COMPARATIVE STUDY ON FREEZE-DRIED LACTIC CHEESE STARTERS AND RIPENING CULTURES FOR THE PRODUCTION OF CAMEMBERT CHEESE

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ABSTRACT

Background and Methodology

The key to success in producing cheeses is the performance of the starter cultures (Parente and Cogan, 2004). Storage of freeze-dried cheese cultures at refrigeration and ambient temperature or higher provides convenience to culture handling and transportation, as well as reduce cost. This study investigated the effects of 4 storage temperatures: -18°C, 4°C, 20°C and 37°C on the stability of mesophilic lactic cheese starters and ripening cultures intended for Camembert production. In phase one, a 2^2 randomized complete block design (RCBD) was used to determine the potential of 14 commercial freeze-dried direct-vat-set (DVS) mixed cultures to produce Camembert after 5 months storage at the 4 temperatures. The cultures used were: O-type: *Lactococcus* (*L.*) *lactis* subsp. *lactis*, *L. lactis subsp. cremoris*; LD-type: *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* species (*Leuconostoc* (*Leuc.*) *lactis* and *Leuc. mesenteroides subsp. cremoris*) and a mould, *Penicillum* (*P.*) *camemberti*. During storage, the cultures were analysed for cell viability, acid production, colour and species composition. The characterised cultures were screened to select the most stable cultures with good potential for Camembert production. In phase two, a 2^3 RCBD design was used to study the potential of the cultures to produce prototype Camembert cheese using I-Make® Limited domestic cheese kits. The prepared cheeses were characterised for acidity, viable cell counts content, texture, volatile aromatic compounds and proteolysis using standard procedures.

Results and Discussion

Viable cell counts and acidification potential of cultures decreased ($P<0.05$) during storage at selected temperatures for 5 months. Cultures stored at 37°C were the most affected. Proportion of citrate-fermenting lactic acid bacteria (LAB) in LD-type starters also decreased in a similar pattern. Cell inactivation at high temperature was probably attributed to high oxidation, browning reactions, lactose crystallization, changes in glass transition temperature (T_g) of culture-lactose matrix and loss of β-galactosidase enzyme activity, which were possibly also affected by water activity (a_w) of the culture during storage (Higl et al., 2007; Kurtmann et al., 2009c). Viability and activities of cultures stored at 4 and 20°C after 5 months were comparable to those of -18°C cultures and levels normally used in industry. Thus, the cultures demonstrated good potential for Camembert cheese production.

Similar patterns of microbial growth (LAB and *P. camemberti*) and acidification were observed in both cheeses (O- and LD-types) during cheese fermentation. However, cheeses fermented with O-type starters had better growth and acidification activity ($P<0.05$), which may be attributed to compositional differences of culture, leading to variable metabolic patterns (Mcsweeney and Fox, 2004). Cheeses produced with cultures stored at 4 and 20°C had lower levels of cell growth and acidity ($P<0.05$), suggesting that the microorganisms could have been affected by prolonged storage at relatively high temperatures.

During cheese ripening, changes in microbial content, acidity, proteolysis, texture and aroma compounds, were similar, and significantly changed ($P<0.05$) with ripening time. Viable cell counts of LAB reduced, while pH and *P. camemberti* counts increased. Increase of pH may result from lactate metabolism by *P. camemberti* creating an alkaline environment due to the deamination activity of the mould (Spinnler and Gripon, 2004). Proteolysis of cheeses was correlated ($P<0.05$) with LAB and *P. camemberti* activity as well as the pH of

samples. Softening of cheese was associated with increased proteolysis and pH due to the growth of *P. camemberti* (Spinnler and Gripon, 2004). A range of volatile organic compounds, dominated by fatty acids, alcohols and aldehydes were identified in cheese samples as reported in other studies (Sable and Cottenceau, 1999). Changes in 3-methylbutanal and 3-methylbutanol profiles of samples reflected the degradation of leucine,, synthesis of the aldehyde and its degradation to branched alcohols as a consequence of peptidolytic activity of LAB (Yvon and Rijene, 2001) and enzymatic activity of *P. camemberti* (Molimard and Spinnler, 1996). Increased concentrations of 2-heptanone, 2-nonanone and butyric acid in cheese samples suggested lipolytic activity in all samples (Molimard and Spinnler, 1996). The activity of *P. camemberti* involved in β-oxidation pathway for producing methyl ketones was also demonstrated confirmed by identified metabolites.

Higher proteolysis and softness in LD-cheeses than O-type, suggested a higher degree of cheese ripening (Ardö, 1999), which may be attributed to proteolytic and peptidolytic activity of LD-starters (Tzanetaki et al., 1993). Higher proteolysis may be also associated with higher pH of cheese curd at draining, which facilitated higher syneresis. Increased whey content of curd may retain higher concentration of coagulant enzyme in the curd (Guinee and Wilkinson, 1992) and effectively stimulate the growth of *P. camemberti*, thus probably allowing proteolysis to occur more readily (Grappin et al., 1985). A relatively higher concentration of 3-methylbutanal was found in O-type cheeses than in LD-type. This suggests that LAB in O-type starters may exhibit higher activity in degrading leucine to 3-methylbutanal than LD-type starters (Yvon and Rijene, 2001). 2,3-butandione was suspected in LD-type cheeses but not in O-type samples, demonstrating the active role of citrate-fermenting bacteria of LD-starters (Mcsweeney and Fox, 2004).

Results indicate that storage temperature of cultures had a significant ($P<0.05$) impact on viable cell counts and acidity of samples. In spite of reduced cell counts, proteolysis, texture and aroma of the prototype cheese samples were not affected ($P<0.05$). Although there were no differences between the Camembert cheeses, 4 and 20°C cultures used in cheese-making may enhance the ripening process (Ardö, 1999) than -18°C cultures, as indicated by relatively higher proteolysis and degree of softening. Lower levels of 3-methylbutanal in samples containing 4 and 20°C cultures was probably due to the reduced aminotransferases activity of LAB (Yvon and Rijene, 2001) after prolonged storage at the two temperatures. The slightly higher levels of 2-heptanone, 2-nonanone and butyric acids in samples with 4 and 20°C cultures were probably due to increased lipolytic activity of enhanced growth of *P. camemberti* (Molimard and Spinnler, 1996) during cheese ripening.

Conclusion

LAB starter cultures and *P. camemberti* can be stored for 5 months at 4 and 20°C without affecting their activities and the quality of prototype Camembert produced. Camembert cheese samples produced in this study had typical characteristics of this type of cheese. Cheese fermented with LD-type starters showed extra flavour enhancement potential and the products had higher degree of softening due pronounced proteolysis. Cultures stored at 37°C for 5 months were characterised by poor viable cells and capability to the produce acid, therefore, they were not suitable for Camembert cheese production.

