.1 pH

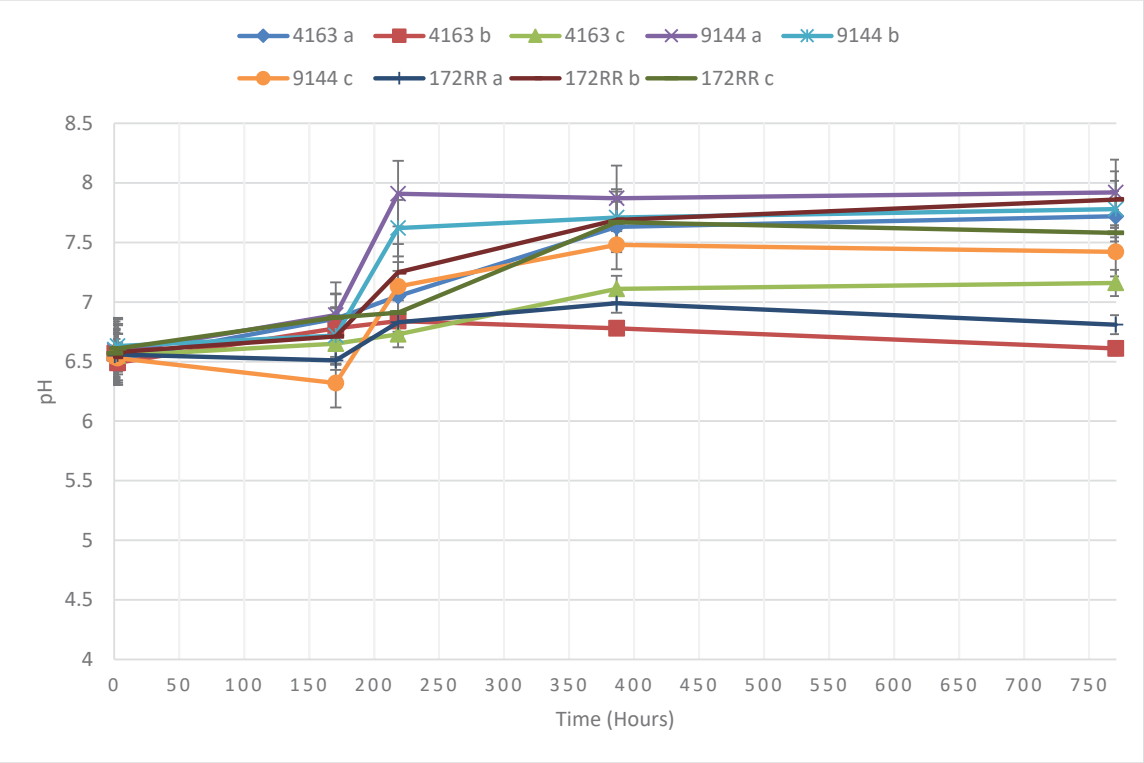


Figure 14 Changes of pH at the mould rind of Camembert cheese throughout manufacture and ripening

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

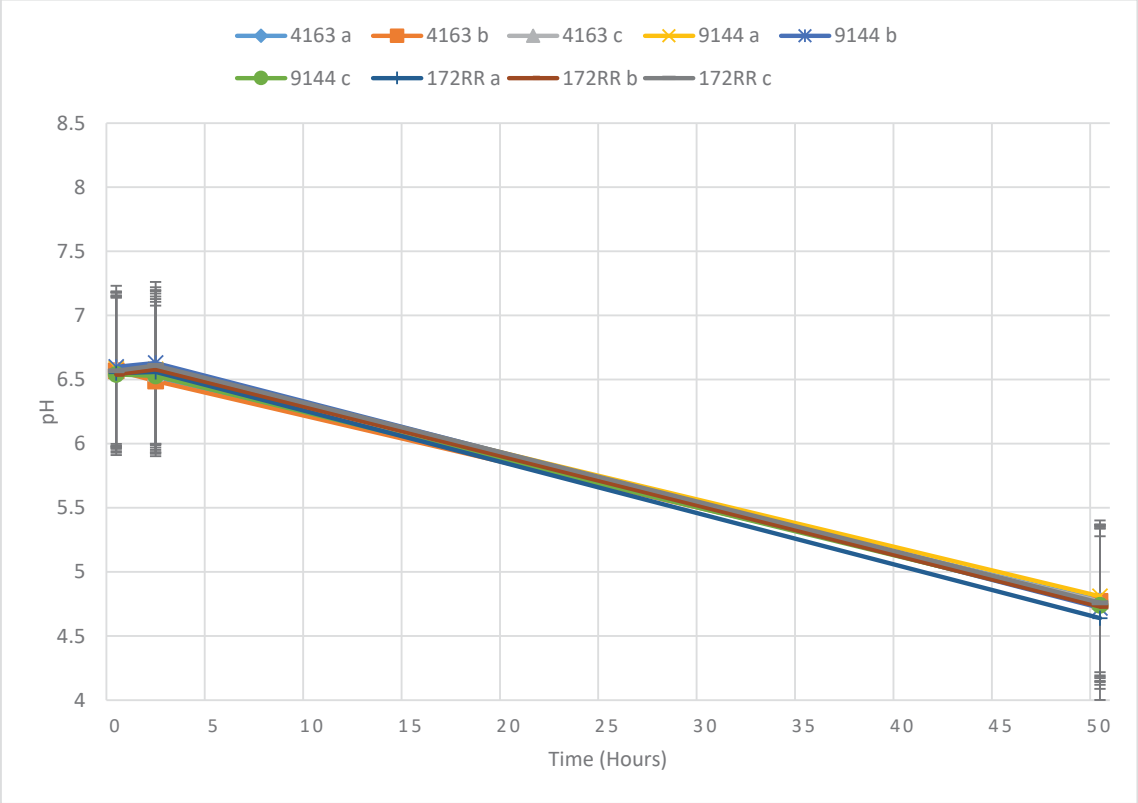


Figure 15 Changes of pH in the rind of Camembert cheese during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

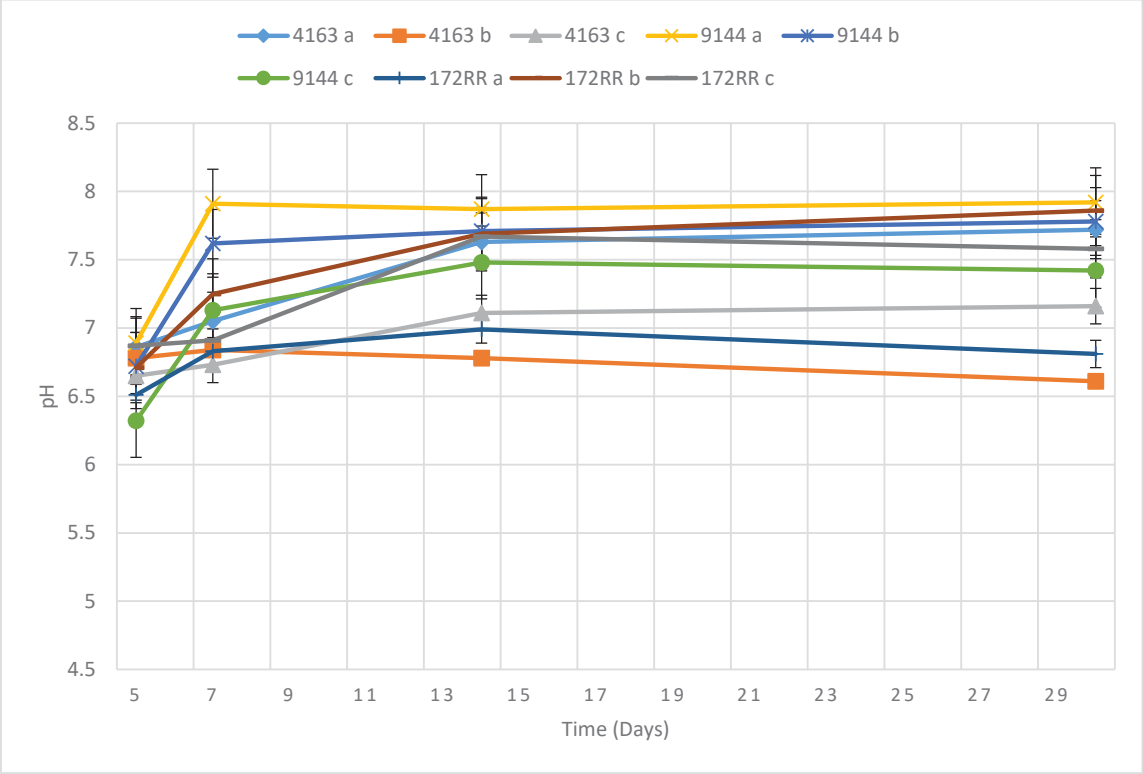


Figure 16 Changes of pH in the rind of Camembert cheese during the ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

4.1.2.1.2.2 Water Activity

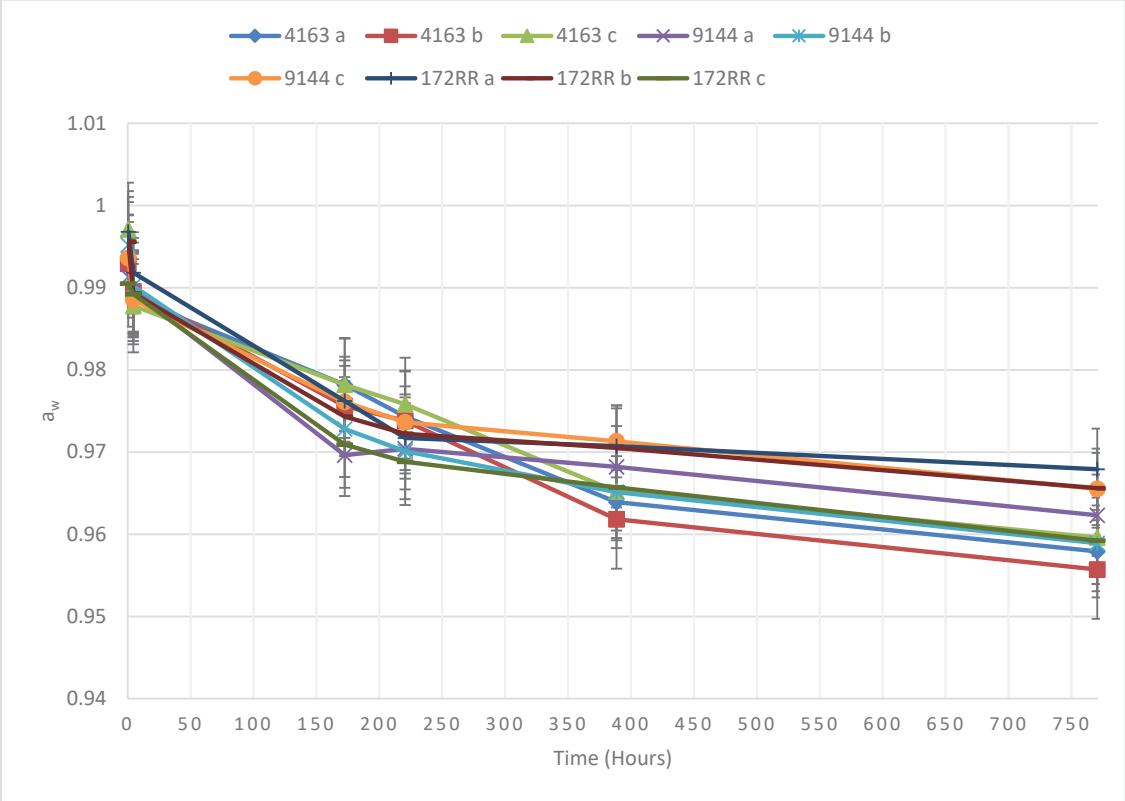


Figure 17 Changes of a_w at the mould rind of Camembert cheese throughout the manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

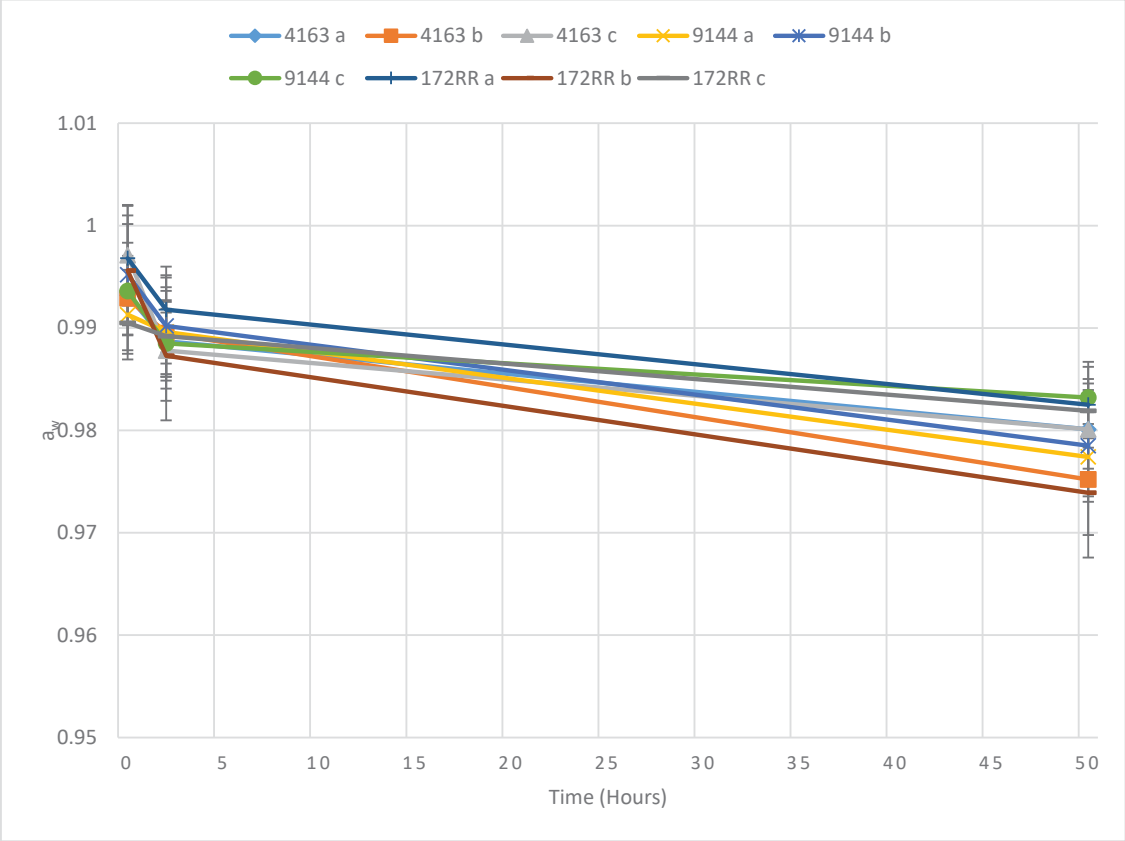


Figure 18 Changes of a_w at the mould rind of Camembert cheese during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

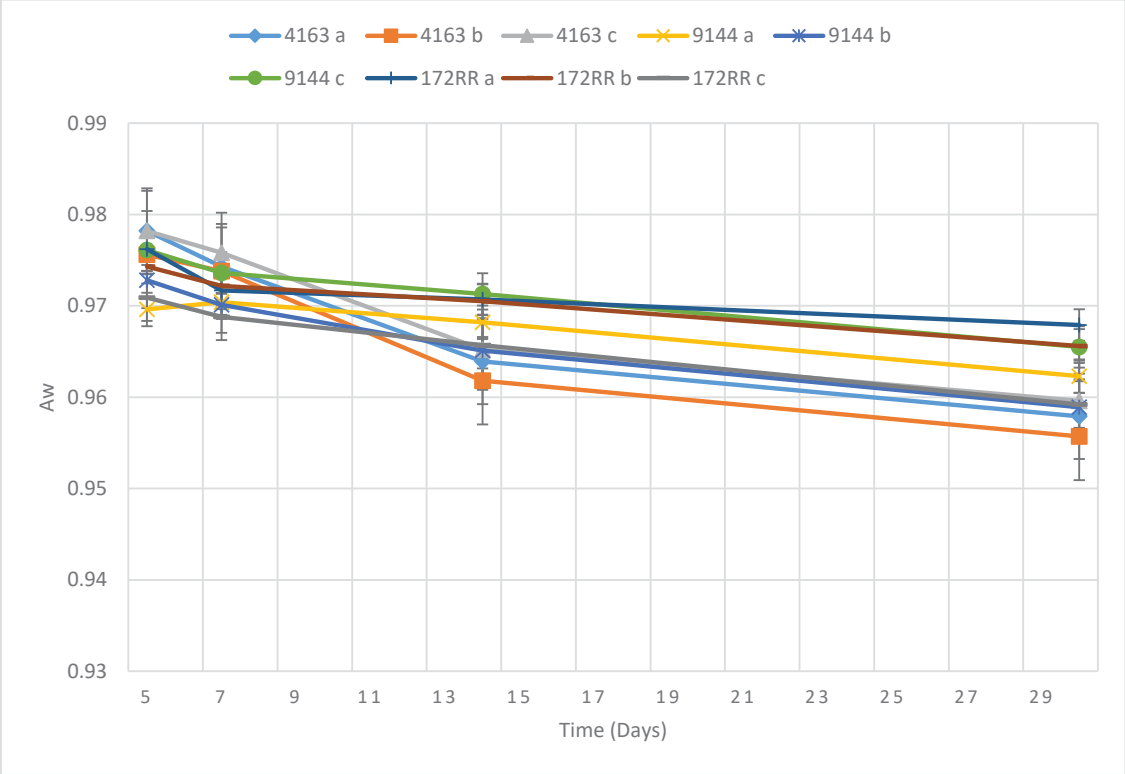


Figure 19 Changes of a_w at the mould rind of Camembert cheese during the ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

Figure 14 and 16 indicate that the pH of mould from all cheese samples increased from day 5 to day 7 at the ripening stage and slightly increased in the following days which end up with a level between 6.61 and 7.86 ($p<0.05$). The pH of cheeses inoculated with ATCC 9144 (a and b) almost remained constant after 7 days ripening, whereas the pH of the cheeses inoculated with most of the other strains increased slightly from day 7 to day 14 and remained stable afterwards. However, the pH of cheeses inoculated with 4163 (b) and 172RR (a) decreased after 7 days and 14 days of ripening respectively. The water activity of cheese samples gradually decreased from the initial stage (0.990 – 0.997) to the end (0.955 – 0.968) with the only exception of cheese inoculated with 9144 (c) which increased slightly from day 5 to day 7 (from 0.9696 to 0.9704). The lower boundary for *S. aureus* growth is 0.88, which is lower

than the values recorded from the cheeses in this study. For both pH and water activity, no significant difference was shown by T-test ($p>0.05$).

4.1.2.2 Surface of Camembert Cheese

4.1.2.2.1 Survival of *S. aureus*

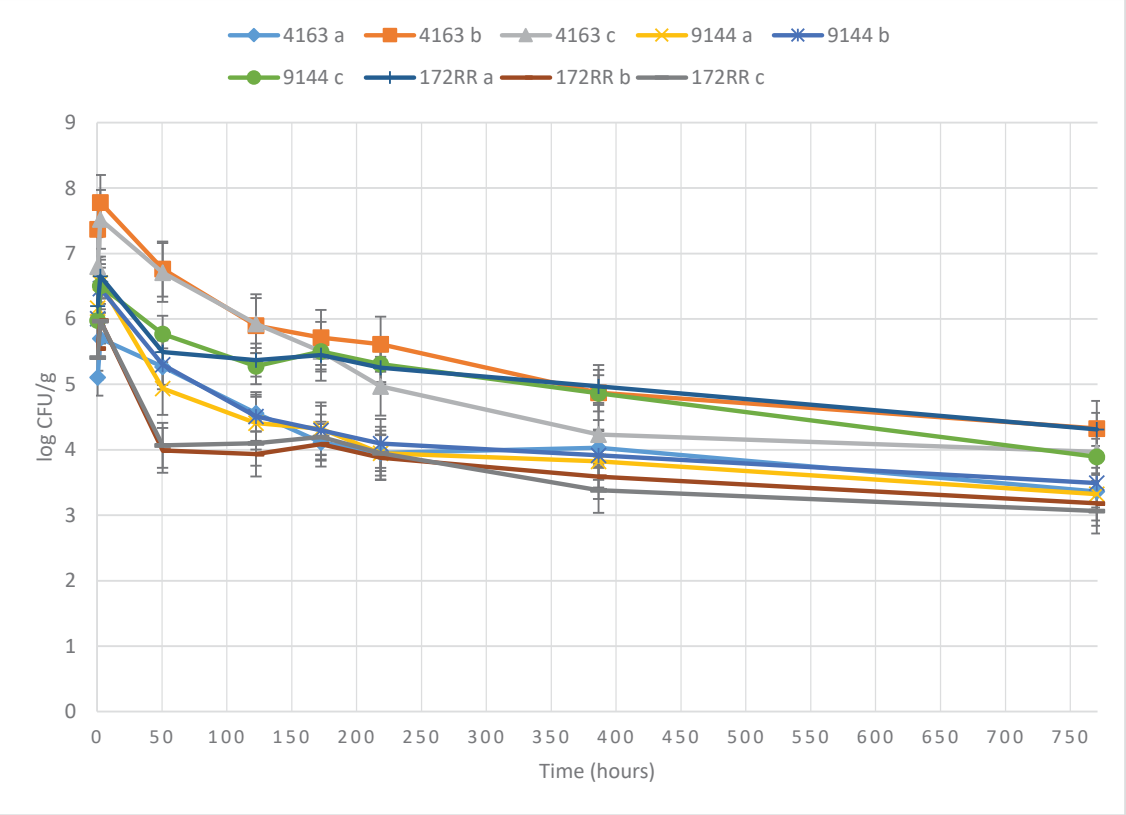


Figure 20 Survival of *S. aureus* at the Surface of Camembert cheese throughout manufacture and ripening stage

