Ultra Filtration (UF) Process Development for the Production of Camembert Cheese

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ABSTRACT

The application of UF technology in cheese production has several potential advantages; product consistency, yield, lower costs and more automation. This study investigated the effects of four processing variables in the manufacture of Camembert cheese using UF and their impact on cheese quality. Using an incomplete block design, sixteen unique treatments were produced with combined processing variables (high-fat or low-fat; brine-salted or retentate-salted; acidified to pH 5.2 or pH 4.9; set in tubular moulds and small moulds). The cheeses were matured for seven weeks at 4±1 ºC and were analysed for total solids, fat, salt, non-protein nitrogen (NPN) and soluble nitrogen (SN) contents during the maturation period (seven weeks). Major defects were evaluated by experienced cheese graders in the fourth week. pH was measured and instrumental analysis was also conducted. Sensory evaluation on consumer acceptance was also conducted in the fourth week.

All the cheese samples exhibited similar increases in rind and core pH, NPN/TN and SN/TN ratios, and were generally characterised by thick rind and softness. The low-fat cheese samples had significantly lower NPN/TN ratio and higher overall acceptance in sensory evaluation. The salt content was also significantly higher. The retentate-salted cheese samples had significantly lower NPN/TN ratios and more defects in rind discolouration and deformation, and saltiness. The cheese samples acidified to pH 5.2 had significantly lower NPN/TN ratios and fewer defects in rind discolouration, softness, sourness, and bitterness. The cheese samples made using tube moulds were significantly firmer with fewer defects in rind deformation, core unevenness, and softness.

The level of fat and extent of acidification was found to have a profound effect on cheese quality, and cheeses produced with low-fat retentate and/or acidified to pH 5.2 generally had superior shelf-life with lower levels of proteolysis. The preference of the two salting methods may be debatable, but considering labour and time, retentate-salting is preferable. Tube mould generally produced better cheese with fewer defects.

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LIST OF ABBREVIATIONS

HB4.9S	High-fat, brine-salted, pH 4.9, small mould treatment
HB4.9T	High-fat, brine-salted, pH 4.9, tube mould treatment
HB5.2S	High-fat, brine-salted, pH 5.2, small mould treatment
HB5.2T	High-fat, brine-salted, pH 5.2, tube mould treatment
HR4.9S	High-fat, retentate-salted, pH 4.9, small mould treatment
HR4.9T	High-fat, retentate -salted, pH 4.9, tube mould treatment
HR5.2S	High-fat, retentate-salted, pH 5.2, small mould treatment
HR5.2T	High-fat, retentate-salted, pH 5.2, tube mould treatment
LAB	Lactic acid bacteria
LB4.9S	Low-fat, brine-salted, pH 4.9, small mould treatment
LB4.9T	Low -fat, brine-salted, pH 4.9, tube mould treatment
LB5.2S	Low -fat, brine-salted, pH 5.2, small mould treatment
LB5.2T	Low -fat, brine-salted, pH 5.2, tube mould treatment
LR4.9S	Low -fat, retentate-salted, pH 4.9, small mould treatment
LR4.9T	Low -fat, retentate -salted, pH 4.9, tube mould treatment
LR5.2S	Low -fat, retentate-salted, pH 5.2, small mould treatment
LR5.2T	Low -fat, retentate-salted, pH 5.2, tube mould treatment
MF	Microfiltration
N	Nitrogen
NF	Nanofiltration
NPN	Non-protein nitrogen
RO	Reverse osmosis
SN	Soluble-nitrogen
TCA	Trichloroacetic acid
TN	Total nitrogen
UF	Ultrafiltration
UHT	Ultra-high temperature

1. INTRODUCTION

Camembert is a surface mould-ripened cheese with a soft consistency in a flat cylindrical form (Scott, 1998). It originated in the Normandy region of France and has been produced industrially since the beginning of the twentieth century (Fox et al., 2004). The manufacture of Camembert and other surface mould-ripened cheeses has since become widespread in France and other regions in Europe.

Traditionally, Camembert is made from raw milk with the addition of a mesophilic starter. Rennet is added at about pH of 6.4 and coagulation occurs for 30-45 min. The coagulum is transferred to the moulds and allowed for whey drainage starting at 26 – 28 ºC and gradually cooled to 20 ºC. At this stage, a curd with pH of 4.6-4.7 and low mineral content is obtained. The cheese is dry-salted and ripened for 21 days or more at 11-13 ºC and 90% relative humidity (Fox et al., 2000). With the development of industrial methods to accommodate large milk throughput, Alpma-type processes and highly mechanised equipments are now used (Fox et al., 2004).

The emergence of membrane processes has unlocked many opportunities in the dairy industry, leading to significant new process and product development (Fox et al., 2004). The introduction of membrane processes in cheese manufacture by the Maubois, Mocquot and Vassal (MMV) process has enabled significant advances in cheese-making (Maubois and Mocquot, 1975). The benefits include improved plant efficiency, increased cheese yield, and the development of automated processes (Fox et al., 2004). Camembert was the first cheese to be made according to the MMV principle using ultrafiltration (UF), and its application has been well accepted in the industry using a continuous moulding and demoulding system ‘Camatic’ (Maubois, 2002; Fox et al., 2004).

Although UF cheeses provide advantages such as yield increase, reduced cost of labour, and increased plant capacity, they possess unique ripening characteristics and texture qualities (Fox et al., 2004). These properties may cause quality defects which impact on the preference of the consumer in purchasing the product (Walstra et al., 2006). It is therefore of paramount importance to study the factors contributing to

undesirable characteristics, and modify the existing manufacturing methods aimed at improving the overall cheese quality and shelf-life.

The main objective of this project was to determine the optimum processing parameters for the production of Camembert cheese from bovine milk utilising a hollow-fibre UF system, with respect to improving the overall quality and shelf-life of the cheese. Four processing variables were studied. The cheeses made were monitored for chemical, microbiological, physical, and sensory aspects during maturation to evaluate the quality of the cheeses.

2. LITERATURE REVIEW

2.1. Introduction

For millennia, bovine milk has been used to produce products such as cream, butter, kefir, yogurt, and cheese. With the advances of modern technology, it has enabled us to produce ice cream, powdered milk and infant formulae, concentrated milks, lactose, casein and functional protein products for food-additive and industrial uses (Walstra et al., 2006).

In many cultures of the world, the consumption of milk by humans continues beyond infancy, especially in the Western world. Animal milk, particularly cows' milk, is used as a food product due to its nutritional value.

The principle components in bovine milk include lactose, fat, protein, minerals, organic acids, and water. Lactose, or milk sugar, is the major carbohydrate of milk and is a disaccharide of glucose and galactose. It is mainly converted into lactic acid in many fermented milk products such as yogurt and cheese (Walstra et al., 2006). The fats in milk are primarily triglycerides, varying in lengths (2 to 20 carbon atoms) and saturation (0 to 4 double bonds). Other milk lipids include diglycerides, monoglycerides, phospholipids, cholesterol, and free fatty acids. Nearly all fat in milk is in fat globules, which varies in diameter (0.1 to 15 μm) and consists of a membrane to prevent them from coalescence (Walstra et al., 2006). Casein, present in micelles, accounts for about four fifths of milk protein. It includes α_{s1}-, α_{s2}-, β-, and κ-casein. The behaviour of casein micelles has great importance in determining the properties of milk and subsequent dairy products, especially in cheese making. Other proteins in milk consists of serum proteins, β-lactoglobulin, and a wide range of enzymes (Walstra et al., 2006). The mineral substances in milk mainly consist of K, Na, Ca, Mg, Cl, and phosphate. Milk also contains partly ionized salts and organic acids, such as citrate.

The chemical composition of milk varies between species, and is affected by the season, stage of lactation, and health of animal. This subsequently determines the

nutritional value of the milk, its flavour, the growth of microorganisms and chemical reactions that can occur in milk, as well as the quality of processed dairy products.

Today, the consumption of milk has become part of a regular human diet in many regions. Countries such as Finland, Sweden, and Ireland are among the top fluid milk drinkers, consuming 183.9 litres, 145.5 litres, and 129.8 litres per capita in 2006, respectively (FIL/IDF, 2007). On the other hand, cheeses in Greece, France, and Italy dominate milk consumption, with 28.9 kg, 23.9 kg, and 23.7 kg of cheese consumed per capita in 2006, respectively. Considering the climate of these regions, it would appear that cultures in warmer climates produce more stable products such as cheese as means of preservation.

2.2. Cheese

The history of cheese is believed to have evolved around 6000-7000 BC when the ancient human learnt to domesticate animals, a period referred to as the "Agricultural Revolution" (Fox, 1993). This allowed human to secure the provision of milk, leading to the discovery of fermented dairy products by accident.

Due to the highly perishable nature of milk, especially in warmer climates, it is prone to microbial contamination. Some species of bacteria utilise milk lactose as a source of energy to produce lactic acid as a by-product. When sufficient acid has been produced to allow the caseins, the principal protein in milk, to reach the region of their isoelectric point (≈ pH 4.6), aggregation occurs and a gel that entraps fat and other milk constituents is formed. As this gel structure is disturbed and broken, the aqueous phase of the gel, the whey, is separated from the curd. Whereas the whey may be consumed immediately, it was realized that the storage of the curd could be extended by dehydration and adding salt. Hence, the result is a preserved milk product that is durable and easily transportable, and perhaps the very first form of cheese (Fox et al., 2000).

Over 2000 varieties of cheeses exist to date and it accounts for about 35% of total milk production (Fox et al., 2000). In 2005, the Food and Agricultural Organisation of the United Nations (FAO) estimated that the worldwide production of cheese was

18.7 million tonnes, and Europe was the largest producer which contributed 9.9 million tonnes of cheese (FAO, 2008).

The definition of cheese given by the Australia New Zealand Food Standards Code is that "Cheese means the ripened or unripened solid or semi-solid milk product which may be coated and is obtained by one or both of the following processes – (a) coagulating wholly or partly milk, and/or materials obtained from milk, through the action of rennet or other suitable coagulating agents, partially draining the whey which results from such coagulation; or (b) processing techniques involving concentration of coagulation of milk and/or materials obtained from milk which give an end-product with similar physical chemical and organoleptic characteristics as the product described in (a)" (FSANZ, 2008).

2.2.1. Types of cheeses

The types of cheeses are generally categorised based on their rheological properties and moisture content. An often used scheme classifies cheese into hard, semi-hard, semi-soft, and soft cheeses. Common examples of hard cheeses include Parmesan and Cheddar; semi-hard cheeses include Gouda, Edam, Emmental, and Roquefort; semi-soft cheeses include Havarti, Mozzerella, and Feta; and soft cheeses include Brie, Camembert, Cottage, and Cream (Scott, 1998).

In many types of cheese, some are consumed immediately after manufacture, namely fresh cheese. This type of cheese undergoes minimal, if any, ripening after the fermentation of lactose. They generally have limited shelf life of about two weeks under refrigeration. Examples of fresh cheese include Quarg and Cottage cheese (Walstra et al., 2006).

In the majority of the cheese varieties other than the fresh cheeses, the cheeses are generally allowed to ripen before consumption. Cheese ripening involves chemical changes in the cheese which often begin before curd making finishes. In non-fresh cheese types, the process of ripening is allowed to continue, which causes change in the composition, structure, and organoleptic properties of the cheese. The phenomenon of cheese ripening involves chemical, physical, and micriobiological

interactions which are highly dynamic and complex, of which the specific reactions and metabolic activities are still not fully understood (Fox et al., 2000).

A common type of hard or semi-hard ripened cheese is Cheddar where the cheddaring process is used during manufacture. In Cheddar cheese production, after a sequence of manufacturing processes which include curd-heating and curd-cutting, cheddaring is applied where the curd is stacked and turned (Walstra et al., 2006). Gouda-type cheeses are semi-hard ripened cheeses involving curd-washing and eye-formation. Curd-washing in these cheeses enables the lowering of lactose content, which affects subsequent acid development. Citric acid fermenting starter bacteria produces CO_2 in the cheese, which causes eye formation and produces a cheese with holes in the cheese matrix (Walstra et al., 2006; Scott, 1998). Swiss-type cheeses utilise high temperatures during curd treatment in manufacture and produces a cheese with low water content and relatively high pH, such as Emmental, a semi-hard cheese with eyes (Walstra et al., 2006; Scott, 1998). Another common type of cheese is white brine cheese. They are rindless, highly salted, drained-curd, and low pH (Scott, 1998). These cheeses originated from the regions in the Middle East where the climate is hot and unsuitable for cheese storage. Hence, they are stored in strong brine for improved shelf-life, producing cheeses such as Feta and Domiati (Scott, 1998).

Soft cheeses comprise of a broad range of different curd types, including very soft Petit Suisse and Gervais, to firmer Camembert and Brie, and firmer Limburger and Romadur (Scott, 1998). These types of cheese are generally discriminated by the method of ripening and moisture content. Camembert is one of the most popular surface mould ripened cheese in France and elsewhere. It has white mould on the rind with a soft and spreadable texture (Scott, 1998; Kristensen, 1999).

Various factors contribute to the development of a type of cheese, which depends on the local conditions, including climate, storage environments, the availability and type of milk. Additionally, the knowledge in traditional methods that have evolved and accumulated experience over generations as well as new findings play a significant role (Walstra et al., 2006).

2.3. Advances in the dairy industry

With the advancement in dairy technology over the past century, many process steps have been developed and employed in manufacturing of various cheese types. Examples of which include the pasteurisation of cheese milk, use of starter cultures, use of industrial coagulants, as well as washing of the curd. New data promotes the use of surface flora inocula, acceleration of ripening, application of latex to cheese rind, and also the use of membrane technology (Walstra et al., 2006).

The emergence of membrane technology has uncovered innovative applications and new products in the dairy industry. Its application in cheese making has become popular since the invention of the MMV process, which produced cheese by UF of the cheese milk (Fox, 1993; Maubois and Mocquot, 1975).

2.3.1. Membrane technology

The applications in membrane and membrane separation techniques have advanced from laboratory to large scale industrial processes, introducing considerable technical and commercial challenges. Membrane technology is currently utilised in many industrial-sized applications, such as the use of reverse osmosis in potable water extraction from the ocean, ultrafiltration in fractionating macromolecular solutions in the food and drug industry, recovery of valuable constituents and cleaning of industrial effluents by electrodialysis, application in artificial kidney using dialysis in removing urea and other toxins in the blood stream, as well as releasing drugs at a predetermined rate in medical treatments (Porter, 1990).

Membrane processes can also be customised to modify their properties in specific separation applications. The basic principle of a membrane process involves a separating barrier and a driving force used to transport chemical components, which is similar in all different modes of operations (Porter, 1990). When compared to conventional separation methods, membrane processes are generally faster, more efficient, and economical. There are numerous advantages associated with the use of membrane technology, which includes lower energy consumption, the ability to combine with other separation processes to form hybrid processing, separation under

mild conditions, the possibility for setting a continuous process, the ability to up-scale easily, the high variability and adjustability in membrane properties, and the use of additives to products are not required (Mulder, 1996).

The range of expertise involved in membrane technology is broad. Examples include the development of membrane structures by polymer chemists; defining transport properties of different membrane and their separation characteristics by physicist and mathematicians; and designing of large scale separation processes for industrial use by chemical engineers (Porter, 1990).

2.3.2. Principles of membrane filtration

Membrane filtration is applied in many processes which is based on the principle of selective permeability of one or more components of a liquid mixture through a membrane barrier (FIL/IDF, 1991; Mulder, 1996). There are a number of ways for mass transportation through a membrane, commonly facilitated by a significant flux of matter such as convection or diffusion, electrical potential differences, concentration, pressure, or temperature gradients (Mulder, 1996; Porter, 1990). The rate of permeation through a membrane is usually proportional to the driving force, hence the flux-force relationship can be described by a linear phenomenological equation (Mulder, 1996).

Most membrane processes are driven by a pressure or concentration difference across the membrane, or by electrical potential difference. Membrane processes which are driven by pressure difference include microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) (FIL/IDF, 1991). Membrane processes driven by concentration include pervaporation, gas separation, vapour permeation, dialysis, diffusion dialysis, and carrier-mediated transport. Membrane processes can also be driven by electrical potential differences known as electrodialysis, electro-osmosis and membrane electrolysis (Mulder, 1996).

2.3.3. Pressure-driven membrane processes

The applications of pressure-driven membrane processes are generally based on concentrating a dilute solution and separating out specific molecules (aqueous or non-aqueous) (FIL/IDF, 1991; Mulder, 1996). In these processes, the solvent is usually in a continuous phase and the concentration of the solute is relatively low (Mulder, 1996). With specific pore size and pore size distribution, the membrane used is dependent on the molecular size and chemical properties of the solute. Hence, the types of membrane processes are defined by the molecular size of the solute and the consequent membrane structure (Porter, 1990; Walstra et al., 2006).

The principle of pressure-driven membrane processes is that due to applied pressure, the solvent and various solute molecules permeate through the membrane, while other molecules are retained depending on the structure of the membrane (Mulder, 1996). Several membrane processes are commonly used, including MF, UF, NF, and RO, where the size of the molecules being separated decreases and the pore size in the membrane becomes smaller, respectively (Porter, 1990).

2.3.4. Microfiltration

Microfiltration (MF) is the separation process which is similar to conventional coarse filtration. The membrane pore sizes are between 10 to 0.05 μm with cross-flow pressure kept at low levels, usually under 2 bars (Mulder, 1996). “Cross flow” or “tangential flow” filtration is used in order to offset deposition on the membrane layers where the fluid flow is tangential to the membrane surface and perpendicular to the permeate flow through the membrane (FIL/IDF, 1991). MF is used to selectively separate particles with molecular weights greater than 200,000 Da, allowing the process to retain suspensions and emulsions. In dairy applications, MF is involved in removing somatic cells, bacteria (Elwell and Barbano, 2006), fat globules, casein micelles, aggregated whey components (β-casein and β-lactoglobulin) in milk (Fox et al., 2004; Saboya and Maubois, 2000).

In other applications, MF is used in sterilisation and clarification of beverages (Hajipour et al., 2010) and pharmaceuticals (Sirkar, 2000), cell harvesting (Roush and Lu, 2008), membrane bioreactors for continuous fermentation (Lee et al., 2008),

separation of oil-water emulsions (Vahid et al., 2006), plasmaphersis (Mulder, 1996), waste-water treatment (Herzberg et al., 2010; Lorain et al., 2010), as well as metal ion recovery (Matis et al., 2005).

2.3.5. Ultrafiltration

Ultrafiltration (UF) membranes generally have pore sizes from 0.05 μm to 1 nm with the cross-flow pressure applied between 1 to 10 bars. UF is often used to retain macromolecules and colloids from a solution, typically with molecular weights between 1000 to 200,000 Da (Mulder, 1996). The application of UF in milk involves separation and fractionation of proteins, fat and colloidal salts from lactose, soluble minerals, non-protein nitrogens (NPN), water-soluble vitamins and water (FIL/IDF, 1991, Fox et al., 2004, Hinrichs, 2001). Other industrial applications of UF include the recovery of whey proteins (Yorgun et al., 2008) and potato starch (Zwijnenberg et al., 2002), production of ultrapure water for semiconductor industries (Kang, 1999), treatment of oil and latex emulsions (Jönsson and Trägårdh, 1990), and paper waste treatment (Porter, 1990).

2.3.6. Nanofiltration and reverse osmosis

Nanofiltration (NF) utilises membranes with pore sizes typically under 2 nm, with cross-flow pressures between 10 to 25 bars. NF is used to separate low molecular weight solutes between 1000 to 200 Da, such as inorganic salts or small organic molecules (e.g. glucose) (Mulder, 1996). Its applications are also dependent on the availability of special membranes, allowing it to selectively reject ions based on their charge. In the dairy industry, NF is often utilised in partial demineralisation of whey-like materials (Suárez et al., 2006). The small pore sizes of the membranes are also sufficient to retain most of the lactose, hence NF is often referred as an ultratight UF or loose RO (FIL/IDF, 1991).

Reverse osmosis (RO) is used to separate molecular weight solutes with 150 Da or less. Hence it is generally utilised in the removal of water or to concentrate a dilute solution (FIL/IDF, 1991; Luce and Rex Dieterle, 2005). The operating cross-flow

pressures in RO are about 15 to 80 bars, which is relatively high due to the tight nature of its membranes (Mulder, 1996).

2.3.7. Application of membrane technology in cheese

The use of membranes in cheese making began in the late 1960s when the MMV process, named after its inventors - Maubois, Mocquot and Vassal, was invented (Maubois and Mocquot, 1975). This innovative discovery allowed an opportunity for advancement in cheese making technology. The benefits from the incorporation of membrane processes in cheese making included improvements in plant efficiency, reduced cost of labour and materials, increased plant capacity (Sharma et al., 1989), increases in cheese yield (Hinrichs, 2001), development of a continuous process, reduced pollution from whey discharge, and development of new cheese varieties (Fox et al., 2004).

Practical applications of membranes in cheese include the manufacture of most types of cheese from the milk of cows, goats, ewes or water buffaloes, as well as production of milk powders with excellent cheese-making properties (Fox et al., 2004). It is also used in restoring rennet coagulation properties of ultrahigh temperature-treated (UHT) milk (Ferron-Baumy et al., 1991), on-farm concentration of milk, removal of somatic cells and bacteria from cheese milk (Maubois, 2002; Thomann et al., 2008), and casein enrichment of cheese milk (Fox et al., 2004; Thomann et al., 2008).

2.3.8. Characteristics of cheeses manufactured using membrane processes

In 1989, it was recorded that over 400,000 tonnes of cheese were made using UF (Mistry and Maubois, 1993), and it is currently the most widely used membrane process for cheese-making. In the manufacture of cheese using UF or MF, the quality of the end cheeses are often determined by specific properties of the protein-enriched product (Fox, 1993).

During the UF of milk at its normal pH (about 6.7), mineral salts bound to casein micelles are concentrated in the same proportion as proteins. This results in a retentate with increased buffering capacity (Fox et al., 2000), which affects a number of basic

parameters in cheese-making, such as acidification by lactic acid bacteria (LAB), final pH value, coagulation by rennet, rheological properties of the curd, activity of ripening enzymes, growth and rate of survival of spoilage micro-organisms, as well as water-holding capacity of the cheese throughout ripening (McSweeney, 2007). Hence, higher lactic acid production is needed by LAB to obtain the optimal pH, which often results in acid-tasting products for most cheese varieties (McSweeney, 2007).

A notable aspect of UF cheese is that whey proteins are incorporated in the curd. The amount of whey proteins retained is dependent on the degree of UF concentrate and variety of the cheese (Lo and Bastian, 1998). The whey proteins account for approximately 20% of the total protein in the cheese if it is retained completely. It substitutes part of the casein and acts as an inert filler, which may soften the cheese (Harper et al., 1989). Additionally, whey proteins have much higher water-binding capacity than casein, thus UF cheeses are often less susceptible to drying than traditionally-made cheeses during their shelf-life (Harper et al., 1989; Walstra et al., 2006).

During the acidification of UF cheese, large quantities of calcium salts are released into the aqueous phase of the cheese curd, which increases ionic strength and modifies casein aggregation. This sometimes results in cheese with a crumbly or sandy texture, as well as poor spreadability and stretching properties (Fox, 1993). Moreover, there is significant difference in the rheological properties of UF retentate and milk. Milk is considered as a Newtonian liquid. Whereas UF retentate exhibits a pseudoplastic behaviour, as its viscosity increases with decreasing shear stress (Walstra et al., 2006). Therefore, specific design and operating parameters of the UF system should be customised for cheese-making, using suitable UF flux and monitoring the degree of membrane fouling (Fox, 1993).

2.3.9. Major approaches in cheese-making using membrane processes

The production of cheese using UF can be divided into three major categories, which include (1) use of protein-standardised cheese milk (Kelly et al., 2008); (2) intermediate or medium concentrate retentates (Schreier et al., 2010); and (3) liquid

pre-cheeses or often highly-concentrated retentates, where the UF retentate possesses the composition of the cheese variety to be made (Fox, 1993).

In the collection of milk at dairy plants, the protein content varies and is affected by multiple factors, such as the stage of lactation (Mioč et al., 2008), weather (Roupas, 2001), feeding and breed of lactating animal (Auldist and Hubble, 1998). These variations in the composition of the incoming milk may require adjustment by cheesemakers in making consistent quality products (Roupas, 2001). For example, low protein content produces weak curds and subsequently high losses of caseins in whey (Walstra et al., 2006). In Europe, protein-standardised milk is applied in the manufacture of Camembert cheeses utilising the Alpma coagulator (Fox, 1993), which produces a continuous supply of curd for moulding.

Various cheese varieties ranging from soft to hard have been made using medium or intermediate concentrated retentates, which include the manufacture of UF Cheddar (Agrawal and Hassan, 2008), Feta (Jana and Thakar, 1996; Karami et al., 2009), and Havarti cheeses (Lo and Bastian, 1998).

When a higher concentration factor is used in UF or MF, a highly-concentrated retentate or liquid pre-cheese is produced. The cheese milk is often concentrated to the composition of the drained curd being made before the addition of rennet in soft cheeses (Maubois, 2002). Hard cheeses on the other hand require a series of UF and MF processes, as UF alone does not sufficiently result in the high retentate concentration required for hard cheeses without membrane fouling (Schreiera et al., 2010). Whey drainage in this process is minimal and the use of cheese vats is not required. This principle was first applied in the manufacture of Camembert cheese (Maubois and Mocquot, 1975), and was successfully developed in producing other cheese varieties (Brandsma and Rizvi, 2001; Schreier et al., 2010). Nowadays, a range of membrane processing modules are commercially available for the manufacture of soft cheeses, and these membranes have been developed based on cellulose acetate (Nagendran and Mohan, 2008), polycarbonates (Ko et al., 1993), polysulphonates, and zirconium oxide (Shaw, 1981). A continuous procedure known as the ‘Camatic’ process is utilised in a Camembert cheese plant by Alfa Laval, which makes use of the

MMV patent and employs a Romicon hollow fibre UF plant with polysulphonate membranes (Shaw, 1981; Fox et al., 2004).

In France, UF Camembert cheeses have been associated with psychological and commercial difficulties. Although there is no significant differences between the organoleptic qualities of UF and traditionally made Camembert cheese, the bulk density of the UF cheese paste is much higher than that of traditional cheese due to the lack of mechanical openings (Fox, 1993). Therefore, French consumers get the impression that they received less cheese for their money, as they are accustomed to buying Camembert cheese by the size of the piece and not by weight (Fox, 1993). Although UF Camembert was not highly successful on the French market, it led to the development of other UF varieties, and the subsequent success in the manufacture of UF Feta. In Denmark, Feta accounts for 35% of the total cheese produced and more than 90% is produced by UF (Fox, 1993).

While UF cheeses offer considerable yield benefits and have been well established on the consumer market, they do inherit unique composition, ripening characteristics, organoleptic and textural qualities (Maubois, 2002). It has been suggested that separate standards of identity should be used for UF cheeses, and new cheese varieties should be developed instead of duplicating traditional varieties (Fox, 1993).

2.4. Camembert cheese

A small proportion of world cheese production consists of mould-ripened cheeses, which have a growing popularity with consumers and increasing demand on the market (Fox et al., 2004). Surface mould-ripened soft cheeses such as Camembert and Brie; and blue veined cheeses such as Roquefort, Gorgonzola, and Danish Blue are examples of mould-ripened cheess. The mould present on the surface (*Penicillium camemberti*) or within the cheese (*P. roqueforti*) gives the products different appearances and produce very distinctive aromas and tastes (Fox et al., 2000). Camembert is well known and is the most widely produced surface mould-ripened soft cheese, with the growth of a white mould species *Penicillium camemberti* attributing to its characteristic surface rind (Shaw, 1981).

2.4.1. Composition, properties and standards

Under French food law, Camembert is a non-scalded and non-pressed cheese. The cheese generally weighs between 210 and 260 g, and must have at least 110 g of total solids (TS). The fat content in dry matter is about 45%, but there may be variants with lower or higher fat contents (Shaw, 1981). The dimensions of a Camembert explained by Shaw (1981) are 10.5 to 11.0 cm diameter with 3.0 cm thickness.

2.4.2. Manufacturing of Camembert

The manufacture of Camembert cheese and most cheese varieties is fundamentally a method of preserving milk over the short and medium term (Shaw, 1981; Walstra et al., 2006). The main purpose of this preservation method is to lower the pH and water activity, which discourages the growth of spoilage microorganisms (Linton et al., 2008). The manufacturing process of Camembert and other soft cheese varieties has been well-documented (Fox et al., 2000; Walstra et al., 2006; Gunasekaran and Ak, 2003; Shaw, 1981; Scott, 1998). A schematic of the manufacturing protocol for Camembert cheese is shown in Figure 2.1. In comparison to other cheese varieties, Camembert has a relatively high pH value and high water activity. Therefore, it results in the product having a short shelf-life with maturation being achieved in a relatively short time (Shaw, 1981). The lowering of the pH is achieved by controlled lactic fermentation, and draining and salting of the curd reduces the water activity. In addition to the control of pH and water activity to produce a stable product, further controlled transformation of the product during maturation is attained by the micro-flora and enzymes (Fox, 1993). Coagulation of milk in the manufacture of Camembert cheese is obtained by a combination of the lactic acid production by lactic acid bacteria (LAB) and a coagulant protease.

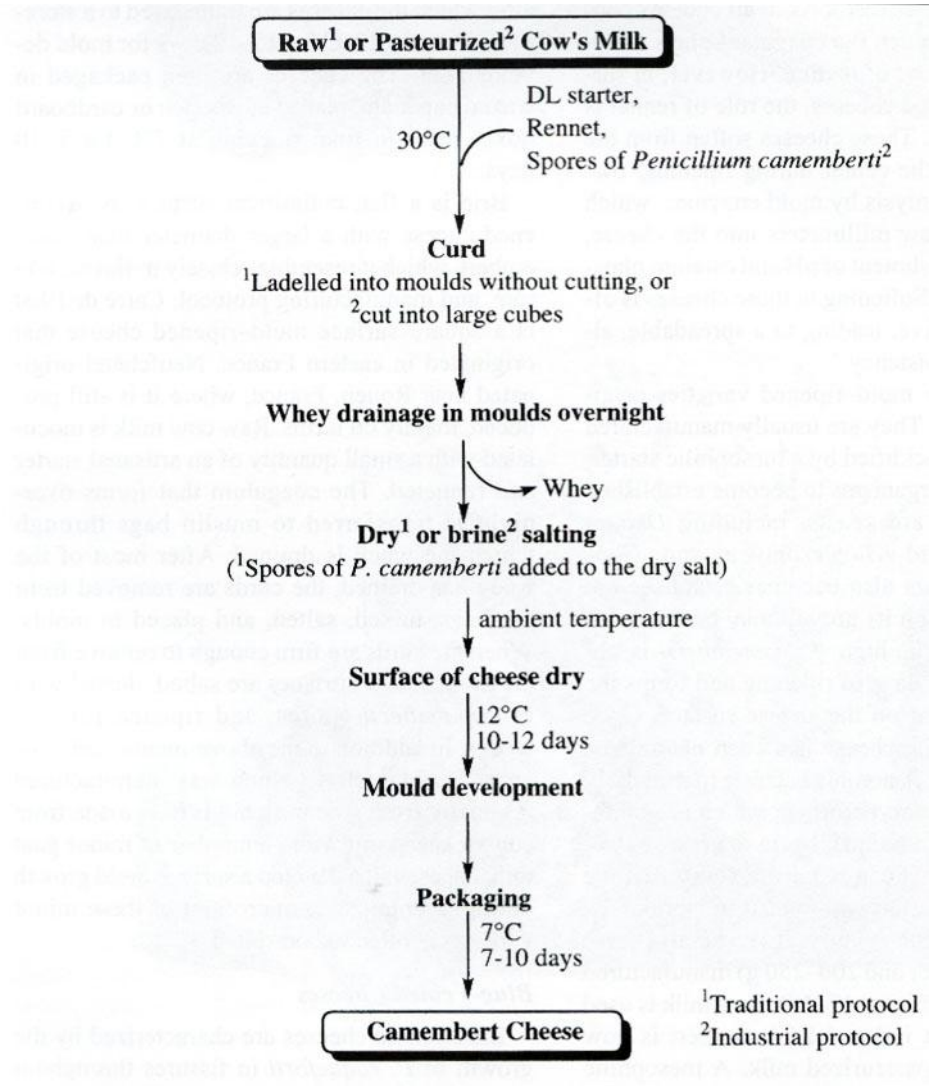


Figure 2.1: Manufacturing protocol for Camembert cheese (Walstra et al., 2006).

In the various stages of manufacture, including milk ripening, coagulation, curd draining, and maturation, the acidification profile is of key importance (Gunasekaran and Ak, 2003). The rate and quantity of lactic acid production affects (1) the level of calcium solubilisation in milk, and consequently the rheological properties of the coagulum; (2) the rate of whey draining in curd cutting; and ultimately (3) the mineral composition and texture of the final cheese (Fox et al., 2000). Additionally, LAB starters are important during the maturation of the cheese, as they contribute to the overall flavour of the cheese. Therefore in cheese-making, the acid production phase should be of a predictable and consistent nature with defined methods of control. Hence, it is crucial to select and use LAB cultures of good quality (Fox et al., 2000).

The level of proteolysis obtained by the coagulating enzymes is another major factor which influences a sequence of events, including the rheological properties of the coagulum, whey drainage, and maturation of the final cheese (Walstra et al., 2006). The ripening or maturation period for Camembert varies between 15 and 60 days (Fox et al., 2000), depending on a number of factors including the moisture content of the cheese, the activity of proteases and lipases derived from the rennet, LAB starters, surface or internal microflora of the cheese (Fox, 1993), temperature as well as the relative humidity of the maturing rooms (Walstra et al., 2006; Gunasekaran and Ak, 2003).

The methods for the manufacture of Camembert and other soft cheeses have evolved over the years, where various recipes may be found showing variations in the amount of LAB starters or rennet addition, time or temperature control in different stages, etc. The addition of various technological innovations to the traditional methods have allowed them to evolve into large-scale industrial processes that are highly sophisticated, mechanised, and automated with large milk throughput (Fox, 1993).

2.4.3. UF in Camembert cheese-making

The application of UF processes in Camembert cheese-making since the invention of the MMV process has led the production of Camembert cheese to industrial scale with high capacity and automation. An example is the "Camatic" process which was developed by Alfa-Laval for continuous cheese manufacture and is located in its Nantes Camembert plant. It has a capacity of 50,000 litres per day or 4,000 Camembert cheeses per hour (Shaw, 1981).

The 'Camatic' consist of three main stages, comprising the preparation of the pre-cheese, automated moulding operations and ripening of the cheese (Fox, 1993). The procedure of manufacture gives about 12 to 15 % yield increase (Shaw, 1981), and involves the following:

- High-temperature short-time pasteurised milk is ultrafiltered at 50 ºC to a pre-cheese retentate of 5:1 and a TS content of 35%;
- The pre-cheese is cooled to 30 ºC and 2 % mesophilic LAB starter and 0.75% NaCl are added;

- The mixture is acidified to pH 5.5 and is filled into forms by an automated process which utilises on-line inoculation with rennet;
- Curd wheels develop rapidly and are continuously and gently moved for 45 minutes;
- The cheeses are turned over once, and a continuous electric current is applied to each cheese between the air-exposed surface in contact with a carbon electrode and the stainless steel cup holding the cheese. Any whey discharged from the cheese is demineralised in this process allowing the whey for uses in other foodstuffs (Harju, 1989);
- Air injector is used to demould the wheels onto cheese trays;
- The fresh Camembert cheeses are brined for approximately 30 minutes and then removed from the brine solution;
- The cheeses are sprayed with *Penicillium camemberti* spores and held for 12 days at 11-12 ºC to allow mould development on the cheese surface (Shaw, 1981).

2.5. Cheese ripening and quality

The ripening of cheese includes all the biochemical changes occurring in cheese, catalysed by the metabolic activity of the microflora and enzymes derived from these organisms or from other sources (Fox et al., 2000). During cheese ripening, the structure and composition of the cheese changes, as well as its organoleptic properties (Walstra et al., 2006), thus affecting the ultimate quality of the cheese.

2.5.1. Effects of lactic fermentation

The growth of LAB is important in the manufacture and maturation of cheese. It has been indicated that in the fresh cheese, starter bacteria replicate only a few times, and their growth in cheese ceases at relatively high pH levels (e.g. 5.7) with variations depending on the species and the strains involved (Walstra et al., 2006). Although the starter culture ceases to grow, fermentation in the cheese continues and allows further reduction in pH. The growth of most LAB may be reduced or stopped by the addition of salt to the cheese, with the exception of salt-tolerant LAB contained in cheese milk added with high salt content (Walstra et al., 2006). The rate of acid production in

cheese is of particular importance, where a predictable and consistent rate of acidification is necessary in eliminating variations in subsequent curd syneresis, moisture content and texture profile of the cheese (Kristensen, 1999; Walstra et al., 2006).

The production of lactic acid from lactose is essential for the preservation of cheese. It reduces pH and lactose content in the cheese, which help prevent the growth of undesirable microorganisms (Walstra et al., 2006).

2.5.2. Proteolysis

In the process of cheese ripening, proteins are broken down by proteolytic enzymes into smaller molecules, ranging from large peptides to free amino acids or ammonia. The concentrations of these products differ depending on the type of cheese, starter and coagulant used, and conditions of ripening (McSweeney, 2007).

Milk proteinases

In cheese, milk proteinases (alkaline and acid proteinases) decompose α_{s1}-, α_{s2}-, and β-casein. Most of the alkaline milk proteinase, or plasmin, is found as inactive plasminogen with a small portion being active (Walstra et al., 2006). It has a high affinity for casein at the pH of milk, and dissociates from casein at low pH (Bastian and Brown, 1996). The enzyme becomes active at high pH and decomposes β-casein more quickly than α_{s1}-casein, and the metabolism of β-casein yields various γ-caseins (γ^1-, γ^2- , γ^3- caseins). Acid milk proteinases have an optimal pH of 5.1 to 5.6 in cheese, and metabolise α_{s1}-casein faster than β-casein (Walstra et al., 2006).

Milk proteinases contribute to the proteolysis in cheese, which increases the amount of soluble nitrogen (SN) compounds, mainly consisting of peptides and small amounts of amino acids (Bastian and Brown, 1996). The proteinase content and its precursors in the milk can vary among milking times. Heat treatments or scalding processes induces partial inactivation of compounds which inhibit plasminogen activators, and consequently increases plasmin activity (Grappin et al., 1985). The pH conditions in cheese are often unfavourable for significant proteinase activity (Bastian and Brown,

1996). However, a high pH cheese such as Camembert due to surface mould activity may activate the milk proteinases (Walstra et al., 2006).

Enzymes from clotting agents

Clotting enzymes not only have a specific function in milk clotting, but also have a significant effect on proteolysis in cheese and the subsequent quality of the product (Fox et al., 2000). The amount of clotting enzymes (e.g. calf rennet) retained in the cheese affects its action on further proteolysis. This is dependent on the adsorption of the enzyme onto the paracaseinate, determined by factors such as the amount of the enzyme added to the milk; the pH during curd making of which a higher quantity of the enzyme is adsorbed onto the paracasein at lower pH; and the scalding temperature of the curd for cheese varieties such as Emmentaler, where higher scalding temperatures inactivates a larger portion of the enzyme (Walstra et al., 2006).

The optimum pH of most clotting enzymes, such as calf rennet is approximately 5. The metabolism of α_{s1}-casein is markedly faster than that of β-casein. Proteolysis by clotting enzymes produces SN compounds with a large portion consisting of products with a relatively high molar mass, and only a small amount of amino acids (Walstra et al., 2006).

Enzymes from lactic acid bacteria

Starter bacteria produce proteolytic enzymes which play an important role in the maturation of ripened cheeses (Grappin et al., 1985). They target large peptides formed by clotting enzymes or plasmin, and produce small peptides and free amino acids which contribute directly or indirectly as precursors to flavour development (Walstra et al., 2006). Generally, any variations in the proteolysis of a cheese type is mostly due to variation in the enzyme activity of the starter bacteria.

In the mesophilic starters, some *Lactococcus* strains are proteinase positive (Prt+), which contain a cell envelope proteinase required for growth of the bacteria in milk (Foucaud and Juillard, 2000; Walstra et al., 2006). In some strains which are proteinase negative (Prt-), they depend on the Prt+ strains to form peptides from milk proteins, primarily casein (Walstra et al., 2006; Foucaud and Juillard, 2000). In cheese, both α_{s1}- and β-casein are targeted, but the rate of peptide formation is slow.

This is aided by action of clotting enzymes such as chymosin, which produces peptides at a rapid rate, and these are further hydrolysed by bacterial enzymes (Walstra et al., 2006). Additionally, smaller peptides are transported into the bacterial cell where specific peptidases immediately hydrolyse the peptides into amino acids, and they are subsequently diffused out of the cell. The peptidases in the bacterial cells include endopeptidases, aminopeptidases, di- and tripeptidases, and proline-specific peptidases (Walstra et al., 2006).

The production of peptides and subsequently the formation of amino acids are of significant importance in the flavour profile of cheese, as accumulation of peptides might lead to a bitter off flavour (Kristensen, 1999). Some cultures have the ability to degrade bitter-tasting peptides, and are often used in the manufacture of various cheese types. This ability has been found to have positive relationship with the sensitivity of the cell to lysis (Walstra et al., 2006).

In order for the bacteria to perform peptidolysis, energy is required to transport the peptides into the cell and convert them into amino acids. The energy source comes from lactose, which is usually fully consumed within 24 h in most cheeses. Therefore, lysis of the starter bacteria is required to release their intracellular peptidases into the cheese (Exterkate, 2006; Hannon et al., 2006). This can be achieved by bacterial autolysins with a subsequent osmotic shock to break down the cell wall, usually induced by a steep salt gradient (Walstra et al., 2006). Membrane permeabilisation techniques have also been studied to release the intracellular peptidases, through delipidating action of n-butanol (Exterkate, 2006).

The quantity of enzymes produced by the starter bacteria can vary depending on the growth conditions. In the case of cheese milk that has been highly concentrated by ultrafiltration or undergone intense heat treatment (10 min at 120 ºC), the amount of cell envelope proteinases and some of the intracellular peptidases are significantly reduced (Walstra et al., 2006). Thermophilic starters, such as *Streptococcus thermophilus* (Simov and Ivanov, 2005) and various *Lactobacillus* species, such as *L. helveticus* (Richoux et al., 2009), *L. delbrueckii* ssp. *bulgaricus* (Moreira et al., 2003),

and *L. casei* (Ong et al., 2007) also demonstrated similar proteolytic activities in cheese.

Enzymes from non-starter microorganism

Contamination of the cheese milk by non-starter LAB often involves the *Lactobacillus* species. They do not utilise sugar as a carbon source but feed on amino acids or the carbohydrate moiety of glycoproteins in the membrane of fat globules (Walstra et al., 2006). These bacteria are often highly proteolytic and can produce a wide variety of flavour compounds in cheese, of which they are usually undesirable. Therefore, milk pasteurisation and good hygienic practices during cheese-making can mostly prevent the growth of such microorganisms (Walstra et al., 2006). However, in the production of certain types of cheese using traditional methods, raw milk is used to enhance the more typical flavours of these cheese types (Fox et al., 2000).

In most cheese varieties, a secondary microflora is inoculated in the cheese milk or curd. An example is *Penicillium camemberti* on surface mould cheeses such as Camembert and Brie (Fox et al., 2000). *P. camemberti* produces aspartyl and metalloproteinases which metabolises α_{s1}- and β-caseins (Fox, 1993). Two extracellular proteinases, including an acid proteinase and a neutral one, are produced by *P. camemberti*. The latter one induces large increases in pH 4.6-SN, but does not have much effect on the production of free amino acids (Boutrou et al., 2006). Additionally, an exocellular acid carboxypeptidase and a neutral carboxypeptidase which is bound mycelially, and an exocellular aminopeptidase are synthesised by *P. camemberti*, of which have impact on the formation of free amino acids in soft cheeses such as Camembert (Boutrou et al., 2006).

2.5.3. Lipolysis

The concentration of active native lipoprotein lipases in milk is dependent on the pasteurisation process. Approximately 10 to 15% of the lipase remains after usual pasteurisation conditions (Walstra et al., 2006). The activity of lipase in raw milk is relatively stable, but acidification and increasing salt content of the milk or curd usually leads to its deactivation (Fox et al., 2000). Lipolysis significantly increases with temperature, and also with the homogenisation of the cheese milk. This is due to

changing of the surface layer of fat globules, of which the rate of lipolysis returns to its normal level when the surface layer stabilises (Walstra et al., 2006).