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ALA	=	α-acetolactate (α-acetolactic acid)
ArAAs	=	Aromatic amino acids
ANOVA	=	Analysis of variance
ASN	=	Acid soluble nitrogen
a_w	=	Water activity
BcAAs	=	Branched chain amino acids
C	=	Carbon
CCP	=	Colloidal calcium phosphate
CAR-PDMS	=	Carboxen/Polydimethylsiloxane
Cit^+	=	Citrate-fermenting
Cit^+	=	Non-citrate-fermenting
cfu/g	=	Colony forming unit per gram
cfu/ml	=	Colony forming unit per milliliter
CN	=	Casein nitrogen
CO_2	=	Carbon dioxide
$(Ca_3(PO_4)_2$	=	Calcium phosphate
Cd	=	Cadmium chloride ($CdCl_2$)
C/F	=	Casein-to-fat ratio
CV%	=	Coefficient of variation
d	=	Day
Da	=	Dalton
DVS	=	Direct vat set
DVI	=	Direct vat inoculation
DMC	=	Dry matter content
DNA	=	Deoxyribonucleic acid
DSS	=	Defined-strain starters
DMS	=	Dimethyl sulfide
DMDS	=	Dimethyl disulfide
DMTS	=	Dimethyl trisulfide
FA	=	Fatty acid
FFA	=	Free fatty acid
FAO/WHO	=	Food and Agriculture Organization/World Health Organization
FSANZ	=	Food Standard Australia New Zealand
FDM	=	Fat-in-day matter
g	=	Gram
GLM	=	General linear model
GLY pathway	=	Glycolytic pathway
h	=	Hour
H_2O	=	Water
HCl	=	Hydrochloric acid
HS-SPME/GC-MS	=	Headspace solid phase microextraction/gas chromatography-mass spectrometer
i.d.	=	Internal diameter
IDF	=	International dairy federation
IMCU	=	International milk clotting units

Ile	=	Isoleucine
k	=	Rate constant
k-casein		Kappa-casein
KHP	=	Potassium hydrogen phthalate Solution
kg	=	Kilogram
LAB	=	Lactic acid bacteria
Leu	=	Leucine
Log cfu/g	=	Logarithm colony forming unit per gram
LPL	=	Lipoprotein lipase
mmol/L	=	millimoles per litre
MRS	=	Molten de Man Rogosa Sharpe
MSS	=	Mixed-strain starters
MNFS	=	Moisture in non-fat substance
Met	=	Methionine
m/*z*	=	Mass-to-charge ratio
mV	=	millivolts
N	=	Nitrogen
n	=	mole
nm	=	nanometer
NaOH	=	Sodium hydroxide
$NaCl_2$	=	Sodium chloride
NSLAB	=	Non-starter lactic acid bacteria
NPN	=	Non-protein nitrogen
NH_3	=	Ammonia
NQ	=	Not quantified
PA	=	Polyacrylate
pH 4.6-SN	=	Soluble nitrogen at pH 4.6
PTA-SN	=	Phosphotungstic acid soluble nitrogen
PCA	=	Principle component analysis
PDA	=	Potato dextrose agar
PK pathway	=	Phosphoketolase
Phe	=	Phenylalanine
pI	=	Isoelectric point
ppm	=	parts per million
Pa.s	=	Pascal-second
r^2	=	Correlation coefficient
RTE	=	Ready-to-eat
RH	=	Relative humidity
RSM	=	Reconstituted skim milk
RT	=	Retention time
RCBD	=	Randomized complete block design
RNA	=	Ribonucleic acid
SD	=	Standard deviation
SN	=	Soluble nitrogen
T_g	=	Glass transition temperature
T.A.	=	Titratable acidity
TCA	=	Trichloroacetic acid
TCA-SN	=	Trichloroacetic acid soluble nitrogen
TN	=	Total nitrogen

Tyr	=	Tyrosine
Trp	=	Tryptophan
TAG	=	Triacylglyceride
Trace	=	Trace amount
µ	=	Micrometer
µg	=	Microgram
U	=	Unit
v/v	=	Volume/volume
Val	=	Valine
VC	=	Volatile compounds
w/v	=	Weight/volume
WSN	=	Water soluble nitrogen
X-gal	=	5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside
Ø	=	Diameter

1 INTRODUCTION

Cheeses are popular household fermented dairy products that, since early history, have formed an integral part of the human diet (Fox et al., 2000). The production of cheese involves complex interactions between milk, rennet and bacteria (Fox and McSweeney, 2004). Originally, the fermentation of milk in cheese manufacture occurred spontaneously by advent microbial population from the indigenous flora in raw milk, milk handler, atmosphere and utensils. This practice, however, often results in an unpredictable fermentation process, leading to economic losses and inconsistent product quality, as well as unsafe products (Fox et al., 2000). In modern industry, an upsurge of interest in providing suitable starter cultures for cheese production has been observed in order to control the fermentation process. Indigenous flora has largely been replaced by defined commercial starter cultures for the production of safe and consistent high quality products with stable shelf life (Spinnler and Gripon, 2004; Parente and Cogan, 2004; Bockelmann, 2010).

Commercial starter cultures of cheese industry are conveniently divided into mainly lactic acid bacteria (LAB) starters and ripening cultures, respectively.

LAB belonging to the genera *Lactococcus* (*L.*), *Streptococcus* (*S.*), *Leuconostoc* (*Leuc.*) and *Lactobacillus* (*Lb.*) are widely used in the cheese industry as starter bacteria due to their ability to produce acid during milk fermentation (Singh et al., 2003; Fox and McSweeney, 2004; Parente and Cogan, 2004; Singh and Cadwallader, 2008). In addition to acid production, LAB also contribute to cheese ripening since their enzymes are involved in various biochemical reactions, including citrate metabolism, proteolysis, lipolysis and conversion of amino acids and free fatty acids to flavor compounds, and gas production in selected products. The development of acid also influences the texture of cheeses and plays a vital role in preservation, thus contributing to the microbial safety of cheese products (Banks, 1998; Fox and McSweeney, 2004; Parente and Cogan, 2004).

For certain cheese varieties, secondary cultures are essential for ripening. Secondary ripening microorganisms do not play any active role during cheese milk fermentation, but are involved with the starter bacteria in the ripening process to induce organoleptic and biochemical changes in or on the cheese (Law, 1997; Bockelmann, 2010). For example, *Penicillum* (*P.*) *camemberti*, a white mould, is used to assist in the ripening of Camembert for developing white, fluffy appearance and producing distinct flavor, as well as promote softening of body texture (Spinnler and Gripon, 2004). In Swiss cheese, propionic bacteria are used to promote eye formation (Fox et al., 2000; Spinnler and Gripon, 2004).

In modern dairy industry, cultures are available in different forms such as liquid, air-dried (spray-dried and vacuum-dried), frozen and freeze-dried (lyophilized) forms. The most

popular cultures are the Direct Vat Set (DVS) which are also called Direct-Vat Inoculation (DVI). The popularity of DVS is attributed to convenience in during handling and retention of high activity (Kindsedt, 2005; Hoier et al., 2011). DVS cultures are preserved in many forms, either as frozen liquid concentrate, or in powdered freeze-dried form. While cultures preserved in frozen form only exhibit maximum survival in frozen environment, the cost of storage conditions limits their use. Instead, preservation of cheese starter cultures by freeze-drying technique has generated much interest due to lower storage costs, transportation and easier handling compared to freezing liquid cultures (Morgan et al., 2006; Santivarangkna et al., 2007; Santivarangkna et al., 2008).

The performance of freeze-dried cultures is vital in cheese production, both technologically and economically. Prior to commercial applications, freeze-dried culture strains are screened to determine cell viability, storage stability and important technological properties (rapid acid production, ability to produce flavor production in milk and bacteriophage resistance) to obtain desired performance in the final cheese manufacture (Parente and Cogan, 2004; Hoier et al., 2011; Nollet and Toldra, 2012). During production of freeze-dried cultures, the process of freezing and drying can damage the bacterial cell membrane (Tamime and Robinson, 1999; Santivarangkna et al., 2008). The damage however can be minimized with the addition of protectants (e.g., lactose and milk) prior to treatment that prevent cellular injury by stabilizing the cell membrane constituents during preservation procedure (Cerrutti et al., 2000; Strasser et al., 2009). The major problem after freeze-drying is the loss of viability during storage (Tamime and Robinson, 1999; Parente and Cogan, 2004). Among the several factors that cause injury and instability of cultures during storage, temperature has been singled out as being critical; inactivation of freeze-dried bacteria has been reported when exposed to high temperatures. As the storage temperature increases, so does the mortality of the culture, resulting in lower acidification activity (Morgan et al., 2006; Santivarangkna et al., 2007; Santivarangkna et al., 2008). Storage temperature, humidity and length of storage have combined negative effects on viability and acidification activity of cultures (Law, 1997; Fox et al., 2000; Parente and Cogan, 2004; Higl et al., 2007; Hoier et al., 2011).