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

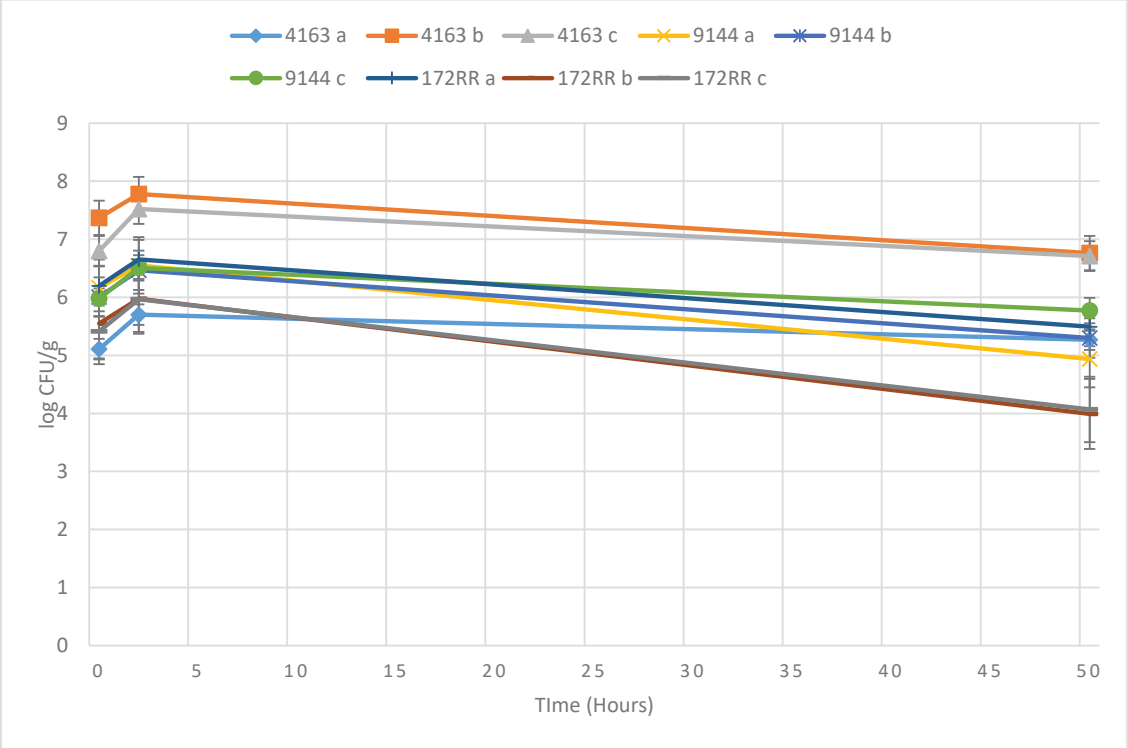


Figure 21 Survival of *S. aureus* at the surface of Camembert cheese during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

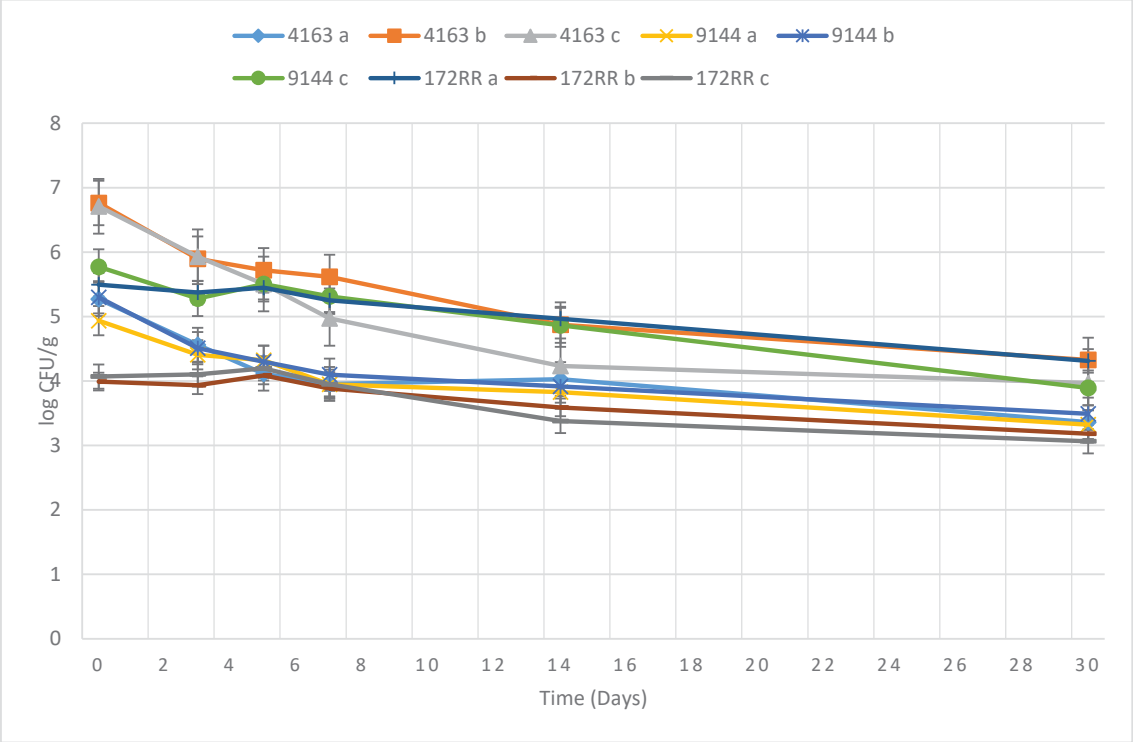


Figure 22 Survival of *S. aureus* at the surface of Camembert cheese throughout ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

The survival of *S. aureus* on the surface of Camembert cheese was examined at the end of cheese moulding and on the 3rd, 5th, 7th, 14th, and 30th days of ripening. The counts of *S. aureus* in the surface of the cheeses showed a similar pattern to the rind with numbers decreasing during manufacture and ripening. The reduction in the number of *S. aureus* at the end of testing was between 1.6 logs (4163 a) and 3.1 logs (4163 b). The maximum yield of *S. aureus* counts occurred at the beginning of cheese curd drainage, where there was approximately a 0.4 to 1 log increase from the initial inoculation. A large number of bacteria were lost during the draining and moulding period which was considered to be caused by the sudden decrease in pH. The bacterial numbers of both two reference strains decreased from the curd stage to the 3rd day of ripening, followed by a stable period between day 3 and day 7 except for 4163 (c), which continued decreasing. During this period, 9144 (c) and 172RR (a, b and c) increased

slightly from day 3 to day 5. Compared to reference strains, the counts of all 172RR samples decreased more during the moulding time and the 4 °C ripening stage. No significant difference was shown by T-test between different strains ($p>0.05$) during the whole period of manufacturing and ripening.

4.1.2.2.2 Physical Parameters

4.1.2.2.2.1 pH

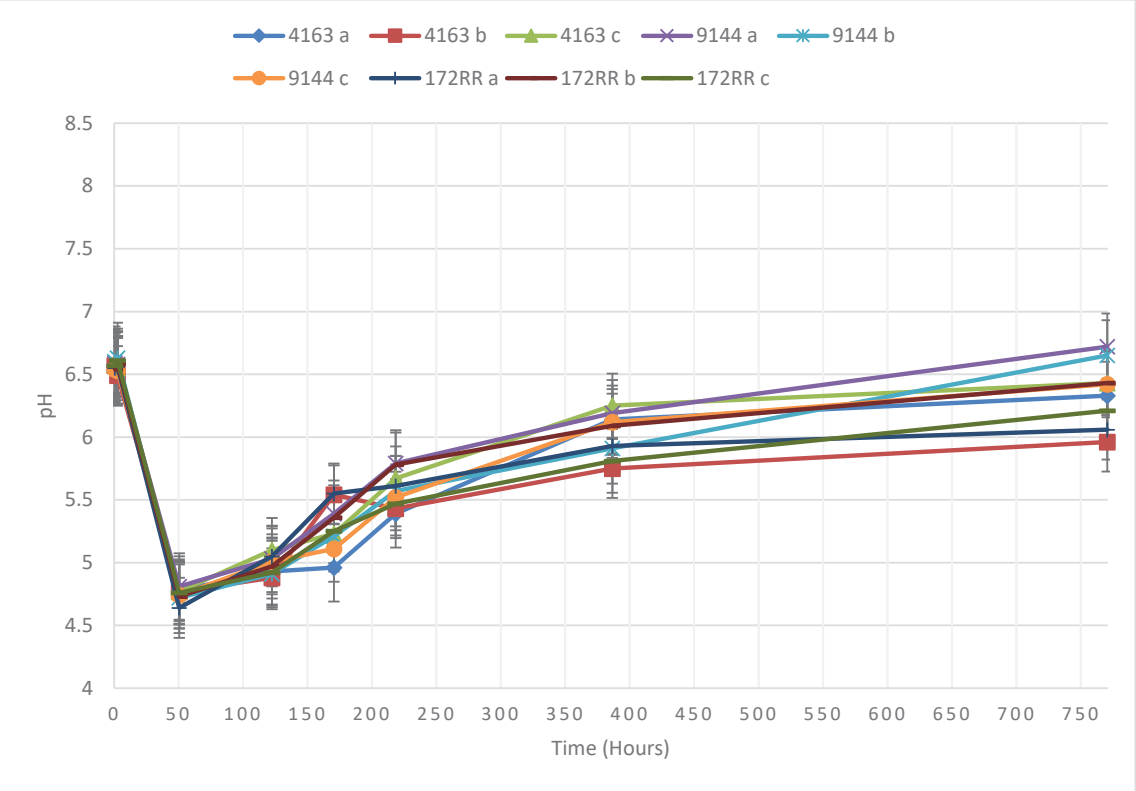


Figure 23 Changes of pH at the surface of Camembert cheese throughout the manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

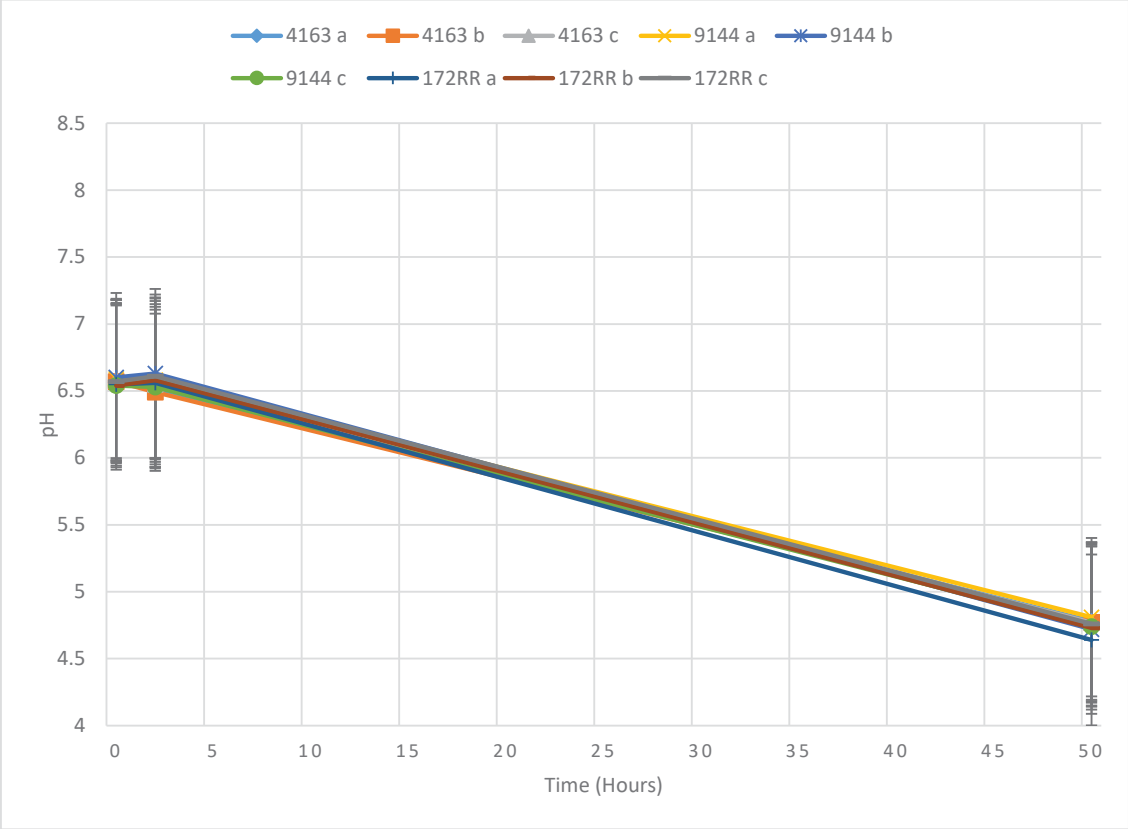


Figure 24 Changes of pH at the surface of Camembert cheese during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

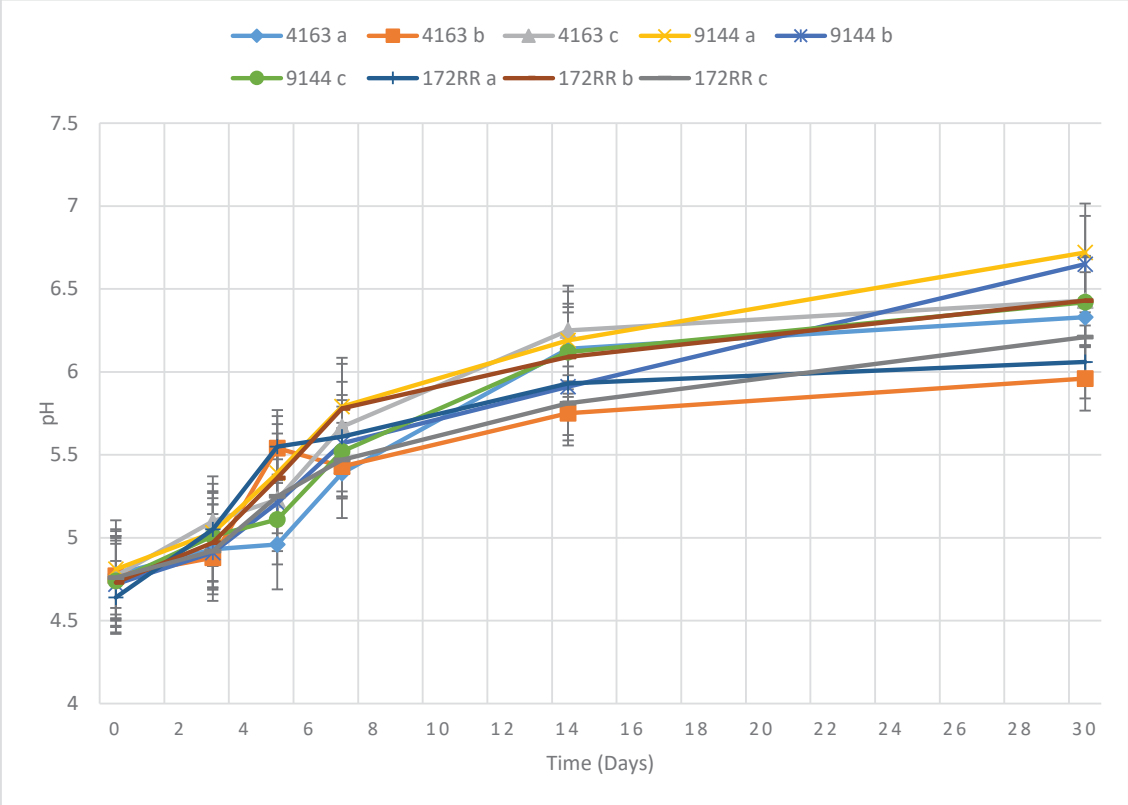


Figure 25 Changes of pH at the Surface of Camembert cheese during ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

4.1.2.2.2.2 Water Activity

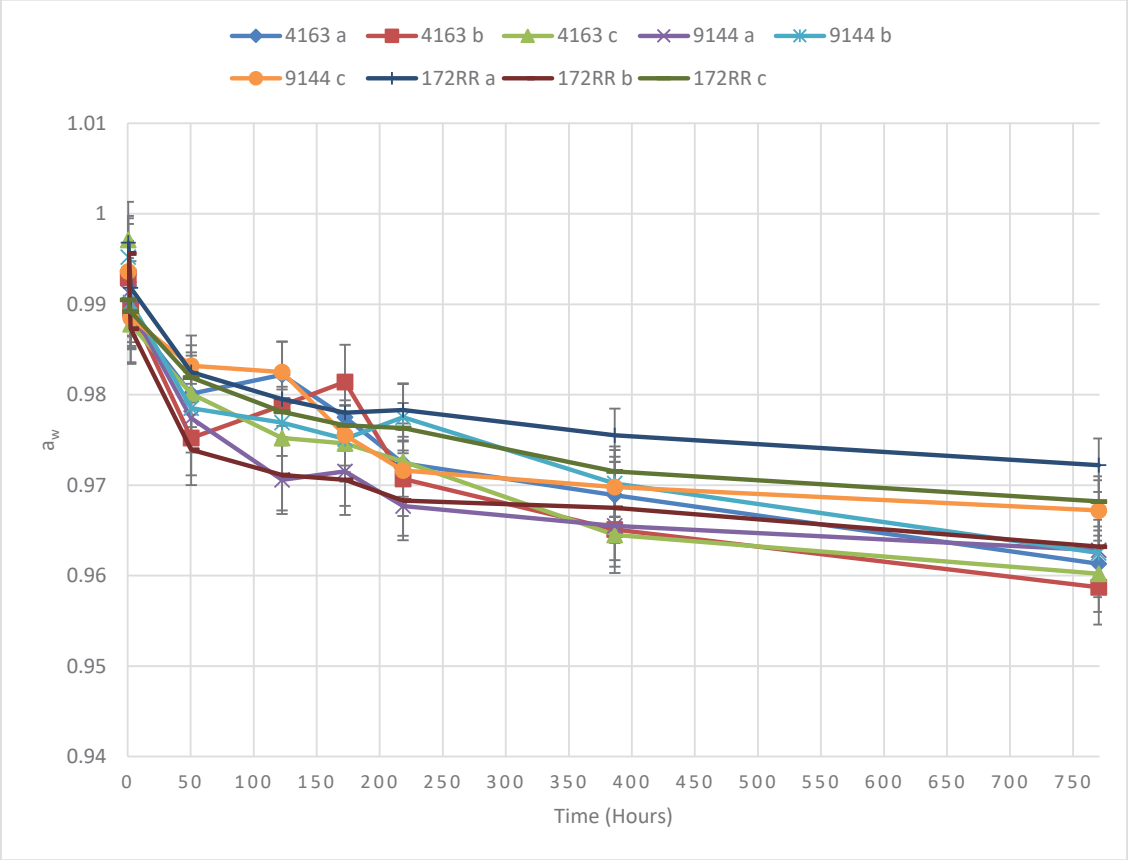


Figure 26 Changes of a_w at the surface of Camembert cheese throughout the manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

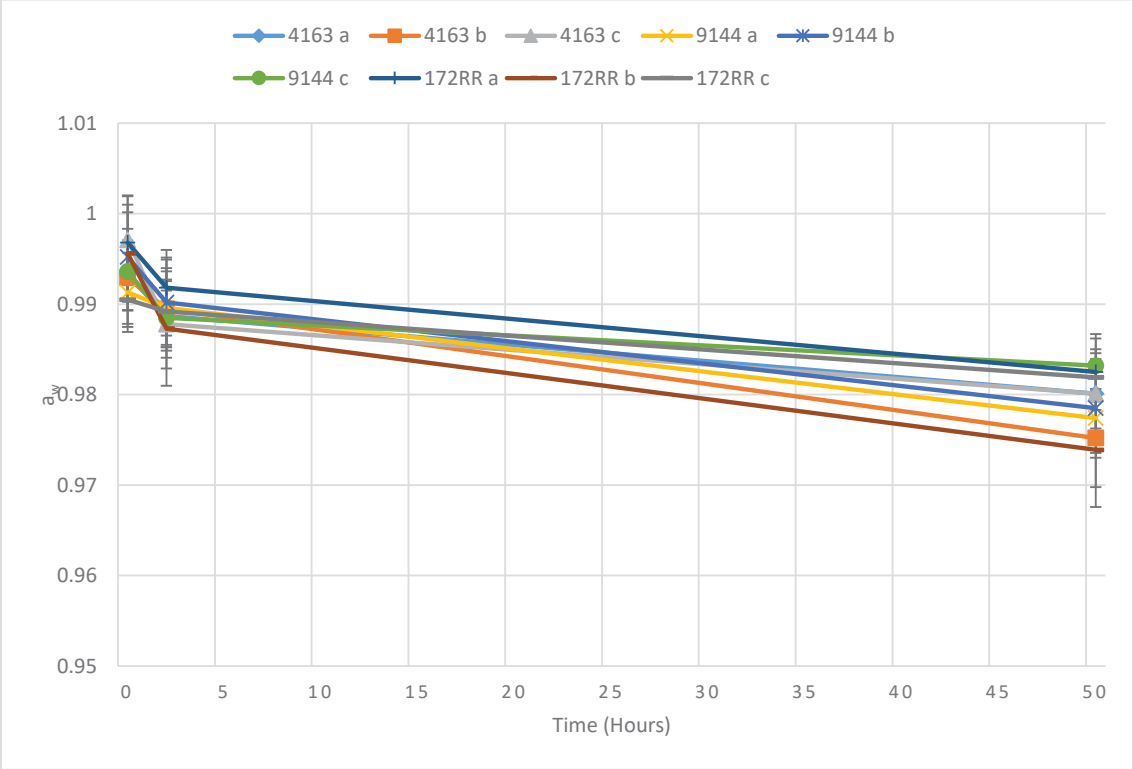


Figure 27 Changes of a_w at the surface of Camembert cheese during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

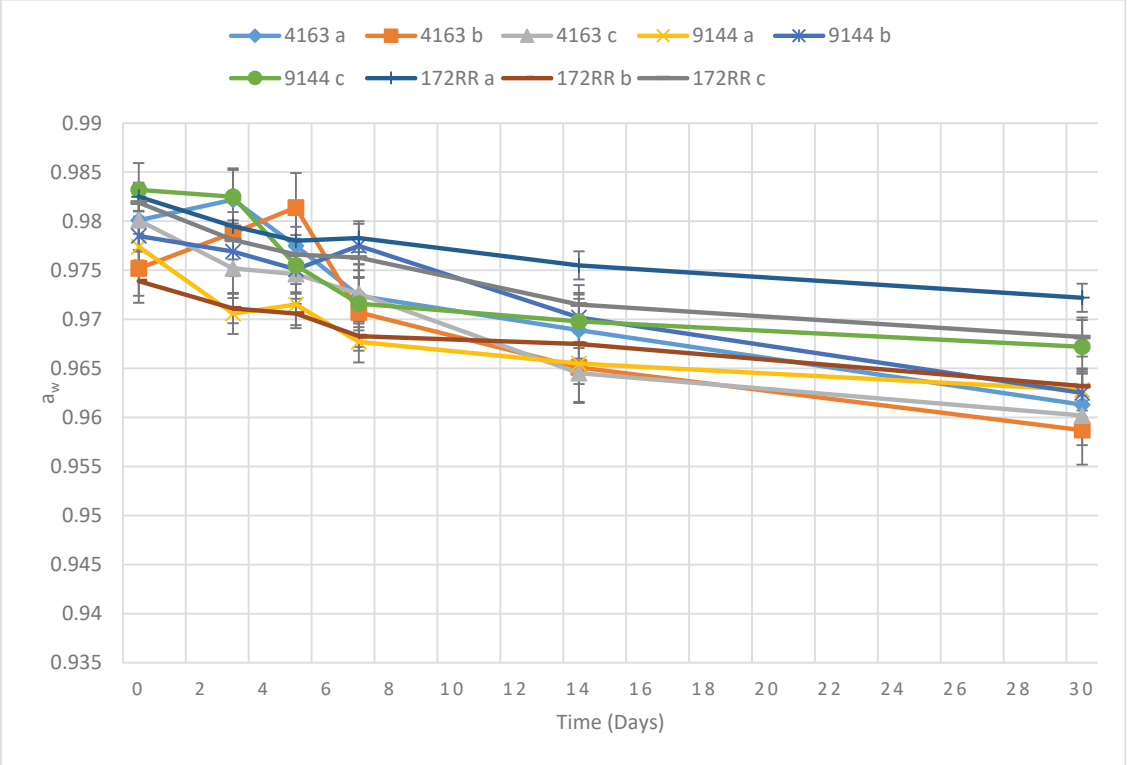


Figure 28 Changes of a_w at the surface of Camembert cheese during the ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

The surface of the cheese is defined as the part just beneath the mould rind of Camembert, which is described in Methods section (Section x). The physical parameters and the survival of *S. aureus* on the surface of Camembert cheese were examined at the end of cheese moulding and on the 3rd, 5th, 7th, 14th, and 30th day of ripening. The pH of all samples from the end of ripening day ranged from 5.96 and 6.72. The minimum pH for all samples occurred at the end of cheese moulding, at which point the pH was controlled to a level lower than pH 4.8. The value of pH increased gradually during the ripening period except for the slight decrease of 4163 (b) from day 5 to day 7. The water activity of most cheese samples kept decreasing throughout at the whole period of manufacture including ripening and storage, ending at a level between 0.958 and 0.975 (Figure 26 and 28). Only two samples, 4163 (a) and (b), showed an increase in water activity at the 3rd day of ripening which is, while an increase in water activity for 4163 (b) occurred from the 5th to the 7th days of ripening. However, the

value of water activity at the end of ripening was still high enough for the *S. aureus* growth. There was no significant difference between different Strains (p>0.05).