Generally, LAB are not very lipolytic when compared to other bacteria such as *Pseudomonas*, and moulds (Fox et al., 2000; Walstra et al., 2006). However, when lactococci and lactobacilli are present in high numbers over a long period, their lipases and esterases become the principal lipolytic agents in Cheddar and Dutch-type cheeses (Fox et al., 2000). In cheese types such as Romano, Parmesan and Blue, they are characterised by extensive lipolysis.

2.5.4. Flavour development

The balance of different flavour compounds is essential to the flavour of the cheese. The flavour compounds originating from the fat and other milk components are weak in the curd. The sweetness from lactose in the curd disappears rapidly due to lactic fermentation, which produces an acidic flavour in the cheese (Walstra et al., 2006). The flavour compounds (e.g. diacetyl) produced by the starter bacteria are particularly important in fresh-type cheeses. In ripened cheeses, salt provides additional flavour, and its concentration varies between cheese varieties (Kristensen, 1999).

Degradation of protein

The flavour of the cheese undergoes major changes during ripening. The degradation of protein results in the formation of short-chain peptides and free amino acids, which contribute to the flavour profile of the cheese (Boutrou et al., 2006). The conditions of ripening can lead to variation in specific tastes and their relative intensities. A bitter flavour may be developed if a specific way of protein degradation is encouraged, resulting in the accumulation of short-chained hydrophobic peptides (Walstra et al., 2006).

The conversion of amino acids by deaminases, decarboxylases, transaminases, and lyases also play an important role in developing more complex flavours in cheese (Figure 2.2) (Walstra et al., 2006). Deaminases and decarboxylases yield amines and ammonia from amino acids. Alpha-keto acids are formed from transamination of amino acids, which may be subsequently converted into aldehydes by

decarboxylation, and then into alcohols or carboxylic acids by dehydrogenation (Walstra et al., 2006; Fox et al., 2000). The majority of these volatile compounds are odour active. Aromatic amino acids, branched-chain amino acids, and methionine are important substrates for the development of flavour in cheese. They can be converted into compounds such as *p*-cresol and indole by lyases, which contribute to off-flavours. Branched-chain amino acids can be converted into compounds such as isobutyrate, isovalerate, and 3-methylbutanal often with the presence of *Lactococcus lactis* strains (Walstra et al., 2006). Sulphur compounds produced from methionine by several lyases contribute to the typical flavour of many cheese varieties. Other notable compounds formed from methionine are thiaalkanes (e.g. 2,4 dithiapentane), which gives a distinctive garlic flavour in Camembert cheese (Fox et al., 2000; Walstra et al., 2006). Additionally, cheese consistency and mouthfeel are affected by protein degradation, where cheese consistency is correlated with flavour perception (Hersleth et al., 2005).

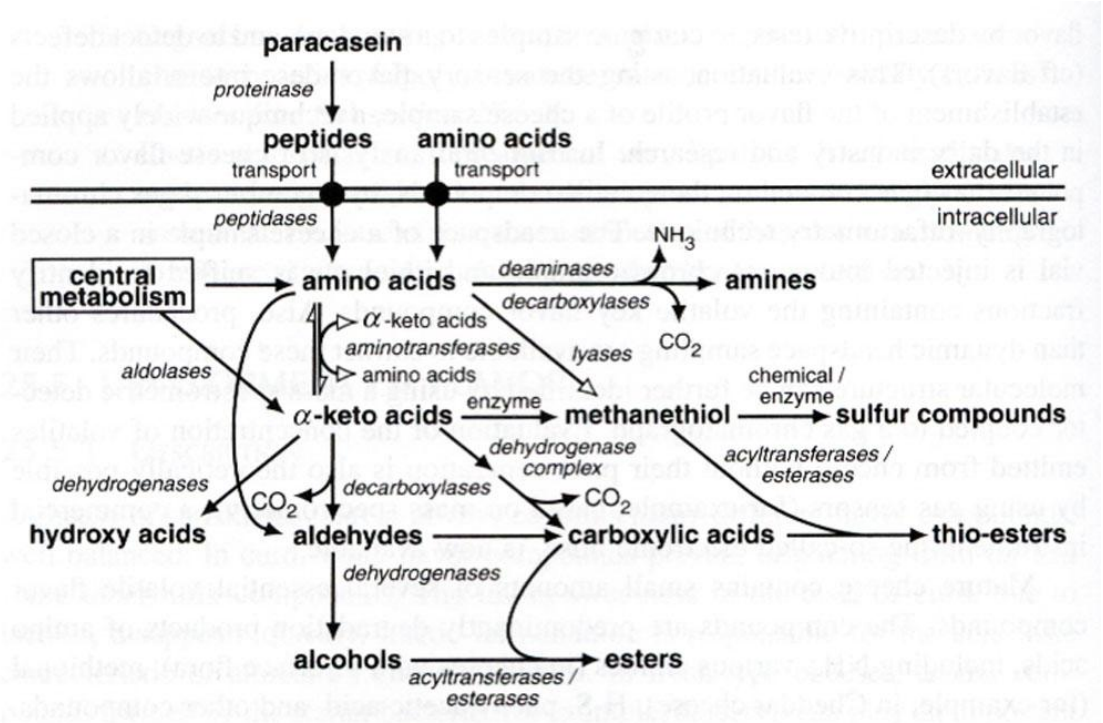


Figure 2.2: Overview of general protein conversion pathways for flavour formation in cheese (Walstra et al., 2006).

Degradation of lipids

Free fatty acids (FFA) produced in the lipolysis of fat are also involved in the flavour perception of cheese. Depending on the concentration and perception threshold of the volatile fatty acids, they can contribute to the aroma of the cheese, or to a rancid

defect (Poveda et al., 2008). The FFA act as precursors of flavour compounds such as methylketones, alcohols, lactones, and esters (Figure 2.3).

The effect of LAB on fatty acids conversion is relatively small, but in mould-ripened cheeses (e.g. Camembert and Roquefort) the fat-derived flavour compounds play a significant role (Figure 2.3) (Walstra et al., 2006). The mould in these cheeses produces β-keto acids through the β-oxidation of fatty acids, which is subsequently decarboxylated to produce methylketone, characterised by its fruity, floral and musty notes. Secondary alcohols can be formed by the reduction of methylketones, including heptan-2-ol and nonan-2-ol. Together with the methylketones, they represent 20 to 30% of all aroma compounds in Camembert-type cheeses (Walstra et al., 2006). Meanwhile, primary alcohols are formed from the metabolism of lactose and amino acids, which is described by its mild and fruity notes. Eight-carbon aroma compounds, such as oct-1-en-3-ol, are derived from linoleic and linolenic acids by lipoxygenase and hydroperoxide lyase found in moulds, which is responsible for a mushroom note in cheese (Walstra et al., 2006). In addition, cheeses consist of a wide range of esters, usually described as fruity and floral notes, and are formed by esterases present in most microorganisms involved in cheese ripening (Fox et al., 2000; Coolbear et al., 2008).

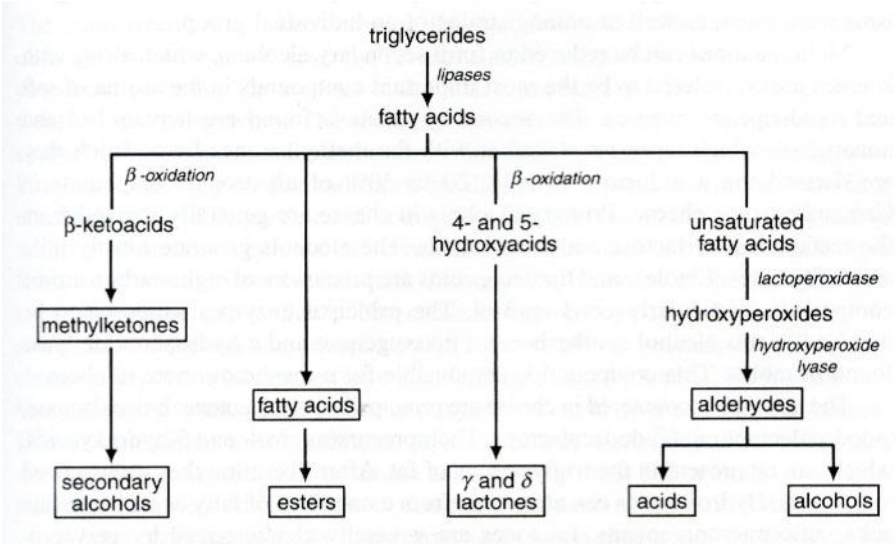


Figure 2.3: Formation of flavour compounds from fat in cheese (Walstra et al., 2006).

2.5.5. Texture development

In the microstructure of a fresh cheese immediately after curd making, the cavities in the matrix of the paracasein micelles are mostly filled with fat globules and a small amount of whey (Gunasekaran and Ak, 2003). The moisture is still able to move freely through the network. However, the matrix alters within a day and becomes more homogeneous. Whey is drained from the cavities and it has become increasingly difficult for moisture to move freely (Walstra et al., 2006). This can be attributed to the dissolution of calcium phosphate and subsequent proteolysis. When the cheese is matured for a long period, fat globules become partly fused and aggregated (Walstra et al., 2006; Gunasekaran and Ak, 2003).

During ripening, various changes in the cheese composition occur. Proteolysis causes the uptake of water and the formation of ionic groups ($-COO^-$ and $-NH_3^+$), production of ammonia and subsequently increase in pH. The rate of such change depends on several conditions, such as the size and shape of the cheese, amount of retained rennet, amount and type of bacteria and enzymes released, pH, surface flora, and ripening conditions (Walstra et al., 2006). The changes in consistency over the ripening period are dependent on the type of cheese. Cheese with a high water content and low initial pH may start off as being short and firm, but as the pH increases over time (e.g. 5.4), the consistency becomes rubbery. This may be attributed to proteolysis which causes the cheese to develop a soft and plastic, and in some cases, liquid-like texture at higher pH levels by degrading the casein responsible for holding the cheese structure (Walstra et al., 2006; Gunasekaran and Ak, 2003).

2.6. Analysis of cheese

2.6.1. Determination of pH

In cheese manufacture and ripening, pH is useful in predicting the activity of the microflora and their enzymes in the milk, curd, and cheese (Gunasekaran and Ak, 2003). The rate of change in pH is usually of a predictable and consistent nature in cheese-making. If, for example, the pH decreases too rapidly before the addition of starter or does not decrease during acidification, it is likely that a contamination is present in the cheese milk (Fox et al., 2004). During the ripening of Camembert, the

cheese usually has an initial pH of about 4.8 which increases to about 7.5 (Fox, 1993). This is due to the catabolism of lactic acid and the production of ammonia by proteolysis, and pH serves as an indicator for the age of the cheese during maturation (Walstra et al., 2006). The measurement of pH is commonly carried out using a pH-meter with various electrodes (Kosikowski and Mistry, 1997). Additionally, specific electrodes designed for the measurement of pH in cheese and dairy products are available.

2.6.2. Total solids

Total solids (TS) in cheese consist of fat, proteins, lactose, minerals and ash (Fox et al., 2000). The TS content in cheese is expected to increase during ripening as a result of surface evaporation, exchange of volatile compounds, and action of proteolysis (Walstra et al., 2006). TS content is determined by the loss of moisture through drying of the cheese sample using a hot-air or vacuum oven at 105 ºC (AOAC International, 2005).

2.6.3. Salt content

In cheese-making, salt is used to inhibit or slow down the growth of microorgasms and the activity of enzymes after curd making. It also reduces moisture content in the cheese and contributes significantly to cheese flavour (Walstra et al., 2006). The effect of salting on the ripening and subsequent flavour and texture of the cheese depends on the cheese variety and the method used (Scott, 1998). The Volhard method is used to determine the salt content in cheese (Krik and Sawyer, 1991). The salt reacts with standard silver nitrate solution in excessive amounts and the unused silver nitrate is titrated against potassium thiocyanate solution.

2.6.4. Fat content

Fat content contributes significantly to the flavour and texture of cheese. The Mojonnier method is commonly used for determining fat in cheese, which is described in the AOAC Official Methods (AOAC International, 2005). The modified Schmid-Bondzynski-Ratzlaff method is also very similar but requires slight

alterations. Generally, the fat in cheese is extracted using ethanol, diethyl ether and petroleum ether, where the solvents are subsequently evaporated and the extracted fat is weighed (AOAC International, 2005).

2.6.5. Nitrogen content

The extent of proteolysis in cheese can be determined by the analysis of the N content in different fractions of the cheese. This includes different N fractions which enables the measurement of different activities in cheese (Ardö, 1999; Butikofer et al., 1993). The fractionation scheme described by Ardö (1999) comprises the total nitrogen (TN); pH 4.4-SN, which consists of all proteins excluding casein, and non-protein nitrogens (NPN) including peptides, amino acids and smaller N compounds soluble at pH 4.4 to 4.6; and TCA-SN, which consists mainly of NPN including medium sized to small peptides, amino acids, and smaller N compounds (Ardö, 1999). Fractionation of the N compounds in cheese allows the monitoring of proteolysis throughout cheese maturation, as the caseins in cheese are continuously broken down into N compounds by enzyme action (Fox et al., 2000). The Kjeldahl method is subsequently used to determine the N content in each fraction, which involves extraction, digestion, and titration (AOAC International, 2005).

2.6.6. Texture analysis

Texture is an important quality which determines the identity of a cheese and its preference by consumers (Antoniou et al., 2000). Texture measurement techniques can be categorised as either subjective or instrumental (Gunasekaran and Ak, 2003). Instrumental methods are often used to mechanically mimic the sensory evaluation of human assessors, without being subjective and biased. Texture profile analysis (TPA) is used to imitate the grading action of the jaw, by inducing two successive deformations in the food sample (Gunasekaran and Ak, 2003). It enables the explanation of primary mechanical properties such as hardness, cohesiveness, viscosity, elasticity, and adhesiveness, and secondary properties such as brittleness, chewiness, and gumminess (Gunasekaran and Ak, 2003; Antoniou et al., 2000).

In the other texture analyses which do not follow the TPA protocol, uniaxial compression test is often used in measuring cheese properties (Gunasekaran and Ak, 2003). It is simple and measures some of the most important properties in cheese, such as firmness and springiness (Fox, 1993). The method usually involves the measurement of the force required to compress or deform the cheese sample to a certain percentage of its original height (Antoniou et al., 2000; Wium et al., 1997). Attributes such as firmness, fracturability, springiness, chewiness and adhesiveness can be calculated based on the peak forces and work required (Fox et al., 2004).

2.6.7. Sensory analysis

The most important sensory properties of cheese ripening are those of which affects the consumer in purchasing the product, including flavour, aroma, texture, and appearance (Fox et al., 2000).

Cheese grading

Sensory analysis of cheese has been traditionally performed by cheese graders or experienced experts in determining the quality of the cheese at the point of sale. In these systems, points are usually deducted for perceived defects with respect to their intensity. The characteristics sought after by expert judges are usually desired by general consumers, but the scores or grades awarded do not always correlate with consumer preference (Meilgaard et al., 2007). The expert grading systems are commonly used in the cheese industry to ensure consistent product quality and may be used for pricing (Fox et al., 2000).

Discriminative analysis

The use of discrimination tests, such as the paired comparison, duo-trio, and triangle tests enables one to assess whether there are differences between cheeses in terms of certain sensory characteristics (Meilgaard et al., 2007). The protocols of these tests are very similar, where two samples (paired comparison test), two samples with a reference sample (duo-trio test), or three samples (triangle test) are evaluated based on specific attributes or overall likings (Meilgaard et al., 2007). This type of sensory analysis requires trained panellists and is dependent on the perception threshold of individual panellists on specific sensory stimuli (Fox et al., 2004).

Descriptive analysis

Descriptive evaluation requires a panel of trained assessors and is used to determine the sensory profile of the cheese tested. It involves three main stages: selection of potential assessors based on their ability to perceive sensory stimuli; determining a list of sensory terminology or 'descriptors' which describe the sensory attributes of the cheese; and defining the processes to quantify the intensity of each descriptor (Fox et al., 2000). Assessors in the panel are required to differentiate between atrributes, give reproducible scores, and agree with other panellists (Meilgaard et al., 2007).

Consumer acceptance

An acceptance test is used to determine the "affective status" of a product, usually measured by a hedonic line, intensity, or magnitude estimation scales with degrees of acceptance or liking. This type of test usually involves untrained consumer groups which are highly diverse, and the tests easily understandable. The tests are similar to attribute difference tests except that the attribute in this case is acceptance or liking (Meilgaard et al., 2007).

2.6.8. Microbiological analysis

The consumption of contaminated cheese can lead to food illnesses. In 2006, contaminated cheese accounted for about 0.4% of the total food outbreaks in Europe (Kousta et al., 2010). Common food pathogens associated with food outbreaks include *Listeria monocytogenes*, *Samonella* sp, *Escherchia coli*, and *Staphylococcus aureus* (Kousta et al., 2010). Cheeses are food products that are ready for direct consumption without any further treatment to ensure their safety. Contamination of cheese by pathogens can occur at several stages and it is therefore important that strict microbiological analyses are carried out to as part of a food control system.

2.7. Conclusions

Membrane technology has become widely used with the invention of the MMV process in producing Camembert cheese. Manufacture of cheese using UF has shown to exhibit different properties compared to traditional processes, including the increase in buffering capacity, incorporation of whey proteins in cheese, and changes in acidification profile. The 'Camatic' procedure employed by Alfa-Laval demonstrated the success in producing UF Camembert cheese using a highly automated and continuous process. The properties of ripened cheeses, especially in those of surface mould-ripened soft cheeses, are highly dynamic and are affected by a number of factors. Learning these fundamentals is essential in understanding the different quality attributes present in UF cheeses.

The factors which affect cheese ripening and its subsequent quality include lactic fermentation of the cheese; proteolysis and lipolysis induced by a variety of enzymes; the development of flavour and texture. Proteolysis in cheese is facilitated by different types of enzymes including milk proteinases, and enzymes of clotting agents, LAB, and non-starter microorganisms, which act on the paracasein structure of the cheese and produce smaller peptides and N compounds. Lipolysis in cheese liberates volatile compounds which contribute greatly to the aroma and flavour of cheese. Some secondary floras such as *Pseudomonas* and moulds have high lipolytic activity which gives certain cheese types their typical flavour. The degradation of protein and fat globules by the fore-mentioned enzymes lead to the production of smaller peptides, amino acids and other N compounds, as well as FFA and other FFA-derivatives which contributes to the flavour and aroma of the final cheese. The breakdown of the cheese microstructure also causes changes in texture and consistency of the cheese, which are of significant importance in consumer preference.

Although production of Camembert using UF has been highly successful, the processing parameters are often confidential for commercial purposes and vary between processing plants, ingredients, and product requirements. This study aims to determine the optimum processing parameters for the production of Camembert cheese from bovine milk utilising a hollow-fibre UF system, with respect to improving the overall quality and shelf-life of the cheese.

3. MATERIALS AND METHODS

3.1. Experimental design

In this study, an incomplete block design was used to investigate the effect of various variables in the manufacture of Camembert cheese on their impact on the final cheese quality. The manufacturing process was carried out at Goodman Fielder NZ Ltd Puhoi Valley Cheese plant. The design involved four variables: level of fat (in fat/protein ratio), salting method, level of acidification (determined by pH), and moulding type. Each variable contained two levels, resulting in sixteen unique treatments for the Camembert cheeses. The treatments were divided into four separate batches (no. 1-4, 5-8, 9-12, and 13-16, respectively) each containing four treatments (Table 3.1) as it was the maximum handling capacity per batch. Ideally, all sixteen treatments should be randomised across the four batches, but different levels of fat cannot be used simultaneously in a single batch and required scheduling of the processing plant to coordinate with mainstream products manufacture. The experiment was partially duplicated with all low fat treatments being identically repeated. The treatments were coded by underlining as follows: Low-fat/High-fat; Brine-salted/Retentate-salted; pH 5.2/pH 4.9; Tube mould/Small mould (e.g. LB5.2T represents low fat, brine salting, pH 5.2, tube mould). The underlined letters and numbers indicate the codes used.

Table 3.1: Incomplete block design of sixteen unique treatments for the manufacture of Camembert cheese

No.	Treatment Codes	Milk F/P Ratio	Salting Method	Extent of Acidification	Mould Type
1	LB5.2T	F/P 1.32:1	Brine-salted	pH 5.2	Tube mould
2	LB4.9S	F/P 1.32:1	Brine-salted	pH 4.9	Small mould
3	LR5.2S	F/P 1.32:1	Retentate-salted	pH 5.2	Small mould
4	LR4.9T	F/P 1.32:1	Retentate-salted	pH 4.9	Tube mould
5	LB5.2S	F/P 1.32:1	Brine-salted	pH 5.2	Small mould
6	LB4.9T	F/P 1.32:1	Brine-salted	pH 4.9	Tube mould
7	LR5.2T	F/P 1.32:1	Retentate-salted	pH 5.2	Tube mould
8	LR4.9S	F/P 1.32:1	Retentate-salted	pH 4.9	Small mould
9	HB5.2T	F/P 1.59:1	Brine-salted	pH 5.2	Tube mould
10	HB4.9S	F/P 1.59:1	Brine-salted	pH 4.9	Small mould
11	HR5.2S	F/P 1.59:1	Retentate-salted	pH 5.2	Small mould
12	HR4.9T	F/P 1.59:1	Retentate-salted	pH 4.9	Tube mould
13	HB5.2S	F/P 1.59:1	Brine-salted	pH 5.2	Small mould
14	HB4.9T	F/P 1.59:1	Brine-salted	pH 4.9	Tube mould
15	HR5.2T	F/P 1.59:1	Retentate-salted	pH 5.2	Tube mould
16	HR4.9S	F/P 1.59:1	Retentate-salted	pH 4.9	Small mould

Notes: Milk F/P Ratio = milk fat : protein ratio

3.1.1. Description of processing variables

Level of fat (Milk fat/protein ratio)

The level of fat in cheese can significantly affect its flavour and texture (Gunasekaran and Ak, 2003). Therefore, fat content in the pre-cheese retentate was adjusted at two levels. The average F/P ratio in the non-standardised milk used in Camembert cheese production was 1.32:1 and this was used as the lower level. The higher level of F/P ratio was adjusted to approximately 1.59:1. This was equivalent to the ratio used for single cream Camembert cheese produced by the conventional method. Adjusting the desired level of milk F/P ratio was achieved by the addition of pasteurised cream to the pre-cheese retentate.

Salting method

The two salting methods used were addition of dry salt to the pre-cheese retentate and brine immersion of the cheese after moulding. Retentate-salting involved the addition of dry salt into the pre-cheese retentate prior to rennet and formation of cheese blocks. Brine salting was done by immersing whole cheese units (3 cm height × 8 cm diameter in brine tanks with about 95% saline solution for 10 minutes after the cheese blocks were formed and the desired pH was achieved by acidification.

Extent of acidification

The acidification stage during cheese production is important as it determines the ultimate pH of the cheese, which in turn affects the growth of starter cultures and formation of the casein network (Fox et al., 2000). Pre-cheese retentate was placed in the acidification chamber at warm temperatures where they were allowed to form blocks of cheese. The amount of time the cheese was left in the chamber determines the ultimate pH, being either pH 5.20 ± 0.05 or 4.90 ± 0.05.

Mould Type

Two types of moulds were used: a cylindrical plastic tube mould measuring 35 cm height × 8 cm diameter (tube moulds); and silicone tray mould measuring 3 cm height × 8 cm diameter (small moulds). The pre-cheese retentate was poured into the cylindrical plastic tube moulds lined with plastic film. When the cheese was formed, they were removed from the moulds and plastic film. Cheese formed in tube moulds

were sliced with a wire cutter, and then loaded onto metal racks for further processing. Meanwhile, cheese formed in small moulds were removed from the moulds using a blunt knife, and they were loaded onto metal racks.

3.2. Production of Camembert

Pilot-scale production of the cheeses was conducted at Goodman Fielder (NZ) Ltd. Puhoi Valley Cheese plant. Seventy-two blocks of Camembert cheeses (125 g) were produced for each treatment.

Preparation of pre-cheese retentate

As part of the quality control system, cheese milk was analysed at the Puhoi Valley Cheese laboratory to ensure that the chemical and microbiological quality were within acceptable limits before being processed (Figure 3.9). The milk was pasteurised by a heat exchanger at 78 ± 2 ^{o}C for 15 seconds. A sample of the pasteurised milk was analysed for coliform count to ensure it was less than 10 cfu ml^{-1}. The milk was then pumped through a hollow fibre ultrafiltration unit and re-circulated until a TS content of 41.5 ± 1.0 % was reached, which was determined by the Lactoscope FTIR Advanced (Delta Instruments BV, Drachten, Netherlands) and its software (FTIR Scope Advanced). The retentate was homogenised at 19.31 bars, re-pasteurised at 78 ± 2 ^{o}C for 15 seconds, and cooled to 38 ± 1 ^{o}C in the cheese vats. A sample of the retentate was tested for coliforms level.

Addition of culture

Commercial freeze dried starter cultures (DELVO-ADD® 100-X DSF; 170 g 100 L^{-1} retentate, and DELVO-TEC® T30A DSF; 186 g 100 L^{-1} retentate, DSM, Heerlen, Netherlands) containing a mixture of *Lactococcus lactis* ssp., *Leuconostoc* sp., and *Streptococcus thermophilus* respectively was rehydrated in ultra-high temperature (UHT) treated milk at 38 ± 1 ^{o}C for 60 minutes. A commercial freeze dried mould culture (CHOOZIT™ PC12; 5 doses 100 L^{-1} retentate, Danisco, Copenhagen, Denmark) containing *Penicillium camemberti* was rehydrated in UHT milk at 4 ± 1 ^{o}C for 24 hours. Both rehydrated starter and mould cultures were added to the retentate and incubated for 30 minutes and the pH decreased to below 6.50.

Introduction of process variables

A trial fraction of the retentate (80 L) was obtained at this stage to conduct the experiment. The portion of retentate was divided into four treatments (20 L) for every batch (Figure 3.2), following the experimental design in Figure 3.1. In treatments involving lower fat contents, the retentate was not adjusted. Treatments involving higher fat content were subjected to the adjustment of fat/protein ratios of approximately 1.59:1 by adding 40% cream. The F/P ratio was analysed with the Lactoscope FTIR Advanced. Consequently, in treatments that involved salting in the retentate, 300 grams of dry salt was added per treatment of retentate (20 L). Salt was not added to treatments intended for brine salting at this stage. Fungal protease (Fromase®, DSM, Heerlen, Netherlands) was added to the retentate. All mixing of ingredients were done in a pour tank (Figure 3.3) and the mixture was then pumped into either silicone tray small moulds (Figure 3.4) or plastic tube moulds (Figure 3.5). A sample of the retentate was analysed for coliform level. The moulded retentate were left to set in a temperature controlled chamber at 30 ± 1 ^{o}C for 6 hours, 27 ± 1 ^{o}C for 18 hours, and then 22 ± 1 ^{o}C for 24 hours. During the 48-hour cheese setting period, treatments with lower acidification were removed from the chamber at pH 5.20 ± 0.05. Treatments with higher acidification were removed at pH 4.90 ± 0.05. Plastic tubes were removed from the young cheese logs and they were sliced by a wire cutter (Figure 3.6) to approximately 125 g units. Young cheeses in the small moulds were removed from the silicone trays. All young cheeses were loaded onto metal racks (Figure 3.7) and treatments which require brine salting were immersed in brine tanks (Figure 3.8) with about 95% salinity for 10 minutes before being placed in the ripening room together with other treatments.

Ripening, packing and storage

All the young cheeses were ripened at 18 ± 1 ^{o}C for two days and then 13 ± 1 ^{o}C for six days in 95% humidity. Each cheese was turned every two days. At the end of the ripening period, the cheeses were cooled to 7 ± 1 ^{o}C for 12 hours before they were packed (Figure 3.10). The cheeses were stored in 4 ± 1 ^{o}C chilling chambers at Massey University for seven weeks. They were sampled on a every 7 days and monitored for pH, TS, salt content, fat content, nitrogen fractions, texture and sensory attributes.

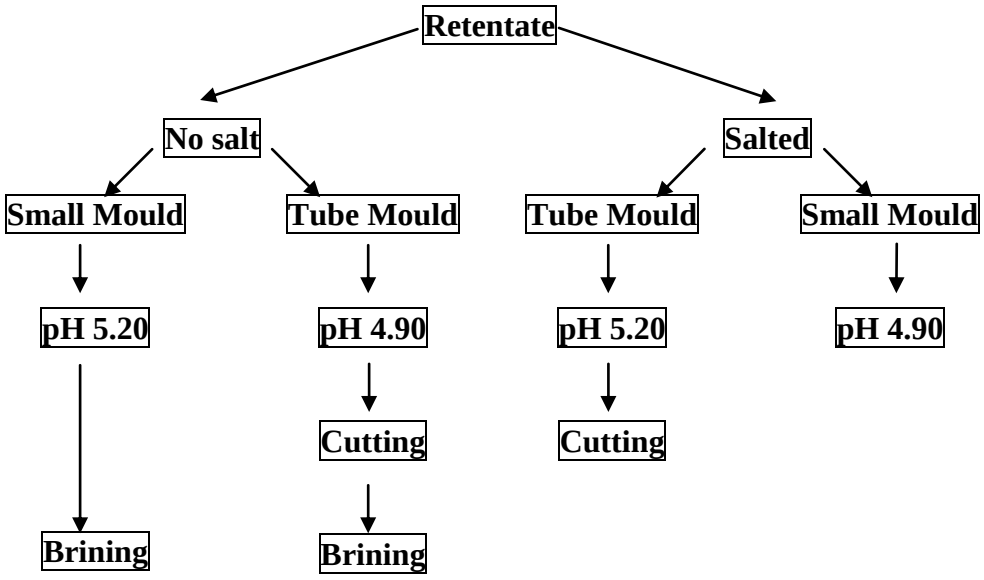


Figure 3.1: Set-up of the four processing variables applied to four treatments per block of Camembert cheese production.

Figure 3.2: A small batch of retentate (80 L) was separated from the main batch to carry out trial experiment with four treatments (20L each).

Figure 3.3: A pour tank with pump which circulates and allows the mixing of retentate with other ingredients. The pre-cheese retentate was then pumped into different moulds.

Figure 3.4: Small silicone tray moulds containing pre-cheese retentate.

Figure 3.5: Tube moulds containing pre-cheese retentate in plastic films.

Figure 3.6: Wire cutter used for slicing young cheese logs removed from tube moulds into approximately 125 g units.

Figure 3.7: Individual young Camembert cheeses loaded onto metal racks.

Figure 3.8: Brine tank used to immerse young cheeses. Stirring of the excess salt at the bottom of the tank was done prior to the immersion of the young cheeses, which ensures the salinity is above 95%.

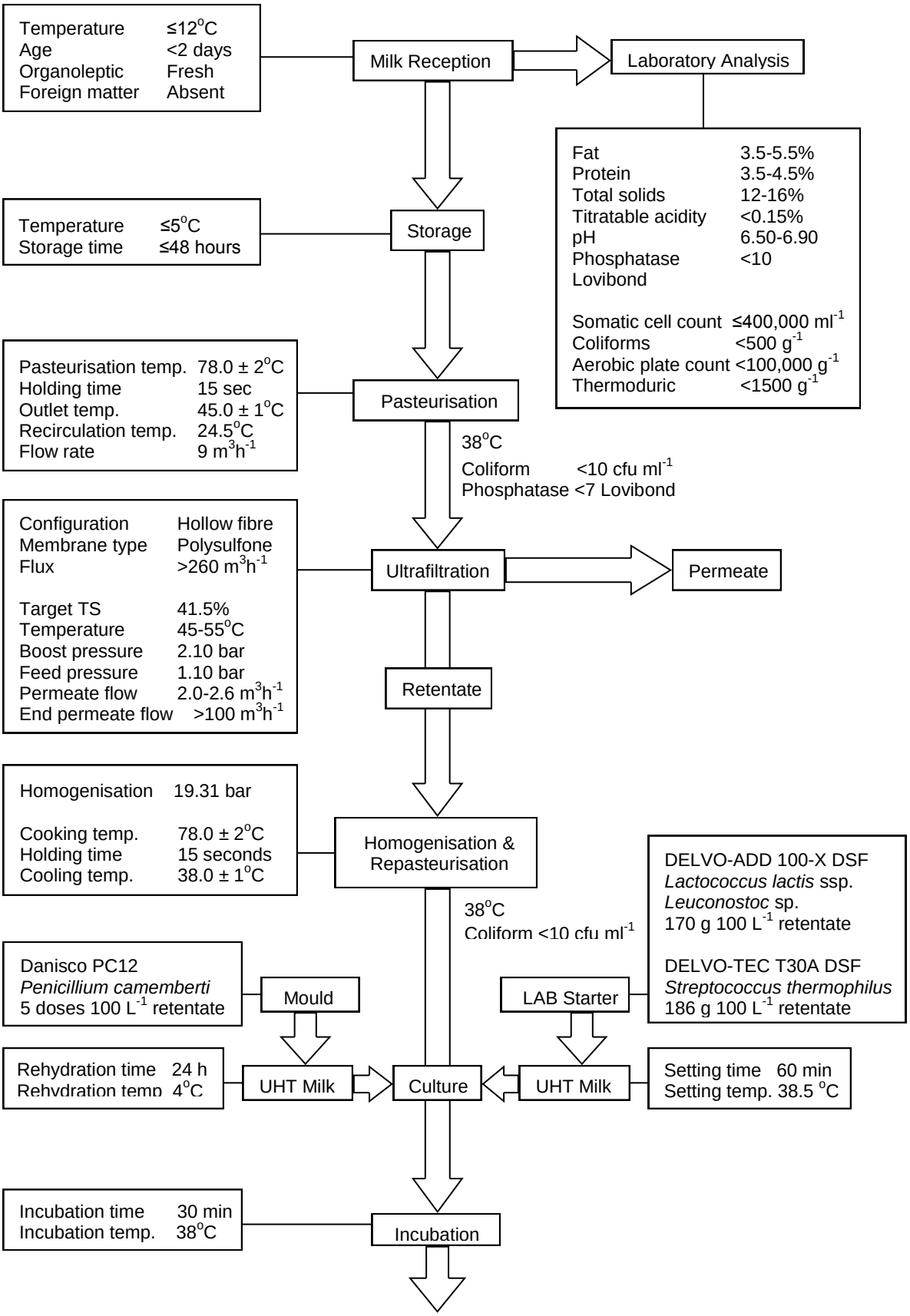


Figure 3.9: Process flow chart of Camembert cheese production using ultrafiltration, from milk reception to culture addition.

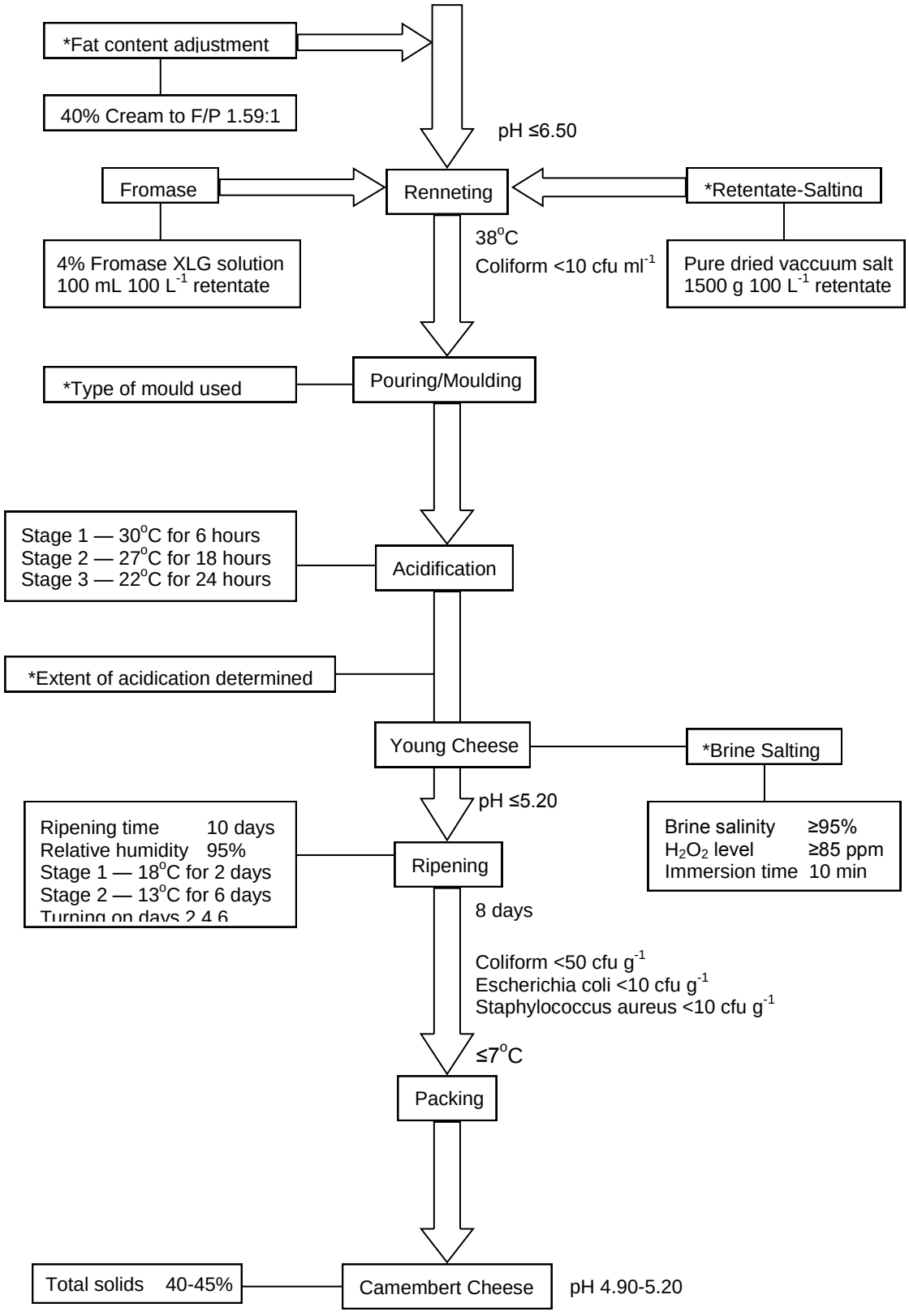


Figure 3.10: Process flow chart of Camembert cheese production using ultrafiltration, continuing from culture addition to the finished product. (*) represents process variables which were manipulated.

3.3. Analysis of nitrogen fractions

Three types of nitrogen content were analysed in order to monitor the release of soluble nitrogen fractions during the maturation of the cheese. These include total nitrogen (TN), soluble nitrogen (SN) at pH 4.4, and non-protein nitrogen (NPN) in 24% trichloroacetic acid (TCA). The method used for separating various nitrogen fractions in cheese has been described by Ardö (1999). The ratios of SN or NPN to TN serve as useful indicators for the extent of proteolysis in cheese.

Fractionation of nitrogen contents

Duplicate cheese from each treatment and one sample from each cheese were analysed at weeks one, three, five, and seven to determine the degree of proteolysis during maturation. A dispersion of the cheese was obtained by adding 80 mL warm 0.5 M tri-sodium citrate solution (AR, LabServ Pronalys) to 17 – 19 grams of grated cheese without rind and stirred for 60 minutes using a magnetic stirrer and hotplate at 80 ^{o}C. The dispersion was cooled to room temperature (20 ^{o}C) and made up to 250 mL with distilled water. A 20 mL sample of the dispersion was used for TN analysis. The pH of the remaining cheese dispersion was adjusted to pH 4.4 using 1.0 M hydrochloric acid (AR, LabServ Pronalys). The dispersion was stirred as the pH was being adjusted, and the pH was constantly monitored by a pH meter (PB-20, Sartorious) with a cheese electrode (model L8880, Schott) attachment. Two hundred millilitres of the pH adjusted dispersion was made up to 250 mL using distilled water. Of the mixture, 80 mL was filtered using quantitative filter paper (model LBS0040.110, LabServ Filtration). A 25 mL sample of the filtrate was used for SN analysis. Then, 50 mL of the unfiltered pH 4.4 dispersion was made up to 100 mL using 24% (w/v) trichloroacetic acid solution (AR, Normapur). The solution was kept overnight at 4 ^{o}C and filtered using quantitative filter paper (model LBS0040.110, LabServ Filtration). A 25 mL sample of the filtrate was used for NPN analysis.

Digestion

The AOAC (2005) method 933.20 was used to determine the nitrogen content of TN, SN, and NPN. All samples to be digested were placed in Kjeldahl tubes and at least one nitrogen recovery test (0.20 g phenylalanine) was included per batch (20 tubes). Two Kjeltabs (Foss™) and 20 mL concentrated sulphuric acid (LabServ Pronalys,

Reagent Grade) were added to each digestion tube. Digestion was carried on a digestion unit (model 2020, Tecator, Sweden) using a temperature scheme of 200 ^{o}C for 60 minutes, 270 ^{o}C for 45 minutes, 330 ^{o}C for 45 minutes, 405 ^{o}C until the samples turned clear and colourless. The samples were cooled after digestion. Subsequently 70 mL of distilled water was added to each tube and they were further allowed to cool prior to distillation.

Distillation

The digestion tubes were placed in an automatic distillation unit (model 1026, Tecator Kjeltec System, Denmark). The settings of the automatic distillation included three injections of 20 mL aliquots of 40% (w/v) sodium hydroxide solution (LabServ, Reagent Grade) and a steam-on time of 3.2 minutes. Samples were distilled against 50 mL of 4% (w/v) boric acid solution (LabServ, Reagent Grade) containing 1% bromocresol green (0.1% w/v dissolved in 95% ethanol) and 0.7% methyl red (0.1% w/v dissolved in 95% ethanol) in a receiver flask. The presence of nitrogen would induce a colour change from red to green in the boric acid during distillation. The nitrogen content was determined by titrating the samples against standardised 0.1 M hydrochloric acid solution to a grey-mauve endpoint.

3.4. Measurement of pH

Determination of pH was carried out on both the centre and outer portions with rind of the cheese samples. Duplicate cheeses from each treatment and duplicate samples from each cheese were tested every week for six weeks to monitor pH changes. A calibrated pH meter (PB-20, Sartorious) with cheese electrode (model L8880, Schott) attachment was used. Cheese (10 g) from the both centre and outer portions were added with 5 mL of distilled water and minced in small sample cups. The temperature of the samples was measured using a thermometer. The pH was measured at 20 ± 1 ^{o}C by inserting the pH electrode into the slurry with gentle mixing; two measurements were recorded for each sample. The pH electrode was rinsed with distilled water after each measurement.

3.5. Determination of total solids

Total solids (TS) content was determined using the hot air oven method (AOAC International, 2005). Duplicate cheeses from each treatment and one sample from each cheese were tested every week for six weeks. A dry aluminium moisture dish was placed in a 105 ^{o}C air oven for 60 minutes to ensure all moisture was removed and then cooled to room temperature (20 ^{o}C) in a desiccator. The weight of the cooled dry moisture dish was recorded and 10 grams of prepared cheese sample without rind were spread uniformly in the dish. The weight of the sample was recorded. The dish was placed into 105 ^{o}C air oven for 8 hours and cooled to room temperature in a desiccator. The weight of the dish and its contents were recorded, then returned into the 105 ^{o}C air oven for 60 minutes and cooled to room temperature in a desiccator. The analysis was performed to within a standardising ± 1 mg.

3.6. Determination of fat content

The AOAC (2005) method was used in the determination of fat in cheese (933.05). Duplicate cheeses from each treatment and one sample from each cheese were tested in the first week after packing. Two to three grams of prepared cheese sample without rind were measured into Mojonnier tubes and 10 mL 25 % hydrochloric acid (w/v) was added. The tubes were placed in 60 ^{o}C water bath for 30 minutes with occasional swirling and cooled for five minutes.

Fat Extraction

Ten mL 95% ethanol (LabServ, Reagent Grade), 25 mL diethyl ether (LabServ Pronalys, Reagent Grade), and 25 mL petroleum ether (boiling range 40 ^{o}C – 60 ^{o}C, LabServ Pronalys, Reagent Grade) were added to the samples successively and mixed thoroughly after adding each reagent. The samples were centrifuged for two minutes at 20 g and the organic solvent layer was decanted carefully into the pre-weighed aluminium dish. The solvents in aluminium dishes were evaporated on hot plates at mild temperatures below 40 ^{o}C.

A second extraction was repeated using 5 mL ethanol, 15 mL diethyl ether, 15 mL petroleum ether. The samples were centrifuged for another 2 minutes at 20 g and the

organic solvent layer was decanted into their respective aluminium dishes. After evaporating all solvents, the dry aluminium dishes were placed in the 105 ^{o}C air oven for 30 minutes. The dishes were allowed to cool to room temperature (20 ^{o}C) in a desiccator. The fat content was determined by weighing the fat in the dry dish.