Camembert cheese is a surface mould-ripened cheese with a soft consistency in a flat cylindrical form and has a soft plastic texture which becomes semi-liquid as the cheese matures (Spinnler and Gripon, 2004; Codex Standard, 2010). In New Zealand, Camembert is made from pasteurized milk with the addition of mesophilic lactic starter bacteria which include lactococci species (*L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* species (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris* and ripening microorganisms which may include yeasts (*Kluyveromyces* (*K.*) *lactis, Saccharomyces* (*S.*) *cerevisiae* and *Debaryomyces* (*D.*) *hansenii*) and moulds (*P. camemberti* and *Geotrichum* (*G.*) *candium*) and smear bacteria (*Corynebacterium* and *Brevibacterium* (*B.*) *linens*) (Fox et al., 2000; Parente and Cogan, 2004; Spinnler and Gripon, 2004). LAB starters produce sufficient acid to reduce pH of milk to desirable levels, followed by addition of rennet at

about pH 6.4 and coagulation occurs for 30-45 min. The coagulum is then transferred to cheese moulds and whey is allowed to drain at 26-28°C with gradual cooling to 20°C. 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 (d) or more at 11-13°C under 90% relative humidity (RH) (Spinnler and Gripon, 2004).

The vast majority of Camembert cheeses in the current market are produced industrially and sold as ready-to-eat (RTE) products (Spinnler and Gripon, 2004). While the commercial products have served the market well for a long time, a common complaint today is that cheese is often boring and bland; and this is a result of pasteurization and standardization of cheese making processes (Law, 1997; Fox et al., 2000). International travel and increased communication about food ingredients and nutritional values have increased people interest for high quality, nutritious, flavoursome food. Many people are now reverting back to preindustrial practises and this has lead an increase in commercial domestic kits that allow consumers to produce own healthy, nutritious, tasty, cost-effective cheese products with no harmful preservatives, which is often described as 'do-it-yourself'. Several 'do-it-yourself' commercial domestic kits are being marketed including equipment for making Camembert cheese. Giving consumers the opportunity to produce own cheese in their own homes has enormous business potential. The main challenge facing domestic production of cheese is maintaining a sufficient cold chain. Optimum storage temperatures are essential to maintaining viable cell population of freeze-dried cheese starters and ripening cultures. The commonly used freeze-dried cultures should be stored at -18 °C or lower to maintain viability of bacteria cells needed for cheese-making (Tamime and Robinson, 1999; Hoier et al., 2011); this may be difficult to maintain at household level, as well as when exporting to distant markets.

In Camembert cheese production, lactic starters and ripening cultures are involved in biochemical activities that are vital to the development of important sensory properties such as appearance, texture, taste, mouth-feel and aroma (Leclercq-Perlat et al., 2004a; Spinnler and Gripon, 2004). The biochemical activities are largely determined by the viability of the cultures, which in turn may be affected by storage and handling conditions (Parente and Cogan, 2004). Generally, there is scanty information on stability of freeze-dried cheese cultures at ambient temperature or higher, nor in refrigerators for prolonged storage time (Gyosheva et al., 1995; Andersen et al., 1999; Tamime and Robinson, 1999; Higl et al., 2007). The effect of cheese culture activity on physicochemical, biochemical and microbiological aspects of Camembert cheese have been investigated with the aim of understanding their behaviour during ripening (Sousa and McSweeney, 2001; Leclercq-Perlat et al., 2004a; Leclercq-Perlat et al., 2004b; Spinnler and Gripon, 2004; Sullivan et al., 2005; Guizani et al., 2007; Lessard et al., 2012). However, no attempts were made to study their ability to make Camembert after prolonged storage at higher temperatures. Thus, there is a gap in the knowledge on the ability of cheese cultures that have been stored at refrigeration, ambient

and higher temperatures for prolonged storage period to produce Camembert. In addition, cheese starter and ripening cultures have also not been investigated in Camembert cheese production using commercial domestic cheese-making kits.

1.1 Aim and objectives

The main objective of the study was to investigate the effects of four selected temperatures on the stability of mesophilic cheese starter and ripening cultures during storage. The experiments were conducted in three integrated phases with the following specific objectives:

1. To analyse the properties (cell viability, acid production, colour and culture composition) of 14 different commercial freeze-dried mesophilic cheese starter (*Lactococcus* and *Leuconostoc* species) and ripening cultures (*Penicillium*) when stored at refrigeration (4˚C), ambient (20˚C) and elevated ambient (37˚C) temperatures for 5 months, using -18˚C as treatment control.

2. To screen and select cheese cultures with potential to produce prototype Camembert cheeses using a commercial domestic kit. The cheese was used as a model to test the capability of potential starter cultures to produce high quality products.

3. To characterise the prepared prototype Camembert during ripening, with respect to microbiological, physicochemical and biochemical properties which comprised analyses of viable cell counts, acidity, rheology, nitrogen fractions, free amino acids and aroma compounds.

2 LITERATURE REVIEW

2.1 General aspects of cheese technology

2.1.1 World production and classification of cheese

The history of cheese making dates back to about 2000 BC (Fox and McSweeney, 2004). Over time, the technology has evolved from a village cottage industry to large-scale, high technology fermentation commercial enterprises. However, the traditional technology is still being practised in communities around the world, both in the developed and developing regions (Kosikowski and Mistry, 1997; Johnson and Law, 2010). The Food and Agriculture Organization/World Health Organization (FAO/WHO) (Shaw, 1981) defines 'cheese' as:

'Cheese is the fresh or matured solid or semi-solid product obtained by coagulating milk, skimmed milk, partly skimmed milk, cream, whey cream, or buttermilk, or any combination of these materials, through the action of rennet or other suitable coagulating agents, and by partially draining the whey resulting from such coagulation'.

Meanwhile, the definition of cheese given by the Food Standard Australia New Zealand (FSANZ, 2008) is that:

'Cheese means the ripened or un-ripened 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, or materials obtained from milk, or both, 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 or coagulation of milk, or materials obtained from milk, or both, which give an end-product with similar physical, chemical and organoleptic characteristics as the product described in paragraph (a)'.

The world produces significant quantities of cheeses, with over 2000 varieties of cheeses accounting for about 35% of total milk production. In 2005, FAO/WHO estimated the world production of cheese at 19 million, with Europe contributing nearly 10 million tonnes of cheese (FAO, 2008). According to Johnson and Law (2010), the major cheese-consuming regions remain the European Union and the United States of America which is about 80% of world consumption. Traditionally, most of the international trade in cheese has been between the developed countries. Trade is dominated by the European Union, New Zealand and Australia which together account for about 75% of the world market (Banks, 1998).

There is no definitive list of cheese varieties. In 1971, a comprehensive study of cheese varieties was undertaken by the International Dairy Federation (IDF) and 395 cheese

varieties were classified (Banks, 1998) as summarised in Table 1. Fox and Mcsweeney (2004) and Johnson and Law (2010) suggested that a classification scheme for cheese should also include an indication of manufacturing procedure, ripening characteristics (e.g. method of coagulation, ripening agent) (Figures 1 and 2).