4.1.2.3 Core of Camembert cheese

4.1.2.3.1 Survival of *S. aureus*

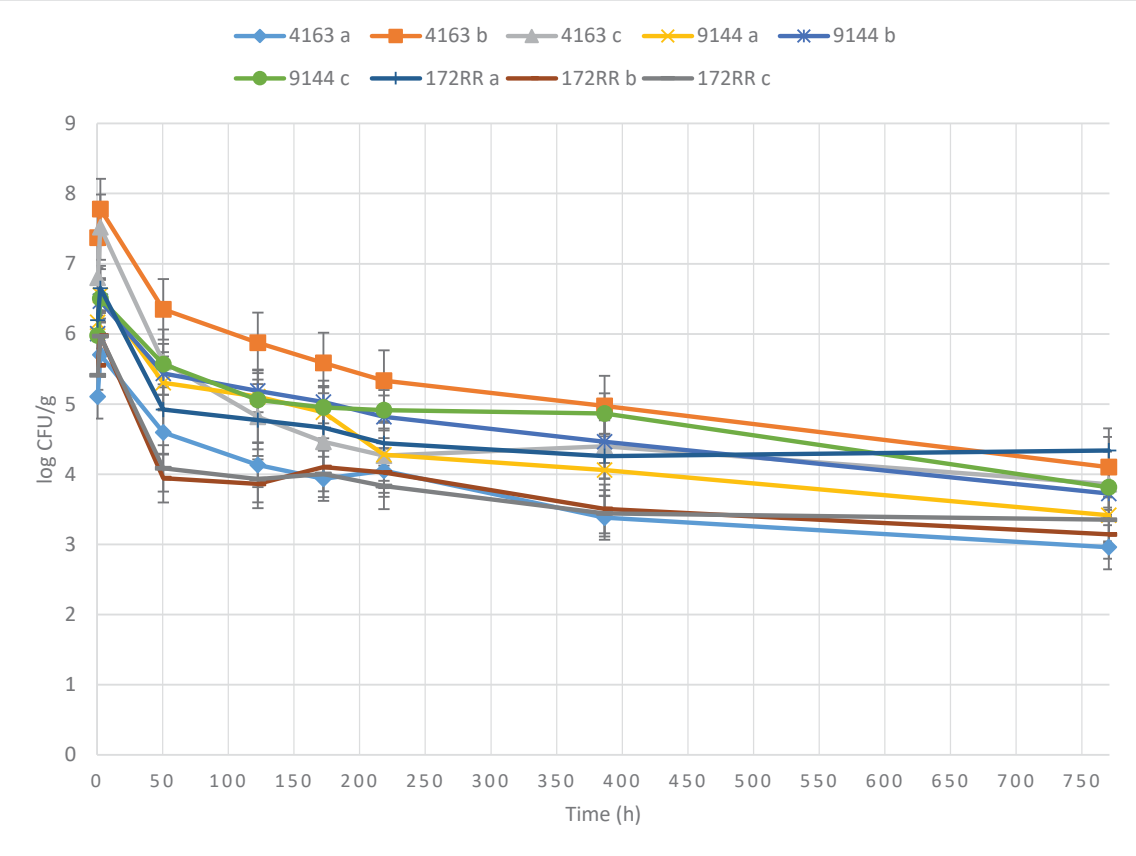


Figure 29 Survival of *S. aureus* at the core of Camembert cheese throughout manufacture and ripening stage

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

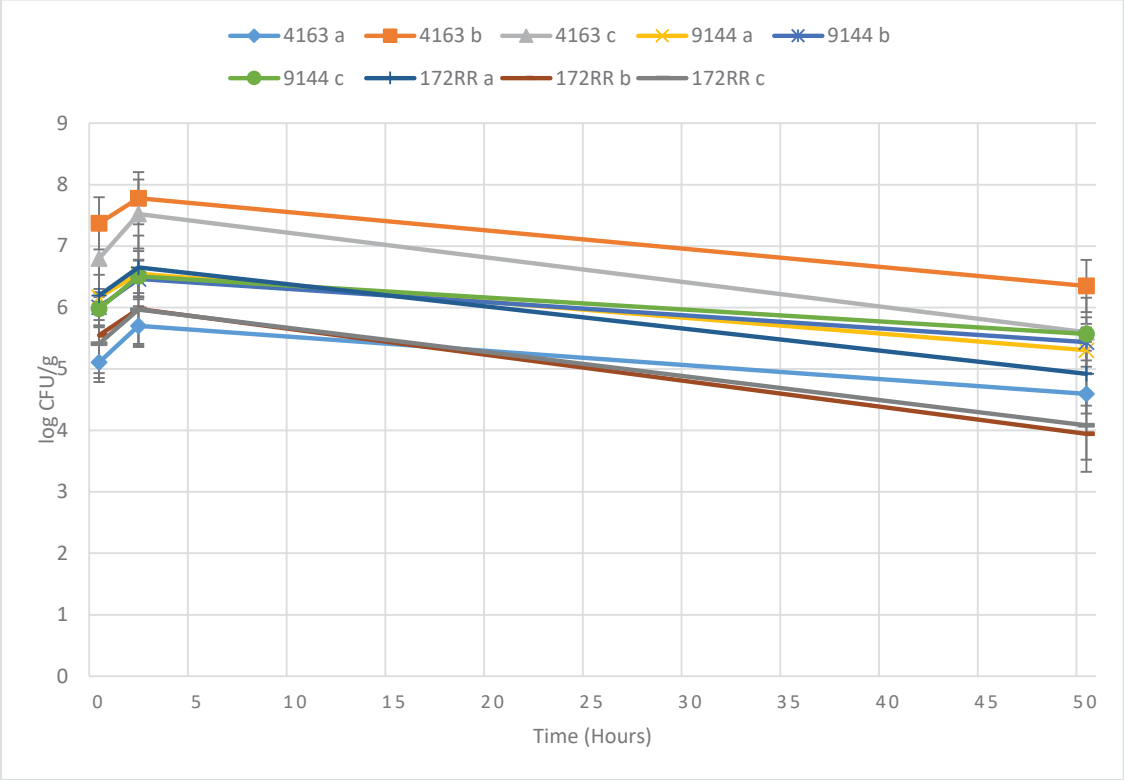


Figure 30 Survival of *S. aureus* at the core of Camembert cheese during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

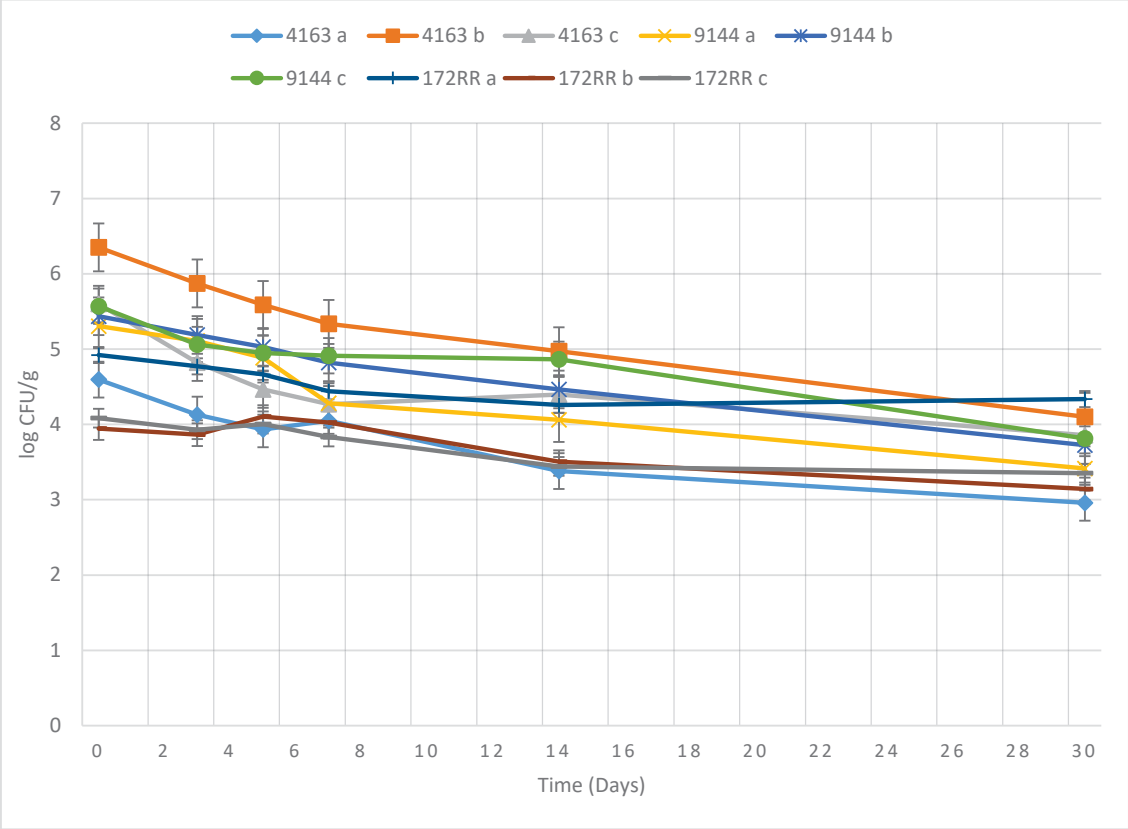


Figure 31 Survival of *S. aureus* at the core of Camembert cheese throughout the ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

The core of each cheese was tested at the same time points as the surface: the end of moulding and the 3rd, 5th, 7th, 14th, and 30th days of ripening. The reduction in the bacteria count in cheese samples was between 1.7 logs (172RR a) and 3.1 logs (4163 b). The maximum yield of *S. aureus* counts occurred at the beginning of cheese curd drainage. The bacterial number kept decreasing from the curd stage to the 3rd day of ripening, followed by a stable period between day 3 and day 7, during which 172RR (b and c) increased slightly from day 3 to day 5. The reduction in numbers during the curd drainage and moulding may be explained by the sudden decrease in pH. From observation, the counts of 172RR decreased more dramatically than the reference strains during the moulding time whereas the difference was

not statistically significant ($p>0.05$). The counts continued to reduce after 7 days of ripening, except for 172RR a, which showed a 0.6 log increase from day 14 to day 30, where a significant difference ($p<0.05$) was identified from others. In general, the death rate of *S. aureus* in cheese samples reduced during the ripening period resulting in numbers that reached a stable level towards the end of the trial. No significant difference was shown by T-test between the different strains ($p>0.05$).

4.1.2.3.2 Physical Parameters

4.1.2.3.2.1 pH

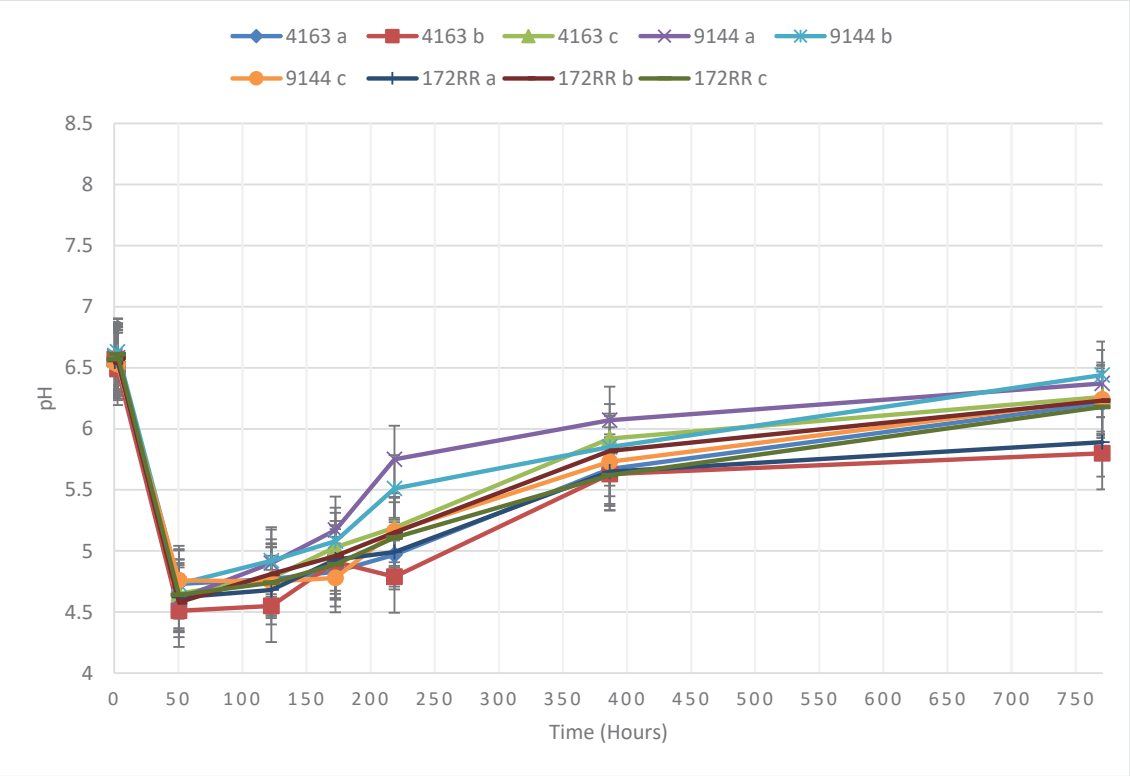


Figure 32 Changes of pH at the core of Camembert cheese throughout the manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

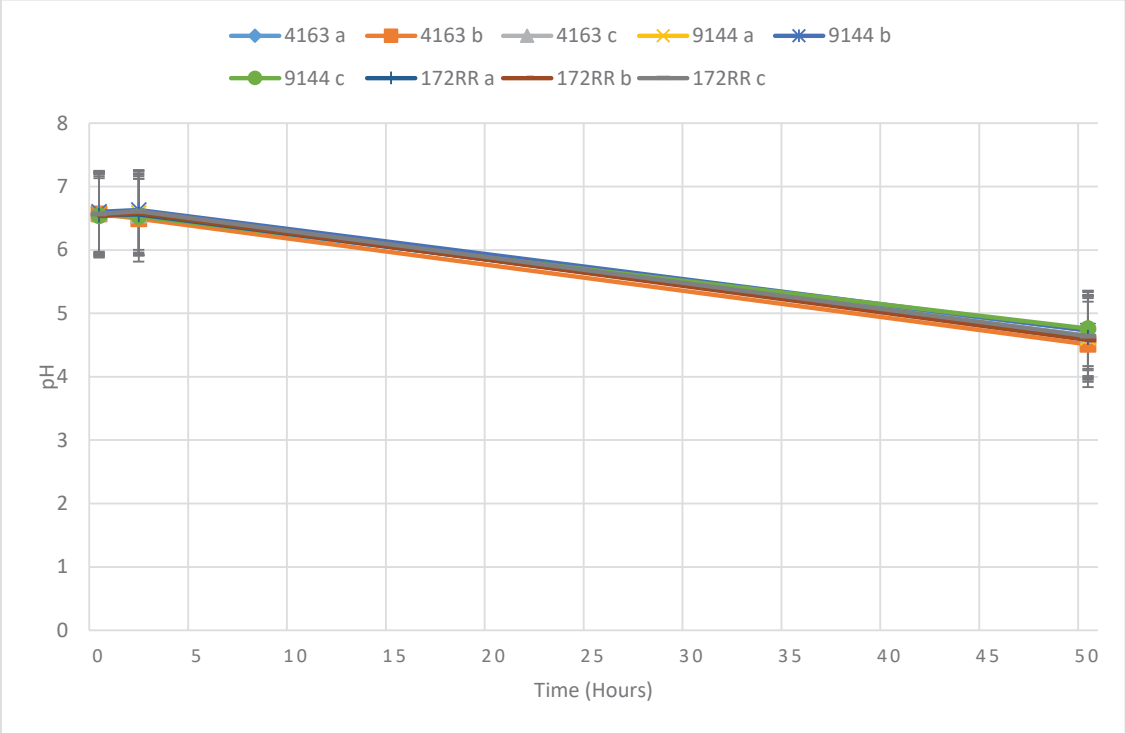


Figure 33 Changes of pH at the core of Camembert cheese during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

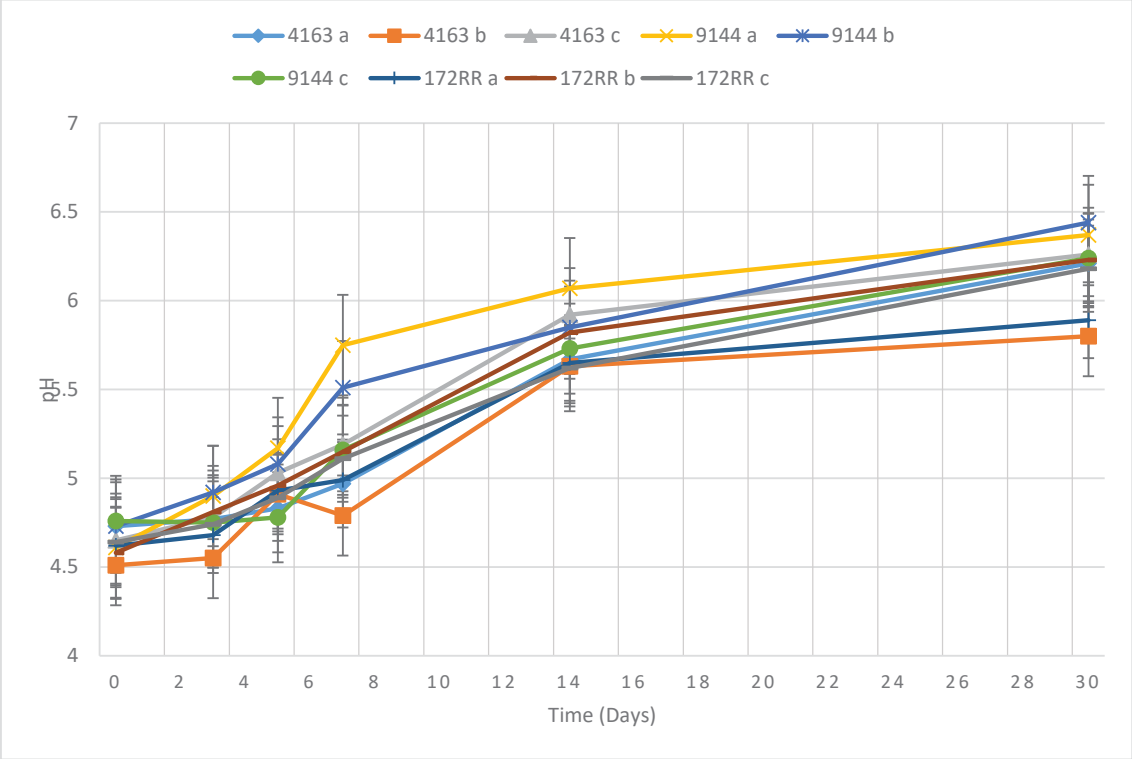


Figure 34 Changes of pH at the core of Camembert cheese during the ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