3.7. Determination of salt content

The determination of the salt content is described in the AOAC (2005) methods 935.43 and Kirk and Sawyer (1991). Duplicate cheeses from each treatment and duplicate samples from each treatment were tested in the first week after packing. Three grams of prepared cheese sample without rind were weighed into a 200 mL conical flask and 10 mL distilled water, 0.05 M silver nitrate solution (Ajax Chemicals, Analytical Reagent Grade, Univar), and 10 mL concentrated nitric acid (Ajax Chemicals, Analytical Reagent Grade, Univar, 70% w/w) were added. The cheese was dispersed by swirling and boiled gently until granular precipitation was present. To the contents of the flask, 50 mL distilled water were added and cooled to room temperature (20 ^{o}C). The excess silver nitrate was titrated with 0.05 M potassium thiocyanate solution (Sigma, ReagentPlus™ Grade, 99.0%) using saturated ferric alum solution (LabServ, Reagent Grade) as an indicator to a reddish-brown endpoint.

3.8. Instrumental analysis

Instrumental texture analysis was performed using a TA-XT2 Texture Analyzer (SMS Stable Micro Systems Ltd, United Kingdom), operating in the compression mode. The test measurements were analysed using the Texture Analysis TE32 software. Duplicate cheeses from each treatment were analysed weekly for six weeks. Duplicate samples from each cheese were prepared by cutting out test pieces (15 mm in diameter) 10 mm from the centre of the cheese unit. The height of the test pieces were approximately 30 to 35 mm with the rind intact. Samples were cut at 4 ^{o}C and left at room temperature (20 ^{o}C) for at least 30 minutes before testing. The samples were analysed with compression up to 50% of initial height at a rate of 10 mm/min using an automatic trigger force of 5.0 gram. The force (N) was recorded for analysis. The measurements were carried out using a 50 mm diameter flat plate probe.

3.9. Cheese grading

Grading system

The cheese grading method used was developed exclusively by Puhoi Valley Cheese to periodically assess their cheese samples (Appendix 2). A list of undesirable attributes (e.g. brown edges, under ripe, too acidic etc.) was divided into four subgroups comprising appearance, cut, texture, and flavour (Table 3.2). The cheeses were given a total score of 40 points, and for each presences of undesired attribute, a point was deducted. Only cheeses with distinctively outstanding attributes were given extra points above 40 to the maximum of 50 points. Additional comments were also recorded on points being deducted or given, where these were extensively used during the grading of UF Camembert.

Grading procedure

Eight cheeses of each treatment type were graded by 3 – 4 experienced cheese graders at Puhoi Valley Cheese, and a consensus of the grading result was recorded. Each cheese was first graded on the exterior appearance. The cheese was then cut across the centre using a knife and the appearance of the cross section (cut) was graded. The cheese was cut up into wedges and evaluated by the graders, and the texture and flavour of the cheese were then graded.

Analysis of cheese grading results

A selective list of attributes as well as commonly used comments was grouped into nine major sensory defects. The defects included rind discolouration (tanning or bolding of the surface mould flora), rind deformation (unevenness and misshaping of the rind), thick rind, core unevenness (a firm core texture which gradually gets softer towards the rind), excessive softness, saltiness, sourness, bitterness, blandness. The percentage of occurrence was calculated based on the defect detected (based on consensus and a yes/no answer) out of eight graded cheese in each treatment type (e.g. 4 out of 8 low-fat cheese had thick rind = 50% occurrence).

Table 3.2: A list of attributes used in cheese grading and the total scores given in each subgroup.

Subgroups	Attributes	Standard Score Total	Maximum Score Total
Appearance	**Crooked or lopsided** **Oversized or undersized** **Bare mould & uneven** **Too moist or wet** **Uneven colour** **Brown edges**	**8**	**10**
Cut	**Dull** **Presence of holes** **Under ripe** **Over ripe** **Undesired rind**	**4**	**5**
Texture	**Too soft** **Too hard or dry** **Chalky**	**12**	**15**
Flavour	**Bitter** **Too acidic** **Ammonia** **Lacking flavour** **Chemical soapiness** **Too salty** **Metallic** **Rancid**	**16**	**20**

3.10. Consumer acceptance

Panellists were recruited across Massey University Oteha Rohe campus which consisted of students, staff and guests. The panel sessions following the incomplete block design and the sixteen treatments of cheeses were divided into four separate sessions, with two extra sessions serving as duplicates. Approximately 30 panellists participated in each consumer acceptance test.

Cheese samples at four weeks of maturation were prepared by cutting into pieces of eighths, coded with random numbers, and served at 18 ± 2 ^{o}C in sensory booths. Each panellist was presented with five cheese samples which comprised four treatments and a duplicate of one of the treatments, which tested for consistency within the repeats. Although it may not be ideal to present all samples simultaneously, this was done to manage the sensory sessions, thereby reducing possible errors.

The panellists were given questionnaires, where they were asked to taste each sample and rate, using a 9-point hedonic scale ranging from "dislike extremely" to "like extremely", for the attributes of appearance, aroma, flavour, texture, and overall acceptance independently. The panellists were also asked to thoroughly rinse their mouth with the water provided between samples and to rate each sample without comparing between them.

3.11. Microbiological analysis

Microbiological analysis was carried out by Goodman Fielder (NZ) Ltd. Puhoi Valley Cheese plant. One sample of cheese from each treatment was tested for coliforms, *E. coli*, and *Staphylococcus aureus* counts on the first day of ripening. For the cheese to be considered safe for human consumption, the counts for each of the above species must be <1 cfu/g (FSANZ, 2009).

3.12. Statistical analysis of data

The data were analysed using the Minitab 15 (Minitab Ltd., Coventry, U.K.). One-way Analysis of Variance (ANOVA) was used to determine significant differences between treatments with significant level set at 5%. Graphical presentations were produced using Microsoft® Office Excel 2003 (Microsoft Corporation, U.S.A.) with means and standard error of the mean (SE_M), n values were calculated by number of treatments × number of cheese in treatment × number of replicates per cheese in analysis (e.g. n = 16 low fat treatments × duplicate cheese per treatment × duplicate pH measurements per cheese = 64).

4. RESULTS

Camembert cheese from the sixteen treatments, during storage at 4 ± 1 ºC, were monitored weekly for changes in TS, pH, and textural properties using the texture analyser (TA); fortnightly for changes in non-protein nitrogen (NPN) and soluble nitrogen (SN). The salt and fat content were analysed a week after packaging to assess the effects of the treatments. The organoleptic qualities of the cheese samples were also evaluated by consumer sensory panels, and experienced cheese tasters in cheese grading. The results are illustrated graphically in Figures 4.1 to 4.50.

For quick references, treatments of the cheese samples were abbreviated in the following manner, explained by the underlined letter as: Low-fat/High-fat; Brine-salted/Retentate-salted; pH 5.2/pH 4.9; Tube mould/Small mould.

4.1. Change in total solids

Table 4.1: p-values ($p \leq 0.05$) for total solids within each type of treatment.

	p-values for weeks 1- 6					
Cheese treatments	**1**	**2**	**3**	**4**	**5**	**6**
Level of fat	0.791	0.994	0.740	0.954	0.039	0.008
Type of salting	0.002	0.301	0.085	0.004	0.986	0.963
Final acidification pH	0.000	0.000	0.000	0.000	0.000	0.000
Mould type	0.226	0.191	0.772	0.451	0.933	0.959

Note: The significant level was set at 5%.

Low-fat versus high-fat

The results obtained for the low-fat cheese samples were combined with the results for the high-fat cheese samples to ascertain if fat content had any impact on TS. In the first four weeks of their shelf life there were no significant differences in the TS content between low-fat and high-fat cheeses (Figure 4.1). From week five onwards the high-fat cheese samples had significantly higher mean TS content than that of the low-fat treatments.

Brine-salted versus retentate-salted

The results obtained for the brine-salted cheese samples were combined with the results for the retentate-salted cheese samples to ascertain if the salting method had any significant impact on TS. The mean TS in brine-salted cheeses were significantly

higher than retentate-salted cheeses at weeks 1 and 4, but at weeks 2, 3, 5 and 6 there was no significant differences between the TS in all of the cheese samples (Figure 4.2). The effects of the two salting methods on cheese TS was only significant during early stages of maturation.

pH 5.2 versus pH 4.9

The results obtained for the cheese samples acidified to pH 5.2 were combined with the results for the cheese samples acidified to pH 4.9 to ascertain if the final acidification pH had any impact on TS. Cheese produced with higher final acidification pH of 5.2 had significantly higher mean TS than the lower acidification final pH of 4.9 cheeses during all six weeks (Figure 4.3). The cheese samples made with a higher final acidification pH exhibited higher moisture loss.

Tube mould versus small mould

The results obtained for the two mould types were combined for each mould type to determine if the mould type had any impact on TS. The TS in the cheese samples from the two mould types were not significantly different from each other (Figure 4.4).

The mean TS of each cheese type during the maturation period (six weeks) are shown in Figures 4.5 to 4.8. Generally, cheese samples acidified to pH 5.2 (LB5.2T, LB5.2S, LR5.2T, LR5.2S, HB5.2T, HB5.2S, HR5.2T, HR5.2S) had higher mean TS than cheese acidified to pH 4.9 (LB4.9T, LB4.9S, LR4.9T, LR4.9S, HB4.9T, HB4.9S, HR4.9T, HR4.9S). When comparing the types of mould used, the cheeses made using the tube moulds had higher mean TS than those made using the small moulds, but only in those acidified to pH 5.2 and not those at pH 4.9. This is not shown in the combined mean TS of mould types shown in Figure 4.4.

In general, the two final acidification pH levels (pH 5.2 and 4.9) produced cheese with significantly different mean TS (Figure 4.2). Samples of cheese at pH 5.2 had higher mean TS during the storage period than those produced at pH 4.9. Treatments with different levels of fat (Figure 4.1) and salting methods (Figure 4.2) had significant differences in the mean TS but only in the early stages of maturation post-packaging.

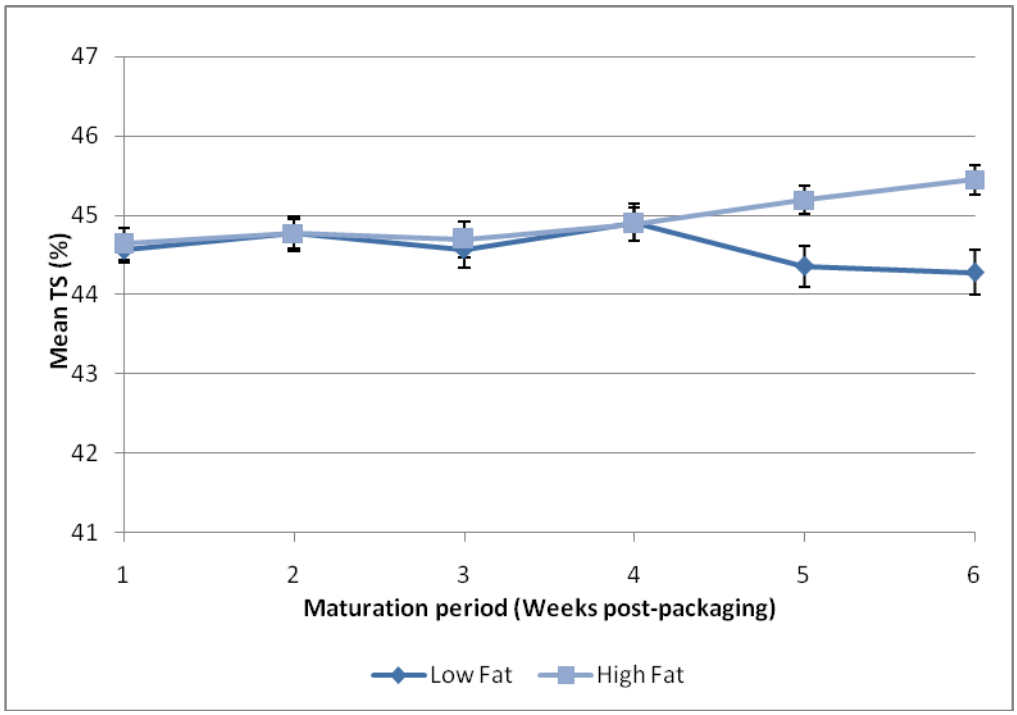


Figure 4.1: Changes in total solids (TS) (mean ± SE_M) of low-fat (n = 32) and high-fat (n = 16) Camembert cheese stored at 4 ± 1 °C for six weeks post-packaging.

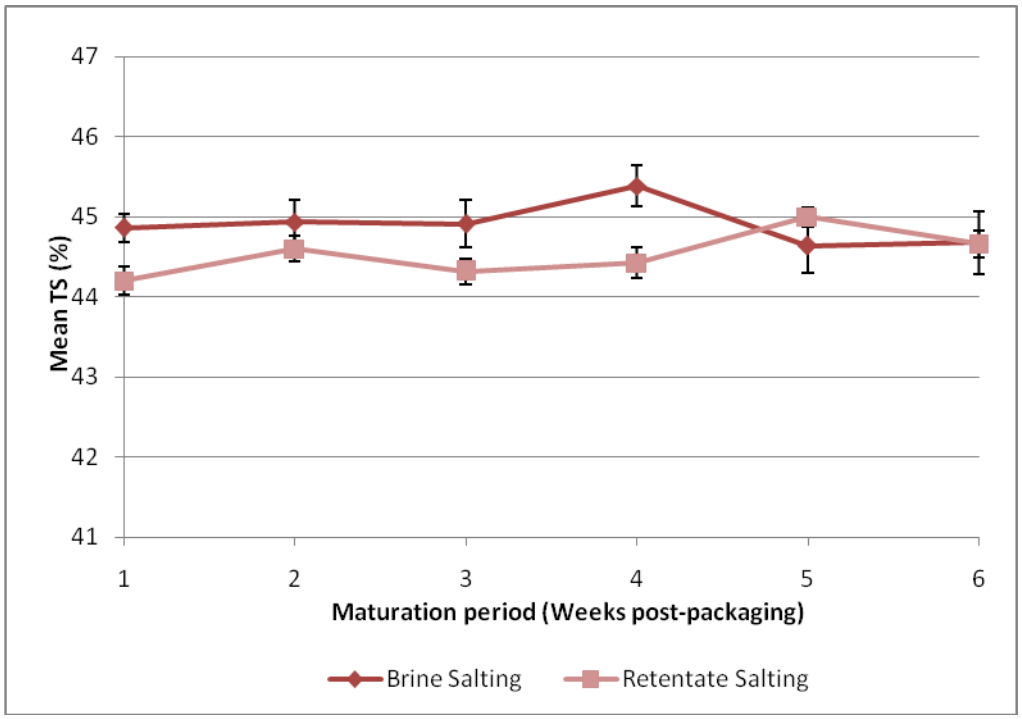


Figure 4.2: Changes in total solids (TS) (mean ± SE_M) of brine-salted (n = 24) and retentate-salted (n = 16) Camembert cheese stored at 4 ± 1 °C for six weeks post-packaging.

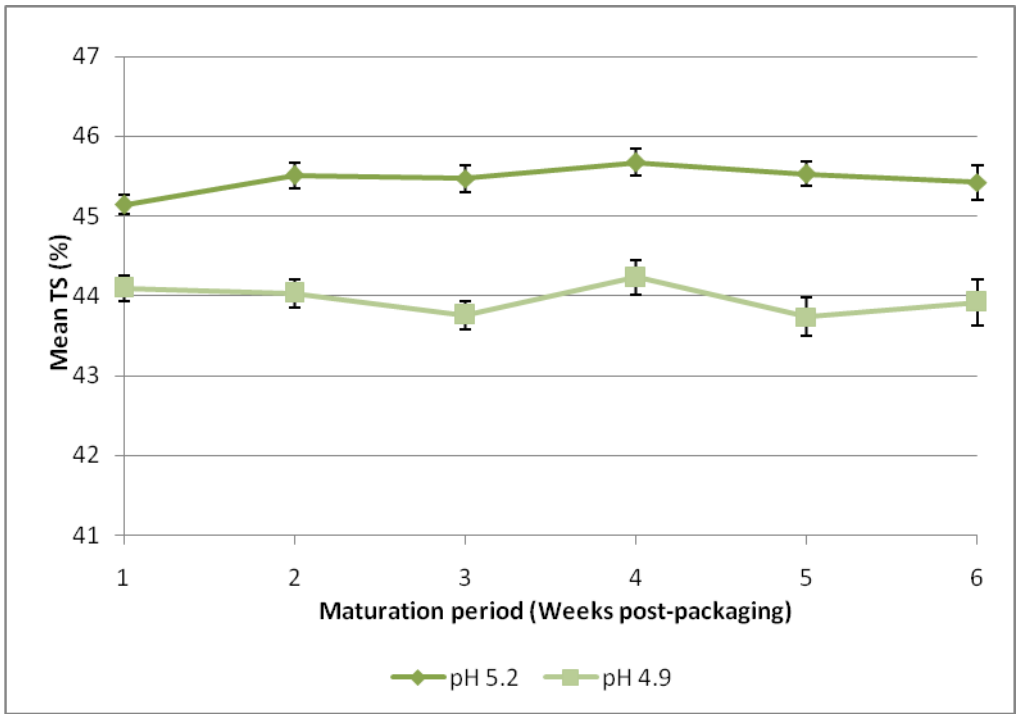


Figure 4.3: Changes in total solids (TS) (mean ± SE_M) of Camembert cheese acidified to pH 5.2 (n = 24) and pH 4.9 (n = 24). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

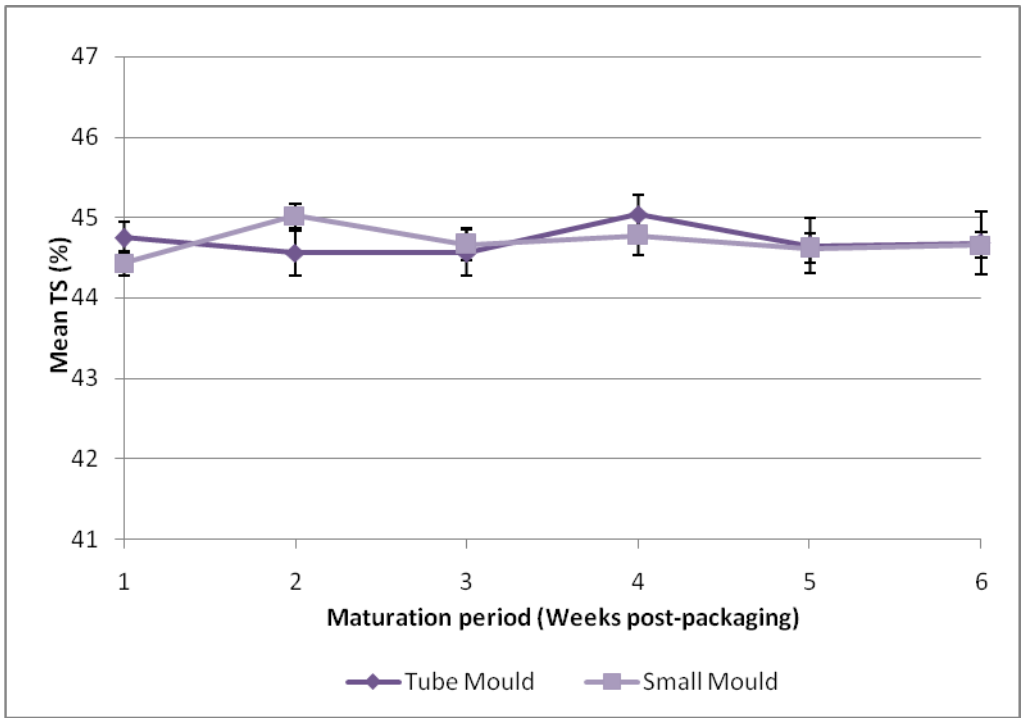


Figure 4.4: Changes in total solids (TS) (mean ± SE_M) of Camembert cheese made using tube moulds (n = 24) and small moulds (n = 24). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

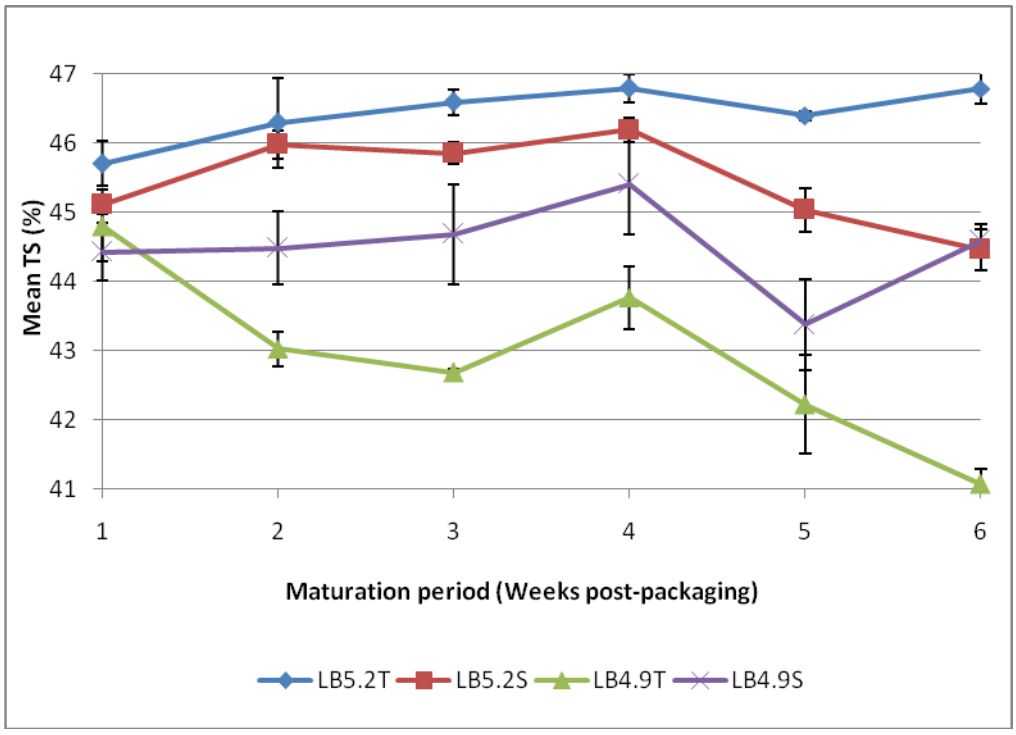


Figure 4.5: Changes in total solids (TS) (mean ± SE_M) of Camembert cheese for low-fat brine-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

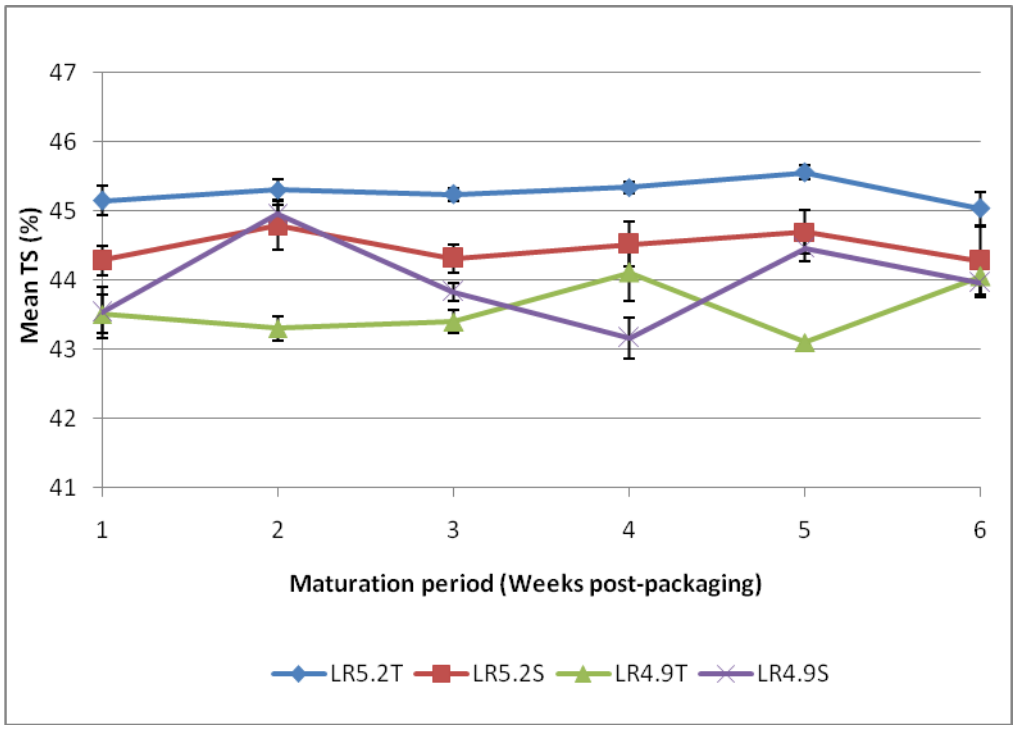


Figure 4.6: Changes in total solids (TS) (mean ± SE_M) of Camembert cheese for low-fat retentate-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

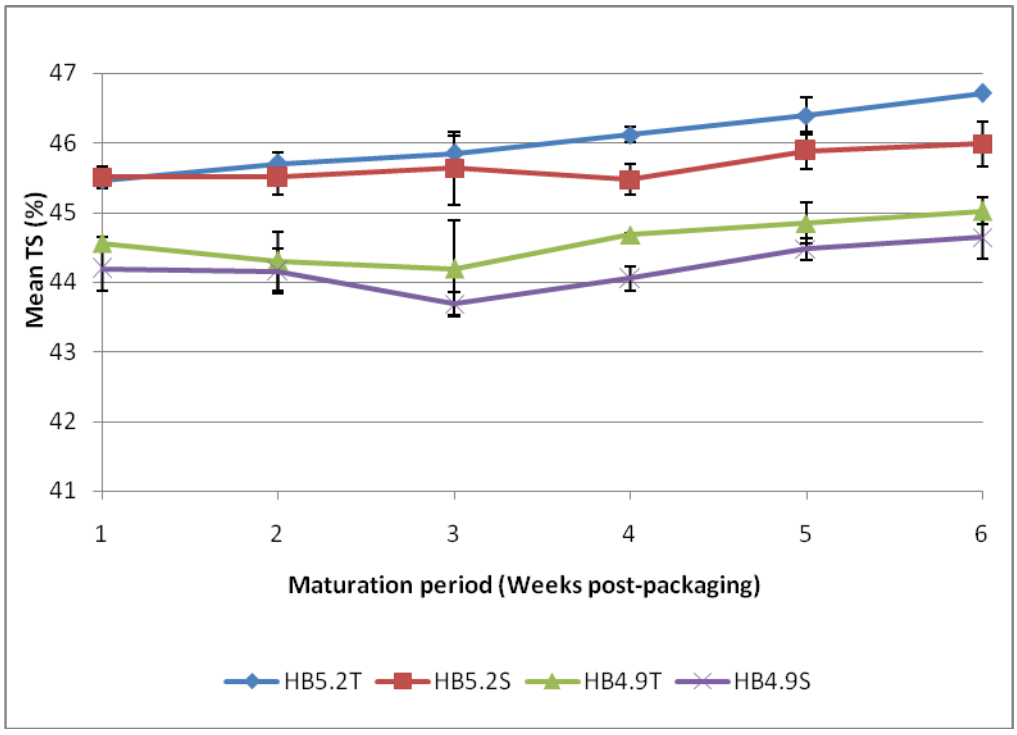


Figure 4.7: Changes in total solids (TS) (mean ± SE_M) of Camembert cheese for high-fat brine-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

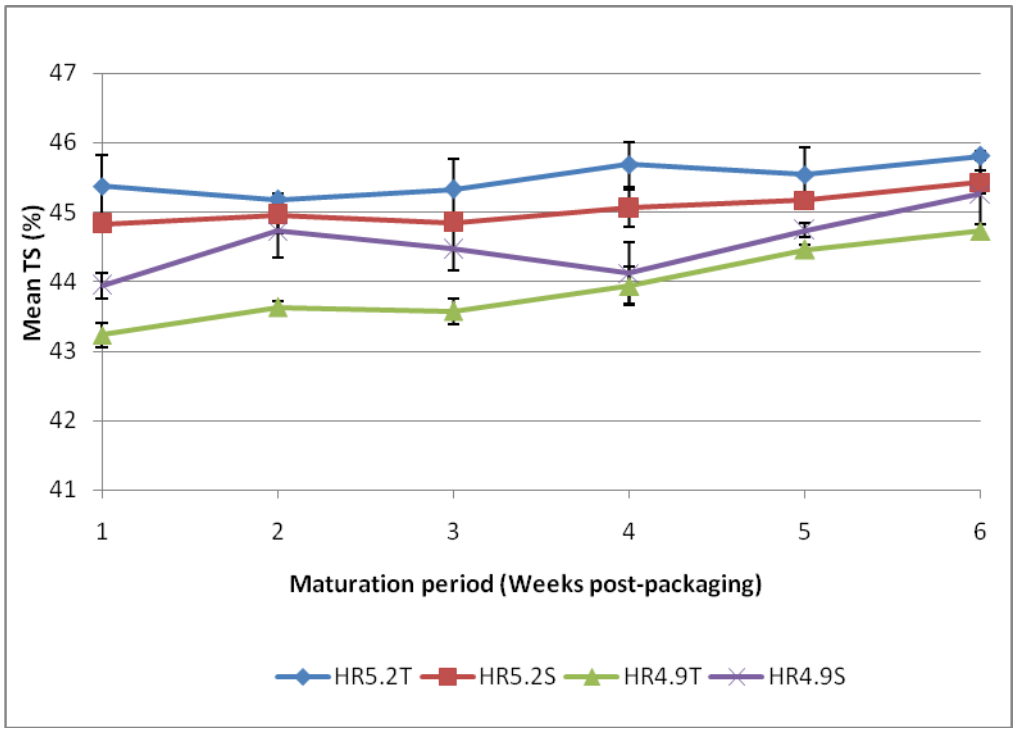


Figure 4.8: Changes in total solids (TS) (mean ± SE_M) of Camembert cheese for high-fat retentate-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

4.2. Salt content

The mean salt content for all cheese samples, consisting of the four variables (level of fat, salting method, final acidification pH, and mould type) and their two levels are shown in Figure 4.9. The low-fat cheese samples had significantly higher mean salt content (1.74 ± 0.02 % $^{w}/_{w}$) than the high-fat samples (1.52 ± 0.04% $^{w}/_{w}$). This was shown in Figure 4.10, where five of the high-fat cheese samples (HB4.9S, HR4.9T, HB4.9T, HR5.2T, and HR4.9S) had noticeably lower salt content when compared to the rest of the cheese samples. The mean salt contents of the cheese samples made using brine-salting and retentate-salting were not significantly different from each other, as shown in Figure 4.9. In the cheese samples ripened to different final acidification pH, the pH 5.2 samples (1.74 ± 0.03 % $^{w}/_{w}$) had higher mean salt content than the pH 4.9 samples (1.52 ± 0.04 % $^{w}/_{w}$). This was observed in Figure 4.10 where almost all the pH 5.2 samples had higher salt content than the pH 4.9 samples made using the same salting method. Finally, cheese samples made using the tube mould were not significantly different to samples made using the small mould (Figure 4.9).

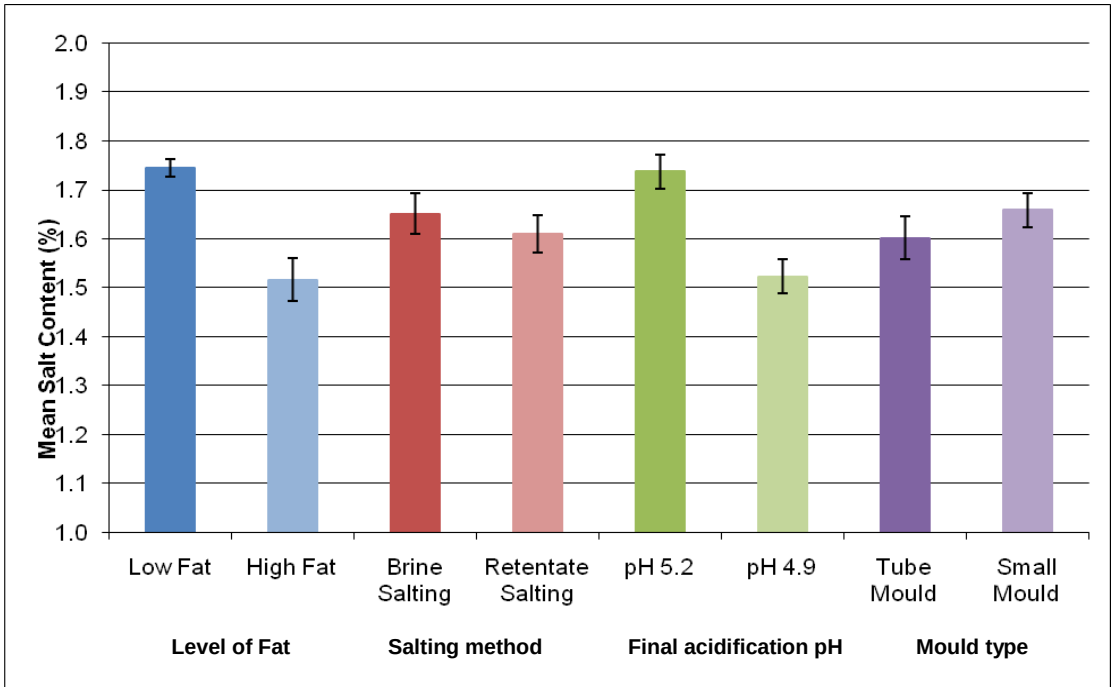


Figure 4.9: Combined mean of salt content (mean ± SE_M) in Camembert cheese samples for each treatment: high-fat (n = 32) and low-fat (n = 32); brine-salted (n = 32) and retentate-salted (n = 32); final acidification pH of 5.2 (n = 32) and pH 4.9 (n = 32); and tube mould (n = 32) and small mould (n = 32). Samples were analysed in the first week post-packaging.

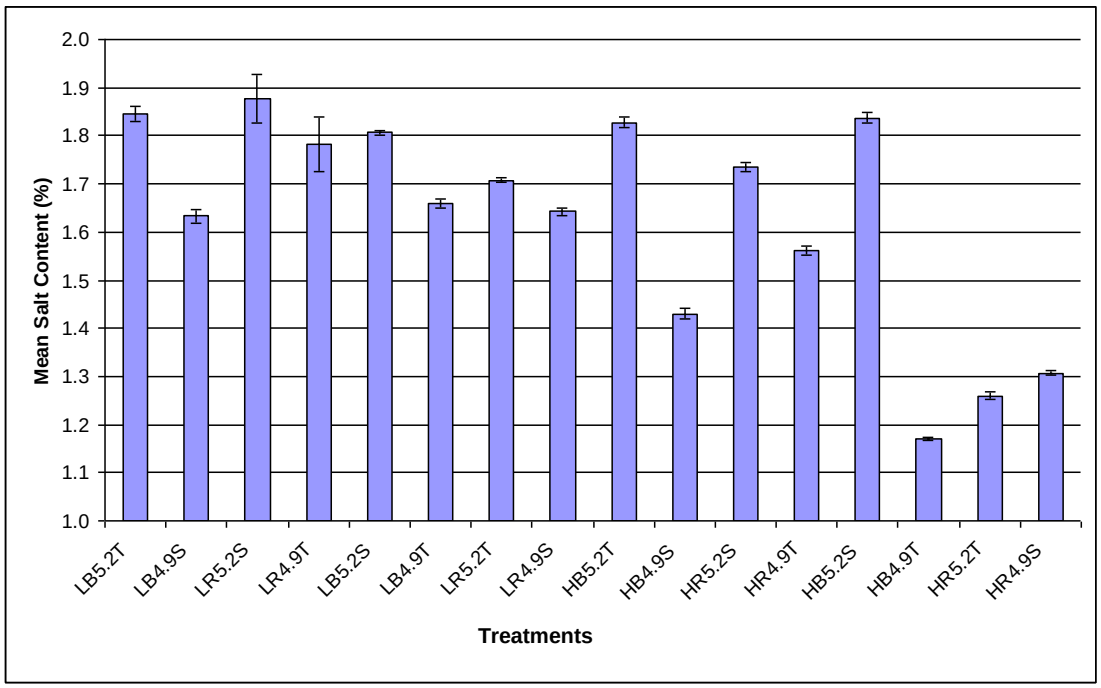


Figure 4.10: Salt content (mean ± SE_M) in Camembert cheese samples of sixteen treatments (n = 4 in each treatment) analysed in the first week post-packaging.

Table 4.2: p-values ($p \leq 0.05$) for salt and fat levels within each type of treatment.

Cheese treatments	Salt content	Fat content
Level of fat	0.000	0.000
Type of salting	0.455	0.689
Final acidification pH	0.000	0.575
Mould type	0.311	0.762

Note: The significant level was set at 5%.

4.3. Fat content

The combined mean fat content of each variable and their two levels are shown in Figure 4.11. The fat content of low-fat cheese samples (24.96 ± 0.23 % $^{w}/_{w}$) was significantly lower than in the high-fat samples (28.03 ± 0.14 % $^{w}/_{w}$). This is also observed in Figure 4.12 where the eight low-fat samples had significantly lower fat contents than the eight high-fat samples. Note that LB5.2S had a slightly higher fat content (26.57 ± 0.14 % $^{w}/_{w}$) than the other low-fat cheeses, but the fat content was still significantly lower than that of high-fat samples (above 27 %). This may be attributed to error in blending of the cheese samples.

No significant differences were observed between the levels of the three other variables: salting method, final acidification pH, and mould type.

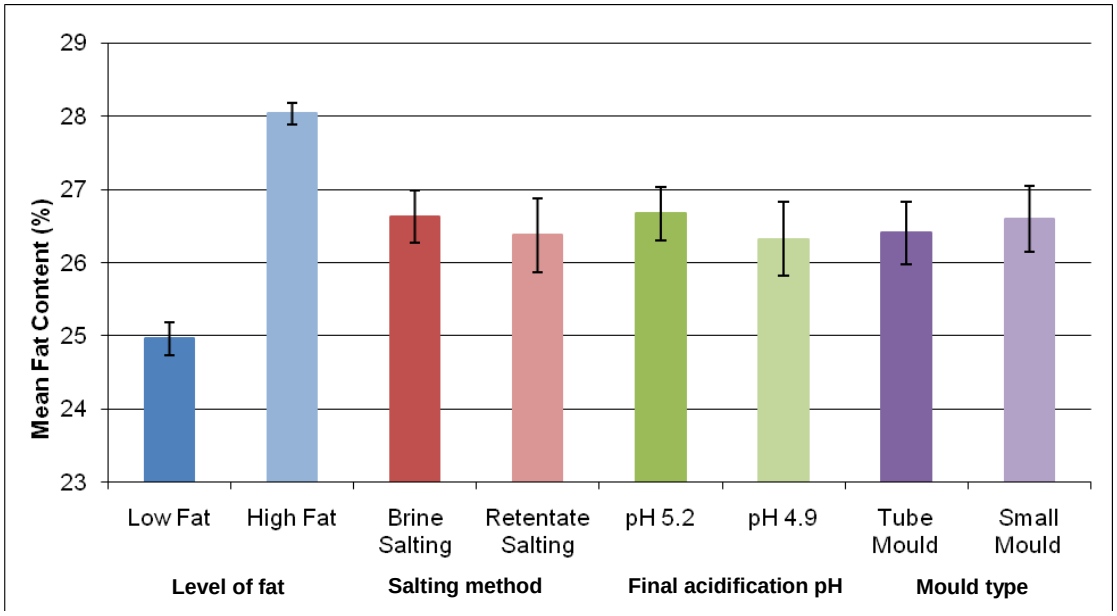


Figure 4.11: Combined mean of fat content (mean ± SE_M) in Camembert cheese samples for each treatment: low-fat (n = 16) and high-fat (n = 16); brine-salted (n = 16) and retentate-salted (n = 16); final acidification pH of 5.2 (n = 16) and pH 4.9 (n = 16); and tube mould (n = 16) and small mould (n = 16). Samples were analysed in the first week post-packaging.

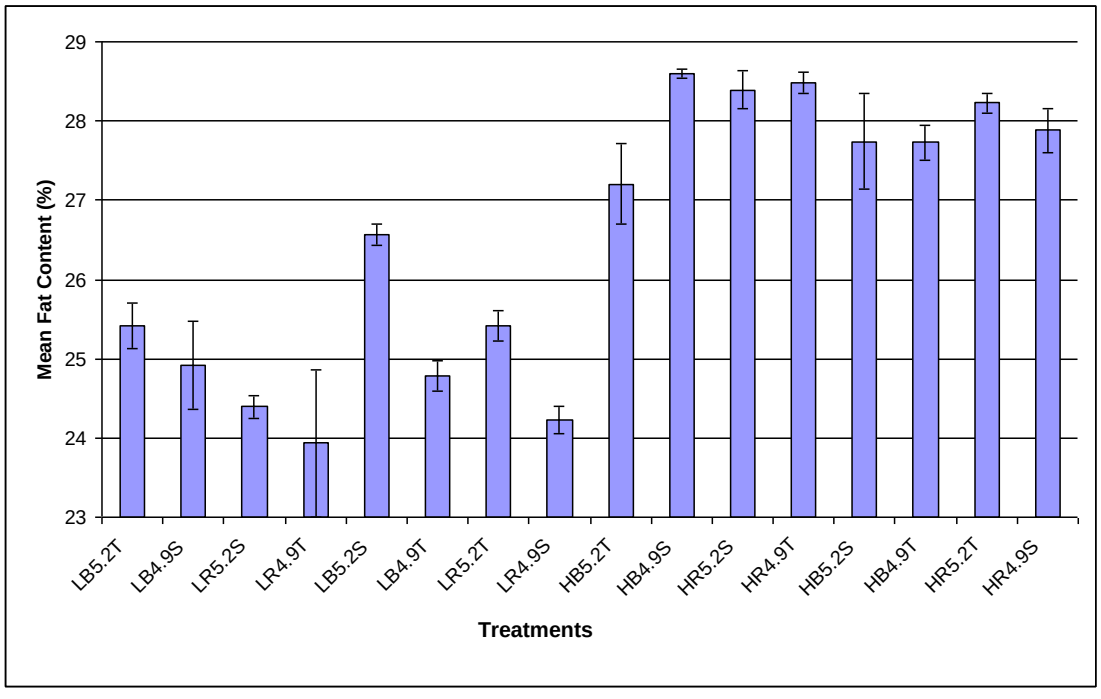


Figure 4.12: Fat content (mean ± SE_M) in Camembert cheese samples of sixteen treatments (n = 4 in each treatment) analysed in the first week post-packaging.

4.4. Changes in pH levels

The pH levels of the cheese samples were determined at the core (centre) and on the rind surface. The mean core pH levels were significantly higher in high-fat samples at weeks 5 and 6 (Figures 4.13), as was in brine-salted samples at weeks 4 and 6 (Figure 4.14), in samples acidified to pH 5.2 at weeks 1, 3 and 5 (Figure 4.15), and in small mould samples at weeks 2 and 5 (Figure 4.16). The rind pH exhibited similar trends as the core pH but only in acidification pH and mould treatments (Figures 4.13 to 4.16). The core pH levels of all treatments had similar trends including the rate of increase (mean = 0.4744, SE_M = 0.1677) starting at the mean pH of 5.28 ± 0.007 up to pH 7.57 ± 0.011 at week 6. Also, the rind pH levels of all treatments had the same trends with similar rates of increase (mean = 0.2497 SE_M = 0.0883) starting at mean pH of 6.62 ± 0.020 up to pH 7.89 ± 0.004) at week 6.

Figures 4.17 to 4.20 show the changes in core pH levels of the 16 cheese samples for each different treatment. Low-fat brine-salted samples acidified to a higher final pH, (LB5.2T and LB5.2S) generally had higher core pH than those acidified to the lower final pH cheeses (LB4.9T and LB4.9S) (Figure 4.17), this was observated when the data was combined with other high-fat or retentate-salted samples to generate mean values in Figure 4.15, suggesting that pH adjustment was somewhat effective. For low-fat retentate-salted samples (Figure 4.18), no conclusive trends were observed in core pH levels between samples acidified to pH 5.2 or 4.9 or in the different mould types. However, from week three to week six, LR5.2T and LR4.9S samples had higher mean core pH values than LR5.2S and LR4.9T. With respect to the rind pH, no significant trends were observed between the low-fat samples (Figures 4.17 and 4.18).

The changes in mean core pH of high-fat brine-salted cheese samples are shown in Figure 4.19. At weeks one and two, the samples acidified to pH 5.2 (HB5.2T and HB5.2S) had higher mean core pH levels than those acidified to pH 4.9 (HB4.9T and HB4.9S). This suggested that a relatively higher pH was maintained after one week of on-site ripening. At weeks three and four, HB5.2S had higher mean core pH levels than HB4.9T, followed by HB4.9S and then HB5.2T. Finally, at week five and six, HB4.9S samples had a noticeably significant increase in core pH. This particular

cheese resulted with the highest pH levels compared to the four high-fat samples after six weeks.