Table 1. Classification of cheese varieties (Banks, 1998).

Raw material	**Cheese type**	**Characteristics**		
		Interior	Exterior	Composition
Cows' milk	Hard	Large-round opening	Hard dry rind	Fat in dry matter
Sheeps' milk	Semi-hard	Medium sized opening	Hard rind with smeary surface	Moisture
Goats' milk	Soft	Small around openings	Soft, dry rind	Moist in non-fat milk solid
Buffaloes' milk	Semi-soft	Irregular openings	Soft rind with smeary surface	
	Acid-curd	No openings	Soft rind with white mould	
	Whey	Blue-green mould veins	Soft rind with green mould	
		White mould veins	Soft rind with paraffin	
		Added spices	No rind	
		Added herbs		

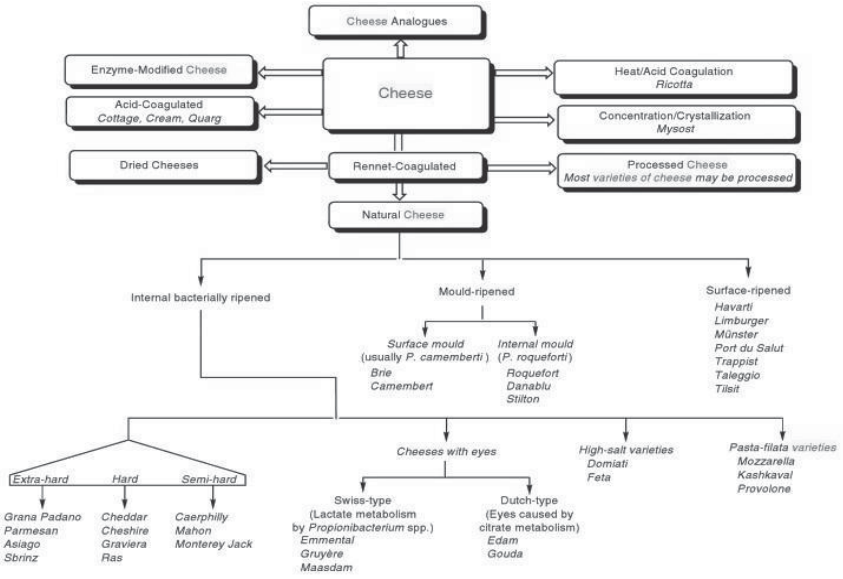


Figure 1. The diversity of cheese (Fox and McSweeney, 2004). Acid-coagulated cheese is made through direct acidification by adding acid to the milk to coagulate the proteins; Rennet-coagulated cheese is made using rennet (enzyme) in addition to starter cultures to coagulate the milk; Process cheese is made using 'natural cheese' plus other ingredients that are cooked together to change the textural and/or melting properties and increase shelf life. The term 'natural cheese' is an industry term referring to cheeses that is made directly from milk. Moisture content for extra hard: < 25%; hard: 25-36%; semi-hard: 36-40% and soft: >40%.

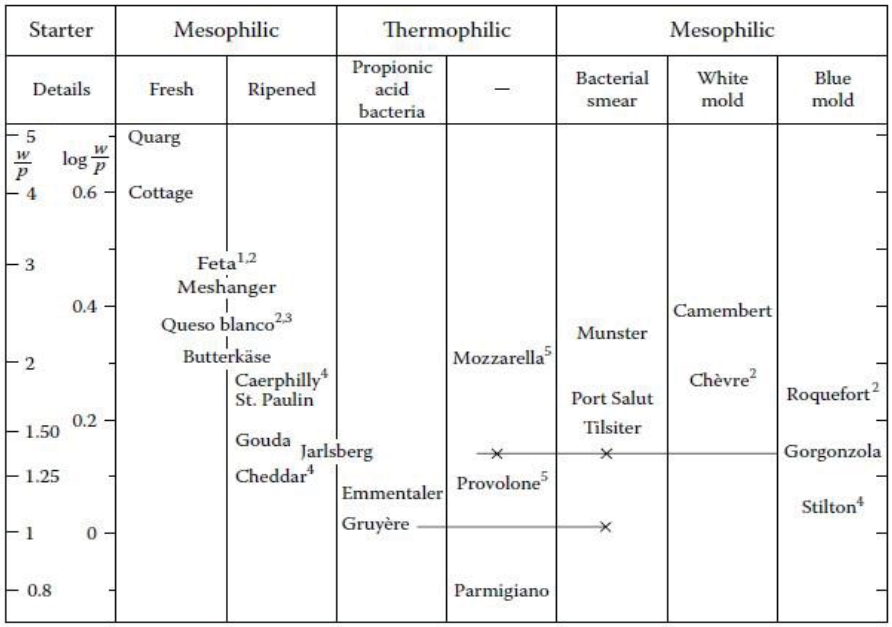


Figure 2. Classification of cheese varieties (Johnson and Law, 2010). *w/p* (water:protein ratio) represents the water content of cheese curd; [1]Kept in brine; [2]Milk may differ from cow's milk; [3]Usually by acid coagulation; [4]Salting of the curd prior to pressing; [5]Stretched curd. '×' means that the parameter involved applies as well.

2.1.2 Cheese-making

Cheese-making is a rather simple process in itself, but the production involves complex chemical and physical interactions between milk, rennet and bacteria (Fox and McSweeney, 2004). The manufacture of cheese is fundamentally a method of preserving milk over the short and medium term. The main purpose of this preservation method is to lower the pH (achieved through acidification by controlled lactic acid fermentation) and water activity (through coagulation, draining and salting of cheese curd). There is no standard method of cheese-making; however the basic technology for the manufacture of all cheese types is similar. The manufacture of cheeses can be divided into two distinct phases: (a) manufacture of cheese curd (conversion of milk to curd), which is essentially completed within 24 h; and (b) cheese curd maturation (ripening of the curd) both of which involve several processes.

2.1.2.1 Manufacturing

The manufacturing phase is generally defined as those operations performed during the first 24 h and the basic steps are: (a) selection, standardization and pasteurization of cheese milk, (b) acidification, (c) coagulation, (d) dehydration, (e) shaping and (f) salting (Kosikowski and Mistry, 1997; Banks, 1998; Fox et al., 2000; Fox and Cogan, 2004; Johnson and Law, 2010).

(a) Selection, standardization and pasteurization of cheese milk

Most cheese varieties commence with the selection and pre-treatment of milk of high microbiological and chemical quality. Cheese can be made using milk of any species (cow, goat, sheep), while cow's milk is most common (Fox and Cogan, 2004; Fox and McSweeney, 2004). Due to major compositional abnormalities, milk from cows in the very early or late stages of lactation and those suffering from mastitis should be excluded. The milk should also be of good microbiological quality in low contamination level, free of chemical taints and free fatty acids, which cause off-flavours in the cheese, and antibiotics, which inhibit starter culture activity (Kosikowski and Mistry, 1997; Fox et al., 2000; Fox and Cogan, 2004; Johnson and Law, 2010). In regions where fresh milk is scarce, cheese has also been successfully made from recombined anhydrous milk fat and reconstituted skim milk powder (Johnson and Law, 2010). Before milk is used for cheese production, it is subjected to several pre-treatments, with various objectives.