4.1.2.3.2.2 Water activity

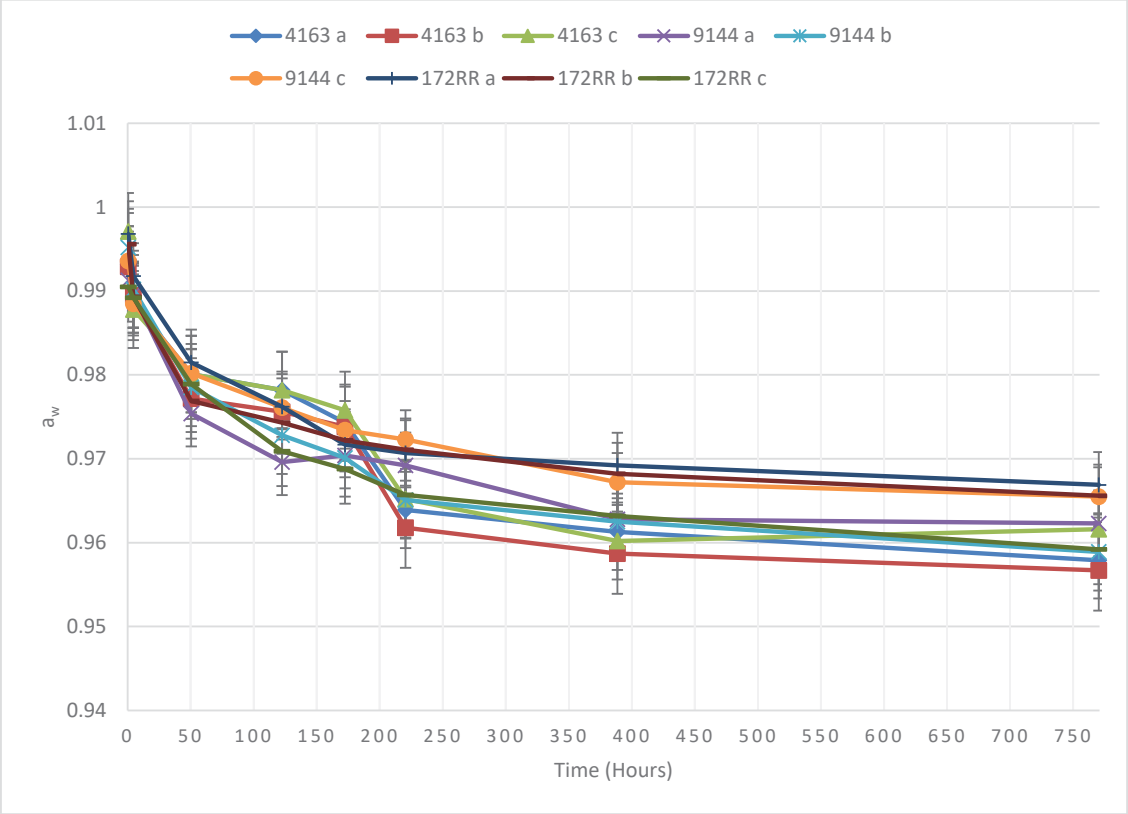


Figure 35 Changes of water activity at the core of Camembert cheese throughout the manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

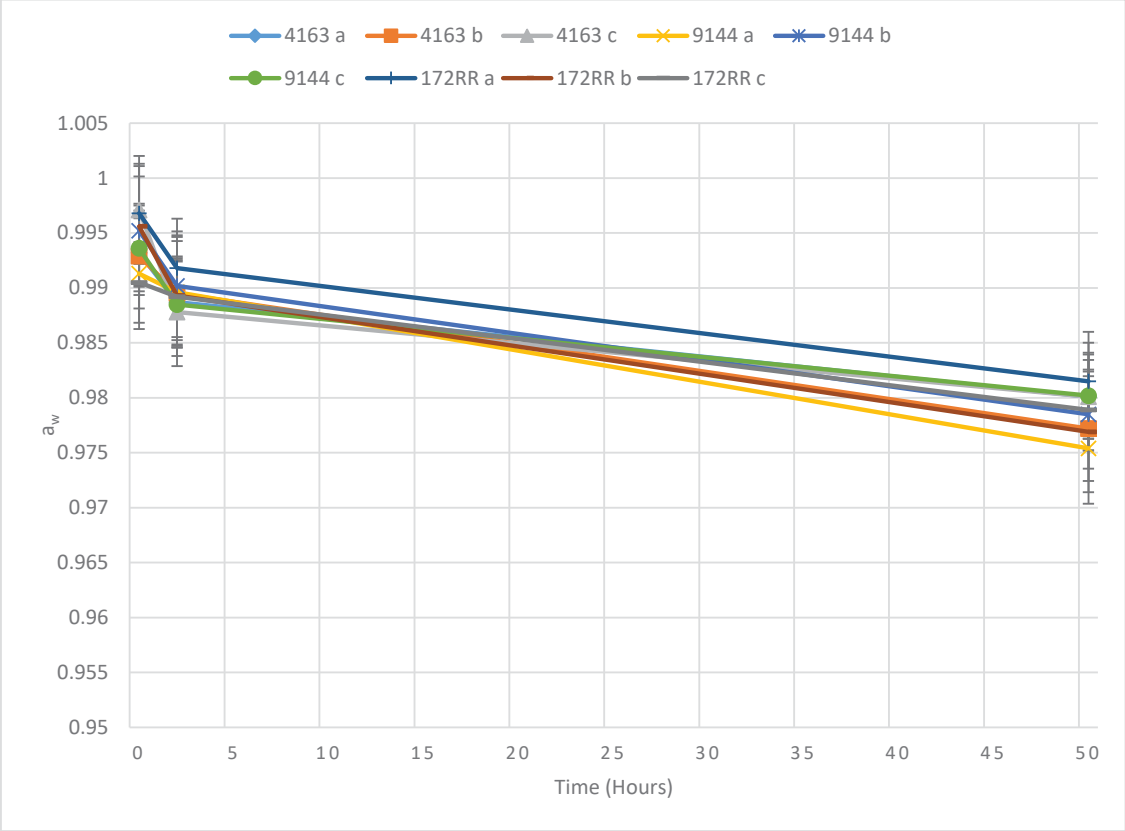


Figure 36 Changes of a_w at the core of Camembert cheese during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

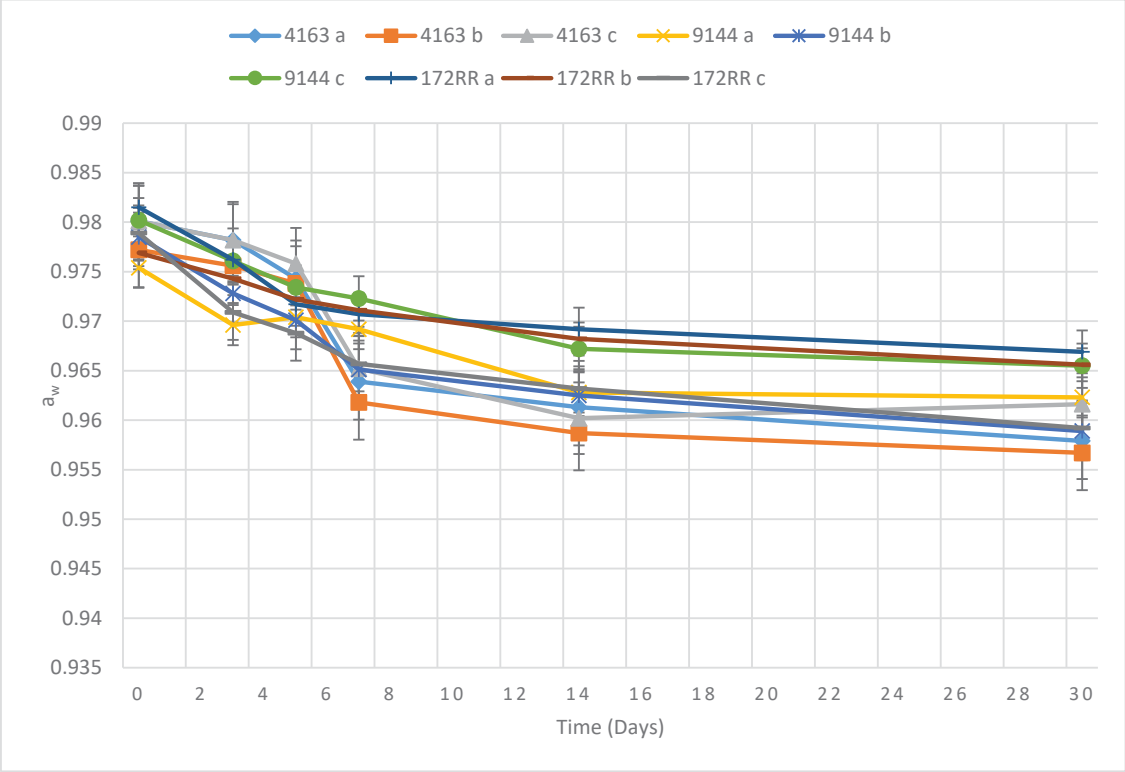


Figure 37 Changes of a_w at the core of Camembert cheese during the ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key; the a,b,c in the figure represent triplicate trials. Error bars are generated by the standard deviation of triplicates in every single trial.)

The core of each cheese was tested at the same time points as the surface: the end of moulding and the 3rd, 5th, 7th, 14th, and 30th days of ripening. The core of cheese pH ranged between 5.80 and 6.44 at the end of the experiment. The minimum pH was recorded at the end of moulding. The pH increased gradually during the ripening period except in sample 4163 (b), the pH of which decreased slightly from day 5 to day 7. The pH of all samples increased after 7 days of ripening with only two samples with a pH lower than 6 (172RR a and 4163 b). The water activity in the core of the cheese decreased in almost every sampling period except for 9144 (a) which increased slightly (0.001) from day 3 to day 5, and 4163 (c) which increased from day 14 to 30. The water activity was not low enough to prevent the growth of *S. aureus*. The T-test results for both pH and water activity did not indicate a significant difference between different strains ($p>0.05$).

4.2 Salt content

The salt content of cheeses was determined after 14 days of ripening. Each group of cheese samples (with three different strains) was tested three times. The results of all samples were at a similar level ranging from 1.31 – 1.44 %, which were significantly lower than the upper limit (25%) for *S. aureus* growth. The salt content in cheese is not able to inhibit the growth of *S. aureus*. For detailed calculation, please refer to section 8.22.

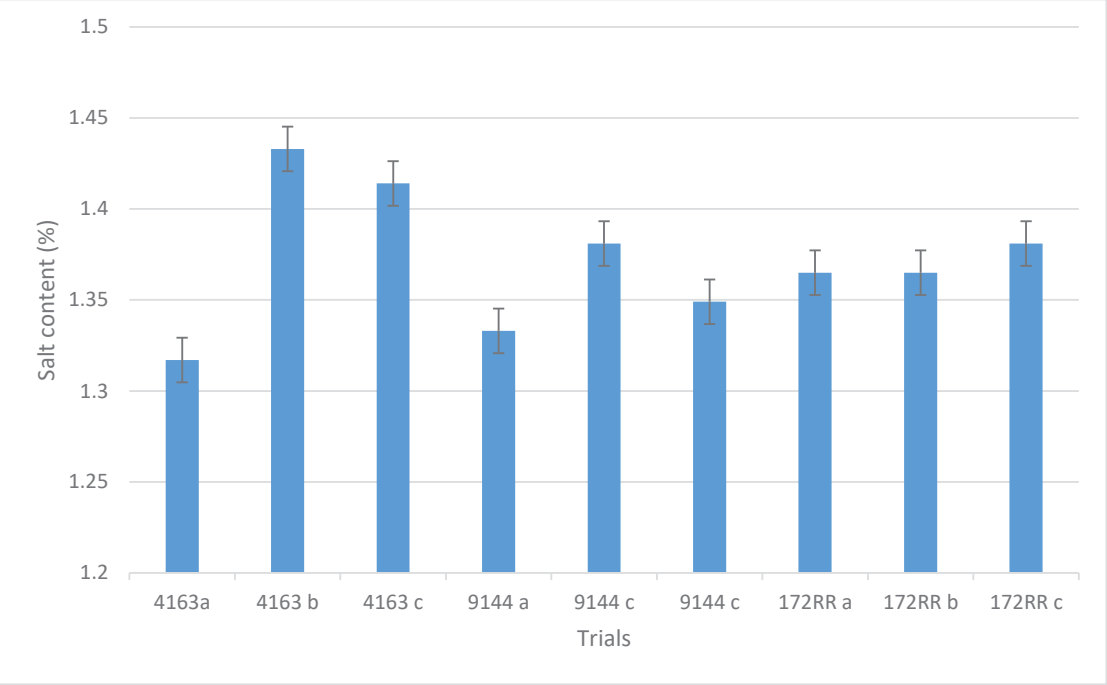


Figure 38 Salt content in cheese samples at the 14 days of ripening

4.3 Control groups (Cheeses Without Culture Blend)

In order to further investigate whether the addition of the Culture Blend is a factor influencing the fate of *S. aureus* in cheese, comparison cheese groups without the addition of starter culture and secondary flora were made for each strain.

4.3.1 Surface of the controlled Cheeses

4.3.1.1 Survival of *S. aureus*

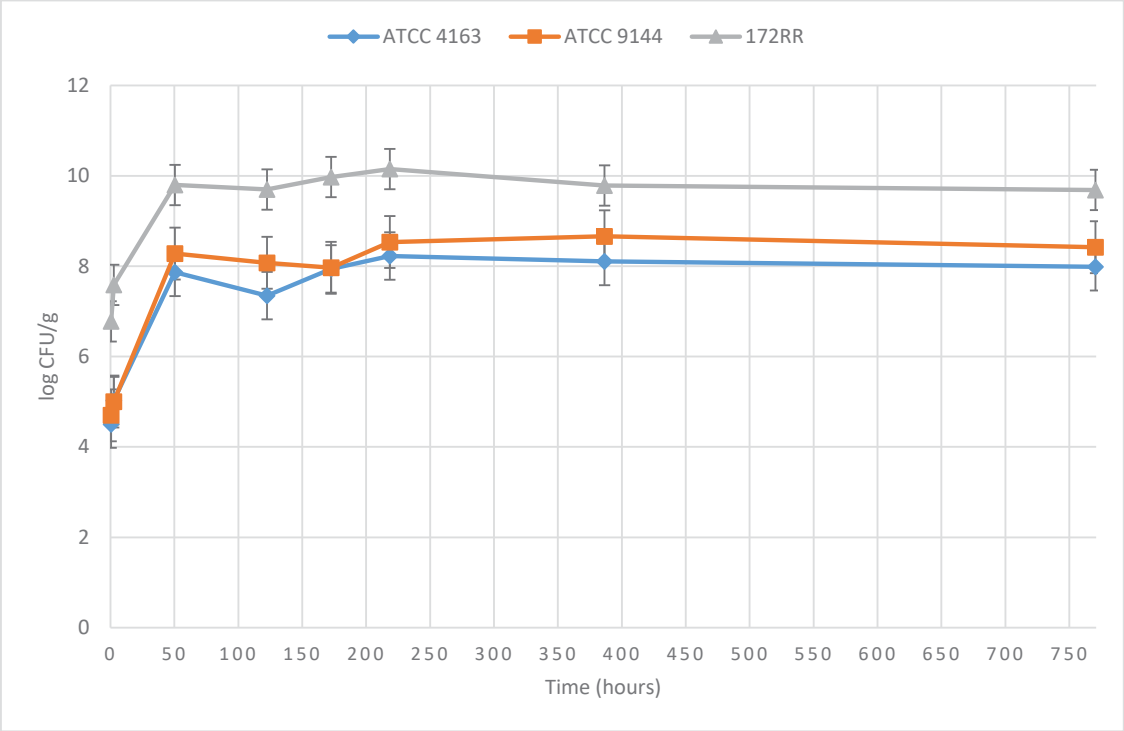


Figure 39 Survival of *S. aureus* at the surface of controlled Camembert cheese (without culture blend) throughout manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

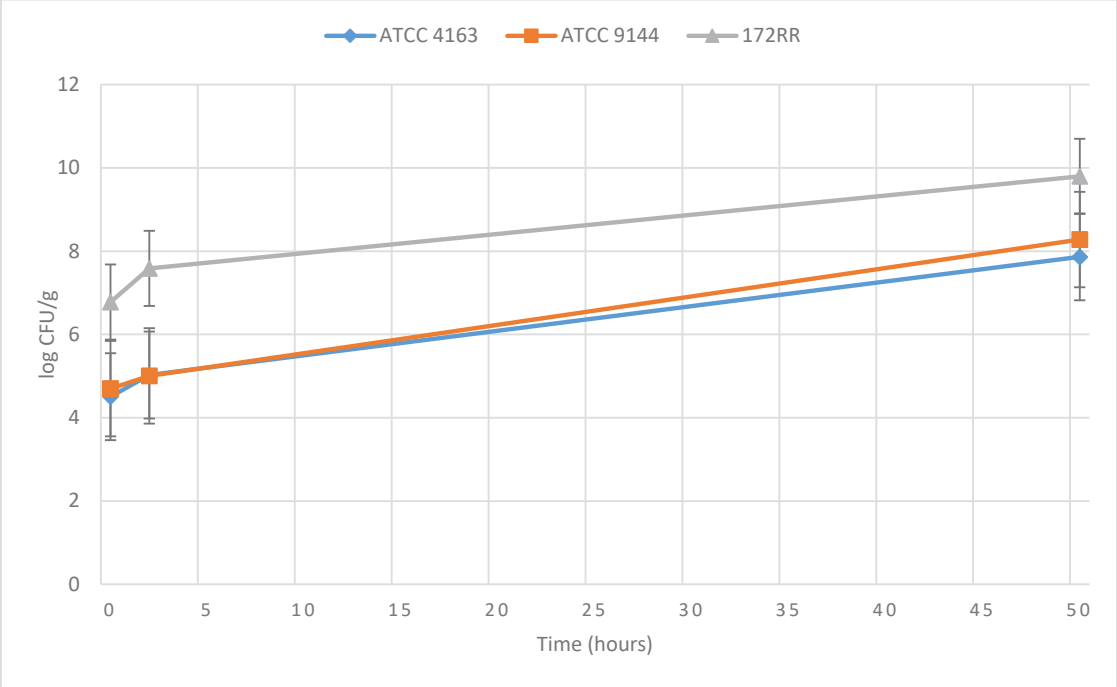


Figure 40 Survival of *S. aureus* at the surface of controlled Camembert cheese (without culture blend) during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

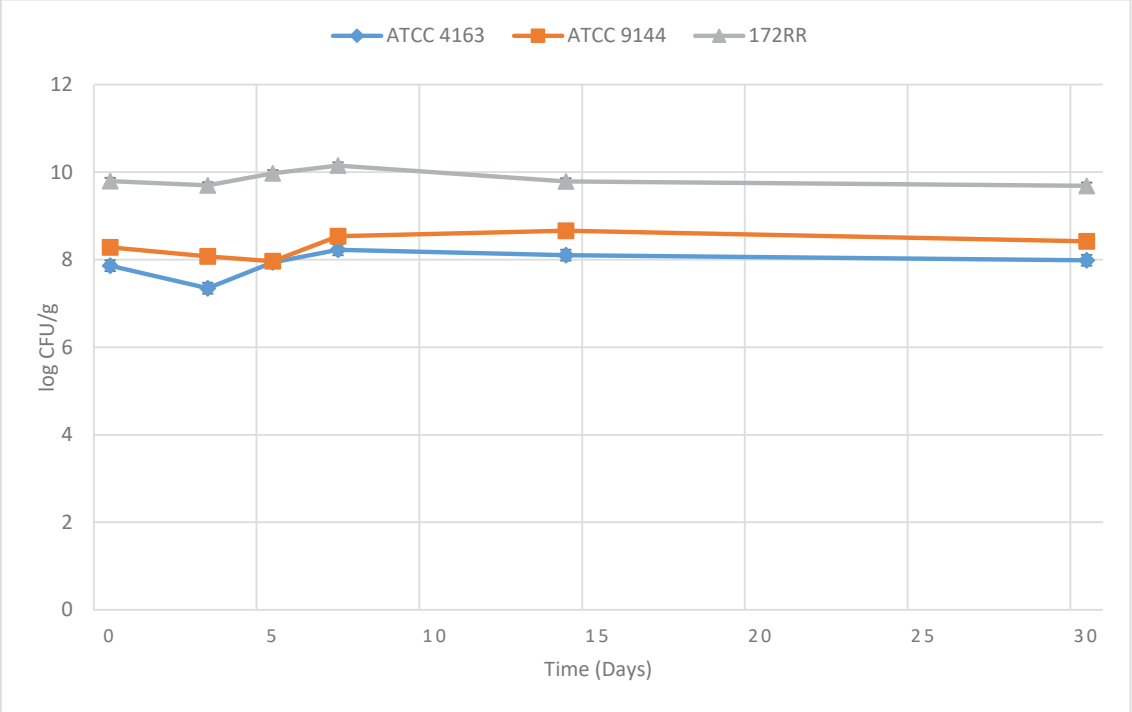


Figure 41 Survival of *S. aureus* at the surface of controlled Camembert cheese (without culture blend) during ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

As we can see from Figure 39 and 40, the counts of all three *S. aureus* strains increased during the manufacturing stage, which was complete opposite to that in standard camembert cheeses. The counts didn't show obvious changes during the ripening period, which was similar to the cheeses in the main trial. The increase in the number of *S. aureus* at the end of testing was between 2.4 – 2.9 logs. The results between two reference strains didn't show a significant difference ($P>0.05$). The differences between each reference strain and the dairy strain (172 RR), however, were statistically significant ($P<0.05$). The reason results in this difference was assumed to be the large difference in initial bacteria level since all three samples showed a similar trend on growth.