The changes in core mean pH levels of high-fat retentate-salted samples are shown in Figure 4.20. At week one, samples acidified to pH 5.2 (HB5.2T and HB5.2S) had higher core mean pH levels than those acidified to pH 4.9 (HB4.9T and HB4.9S). No obvious trends could be observed from the four samples, but at week six, cheese samples with lower final acidification pH (HB4.9T and HB4.9S) also had higher mean core pH levels than those with higher final acidification pH levels (HB5.2T and HB5.2S). These observations are however lost, when the data are combined with other low-fat and brine-salted samples as shown in Figure 4.15. In terms of the rind pH levels, no significant trends were found between the high-fat samples (Figures 4.19 and 4.20). This suggests that the growth of surface-mould flora altered the rind pH of the cheese in a consistent manner (Fox et al., 2000), regardless of the four variables implemented (level of fat, salting method, final acidification pH, and mould type).

Table 4.3: p-values ($p \leq 0.05$) for core pH within each type of treatment.

	p-values for weeks 1-6					
Cheese treatments	**1**	**2**	**3**	**4**	**5**	**6**
Level of fat	0.860	0.828	0.323	0.923	0.019	0.118
Type of salting	0.632	0.271	0.849	0.005	0.170	0.004
Final acidification pH	0.005	0.470	0.001	0.044	0.018	0.073
Mould type	0.056	0.002	0.369	0.435	0.003	0.706

Note: The significant level was set at 5%.

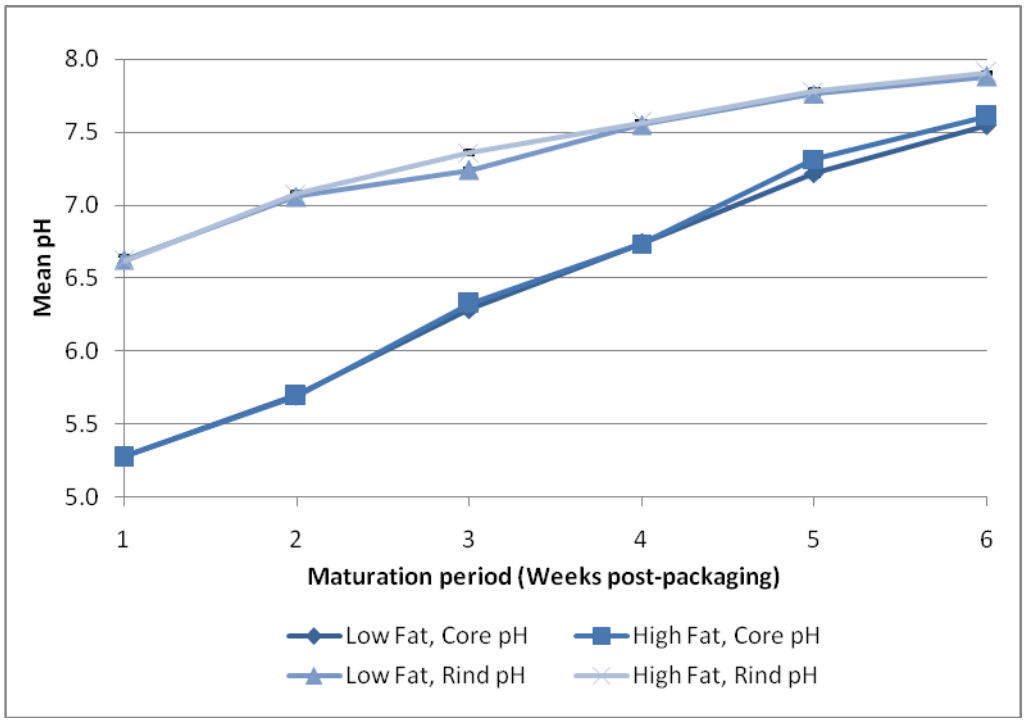


Figure 4.13: Changes in the core and rind pH (mean ± SE_M) of low-fat (n = 64) and high-fat (n = 32) Camembert cheese stored at 4 ± 1 ºC for six weeks post-packaging.

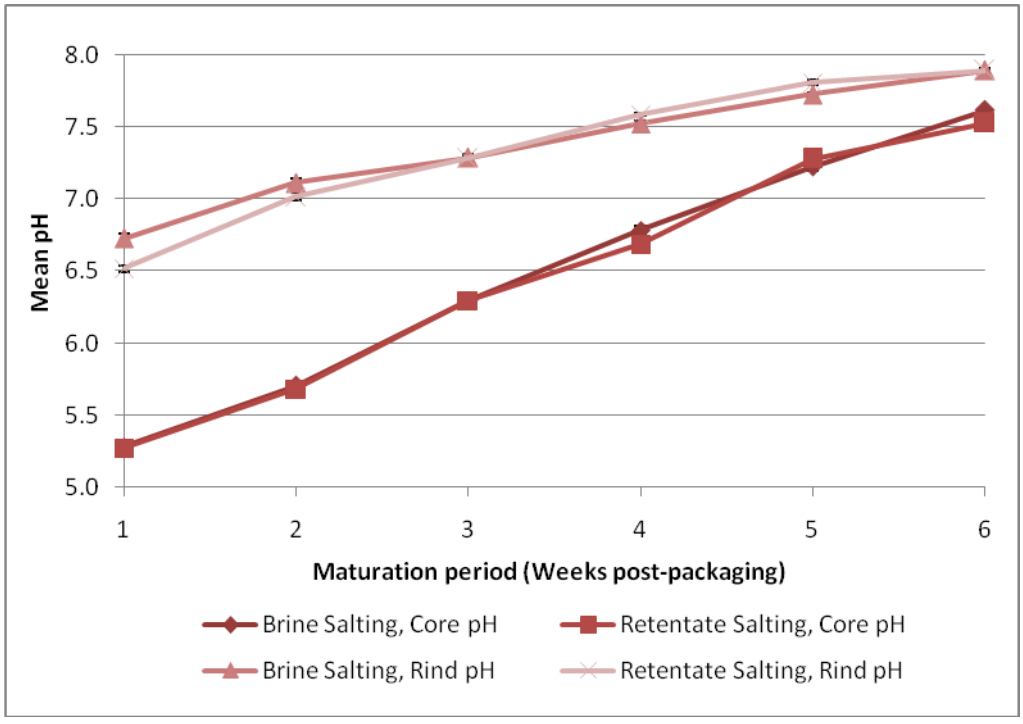


Figure 4.14: Changes in the core and rind pH (mean ± SE_M) of brine-salted (n = 48) and retentate-salted (n = 48) Camembert cheese stored at 4 ± 1 ºC for six weeks post-packaging.

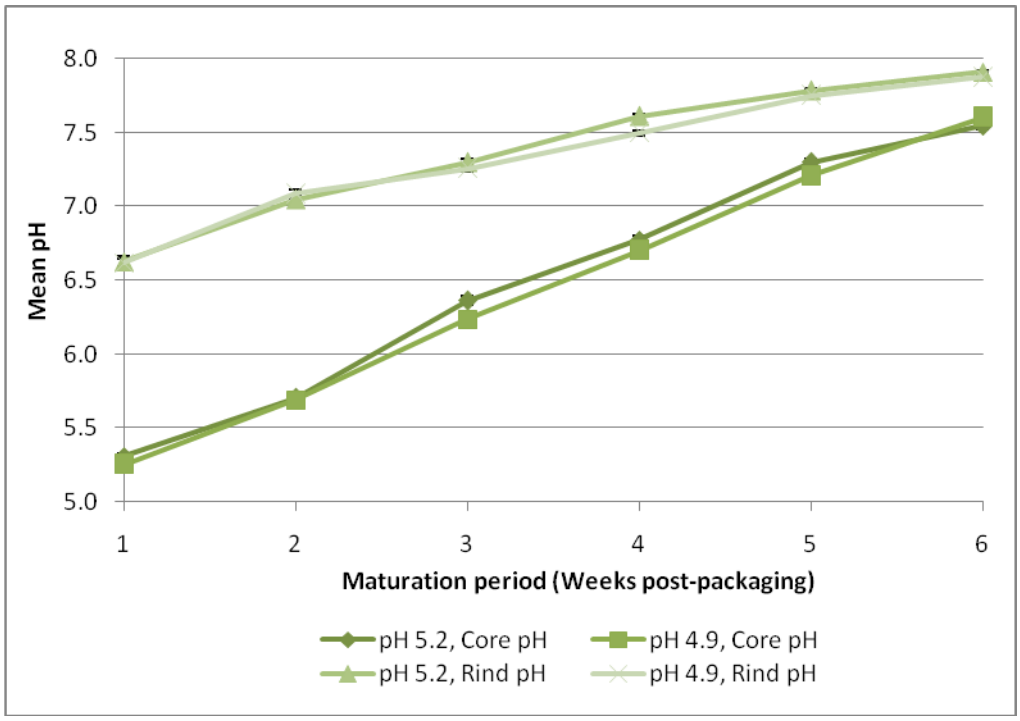


Figure 4.15: Changes in the core and rind pH (mean ± SE_M) of Camembert cheese acidified to pH 5.2 (n = 48) and pH 4.9 (n = 48). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

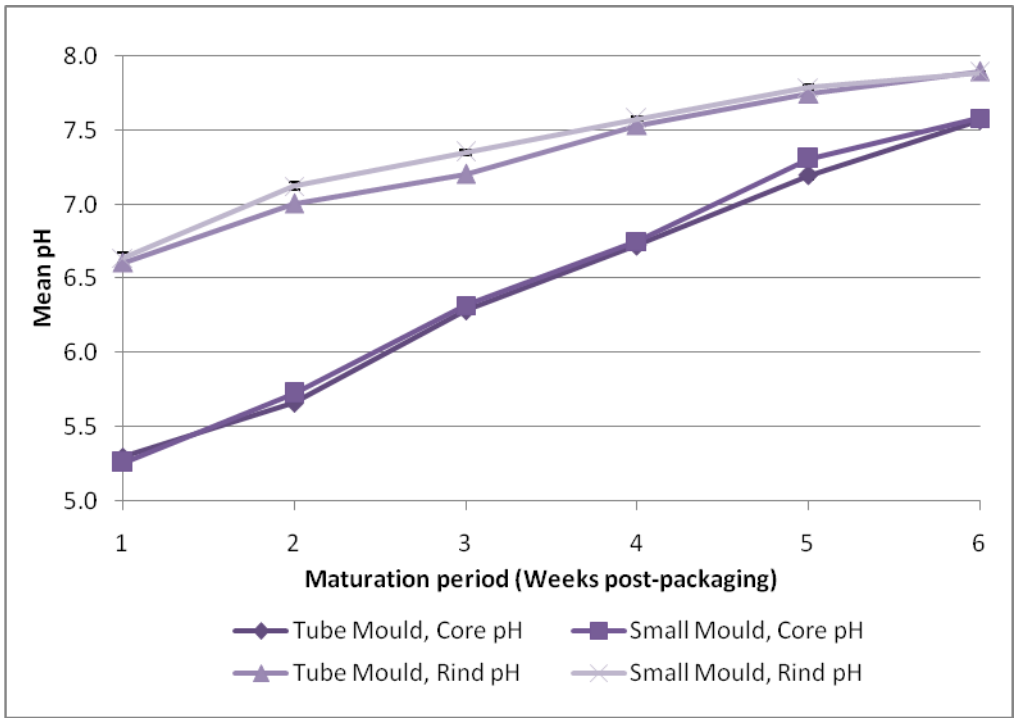


Figure 4.16: Changes in the core and rind pH (mean ± SE_M) of Camembert cheese made using tube moulds (n = 48) and small moulds (n = 48). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

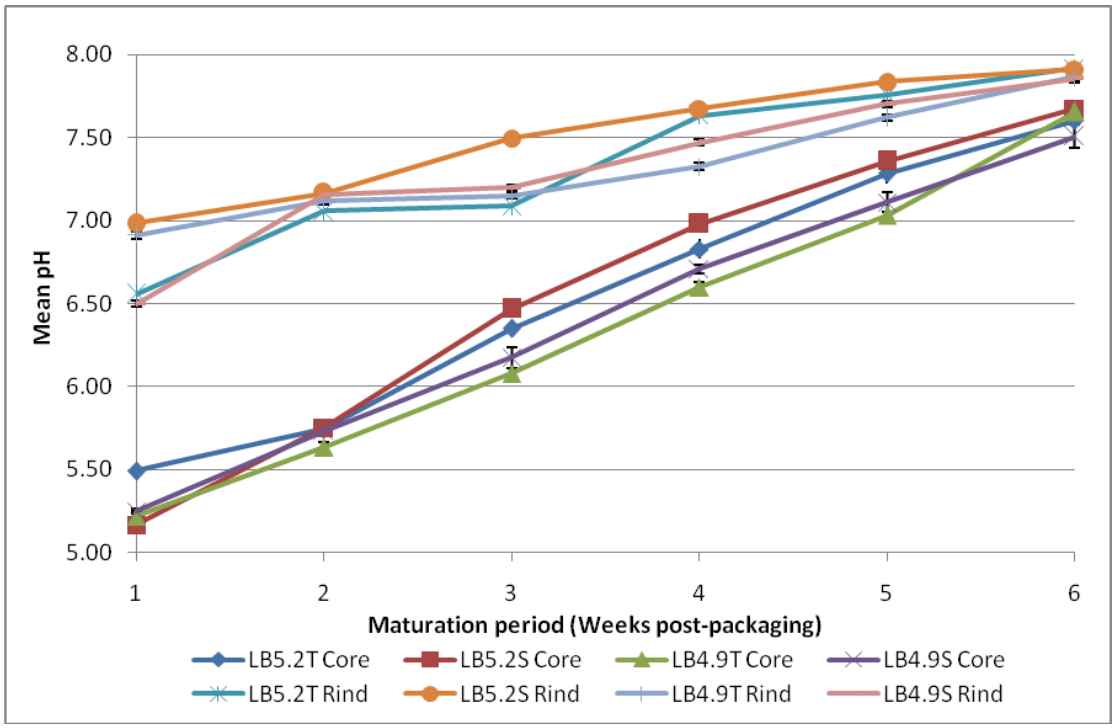


Figure 4.17: Changes in the core and rind pH (mean ± SE_M) of Camembert cheese for low-fat brine-salted samples (n = 8 in each treatment). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

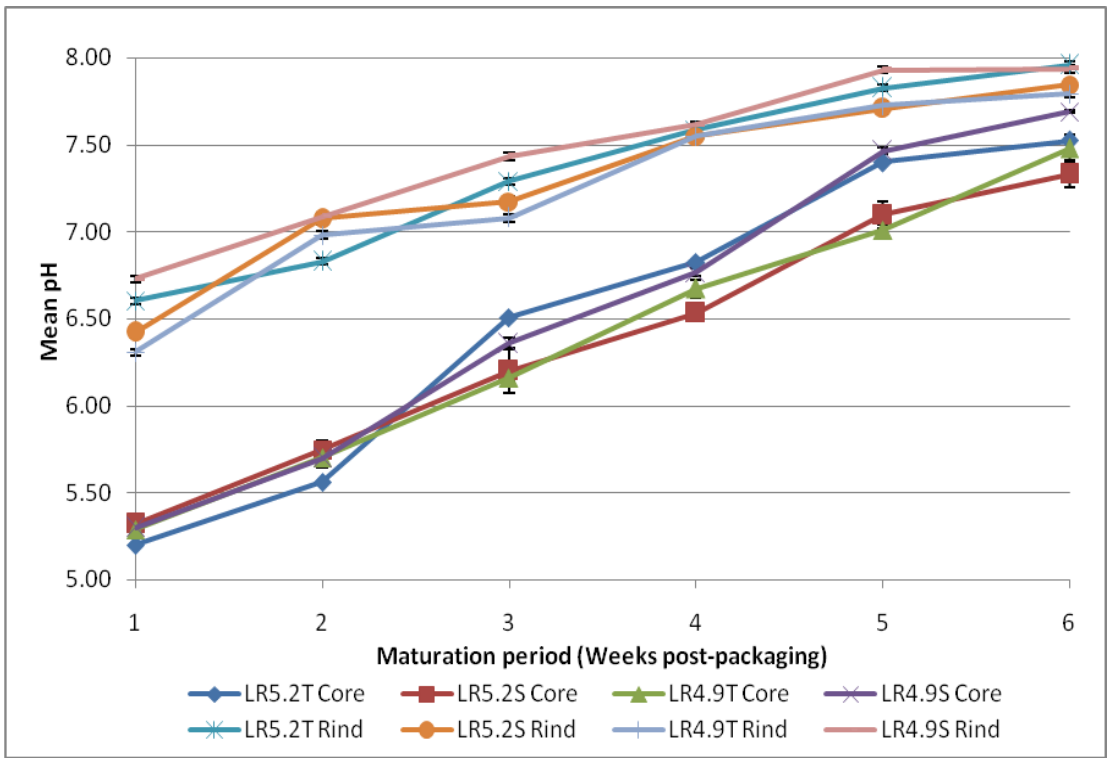


Figure 4.18: Changes in the core and rind pH (mean ± SE_M) of Camembert cheese for low-fat retentate-salted samples (n = 8 in each treatment). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

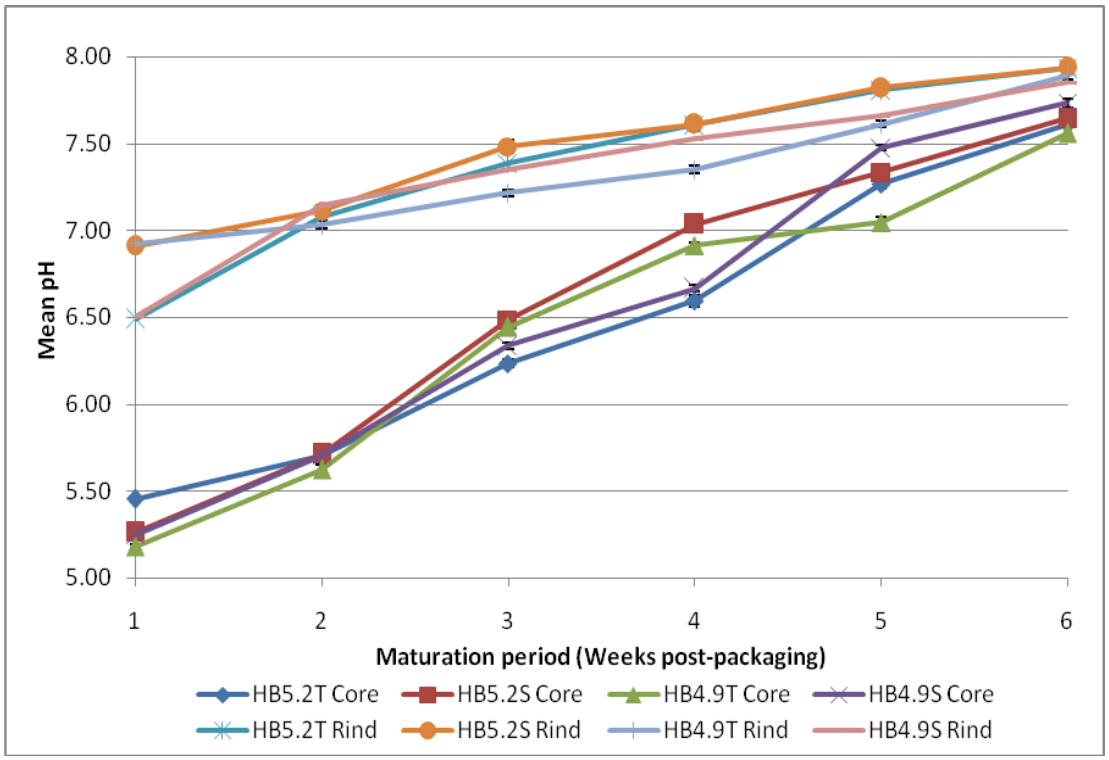


Figure 4.19: Changes in the core and rind pH (mean ± SE_M) of Camembert cheese for high-fat brine-salted samples (n = 8 in each treatment). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

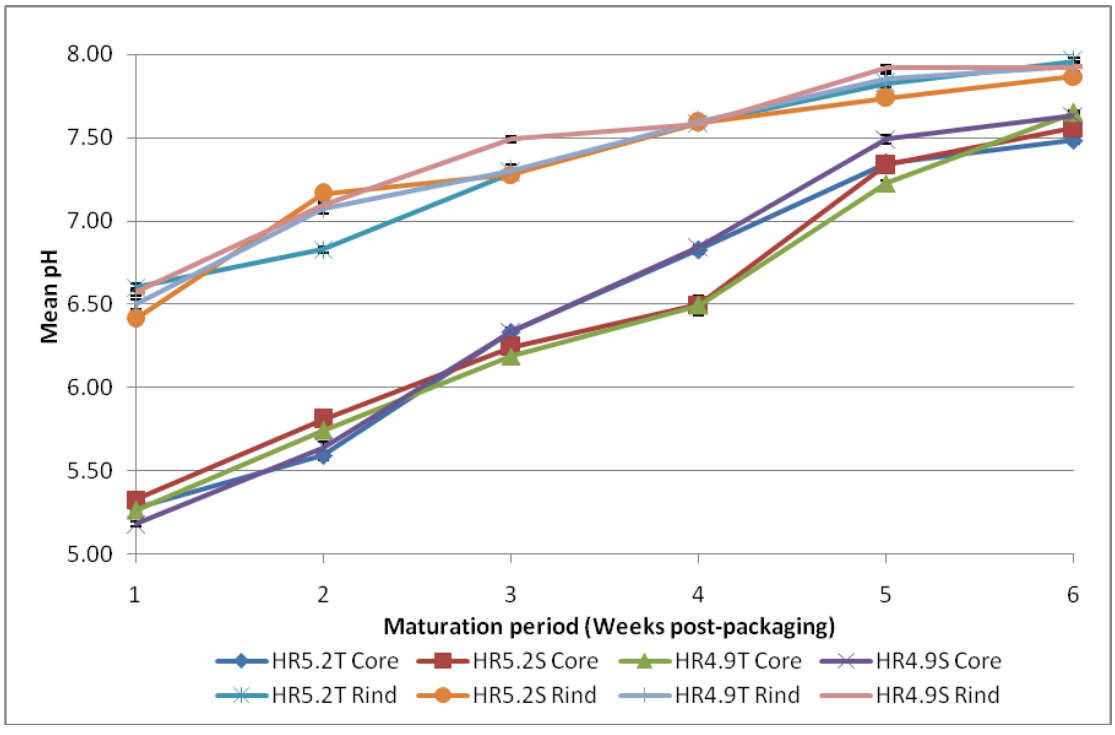


Figure 4.20: Changes in the core and rind pH (mean ± SE_M) of Camembert cheese for high-fat retentate-salted samples (n = 8 in each treatment). The samples were stored at 4 ± 1 ºC for six weeks post-packaging.

4.5. Level of proteolysis

4.5.1. Non-protein nitrogen (NPN)

Total nitrogen (TN) values and non-protein nitrogen (NPN) fractions of the cheese samples were determined by the Kjeldahl method. The portion of NPN in TN serves as an indicator for the level of proteolysis at different stages during the maturation of the cheese.

Table 4.4: p-values ($p \leq 0.05$) for NPN/TN ratios within each type of treatment.

	p-values for selected periods (weeks)			
Cheese treatments	**1**	**3**	**5**	**7**
Level of fat	0.030	0.000	0.502	0.000
Type of salting	0.003	0.000	0.000	0.000
Final acidification pH	0.046	0.041	0.034	0.009
Mould type	0.138	0.372	0.193	0.682

Note: The significant level was set at 5%.

High-fat versus low-fat

In Figure 4.21, the mean NPN/TN ratios for high-fat cheese samples were significantly higher than those of low-fat samples at weeks one, three and seven, suggesting that in general, high-fat samples had a higher level of proteolysis than low-fat samples.

Brine-salted versus retentate-salted

In Figure 4.22, the mean NPN/TN ratios for brine-salted samples were significantly higher than those of retentate-salted samples for all four measurements at weeks one to seven, suggesting a consistently higher level of proteolysis in brine-salted cheese.

pH 5.2 versus pH 4.9

NPN/TN ratios for cheese samples produced with the two final acidification pH levels are shown in Figure 4.23. Samples acidified to pH 4.9 had significantly higher NPN/TN ratios than samples acidified to pH 5.2 from week one to seven. This suggested that the levels of proteolysis were higher in cheese acidified to pH 4.9 than those acidified to pH 5.2 in a consistent manner for seven weeks during maturation.

Tube mould versus small mould

The differences of the mean NPN/TN ratios in cheese samples made using tube moulds and small moulds were not significant (Figure 4.24).

The mean NPN/TN ratios for each of the sixteen treatments are presented in Figures 4.25 to 4.28. When comparing low-fat (Figures 4.25 and 4.26) with high-fat cheese samples (Figures 4.27 and 4.28), the high-fat samples generally had higher mean NPN/TN ratios than the low-fat samples with the exception of LB4.9S in Figure 4.25. The mean NPN/TN ratios of the low-fat retentate-salted treatments shown in Figure 4.26 were generally lower than other treatments shown in Figures 4.25, 4.27 and 4.28, which probably gave rise to lower overall means of NPN/TN ratio for retentate-salted cheeses.

In the case of mean NPN/TN ratios for the high-fat cheese samples acidified to the two final acidification pH levels (pH 5.2 and pH 4.9), results in Figure 4.27 show that the pH 4.9 samples (HB4.9T and HB4.9S) were clearly above the pH 5.2 samples (HB5.2T and HB5.2S) for all seven weeks. Low-fat cheese samples, LB4.9S (Figure 4.25) and LR4.9S (Figure 4.26) had slightly higher mean NPN/TN ratios than other treatments in Figures 4.25 and 4.26, respectively. Regarding the two types of moulds used in terms of mean NPN/TN ratios, no apparent trends were observed. Therefore, there were no significant differences between the two types of moulds used with respect to the NPN/TN ratios in the cheese samples (Figure 4.24).

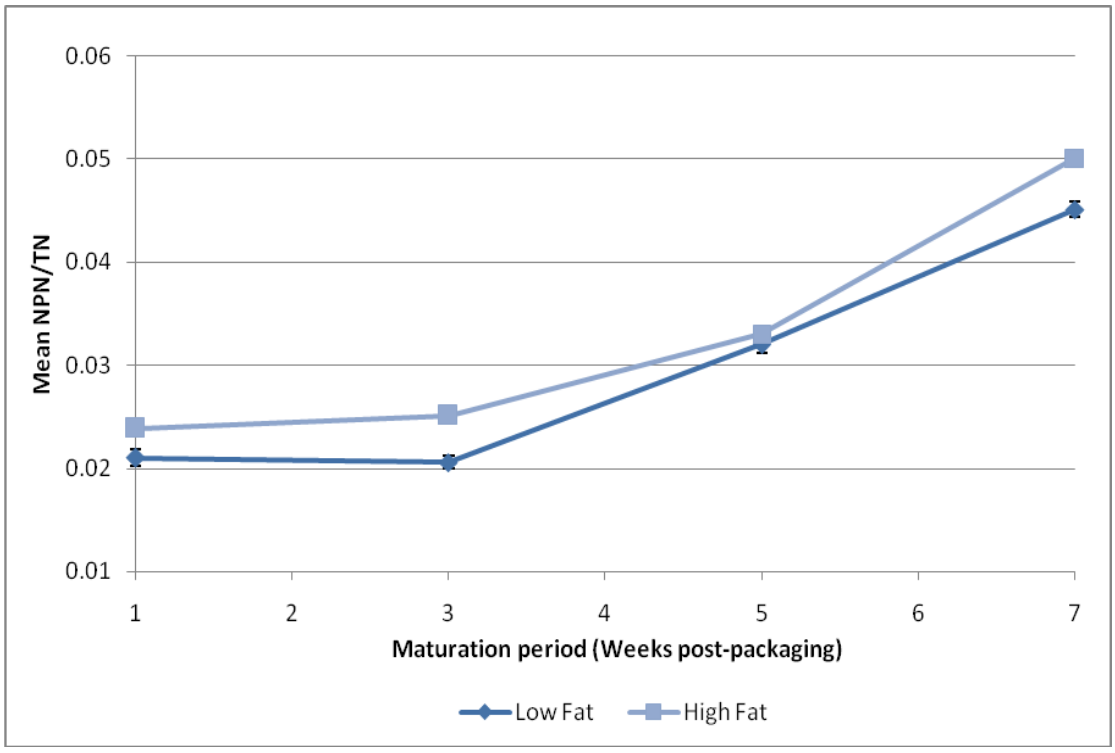


Figure 4.21: Changes in the NPN/TN ratios (mean ± SE_M) of low-fat (n = 32) and high-fat (n = 16) Camembert cheese stored at 4 ± 1 ºC for seven weeks post-packaging.

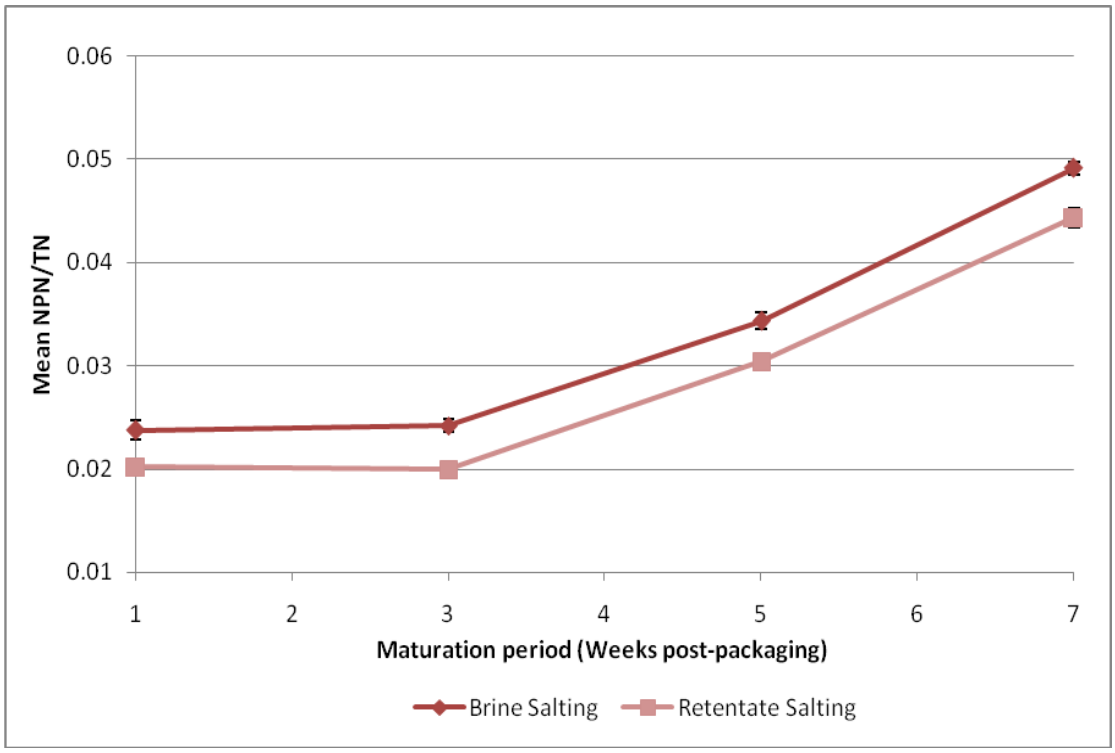


Figure 4.22: Changes in the NPN/TN ratio (mean ± SE_M) of brine-salted (n = 24) and retentate-salted (n = 24) Camembert cheese stored at 4 ± 1 ºC for seven weeks post-packaging.

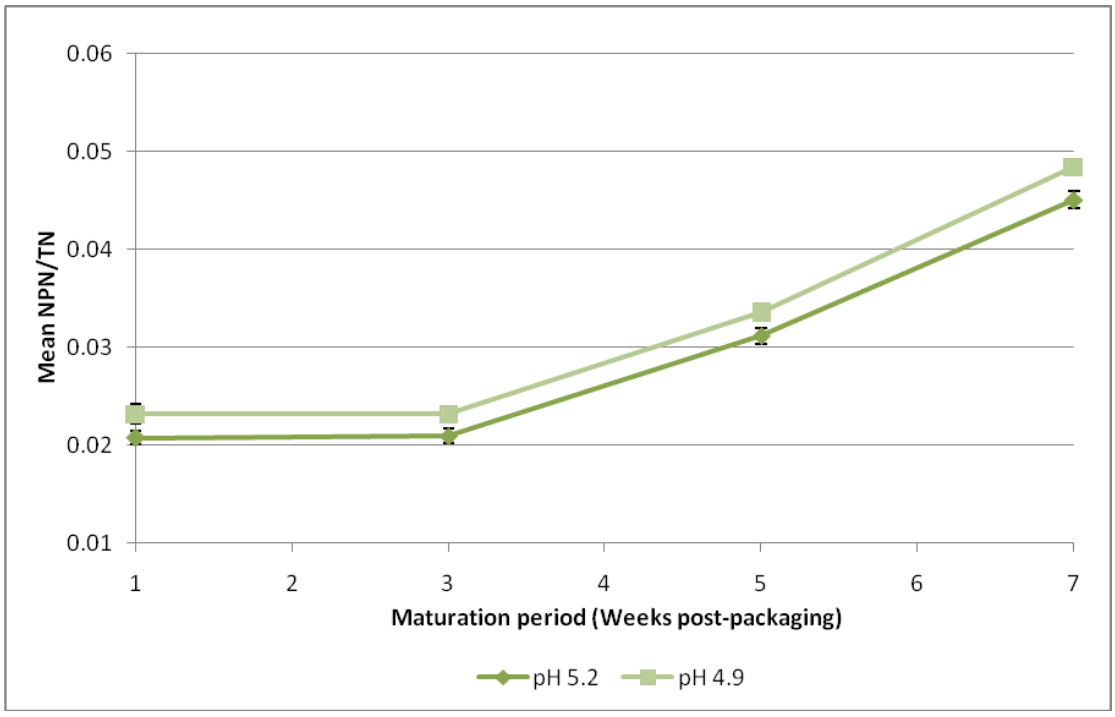


Figure 4.23: Changes in the NPN/TN ratio (mean ± SE_M) of Camembert cheese acidified to pH 5.2 (n = 24) and pH 4.9 (n = 24). The samples were stored at 4 ± 1 ºC for seven weeks post-packaging.

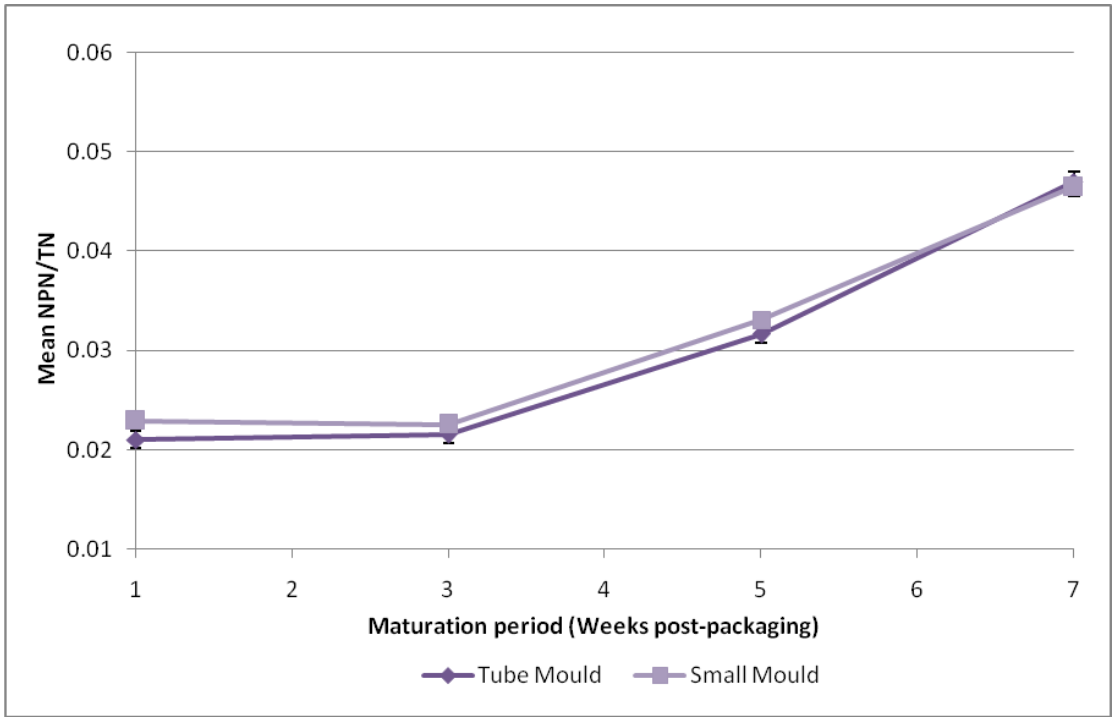


Figure 4.24: Changes in the NPN/TN ratio (mean ± SE_M) of Camembert cheese made using tube moulds (n = 24) and small moulds (n = 24). The samples were stored at 4 ± 1 ºC for seven weeks post-packaging.

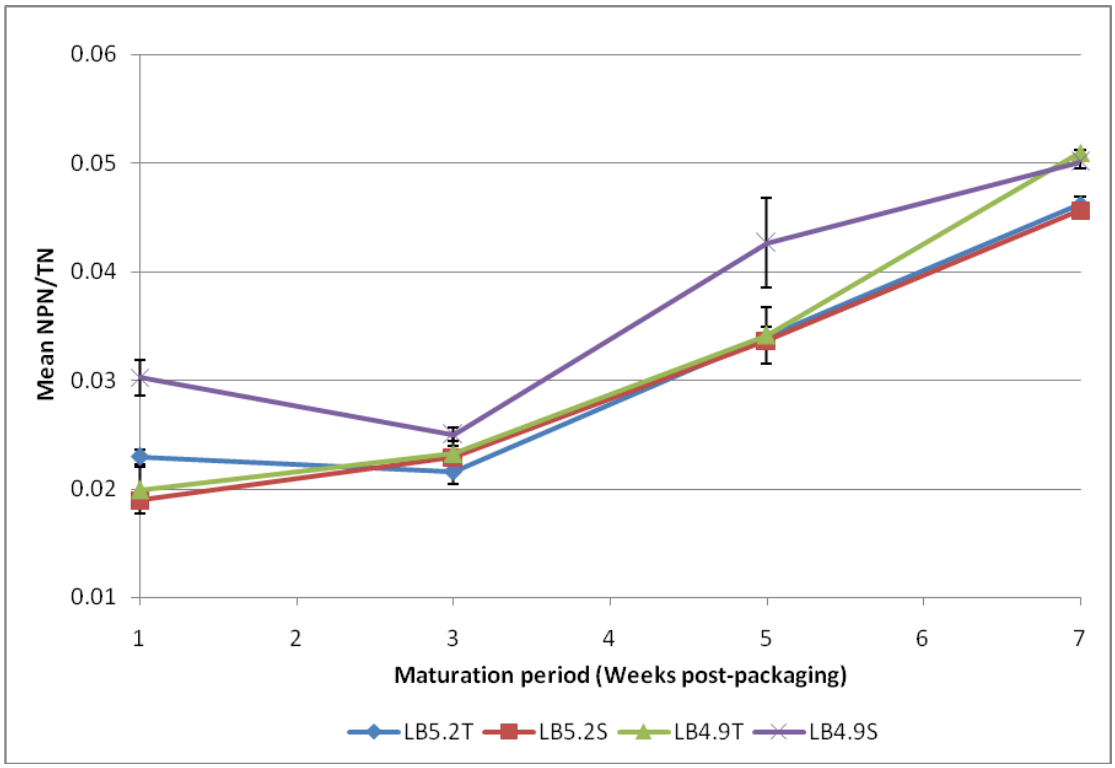


Figure 4.25: Changes in the NPN/TN ratio (mean ± SE_M) of Camembert cheese for low-fat brine-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 ºC for seven weeks post-packaging.

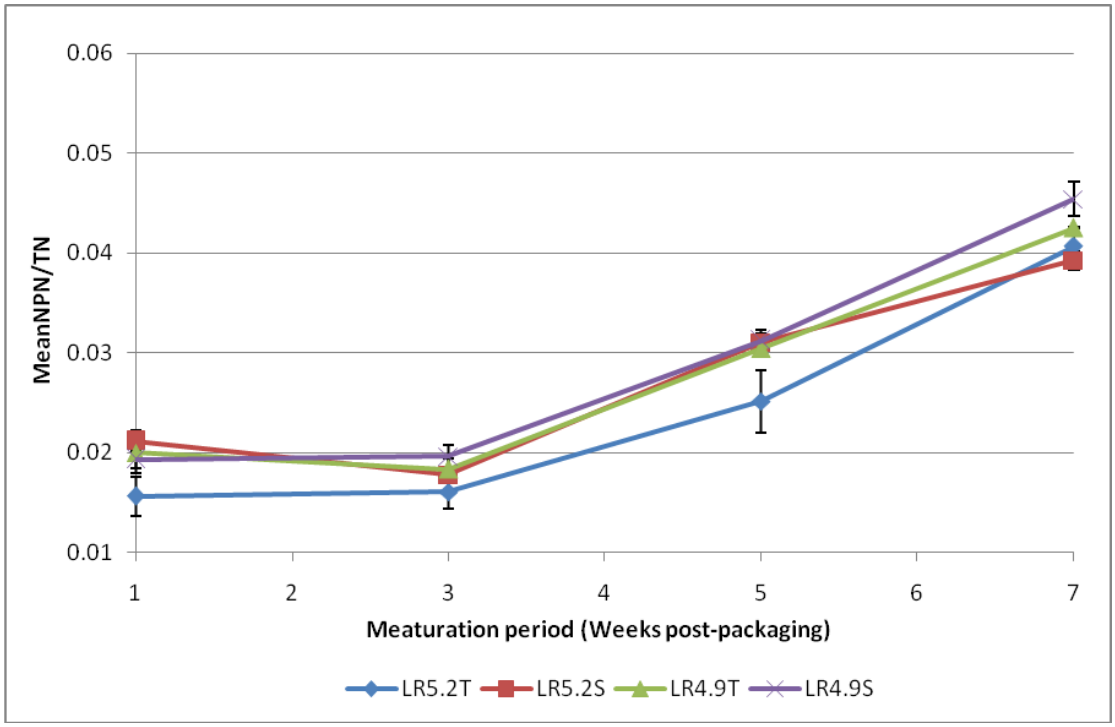


Figure 4.26: Changes in the NPN/TN ratio (mean ± SE_M) of Camembert cheese for low-fat retentate-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 ºC for seven weeks post-packaging.

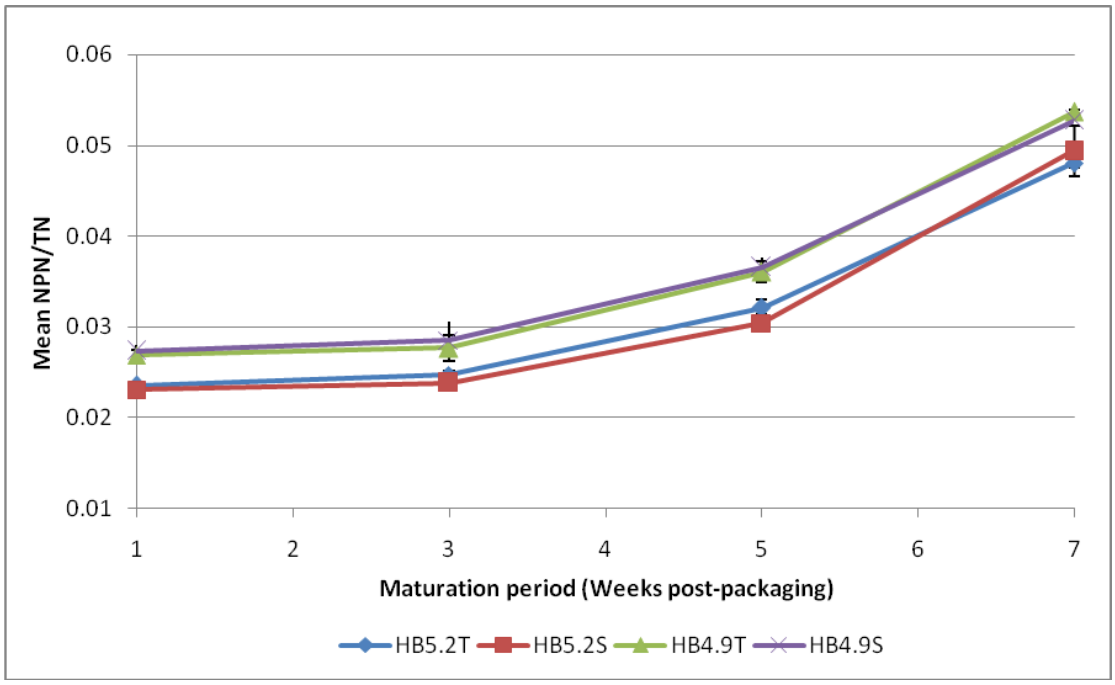


Figure 4.27: Changes in the NPN/TN ratio (mean ± SE_M) of Camembert cheese for high-fat brine-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 ºC for seven weeks post-packaging.