Fat and protein are important components of cheese milk that have large influence on fat-in-dry matter (FDM) and moisture in non-fat substances (MNFS; which in turn the ratio of moisture to protein), which are important compositional factors of cheese. The ratio of casein-to-fat (C/F) in milk determines FDM; and the total amount of casein and fat, in part, determines the yield potential of milk (Fox et al., 2000; Fox and McSweeney, 2004; Johnson and Law, 2010). Due to inevitable variations in milk composition caused by weather, animal-feeding, breed, the cheese milk needs to be standardised with respect to fat and casein prior to cheese-making to ensure the production of consistently high quality products. Based on the definitions for cheeses specified by FAO, standardization of milk is necessary for cheeses with FDM (g 100 g^{-1}) < 50 or > 57. If a higher FDM (or lower C/F) is required, cream is added; if a lower FDM (or higher C/F) is desired, either cream is removed or condensed skim milk powder is added (Johnson and Law, 2010).

Raw milk has a long tradition for cheese-making. The adventitious micro-flora of raw milk, especially the indigenous lactic acid bacteria, may contribute to the acidification and ripening of raw milk cheese (Johnson and Law, 2010). Some of the best cheeses are made from raw milk and the practice is widely used in France (Kosikowski and Mistry, 1997; Johnson and Law, 2010). However, food safety may compromise because the microbial quality of raw milk is difficult to maintain and often results in uncontrolled fermentations. Camembert cheese made from raw milk have been implicated with contamination by pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Clostridium perfringens*, *Salmonella* spp., *Listeria* (*L.*) *monocytogens*, *Yersinia enterocolitica* and *Campylobacter* spp. (Bockelmann, 2010). For modern large-scale cheese-making operations, pasteurized (72°C/15sec) milk is preferred and selected starter LAB cultures are used for inoculation, for more uniform

bacteriological quality of products.

Pasteurisation is not intended to kill all micro-organisms in milk. Although the majority of psychrotrophic spoilage bacteria and several pathogens (*Campylobacter*, *Salmonella*, etc) are effectively eliminated by pasteurization, spore-forming bacteria such as *Clostridium* and *Bacillus* were reported in pasteurized milk (Banks, 1998). Some species of *Lactobacillus* and *Streptococcus* can also survive. Pasteurization can inactive indigenous milk enzymes (except for heat-stable) that may cause off-flavours. Pasteurization process also partially denatures whey proteins and alters the mineral constituent of milk that interferes with gel formation in cheese manufacture (Johnson and Law, 2010). Pasteurization of milk also influences the characteristics of proteolysis during cheese ripening (Singh et al., 2003). Milk pasteurization causes very limited heat-induced interaction of whey proteins with casein and therefore results in the retention of additional whey proteins in cheese beyond the normal amount which is soluble in the aqueous phase of cheese. The presence of heat-denatured whey proteins in cheese can decrease the accessibility of caseins to proteinases during ripening (Singh et al., 2003).

Most cheese milk is not homogenised as the process reduces the fat globules and increases the fat surface area where casein particles adsorb. This results in a soft, weak curd at renneting and increases hydrolytic rancidity, which is a defect in cheese (Singh et al., 2003; Johnson and Law, 2010).

(b) Coagulation of milk

After the cheese milk has been standardized and pasteurized, it is transferred to cheese vats, where it is coagulated to cheese curd. Coagulation is essentially the formation of a gel by destabilizing the casein micelles, causing them to aggregate and form a network which partially immobilizes the water and traps the fat globules if present (Guinee and Wilkinson, 1992; Fox et al., 2000; Dalgleish and Corredig, 2012). Coagulation in various cheese productions is derived from acid-coagulation or enzymatic (rennet)-coagulation, or by a combination of two activities (Fox and McSweeney, 2004).

(c) Microorganisms

Acidification (lowering of pH) achieved by controlled lactic acid fermentation is essential in the formation of a gel from the milk casein; the organisms responsible for acid production during cheese-making are the LAB (Singh et al., 2003; Fox and McSweeney, 2004; Parente and Cogan, 2004; Singh and Cadwallader, 2008).

LAB used as starter cultures in the cheese industry belong to the genera *Lactococcus*,

Streptococcus, *Leuconostoc* and *Lactobacillus*. Of these microorganisms, the lactococci are the most widely used (Fox et al., 2000; Parente and Cogan, 2004). The choice of starters is primarily based on the temperature used in cheese-making and their acid profile. Desired lactic starters reduce milk pH to less than 5.0-5.3 within 6-12 h at 30-37^{o}C depending on cheese variety (Fox et al., 2000; Parente and Cogan, 2004). Mesophilic starter cultures (e.g., *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*) are used in the production of a variety of soft cheeses in which the processing techniques incorporate only moderate scald temperatures of up to about 40^{o}C. In the production of hard cheeses such as Parmesan and Emmental, the processing techniques require the use of high scald temperatures up to about 53°C and thermophilic cultures (e.g., *S. thermophilus*, *Lb. helveticus* or *Lb. delbrueckii* subsp. *bulgaricus*) are used (Banks, 1998; Fox and McSweeney, 2004; Singh and Cadwallader, 2008). Acid-damaged and bacteriophage-attacked LAB starters however have the potential to slow and even stop the rate of acid development (Hoier et al., 2011).

In addition to acid production, starter cultures also contribute to cheese ripening since their enzymes are involved in various biochemical reactions, including citrate metabolism, proteolysis, lipolysis and conversion of amino acids and free fatty acids to flavor compounds, and gas production. The development of acid also influences the texture properties of the cheeses by encouraging the contraction of rennet-curds and the expulsion of moisture from the cheese curd by syneresis. Furthermore, by lowering the pH and producing antimicrobial compounds (e.g., nisin), lactic starters also have a role in preservation and contribute to the microbial safety of cheese products (Banks, 1998; Parente and Cogan, 2004; Fox and McSweeney, 2004).

Secondary ripening microorganisms are also used in cheeses, for certain varieties, to produce distinct flavour and texture properties. For example, *P. camemberti*, a white mould, is used to assist in the ripening of Camembert, for developing white, fluffy appearance and producing distinct flavor, as well as promotes a softening of body texture. In Swiss cheese, propionic bacteria are used to promote eye formation (Fox et al., 2000; Spinnler and Gripon, 2004). In spite of their significant roles in cheese ripening, secondary bacteria do not play an active role in acidification during fermentation.

(d) Coagulation

Milk contains on average 3.3% protein, which exists in two forms: as a suspension/colloidal (casein) and in a soluble form (whey protein), which are distinguished by their behaviour on lowering the pH to 4.6. The casein proteins will precipitate at pH 4.6, whereas the whey proteins will not (Fox et al., 2000). The casein is composed of several constituents designated as α_{s1}-, α_{s2}-, β- and κ-caseins, all of which show differences in their polypeptide chain structure. In milk at normal pH (about 6.8), the caseins are bound together with colloidal

calcium phosphate (CCP) in the form of spherical particles, known as casein micelles (Fox et al., 2000; Dalgleish and Corredig, 2012). According to Dalgleish and Corredig (2012), the structure of casein micelle is that the α_{s1}, α_{s2} and β-caseins are surrounded by a layer of κ-casein. The behaviour of casein micelles has great importance in determining the properties of milk. In milk at normal pH, κ-casein, which is located on the surface of casein micelles, provides steric stabilization to the micelle so that they remain soluble. Coagulation occurs when casein micelles destabilize, and the stability of the micelles depends on the loss of electrostatic- and/or stearic-repulsions, which is related to rate of pH changes and the presence of chymosin (Banks, 1998; Singh et al., 2003; Singh and Cadwallader, 2008). When caseins are aggregate, fat globules and microorganisms are trapped in the developing curd.