4.3.1.2 Physical parameters

4.3.1.2.1 pH

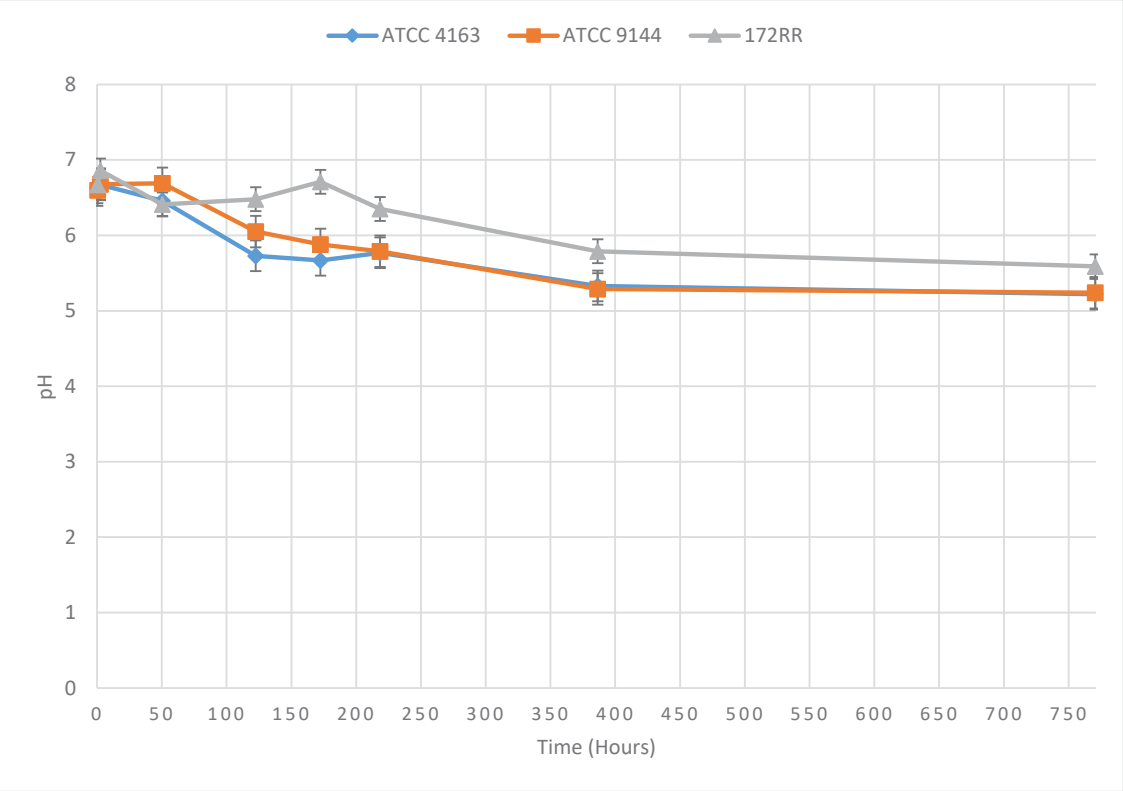


Figure 42 Changes of pH at the surface of controlled Camembert cheese (without culture blend) throughout manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

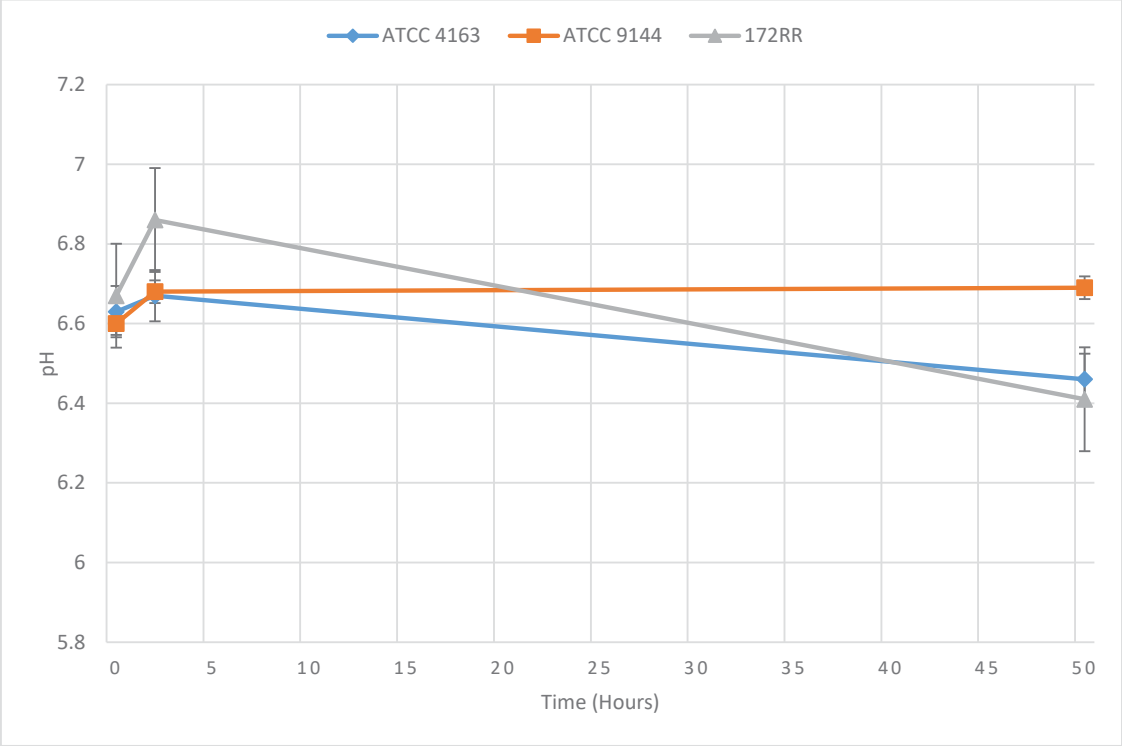


Figure 43 Changes of pH at the surface of controlled Camembert cheese (without culture blend) during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

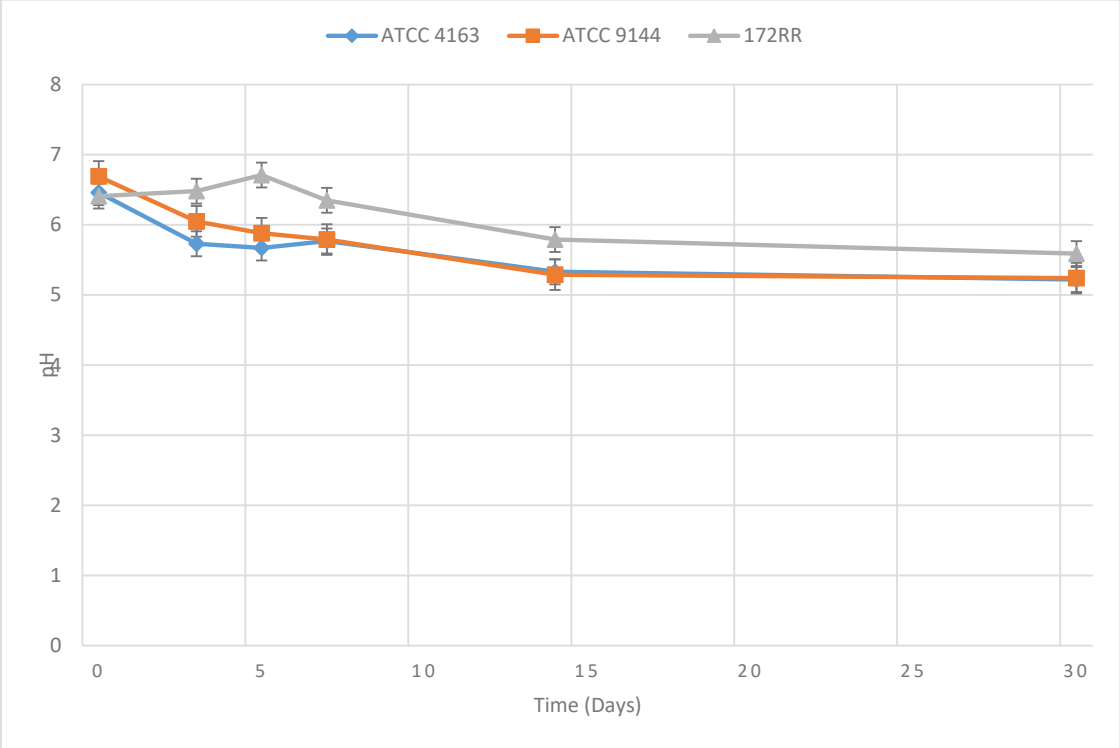


Figure 44 Changes of pH at the surface of controlled Camembert cheese (without culture blend) during ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

4.3.1.2.2 Water activity

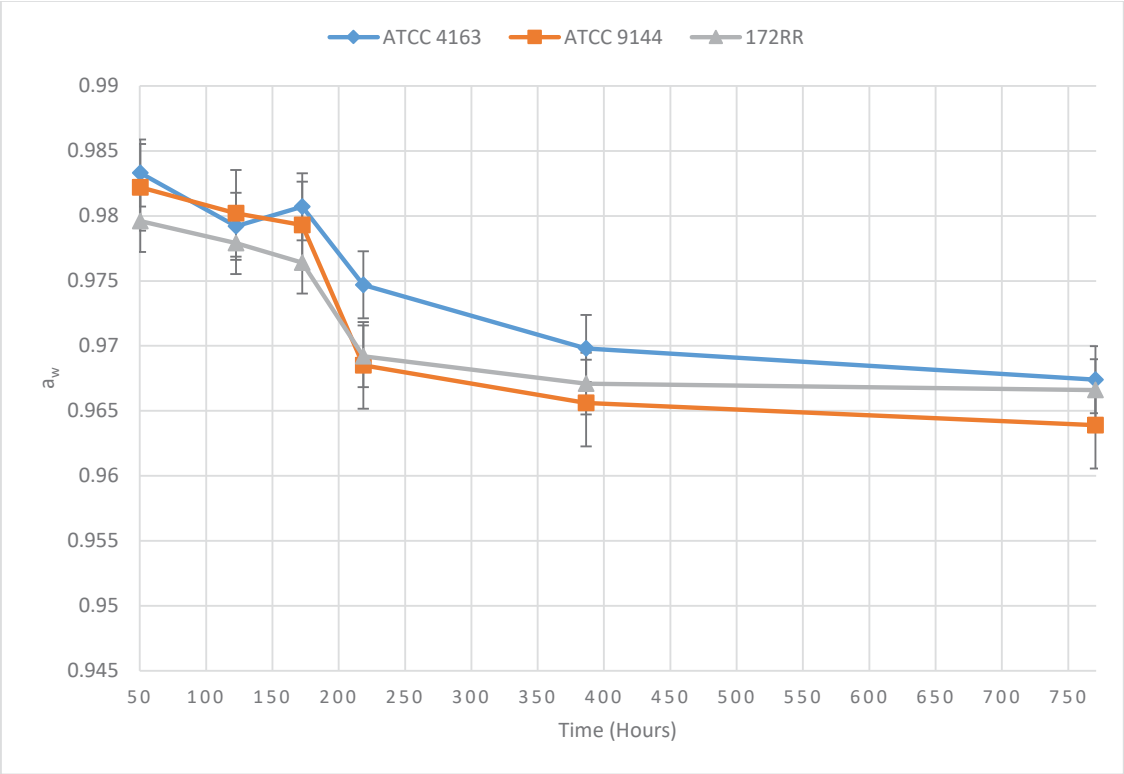


Figure 45 Changes of a_w at the surface of controlled Camembert cheese (without culture blend) throughout manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

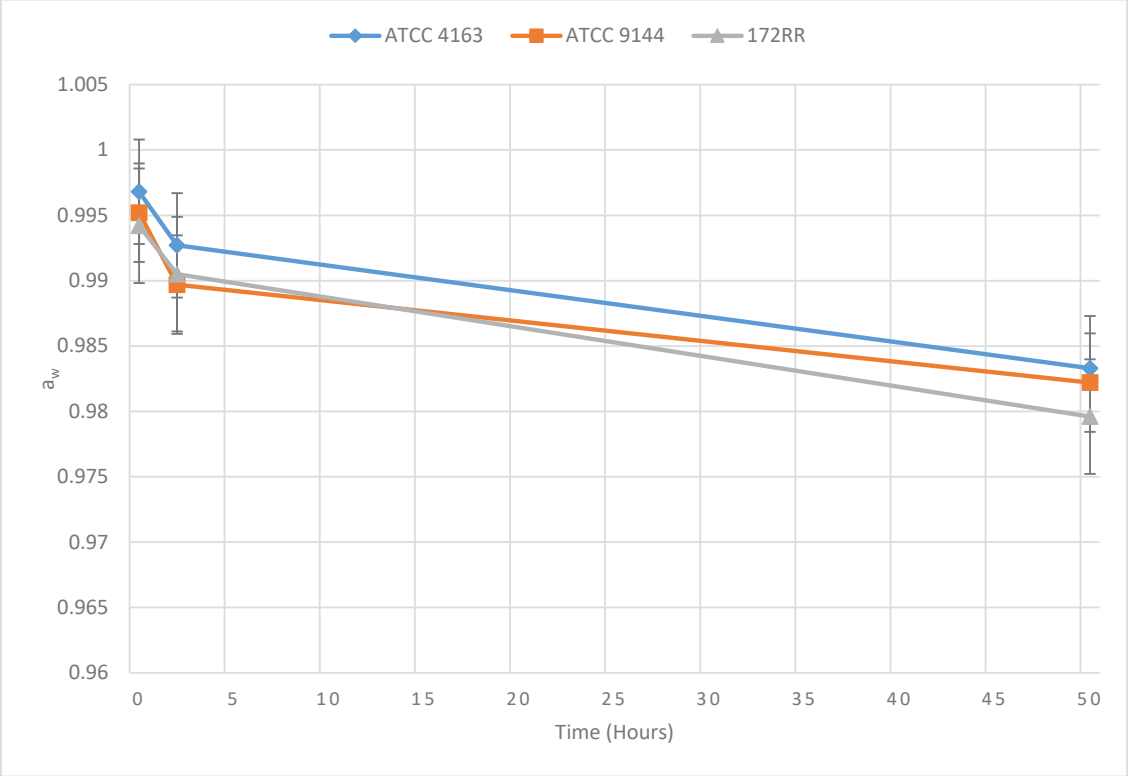


Figure 46 Changes of pH at the surface of controlled Camembert cheese (without culture blend) during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

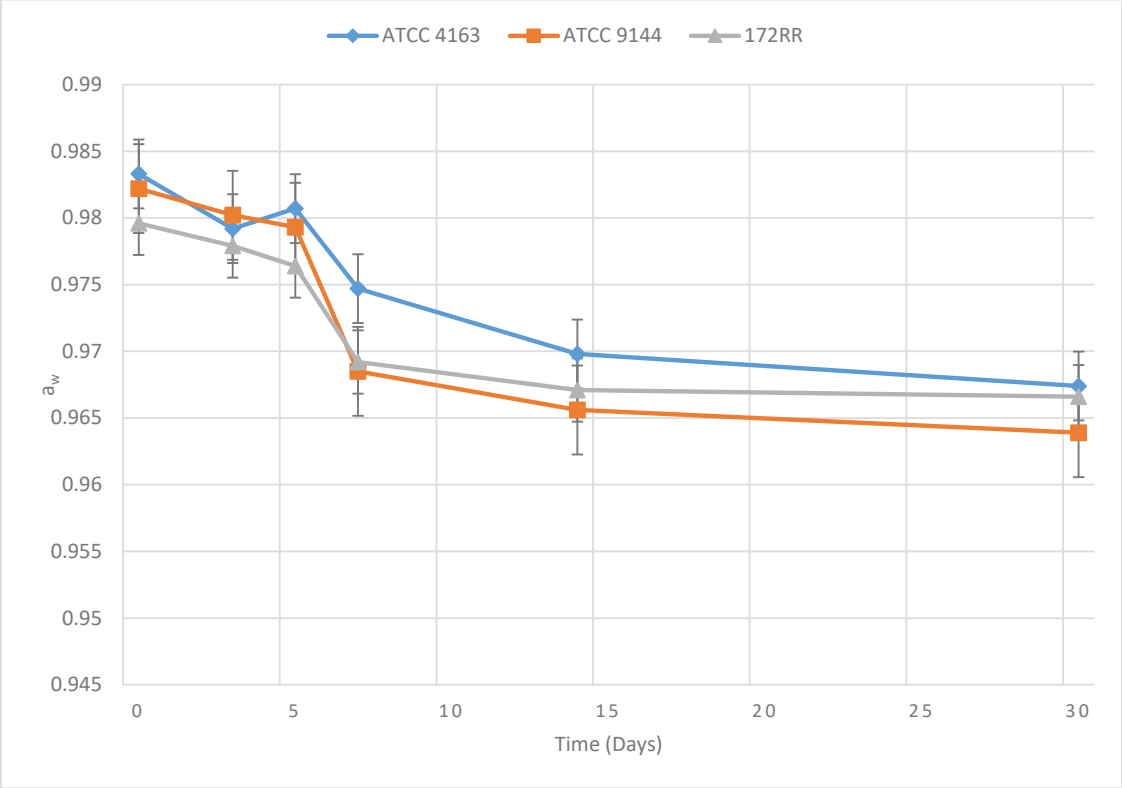


Figure 47 Changes of a_w at the surface of controlled Camembert cheese (without culture blend) during ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

These control cheeses did not show a dramatic decrease in pH during the draining time, but decreased gradually until the end of ripening. The pH of all control samples resulted in a pH range between 5.2 -5.6, which was also the minimum value of pH in the whole period. The water activity of samples kept decreasing and ended up at a level from 0.96 – 0.97. Both pH and water activity values were suitable for *S. aureus* growth throughout the manufacture and ripening period. No significant difference was identified between different strains ($P>0.05$).

4.3.2 Core of the Controlled cheeses

4.3.2.1 Survival of *S. aureus*

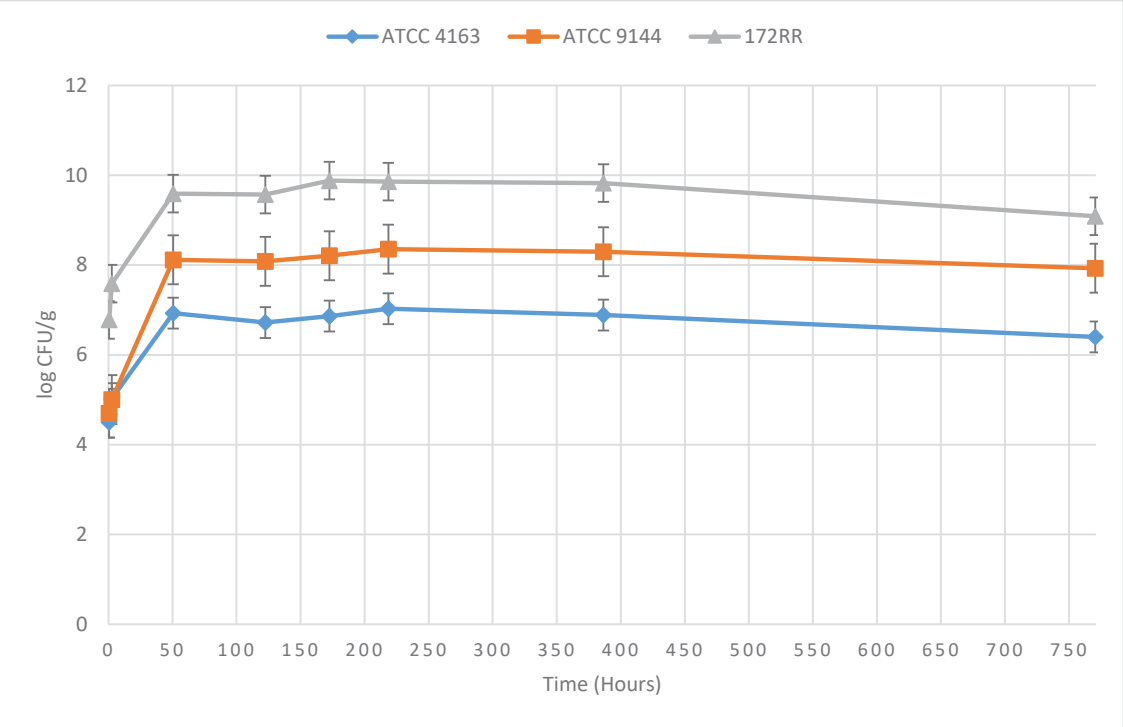


Figure 48 Survival of *S. aureus* at the core of control Camembert cheese (without culture blend) throughout manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

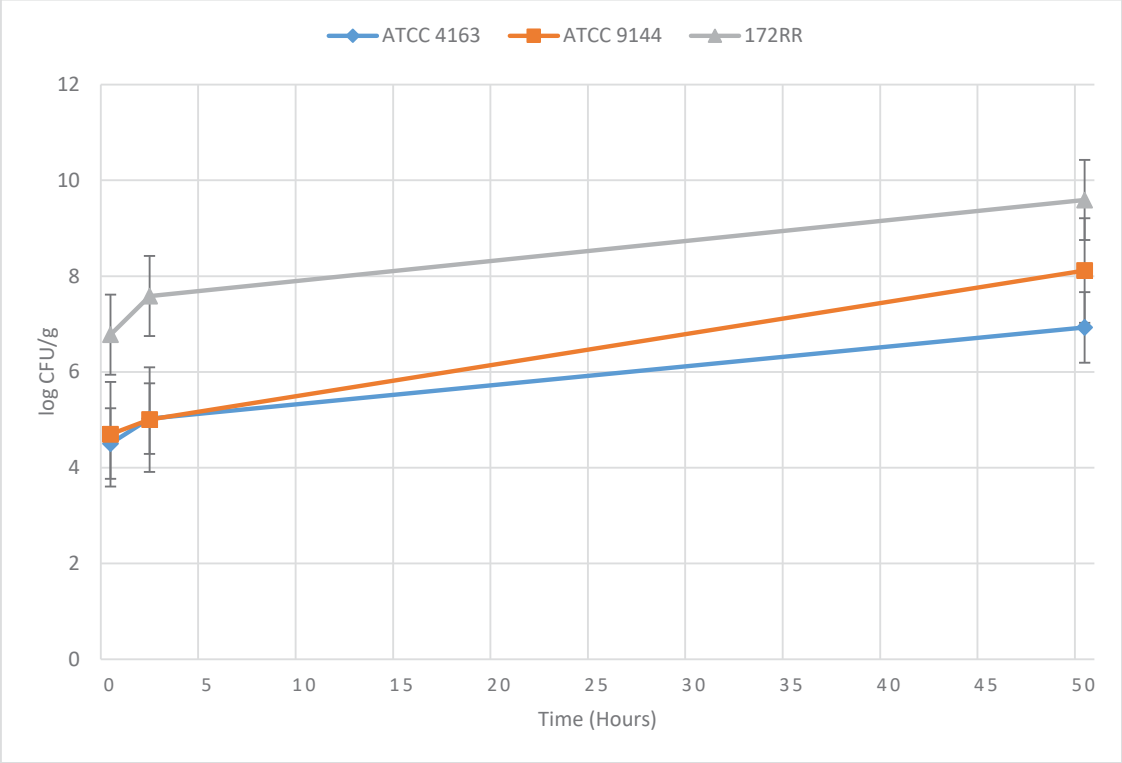


Figure 49 Survival of *S. aureus* at the core of control Camembert cheese (without culture blend) during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

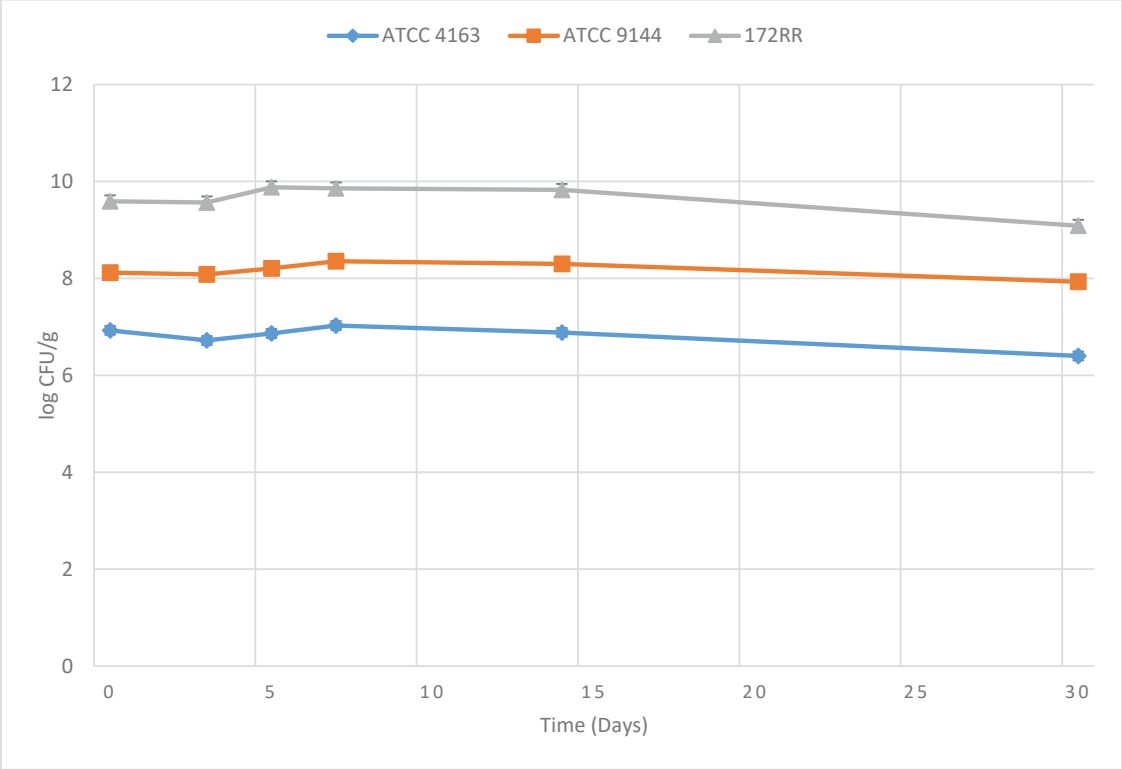


Figure 50 Survival of *S. aureus* at the core of control Camembert cheese (without culture blend) during the ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

Similar to the results from the surface, the counts of *S. aureus* in the core of control cheeses increased during manufacture followed by a stable period until the end of the testing day. The increase of *S. aureus* counts until the end of testing was between 1.8 – 2.3 logs. The T-test results were similar to the surface part. No significant difference was found between reference strains ($P>0.05$), while the differences between reference and dairy strain (172 RR), were significant ($P<0.05$), which may be caused by the different level of initial inoculum.