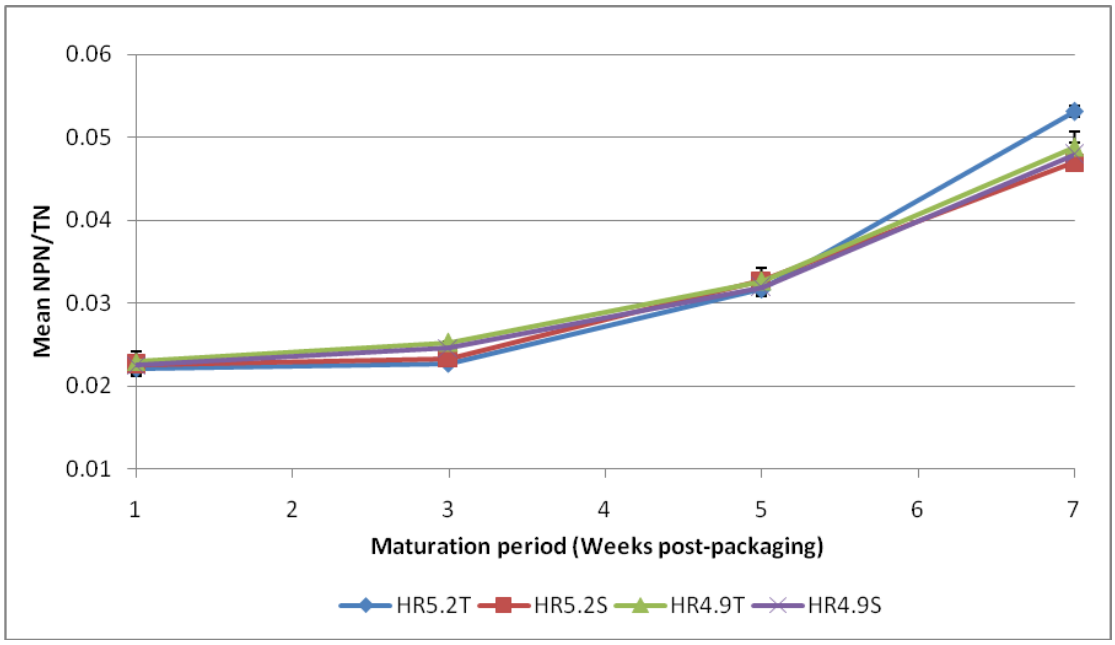


Figure 4.28: Changes in the NPN/TN ratio (mean ± SE_M) of Camembert cheese for high-fat retentate-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 ºC for seven weeks post-packaging.

4.5.2. pH 4.4 soluble nitrogen (SN)

The mean soluble nitrogen/total nitrogen SN/TN ratios were determined by the Kjeldahl method, which also indicate the level of proteolysis at different stages of cheese ripening. The SN includes protein fractions which are soluble in pH 4.4 as well as NPN.

Table 4.5: p-values ($p \leq 0.05$) for SN/TN ratios within each type of treatment.

	p-values for selected periods (weeks)			
Cheese treatments	**1**	**3**	**5**	**7**
Level of fat	0.709	0.176	0.351	0.000
Type of salting	0.639	0.916	0.001	0.912
Final acidification pH	0.529	0.097	0.050	0.000
Mould type	0.329	0.439	0.450	0.695

Note: The significant level was set at 5%.

The mean SN/TN ratios of high-fat cheese samples were significantly higher than those of the low-fat samples at week seven of maturation only as shown in Figure 4.29. There were also no significant differences between brine-salted cheeses and retentate-salted cheeses in terms of the SN/TN ratio, except at week five, where the brine-salted samples had a higher mean SN/TN ratio than the retentate-salted samples (Figure 4.30).

Cheese samples acidified to pH 4.9 had significantly higher mean SN/TN ratios than samples acidified to pH 5.2 only significant at week 7 of storage (Figure 4.31). The mean SN/TN ratios of cheese samples made using tube moulds and small moulds are shown in Figure 4.32, where both treatments were not significantly different from each other.

In comparing low-fat (Figures 4.33 and 4.34) and high-fat (Figure 4.35 and 4.36) treatments, generally higher mean SN/TN ratio was observed in high-fat treatments at week seven. No trends were observed between brine-salted (Figures 4.33 and figure 4.35) and retentate-salted treatments (Figures 4.34 and 4.36). In the case of cheese samples made at two final acidification pH levels, no significant observations could be drawn for the first five weeks of maturation for the SN/TN ratios (Figures 4.33 to 4.36). However, at week seven, the majority of the samples acidified to pH 4.9 had significantly higher mean SN/TN ratios than their pH 5.2 equivalent treatments with

other variables remaining constant. The exception was HB4.9T, the SN/TN ratio for this treatment was not significantly higher than its equivalent HB5.2T (Figure 4.35). No apparent trends could be observed in tube mould and small mould treatments shown in Figures 4.33 to 4.36, suggesting the use of different moulds had no apparent impact in cheese proteolysis.

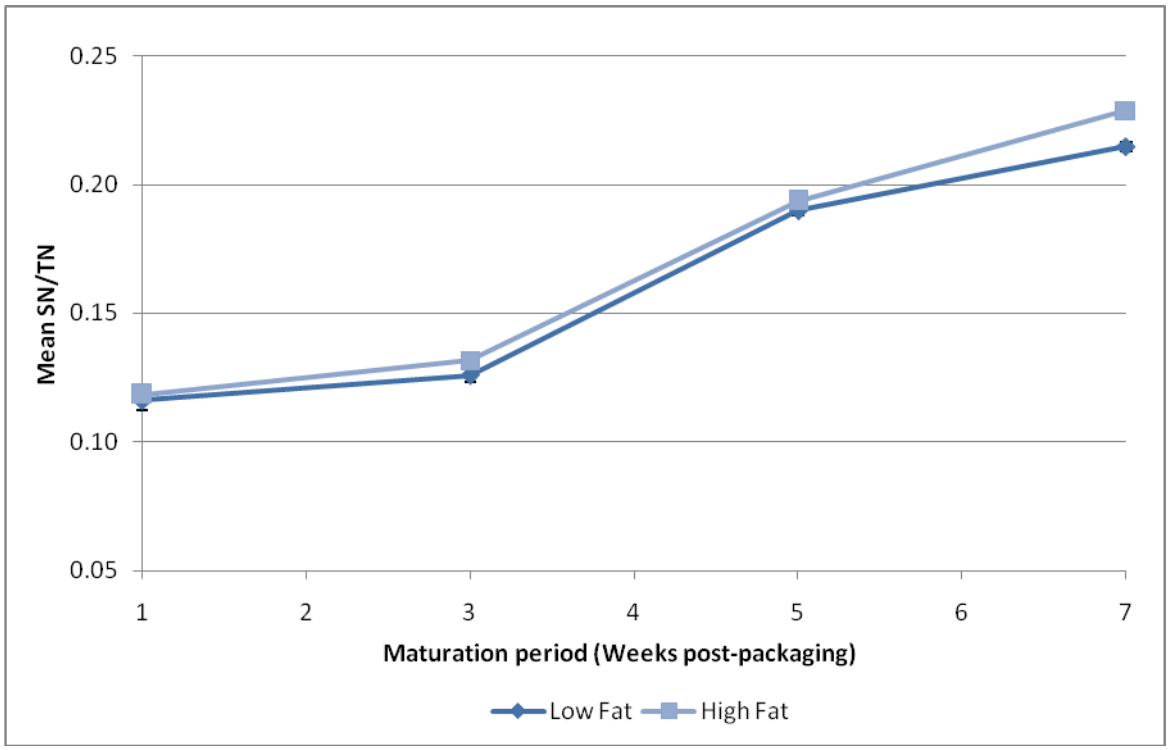


Figure 4.29: Changes in the SN/TN ratio (mean ± SE_M) of low-fat (n = 32) and high-fat (n = 16) Camembert cheese stored at 4 ± 1 ºC for seven weeks post-packaging.

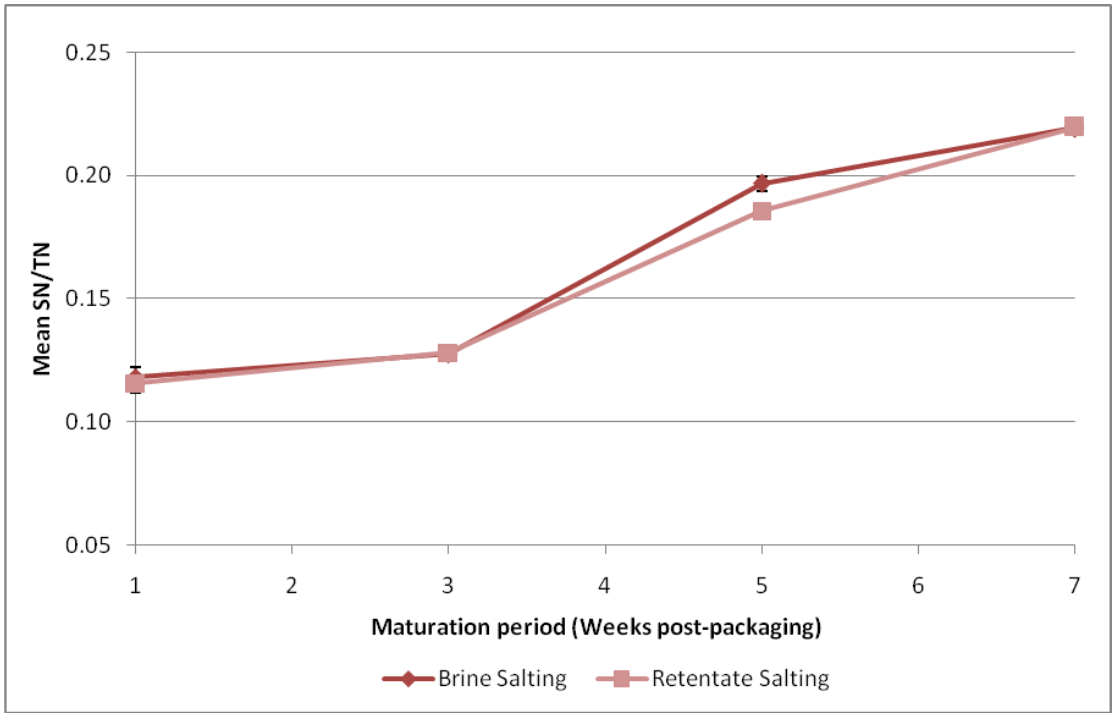


Figure 4.30: Changes in the SN/TN ratio (mean ± SE_M) of brine-salted (n = 24) and retentate-salted (n = 24) Camembert cheese stored at 4 ± 1 ºC for seven weeks post-packaging.

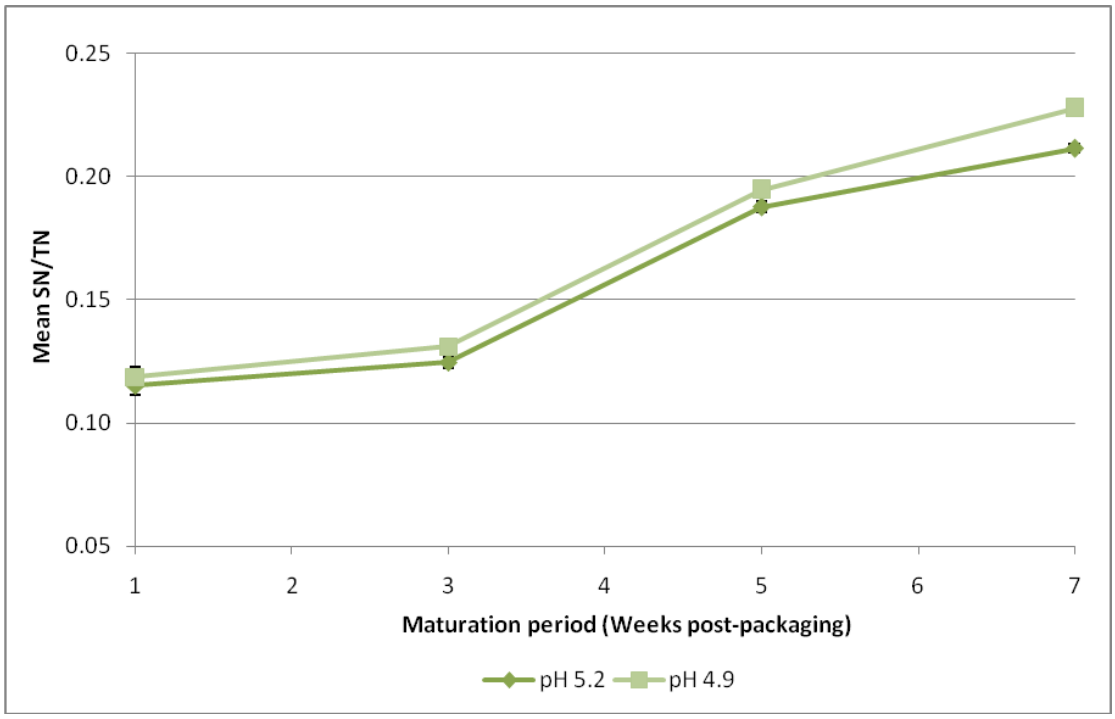


Figure 4.31: Changes in the SN/TN ratio (mean ± SE_M) of Camembert cheese acidified pH 5.2 (n = 24) and pH 4.9 (n = 24). The samples were stored at 4 ± 1 ºC for seven weeks post-packaging.

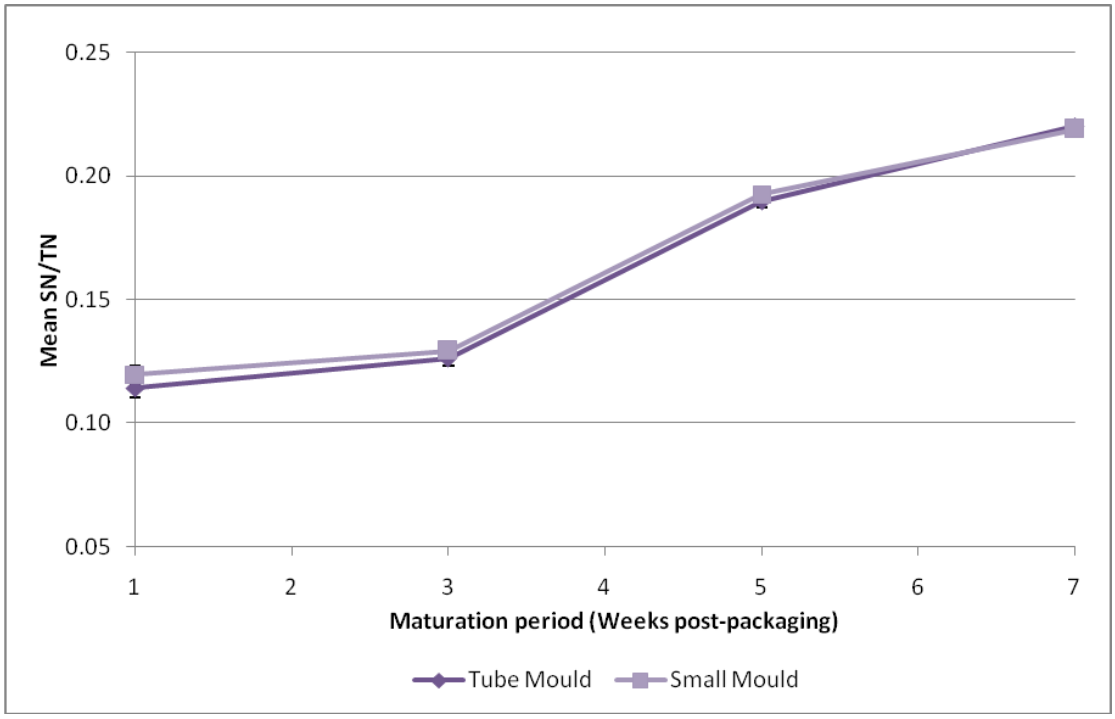


Figure 4.32: Changes in the SN/TN ratio (mean ± SE_M) of Camembert cheese made using tube moulds (n = 24) and small moulds (n = 24). The samples were stored at 4 ± 1 ºC for seven weeks post-packaging.

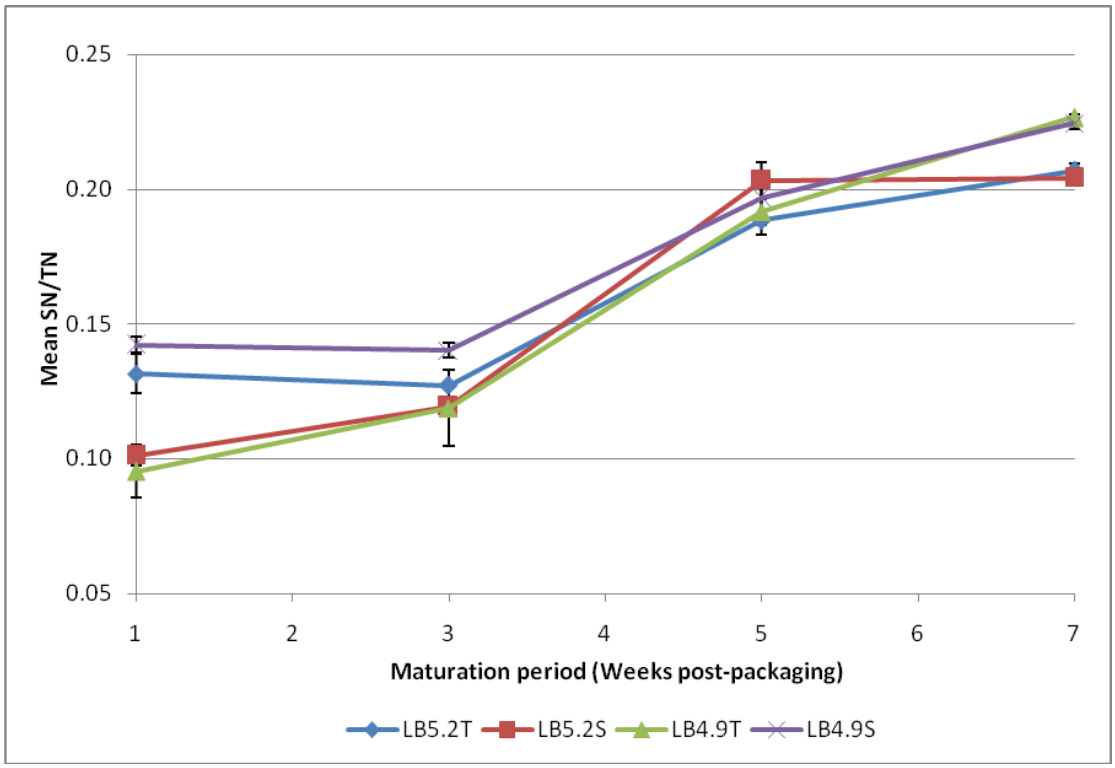


Figure 4.33: Changes in the SN/TN ratio (mean ± SE_M) of Camembert cheese for low-fat brine-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 °C for seven weeks post-packaging.

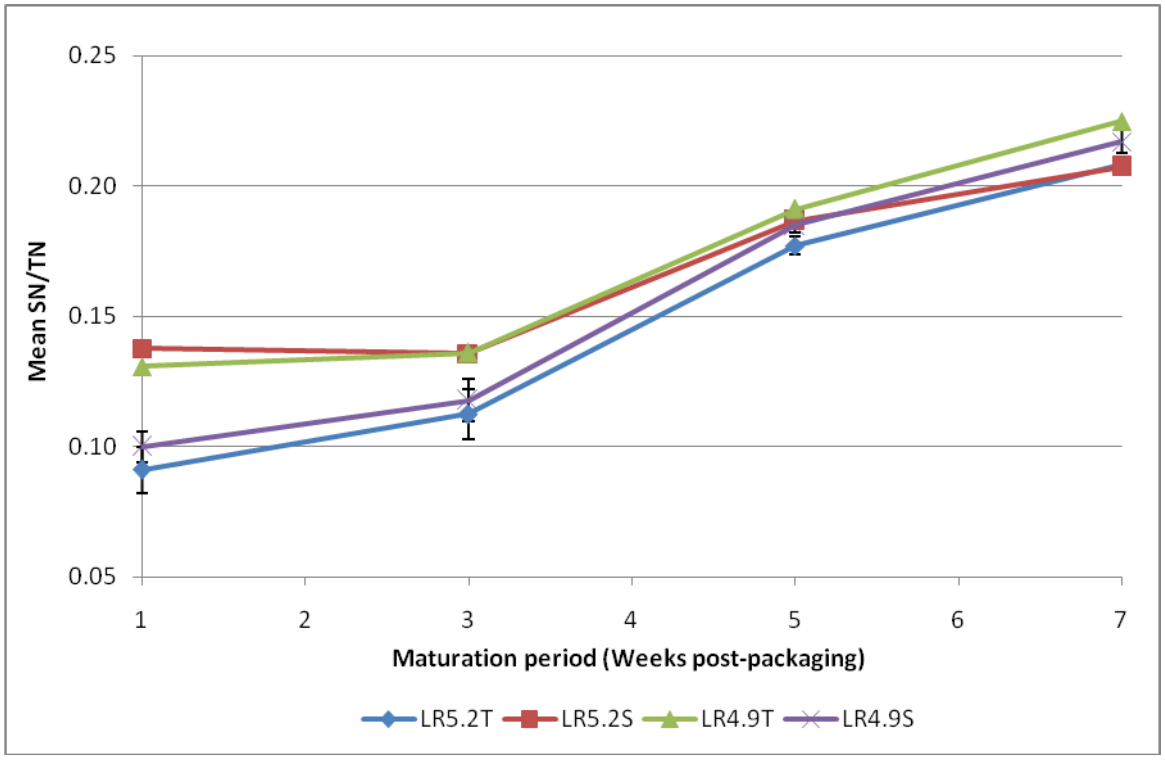


Figure 4.34: Changes in the SN/TN ratio (mean ± SE_M) of Camembert cheese for low-fat retentate-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 °C for seven weeks post-packaging.

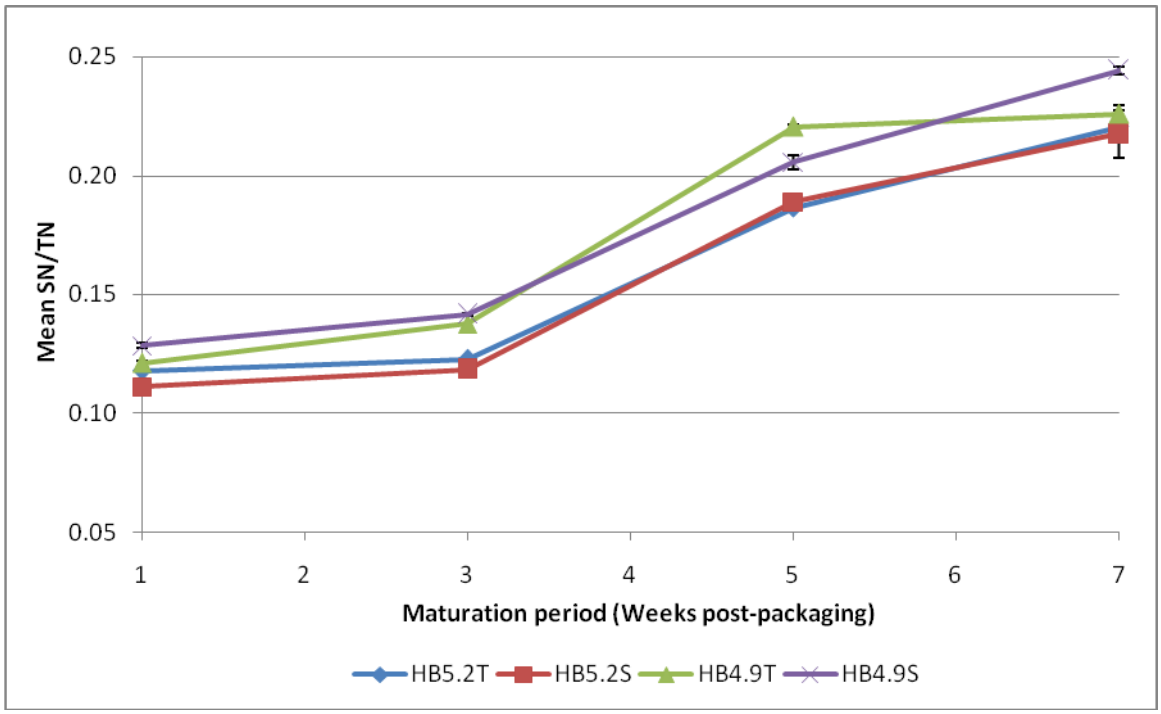


Figure 4.35: Changes in the SN/TN ratio (mean ± SE_M) of Camembert cheese for high-fat brine-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 ºC for seven weeks post-packaging.

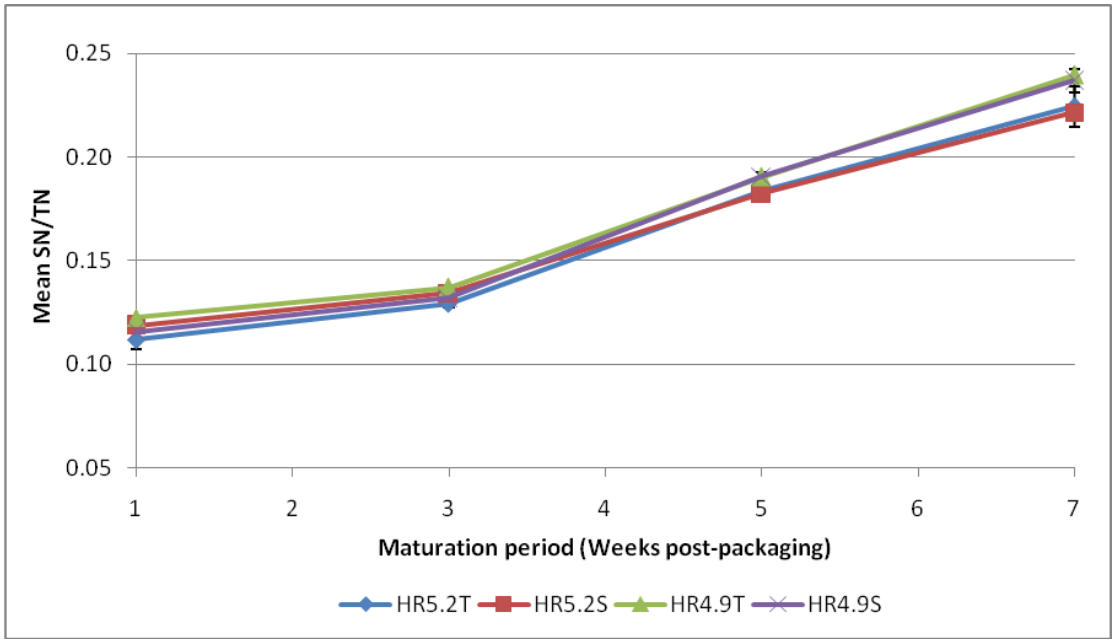


Figure 4.36: Changes in the SN/TN ratio (mean ± SE_M) of Camembert cheese for high-fat retentate-salted samples (n = 4 in each treatment). The samples were stored at 4 ± 1 ºC for seven weeks post-packaging.

4.6. Texture analysis

Instrumental texture analysis was used to measure the force required to compress a cheese sample to 50% of its original height. A higher deformation force indicated the cheese sample was relatively firmer and a lower deformation force indicated the sample was softer. Figures 4.37 to 4.40 show the deformation force for the sixteen treatments analysed. In Figure 4.37, samples LB5.2T and LB5.2S were significantly firmer (higher deformation forces) than LB4.9T and LB4.9S at weeks one and two. At weeks five and six, LB5.2T was significantly firmer than the other three samples. However, this does not suggest that samples acidified to pH 5.2 were necessarily firmer than those acidified to pH 4.9 as LB5.2S had similar low deformation forces similar to pH 4.9 samples at week three to six. In Figure 4.38, no meaningful trends can be observed, with the exception of LR5.2T, which was significantly firmer at week two to four than other samples. In comparing the brine-salted samples in Figure 4.37 and retentate-salted samples in Figure 4.38, the two salt treatments did not result in any significant differences in the case of texture analysis measurements.

Texture analysis results for the high-fat cheeses are shown in Figure 4.39 and Figure 4.40. In Figure 4.39, samples acidified to pH 5.2, HB5.2T and HB5.2S were significantly firmer than HB4.9T and HB4.9S at weeks one and two. At week four to six, HB5.2T was significantly firmer than the rest of the samples, which was similar to LB5.2T in Figure 4.37. This suggests that the combination of high pH and tube mould treatment, regardless of fat content, contribute to a firmer cheese during its maturation period. In Figure 4.40, HR4.9T was significantly firmer than the samples acidified to pH 5.2 at week one and also in weeks five and six. This is apparent as the samples acidified to pH 4.9 were softer (lower deformation forces) than those acidified to pH 5.2 in the first two weeks of the brine-salted samples (Figures 4.37 and 4.39), suggesting the influence of salt in cheese firmness. Sample HR5.2T was firmer than the other samples at week two to four (Figure 4.40). This was an interesting observation as pH 5.2-tube mould samples were always firmer at week two in all texture analysis results. Sample HR4.9S was generally softer than all samples and was consistent in all texture analysis measurements for being softer than other samples in the first two weeks. No trends could be observed when comparing between samples of the two salt treatments for high-fat cheese samples in Figures 4.39 and 4.40.

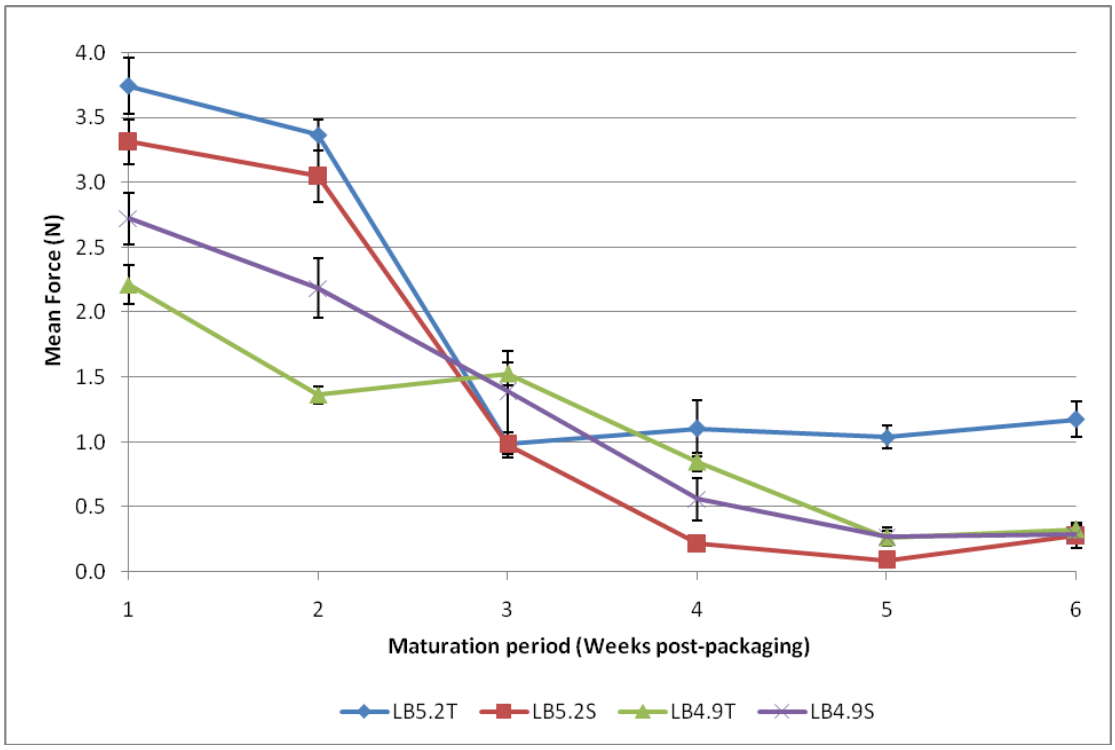


Figure 4.37: Change in force (N) (mean ± SE_M) at 50% deformation for low-fat brine-salted cheese samples (n = 8 in each treatment) stored at 4 ± 1 °C for six weeks post-packaging.

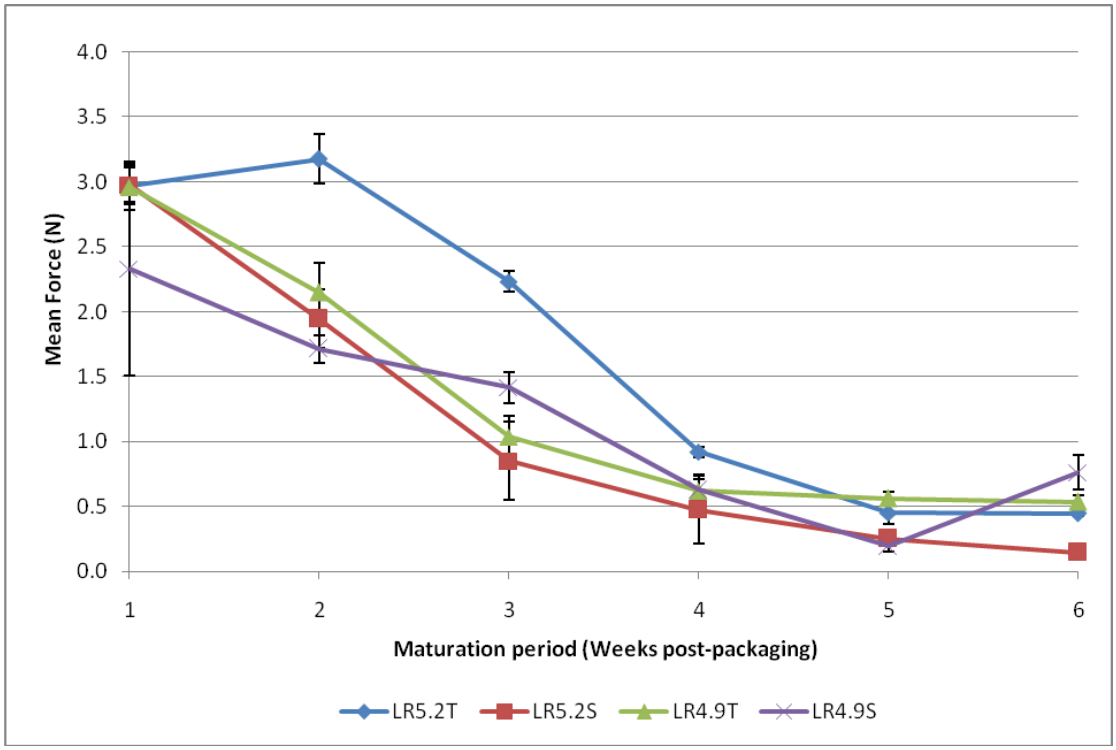


Figure 4.38: Change in force (N) (mean ± SE_M) at 50% deformation for low-fat retentate-salted cheese samples (n = 8 in each treatment) stored at 4 ± 1 °C for six weeks post-packaging.

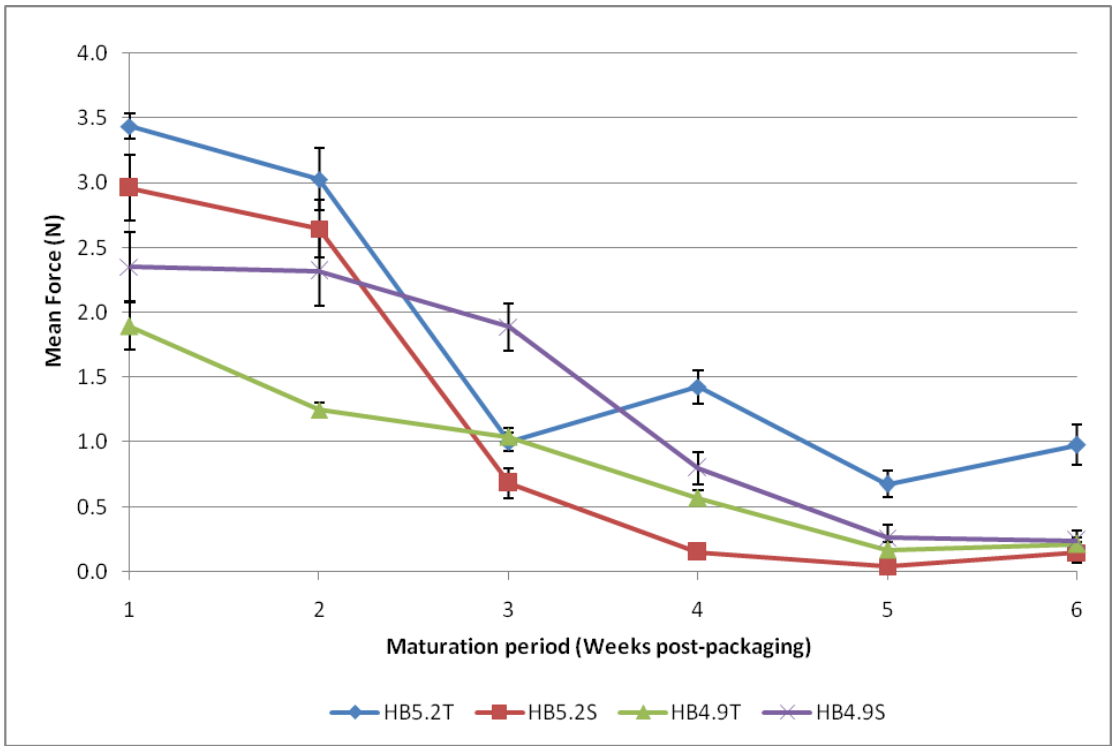


Figure 4.39: Change in force (N) (mean ± SE_M) at 50% deformation for high-fat brine-salted cheese samples (n = 8 in each treatment) stored at 4 ± 1 ºC for six weeks post-packaging.

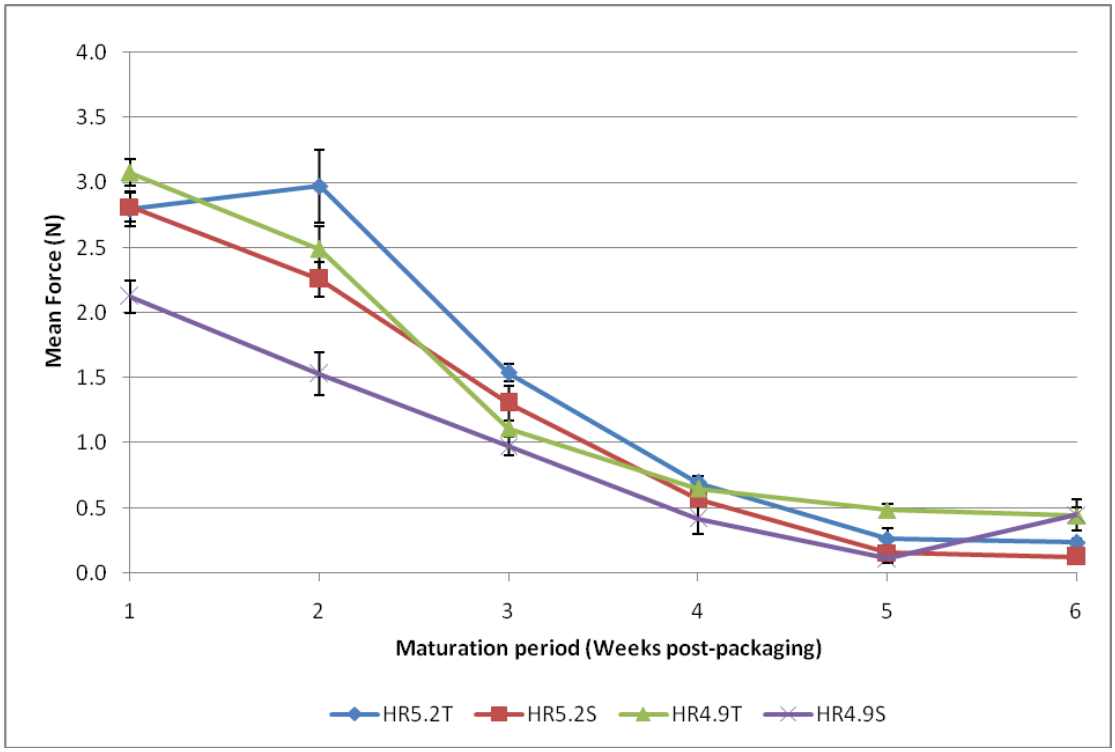


Figure 4.40: Change in force (N) (mean ± SE_M) at 50% deformation for high-fat retentate-salted cheese samples (n = 8 in each treatment) stored at 4 ± 1 ºC for six weeks post-packaging.

4.7. Cheese grading

The week four cheese grading data are presented in Figures 4.41 to 4.44. Nine major sensory defects were plotted on a graph against the percentage of occurrence when each sample from the 16 treatments was graded. The defects included rind discolouration (tanning or bolding of the surface mould flora), rind deformation (unevenness and misshaping of the rind), thick rind, core unevenness (a firm core texture which gradually gets softer towards the rind), excessive softness, saltiness, sourness, bitterness, and blandness. The percentage of occurrence indicated the frequency of detecting certain detects within the same group of cheese samples at the age of four and six weeks as determined by cheese judges.

Low-fat versus high-fat

The occurrence of rind discolouration was more prominent among low-fat cheese samples with 100% occurrence compared to 50% in the high-fat samples (Figure 4.41). Twenty-five percent of the low-fat samples graded were affected by core unevenness whereas the high-fat samples did not have this defect. Twenty-five percent of the low-fat samples and 12.5% of the high-fat samples were judged to have excessive sourness. Both treatments were often affected by thick rind, with 87.5% and 100% occurrence in the high-fat and low-fat samples, respectively. The majority of cheese samples from both treatments were also affected by excessive softness, with 87.5% and 75% occurrence in the high-fat and low-fat samples, respectively. Rind deformation at week four was apparent in the high-fat samples, occurring in 37.5% compared to 12.5% in the low-fat samples. The high-fat samples also had excessive saltiness and bitterness with 75% and 50% occurrence, respectively, whereas in the low-fat samples their occurrences were only 50% and 25%, respectively. Forty percent of both the high-fat and low-fat samples had excessive blandness.

Brine-salted versus retentate-salted

The retentate-salted cheese samples had higher occurrences in rind discolouration, rind deformation, and excessive saltiness than the brine-salted samples (Figure 4.42). The occurrence of rind discolouration in the retentate-salted samples was 87.5% compared to 62.5% in the brine-salted samples. Thirty-eight percent of the retentate-salted cheese samples exhibited rind deformation, whereas only 12.5% of the brine-

salted samples were affected. The majority of the retentate-salted samples had excessive saltiness with 87.5% occurrence and only 37.5% occurrence in the brine-salted samples. In contrast, defects such as thick rind, excessive softness, sourness, bitterness and blandness had higher occurrence in the brine-salted samples compared to the retentate-salted samples. All the graded brine-salted samples (100%) were found to have thick rinds and 87.5% had excessive softness, whereas only 87.5% of the retentate-salted samples had thick rind and 75% had excessive softness. In terms of flavour defects, 25% of the brine-salted samples had excessive sourness, 62.5% had excessive bitterness, and 50% had excessive blandness, compared to 12.5%, 12.5% and 25% of the retentate-salted samples, respectively.

pH 5.2 versus pH 4.9

A comparison of grading defects in cheese samples acidified to pH 5.2 or pH 4.9 showed that those acidified to pH 4.9 were more often affected by rind discolouration, thick rind, excessive softness, sourness, and bitterness (Figure 4.43). Rind discolouration occurrence in the pH 4.9 samples was 87.5%, while it was only 62.5% in the pH 5.2 samples. Both pH treatments of the cheese samples had high occurrences of the thick rind defect, with 100% in the pH 4.9 samples and 87.5% in the pH 5.2 samples. All the pH 4.9 samples (100%) had excessive softness during grading, whereas only 62.5% of the pH 5.2 samples had excessive softness after four weeks maturation. In terms of flavour, 37.5% of the pH 4.9 samples had excessive sourness and 50% had excessive bitterness, compared to no occurrence in excessive sourness and only 25% had excessive bitterness in the pH 5.2 samples. However, 75% of the pH 5.2 samples had excessive saltiness compared to 50% of the pH 4.9 samples. A small portion of both pH 5.2 and pH 4.9 samples were affected by rind deformation (25%) and core unevenness (12.5%). Thirty-eight percent of samples from both treatments were considered to be too bland (Figure 4.49).