Acid-coagulation

During acid-coagulation, acid-production by starter cultures causes casein micelles to destabilize or aggregate by decreasing their high negative electric charge on the κ-casein layer of casein micelle to nearly no net charge, as their isoelectric point (pI) is approached (pI = 4.6). Meanwhile, acidity increases the solubility of CCP, a structural unit within casein micelles which acts as a neutralizing bridge between two negatively charged phosphoseryl groups. As pH drops below 6, calcium phosphate is cleaved from the inside of casein particles and gradually become soluble in the aqueous phase of milk, causing a 'loosening' of the casein network. At pH 5.3-5.2 (≥ 20°C), gelation starts as casein micelles become loose in their structural integrity and unstable to form the beginning of agglomeration. Below pH 5, gel firmness increases considerably, which continues to increase, reaching maximum around the pI. While at pH 4.6 (≥ 20°C), the pI of casein is reached and the micelle no longer carrying any charge to keep it suspended by repelling forces, casein becomes completely insoluble and precipitates out. This results in the formation of a fragile coagulum which is a 3-dimensional heterogeneous casein network held together by covalent and protein-to-protein interactions (Banks, 1998; Fox et al., 2000; Singh et al., 2003; Singh and Cadwallader, 2008; Dalgleish and Corredig, 2012). Since acidity influences the onset of gelation and coagulum formation, the rate and the extent of acid production by cheese starter cultures is critical for textural attributes of finished cheese products.

Enzymatic (rennet) coagulation

In cheese manufacture, a coagulant is primarily used to coagulate milk. The predominant coagulant used in cheese manufacture is calf rennet, with the active enzyme component, chymosin, which is secreted by the young of several mammalian species. Lamb and kid rennet pastes are also used in cheese manufacture as coagulants. Microbial proteases secreted by *Cryphonectria parasitica, Rhizomucor pusillus* and *Rhizomucor miehei* are other sources of coagulants (Banks, 1998; Fox et al., 2000; Singh et al., 2003; Singh and Cadwallader, 2008; Dalgleish and Corredig, 2012). Many plants also have coagulating properties. Ancient

Greeks used ficin from the fig tree to coagulate milk. Other examples include papain from papaya tree, and bromelin from pineapple (Guinee and Wilkinson, 1992).

The milk clotting activity of rennet is due to the presence of the enzyme chymosin, which is a two-phase reaction (Figure 3). Phase one involves enzymatic hydrolysis of κ-casein by chymosin through converting κ-casein to para-casein. Chymosin specifically cleaves κ-casein at the Phe_{105}-Met_{106} position and releases the C-terminal region resulting in the formation of soluble CCP, which diffuses from the micelle and para-κ-casein, leaving a distinctly hydrophobic peptide on the micelles. Phase two is the coagulation (aggregation) of the altered casein micelles by Ca^{2+} at >20°C. The micelles aggregate due to the loss of stearic repulsion of the para-κ-casein and the surface potential which inhibits the micelles from coming close together (Banks, 1998; Fox et al., 2000; Dalgleish and Corredig, 2012). When acid-coagulation is also involved, the aggregation of para-κ-casein micelles aggregates further contributing to the loss of electrostatic repulsion due to the decrease in pH as it approaches its isoelectric point (pH 4.6). Once a critical degree of proteolysis has been reached (80%), then aggregation can occur, and a coagulum is formed. In the second phase, coagulation requires a temperature in excess of 20°C; the rate of rennet reaction was reported to increase with increasing temperature up to 40°C (Dalgleish and Corredig, 2012).

Enzymes present in rennet also play an important role in the generation of flavour compounds during cheese ripening (Section 2.6.2).

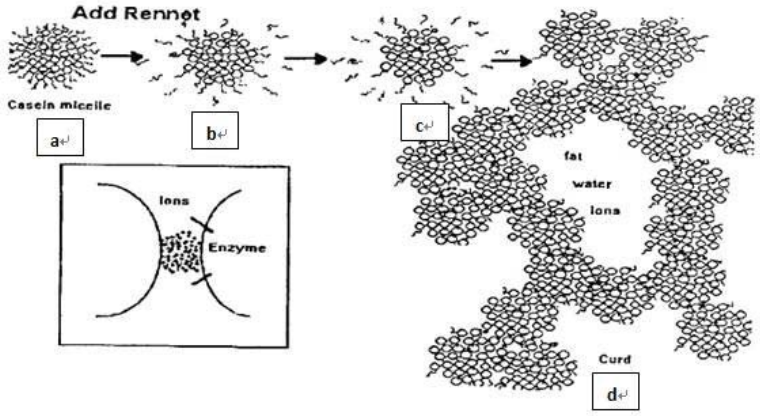


Figure 3. Action of the principle enzyme of rennet (chymosin) on the casein micelles during milk coagulation (Dalgleish and Corredig, 2012). (a, b) casein micelles with intact k-casein layer being attacked by chymosin; (c) micelles partially denuded of k-caseins; (d) extensively denuded micelles during aggregation.

(e) Syneresis

Cheese coagulum is stable if maintained under quiescent conditions, but it rapidly undergoes syneresis (expulsion of whey from cheese coagulum) when cut or broken (Fox et

al., 2000). Syneresis occurs when casein molecules re-arrange after a coagulum has formed resulting in tightening of the casein network, thereby removing moisture from the casein network; water activity of the curd is thus reduced (Fox et al., 2000; Fox and McSweeney, 2004; O'Callaghan and Guinee, 2004).

When first formed, the cheese coagulum has a water content of about 87%. Due to dehydration, syneresis essentially concentrates the fat and casein of milk by a factor of 6-12 with the water content of coagulum being reduced to between 20-56%, depending on cheese variety (Banks, 1998; Singh et al., 2003; Fox and McSweeney, 2004; Singh and Cadwallader, 2008). Moisture retention in the coagulum is influenced by variations in the processing conditions following curd formation. Of particular significance, is the rate of acid development which increases syneresis resulting in a firmer curd. The development of acidity during cheese syneresis is affected by the type of starters used (Banks, 1998). Increasing the temperature (cooking) of the curd after cutting additionally increases the rate of moisture removal from the curd, depending on the cheese variety (Fox et al., 2000). The extent to which the coagulum is cut, the agitation of the curd in the whey, together with the level of salting also determine the moisture level in the cheese (Banks, 1998).

(f) Salting

When cheese curds are salted, the concentration of the salt diffuses rapidly throughout the curd from the surface to the centre until equilibrium is reached, while the cheese curd undergoes intense syneresis.

The concentration of salt-in-cheese moisture has a major effect on the growth of microorganisms in cheese (Mcsweeney and Fox, 2004; Parente and Cogan, 2004). Salt increases the osmotic pressure of the aqueous phase of cheese, causing dehydration of bacterial cells and retard the growth of microorganisms, including pathogenic and spoilage bacteria, cheese starter cultures and secondary ripening microorganisms if applicable. However, although the growth of the starter reduces shortly after salting, metabolism of lactose continues, and the concentration of lactic acid gradually increases (Fox et al., 2000). The growth of *Lactococcus* strains used as starters is stimulated by low levels of NaCl but is strongly inhibited at levels above 5%, when metabolism of lactose may also terminate (Banks, 1998; Fox et al., 2000; Fox and McSweeney, 2004; Spinnler and Gripon, 2004). If starter activity (cell growth and lactose metabolism) is inhibited after manufacture owing to excessive level of salt-in-moisture (>5%), residual lactose will be metabolised relatively late during ripening, when the number of non-starter lactic acid bacteria (NSLAB) is high. Furthermore, some secondary ripening microorganisms are salt-tolerant and their growth is stimulated by low levels of NaCl. For example, the growth of *P. camemberti* on Camembert cheese is poor and patchy at levels below 0.8% NaCl.