4.3.2.2 Physical Parameters

4.3.2.2.1 pH

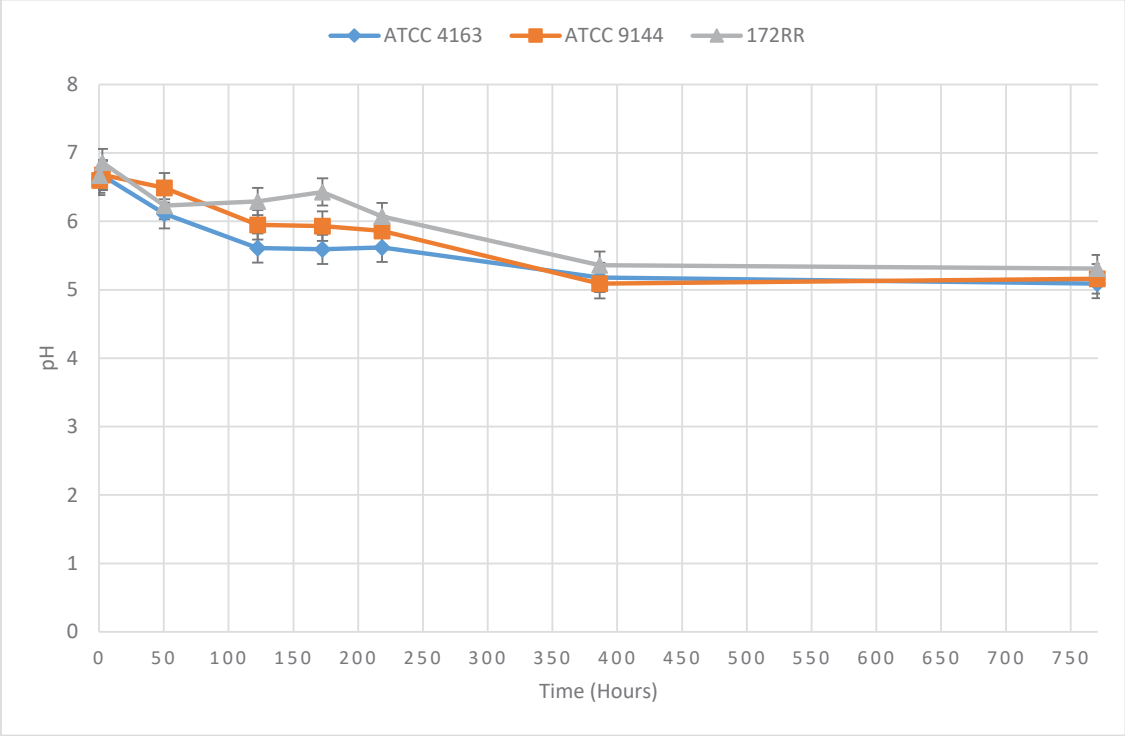


Figure 51 Changes of pH at the core of control Camembert cheese (without culture blend) throughout manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

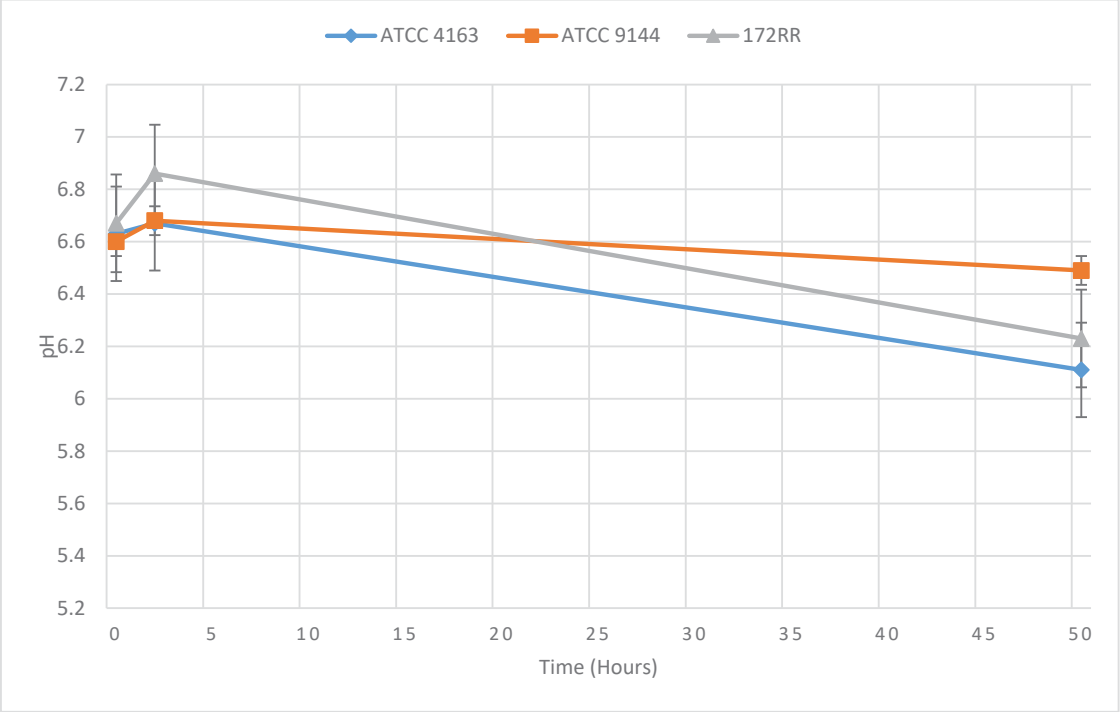


Figure 52 Changes of pH at the core of control Camembert cheese (without culture blend) during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

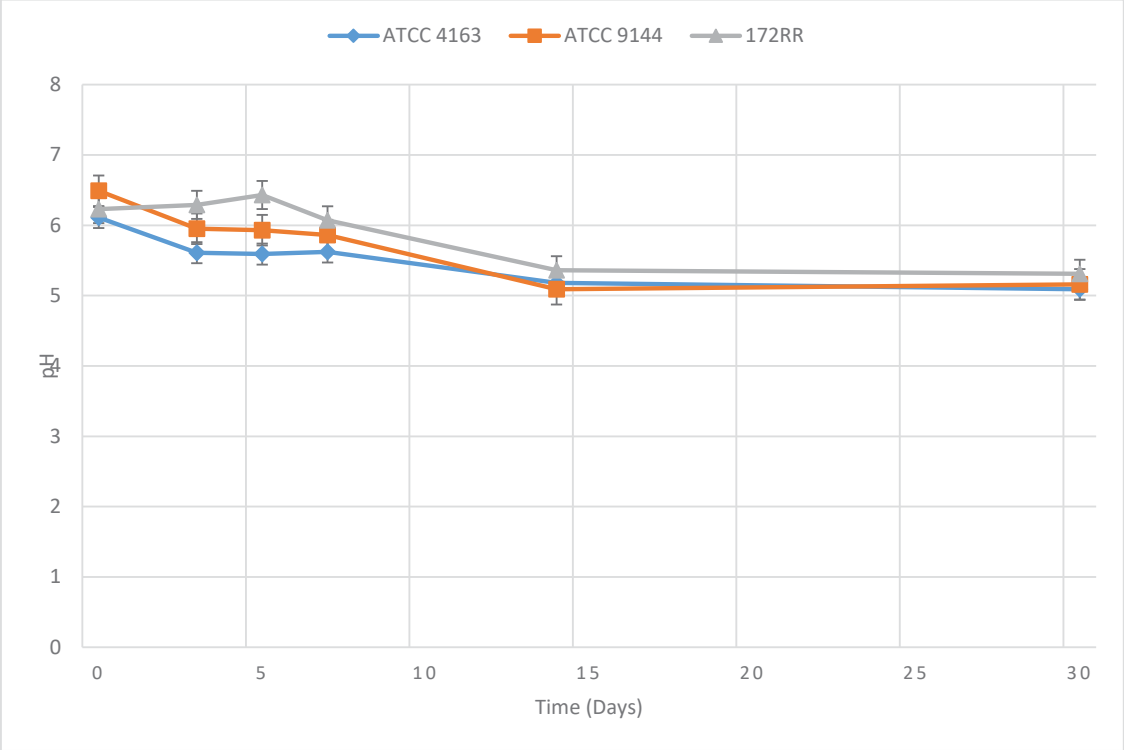


Figure 53 Changes of pH at the core of control Camembert cheese (without culture blend) during the ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

4.3.2.2.2 Water activity

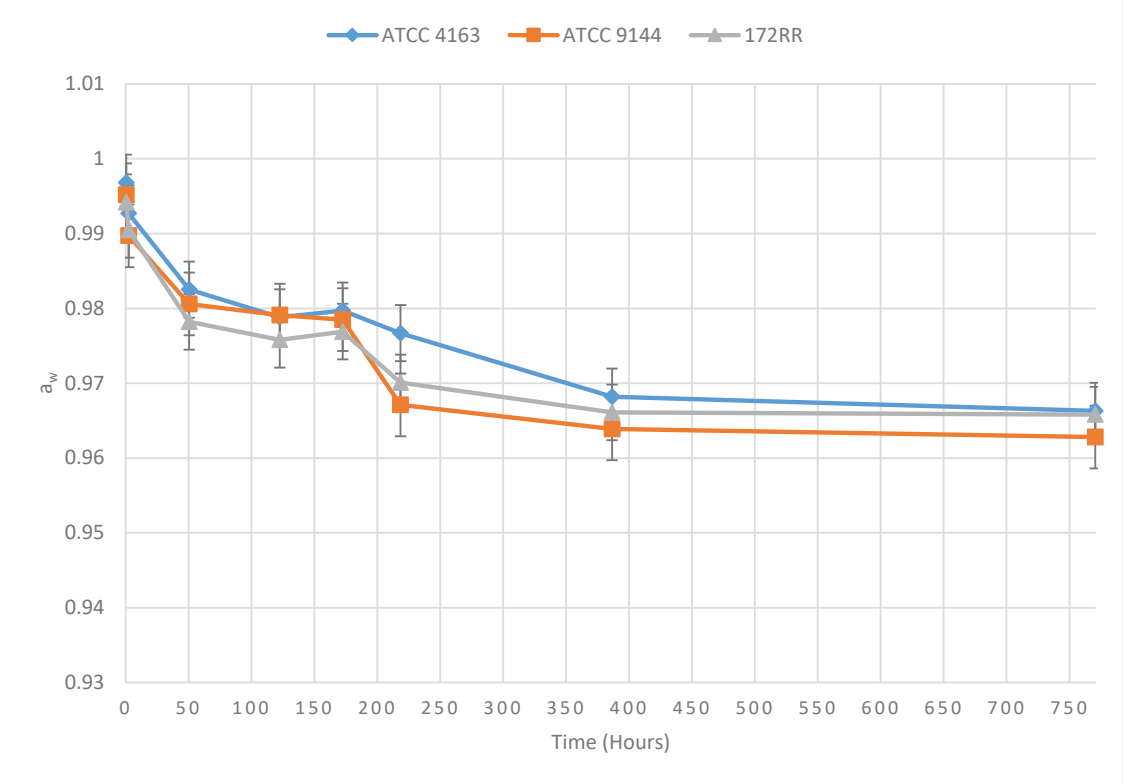


Figure 54 Changes of a_w at the core of control Camembert cheese (without culture blend) throughout manufacture and ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

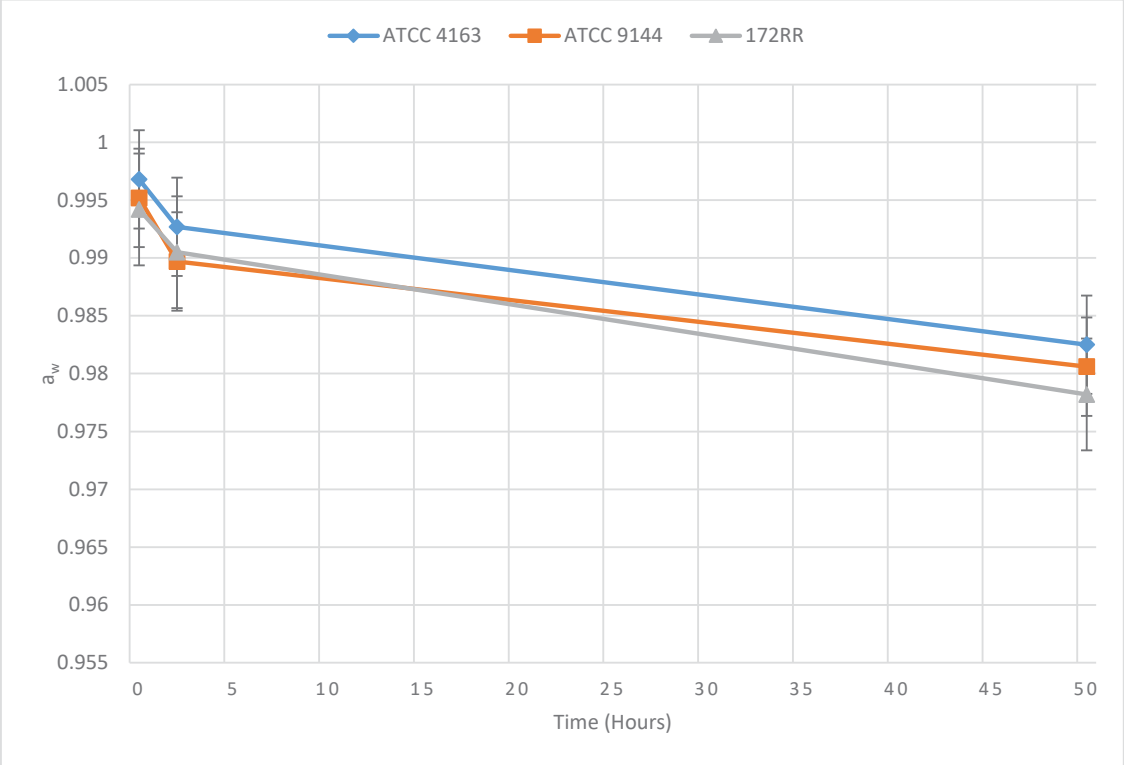


Figure 55 Changes of a_w at the core of controlled Camembert cheese (without culture blend) during manufacture

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

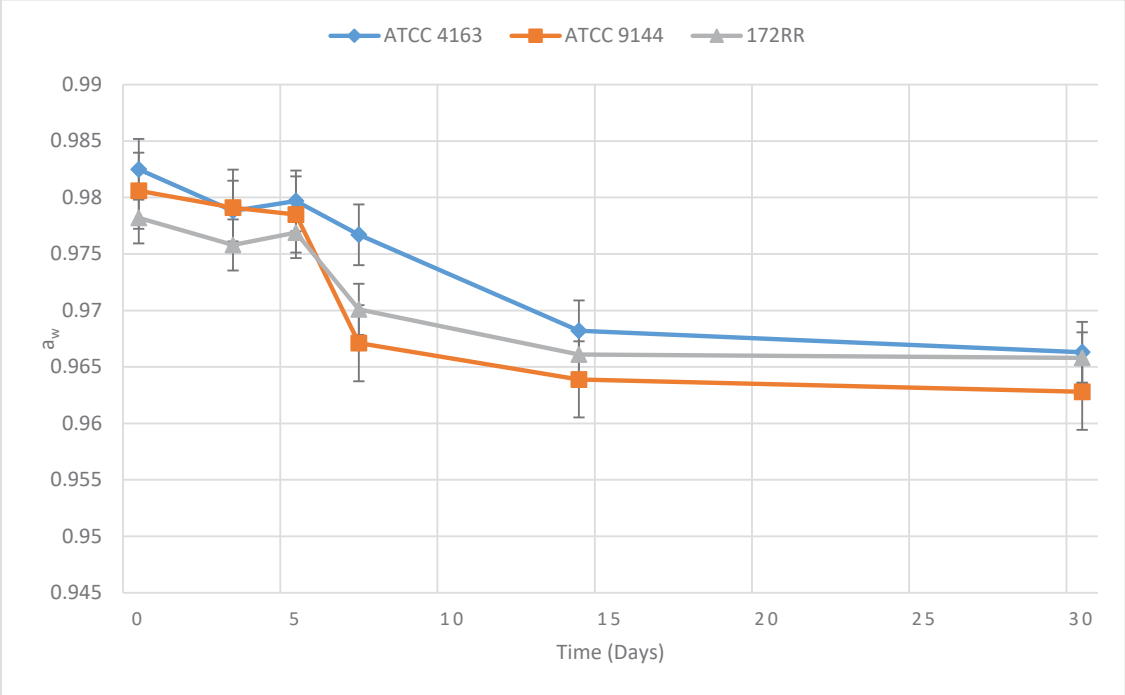


Figure 56 Changes of a_w at the core of control Camembert cheese (without culture blend) during the ripening period

(Coloured line represent different strains of *S. aureus* inoculated into the cheese as indicated in the key. Error bars are generated by the standard deviation of triplicates in every single trial.)

Both the pH and water activity at the core parts of control cheeses gradually and slowly decreased until the end of testing. The decreasing pH and water activity were similar to that on the surface. The pH of samples decreased to a level between 5.09 and 5.31 while the water activity ranged from 0.96 to 0.97. The pH and water activity are suitable for *S. aureus* growth. The difference between strains was not significant (P>0.05).

5 Discussion

Only fresh quality pasteurized milk was used in this study, and none of the milk samples was found to be contaminated before the experiment. The focus of the investigation was the effect of the cheese manufacture and ripening on the survival of *S. aureus*. Generally, the results of this study indicate a 1.6 to 3.1 log reduction of *S. aureus* during the whole manufacturing process and ripening period. The results may vary from different parts of cheeses, different levels of bacteria inoculations and different strains. More specifically, the log reductions of *S. aureus* on the rind, surface and core parts of cheeses are 1.9 – 3.0, 1.6 – 3.1 and 2.4 – 2.9, respectively. Further, the results indicate that 172 RR, the strain retrieved from cattle, has more difficulty than the other strains tested in this study in surviving in Camembert cheeses, especially before the ripening stage.

5.1 Results discussion

5.1.1 Survival of *S. aureus* During the Manufacture and Ripening of Camembert cheese

5.1.1.1 Manufacture stage

When the cheese curd was formed, after the addition of the culture blend and rennet, the counts of all three strains of samples increased, similar to the control groups that did not contain the culture blend, as shown in Figure 9 and 39. The slight increase of *S. aureus* during the curd formation was considered to be due to the loss of volume and the normal growth of *S. aureus*, which, in line with the hypothesis for this study and reported in previous studies in terms of semi-hard cheeses (Delbes et al., 2006; Meyrand et al., 1998). The increased density of *S. aureus* during coagulation and drainage may be caused by the physical entrapment of cells as well as microbial growth.

During draining, the volume of cheese kept decreasing until the whey was fully expelled. It was hypothesized that the counts of *S. aureus* may still increase due to physical entrapment. In fact, the count of bacteria, however, decreased at this stage. During the period of drainage,

the acidification continued until the pH was between 4.4 and 4.8, which is not low enough to cause the death of *S. aureus*. It was assumed that the bacterial death was probably related to the combination of decreasing pH and the activity of culture blend.

In order to identify the reason for bacterial death, further experiments with control groups in different conditions were carried out. As presented in Section 4.3, the changes of *S. aureus* count in cheeses without culture blend were documented and compared. Between 2 to 50 hours (equal to the time length before cheese drainage), the counts in all control group cheeses, without culture blend, increased and the pH decreased. In this case, however, the pH of samples without culture blend resulted in levels between 6.4 – 6.7 after 50 hours, which is suitable for *S. aureus* growth. It can now be assumed that the death of *S. aureus* during the draining period was due to the combined effect of culture blend activity and low pH value. Similar results were identified in previous research, which demonstrates that the growth rate of *S. aureus* was inhibited by *L. lactis,* even when the pH was around 6.4. Strong inhibition of growth was identified when pH was lowered to 5 by adding *L. lactis* and no growth occurred when pH was lower than 4.5 (Charlier, Even, Gautier, & Le Loir 2008). However, until now, no study has found a reduction of *S. aureus* counts in a pasteurized milk Camembert cheese.

For the early stages of manufacture of Camembert cheese, *S. aureus* numbers decrease due to the acid pH and the activity of culture blend.

5.1.1.2 Ripening Stage One (0 – 14 days)

The count of *S. aureus* continued to decrease during the first ripening stage (14 days at 12 °C). The cheese samples were kept at 12 °C in an incubator, which was still suitable for *S. aureus* growth. The counts of *S. aureus* in all three parts of the cheese decreased slower than during manufacture. The water activity of all cheese samples at this stage was higher than 0.95, ideal for the growth of *S. aureus*. The lower death rate compared to the drainage time was assumed to be due to the increase of pH value caused by the consumption of lactate and lactose (McSweeney, 2007). During this period of ripening the pH of the surface of the cheese increased from a starting point of 4.6 and 4.8 to between 5.8 and 6.3 while the core parts increased from 4.5 - 4.8 to 5.6 - 6.1. Since the mould rind of cheese was not well grown until the 5th ripening day, further testing was done after that day when the pH of cheese rind increased. The pH level of all parts of cheeses during the first ripening stage was suitable for

S. aureus growth. During the first 7 ripening days, the rate of decrease in counts in the surface and core part of the cheese were relatively low compared to the that during drainage. From day 3 to day 5 of ripening, a slight increase of bacterial counts was identified in some cheese samples. According to the results from the control group cheeses (without adding culture blend), the counts of *S. aureus* during this period were relatively stable, which was similar to the regular Camembert cheese samples.