Tube mould versus small mould

Rind discolouration in cheese samples made using tube moulds occurred more often than those made using small moulds, with 87.5% and 62.5% occurrence, respectively (Figure 4.44). All the graded tube mould samples (100%) were affected with thick rind, as a high number of the small mould samples also suffered from the same defect

at 87.5% occurrence. In contrast, the small mould samples had higher occurrence in defects including rind deformation, core unevenness, excessive softness, sourness, bitterness and blandness. In the samples made using small moulds, 50% were affected by rind deformation and 25% had core unevenness, whereas there were no occurrences of these two defects in those made using tube moulds. All of the small mould samples (100%) graded had excessive softness at four weeks post-packaging, compared to 62.5% of the tube mould samples. For flavour defects, 62.5 % of the samples from both treatments were described to have excessive saltiness, and the small mould samples were more often perceived to have excessive sourness at 25%, excessive bitterness at 50%, and excessive blandness at 62.5% occurrence compared to 12.5%, 25%, and 12.5% in the tube mould samples, respectively.

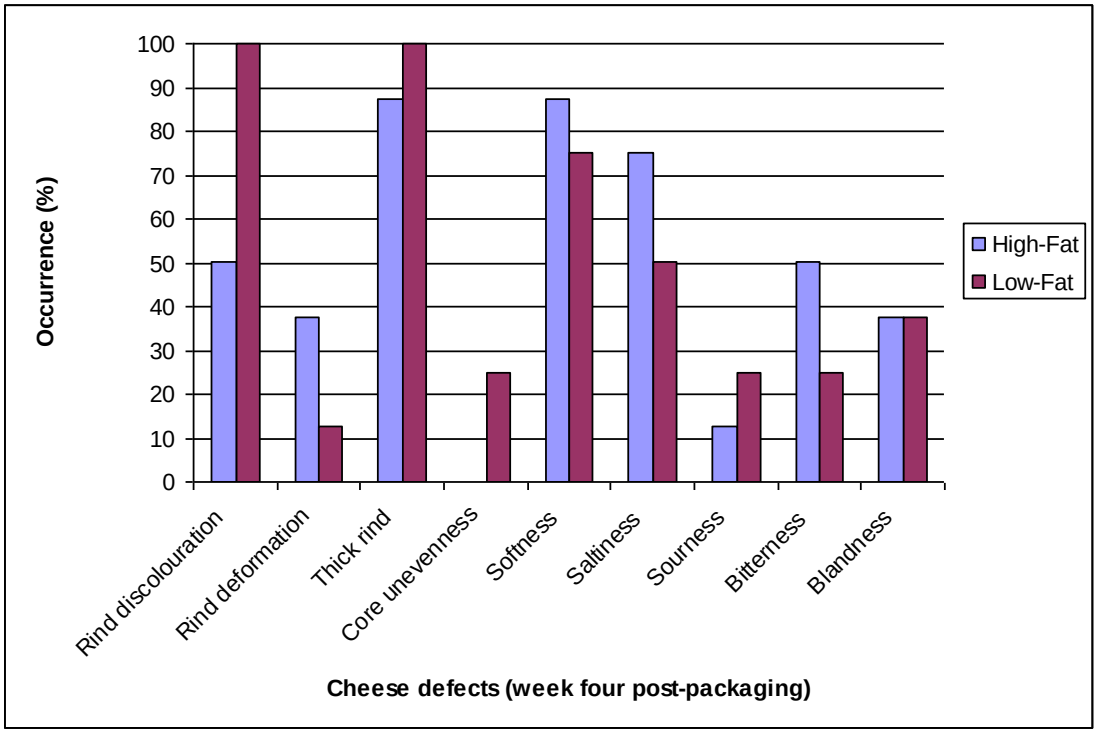


Figure 4.41: Percentage of occurrence in nine major sensory defects for high-fat (n = 8) and low-fat (n = 8) Camembert cheese at the age of four weeks. The samples were stored at 4 ± 1 ºC post-packaging.

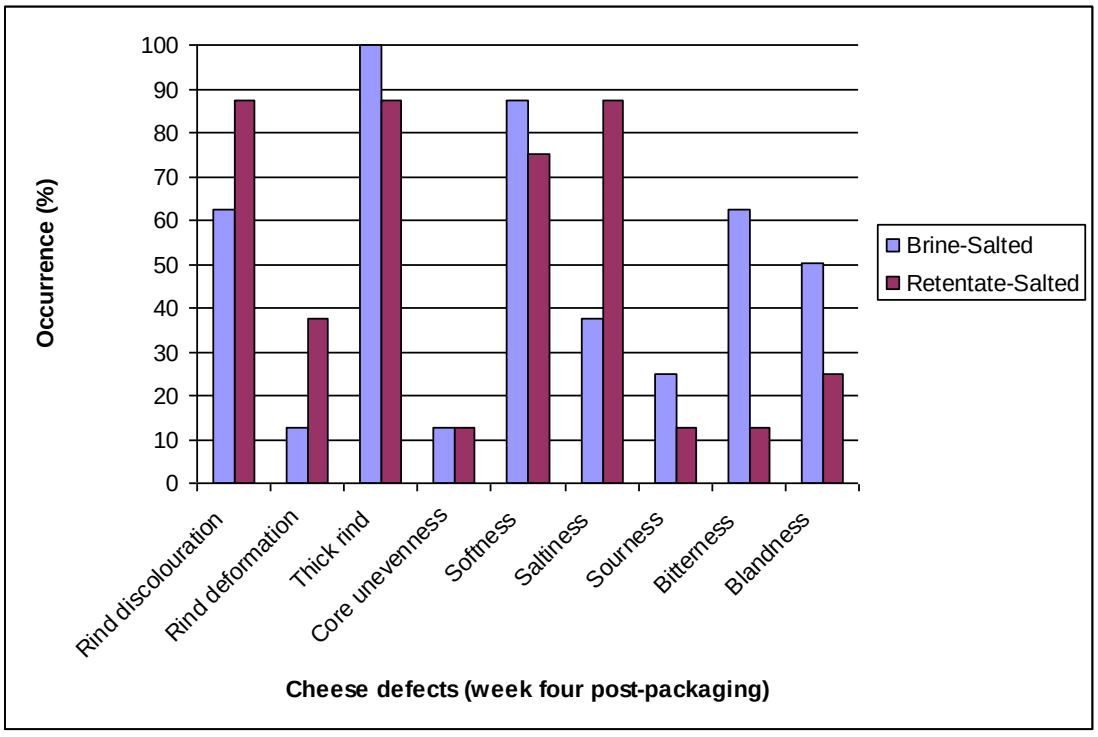


Figure 4.42: Percentage of occurrence in nine major sensory defects for brine-salted (n = 8) and retentate-salted (n = 8) Camembert cheese at the age of four weeks. The samples were stored at 4 ± 1 ºC post-packaging.

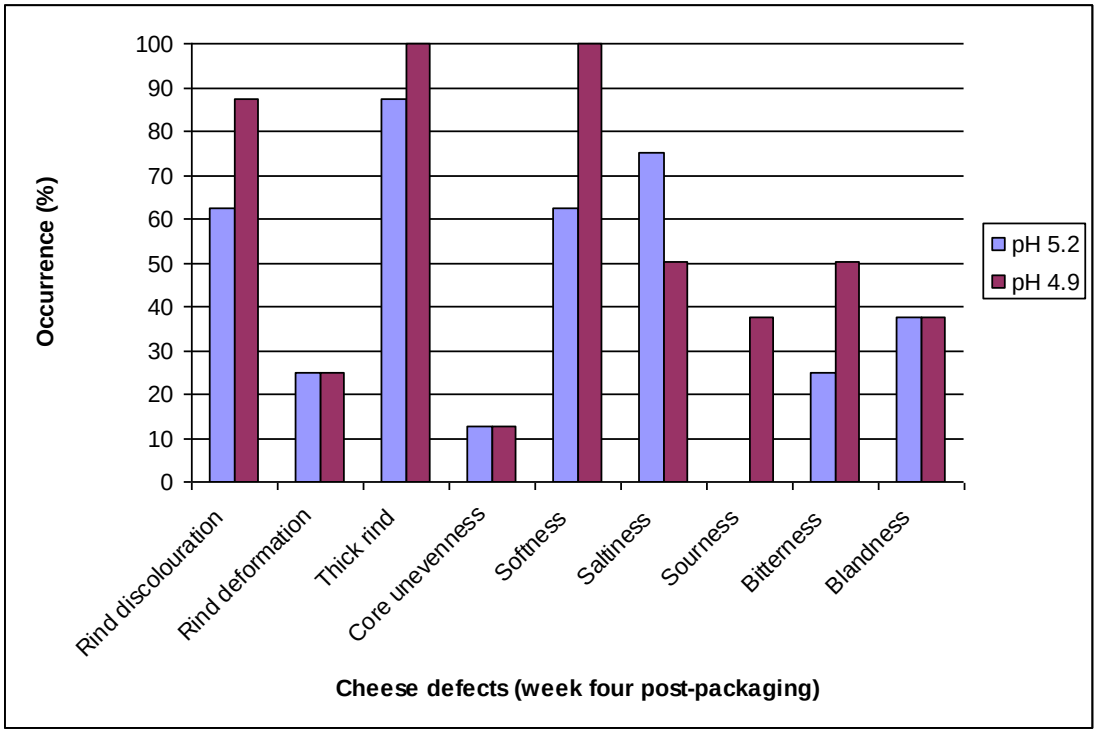


Figure 4.43: Percentage of occurrence in nine major sensory defects for Camembert cheese acidified to pH 5.2 (n = 8) and pH 4.9 (n = 8) at the age of four weeks. The samples were stored at 4 ± 1 ºC post-packaging.

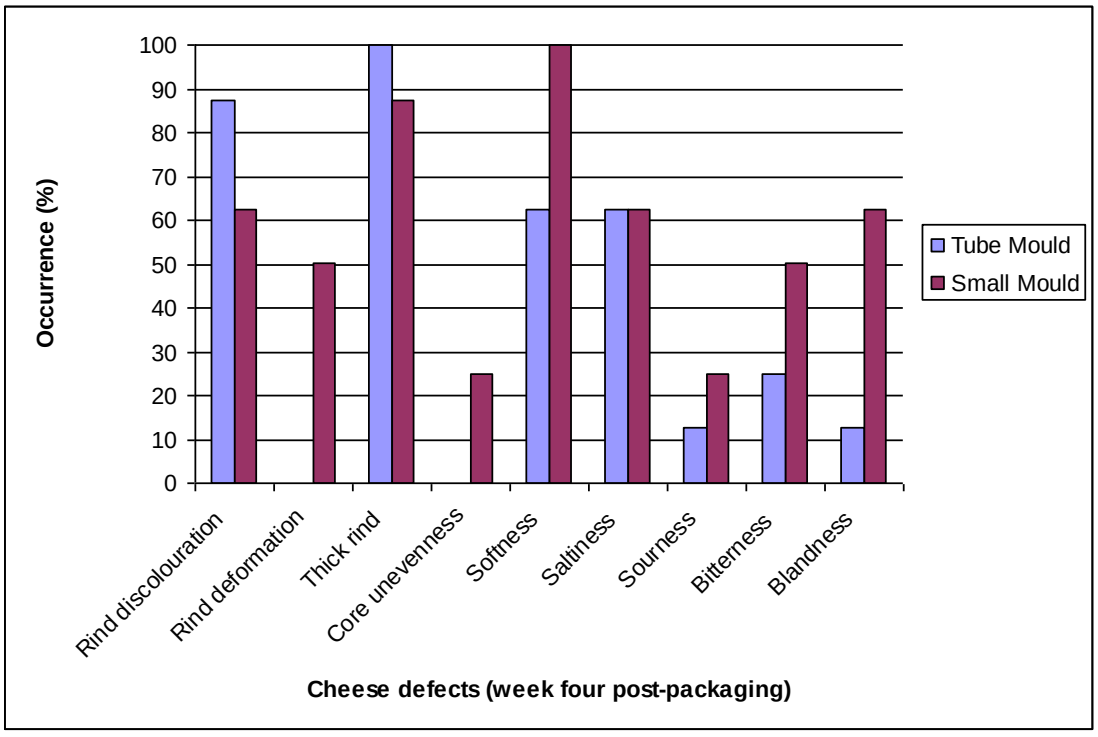


Figure 4.44: Percentage of occurrence in nine major sensory defects for Camembert cheese made using tube moulds (n = 8) and small moulds (n = 8) at the age of four weeks. The samples were stored at 4 ± 1 ºC post-packaging.

4.8. Consumer acceptance

Cheese samples were stored at 4 ± 1 ºC and after four weeks they were evaluated for consumer acceptability with consumer sensory panels. Sixteen cheese treatments inclusive of the four variables (level of fat, salting method, final acidification pH, and mould type) were evaluated by consumer sensory panellists, which consisted of four sensory sessions. Two of the four sensory sessions were done in duplicate, resulting in a total of six sessions. Each session included four different cheese samples at the age of four weeks with 21 to 43 panellists varying in each session. The panellists consisted of students, guests and staff of Massey University, Albany campus.

Table 4.6 shows the mean and standard deviations in the sensory scores of the four treatments (level of fat, salting method, final acidification pH, and mould type) in terms of appearance, odour, flavour, texture, and overall acceptance, and Table 4.7 shows the significant levels (p-values) between the treatments. The tables shows that the low-fat cheese samples were significantly more preferred in terms of appearance, texture, and overall acceptance; the brine-salted samples were significantly more preferred in appearance; the retentate-salted samples were significantly more preferred in flavour; and the tube mould samples were significantly more preferred in appearance and odour.

Figures 4.45 to 4.48 show the combined overall sensory acceptance scores of the four treatments (level of fat, salting method, final acidification pH, and mould type) and their two levels. Apart from the level of fat, no significant differences were found between the consumer panels for the two levels of each treatment. This may be due to the high variation in consumers' preferences of the Camembert cheese, resulting in a wide spread of results (Appendix 1).

The overall consumer acceptance of the sixteen cheese samples are shown in Figure 4.49, providing more information about the consumer acceptance results for the overall acceptance of each treatment. All samples with the exception of HR5.2T scored above a score of five ('neither like nor dislike'), suggesting that most samples were acceptable to the sensory panellists (Figure 4.49). The cheese samples that were

'slightly liked' (scored 6 -7) by the panellists were HB5.2S, HR4.9T, HR5.2S, LB4.9S, LB5.2T, LR4.9T and LR5.2T.

It should be noted that from the results shown in Figure 4.49, the scores for most treatments covered a wide range, with upper quartiles of consumer acceptance scores for most cheese samples lying between six and eight, and lower quartiles of scores lying between four and six. It was also observed that LR4.9T had a large number of outliers as a cluster of the data were plotted outside the quartile range, suggesting two distinctive consumer groups were present during these sensory sessions.

Table 4.6: Mean scores and standard deviations of the combined of the five sensory attributes.

Cheese treatments	n	Appearance	Odour	Flavour	Texture	Overall Acceptance
Low-fat	620	5.96 ± 1.71	5.56 ± 1.40	6.11 ± 1.68	6.08 ± 1.74	6.00 ± 1.60
High-fat	225	5.24 ± 2.17	5.76 ± 1.57	6.02 ± 1.90	5.45 ± 2.20	5.61 ± 1.83
Brine-salted	420	5.97 ± 1.75	5.67 ± 1.44	5.96 ± 1.78	5.94 ± 1.79	5.92 ± 1.64
Retentate-salted	425	5.57 ± 1.96	5.56 ± 1.46	6.21 ± 1.70	5.88 ± 1.99	5.88 ± 1.71
pH 5.2	413	5.85 ± 1.84	5.60 ± 1.48	6.10 ± 1.71	6.00 ± 1.87	5.91 ± 1.71
pH 4.9	432	5.69 ± 1.90	5.63 ± 1.41	6.07 ± 1.78	5.82 ± 1.90	5.89 ± 1.63
Tube Mould	431	5.90 ± 1.79	5.72 ± 1.45	6.09 ± 1.72	6.01 ± 1.86	5.97 ± 1.64
Small Mould	414	5.63 ± 1.94	5.50 ± 1.44	6.08 ± 1.77	5.81 ± 1.92	5.82 ± 1.71

Table 4.7: p-values ($p \leq 0.05$) of the four processing variables of the five sensory attributes.

Variables	Appearance	Odour	Flavour	Texture	Overall Acceptance
Level of Fat	0.000	0.069	0.506	0.000	0.003
Salting Method	0.002	0.253	0.035	0.616	0.719
Final Acidification pH	0.227	0.716	0.774	0.176	0.869
Mould Type	0.039	0.028	0.913	0.138	0.204

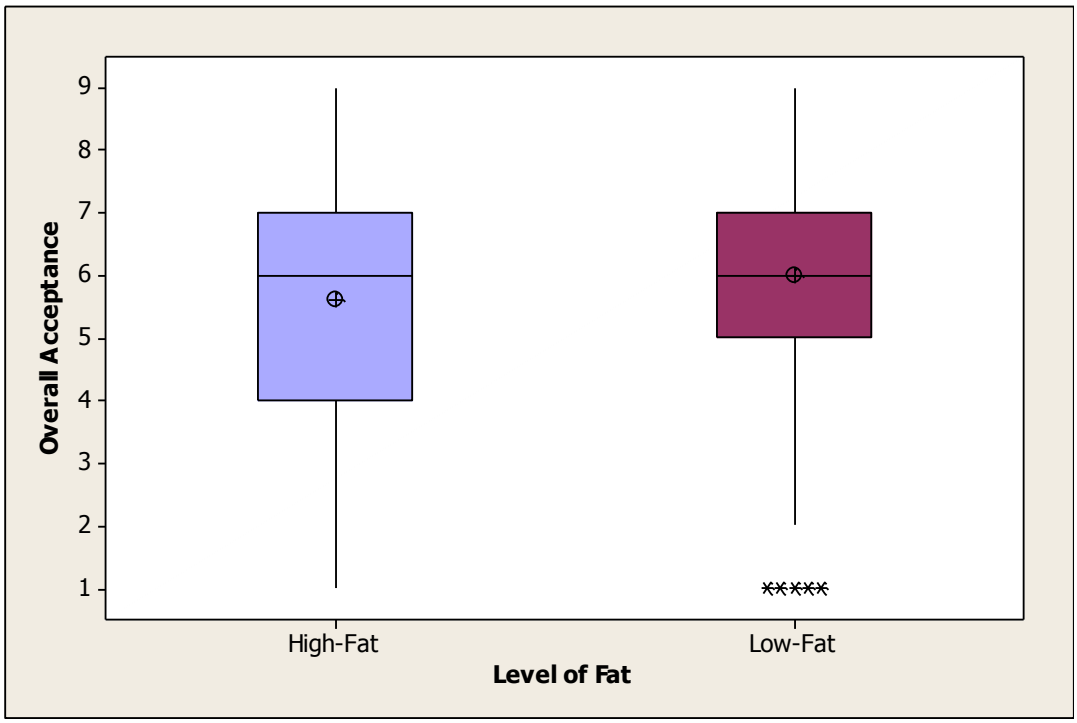


Figure 4.45: Consumer overall acceptance of high-fat (n = 225) and low-fat (n = 620) Camembert cheese at the age of four weeks. The cheese samples were stored at 4 ± 1 ºC post-packaging. ⊕ represents the mean; horizontal lines represent upper quartile, median, and lower quartile respectively from top to bottom; * represents outliers.

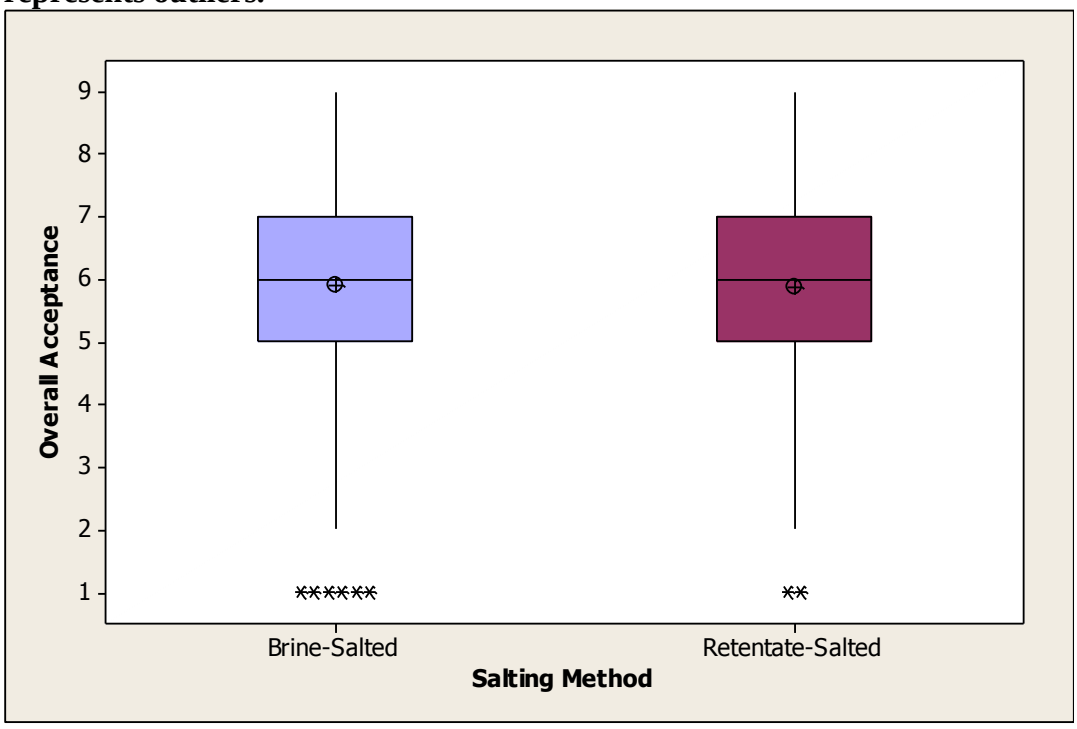


Figure 4.46: Consumer overall acceptance of brine-salted (n = 420) and retentate-salted (n = 425) Camembert cheese at the age of four weeks. The cheese samples were stored at 4 ± 1 ºC post-packaging. ⊕ represents the mean; horizontal lines represent upper quartile, median, and lower quartile respectively from top to bottom; * represents outliers.

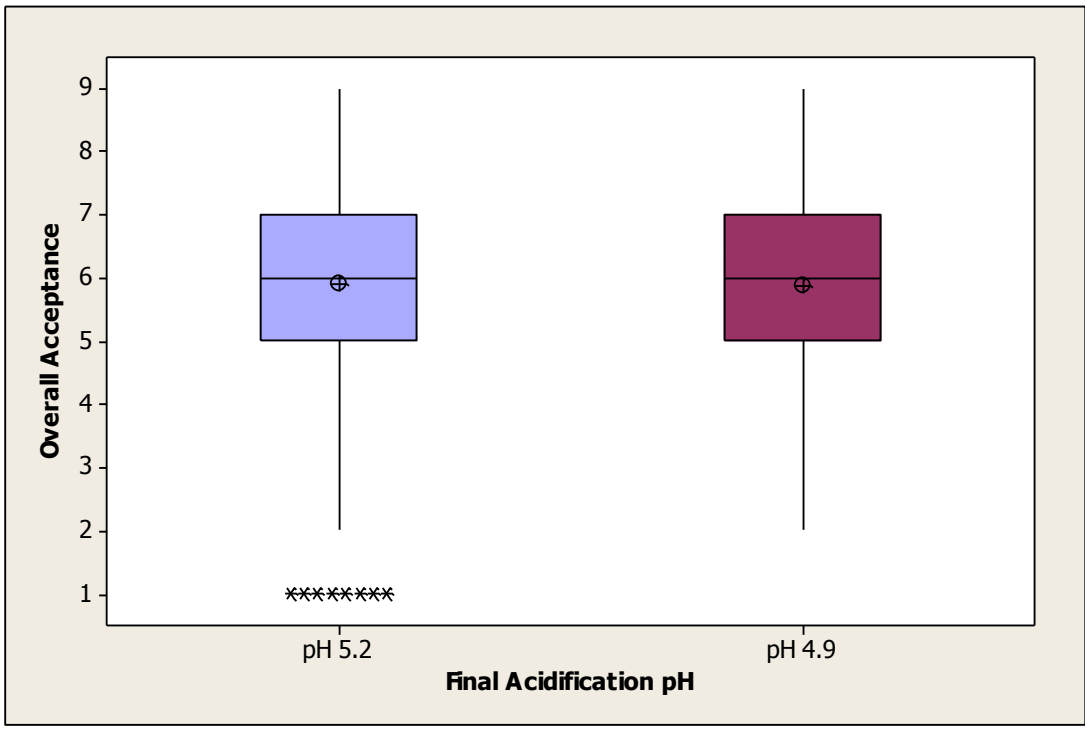


Figure 4.47: Consumer overall acceptance of Camembert cheese acidified to pH 5.2 (n = 413) and pH 4.9 (n = 432) at the age of four weeks. The cheese samples were stored at 4 ± 1 ºC post-packaging. ⊕ represents the mean; horizontal lines represent upper quartile, median, and lower quartile respectively from top to bottom; * represents outliers.

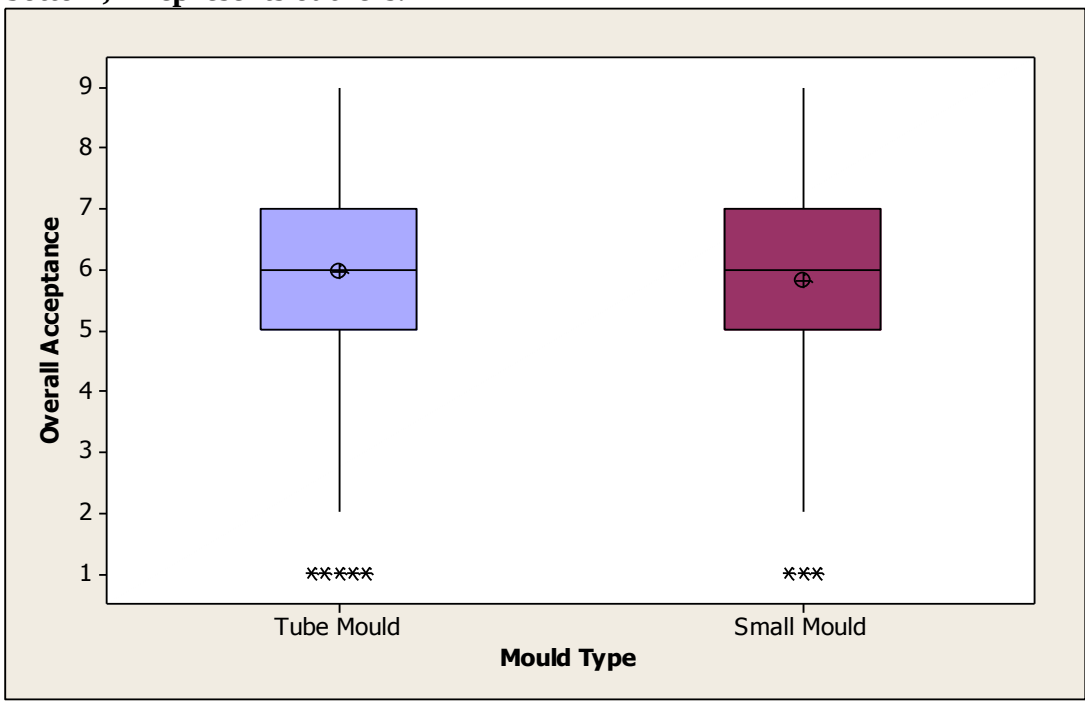


Figure 4.48: Consumer overall acceptance of Camembert cheese made using small mould (n = 414) and tube mould (n = 431) at the age of four weeks. The cheese samples were stored at 4 ± 1 ºC post-packaging. ⊕ represents the mean; horizontal lines represent upper quartile, median, and lower quartile respectively from top to bottom; * represents outliers.

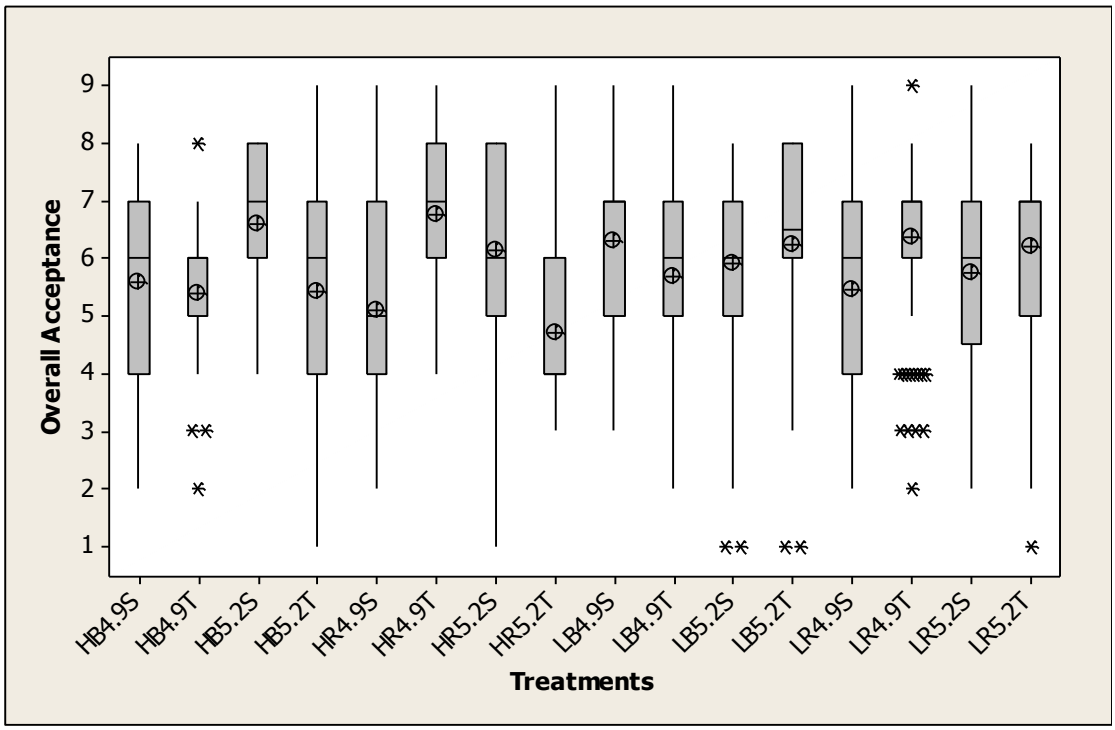


Figure 4.49: Consumer overall acceptance of sixteen treatments of Camembert cheese at the age of four weeks. The cheese samples were stored at 4 ± 1 ºC post-packaging. The sample size of consumer panellists varied between 21 and 43 panellists per treatment. ⊕ represents the mean; horizontal lines represent upper quartile, median, and lower quartile respectively from top to bottom; * represents outliers.

5. DISCUSSION

The impact of four processing variables (level of fat, salting method, final acidification pH, and mould type) on the quality and ripening characteristics of UF Camembert cheese were investigated in this study. It should be noted that factors affecting the cheese quality are not limited to the variables studied as other processing parameters play significant roles in cheese production.

5.1. Moisture retention

The moisture content and water-binding characteristics in cheese during ripening have significant impact on the rheology and texture of the cheese (Fox et al., 2000). High moisture retention by sorption of water in protein reduces firmness and contributes to a more elastic behaviour in cheese texture (Kneifel et al., 1991). During cheese ripening, dehydration of the cheese occurs as water evaporates from the cheese surface to the surrounding atmosphere (Riahi et al., 2007; Schlesser et al., 1992). Although Camembert is categorised as a type of cheese with high moisture content (Scott, 1998), the rate of dehydration during ripening is also attributed to the state of water and water-binding capacity of the protein network (Geurts et al., 1974; Kneifel et al., 1991). The incorporation of whey proteins in the cheese by UF increases the water-binding capacity, and influences the ripening of cheese as they act as inert filler within the paracasein network (Hinrichs, 2001; Harper et al., 1989; FIL/IDF, 1991). Analysis of TS reveals the changes in cheese moisture during the maturation period. Measurements of pH and proteolysis also act as useful indicators for the level of moisture retention, and the two factors are valuable in determining the quality of the cheese.

Results from this study did not show significant increase in the mean TS for the cheese treatments from the four processing variables. This can be partly attributed to the moisture loss being located mostly in the rind and outer portion of the cheese, where the cheeses were sampled without the rind during TS determination. The absence of TS increase can also be attributed to the increased water-binding capacity in cheese due to proteolysis (Kneifel et al., 1991). In a study by Schlesser et al. (1992), TS in Camembert cheese increased during the first 15 d of maturation due to

surface evaporation. The values of TS varied only slightly after 15 d, suggesting that the water-binding properties of the cheese changed as the cheese matured (Schlesser et al., 1992).

As the paracasein is hydrolysed during ripening, more water-binding sites become available from the carboxylic acid and amino groups formed (Tarakci and Tuncturk, 2008), thereby increasing water sorption (Schlesser et al., 1992). Studies on Emmentaler (Malthlouthi et al., 1981) and Gruyère (Ruegg and Blanc, 1976) cheeses have shown that water sorption increases with a higher level of proteolysis. Hence, the lack of increase in TS can also be attributed to the general increase in NPN/TN and SN/TN ratios of the cheese treatments studied, which enables better water-binding capacity due to increased proteolysis. Additionally, the depletion of lactic acid and subsequent increase in pH from the isoelectric point of paracasein can also contribute to the increase in water sorption (Ruegg and Blanc, 1976).

Effect of the level of fat

The relatively lower TS in low-fat cheese samples from week five may be due to measuring error. However, it has been indicated that increase in cheese fat content leads to the replacement of water-binding proteins by fat globules. Subsequently, moisture is lost more readily by evaporation (Kneifel et al., 1991).

Effect of the Salting Method

Results from the two salting methods did not significantly differ in the cheese TS. Retentate-salting of the cheese was expected to have significant impact on the properties of cheese, as it affects starter growth, the duration of curd formation, and the subsequent texture of the formed curd (Walstra et al., 2006). When observing the level of proteolysis between the brine-salted and retentate-salted cheese samples, NPN/TN and SN/TN ratios were higher in those samples which were brine-salted, suggesting that they had better water-binding capacity. However, it should be noted that retentate-salted cheese samples had significantly lower TS at some point (weeks 1 and 4) than those which were brine-salted during maturation. This is perhaps due to moisture entrapped within the cheese curd during coagulation of the salted retentate,

as drainage of moisture would be minimal compared to those seen in brine-salting (Walstra et al., 2006).

Effect of the final acidification pH

Published data suggest that the water-binding capacity of casein increases as the pH is increased from the isoelectric point (Schlesser et al., 1992). This may be due to the net charge of the casein becoming more negative, which allows two to three times more water sorption than at the isoelectric point (Schlesser et al., 1992; Marchesseau et al., 1997). The cheese samples that were acidified to pH 4.9 had significantly lower TS than samples acidified to pH 5.2, which suggested a higher moisture loss. It should be noted that these pH values were only used as end-points for the acidification stage during cheese manufacture. Subsequently, the cheeses were subjected to brine-salting or directly transferred to ripening, packaging, and some storage post-manufacture before the analysis were conducted. The pH levels of the cheeses were expected to considerably change during this period. Therefore, the lower TS in cheese samples acidified to pH 4.9 can be explained by a high level of proteolysis, indicated by higher NPN/TN and SN/TN ratios, which resulted in increased water-binding capacities of the proteins.

Effect of the mould type

The type of mould did not have a significant impact on cheese maturation in terms of TS content, NPN and SN indicators. Both tube and small moulds had similar dimensions, which enabled comparable surface evaporation (Riahi et al., 2007). Further, their effects on the maturation of cheese, particularly in proteolysis, are considered minor. In the cheese samples acidified to pH 5.2, relatively lower TS were shown in those samples made using small moulds. This may be explained by the increased risk of contamination by the non-starter bacteria, which become apparent only when the initial level of proteolysis is relatively low (Kousta et al., 2010).

5.2. Salt uptake

The uptake of salt is fundamentally based on the concentration gradient between the cheese moisture and the source of salting, conventionally the brine solution or dry salt crystals. The model for salt uptake is primarily based on brine-salting, but it also implies other salting methods (McSweeney, 2007). The rate of salt penetration depends on several factors, including the porosity of the cheese (Geurts et al., 1980), tortuosity of the routes of water within the cheese in bypassing obstructing particles (Walstra et al., 2006), the proportion of the total water bound (Melilli et al., 2006), the viscosity of the free water phase allowing for transport (Guo et al., 1997; Payne and Morison, 1999), and the interaction of sodium with the protein network (Payne and Morison, 1999).

Effect of the level of fat

The high-fat cheese samples were shown to have significantly lower salt uptake. Fat globules act as obstacles in the water channels within the protein network (Melilli et al., 2006). This decreases the effective diffusion coefficient by reducing the effective distance covered by the diffusing ions (Walstra et al., 2006). The effect of this seems to be milder in the high-fat samples combined with higher final acidification pH.

Effect of the salting method

The manufacturer produces two types of Camembert cheese by the conventional method and the UF method, using brine-salting and retentate-salting respectively. It is predictable that the manufacturer would have determined the salting parameters in both methods, to produce cheese with similar salt levels based on experiments in order to achieve consistency. This study applied both types of salting methods in the manufacture of UF Camembert, and surprisingly, no significant differences in salt uptake were observed. The major differences between the two salting methods are that retentate-salting affects the growth of the LAB starters and lactic acid production (Fox et al., 2004) and prolongs the acidification period up to 48 h, compared to a maximum of 24 h in brine salting. In contrast to the extensive acidification time in UF cheese due to increased buffering capacity (Walstra et al., 2006), acidification and curd formation in conventionally-made Camembert only takes about 30 min.

The behaviour of the salt gradient in retentate-salted cheese is also different to those that were brine-salted, where a salt gradient is created from the outer portion of the cheese block and diffuses inwards (Fox et al., 2000). Instead, the concentration gradient in retentate-salted cheese exists between dispersed salt crystals and the surrounding retentate. This creates patches of highly salted areas in the retentate, where clotting occurs simultaneously in this stage. The displacement rate of moisture within the retentate may be decreased as paracasein linkages form, but small drainage can occur and the salt eventually diffuses evenly within the fresh cheese (Geurts et al., 1980).

The growth of LAB starters is generally inhibited by the introduction of NaCl (Scott, 1998; Fox et al., 2000). However, it has been reported that *Lactococcus* spp. have higher NaCl tolerance than thermophilic *Streptococcus thermophilus* and *Lactobacillus* spp. such as *Lb. helveticus* and *Lb. delbrueckii* subsp. *lactis*. (McSweeney, 2007). Therefore, retentate-salting may have a profound effect on the LAB starter profile during acidification and curd formation in UF Camembert, which subsequently affects proteolysis during cheese ripening. In this study, however,

Effect of the final acidification pH

It has been indicated that the increase in pH during curd formation allows better hydration of the casein micelles due to the higher negative charges. Hence, there is a better solubilisation of proteins which facilitates the diffusion of salt (Floury et al., 2009). This probably explains the significantly higher salt uptake in cheese samples that were acidified to pH 5.2, as the rate of ion exchange was considerably higher (Walstra et al., 2006).

Effect of the mould type

The salt content in both tube and small moulds was not significantly different. The similar dimensions of the cheese samples produced using both moulds would have allowed comparable salt uptake during brining; and the amount of salt added per volume of retentate were the same in retentate-salted treatments.

5.3. Proteolysis

During the ripening of cheese, casein is broken down into smaller peptides and N compounds by a variety of enzymes (Weimer, 2007). The degradation of protein plays a significant role in determining the biochemistry, texture, and organoleptic properties of the cheese (Walstra et al., 2006). The assessment of proteolysis by chemical analysis involves estimating different N fractions in the cheese samples. These include SN which consists of non-casein proteins and peptides, extracted at the isoelectric pH of paracasein (pH 4.6); and NPN which consists mainly of medium to short peptides and amino acids, obtained by treating the cheese extracts with TCA (e.g. 12%) solutions or with 70% aqueous ethanol (Ardö, 1999). The determination of N in these fractions can be carried out using the Kjeldahl method (Ardö, 1999), several forms of gel electrophoresis (Yetişmeyen et al., 2006; Hayaloglu et al., 2008), high performance liquid chromatography (Piraino et al., 2008), and gas chromatography (Hayaloglu et al., 2008).

In this study, the NPN/TN and SN/TN ratios were used to estimate the level of proteolysis in cheese. All results showed increasing trends in NPN/TN and SN/TN ratios as the cheeses matured. It is apparent that the levels of proteolysis increases with the age of cheese. It can be observed that the differences in NPN/TN ratios between treatments of each processing variable were consistently larger than those observed in SN/TN, where significant differences in SN/TN between treatments were only present at a certain period during maturation. It may be reasonable to mention that SN produced mostly by the surface mould flora, *Penicillium camemberti*, were more readily degraded by bacterial enzymes, which produce smaller NPN molecules (Walstra et al., 2006).

Effect of the level of fat

It is generally acknowledged that having higher fat content increases the softness of cheese due to disruption of the casein matrix (Gunasekaran and Ak, 2003). The direct effects of fat content on the level of proteolysis in UF Camembert have not been reported much. However, studies on Cheddar cheese indicated that the fat content of cheese did not influence proteolysis (Kilcawley et al., 2007; Fenelon et al., 1999). In contrast, results in this study showed that the high-fat cheese samples had

significantly higher NPN/TN and SN/TN (week 7) ratios, suggesting higher activities of proteolysis. This may be a result of reduced paracasein linkages due to increased fat content, allowing proteolysis to occur more readily (Schlesser et al., 1992).

Effect of the salting method

The two salting methods were presumed to have profound effects on the level of proteolysis, as discussed earlier. Retentate-salted cheese samples experienced a significantly longer acidification period during curd formation in order to reach desired acidification (21 ± 2 h for pH 5.2 and 45 ± 2 h for pH 4.9 compared to 7 ± 2 h and 20 ± 2 h respectively in brine-salted treatments). This can be attributed to the presence of salt in the clotting retentate, which effectively slows the growth of LAB starters. As a result, lactic acid production was significantly hindered by the presence of salt in addition to the already high buffering capacity of UF retentate (Walstra et al., 2006). The NPN/TN and SN/TN (week 5) ratios were significantly higher in the brine-salted cheese samples. This may be explained by the relatively better growth conditions for the LAB starters in the clotting retentate. In contrast, starter growth in the salted retentate could have been suppressed, thus reducing the production of proteolytic enzymes.

Effect of the final acidification pH

The interaction between clotting enzymes and caseins is important in cheese manufacture. Clotting enzymes specifically cleave the Phe_{105}-Met_{106} bond of κ-casein during the clotting of the milk (Walstra et al., 2006; Larsson and Andren, 1997). At the same time, some of the clotting enzymes become adsorbed onto paracasein, becoming part of the cheese (Walstra et al., 2006). During cheese ripening, the clotting enzymes retained promote proteolysis in addition to the indigenous milk enzymes and enzymes from the starter cultures and secondary flora (Larsson and Andren, 1997; Trujillo et al., 2000).

Cheese samples acidified to pH 4.9 had higher NPN/TN and SN/TN (week 7) ratios than those acidified to pH 5.2. The casein was readily coagulated at about pH 6.3 with the presence of Fromase, an aspartyl protease from *Rhizomucor miehei* commonly used as a chymosin substitute (Preetha and Boopathy, 1997). Further acidification

occurs when LAB starters deplete the available lactose and produce higher levels of lactic acid (Fox et al., 2000). The time required for the cheese pH to achieve 4.9 is also considerably longer compared to pH 5.2. The increased proteolysis in cheese samples acidified to 4.9 can be attributed to a combination of increased retention of the clotting enzyme and prolonged acidification time. When the clotting enzyme is mixed with κ-casein, the highly negatively charged casein macropeptide (Met_{106}-Val_{169}) is cleaved (Walstra et al., 2006). This forms para- κ-casein with an isoelectric pH >7, hence it is positively charged at pH below 7 (Larsson and Andren, 1997). The acid aspartyl protease from *R. miehei*, Fromase, and other common clotting enzymes such as chymosin have an acidic isoelectric pH (~4.6) (Larsson and Andren, 1997; Preetha and Boopathy, 1997), and are negatively charged at pH above 4.6. Therefore, there is a higher affinity of the protease on the paracasein at lower pH, which increases its retention in cheese and subsequently encourages proteolysis during cheese ripening (Preetha and Boopathy, 1997).

The prolonged acidification time required to achieve pH 4.9 in the cheese would have allowed extensive growth of LAB starters and the production of cell envelope proteinases (Walstra et al., 2006). Proliferation of the starter cultures may get to a point where growth ceases and autolysis occurs, enabling the liberation of intracellular peptidases (Walstra et al., 2006). Additionally, the initial salt content in cheese samples acidified to pH 4.9 was lower. Therefore, the inhibitory effect of salt on the many of the cheese non-starter microflora decreases and their proteolytic action may be subsequently higher (McSweeney, 2007).