2.1.2.2 Ripening

The control of acid production in milk and water activity of cheese curd during manufacture is not the only factor to be considered in producing cheese. For majority of cheese varieties, further controlled transformation of the product must be achieved during maturation. Ripening of cheese under controlled conditions of temperature and humidity determines the final flavour and body characteristics of cheese product through a complex set of biochemical reactions (Banks, 1998; Singh et al., 2003; Fox and McSweeney, 2004; Singh and Cadwallader, 2008). The primary biochemical changes involve the metabolism of residual lactose, lactate, citrate, lipolysis and proteolysis (Section 2.5.3). This is achieved by the activity of indigenous milk enzymes, protease and lipase derived from the rennet, as well as activity of LAB starters, and surface or internal micro-flora and their enzymes.

2.2 Camembert cheese

2.2.1 World consumption of surface mould-ripened cheeses

Surface mould-ripened soft cheeses are characterised by the presence of a felt-like coating of white mycelia due to the growth of *P. camemberti* on the surface (Spinnler and Gripon, 2004). Camembert and Brie are examples of surface mould-ripened cheeses, with Camembert being the most widely produced (Spinnler and Gripon, 2004). Compared to that of hard and semi-hard cheese products, the world production of surface mould-ripened soft cheeses may be low, but popularity and demand are growing among consumers (Fox and McSweeney, 2004). It was estimated that in 1999, surface mould-ripened cheeses represented 7-8% of the total production of cheese in 15 countries of the European Union and 2-3% of the world production (Banks, 1998; Spinnler and Gripon, 2004). The level of Camembert consumption is highest in France where soft cheeses constitute more than one-third of total production (Spinnler and Gripon, 2004; Johnson and Law, 2010). Meanwhile in India, the use of animal rennet is prohibited for religious reasons and this has kept production of surface mould-ripened cheese at very low levels.

2.2.2 Industry definition of Camembert cheese

Camembert is a soft mould-surface ripened cheese (Spinnler and Gripon, 2004; Codex Standard, 2010). It is classified as 'soft cheese', which is generally un-pressed, contains high moisture. At present, there is no definition for Camembert cheese in the New Zealand Food Law. Under Codex Standard (2010) and French Food Law (Shaw, 1981), typical Camembert cheese has a flat cylindrical form of approximately 10.5-11 cm in diameter and 2.5-3.0 cm

thick. The interior of the cheese body is yellowish, its surface is covered entirely with a white fluffy-like mycelia due to the growth of *P. camemberti* (dominant ripening mould), but it may also have red, brownish or orange coloured spots (Kosikowski and Mistry, 1997; Spinnler and Gripon, 2004; Codex Standard, 2010). Gas holes are generally absent, but few openings and splits may be present (Codex Standard, 2010). The cheese is ripened from the surface to the centre producing a soft-textured (when pressed by thumb), but not crumbly.

Under French Food Law (Shaw, 1981), Camembert weighs between 210-260 g, and must have at least 110 g of total solids. The FDM is about 45%, but there may be variants with lower or higher fat contents. Compared to other cheese varieties, Camembert has higher water activity (about 0.98), with a comparatively low pH value (about 4.6).

Compared to hard and semi-hard cheese products, maturation of Camembert is achieved in a relatively short time (about 21 d). Ripened Camembert cheese was generally a soft plastic texture with a pronounced core which becomes semi-liquid as the cheese matures (Kosikowski and Mistry, 1997). However, some consumer markets prefer an older cheese ripened for 27-35 d characterised by a running liquid layer at the core when cut. The cheese has a delicate aromatic mushroom flavour and an ammonical aroma when fully ripe. Pronounced ammonia aroma, detectable through the package, however, indicates that the cheese is over-ripe and should be rejected.

2.2.3 Technology

A typical process for commercial manufacture of Camembert is illustrated in Figure 4 (Walstra et al., 2006) and Table 2 (Banks, 1998).

Camembert, without designation of origin, is made from raw milk with the addition of mesophilic starter. To facilitate the production of consistent high quality Camembert cheese throughout the year that complies with specific regulations, the cheese milk is pasteurized and standardized to appropriate protein-to-fat ratios prior to use.

The mesophilic starter bacteria used to make Camembert are mixed homofermentative and heterofermentative strains, including *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris* (Fox et al., 2000; Josephsen and Jespersen, 2004; Spinnler and Gripon, 2004). The starter cultures are added to the milk at 0.2-2% (Banks, 1998; Fox et al., 2000; Parente and Cogan, 2004; Josephsen and Jespersen, 2004; Spinnler and Gripon, 2004), and the milk is ripened for 7-9 h at 30-33°C. Secondary ripening microorganisms are important for Camembert production, especially a mould culture consisting of *P. camemberti*. In modern Camembert production, there are three ways of inoculating secondary ripening cultures, by adding the culture

spores to the milk, spraying a solution of the mould spores onto the cheese surfaces, or applying dry mould spores along with salt to the cheese surface (Kosikowski and Mistry, 1997; Bockelmann, 2010).

In the manufacture of Camembert, milk coagulation is achieved by acid-coagulation through lactic starter activity followed by enzymatic (rennet) coagulation. Acid-coagulation assists with enzymatic coagulation by lowering the pH prior to rennet addition (Fox et al., 2000; Dalgleish and Corredig, 2012). Rennet is added at about 0.017% and the pH at renneting is approximately 6.4. The coagulation time is 30-45 min and curd formation is allowed to proceed for approximately 2 hour (h) (Kosikowski and Mistry, 1997; Spinnler and Gripon, 2004).

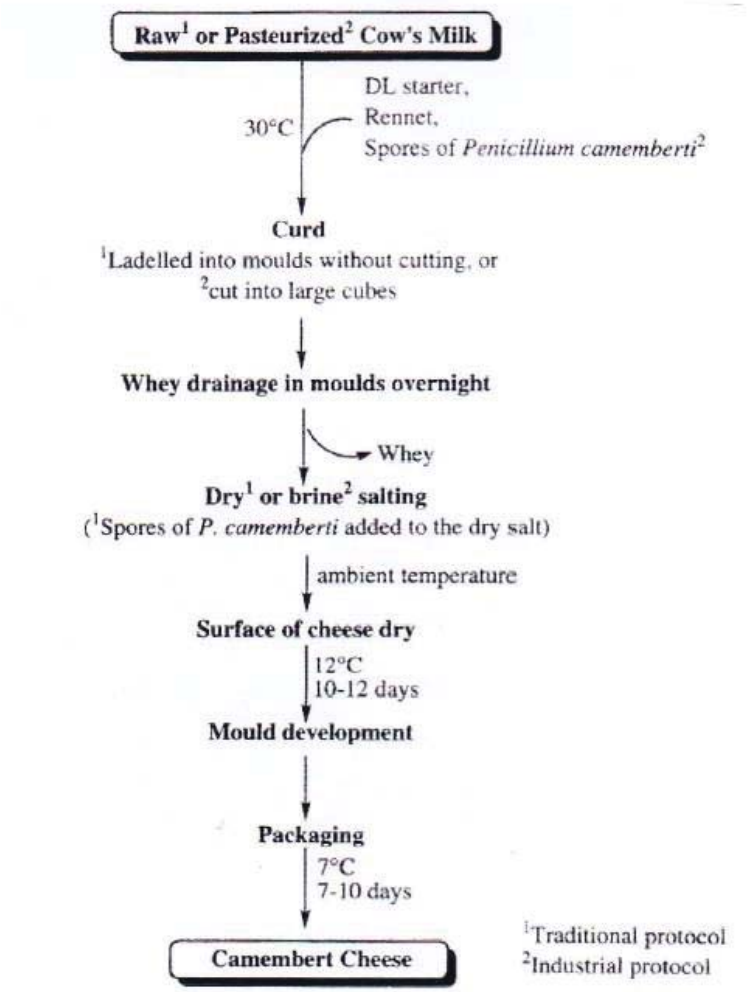


Figure 4. Manufacturing protocol for Camembert cheese (Walstra et al., 2006).