5.1.1.3 Ripening Stage Two (15 – 30 days)

At this stage, the number of *S. aureus* in cheese remained stable or showed a slight decrease. Reason for this was assumed to be the low temperature. Cheeses at this stage were being kept at 4 °C for further ripening and storage. Since the growth boundary of *S. aureus* is 6 °C – 48 °C, the temperature during this period was not able to support growth. Although the pH of all parts of cheeses kept increasing during this period, *S. aureus* could not grow at this temperature.

5.2 Importance and Methods for Preventing the Contamination of Camembert cheese with *S. aureus*

According to previous research, the presence of *S. aureus* in dairy products has been a problem for a long time. Staphylococcal food poisoning (SFP) outbreaks have been reported due to the consumption of Minas cheeses in Brazil (do Carmo et al., 2002) and Monte Veronese (a kind of semi-hard cheese) in Italy (Poli et al., 2007). In Switzerland, from 1996 to 2006, 7 out of 11 reports of SFP outbreaks were caused by the consumption of cheeses (Baumgartner, 2008; Hummerjohann, Naskova, Baumgartner, & Graber, 2014). The primary contamination sources leading to these outbreaks are the food handlers, cattle mastitis and temperature abuse.

This current study, however, showed a 1.5 – 3 log death of *S. aureus* during the manufacture and ripening process, which provides some confidence in the safety of cheese that may become contaminated with *S. aureus*. The cheese-making process reduced *S. aureus* numbers

through pH reduction during drainage, the activity of cheese culture blend and the low temperature (4 °C) for ripening and storage. Similar results were reported from previous work in dairy products. Charlier et al. (2008) investigated the inhibition of *S. aureus* growth in milk and indicated that no growth occurred when the pH level was adjusted to 4.4 and 4.5 by adding *L. lactis* (Charlier et al., 2008). Another study, which was also carried out in milk, reported that neither the growth of *S. aureus* nor enterotoxin production occurs when the pH of the environment is acidified to 4.5 by lactic acid (Tatini et al., 1971). Many other studies investigating the inhibition of *S. aureus* growth other than in dairy products had also confirmed a significant difference in the lower limit growth when the pH was adjusted by different acids (Tatini, 1973). The growth of *S. aureus* and enterotoxin production can be strongly inhibited by the presence of lactic acid or organic acids like citric and acetic acid even when the pH is at 5 (de Oliveira, Stamford, Neto, & de Souza, 2010; Lancette & Bennett, 2001; Le Loir, Baron, & Gautier, 2003; Tatini, 1973)

Apart from the effect of pH, the cheese culture blend and temperature, some other factors may also contribute to the death of *S. aureus* in Camembert cheese. A well-demonstrated feature of *S. aureus* is its high sensitivity of *S. aureus* to microbial competition, especially in milk and fermented products (Le Loir et al., 2003). One study indicated that the *S. aureus* growth and enterotoxin production in milk would be inhibited with the high numbers of competing microorganisms (Genigeorgis, 1989). The competition between *S. aureus* and lactic acid bacteria has been investigated in cheeses (Otero, Garcia, & Moreno, 1988; Vernozy-Rozand et al., 1998) and milk (Charlier et al., 2008). The inhibition effects provided by lactic acid starter bacteria are primarily due to the nutritional competition, lowered pH, hydrogen peroxide and lactic acid production (Charlier et al., 2009; Charlier et al., 2008; Haines & Harmon, 1973; Le Loir et al., 2003). Sometimes, the production of other antimicrobial substances like organic acids and bacteriocins may also contribute to the inhibition of *S. aureus* growth (Charlier et al., 2009; Le Loir et al., 2003).

Staphylococci need to grow to approximately 10^6 CFU/g to produce sufficient enterotoxin (do Carmo et al., 2002) to cause food poisoning. The level of the inoculum used in this study was much greater than would be expected from contamination that may occur during manufacture and the reduction in numbers during manufacture will ensure minimal risk from any residual contamination in the product stored under refrigeration. The only growth fact

was found during curd formation, which was assumed to be caused by the physical entrapment according to previous studies (Delbes et al., 2006). Toxin production will be significantly inhibited when pH is lowered to 5 by lactic acid (Charlier et al., 2009; Charlier et al., 2008; Le Loir et al., 2003; Tatini, 1973). Thus, only highly contaminated cheese (>10^6 CFU/g) is likely to pose a risk of producing enterotoxin, and this seems highly unlikely. Nevertheless, although the chance is minimal, it is still possible that cheeses may be contaminated by food handlers during manufacture and some other processes.

Several CCPs during the cheese production are identified:

a. Milk before and after the pasteurization (enterotoxin may be produced before pasteurization);
b. Cheese after being salted by food handlers (humans are a reservoir for *S. aureus);*
c. Correct temperature at the second ripening stage and storage period (*S. aureus* may grow if the temperature is over 6 °C).
d. Packing and distribution under refrigeration.

Good hygiene is required for Camembert production. Preventing the contamination of *S. aureus* is essential to ensure that there is no risk from SFP. The number of *S. aureus* may not be precise enough to indicate whether the product is actually harmful. Enterotoxin production in abused milk before pasteurization is a potential risk that cannot be mitigated by the manufacturing process. Thus, it is recommended that testing for *S. aureus* may be better to be replaced by the detection of Staphylococcal enterotoxins.

Interestingly, the dairy strain of *S. aureus* cell numbers showed a greater reduction than the reference strains during the manufacture of the cheese. This shows that the dairy strain chosen for this trial is more sensitive to the pH and starter cultures than the reference strains. Strains from a natural environment often differ from those of reference cultures that have been cultivated in a laboratory for many generations. It would be interesting to repeat with other dairy isolates to see if this higher sensitivity of these strains is universal for the dairy isolates or if this is simply a property of this particular isolate.

6 Conclusion

Two major conclusions can be drawn from this study. First, *S. aureus* will decrease during the manufacturing and ripening of Camembert Cheeses. The greatest decrease occurs at the end of drainage, at which point a 0.8 -2 log decrease was observed. Second, the combined effect of culture blend (starter and secondary flora) activity and low pH are believed to contribute to the death of *S. aureus.*

Although the *S. aureus* decreased, some survived until the end of the last test day. Good hygiene and enterotoxin detection are necessary to ensure safety during the manufacturing process and before packaging.

6.1 Limitations and Further recommendations

There are still some issues have not been included in this study. Only pasteurized milk was used here. Internationally, there are plenty of factories using raw milk for cheese production, in particular in France. Cattle mastitis is a source of *S. aureus*, which was also the origin of the 172 RR strain used in this trial. The introduction of raw milk into the manufacture of Camembert cheese will increase the risk of the of *S. aureus* contamination and enterotoxin presence in the product. Further study is recommended for three purposes:

a. Detection of enterotoxin during the whole manufacture processes: milk (raw or pasteurized), cheese curd, after drainage, during ripening and packaging;
b. Identification of the specific strains in the culture blend affecting the survivability of *S. aureus* with pH controlled (factorial design with different combinations of culture strains and pH levels)
c. Repeat a similar study with a wider range of *S. aureus* isolates from milk, cattle and Staphylococcal food poisoning (SFP) sources.

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8 Appendix

8.1 Bacterial Enumeration

In following tables, the numbers represent hours: (1) the origin (0 h) is corresponding to the time before adding rennet; (2) 2 h is curd at the start of drainage; (3) 50 h represents the end of moulding; (4) 122 h, 172 h, 218 h and 386 h are the 3rd day, 5th day, 7th day and 14th day of ripening at 12 °C (1 - 14 ripening days), respectively; (5) 770 h represents the 30th day of ripening at 4 °C (14 - 30 ripening days).

8.1.1 Regular Camembert Cheeses

8.1.1.1 Survival of *S. aureus* in All Trials Inoculated by ATCC 4163

8.1.1.1.1 Initial Stages

Table 3 Viable Cells (CFU/g) at the beginning stages of Camembert cheese manufacture

	BAR*	Curd	Whey
Trial 1	1.28E+05	5.03E+05	3.93E+04
Trial 2	9.50E+05	5.21E+06	6.70E+05
Trail 3	6.31E+06	3.32E+07	3.96E+05
Trial 4	7.94E+06	5.83E+07	4.10E+06
Trial 5	8.66E+04	4.60E+05	3.00E+03
Trial 6	3.65E+05	5.10E+05	9.70E+03
Trial 7	2.34E+07	6.01E+07	2.24E+06
Trial 8	2.31E+04	7.63E+04	1.40E+04

Table 4 Log Viable Cell (log CFU/g) at the beginning stages of Camembert cheese manufacture

	BAR*	Curd	Whey
Trial 1	5.10721	5.701568	4.594393
Trial 2	5.977724	6.716838	5.826075
Trail 3	6.800029	7.521138	5.597695
Trial 4	6.899821	7.765669	6.612784
Trial 5	4.937518	5.662758	3.477121
Trial 6	5.562293	5.70757	3.986772
Trial 7	7.369216	7.778874	6.350248
Trial 8	4.363612	4.882525	4.146128

8.1.1.2 Mould Rind

Table 5 Viable Cells (CFU/g) at the mould Rind of Camembert cheese during manufacture and ripening

	0	2	172	218	386	770
Trial 1	1.28E+05	5.03E+05	5.52E+05	2.50E+05	6.60E+03	2.40E+03
Trial 2	9.50E+05	5.21E+06	1.31E+05	6.25E+04	1.69E+04	7.50E+03
Trail 3	6.31E+06	3.32E+07	5.65E+05	8.63E+04	2.30E+04	1.08E+04
Trial 4	7.94E+06	5.83E+07	2.07E+05	1.26E+05	3.73E+04	6.30E+03
Trial 5	8.66E+04	4.60E+05	2.80E+04	1.61E+04	5.50E+03	2.90E+03
Trial 6	3.65E+05	5.10E+05	1.43E+04	1.15E+04	7.50E+03	2.75E+03

Trial 7	2.34E+07	6.01E+07	1.25E+06	7.53E+05	1.09E+05	2.13E+04
Trial 8	2.31E+04	7.63E+04	9.60E+03	7.40E+03	5.33E+03	1.39E+03

Table 6 Log Viable Cell (log CFU/g) at the mould Rind of Camembert cheese during manufacture and ripening

	0	2	172	218	386	770
Trial 1	5.10721	5.701568	5.741939	5.39794	3.819544	3.380211
Trial 2	5.977724	6.716838	5.117271	4.79588	4.227887	3.875061
Trail 3	6.800029	7.521138	5.752048	4.936011	4.361728	4.033424
Trial 4	6.899821	7.765669	5.31597	5.100371	4.571709	3.799341
Trial 5	4.937518	5.662758	4.447158	4.206826	3.740363	3.462398
Trial 6	5.562293	5.70757	4.155336	4.060698	3.875061	3.439333
Trial 7	7.369216	7.778874	6.09691	5.876795	5.03583	4.32838
Trial 8	4.363612	4.882525	3.982271	3.869232	3.726727	3.143015

8.1.1.2.1 Surface

Table 7 Viable Cells (CFU/g) at the Surface of Camembert cheese during manufacture and ripening

Time	0	2	50	122	172	218	386	770
Trial 1	1.28E+05	5.03E+05	1.87E+05	3.65E+04	1.30E+04	9.00E+03	1.07E+04	2.30E+03
Trial 2	9.50E+05	5.21E+06	4.91E+05	1.90E+05	3.20E+05	2.05E+05	7.30E+04	7.80E+03

Trail 3	6.31E+06	3.32E+07	5.13E+06	8.46E+05	3.20E+05	9.37E+04	1.72E+04	9.30E+03
Trial 4	7.94E+06	5.83E+07	1.43E+06	3.25E+05	1.81E+05	9.15E+04	4.55E+04	1.13E+04
Trial 5	8.66E+04	4.60E+05	5.30E+04	2.80E+04	1.90E+04	1.35E+04	8.50E+03	2.10E+03
Trial 6	3.65E+05	5.10E+05	3.35E+04	2.75E+04	9.50E+03	5.50E+03	6.50E+03	7.60E+03
Trial 7	2.34E+07	6.01E+07	5.78E+06	7.90E+05	5.21E+05	4.11E+05	7.50E+04	2.12E+04
Trial 8	2.31E+04	7.63E+04	2.27E+04	2.13E+04	1.64E+04	1.66E+04	9.40E+03	1.36E+03

Table 8 Log Viable Cell (log CFU/g) at the Surface of Camembert cheese during manufacture and ripening

Time	0	2	50	122	172	218	386	770
Trial 1	5.10721	5.701568	5.270679	4.562293	4.113943	3.954243	4.029384	3.361728
Trial 2	5.977724	6.716838	5.691081	5.278754	5.50515	5.311754	4.863323	3.892095
Trail 3	6.800029	7.521138	6.710117	5.92737	5.50515	4.97174	4.235528	3.968483
Trial 4	6.899821	7.765669	6.155336	5.511883	5.257679	4.961421	4.658011	4.053078
Trial 5	4.937518	5.662758	4.724276	4.447158	4.278754	4.130334	3.929419	3.322219
Trial 6	5.562293	5.70757	4.525045	4.439333	3.977724	3.740363	3.812913	3.880814
Trial 7	7.369216	7.778874	6.761928	5.897627	5.716838	5.613842	4.875061	4.326336
Trial 8	4.363612	4.882525	4.356026	4.32838	4.214844	4.220108	3.973128	3.133539

8.1.1.2.2 Core

Table 9 Viable Cells (CFU/g) at the Core of Camembert cheese during manufacture and ripening

	0	2	50	122	172	218	386	770
Trial 1	1.28E+05	5.03E+05	3.93E+04	1.35E+04	8.60E+03	1.12E+04	2.40E+03	9.10E+02
Trial 2	9.50E+05	5.21E+06	3.71E+05	1.15E+05	8.90E+04	1.16E+05	7.32E+04	8.60E+03
Trail 3	6.31E+06	3.32E+07	3.96E+05	6.60E+04	2.90E+04	1.85E+04	2.50E+04	7.20E+03
Trial 4	7.94E+06	5.83E+07	1.17E+06	2.36E+05	8.90E+04	1.19E+05	4.79E+04	9.10E+03
Trial 5	8.66E+04	4.60E+05	4.90E+04	2.10E+04	1.45E+04	1.28E+04	6.00E+03	1.70E+03
Trial 6	3.65E+05	5.10E+05	3.80E+04	2.30E+04	9.50E+03	4.50E+03	3.50E+03	1.05E+03
Trial 7	2.34E+07	6.01E+07	2.24E+06	7.45E+05	3.85E+05	2.16E+05	9.36E+04	1.26E+04
Trial 8	2.31E+04	7.63E+04	1.85E+04	1.51E+04	1.43E+04	1.10E+04	6.66E+03	8.90E+02

Table 10 Log Viable Cell (log CFU/g) at the Core of Camembert cheese during manufacture and ripening

	0	2	50	122	172	218	386	770
Trial 1	5.10721	5.701568	4.594393	4.130334	3.934498	4.049218	3.380211	2.959041
Trial 2	5.977724	6.716838	5.569374	5.060698	4.94939	5.064458	4.864511	3.934498
Trail 3	6.800029	7.521138	5.597695	4.819544	4.462398	4.267172	4.39794	3.857332
Trial 4	6.899821	7.765669	6.068186	5.372912	4.94939	5.075547	4.680336	3.959041
Trial 5	4.937518	5.662758	4.690196	4.322219	4.161368	4.10721	3.778151	3.230449

Trial 6	5.562293	5.70757	4.579784	4.361728	3.977724	3.653213	3.544068	3.021189
Trial 7	7.369216	7.778874	6.350248	5.872156	5.585461	5.334454	4.971276	4.100371
Trial 8	4.363612	4.882525	4.267172	4.178977	4.155336	4.042576	3.823474	2.94939

8.1.1.3 Survival of *S. aureus* in Different Stages and Parts of the Cheeses

8.1.1.3.1 Initial Stages

Table 11 Viable Cells (CFU/g) at the beginning stages of Camembert cheese manufacture

	BAR*	Curd	Whey
4163 a	1.28E+05	5.03E+05	1.10E+05
4163 b	2.34E+07	6.01E+07	1.18E+07
4163 c	6.31E+06	3.32E+07	3.93E+06
9144 a	1.46E+06	3.55E+06	1.35E+05
9144 b	1.01E+06	2.92E+06	1.05E+05
9144 c	9.50E+05	3.21E+06	6.70E+05
172RR a	1.57E+06	4.49E+06	4.72E+05
172RR b	3.55E+05	9.45E+05	2.34E+05
172RR c	2.58E+05	9.27E+05	1.99E+05

Table 12 Table Log Viable Cell (log CFU/g) at the beginning stages of Camembert cheese manufacture

	BAR*	Curd	Whey
4163 a	5.10721	5.701568	5.041393
4163 b	7.369216	7.778874	7.071882
4163 c	6.800029	7.521138	6.594393
9144 a	6.164353	6.550228	5.130334
9144 b	6.004321	6.465383	5.021189
9144 c	5.977724	6.506505	5.826075
172RR a	6.1959	6.652246	5.673942
172RR b	5.550228	5.975432	5.369216
172RR c	5.41162	5.96708	5.298853

Table 13 Mean Values of all three trials of Each Strain at different manufacturing stages

	BAR*	Curd	Whey
4163	6.425485	7.000527	6.235889
9144	6.048799	6.507372	5.325866
172RR	5.719249	6.198253	5.447337

8.1.1.3.2 Mould Rind

Table 14 Viable Cells (CFU/g) at the mould Rind of Camembert cheese during manufacture and ripening

Time (h)	0	2	172	218	386	770
4163 a	1.28E+05	5.03E+05	5.52E+05	2.50E+05	6.60E+03	2.40E+03
4163 b	2.34E+07	6.01E+07	1.25E+06	7.53E+05	1.09E+05	2.13E+04
4163 c	6.31E+06	3.32E+07	5.65E+05	8.63E+04	2.30E+04	1.08E+04
9144 a	1.46E+06	3.55E+06	8.53E+04	5.50E+04	3.26E+04	2.09E+04
9144 b	1.01E+06	2.92E+06	5.51E+04	2.05E+04	2.50E+04	1.25E+04
9144 c	9.50E+05	3.21E+06	7.90E+05	1.88E+05	4.80E+03	3.00E+03
172RR a	1.57E+06	4.49E+06	1.91E+05	2.09E+05	1.16E+04	2.71E+04
172RR b	3.55E+05	9.45E+05	1.30E+04	1.01E+04	3.70E+03	1.66E+03
172RR c	2.58E+05	9.27E+05	8.90E+03	7.60E+03	2.30E+03	1.96E+03

Table 15 Log Viable Cell (log CFU/g) Viable Cells (CFU/g) at the mould rind of Camembert cheese during manufacture and ripening

Time (h)	0	2	172	218	386	770
4163 a	5.10721	5.701568	5.741939	5.39794	3.819544	3.380211
4163 b	7.369216	7.778874	6.09691	5.876795	5.03583	4.32838
4163 c	6.800029	7.521138	5.752048	4.936011	4.361728	4.033424
9144 a	6.164353	6.550228	4.930694	4.740363	4.513218	4.320146

9144 b	6.004321	6.465383	4.741152	4.311754	4.39794	4.09691
9144 c	5.977724	6.506505	5.897627	5.274158	3.681241	3.477121
172RR a	6.1959	6.652246	5.281033	5.320146	4.064458	4.432969
172RR b	5.550228	5.975432	4.113943	4.004321	3.568202	3.220108
172RR c	5.41162	5.96708	3.94939	3.880814	3.361728	3.292256

Table 16 Mean Values of all three trials from Each Strain at different time points

Time (h)	0	2	172	218	386	770
4163	6.425485	7.000527	5.863633	5.403582	4.405701	3.914005
9144	6.048799	6.507372	5.189824	4.775425	4.197466	3.964726
172RR	5.719249	6.198253	4.448122	4.40176	3.664796	3.648444

8.1.1.3.3 Surface

Table 17 Viable Cells (CFU/g) at the surface of Camembert cheese during manufacture and ripening

Time (h)	0	2	50	122	172	218	386	770
4163 a	1.28E+05	5.03E+05	1.87E+05	3.65E+04	1.30E+04	9.00E+03	1.07E+04	2.30E+03
4163 b	2.34E+07	6.01E+07	5.78E+06	7.90E+05	5.21E+05	4.11E+05	7.50E+04	2.12E+04
4163 c	6.31E+06	3.32E+07	5.13E+06	8.46E+05	3.20E+05	9.37E+04	1.72E+04	9.30E+03
9144 a	1.46E+06	3.55E+06	8.63E+04	2.55E+04	2.10E+04	8.90E+03	6.70E+03	2.10E+03
9144 b	1.01E+06	2.92E+06	1.99E+05	3.23E+04	1.99E+04	1.25E+04	8.20E+03	3.10E+03

9144 c	9.50E+05	3.21E+06	5.91E+05	1.90E+05	3.20E+05	2.05E+05	7.30E+04	7.80E+03
172RR a	1.57E+06	4.49E+06	3.12E+05	2.35E+05	2.81E+05	1.79E+05	9.30E+04	2.05E+04
172RR b	3.55E+05	9.45E+05	9.80E+03	8.60E+03	1.22E+04	7.60E+03	3.90E+03	1.52E+03
172RR c	2.58E+05	9.27E+05	1.17E+04	1.26E+04	1.57E+04	8.90E+03	2.40E+03	1.16E+03

Table 18 Log Viable Cell (log CFU/g) at the surface of Camembert cheese during manufacture and ripening

Time (h)	0	2	50	122	172	218	386	770
4163 a	5.10721	5.701568	5.270679	4.562293	4.113943	3.954243	4.029384	3.361728
4163 b	7.369216	7.778874	6.761928	5.897627	5.716838	5.613842	4.875061	4.326336
4163 c	6.800029	7.521138	6.710117	5.92737	5.50515	4.97174	4.235528	3.968483
9144 a	6.164353	6.550228	4.936011	4.40654	4.321308	3.94939	3.826075	3.322219
9144 b	6.004321	6.465383	5.298853	4.509203	4.298853	4.09691	3.913814	3.491362
9144 c	5.977724	6.506505	5.771587	5.278754	5.50515	5.311754	4.863323	3.892095
172RR a	6.1959	6.652246	5.494155	5.371068	5.448706	5.252853	4.968483	4.311754
172RR b	5.550228	5.975432	3.991226	3.934498	4.08636	3.880814	3.591065	3.181844
172RR c	5.41162	5.96708	4.068186	4.100371	4.1959	3.94939	3.380211	3.064458