Effect of the mould type.

The effects of 'mould type' on proteolysis during cheese maturation would have been thought to be either none or minimal, as the types of moulds had little to do with the biochemistry of the cheese. No apparent differences were shown in the NPN/TN and SN/TN ratios between the two mould types, as expected.

5.4. Textural and organoleptic properties

The ripening of cheese contributes to the final texture due to the chemical and structural changes over time, which is primarily related to the hydrolysis of proteins. The paracasein network is degraded over time, resulting in a softer and more deformable cheese (Gunasekaran and Ak, 2003). It has been reported that proteolysis generally occurs in two stages, where about 20% of the casein network is hydrolysed in the initial stage, and more gradual breakdown takes place in the second stage (Rogers et al., 2009). Degradation of the paracasein in Camembert is facilitated mainly by the surface mould, such as *Geotrichum* and *P. camemberti* (Rash and Kosikowski, 1982). Extracellular proteases produced by these organisms hydrolyse proteins and lipids to peptides, amino acids, and fatty acids, which cause inward migration of softening in the cheese body (Walstra et al., 2006). In this study, softening in the UF Camembert (<7 weeks of shelf-life) occurred too rapidly compared to Camembert produced conventionally by the manufacturer (9 weeks of shelf-life), which effectively reduced its shelf-life and ability to meet consumer expectations. Other defective attributes such as rind discolouration, rind deformation, and thick rind also became apparent as the samples matured.

5.4.1. Instrumental analysis

Uniaxial compression tests were conducted to measure the firmness of the UF Camembert samples by measuring the force required to achieve 50% deformation during the maturation period. Results found no significant differences between treatments in each processing variable. Similar testing parameters were used in other studies on the texture of Camembert (Antoniou et al., 2000; Schlesser et al., 1992), with variations in the extent of height deformation. The major difference which differentiated the UF Camembert from the other cheeses such as Emmentaler or Cheddar was the rapid development of softness during maturation. Results showed that the force required to compress test samples to 50% height deformation decreased over time and then started to stabilise at week four. The cheese samples softened to an extent where the inner body of the cheese had obtained a continuous, flowing core texture. This texture was generally observed at weeks five and six for all cheese treatments. This posed an issue during the measurement of cheese firmness, as the

sample pieces could no longer support their own weight after the sample had attained ambient temperature.

Antoniou et al. (2000) measured Camembert cheese firmness at 10% height deformation, and Schlesser et al. (1992) at 20% height deformation, whereas other studies have measured firmness of other cheese types at a much higher height deformation (80%) (Gaya et al., 1990; Vinas et al., 2007). In this study, 50% height deformation was used, as neither the low nor high deformation heights adequately measured the cheese firmness. The thick rind of UF Camembert prevented accurate measurements of firmness on the cheese body at 10 and 20 % height deformations. At 80% height deformation, the measurements obtained only represented the rind compaction. Hence, measurements of 50% height deformation were used as they adequately represented firmness of the cheese body until a flowing core texture was developed, where accuracy was compromised. Although the purpose of instrumental analysis was to mimic the behaviour of human panels, changes should be made depending on the prevailing conditions. It is therefore suggested that instrumental measurements of cheese with such extensive softness should be conducted at a cooler temperature, for example at 15 ºC. This would allow more accurate comparison between samples of different treatments and eliminate variations caused by inconsistent cheese texture at high temperatures.

Effect of the level of fat

It has been reported that lower fat content contributes to a firmer texture due to a denser protein network (McSweeney, 2007). In Cheddar cheese, it has been shown that low-fat Cheddar exhibited firmer texture when compared to full-fat Cheddar throughout maturation (Rogers et al., 2009). This behaviour is generally applicable to most cheese varieties, as more casein is replaced by fat globules, as less paracasein linkages are present. Even though fat globules have demonstrated the ability to be adsorbed in κ-casein and establish hydrophobic interactions (Su and Everett, 2003), they would still replace the stronger paracasein linkages. During cheese ripening, the reduced amount of paracasein is further degraded, resulting in a softer cheese texture. In the case of UF Camembert, the whey proteins incorporated in the cheese act

similarly to fat globules, which further reduces the amount of paracasein linkages (Hinrichs, 2001; Harper et al., 1989).

Effect of the salting method

The salting methods did not show significant differences in terms of firmness. There is a lack of studies which uses retentate-salting in the manufacture of UF cheeses. This is not surprising as salting is known to have inhibitory effects on the starter growth during acidification (Fox et al., 2000). The combined mean of cheese produced using the two salting methods were not significantly different. However, results on the N fractions from this study showed that brine-salted cheese exhibited higher levels of proteolysis, and should decrease firmness of the cheese. This, however did not occur as the number of replicates may not be sufficient to produce significant texture results.

Effect of the final acidification pH

Although textural results did not indicate any significant differences between the final acidification pH, proteolysis in cheese samples which were acidified to pH 4.9 was significantly higher at weeks 5 and 7. This in theory indicates that a change in texture is probable, and however not exhibited in the results due to high variations.

Effect of the mould type

In terms of the mould type, no significant difference was observed between the mould types. The initial hypothesis was that cheese formed in separate small moulds rather than long tube moulds would allow more whey drainage during acidification of the retentate, thereby attaining a firmer cheese texture. However, this was not the case and TS between the two mould types did not differ significantly.

5.4.2. Cheese grading

Expert grading systems have been used in the industry by cheese manufacturers to ensure the quality of their products are consistent and 'fit for purpose' (Walstra et al., 2006). Generally, most grading systems assess products based on two elements, including a descriptive analysis to determine the sensory attribute profile of the product; and whether the attribute profile meets the final product specifications

(Dodds et al., 2002). Cheese is often graded periodically until the end of its shelf-life specified by the manufacturer, as this reflects the quality of the cheese on the market and in the hands of the consumers. This is of particular importance in the types of cheese which require extensive ripening as their sensory properties can change drastically.

Cheese grading results at week four of the maturation period was used to investigate the quality of UF Camembert when it is likely to be consumed. Initially, the grading system used by the manufacturer was based on an exhaustive list of defective attributes, where scores were deducted when a defect was present in a cheese. For the purpose of this study, nine of the significant defects were presented as percentages of occurrences of all graded samples. The rate at which UF Camembert cheeses deteriorated appeared to have occurred fairly rapidly with the emergence of undesirable characteristics such as rind discolouration, rind deformation, core unevenness, excessive softness, and other flavour defects; hence effectively reducing the shelf-life of the product. This section attempts to discuss various defects detected in the cheese and associate them with effects of the processing variables used.

Rind discolouration

The defect of rind discolouration includes browning and bolding of the surface mould (Figure 5.1). A study with respect to mould browning in Camembert has been reported (Carreira et al., 2002). It was reported that *P. camemberti* was able to produce brown pigments in the presence of tyrosine and Mn^{2+}, and was more intense in the more proteolytic strain. Additionally, the inoculation of a competing microflora, *Yarrowia lipolytica*, showed less browning. This suggests that browning is dependent on the proteolytic activity, the growth rate and density of the *P. camemberti* (Carreira et al., 2002).

Rind discolouration occurred more in low-fat cheese samples, retentate-salted samples, and tube mould samples than their alternatives, as NPN/TN ratios suggested otherwise. The higher occurrence in cheese samples acidified to pH 4.9 was in agreement with proteolysis results. Rind discolouration was observed with >50% occurrence in all treatments. The high occurrence of this defect indicates that it is

generally common among UF cheeses and an attempt to explain difference between the treatments may be difficult. The development of browning gives the consumers an unclean impression, and discolouration of the cheese affects the uniformity of the white mould.

Figure 5.1: The rind discolouration defect, shown by browning of the mould, with some discolouration on the edges.

Rind deformation

Rind deformation includes unevenness, misshaping, and sometimes peeling of the rind (Figure 5.2). This may be attributed to the presence of calcium phosphate deposition at the surface of the cheese (Hannon et al., 2009). Large quantities of calcium salts are released into the retentate after UF, and they assist in the linkages of paracasein during curd formation. When the proteolysis of casein occurs, the calcium ion precipitates onto the surface of the cheese as calcium phosphate due to higher pH (Fox et al., 2000). This acts as a barrier and significantly reduces the rate of diffusion for various substrates required for surface mould growth (Hannon et al., 2009).

Rind deformation was shown to be more apparent in high-fat cheese samples, which is in agreement with the corresponding proteolysis results. Retentate-salted samples and small mould samples also had higher occurrence of rind deformation, but they were not correlated with proteolysis results. The small mould cheese samples were the only treatment with 50% occurrence with this defect, the rest had <50% occurrence. Rind deformation is a major concern in the appearance of the cheese, as unevenness

and peeling of the rind give consumers the impression that the cheese is not in optimal condition for consumption.

Figure 5.2: The rind deformation defect, shown by unevenness and concaving of the rind.

Thick rind

Thick rind is a defect associated with the extensive thickness and height of the surface mould mycelium, *P. camemberti*. In UF Camembert, the thickness of the rind was higher than the thickness observed in Camembert of the same age manufactured conventionally (Figure 5.3). Thick rind was present in all treatments with >80% occurrence. This may be attributed to the higher growth rate of the surface mould in UF Camembert. This contributes to a hard papery chewing sensation as described by the judges during cheese grading which sometimes associated with bitterness.

Figure 5.3: The thick rind defect, shown in the UF Camembert cheese (left) comparing to the Camembert made conventionally (right).

Core unevenness

The growth of the surface mould flora produces extracellular proteases which hydrolyse proteins and lipids into molecules with lower molecular weight. These enzymes soften the cheese inwards from the outer portion (Walstra et al., 2006). As the structure of the protein network in UF Camembert is already weak, the degraded portion of the cheese becomes apparent. This was shown by the flowing texture of the cheese paste in the outer portion, and a core with a texture which remained relatively firm or sometimes chalky (Figure 5.4).

Core unevenness is often observed in young UF Camembert during the first few weeks of its shelf-life, and gradually disappears over time as the entire cheese paste obtains a flowing texture. Results showed that retentate-salted cheese samples and small mould samples had higher occurrences of core unevenness than their alternative treatments. Presumably at week four of the maturation period, most cheese samples would have obtained considerable softness throughout the cheese paste, shown by <30% occurrence in all treatments. However, samples from retentate-salting and small mould seemed to have resisted such textural changes in the core at this age, suggesting that the level of proteolysis had not attained full potential within the centre of these cheeses.

Figure 5.4: The core unevenness defect, shown by the overripened soft and flowing texture in the outer portion and firm (still chalky) texture in the inner portion of the cheese paste.

Softness

The softness of the cheese results from a weak casein network structure. The compactness of the casein network and the amount of casein linkages present play a significant role (McSweeney, 2007). The compactness of the casein network is affected by the amount of casein compared with levels of fat, moisture, and other filler molecules such as whey. This influences the amount of paracasein linkages formed and therefore the overall firmness of the cheese (Walstra et al., 2006).

Secondary factors include the retention of calcium levels, which is involved in the formation of paracasein linkages, by increasing the pH at whey drainage (McSweeney, 2007). The levels of clotting enzymes retained, proteases from lactic starters and secondary flora, and milk proteinases also influence the degree of breakdown in paracasein linkages during cheese ripening (Larsson and Andren, 1997; Grappin et al., 1985).

In the UF Camembert cheese produced, the incorporation of whey proteins and the higher levels of proteolytic enzymes retained due to lack of the whey drainage step is likely to be the main factors contributing to the initial weak casein network and rapid protein degradation during maturation. Although the amount of retained whey proteins and proteolytic enzymes in the fresh cheese had not been analysed in this study, the rapid development of overripening characteristics and softness appeared to support this assumption. Softness in the cheese at week four was shown to be prevalent in all cheese treatments (>50% occurrence). Extreme cases of softening results in a flowing texture of the cheese paste (Figure 5.5).

Occurrence of softness in cheese samples of high-fat and brine-salted treatments were slightly higher than their alternatives. This corresponds with their relatively higher NPN/TN and SN/TN ratios, indicating a higher level of proteolysis. Occurrence of softness in cheese samples acidified to pH 4.9 was much higher than those acidified to pH 5.2, which is in agreement with high NPN/TN and SN/TN ratios. The association of the final pH during the acidification of UF Camembert with the retention of calcium and clotting enzymes discussed earlier may contribute to such softness (McSweeney, 2007). Cheese samples made using small moulds also had higher occurrence in softness. This was also observed in the uniaxial compression test where small mould samples had significantly softer texture.

Figure 5.5: Results of softening at a mild degree (left), and over softening (right) with the development of a flowing texture of the cheese paste.

Saltiness

This is a defect where excessive saltiness is perceived in the cheese. Although published data suggest that the concentration of salt is directly related to the degree of saltiness (McSweeney, 2007), some results obtained from cheese grading suggested otherwise. It was observed that the initial salt content was significantly high in the low-fat cheese samples. However, the saltiness defect perceived in the high-fat samples had higher occurrence than that in the low-fat samples.

The retentate-salted cheese samples had higher occurrence of saltiness than those which were brine-salted, although the initial salt content between the two treatments were not significant. It is possible that the distribution of salt can influence the overall saltiness. The brine-salted cheeses had initially higher concentrations of salt in the outer portion of the cheese body, which gradually diffused inwards (Melilli et al., 2006; Walstra et al., 2006). Meanwhile, retentate-salted cheese had initially relatively homogeneous distribution of salt. It can therefore be stated that the inner portion of the brine-salted cheeses would be less salty due to a lower salt concentration, depending on the rate of salt diffusion (Fox et al., 2004); and the perception of saltiness in the relatively salt-concentrated outer portion of the cheese can be influenced by other flavours from the surface flora (McSweeney, 2007). In comparison to the more homogenous distribution of salt in retentate-salted cheese, the brine-salted cheese may overall be perceived less salty.

The occurrence of saltiness in cheese samples acidified to pH 5.2 was higher than those acidified to pH 4.9. This is in agreement with the significantly higher initial salt content in pH 5.2 cheese samples. Furthermore, the occurrence of saltiness was equal in both mould types, which is also in agreement with their similar levels of initial salt content, suggesting that the type of mould does not influence saltiness.

Sourness

The presence of sourness in cheese may be influenced by the presence of lactate (Weimer, 2007; Fox et al., 2004). Depending on the rate of lactate metabolism which is affected by various factors, the residual lactate and the perceived sourness may reveal the age of the cheese.

The occurrence of sourness in the UF Camembert produced were generally low (<30%), with the exception of retentate-salted samples, as it is expected that the amount of residual lactate in the cheese at four weeks of maturation would be relatively low. Compared to their alternatives, the low-fat cheese samples had relatively higher occurrences in sourness. This is in agreement with the corresponding NPN/TN and SN/TN ratios, suggesting that lower levels of proteolysis in the low-fat cheese exhibited slower rate of lactic acid metabolism (Grappin et al., 1985). The higher occurrences of sourness in the brine-salted cheese may be explained by the better growing environment for LAB starters, compared to the salty environment during curd formation in retentate-salted cheese. Although the salt levels were not significantly different between the salting methods, the process and environments created by the two methods differ. The cheese samples acidified to pH 4.9 had higher occurrences of sourness, which could be attributed to more extensive acid fermentation to achieve the lower pH (Fox et al., 2004). Furthermore, there were higher occurrences of sourness in the small mould cheese samples, which were not expected. However, the differences were small and the occurrence of the defect in both mould types were below 30%.

Bitterness

Bitterness is a serious defect in cheese which results from the excessive accumulation of hydrophobic peptides derived from caseins (McSweeney, 2007; Engel et al., 2001). The bitter peptides usually have a molecular mass of < 6 kDa and a mean hydrophobicity of > 1400 cal per residue. The development of bitterness in cheese may be caused by the undesirable patterns of proteolysis which causes the excessive production of bitter peptides, often by the clotting enzymes (Lemieux and Simard, 1991); or insufficient peptidase activity to degrade hydrophobic peptides into free amino acids (McSweeney, 2007; Broadbent et al., 1998).

Generally, all samples except brine-salted cheese had ≤50% occurrence of bitterness. Published data indicate that low salt level leads to low ionic strength that weakens hydrophobic interactions between the caseins (Pastorino et al., 2003). This facilitates the action of clotting enzymes on hydrophobic regions of the caseins, which results in

the excessive production of hydrophobic peptides (Broadbent and Steele, 2007; Broadbent et al., 1998; McSweeney, 2007). The occurrence of bitterness was higher in the high-fat cheese samples, as well as those acidified to pH 4.9. This is in agreement with the report of McSweeney (2007) as both treatments had significantly lower initial salt content. The relatively higher occurrence of bitterness in brine-salted samples could be associated with the distribution of salt, as lower salt concentration in the centre could have impacted on the levels of bitter peptides produced. The small mould cheese samples had higher occurrences in bitterness. The cause of this defect was unclear as it was unlikely that the mould type affected the proteolytic activities of clotting enzymes (Lemieux and Simard, 1991), the action of peptidases (Broadbent et al., 1998), and the salt content (McSweeney, 2007), which are the primary factors that influence bitter peptide production.

Blandness

Blandness is due to the lack of typical Camembert flavours and aromas which causes the cheese to be uninteresting and flat in taste (Weimer, 2007). Flavour compounds are generated from the action of enzymes from clotting agents, milk, the starter and non-starter bacteria, as well as non-enzymatic conversions. The three main pathways of flavour formation include glycolysis, lipolysis, and proteolysis. The main sources of enzymes involved in these pathways are the starter cultures, such as *Lactococcus lactis*, *Lactobacillus* spp., *Streptococcus thermophilus*, *Leuconostoc* spp.; as well as secondary cultures used in surface-ripened cheese including Camembert and Brie, such as *Penicillium camemberti* and *Geotrichum candidum* (Smit et al., 2005).

The fermentation of lactose mainly produces lactate by LAB, but a portion of the intermediate pyruvate can be converted to various flavour compounds such as diacetyl, acetoin, acetaldehyde, and acetic acid (Walstra et al., 2006). Free fatty acids produced from lipolysis act as precursors of flavour compounds such as methylketones, secondary alcohols, esters and lactones. These are mainly produced by additional cultures such as moulds in surface-ripened cheeses as they have high activities in fat conversion (Smit et al., 2005). A secondary alcohol, 1-octen-3-ol for example, has a mushroom type odour with a perception threshold of 0.01 mg per kg and is a key aromatic compound in Camembert cheese (Weimer, 2007). The

degradation of casein yields small peptides and free amino acids, of which are further converted to various alcohols, aldehydes, acids, esters and sulphur compounds.

Table 5.1: Examples of important flavour components in Camembert cheese (Fox et al., 2004).

Type Metabolism	Flavour Components
Amino acid	3-Methylbutyrate
	3-Methylbutanal
	Methional
	Methanethiol
	Dimethylsulphide
	Benzaldehyde
	Phenylacetaldehyde
Sugar	2,3-Butanedione
Fat	1-Octen-3-ol
	Butyric acid
	1-Octen-3-one
	2-Undecalactone
	γ-Decalactone
Combined pathways	Phenylethyl acetate

Table 5.1 shows examples of important flavour components in Camembert cheese. Blandness is associated with insufficient formation of such typical flavour compounds in the cheese, which is a concern in the UF Camembert. Characterisation of the flavour and volatile compounds in the UF Camembert has not been conducted in this study to investigate the associated metabolic pathways. However, it is speculated that as the UF Camembert has a short shelf-life due to the rapid breakdown of its weak protein structure, proper flavour development has yet to reach its fullest potential.

It was assumed that higher fat levels and longer acidification times (lower pH) would allow the production of more flavour compounds, due to the availability of more substrate for lipolysis and higher levels of LAB enzymes, respectively. The higher proteolysis in brine-salted samples is also expected to have higher levels of flavour formation. However, the occurrence of blandness for these treatments indicated otherwise. This suggests the complexity in flavour development of cheese is rather high, and can not be sufficiently manipulated by altering a few basic manufacturing parameters. It has been suggested that the profile of starter cultures and strains of mould flora used can significantly influence the flavour development in Camembert

cheese (Smit et al., 2005; Boutrou et al., 2006). This is mainly associated with the production of peptidases, which facilitate the conversion of peptides into free amino acids and various flavour compounds (Smit et al., 2005; Weimer, 2007).

5.5. Consumer acceptance

Consumer perception of a cheese consists of several factors, including appearance, odour, flavour, and texture (Marchesseau et al., 1997). Panellists in this study rated the cheese samples according to appearance, odour, flavour, texture, and overall acceptance on a nine-point scale ranging from 'extremely dislike' to 'extremely like'. It was observed that the scores were not normally distributed, suggesting that there is a wide variation in preference. Although the comments from the sensory evaluation were not analysed, a large portion of the panellists defined the cheese samples as "too soft" or "too runny". Most panellists in this group generally do not consume Camembert regularly and are accustomed to cheeses with the Cheddar-type texture. A small group of panellists defined the cheese samples to have good texture but "lacked Camembert flavour". This group of panellists were generally knowledgeable in specialty cheeses. They had a preference for soft and strong-flavoured Camembert, whereby blandness of the UF cheeses became an issue. Generally, all cheese samples were acceptable as their mean overall acceptance score were above 'neither like nor dislike', with one exception, the HB5.2T.

Appearance

The appearance was significantly different between samples produced with different levels of fat, salting method, and mould type; with preference for the low-fat, brine-salted, and tube mould samples. During sensory evaluation, the cheese samples were cut into wedges; appearance of the cheese generally involved examining the colour of the rind, shape of the cheese, as well as the colour and texture of the cheese paste, as revealed by the cut.

The high-fat cheese samples were less preferred, possibly due to more apparent rind deformation and softness described in cheese grading. A characteristic in the overripened cheese is the flowing texture of the cheese paste, often described as 'too runny'. At serving temperature (18 ± 2 ^{o}C), cheese samples with a flowing cheese

paste tended to collapse as they could no longer support their shape, which was undesirable. The retentate-salted cheese samples were less preferred, possibly owing to higher occurrence in rind discolouration and deformation according to cheese grading. Although the dimensions of the small mould cheese samples were similar to the tube mould samples, they were not exactly identical. Furthermore, the risk of contamination in small mould cheeses was higher due to a higher exposed surface area during acidification and curd formation, which could have contributed to the low average sensory scores.

Odour and flavour

The UF Camembert cheeses were generally described as lacking odour and flavour, especially the typical mushroom aroma in Camembert described by the judges from cheese grading. Although the level of proteolysis in UF Camembert seemed to be high, due to the combination of an initially weak protein network (Hinrichs, 2001) and increased enzyme retention during manufacture, specific metabolic pathways are required to produce the right volatile and flavour components (Smit et al., 2005). The odour of the cheese samples was found to be significantly different between the mould types. This was surprising as the type of mould was not expected to play a significant role in the formation of flavour compounds due to the only variable was surface area. The salting method had a significant impact on the panellists' perception of the flavour of the cheese. The high occurrence of saltiness in retentate-salted samples seemed to be relatively less desirable for the consumers, where excessive saltiness masks the complexity of flavours leading to an undesirable cheese.

Texture

The level of fat significantly influenced the consumers' perception in the texture of the cheese. Higher fat content contributes to a weaker protein structure, and hence a softer cheese, whereas in extreme cases, it is undesirable to consumers who prefer firmer cheeses. This corresponded to the higher occurrence of softness in the high-fat cheese samples, and higher NPN/TN ratios. The texture of high-fat samples were generally described as "too soft" or "too runny", which also resulted in the collapsing of the 'cut sample' during sensory evaluation.

Overall acceptance

In terms of overall acceptance of the various UF Camembert treatments, the low-fat cheese samples were significantly more preferred than the high-fat samples. Although two of the high-fat treatments (HR4.9T and HB5.2S), grouped by cluster analysis, had relatively high scores, the quartile ranges of the other high-fat treatments were rather broad. In the low-fat treatments, there was generally less variation in the sensory scores given, shown by the smaller quartile ranges. This observation suggested that the low-fat samples were consistently scored higher, which is desired by manufacturers as consistent consumer acceptability is key to product success.

6. CONCLUSIONS

The application of UF in Camembert cheese-making has created several advantages such as improved yield, reduced cost of labour, improved plant efficiency, reduced pollution, and the possibility for automation. However, adjustments in the manufacturing processes and parameters are required in order to realise the benefits of UF. This includes adapting to the high buffering capacity of the retentate, determining new quantities of clotting enzymes and starter cultures, and in particular, the removal of the whey drainage step. The lack of whey drainage not only allows the incorporation of whey proteins into the cheese which improves yield, but it also has significant consequences in the quality and ripening characteristics of the product.

In the UF cheese, whey proteins act as a filler component which weakens the cheese structure by reducing the compactness of the casein network. The water-binding capacity of the proteins in cheese is also altered by the increased whey content. Prolonged acidification due to increased buffering capacity influences the profile of the starter culture and the composition of their metabolites. Enzymes, particularly those from the clotting agent and starter cultures are retained in the cheese due to the lack of whey drainage, which contribute to the breakdown of the casein network during cheese ripening. The combination of these factors results in a cheese which tends to ripen fairly rapidly, and has a short shelf-life with apparent emergence of various quality defects.

In this study, manipulation of the process variables has shown that the use of high-fat milk or retentate is not desirable. The increased fat content has been indicated to reduce the amount of paracasein linkages by acting as filler, which reduces the compactness of the casein network. This weakens the structure of the UF cheese, causing the cheese to soften relatively quickly with more rapid proteolysis. Salt uptake is also hindered as the tortuosity is reduced by fat globules.

The method of salting had significant effects on the quality and ripening characteristics of the cheese. Retentate-salted cheese had lower levels of proteolysis, possibly due to impaired growth of the starter cultures in the salted retentate.

However, cheese grading showed higher occurrences of rind discolouration and deformation in retentate-salted cheeses, as well as being significantly less preferred in terms of flavour by consumers. However, no significant differences were found in the overall acceptance of the cheeses when compared to brine-salted cheese. Considering the labour-intensiveness in brining and brine maintenance, it is more preferable to use retentate-salting over brine-salting. It is however important to be aware that retentate-salting can influence the growth of starter cultures, such as selectively encouraging salt-tolerant strains or specific metabolic pathways, which can consequently influence the cheese quality.

The extent of acidification in UF Camembert had a significant influence on cheese quality. The higher final acidification pH of 5.2 is more preferable as it is associated with lower levels of proteolysis and lower occurrences of sensory defects including softness, rind discolouration and deformation. The extent of lactic starter growth is not as high due to a shorter acidification time, which leads to a relatively lower production in LAB proteases that contributes to proteolysis during cheese ripening. The advantage of using pH 5.2 over pH 4.9 as an acidification endpoint significantly reduced acidification time, possibly leading to high throughput in production.

The effect of mould type on cheese ripening seems to be insignificant. Although the use of small moulds is associated with softer texture and several other defects, it offers the possibility for an optimised or automated demoulding process, as well as producing Camembert with new shapes. However, the use of tube moulds is still recommended as an existing demoulding and cheese cutting system is already in place. Cheeses produced from tube moulds also seemed to be more stable with fewer defects and had firmer texture.

7. RECOMMENDATIONS

It is recommended that processing variables which involves improving the protein network of the cheese or reducing the level of proteolysis during cheese ripening should be studied further. This primarily involves manipulating the enzymes retained in the curd by investigating the optimal type and amount of clotting agent used, as well as the proteolytic properties of the lactic starter and mould cultures used. This is expected to reduce degradation of the protein network in the cheese and hence produce a firmer texture. The lactic starter and mould cultures also play an important role in flavour development. It has been suggested that strains of *L. lactis* have varying levels of proteolytic and peptidolytic activities. It would be interesting to use strains with different proteolytic and peptidolytic profile in the production of UF Camembert to find ways of improving flavour formation without encouraging too much structural degradation. Additionally, the use of *Geotrichum candidum* along with *P. camemberti* has been suggested to improve the sensory qualities of Camembert cheese and reduce bitterness. It is possible for *G. candidum* to influence the extensive growth of the mould and the thick rind defect of UF Camembert cheeses. It may also be able to introduce a new flavour profile to the blandness of the cheese.

Shelf-life of the cheese is generally dependent on the degradation of proteins and formation of flavour compounds. The metabolic pathways involved are highly complex and dynamic, where the result depends on the net effect of numerous cascading processes. In UF Camembert, the shelf-life can generally be improved by strengthening the protein network and reducing the level of proteolysis during cheese ripening. In the context of commercial aspects, considering implementation of processes, time, and ease of handling, it is recommended that UF Camembert is produced using these factors: low-fat, retentate-salting, final acidification pH of 5.2, and tube mould.

8. REFERENCES

AGRAWAL, P. & HASSAN, A. N. (2008) Characteristics of reduced fat Cheddar cheese made from ultrafiltered milk with an exopolysaccharide-producing culture. *Journal of Dairy Research,* 75**,** 182-188.

ANTONIOU, K. D., PETRIDIS, D., RAPHAELIDES, S., BEN OMAR, Z. & KESTELOOT, R. (2000) Texture Assessment of French Cheeses. *Journal of Food Science,* 65**,** 168-172.

AOAC INTERNATIONAL (2005) *AOAC Official Methods of Analysis,* Gaithersburg, AOAC International.

ARDÖ, Y. (1999) Evaluating Proteolysis by Analysing the N Content of Cheese Fractions. *Bulletin of the IDF,* 337**,** 4-9.

AULDIST, M. J. & HUBBLE, I. B. (1998) Effects of mastitis on raw milk and dairy products. *Australian Journal of Dairy Technology,* 53**,** 28-36.

BASTIAN, E. D. & BROWN, R. J. (1996) Plasmin in milk and dairy products: An update. *International Dairy Journal,* 6**,** 435-457.

BOUTROU, R., AZIZA, M. & AMRANE, A. (2006) Enhanced proteolytic activities of *Geotrichum candidum* and *Penicillium camembertii* in mixed culture. *Enzyme and Microbial Technology,* 39**,** 325-331.

BRANDSMA, R. L. & RIZVI, S. S. H. (2001) Manufacture of Mozzarella cheese from highly-concentrated skim milk microfiltration retentate depleted of whey proteins. *International Journal of Food Science and Technology,* 36**,** 611-624.

BROADBENT, J.R. and STEELE, J.L. (2007). Proteolytic enzymes of lactic acid bacteria and their influence on bitterness in bacterial-ripened cheeses. *ACS Symposium Series, 971,* 193-203.

BROADBENT, J. R., STRICKLAND, M., WEIMER, B. C., JOHNSON, M. E. & STEELE, J. L. (1998) Peptide Accumulation and Bitterness in Cheddar Cheese Made Using Single-Strain Lactococcus lactis Starters with Distinct Proteinase Specificities. *Journal of Dairy Science,* 81**,** 327-337.

BUTIKOFER, U., RUEGG, M. & ARDÖ, Y. (1993) Determination of Nitrogen Fractions in Cheese: Evaluation of a Collaborative Study. *Food Science and Technology,* 26**,** 271-275.

CARREIRA, A., DILLINGER, K., ELISKASES-LECHNER, F., LOUREIRO, V., GINZINGER, W. & ROHM, H. (2002) Influence of selected factors on browning of Camembert cheese. *Journal of Dairy Research,* 69**,** 281-292.

COOLBEAR, T., CROW, V., HARNETT, J., HARVEY, S., HOLLAND, R. & MARTLEY, F. (2008) Developments in cheese microbiology in New Zealand-Use of starter and non-starter lactic acid bacteria and their enzymes in determining flavour. *International Dairy Journal,* 18**,** 705-713.

DODDS, T. M., GREGORY, S. P. & GREENWOOD, S. L. (2002) Streamlining the grading function from market to manufacture. *Australian Journal of Dairy Technology,* 57**,** 164.

ELWELL, M. W. & BARBANO, D. M. (2006) Use of microfiltration to improve fluid milk quality. *Journal of dairy science.,* 89 Suppl 1.

ENGEL, E., NICKLAUS, S., SEPTIER, C., SALLES, C. & LE QUERE, J. L. (2001) Evolution of the Taste of a Bitter Camembert Cheese during Ripening: Characterization of a Matrix Effect. *Journal of Agricultural and Food Chemistry,* 49**,** 2930-2939.

EXTERKATE, F. A. (2006) Controlled permeabilization of Lactococcus lactis cells as a means to study and influence cheese ripening processes. *International Dairy Journal,* 16**,** 788-796.
FAO (2008) Dairy Summit. Food and Agricultural Organisation of the United Nations. Rome, Italy.
FENELON, M. A., GUINEE, T. P., DELAHUNTY, C., MURRAY, J. & CROWE, F. (1999) Composition and sensory attributes of retail Cheddar cheese with different fat contents. *Journal of Food Composition and Analysis,* 13**,** 13-26.
FERRON-BAUMY, C., MAUBOIS, J. L., GARRIC, G. & QUIBLIER, J. P. (1991) Milk and retentate rennet coagulation: Effects of several heat treatments. *Le Lait,* 71**,** 423-434.
FIL/IDF (1991) *New Applications of Membrane Processes.* International Dairy Federation, Brussels, Belgium.
FIL/IDF (2007) *World Dairy Situation 2007.* International Dairy Federation, Brussels, Belgium.
FLOURY, J., ROUAUD, O., LE POULLENNEC, M. & FAMELART, M.-H. (2009) Reducing salt level in food: Part 2. Modelling salt diffusion in model cheese systems with regards to their composition. *LWT - Food Science and Technology,* 42**,** 1621-1628.
FOUCAUD, C. & JUILLARD, V. (2000) Accumulation of casein-derived peptides during growth of proteinase-positive strains of Lactococcus lactis in milk: Their contribution to subsequent bacterial growth is impaired by their internal transport. *Journal of Dairy Research,* 67**,** 233-240.
FOX, P. F. (Ed.) (1993) *Cheese: Chemistry, Physics and Microbiology,* London, Chapman & Hall.
FOX, P. F., GUINEE, T. P., COGAN, T. M. & MCSWEENEY, P. L. H. (Eds.) (2000) *Fundamentals of Cheese Science,* Gaithersburg, Aspen Publishers, Inc.
FOX, P. F., MCSWEENEY, P. L. H., COGAN, T. M. & GUINEE, T. P. (Eds.) (2004) *Cheese - Chemistry, Physics and Microbiology,* Oxford, Elsevier Acedemic.
FSANZ (2008) Australia New Zealand Food Standard Code. *Fermented Milk Products.* Melbourne, Anstat Pty Ltd.
FSANZ (2009) Microbiological Risk Assessment of Milk Cheese. *Risk Assessment Microbiology Section.* Food Standards Australia New Zealand.
GAYA, P., MEDINA, M., RODRIGUEZ-MARIN, M. A. & NUNEZ, M. (1990) Accelerated ripening of ewe's milk Manchego cheese: The effect of elevated ripening temperatures. *Journal of Dairy Science,* 73**,** 26-32.
GEURTS, T. J., WALSTRA, P. & MULDER, H. (1974) Water binding to milk protein, with particular reference to cheese. *Netherlands Milk Dairy Journal,* 28**,** 64-72.
GEURTS, T. J., WALSTRA, P. & MULDER, H. (1980) Transport of salt and water during salting of cheese. 2. Quantities of salt taken up and moisture lost. *Netherlands Milk Dairy Journal,* 34**,** 229-254.
GRAPPIN, R., RANK, T. C. & OLSON, N. F. (1985) Primary Proteolysis of Cheese Proteins During Ripening. A Review. *Journal of Dairy Science,* 68**,** 531-540.
GUNASEKARAN, S. & AK, M. M. (Eds.) (2003) *Cheese Rheology and Texture,* Boca Raton, CRC Press.
GUO, M. R., GILMORE, J. A. & KINDSTEDT, P. S. (1997) Effect of sodium chloride on the serum phase of Mozzarella cheese. *Journal of Dairy Science,* 80**,** 3092-3098.

HAJIPOUR, M., SOLTANIEH, M. & YAZDANSHENAS, M. (2010) Investigation of membrane fouling in cross flow microfiltration of non-alcoholic beer and modeling of tubular membrane flow. *Desalination,* 251**,** 20-28.

HANNON, J. A., DEUTSCH, S.-M., MADEC, M.-N., GASSI, J.-Y., CHAPOT-CHARTIER, M.-P. & LORTAL, S. (2006) Lysis of starters in UF cheeses: Behaviour of mesophilic lactococci and thermophilic lactobacilli. *International Dairy Journal,* 16**,** 324-334.

HANNON, J. A., LORTAL, S., TISSIER, J.-P. & FAMELART, M.-H. (2009) Limited ripening of low-fat UF-cheese due to CaPO4 barrier? *Dairy Science and Technology,* 89**,** 555-568.

HARJU, M., HEIKONEN, MATTI (1989) A process for the demineralization of whey or a liquid based on whey and for producing an acid mixture and a base mixture useful in food industries., Valio, Meijerien, Finland.

HARPER, J., IYER, M., KNIGHTON, D. & LELIEVRE, J. (1989) Effects of Whey Proteins on the Proteolysis of Cheddar Cheese Slurries (A Model for the Maturation of Cheeses Made from Ultrafiltered Milk). *Journal of Dairy Science,* 72**,** 333-341.

HAYALOGLU, A. A., BRECHANY, E. Y., DEEGAN, K. C. & MCSWEENEY, P. L. H. (2008) Characterization of the chemistry, biochemistry and volatile profile of Kuflu cheese, a mould-ripened variety. *LWT - Food Science and Technology,* 41**,** 1323-1334.

HERSLETH, M., ILSENG, M. A., MARTENS, M. & NÃ¦S, T. (2005) Perception of Cheese: A comparison of quality scoring, descriptive analysis and consumer responses. *Journal of Food Quality,* 28**,** 333-349.

HERZBERG, M., BERRY, D. & RASKIN, L. (2010) Impact of microfiltration treatment of secondary wastewater effluent on biofouling of reverse osmosis membranes. *Water Research,* 44**,** 167-176.

HINRICHS, J. (2001) Incorporation of whey proteins in cheese. *International Dairy Journal,* 11**,** 495-503.

JANA, A. H. & THAKAR, P. N. (1996) Recombined milk cheese - A review. *Australian Journal of Dairy Technology,* 51**,** 33-43.

JÖNSSON, A.-S. & TRÄGÅRDH, G. (1990) Ultrafiltration applications. *Desalination,* 77**,** 135-179.

KANG, Y. S. (1999) Membrane industries and R and D in Korea. *Proceedings of the 1998 Membrane Technology/Separations Planning Conference, Newton, Massachusetts, USA, 7-9 December 1998***,** 59-63.

KARAMI, M., EHSANI, M. R., MOUSAVI, S. M., REZAEI, K. & SAFARI, M. (2009) Microstructural properties of fat during the accelerated ripening of ultrafiltered-Feta cheese. *Food Chemistry,* 113**,** 424-434.

KELLY, A. L., HUPPERTZ, T. & SHEEHAN, J. J. (2008) Pre-treatment of cheese milk: Principles and developments. *Dairy Science and Technology,* 88**,** 549-572.

KILCAWLEY, K. N., O'CONNEL, P. B., HICKEY, D. K., SHEEHAN, E. M., BERESFORD, T. P. & MCSWEENY, P. L. H. (2007) Influence of composition on the biochemical and sensory characteristics of commercial Cheddar cheese of variable quality and fat content. *International Journal of Dairy Technology,* 60**,** 81-88.

KNEIFEL, W., PAQUIN, P., ABERT, T. & RICHARD, J.-P. (1991) Water-Holding Capacity of Proteins with Special Regard to Milk Proteins and Methodological Aspects - A Review. *Journal of Dairy Science,* 74**,** 2027-2041.

KO, M. K., PELLEGRINO, J. J., NASSIMBENE, R. & MARKO, P. (1993) Characterization of the adsorption-fouling layer using globular proteins on ultrafiltration membranes. *Journal of Membrane Science,* 76**,** 101-120.
KOSIKOWSKI, F. V. & MISTRY, V. V. (Eds.) (1997) *Cheese and fermented milk foods,* Westport, Edwards Brothers.
KOUSTA, M., MATARAGAS, M., SKANDAMIS, P. & DROSINOS, E. H. (2010) Prevalence and sources of cheese contamination with pathogens at farm and processing levels. *Food Control,* 21**,** 805-815.
KRIK, R. S. & SAWYER, R. (Eds.) (1991) *Pearson's Composition and Analysis of Foods,* Edinburgh, Longman Group UK Limited.
KRISTENSEN, J. M. B. (1999) *Cheese Technology - A Northern European Approach,* Aarhus, International Dairy Books.
LARSSON, K. I. & ANDREN, A. (1997) Affinity Between Chymosin and Individual Caseins at Varying pH-values. *International Dairy Journal,* 7**,** 615-618.
LEE, D. Y., LI, Y. Y., NOIKE, T. & CHA, G. C. (2008) Behavior of extracellular polymers and bio-fouling during hydrogen fermentation with a membrane bioreactor. *Journal of Membrane Science,* 322**,** 13-18.
LEMIEUX, L. & SIMARD, R. E. (1991) Bitter flavour in dairy products. I. A review of the factors likely to influence its development, mainly in cheese manufacture. *Lait,* 71**,** 599-636.
LINTON, M., MACKLE, A. B., UPADHYAY, V. K., KELLY, A. L. & PATTERSON, M. F. (2008) The fate of Listeria monocytogenes during the manufacture of Camembert-type cheese: A comparison between raw milk and milk treated with high hydrostatic pressure. *Innovative Food Science and Emerging Technologies,* 9**,** 423-428.
LO, C. G. & BASTIAN, E. D. (1998) Incorporation of Native and Denatured Whey Proteins into Cheese Curd for Manufacture of Reduced Fat, Havarti-type Cheese. *Journal of Dairy Science,* 81**,** 16-24.
LORAIN, O., DUFAYE, P. E., BOSQ, W. & ESPENAN, J. M. (2010) A new membrane bioreactor generation for wastewater treatment application: Strategy of membrane aeration management by sequencing aeration cycles. *Desalination,* 250**,** 639-643.
LUCE, C. & REX DIETERLE, R. D. (2005) Membrane applications for recovery & renewable energy. *AIChE Annual Meeting, Conference Proceedings.*
MALTHLOUTHI, M., MICHEL, J. F. & MAITENAZ, P. *C.* (1981) Study of Some Factors Affecting Water Vapor Sorption of Gruyere Cheese. *Food Science and Technology,* 14**,** 163.
MARCHESSEAU, S., GASTALDI, E., LAGAUDE, A. & CUQ, J.-L. (1997) Influence of pH on Protein Interactions and Microstructure of Process Cheese. *Journal of Dairy Science,* 80**,** 1483-1489.
MATIS, K. A., LAZARIDIS, N. K., ZOUBOULIS, A. I., GALLIOS, G. P. & MAVROV, V. (2005) A hybrid flotation - Microfiltration process for metal ions recovery. *Journal of Membrane Science,* 247**,** 29-35.
MAUBOIS, J. L. (2002) Membrane microfiltration: a tool for a new approach in dairy technology. *Australian Journal of Dairy Technology,* 57**,** 92-96.
MAUBOIS, J. L. & MOCQUOT, G. (1975) Application of Membrane Ultrafiltration to Preparation of Various Types of Cheese. *Journal of Dairy Science,* 58**,** 1001-1007.
McSWEENEY, P. L. H. (Ed.) (2007) *Cheese Problems Solved,* Woodhead Publishing Limited.

MEILGAARD, M. C., CARR, B. T. & CIVILLE, G. V. (Eds.) (2007) *Sensory Evaluation Techniques,* Boca Raton, Taylor & Francis.
MELILLI, C., BARBANO, D. M., CACCAMO, M., TUMINELLO, L., CARPINO, S. & LICITRA, G. (2006) Interaction of Brine Concentration, Brine Temperature, and Presalting on Salt Penetration in Ragusano Cheese. *Journal of Dairy Science,* 89**,** 1420-1438.
MIOČ, B., PRPIĆ, Z., VNUČEC, I., BARAĆ, Z., SUŠIĆ, V., SAMARŽIJA, D. & PAVIĆ, V. (2008) Factors affecting goat milk yield and composition. *Mljekarstvo,* 58**,** 305-313.
MISTRY, V. V. & MAUBOIS, J.-L. (Eds.) (1993) *Application of Membrane Separation Technology to Cheese Production,* London, Chapman & Hall.
MOREIRA, M., STORANI, E., BEVILACQUA, A. & DE ANTONI, G. (2003) Effects of starter culture on the characteristics of tybo cheese. *Italian Journal of Food Science,* 15**,** 287-294.
MULDER, M. (Ed.) (1996) *Basic principles of membrane technology.* Kluwer Academic. Boston.
NAGENDRAN, A. & MOHAN, D. (2008) Protein separation by cellulose acetate/sulfonated poly(ether imide) blend ultrafiltration membranes. *Journal of Applied Polymer Science,* 110**,** 2047-2057.
ONG, L., HENRIKSSON, A. & SHAH, N. P. (2007) Chemical analysis and sensory evaluation of Cheddar cheese produced with Lactobacillus acidophilus, Lb. casei, Lb. paracasei or Bifidobacterium sp. *International Dairy Journal,* 17**,** 937-945.
PASTORINO, A.J., HANSEN, C.L., and MCMAHON, D.J. (2003). Effect of salt on structure-function relationships of cheese. *Journal of Dairy Science, 86* (1), 60-69.
PAYNE, M. R. & MORISON, K. R. (1999) A multi-component approach to salt diffusion in cheese. *International Dairy Journal,* 9**,** 887-894.
PIRAINO, P., ZOTTA, T., RICCIARDI, A., MCSWEENEY, P. L. H. & PARENTE, E. (2008) Acid production, proteolysis, autolytic and inhibitory properties of lactic acid bacteria isolated from pasta filata cheeses: A multivariate screening study. *International Dairy Journal,* 18**,** 81-92.
PORTER, M. C. (Ed.) (1990) *Handbook of industrial membrane technology,* New Jersey, Noyes Publications.
POVEDA, J. M., SÁNCHEZ-PALOMO, E., PÉREZ-COELLO, M. S. & CABEZAS, L. (2008) Volatile composition, olfactometry profile and sensory evaluation of semi-hard Spanish goat cheeses. *Dairy Science and Technology,* 88**,** 355-367.
PREETHA, S. & BOOPATHY, R. (1997) Purification and characterization of a milk clotting protease from *Rhizomucor miehei*. *World Journal of Microbiology and Biotechnology,* 13**,** 573-578.
RASH, K. E. & KOSIKOWSKI, F. V. (1982) Influence of Lactic Acid Starter Bacteria on Enteropathogenic *Escherichia coli* in Ultrafiltration Prepared Camembert Cheese. *Journal of Dairy Science,* 65**,** 537-543.
RIAHI, M. H., TRELEA, I. C., LECLERCQ-PERLAT, M. N., PICQUE, D. & CORRIEU, G. (2007) Model for changes in weight and dry matter during the ripening of a smear soft cheese under controlled temperature and relative humidity. *International Dairy Journal,* 17**,** 946-953.
RICHOUX, R., AUBERT, L., ROSET, G. & KERJEAN, J. R. (2009) Impact of the proteolysis due to lactobacilli on the stretchability of Swiss-type cheese. *Dairy Science and Technology,* 89**,** 31-41.

ROGERS, N., DRAKE, M., DAUBERT, C., MCMAHON, D., BLETSCH, T. & FOEGEDING, E. (2009) The effect of aging on low-fat, reduced-fat, and full-fat Cheddar cheese texture. *Journal of Dairy Science,* 92**,** 4756-4772.
ROUPAS, P. (2001) On-farm practices and post farmgate processing parameters affecting composition of milk for cheesemaking. *Australian Journal of Dairy Technology,* 56**,** 219-232.
ROUSH, D. J. & LU, Y. (2008) Advances in primary recovery: Centrifugation and membrane technology. *Biotechnology Progress,* 24**,** 488-495.
RUEGG, M. & BLANC, B. (1976) Effects of pH onwater vapor sorption by caseins. *Journal of Dairy Science,* 59**,** 1019.
SABOYA, L. V. & MAUBOIS, J. L. (2000) Current developments of microfiltration technology in the dairy industry. *Le Lait,* 80**,** 541-553.
SCHLESSER, J. E., SCHMIDT, S. J. & SPECKMAN, R. (1992) Characterization of Chemical and Physical Changes in Camembert Cheese During Ripening. *Journal of Dairy Science,* 75**,** 1753-1760.
SCHREIER, K., SCHAFROTH, K. & THOMET, A. (2010) Application of cross-flow microfiltration to semi-hard cheese production from milk retentates. *Desalination,* 250**,** 1091-1094.
SCOTT, R. (1998) *Cheesemaking Practice,* Gaithersburg, Aspen Publishers, Inc.
SHARMA, S. K., FERRIER, L. K. & HILL, A. R. (1989) Effect of Modified Manufacturing Parameters on the Quality of Cheddar Cheese made from Ultrafiltered (UF) Milk. *Journal of Food Science,* 54**,** 573-577.
SHAW, M. B. (1981) The manufacture of soft, surface mould, ripened cheese in France with particular reference to Camembert. *International Journal of Dairy Technology,* 34**,** 131-138.
SIMOV, Z. I. & IVANOV, G. Y. (2005) Growth and proteolytic activity of Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus in reduced sodium Kashkaval cheese. *World Journal of Microbiology and Biotechnology,* 21**,** 1285-1289.
SIRKAR, K. K. (2000) Application of membrane technologies in the pharmaceutical industry. *Current Opinion in Drug Discovery and Development,* 3**,** 714-722.
SMIT, G., SMIT, B. A. & ENGELS, W. J. M. (2005) Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiology Reviews,* 29**,** 591-610.
SU, J. and EVERETT, D.W. (2003). Adsorption of κ-casein onto native milk fat globule, latex particle, and emulsion surfaces. *Food Hydrocolloids, 17* (4), 529-537.
SUÁREZ, E., LOBO, A., ÁLVAREZ, S., RIERA, F. A. & ÁLVAREZ, R. (2006) Partial demineralization of whey and milk ultrafiltration permeate by nanofiltration at pilot-plant scale. *Desalination,* 198**,** 274-281.
TARAKCI, Z. & TUNCTURK, Y. (2008) The effect of adjunct cultures on some chemical and biochemical properties of white-brined cheese. *Journal of Food Biochemistry,* 32**,** 490-505.
THOMANN, S., SCHENKEL, P. & HINRICHS, J. (2008) The Impact of Homogenization and Microfiltration on Rennet-Induced Gel Formation. *Journal of Texture Studies,* 39**,** 326-344.
TRUJILLO, A. J., GUAMIS, B., LAENCINA, J. & LÓPEZ, M. B. (2000) Proteolytic activities of some milk clotting enzymes on ovine casein. *Food Chemistry,* 71**,** 449-457.

VAHID, A., MOHAMMADI, T., PAK, A., KARBASSIAN, M. & GOLSHAN, M. (2006) Dimensional analysis of steady and unsteady state permeation flux for microfiltration of oil/water emulsion by a ceramic membrane. *AIChE Annual Meeting, Conference Proceedings.*

VINAS, M. A. G., BALLESTEROS, C., MARTIN-ALVAREZ, P. J. & CABEZAS, L. (2007) Relationship between Sensory and Instrumental Measurements of Texture for Artisanal and Industrial Manchego Cheeses. *Journal of Sensory Studies,* 22**,** 462-476.

WALSTRA, P., WOUTERS, J. T. M. & GEURTS, T. J. (2006) *Dairy Science and Technology,* Boca Raton, CRC Press, Taylor & Francis Group.

WEIMER, B. C. (Ed.) (2007) *Improving the flavour of cheese*, Cambridge, Woodhead Publishing Limited.

WIUM, H., GROSS, M. & QVIST, K. B. (1997) Uniaxial Compression of UF-Feta Cheese Related to Sensory Texture Analysis. *Journal of Texture Studies,* 28**,** 445-476.

YETIŞMEYEN, A., GENÇER, N., GÜRSOY, A., DEVECI, O., ŞENEL, E., ŞANLI, E. & ÖZTEKIN, F. S. (2006) Protein properties of UF Teleme produced from various types of milk. *Journal of Food and Drug Analysis,* 14**,** 279-283.

YORGUN, M. S., BALCIOGLU, I. A. & SAYGIN, O. (2008) Performance comparison of ultrafiltration, nanofiltration and reverse osmosis on whey treatment. *Desalination,* 229**,** 204-216.

ZWIJNENBERG, H. J., KEMPERMAN, A. J. B., BOERRIGTER, M. E., LOTZ, M., DIJKSTERHUIS, J. F., POULSEN, P. E. & KOOPS, G. H. (2002) Native protein recovery from potato fruit juice by ultrafiltration. *Desalination,* 144**,** 331-334.

APPENDICES

APPENDIX 1

Table 1: Descriptive statistics in cheese compositions (mean ± stdev) for each type of treatment. Significant differences are highlighted in bold ($p \leq 0.05$).

Cheese Treatments	TS Content	Salt Content	Fat Content (%)	Protein Content (%)
Low-Fat	44.56 ± 0.94	1.74 ± 0.10	24.96 ± 0.92	15.09 ± 0.45
High-Fat	44.64 ± 0.83	1.52 ± 0.25	28.03 ± 0.57	14.10 ± 0.58
p-value	0.791	**0.000**	**0.000**	**0.000**
Brine-Salted	44.82 ± 0.88	1.65 ± 0.23	26.62 ± 1.44	14.84 ± 0.72
Retentate-Salted	44.36 ± 0.87	1.61 ± 0.22	26.37 ± 2.02	14.67 ± 0.65
p-value	0.081	0.455	0.689	0.381
pH 5.2	44.91 ± 0.69	1.74 ± 0.19	26.67 ± 1.46	14.86 ± 0.74
pH 4.9	44.27 ± 0.98	1.52 ± 0.20	26.32 ± 2.00	14.66 ± 0.62
p-value	**0.013**	**0.000**	0.575	0.321
Tube Mould	44.56 ± 0.83	1.60 ± 0.25	26.40 ± 1.71	14.75 ± 0.70
Small Mould	44.62 ± 0.97	1.66 ± 0.19	26.59 ± 1.80	14.76 ± 0.68
p-value	0.820	0.311	0.762	0.941

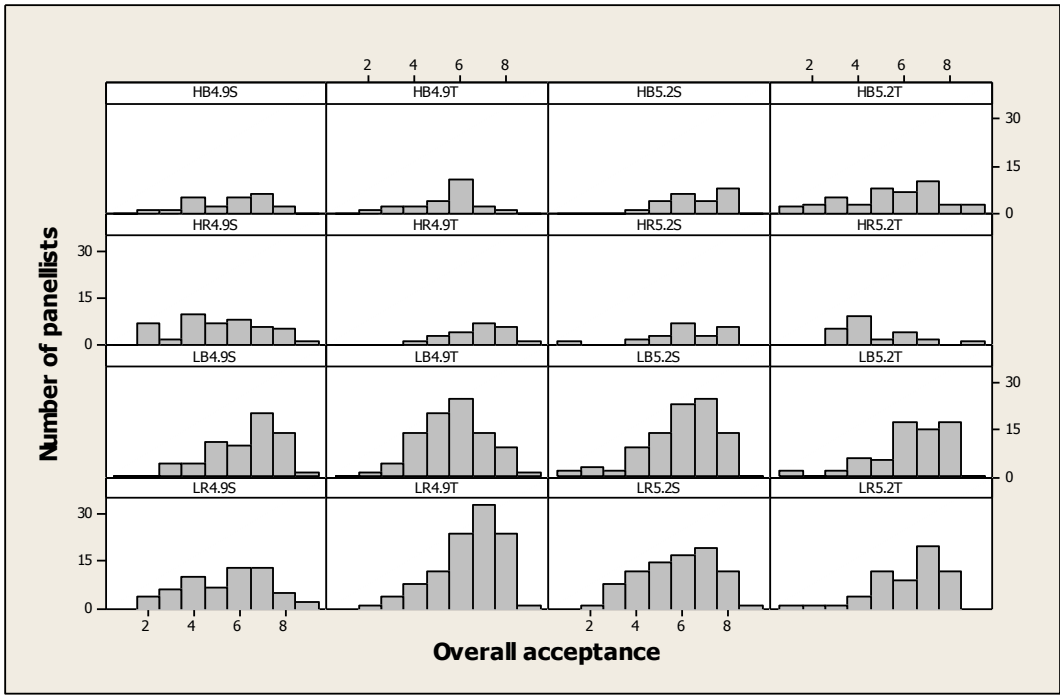
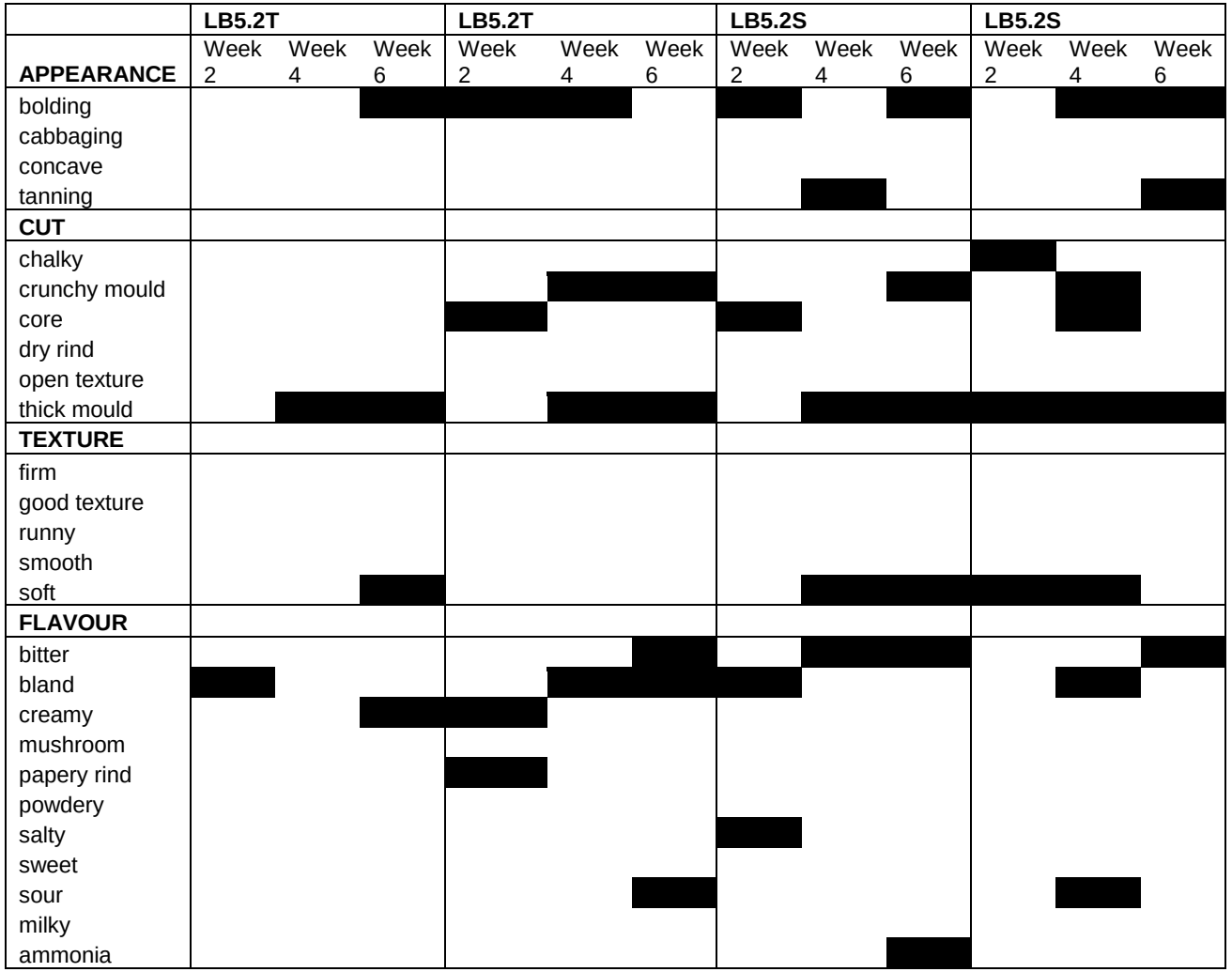


Figure 1: The frequency of overall acceptance scores by sensory panellists for 16 cheese treatments.

APPENDIX 2

CHEESE GRADING

Collaborated cheese grading results



	LR5.2T			LR5.2T			LR5.2S			LR5.2S		
APPEARANCE	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
bolding	■	■	■		■	■		■		■		
cabbaging												
concave												
tanning			■			■	■					
CUT												
chalky												
crunchy mould			■			■				■	■	
core		■										
dry rind												
open texture												
thick mould	■	■	■	■	■			■	■		■	■
TEXTURE												
firm		■										
good texture												
runny												
smooth										■		
soft	■		■		■			■		■	■	■
FLAVOUR												
bitter												
bland												
creamy		■					■					
mushroom									■			
papery rind												
powdery												
salty					■			■			■	■
sweet								■				
sour										■		
milky												
ammonia												■

	LB4.9T			LB4.9T			LB4.9S			LB4.9S		
APPEARANCE	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
bolding	■	■	■		■	■	■	■		■	■	
cabbaging												■
concave												
tanning			■			■						
CUT												
chalky				■						■		
crunchy mould						■						
core							■					
dry rind												
open texture												
thick mould		■			■			■	■			■
TEXTURE												
firm	■	■										
good texture												
runny												
smooth												
soft			■	■	■	■		■	■		■	■
FLAVOUR												
bitter								■		■		■
bland	■				■	■	■					
creamy		■			■							
mushroom									■			
papery rind												
powdery							■					
salty	■		■		■							■
sweet						■						
sour										■	■	
milky												
ammonia			■									

	LR4.9T			LR4.9T			LR4.9S			LR4.9S		
APPEARANCE	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
bolding							■	■	■		■	■
cabbaging												
concave											■	
tanning	■	■	■	■	■	■						■
CUT												
chalky												
crunchy mould									■		■	
core												
dry rind												
open texture	■			■								
thick mould		■			■		■	■	■	■		■
TEXTURE												
firm												
good texture												
runny												
smooth												
soft	■					■	■	■	■			■
FLAVOUR												
bitter			■							■		
bland	■											
creamy				■			■					
mushroom												
papery rind												
powdery												
salty					■	■						■
sweet		■										
sour				■			■			■		
milky												
ammonia						■						■

	HB5.2T			HB5.2S			HR5.2T			HR5.2S		
APPEARANCE	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
bolding			■	■			■					■
cabbaging								■	■			
concave							■					
tanning			■								■	
CUT												
chalky				■								
crunchy mould	■		■		■				■			
core	■						■					
dry rind												
open texture												
thick mould		■			■						■	■
TEXTURE												
firm												
good texture												
runny												
smooth												
soft	■	■	■		■		■	■	■		■	■
FLAVOUR												
bitter	■	■	■		■	■						
bland					■			■				■
creamy				■								
mushroom												
papery rind												
powdery												
salty								■			■	■
sweet												
sour									■			
milky				■					■		■	
ammonia			■									■

	HB4.9T			HB4.9S			HR4.9T			HR4.9S		
APPEARANCE	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6	Week 2	Week 4	Week 6
bolding	■			■		■	■	■				
cabbaging		■	■						■		■	■
concave												
tanning					■	■			■	■	■	■
CUT												
chalky	■						■			■		
crunchy mould		■	■			■						
core										■		
dry rind												
open texture												
thick mould					■			■	■		■	
TEXTURE												
firm												
good texture												
runny												
smooth												
soft		■	■		■	■			■		■	■
FLAVOUR												
bitter		■									■	
bland										■	■	
creamy						■						
mushroom												
papery rind												
powdery												
salty		■		■	■	■		■	■		■	
sweet												
sour	■						■			■	■	
milky			■		■	■		■				
ammonia									■			■

Grading Sheet - White Mould Cheeses

Date Graded	
Grading Team	

WEEK													
No of Cheeses		1	2	3	4	5	6	7	8	9	10		
Cheese													
Batch													
BB Date												**Total**	**% Defect**
Appearance	Crooked or Lopsided												
Max score	Oversized/Undersized												
8	Bare Mould & Uneven												
	Too Moist or Wet												
	Uneven Colour												
	Brown Edges												
	SCORE												
Cut	Dull												
Max Score	Holes												
4	Under Ripe												
	Over Ripe												
	Rind												
	SCORE												
Texture	Soft												
Max Score	Hard/Dry												
12	Chalky												
	SCORE												
Flavour	Bitter												
Max Score	Too Acidic												
16	Ammonia												
	Lacking Flavour												
	Chemical Soapy												
	Too Salty												
	Metallic												
	Rancid												
	SCORE												
Max Score = 40	**OVERALL SCORE**												

APPENDIX 3

CONSUMER SENSORY EVALUATION

INFORMATION SHEET

Massey University
INSTITUTE OF FOOD, NUTRITION AND HUMAN HEALTH

Ultrafiltration Process Development for the Production of Camemberti Cheese: Consumer Sensory Evaluation

Researcher(s) Introduction

Researchers Name:	Edwin Law	Supervisors Name:	Dr Tony Mutukumira Dr Marie Wong
Contact Details:	021418911	Contact Details:	094140800 ext 41203 ext 41204

You are invited to take part in a *consumer sensory evaluation* to assist a project based on Camembert cheese.

Your participation in this activity will take approximately 10 minutes.

We are selecting people for this exercise who meet the following criteria:
General consumer

The foods you will be tasting contain the following components that can be harmful or cause allergic reactions with certain groups of people. You are requested not to partake if you may be adversely affected by the following

- Alcohol in small quantities
- Aspartame
- Quinine
- **Milk and milk derivatives**
- Added Sulphites

People who are pregnant are also requested not to participate in this sensory evaluation.

The information collected in this study will be used to complete an assignment in partial fulfilment of the Master of Technology in Food Technology. No data linked to an individual's identity will be collected.

If you have any questions about this work, please contact one of the people indicated above.

CONSENT FORM

Ultrafiltration Process Development for the Production of Camemberti Cheese: Consumer Sensory Evaluation

CONSENT FORM

THIS CONSENT FORM WILL BE HELD FOR 12 MONTHS FROM DATE OF SIGNING
(For minors aged 8-15 consent form to be signed by parent or guardian)

- I have read and understood the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

- I agree to voluntarily participate in this study under the conditions set out in the Information Sheet.

- I understand I have the right to withdraw from the study at any time and to decline to answer any particular questions.

- I have advised and discussed with the Researcher any potentially relevant cultural, religious or ethical beliefs that may prevent me from consuming the Foods under consideration.

Participants Signature: ..... **Date:** .....

Full Name - printed

.....

Sensory Evaluation of Camembert Cheese

Instructions

- **FIVE** samples are presented.
- Please rate one sample at a time from left to right **WITHOUT COMPARING** them.

1. First, observe the APPEARANCE of each sample. Indicate your degree of likeness/dislikeness by placing an X in the square.

2. Then smell the sample. Indicate your degree of likeness/dislikeness for ODOUR.

3. Then take a bite of the sample and chew three times.

4. Indicate your degree of likeness/dislikeness for FLAVOUR in terms of aroma and taste (sweet, sour, bitter, salty, umami).

5. Indicate your degree of likeness/dislikeness for TEXTURE in terms of mouth-feel/structure (firmness, consistency etc.)

6. Finally, rate your OVERALL ACCEPTANCE of the sample.

SAMPLE 1									Code: 187
Attributes	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Odour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Texture	☐	☐	☐	☐	☐	☐	☐	☐	☐
Overall Acceptance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments (if applicable)									

Please turn over >>

SAMPLE 2									Code: 142
Attributes	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Odour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Texture	☐	☐	☐	☐	☐	☐	☐	☐	☐
Overall Acceptance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments (if applicable)									

SAMPLE 3									Code: 313
Attributes	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Odour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Texture	☐	☐	☐	☐	☐	☐	☐	☐	☐
Overall Acceptance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments (if applicable)									

Please turn over >>

SAMPLE 4									Code: 521
Attributes	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Odour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Texture	☐	☐	☐	☐	☐	☐	☐	☐	☐
Overall Acceptance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments (if applicable)									

SAMPLE 5									Code: 851
Attributes	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Odour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Flavour	☐	☐	☐	☐	☐	☐	☐	☐	☐
Texture	☐	☐	☐	☐	☐	☐	☐	☐	☐
Overall Acceptance	☐	☐	☐	☐	☐	☐	☐	☐	☐
Comments (if applicable)									

THE END

Thank you for your participation!

Hope you've enjoyed the cheese!

APPENDIX 4

ADDITIONAL INFORMATION

DELVO-TEC® TS-30

DIRECT-SET® Range

Description and Composition

DIRECT-SET® highly concentrated, free-flowing, deep-frozen pelletised (DSF) lactic acid bacteria starters, ready to be added straight to the vat.

The **DELVO-TEC® TS-30 DSF** DIRECT-SET® range consists of defined strains of *Streptococcus thermophilus.*

The **TS-30** cultures have proven to be phage robust. Two bacteriophage unrelated cultures are included in the range:

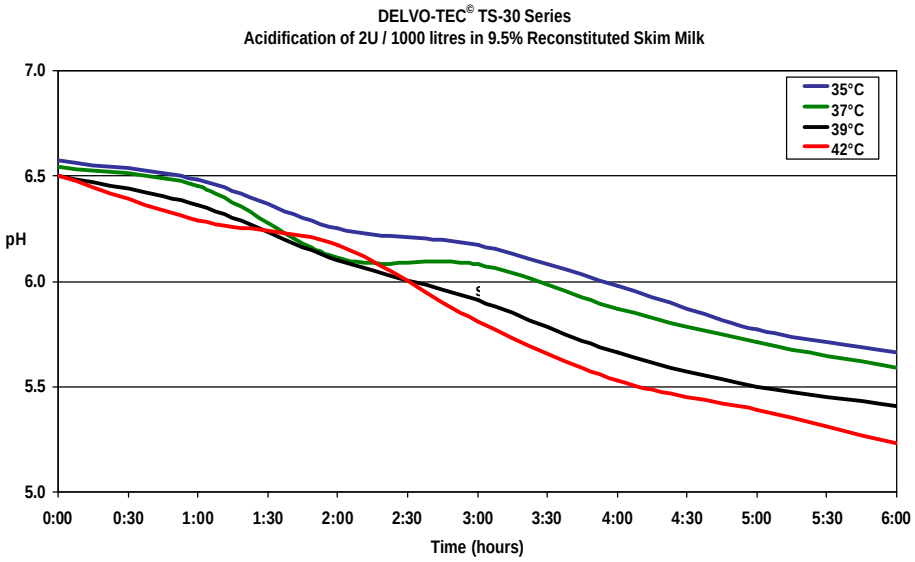
DELVO-TEC® TS-30A
DELVO-TEC® TS-30B

These cultures were not genetically modified according to the European Directive 90/220/CEE.
They are certified as Kosher.

Acidification performance

Test conditions	Substrate: Temperature: Dosage:	70°C - RSM 9.5%-solids 38°C 2 Units/1000 litres
Performance	Time: pH:	5.0 hours 5.5 ± 0.5

Acidification curves



NB. The curves above are obtained in experimental laboratory conditions and are only shown for illustration purpose.

Applications

Examples	Indicative Dosage	Possibly together with
Various stabilised soft cheeses	1-2 Units /1000 L	various DELVO-ADD® cultures

Microbiological properties

Microorganisms	Specifications
Coliforms	Absent in 1 g
Escherichia coli	Absent in 25 g
Salmonella	Absent in 25 g
Listeria monocytogenes	Absent in 25 g
Staphylococcus aureus (coagulase+)	Absent in 1 g
Yeasts & Moulds	< 10 in 1 g
Enterococci	< 10 in 1 g
Non lactic acid bacteria	< 500 in 1 g

Distribution and Storage

The **DELVO-TEC® TS-30 DSF** cultures are packed in metallised laminated sachets in standard pack sizes and shipped in polystyrene boxes with dry.
Minimum Order: 300 units
Storage Conditions: below -45°C. The shelf life is 9 months at -45°C from the date of manufacture.
The "Best Before" date is printed on each pack.

Name	Available Pack Sizes		
	2 units	5 units	10 units
All DELVO-TEC® TS-30 DSF cultures	■	■	■

Technical Service

Our Technical Service Teams can help you in selecting the most suitable starter solutions for your application by designing, supervising and discussing the results of laboratory and plant trials.

Quality Assurance

- DSM Food Specialties has production facilities worldwide, which comply with ISO 9001:2000 requirements, and employ cGMP and HACCP throughout.
- DELVO-TEC® products are manufactured to meet IDF-standards and can be provided with a certificate of conformity and specification sheets.

For further information please contact:
DSM Food Specialties
Dairy Ingredients
P.O. Box 1 2600 MA Delft
The Netherlands
Tel.: +31-15-2792355
Fax: +31-15-2793200
E-mail: info.dairy-ingredients@dsm.com
www.dsm-dairy.com

or our local representative:

DELVO-ADD® 100-X DSF

DIRECT-SET® Range

Description and Composition

DIRECT-SET® highly concentrated, free-flowing, deep-frozen pelletised (DSF) lactic acid bacteria starter, ready to be added straight to the vat.

DELVO-ADD® 100-X DSF is an undefined mixed culture of:

Lactococcus lactis ssp *lactis*
Lactococcus lactis ssp *cremoris*
Lactococcus lactis ssp *lactis* biovar *diacetylactis*
Leuconostoc sp.

This culture was not genetically modified according to the European Directive 90/220/CEE.
It is certified as Kosher.

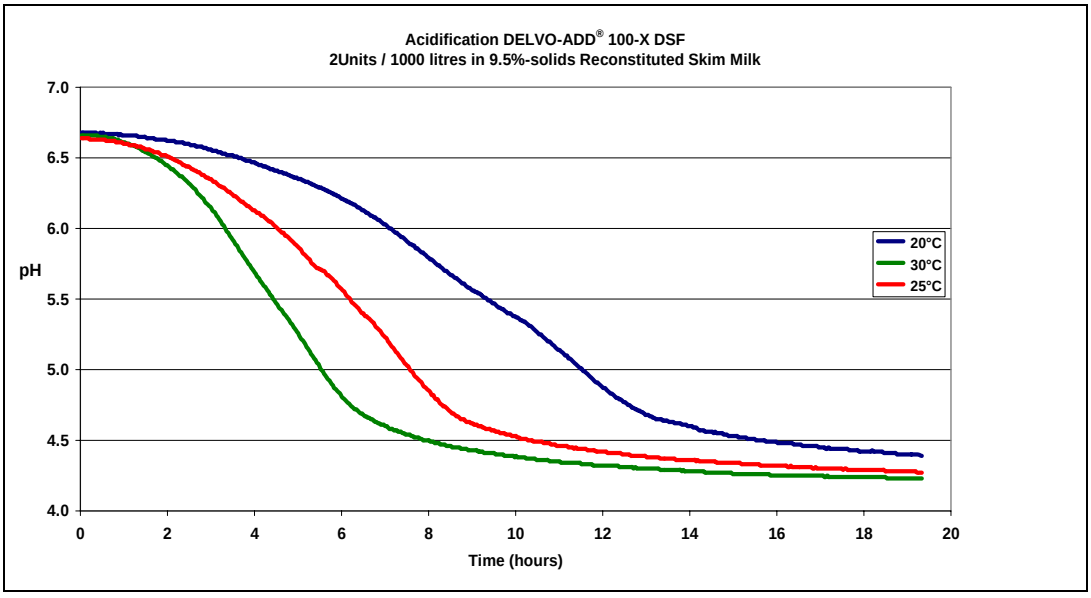
Unit value

1 unit = 150 g

Technological properties and benefits

DELVO-ADD® 100-X DSF is suitable flavour adjunct in various heterofermentative fermented milk and cheese applications, for flavour enhancement.
In cheese applications DELVO-ADD® 100-X DSF may also enhance openness depending on the cheese making process and the specific cheese characteristics.

Even though this culture is not primarily used for acidification, the graph below illustrates its acidification properties.



NB. The curves above are obtained in experimental laboratory conditions and are only shown for illustration purpose.

Applications

Examples	Indicative Dosage	Possibly together with
Sour cream, buttermilk	0.5-1.0 unit / 1000 L	Various DELVO-TEC®, other DELVO-ADD® cultures, and/or LAFTI® probiotics
Fresh cheeses, e.g. quark	0.5-1.0 unit / 1000 L	
Edam, Gouda	0.1-0.3 unit / 1000 L	
Cheddar (Mild, Mature, etc)	0.1-0.3 unit / 1000 L*	

* possibly in combination with a cooking temperature of 40°C

Microbiological properties

Microorganisms	Specifications DSF
Coliforms	Absent in 1 g
Escherichia coli	Absent in 25 g
Salmonella	Absent in 25 g
Listeria monocytogenes	Absent in 25 g
Staphylococcus aureus (coagulase+)	Absent in 1 g
Yeasts & Moulds	< 10 in 1 g
Enterococci	< 10 in 1 g
Non lactic acid bacteria	< 500 in 1 g

Distribution and Storage

DELVO-ADD® 100-X DSF is packed in metallised laminated sachets in standard pack sizes and shipped in polystyrene boxes with dry ice.
Minimum Order: 300 units
Storage Conditions: below -45°C. The shelf life is 9 months at -45°C from the date of manufacture.
The "Best Before" date is printed on each pack.

Name	Available Pack Sizes		
	1 unit	5 units	10 units
DELVO-ADD® 100-X DSF	■	■	■

Technical Service

Our Technical Service Teams can help you in selecting the most suitable starter solutions for your application by designing, supervising and discussing the results of laboratory and plant trials.

Quality Assurance

- DSM Food Specialties has production facilities worldwide, which comply with ISO 9001:2000 requirements, and employ cGMP and HACCP throughout.
- DELVO-ADD® products are manufactured to meet IDF-standards and can be provided with a certificate of conformity and specification sheets.

For further information please contact: **or our local representative:**
DSM Food Specialties
Dairy Ingredients
P.O. Box 1 2600 MA Delft
The Netherlands
Tel.: +31-15-2792355
Fax: +31-15-2793200
E-mail: info.dairy-ingredients@dsm.com
www.dsm-dairy.com

CULTURES DIVISION
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PRODUCT DESCRIPTION - PD 205651-6.0EN

Material no. 61993

PC 12 LYO 50 D

CHOOZIT™ Cheese Cultures

Description

Maturation/ripening culture made up of Penicillium candidum spores.

Penicillium candidum is the ordinary name of Penicillium camemberti.

Usage levels

Product

Camembert	3 - 5 doses / 1,000 l of milk
Stabilized Brie	5 - 8 doses / 1,000 l of milk

The quantities of inoculation indicated should be considered as guidelines. Supplement cultures may be required depending on technology, fat content and product properties desired.

We do not accept any liability in case of undue application.

Directions for use

Direct inoculation of cheese milk
Dilution for use in spray just before use: rehydrate the freeze-dried powder on the enriched tryptone medium before use (sodium chloride, tryptone, glucose for 16 h at 4 °C).
We do not accept any liability in case of undue application.

Composition

Penicillium candidum

Properties

PC 12 LYO 50 D is a maturation/ripening culture made up of Penicillium candidum spores from strains which are specifically selected and drawn up to ensure the ageing without flavour and aspect defects, for soft body cheese, surface mould.
Specially adapted to soluble products with 70 to 90 days shelf life. More, it suits to more traditional curds. Strain of Penicillium candidum allows to get a good mycelium cover stability on soft cheese along the shelf life of the cheese. No flavour defects along the shelf life of cheese: good biochemical, stability due to low enzymatic activities. Whiteness and homogeneous appearance, on faces, heels and rims until 90 days. Selected strains drawn up to be compatible with the different wrappings on market.

Microbiological specifications

Microbiological quality control - standard values and methods

Cell count	2.0E+09 CFU / dose
Tolerance:	from 1.8E+09 to 4.0E+09 CFU

Enterobacteria	< 10 / g [8]
Enterococci	< 10 / g [2]
Staphylococci coagulase positive	< 10 / g [12]
Anaerobic sulphite reducing spores	< 10 / g [9]
Yeasts	< 10 / g [10]
Foreign moulds	< 10 / g [10]
Aerobic mesophilic total count	< 100 / g [11]
Salmonella	neg. / 25 g [14]
Listeria monocytogenes	neg. / 25 g [13]

[8] V08-054 Feb.1999 (reading 48 hours)
[2] Gelose bile esculine sodium azide / 48 h at 37 °C
[12] NF V08-057 Nov. 1994 part 1
[9] V08-061 Oct. 1996 (With Meat Leaver SR medium)
[10] V08-059 Nov. 1995
[11] V08-051 Feb. 1999 (PCA + 9 % milk + 0.02 % pimaricin)
[13] NF V08-055, August 1997
[14] NF V08-052, May 1997

Storage

18 months from date of production at <= -18 °C
6 months from shipment date at + 4°C

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PRODUCT DESCRIPTION - PD 205651-6.0EN

Material no. 61993

PC 12 LYO 50 D

CHOOZIT™ Cheese Cultures

Packaging

These freeze-dried cultures are packaged in sachets. The following information is printed on each sachet: Product name, dosage, batch no and shelf life at -18°C.

Quantity

Unit pack: box of 20 sachets

Purity and legal status

PC 12 LYO 50 D meets the specification laid down by the EU legislation.

Label food regulations should always be consulted concerning the status of this product, as legislation regarding its use in food may vary from country to country.

Safety and handling

MSDS is available on request.

Kosher status

KOSHER O-U-D

Allergens

Below table indicates the presence of the following allergens and products thereof:

Yes	No	Allergens	Description of components
	X	wheat	
	X	other cereals containing gluten	
	X	crustacean shellfish	
	X	eggs	
	X	fish	
	X	peanuts	
	X	soybeans	
X		milk (including lactose)	
	X	nuts	
	X	celery	
	X	mustard	
	X	sesame seeds	
	X	sulphur dioxide and sulphites (> 10 mg/kg)	
	X	lupin	
	X	molluscs	

Local regulation has always to be consulted as allergen labelling requirements may vary from country to country.

Additional information

ISO 9001 certified

GMO status

PC 12 LYO 50 D does not consist of, nor contains, nor is produced from genetically modified organisms according to the definitions of Regulation (EC) 1829/2003 and Regulation (EC) 1830/2003 of the European Parliament and of the Council of 22 September 2003. For raw materials having the potential of being produced from genetically modified organisms, we have obtained written information from our suppliers stating that the raw materials are not produced from genetically modified organisms according to the definitions of above mentioned EC Regulations.

Fromase® XL

Extra thermolabile coagulant for cheese

Introduction

Fromase® XL is an extra thermolabile variant of the microbial coagulant derived from the soil fungus *Rhizomucor miehei*.
In most cases the standard Fromase® TL meets all the requirements of the cheesemaker. In a number of cases however and for various reasons a product with an increased thermolability is needed. Such reasons include for instance the need to use lower whey pasteurisation temperatures when the whey is used for whey protein manufacturing or the need to be able to destroy residual clotting activity in slightly acidic whey.
In those cases the normal characteristics of Fromase® TL may not be the right ones to satisfy the technological requirements.
Research into increased thermolability led to the development of Fromase® XL.

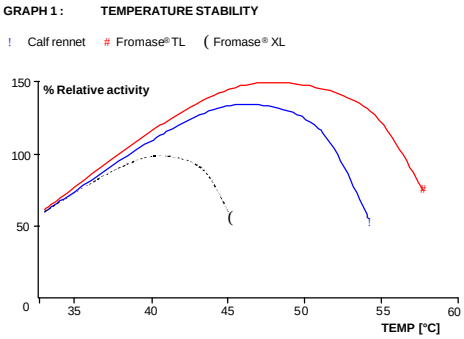
The increased thermolability of Fromase® XL has also been shown to be an advantage in the production of some cheese varieties such as Emmentaler, where the enzyme is already partly inactivated during the cooking of the curds. This has been shown to lead to a better controlled eye formation. Other varieties of cheeses have also been produced with good results using Fromase® XL.

Technical characteristics

Fromase® XL is an acid fungal protease with a molecular weight of approx. 40,000.
The overall amino acid composition of Fromase® XL is very similar to that of animal rennet. Its stability in solution is excellent between pH 3.0 and 6.5. The specificity of Fromase® XL on the beta chain of insulin is very similar to that of animal rennet, with a preference for those linkages involving aromatic amino acids.
Like animal rennet, Fromase® XL induces milk clotting through hydrolytic cleavage of the phenylananine-methionine linkage of kappa casein.

1. Effect of temperature

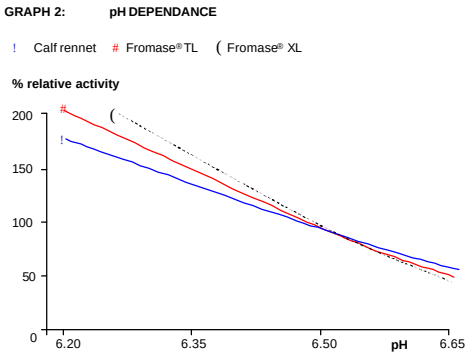
The relative activity of animal rennet, Fromase® TL and Fromase® XL is given in graph 1.



Fromase® XL has the same effect as calf rennet and Fromase® TL in the usual clotting temperature range. At higher temperatures the effect of the enzymes show marked differences.
The thermolability of Fromase® XL is clearly superior to that of calf rennet. Fromase® XL is, like calf rennet, partly inactivated during scalding.

2. Effect of pH

Graph 2 shows that Fromase® XL is somewhat more sensitive with reference to the pH than Fromase® TL or calf rennet in the important pH range for cheese manufacturing.

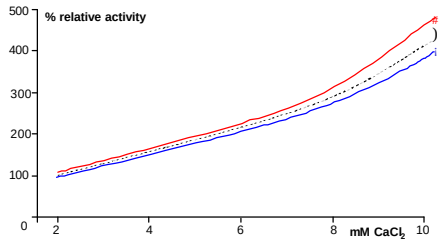


3. Effect of calcium ion concentration

Fromase® XL and calf rennet have the same sensitivity to the calcium ion concentration, whereas Fromase® TL is slightly more sensitive. These studies were carried out using Berridge substrate, the concentration of 10 mM of $CaCl_2$ corresponds to 1.11 g of anhydrous $CaCl_2$ per liter.

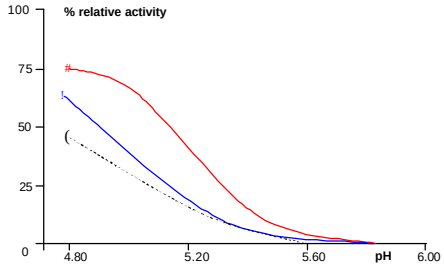
GRAPH 3: EFFECT OF CALCIUM ION CONCENTRATION

! Calf rennet # Fromase® TL (Fromase® XL



GRAPH 4: INACTIVATION IN WHEY, PASTEURISATION: 15 sec. / 73°C

! Calf rennet # Fromase® TL (Fromase® XL



4. Flocculation times and enzyme concentration

Animal rennet is known to follow the law of Storch and Segelke:

$$t = C_1 * \frac{1}{[E]} + C_2$$

In which:

t = flocculation time

[E] = enzyme concentration

C_1 = constant

C_2 = constant depending on the type of milk used and the type of rennet.

This is also the case for Fromase® XL and TL. However, when raw milk is used, Fromase® XL and TL do not follow this law and the deviation observed varies with different types of milk. It has been shown that all the *Rhizomucor miehei* enzymes deviate from the law of Storch and Segelke, corresponding in fact to competitive inhibition by inhibitors present in raw milk that are usually destroyed at temperatures above 68°C. This characteristic is especially evident in the manufacture of Emmental cheese from raw milk. Due to this inhibition the addition rates of Fromase® XL and TL in raw milk have to be increased about 20-25% compared to calf rennet to obtain a coagulation time of 25-30 minutes.

5. Inactivation of Fromase® XL in whey

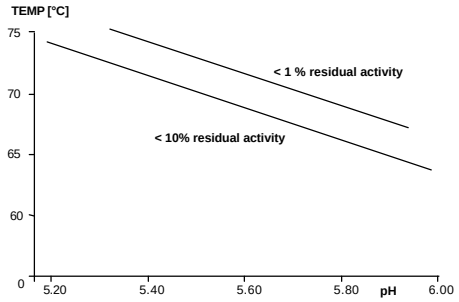
It is essential to ensure that Fromase® is inactivated in whey. Graph 4 shows the activity of Fromase® XL and TL with reference to the whey pH.

As long as storage acidification has been avoided before pasteurisation, Fromase® TL will be inactivated by normal whey pasteurisation conditions (15 sec./73°C). Fromase® XL is even superior to calf rennet in this respect, when Fromase® XL is added directly to reconstituted whey at pH 6.0, less than 1% residual activity is found after 15 sec. pasteurization at 68°C.

To achieve the same degree of inactivation of calf rennet or bovine rennet the temperature must be 2°C higher i.e 15 sec. at 70°C.

Graph 5 shows the influence of pH and temperature on the residual activity of Fromase® XL in whey after pasteurization.

GRAPH 5: pH AND TEMPERATURE EFFECT ON RESIDUAL ACTIVITY IN Fromase® XL



Method of Analysis.

The activity of Fromase® is expressed in International Milk Clotting Units (IMCU) in accordance with the Relative Milk Clotting Activity Test (REMCAT) of the International Dairy Federation (IDF). In the case of Fromase® the IDF method 176:1996 applies. A copy of the method can be obtained from the IDF: 41, Square Vergote, 1030 Brussels, Belgium or request at your local DSM Dairy Ingredients Office

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