Table 19 Mean Values of all three trials from Each Strain at different manufacturing stages

Time (h)	0	2	50	122	172	218	386	770
4163	6.425485	7.000527	6.247575	5.46243	5.111977	4.846608	4.379991	3.885516
9144	6.048799	6.507372	5.335484	4.731499	4.708437	4.452685	4.201071	3.568559
172RR	5.719249	6.198253	4.517856	4.468646	4.576989	4.361019	3.97992	3.519352

8.1.1.3.4 Core

Table 20 Viable Cells (log CFU/g) at the core of Camembert cheese during manufacture and ripening

Time (h)	0	2	50	122	172	218	386	770
4163 a	1.28E+05	5.03E+05	3.93E+04	1.35E+04	8.60E+03	1.12E+04	2.40E+03	9.10E+02
4163 b	2.34E+07	6.01E+07	2.24E+06	7.45E+05	3.85E+05	2.16E+05	9.36E+04	1.26E+04
4163 c	6.31E+06	3.32E+07	3.96E+05	6.60E+04	2.90E+04	1.85E+04	2.50E+04	7.20E+03
9144 a	1.46E+06	3.55E+06	2.02E+05	1.28E+05	7.67E+04	1.90E+04	1.15E+04	2.60E+03
9144 b	1.01E+06	2.92E+06	2.73E+05	1.54E+05	1.07E+05	6.58E+04	2.91E+04	5.30E+03
9144 c	9.50E+05	3.21E+06	3.71E+05	1.15E+05	8.90E+04	8.16E+04	7.32E+04	6.50E+03
172RR a	1.57E+06	4.49E+06	8.34E+04	5.90E+04	4.60E+04	2.75E+04	1.81E+04	2.17E+04
172RR b	3.55E+05	9.45E+05	8.80E+03	7.30E+03	1.27E+04	1.06E+04	3.20E+03	1.39E+03
172RR c	2.58E+05	9.27E+05	1.21E+04	8.50E+03	1.01E+04	6.80E+03	2.77E+03	2.25E+03

Table 21 Log Viable Cell (log CFU/g) at the core of Camembert cheese during manufacture and ripening

Time (h)	0	2	50	122	172	218	386	770
4163 a	5.10721	5.701568	4.594393	4.130334	3.934498	4.049218	3.380211	2.959041
4163 b	7.369216	7.778874	6.350248	5.872156	5.585461	5.334454	4.971276	4.100371
4163 c	6.800029	7.521138	5.597695	4.819544	4.462398	4.267172	4.39794	3.857332
9144 a	6.164353	6.550228	5.305351	5.10721	4.884625	4.278754	4.060698	3.414973
9144 b	6.004321	6.465383	5.436163	5.187521	5.029384	4.818226	4.463893	3.724276
9144 c	5.977724	6.506505	5.569374	5.060698	4.94939	4.91169	4.864511	3.812913
172RR a	6.1959	6.652246	4.921166	4.770852	4.662758	4.439333	4.257679	4.33646
172RR b	5.550228	5.975432	3.944483	3.863323	4.103804	4.025306	3.50515	3.143015
172RR c	5.41162	5.96708	4.082785	3.929419	4.004321	3.832509	3.44248	3.352183

Table 22 Mean Values of all three trials from Each Strain at different manufacturing stages

Time (h)	0	2	50	122	172	218	386	770
4163	6.425485	7.000527	5.514112	4.940678	4.660786	4.550281	4.249809	3.638915
9144	6.048799	6.507372	5.436963	5.118476	4.954466	4.669557	4.463034	3.650721
172RR	5.719249	6.198253	4.316145	4.187865	4.256961	4.099049	3.735103	3.610552

8.1.1.4 Physical Parameters in Different Stages and Parts of the Cheeses

8.1.1.4.1 pH

8.1.1.4.1.1 Initial Stages

Table 23 pH at the beginning stages of Camembert cheese manufacture.

	BAR*	Curd	Whey
4163 a	6.56	6.54	6.51
4163 b	6.57	6.49	6.45
4163 c	6.58	6.52	6.53
9144 a	6.6	6.61	6.46
9144 b	6.59	6.58	6.52
9144 c	6.54	6.53	6.52
172RR a	6.55	6.56	6.53
172RR b	6.54	6.58	6.56
172RR c	6.57	6.61	6.5

Table 24 Mean Values of all three trials from Each Strain at different manufacturing stages

	BAR*	Curd	Whey
4163	6.57	6.516667	6.496667
9144	6.576667	6.573333	6.5
172RR	6.553333	6.583333	6.53

8.1.1.4.1.2 Mould Rind

Table 25 pH of Camembert cheese mould rind during manufacture and ripening.

Time (h)	0	2	170	218	386	770
4163 a	6.56	6.52	6.86	7.05	7.63	7.72
4163 b	6.57	6.49	6.78	6.84	6.78	6.61
4163 c	6.58	6.55	6.65	6.73	7.11	7.16
9144 a	6.59	6.58	6.89	7.91	7.87	7.92
9144 b	6.6	6.63	6.72	7.62	7.71	7.78
9144 c	6.54	6.53	6.32	7.13	7.48	7.42
172RR a	6.55	6.56	6.51	6.83	6.99	6.81
172RR b	6.54	6.58	6.71	7.25	7.69	7.86
172RR c	6.57	6.61	6.87	6.91	7.67	7.58

Table 26 Mean Values of all three trials from Each Strain at different manufacturing stages

Time (h)	0	2	170	218	386	770
4163	6.57	6.52	6.763333	6.873333	7.173333	7.163333
9144	6.576667	6.58	6.643333	7.553333	7.686667	7.706667
172RR	6.553333	6.583333	6.696667	6.996667	7.45	7.416667

8.1.1.4.1.3 Surface

Table 27 pH at the surface of Camembert cheese during manufacture and ripening.

Time (h)	0	2	50	122	170	218	386	770
4163 a	6.56	6.52	4.78	4.93	4.96	5.39	6.14	6.33
4163 b	6.57	6.49	4.77	4.88	5.54	5.43	5.75	5.96
4163 c	6.58	6.55	4.77	5.1	5.23	5.67	6.25	6.43
9144 a	6.59	6.58	4.81	5.03	5.39	5.79	6.19	6.72
9144 b	6.6	6.63	4.72	4.91	5.21	5.57	5.91	6.65
9144 c	6.54	6.53	4.74	5.01	5.11	5.52	6.12	6.42
172RR a	6.55	6.56	4.64	5.05	5.55	5.61	5.93	6.06
172RR b	6.54	6.58	4.73	4.97	5.36	5.78	6.09	6.43
172RR c	6.57	6.61	4.76	4.92	5.25	5.47	5.81	6.21

Table 28 Mean Values of all three trials from Each Strain at different manufacturing stages

Time (h)	0	2	50	122	170	218	386	770
4163	6.57	6.52	4.773333	4.97	5.243333	5.496667	6.046667	6.24
9144	6.576667	6.58	4.756667	4.983333	5.236667	5.626667	6.073333	6.596667
172RR	6.553333	6.583333	4.71	4.98	5.386667	5.62	5.943333	6.233333

8.1.1.4.1.4 Core

Table 29 pH at the core of Camembert cheese during manufacture and ripening

Time (h)	0	2	50	122	172	218	386	770
4163 a	6.56	6.52	4.73	4.77	4.83	4.97	5.67	6.21
4163 b	6.57	6.49	4.51	4.55	4.91	4.79	5.63	5.8
4163 c	6.58	6.55	4.65	4.78	5.03	5.19	5.92	6.26
9144 a	6.59	6.58	4.61	4.9	5.17	5.75	6.07	6.37
9144 b	6.6	6.63	4.73	4.92	5.08	5.51	5.85	6.44
9144 c	6.54	6.53	4.76	4.75	4.78	5.16	5.73	6.24
172RR a	6.55	6.56	4.62	4.68	4.93	4.99	5.65	5.89
172RR b	6.54	6.58	4.58	4.81	4.96	5.15	5.82	6.23
172RR c	6.57	6.61	4.64	4.74	4.89	5.11	5.62	6.18

Table 30 Mean Values of all three trials from Each Strain at different manufacturing stages

Time (h)	0	2	50	122	172	218	386	770
4163	6.57	6.52	4.63	4.7	4.923333	4.983333	5.74	6.09
9144	6.576667	6.58	4.7	4.856667	5.01	5.473333	5.883333	6.35
172RR	6.553333	6.583333	4.613333	4.743333	4.926667	5.083333	5.696667	6.1

8.1.1.4.2 Water Activity

8.1.1.4.2.1 Initial Stages

Table 31 Water activity at the beginning stages of Camembert cheese manufacture.

	BAR*	Curd	Whey
4163 a	0.9932	0.9887	0.9936
4163 b	0.9929	0.9895	0.9961
4163 c	0.9971	0.9878	0.9947
9144 a	0.9913	0.9896	0.9926
9144 b	0.9952	0.9902	0.9938
9144 c	0.9936	0.9885	0.9956
172RR a	0.9968	0.9918	0.9931
172RR b	0.9956	0.9873	0.9915
172RR c	0.9905	0.9892	0.9895

Table 32 Mean Values of all three trials from Each Strain at different manufacturing stages

	BAR*	Curd	Whey
4163	0.9944	0.988667	0.9948
9144	0.993367	0.989433	0.994
172RR	0.9943	0.989433	0.991367

8.1.1.4.2.2 Mould Rind

Table 33 Water activity at the mould Rind of Camembert cheese during manufacture and ripening

Time (h)	0	4	172	220	388	770
4163 a	0.9932	0.9887	0.9782	0.9743	0.9639	0.9579
4163 b	0.9929	0.9895	0.9756	0.9738	0.9618	0.9557
4163 c	0.9971	0.9878	0.9782	0.9758	0.9652	0.9596
9144 a	0.9913	0.9896	0.9696	0.9704	0.9682	0.9623
9144 b	0.9952	0.9902	0.9728	0.9701	0.9651	0.9589
9144 c	0.9936	0.9885	0.9761	0.9736	0.9713	0.9655
172RR a	0.9968	0.9918	0.9762	0.9717	0.9707	0.9679
172RR b	0.9956	0.9893	0.9743	0.9722	0.9705	0.9656
172RR c	0.9905	0.9892	0.9709	0.9688	0.9657	0.9592

Table 34 Mean Values of all three trials from Each Strain at different manufacturing stages

Time (h)	0	4	172	220	388	770
4163	0.9944	0.988667	0.977333	0.974633	0.963633	0.957733
9144	0.993367	0.989433	0.972833	0.971367	0.9682	0.962233
172Rr	0.9943	0.9901	0.9738	0.9709	0.968967	0.964233

8.1.1.4.2.3 Surface

Table 35 Water activity at the mould Rind of Camembert cheese during manufacture and ripening

Time (h)	0	2	50	122	172	218	386	770
4163 a	0.9932	0.9887	0.9801	0.9822	0.9775	0.9724	0.9689	0.9613
4163 b	0.9929	0.9895	0.9752	0.9788	0.9814	0.9707	0.9651	0.9587
4163 c	0.9971	0.9878	0.9801	0.9752	0.9746	0.9726	0.9645	0.9602
9144 a	0.9913	0.9896	0.9774	0.9706	0.9715	0.9677	0.9655	0.9628
9144 b	0.9952	0.9902	0.9785	0.9769	0.9751	0.9775	0.9702	0.9625
9144 c	0.9936	0.9885	0.9832	0.9825	0.9755	0.9716	0.9698	0.9672
172RR a	0.9968	0.9918	0.9825	0.9795	0.978	0.9783	0.9755	0.9722
172RR b	0.9956	0.9873	0.9739	0.9711	0.9706	0.9683	0.9675	0.9632
172RR c	0.9905	0.9892	0.9819	0.9781	0.9766	0.9763	0.9715	0.9682

Table 36 Mean Values of all three trials from Each Strain at different manufacturing stages

Time (h)	0	2	50	122	172	218	386	770
4163	0.9944	0.988667	0.978467	0.978733	0.977833	0.9719	0.966167	0.960067
9144	0.993367	0.989433	0.9797	0.976667	0.974033	0.972267	0.9685	0.964167
172RR	0.9943	0.989433	0.979433	0.976233	0.975067	0.9743	0.9715	0.967867

8.1.1.4.2.4 Core

Table 37 Water activity at the mould Rind of Camembert cheese during manufacture and ripening

Time (h)	0	2	50	122	172	218	386	770
4163 a	0.9932	0.9887	0.9801	0.9782	0.9743	0.9639	0.9613	0.9579
4163 b	0.9929	0.9895	0.9772	0.9756	0.9738	0.9618	0.9587	0.9567
4163 c	0.9971	0.9878	0.9801	0.9782	0.9758	0.9652	0.9602	0.9616
9144 a	0.9913	0.9896	0.9754	0.9696	0.9704	0.9692	0.9628	0.9623
9144 b	0.9952	0.9902	0.9785	0.9728	0.9701	0.9651	0.9625	0.9589
9144 c	0.9936	0.9885	0.9802	0.9761	0.9734	0.9723	0.9672	0.9655
172RR a	0.9968	0.9918	0.9815	0.9762	0.9717	0.9707	0.9692	0.9669
172RR b	0.9956	0.9893	0.9769	0.9743	0.9722	0.9711	0.9682	0.9656
172RR c	0.9905	0.9892	0.9789	0.9709	0.9688	0.9657	0.9632	0.9592

Table 38 Mean Values of all three trials from Each Strain at different manufacturing stages

Time (h)	0	2	50	122	172	218	386	770
4163	0.9944	0.988667	0.979133	0.977333	0.974633	0.963633	0.960067	0.958733
9144	0.993367	0.989433	0.978033	0.972833	0.9713	0.968867	0.964167	0.962233
172RR	0.9943	0.9901	0.9791	0.9738	0.9709	0.969167	0.966867	0.9639

8.1.2 Control groups

8.1.2.1 Survival of *S. aureus*

8.1.2.1.1 Surface

Table 39 Viable Cells (CFU/g) at the surface of controlled Camembert cheese (without culture blend) during manufacture and ripening.

	0	2	50	122	172	218	386	770
4163	3.20E+04	1.06E+05	7.30E+07	2.23E+07	8.70E+07	1.68E+08	1.27E+08	9.70E+07
9144	5.00E+04	1.01E+05	1.90E+08	1.19E+08	9.20E+07	3.43E+08	4.59E+08	2.63E+08
172RR	6.00E+06	3.86E+07	6.25E+09	4.97E+09	9.40E+09	1.41E+10	6.10E+09	4.87E+09

Table 40 Log Viable Cells (log CFU/g) at the surface of controlled Camembert cheese (without culture blend) during manufacture and ripening.

	0	2	50	122	172	218	386	770
4163	4.50515	5.025306	7.863323	7.348305	7.939519	8.225309	8.103804	7.986772
9144	4.69897	5.004321	8.278754	8.075547	7.963788	8.535294	8.661813	8.419956
172RR	6.778151	7.586024	9.79588	9.696356	9.973128	10.14922	9.78533	9.687529

8.1.2.1.2 Core

Table 41 Viable Cells (CFU/g) at the core of controlled Camembert cheese (without culture blend) during manufacture and ripening

	0	2	50	122	172	218	386	770

4163	3.20E+04	1.06E+05	8.50E+06	5.23E+06	7.31E+06	1.07E+07	7.69E+06	2.51E+06
9144	5.00E+04	1.01E+05	1.31E+08	1.21E+08	1.61E+08	2.26E+08	1.98E+08	8.52E+07
172RR	6.00E+06	3.86E+07	3.90E+09	3.71E+09	7.60E+09	7.20E+09	6.70E+09	1.22E+09

Table 42 Log Viable Cells (CFU/g) at the core of controlled Camembert cheese (without culture blend) during manufacture and ripening

	0	2	0	3	5	7	14	30
4163	4.50515	5.025306	6.929419	6.718502	6.863917	7.02735	6.885926	6.399674
9144	4.69897	5.004321	8.117271	8.082785	8.206826	8.354108	8.296665	7.93044
172RR	6.778151	7.586024	9.591065	9.569374	9.880814	9.857332	9.826075	9.08636

8.1.2.2 Physical Parameters

8.1.2.2.1 pH

Table 43 pH at the surface of controlled Camembert cheese (without culture blend) during manufacture and ripening

	0	2	0	3	5	7	14	30
4163	6.63	6.67	6.46	5.73	5.67	5.77	5.33	5.22
9144	6.6	6.68	6.69	6.05	5.88	5.79	5.29	5.24
172RR	6.67	6.86	6.41	6.48	6.71	6.35	5.79	5.59

Table 44 pH at the core of controlled Camembert cheese (without culture blend) during manufacture and ripening

	0	2	0	3	5	7	14	30
4163	6.63	6.67	6.11	5.61	5.59	5.62	5.18	5.09
9144	6.6	6.68	6.49	5.95	5.93	5.86	5.09	5.16
172RR	6.67	6.86	6.23	6.29	6.43	6.07	5.36	5.31

8.1.2.2.2 Water Activity

Table 45 Water activity at the surface of controlled Camembert cheese (without culture blend) during manufacture and ripening

	0	2	50	122	172	218	386	770
4163	0.9968	0.9927	0.9833	0.9792	0.9807	0.9747	0.9698	0.9674
9144	0.9952	0.9897	0.9822	0.9802	0.9793	0.9685	0.9656	0.9639
172RR	0.9942	0.9905	0.9796	0.9779	0.9764	0.9692	0.9671	0.9666

Table 46 Water activity at the core of controlled Camembert cheese (without culture blend) during manufacture and ripening

	0	2	0	3	5	7	14	30
4163	0.9968	0.9927	0.9825	0.9788	0.9797	0.9767	0.9682	0.9663
9144	0.9952	0.9897	0.9806	0.9791	0.9785	0.9671	0.9639	0.9628
172RR	0.9942	0.9905	0.9782	0.9758	0.9769	0.9701	0.9661	0.9658

8.2 Calculations

8.2.1 T-tests Results Between Strains

8.2.1.1 Survival of *S. aureus*

8.2.1.1.1 Experimental Groups

Table 47 T-tests results of *S. aureus* between different strains at the mould rind of cheeses

	9144	172RR
4163	0.276995	0.116456
9144		0.242194

Table 48 T-tests results of *S. aureus* between different strains at the surface of cheeses

	9144	172RR
4163	0.18409	0.073585
9144		0.279931

Table 49 T-tests results of *S. aureus* between different strains at the core of cheeses

	9144	172RR
4163	0.487424	0.130228
9144		0.109513

8.2.1.1.2 Control Groups (without culture blend)

Table 50 T-tests results of *S. aureus* between different strains at the surface of controlled cheeses (without culture blend)

	9144	172RR
4163	0.338946	0.004922
9144		0.016226

Table 51 T-tests results of *S. aureus* between different strains at the core of controlled cheeses (without culture blend)

	9144	172RR
4163	0.064062	0.000091
9144		0.013938

8.2.1.2 pH

8.2.1.2.1 Experimental Groups

Table 52 T-tests results of pH between different strains at the mould rind of cheeses

	9144	172RR
4163	0.155013	0.306308
9144		0.278664

Table 53 T-tests results of pH between different strains at the surface of cheeses

	9144	172RR
4163	0.424205	0.487721
9144		0.435842

Table 54 T-tests results of pH between different strains at the core of cheeses

	9144	177RR
4163	0.347202	0.482604
9144		0.363202

8.2.1.2.2 Control Groups (without culture blend)

Table 55 T-tests results of pH between different strains at the surface of controlled cheeses (without culture blend)

	9144	172RR
4163	0.377745	0.061689
9144		0.114055

Table 56 T-tests results of pH between different strains at the core of controlled cheeses (without culture blend)

	9144	172RR

4163	0.305943	0.131431
9144		0.272175

8.2.1.3 Water Activity

8.2.1.3.1 Experimental Groups

Table 57 T-tests results of water activity between different strains at the mould rind of cheeses

	9144	172RR
4163	0.491234	0.449842
9144		0.455612

Table 58 T-tests results of water activity between different strains at the surface of cheeses

	9144	172RR
4163	0.482441	0.38684
9144		0.398155

Table 59 T-tests results of water activity between different strains at the surface of cheeses

	9144	172RR
4163	0.470911	0.4076
9144		0.430998

8.2.1.3.2 Control Groups (without culture blend)

Table 60 T-tests results of water activity between different strains at the surface of controlled cheeses (without culture blend)

	9144	172RR
4163	0.325771	0.292425
9144		0.47204

Table 61 T-tests results of water activity between different strains at the core of controlled cheeses (without culture blend)

	9144	172RR
4163	0.295133	0.28868
9144		0.493876

8.2.2 Salt content

8.2.2.1 Volume Usage of Ammonium Thiocyanate

Table 62 Volume (ml) usage of ammonium thiocyanate during titration

	4163a	4163 b	4163 c	9144 a	9144 c	9144 c	172RR a	172RR b	172RR c
test 1	8.6	8.5	8.6	8.7	8.55	8.65	8.6	8.55	8.6
test 2	8.7	8.55	8.5	8.6	8.6	8.6	8.6	8.6	8.55
test 3	8.65	8.55	8.55	8.6	8.6	8.6	8.6	8.65	8.6
Average	8.65	8.5333	8.55	8.6333	8.5833	8.6167	8.6	8.6	8.5833

8.2.2.2 Salt Content Calculation

Take 4163 (a) as an example. The average volume of ammonium thiocyanate used in the titration was 8.65ml, the moles of which then be calculated to be as 0.000865 mol. The equation: $Ag^+ + SCN^- \rightarrow AgSCN$ was applied for calculating the moles of unreacted silver nitrate in the 100 ml tested solution, which was the same as the moles of ammonium thiocyanate, 0.000865 mol. The number was multiplied by 5 for calculating the total unreacted silver nitrate in the volumetric flask, the result of which was 0.004325 mol. The total moles of silver nitrate added initially was 0.005 mol (50 ml 0.1 mol/L). The total moles of reacted silver nitrate can then be calculated by subtracting 0.004325 from 0.005. According to the equation: $Ag^+ + Cl^- \rightarrow AgCl$, the moles of chloride ions were the same as reacted silver nitrate, which was 0.000675 mol. The mass of salt was calculated: $0.000675 \times 58.5 = 0.0395$ g. The mass of tested cheese sample was 3 g, so the salt content (g per 100 g cheese) can be calculated: $0.0395 / 3 \times 100\% = 1.317\%$. The salt contents of other trials were calculated using the same method. The results are presented in table 63.

	4163a	4163 b	4163 c	9144 a	9144 c	9144 c	172RR a	172RR b	172RR c
Salt content	1.317	1.433	1.414	1.333	1.381	1.349	1.365	1.365	1.381