The extent of syneresis of Camembert is mainly influenced by how finely the coagulum is cut, pH after the curd is cut, and the coagulation time. The coagulum is cut into cubes of 2-2.5 cm/side and moulded 30-35 min after cutting to allow for faster syneresis. The pH of the curd at this stage is about 5.2-5.55. After transfer of the coagulum to mould containers,

draining takes place spontaneously through the perforated sides of the moulds during draining (16-24 h). Camembert cheeses achieve their shape during draining in mould containers. A typical container for moulding Camembert is in a flat cylindrical form (Ø = 10.5-11 cm; thickness = 2.5-3.0 cm) (Shaw, 1981; Kosikowski and Mistry, 1997; FSANZ, 2008; Codex Standard, 2010).

During the first hour of draining, curds are heated at 26-28°C, and then the temperature is gradually reduced to approximately 20 °C by end of draining while the curd is turned at intervals over the 3 h period of draining. During draining, intense acidification occurs in Camembert cheese curd and the ultimate pH of the curd when taken from the mould is 4.6-4.7 (Kosikowski and Mistry, 1997; Spinnler and Gripon, 2004). Generally, the higher the drop in pH after cutting the coagulum, the more moisture will be squeezed out of the curd (Fox et al., 2000).

Table 2. Manufacture of Camembert (Banks, 1998).

Milk preparation		
Day 1	Raw milk	(a) Pasteurize (b) Standardize protein to fat ratio (c) Add 0.2-0.5% starter at 8-12°C
Cheese manufacture		
Day 2	Milk starter	(a) Heat to 32-33°C (b) Add mould culture 0.001% (v/v) (c) Add rennet, 0.017% (v/v) (d) Curd formation, (120 min) (e) Cut curd, allow syneresis (15-30 min) (f) Transfer to mould, (pH 5.2-5.5) (g) Turn curd in moulds (1) First turn after 10 min (2) Second turn 40 min after (1) (3) Third turn 80 min after (2) (h) Leave for overnight (16 h)
Salting/Brining and drying		
Day 3	pH of cheese(4.9-4.95)	(a) Immerse in brine, 75-80 min (b) Remove cheese from mould, store in drying room at 16-17°C, with 70-80% RH, turn once
Ripening		
Days 4-10		Transfer curd to ripening room at 15°C, RH 70-80%, turn 3-4 times
Day 11		Dry surface mould, turn once, at 13°C, RH 90%
Day 12		Packing
Days 12-15		Cold store, at 3-4°C, RH 70%

After whey draining, curds are salted in two ways, by either dry salting or immersed in brine. At the brining stage, the curd is brined for 70-85 min, re-moulded for draining and placed in a dry room for 24 h. During draining and salting, the curd reduces water activity

and loses considerable amount of moisture. Under the French Food Law (Shaw, 1981), Camembert is a non-scalded and non-pressed cheese.

Camembert cheese is generally matured for a minimum of 21 d in controlled conditions at 11-13 ˚C with 90% RH (Kosikowski and Mistry, 1997; Law, 1997; Fox et al., 2000). The moisture-laden air prevents the cheese from drying as it ripens. Temperatures are kept cool, not only to encourage the activity of the ripening microorganisms, but to inhibit the growth of harmful bacteria that could spoil the cheese. During ripening, the surface micro-flora, in particular *P. camemberti*, starts to grow on day 5-6 and appears on curd mass on day 10. On day 12-14, the surface of curd is entirely covered with the white-like mycelia of the mould. There is, as a result, a marked increase in the external and internal pH of the curd during ripening. Curd surface increases steadily to about 7.0 at the end of maturation, whereas the increase is slower in the interior where the final pH is about 6.0. The ripened cheese has a shelf life of 4-6 weeks when stored under refrigeration temperatures.

2.3 Characteristics of lactic starter cultures and ripening bacteria important for Camembert cheese production

2.3.1 Introduction

The microflora associated with Camembert cheese production is extremely diverse, particularly when raw milk is used, which may be conveniently divided into two groups: (1) the lactic acid starter bacteria (lactic starters), and (2) the secondary microflora.

Mesophilic lactic acid bacteria have traditionally been associated with production of Camembert cheese and consist of 'starter' organisms as they initiate the production of acid in early cheese production. The common LAB genera used for Camembert cheese production are: (1) lactococci (*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*) and (2) *Leuconostoc* (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris)* species (Fox et al., 2000; Parente and Cogan, 2004; Spinnler and Gripon, 2004). LAB starters produce sufficient acid to reduce the pH of milk to desirable levels. Generally, milk pH<5.3 in 6 h at 30-37^{o}C, may be achieved depending on the cheese variety (Parente and Cogan, 2004). In addition to acid production, the starters also contribute to Camembert cheese ripening since their enzymes are involved in citrate metabolism, proteolysis and conversion of amino acids to flavor compounds and gases. Furthermore, by lowering pH and producing antimicrobial compounds (e.g., nisin), lactic starters also have a role in preservation and contribute to the microbial safety of cheese products (Parente and Cogan, 2004).

The presence of secondary microorganisms is essential in producing good quality

Camembert cheeses (Bockelmann, 2010). The composition of micoflora can be diverse when raw milk is used, which may include yeasts, (*Kluyveromyces lactis, Saccharomyces cerevisiae* and *Debaryomyces hansenii*), and moulds, (*P. camemberti* and *G. candidum*) and smear bacteria (*Corynebacterium* and *Brevibacterium linens*). Secondary ripening microorganisms do not play any active role during cheese fermentation, but are involved with starter bacteria in the ripening process to produce organoleptic and biochemical changes in or on the cheese (Law, 1997; Fox et al., 2000; Fox and McSweeney, 2004; Bockelmann, 2010). Traditionally, secondary microflora of Camembert originates from the milk, the cheese-making utensils and the factory environment. In modern production, use of pasteurized milk and high level of hygiene in equipment plant have reduced the sources of indigenous secondary flora. In an effort to produce good quality cheeses, the demand for secondary starters has increased (Parente and Cogan, 2004; Spinnler and Gripon, 2004; Bockelmann, 2010).

2.3.2 Lactic acid starter bacteria

2.3.2.1 Taxonomy of lactic acid bacteria

All dairy fermentations use LAB for acidification and flavor production. The term 'lactic acid bacteria' is used synonymously with 'milk souring microorganisms'. Most of the genera generally included in the LAB are *Aerococcus, Alloiococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus,* and *Weissella.* Only five genera *(Lactococcus, Enterococcus, Leuconostoc, Streptococcus, Lactobacillus*) contain organisms used as cheese starter cultures (Wright and Axelsson, 2012). This diverse group of Gram-positive bacteria is catalase negative, usually non-motile, and generally characterized as non-spore forming, non-respiring cocci and rods, which produce lactic acid as the major end-product during the fermentation of carbohydrates, and sometimes also volatile acids, ethanol and CO_2 (Fox et al., 2000; Parente and Cogan, 2004; Hoier et al., 2011). LAB can tolerate low pH environment and aerotolerant anaerobes that grow best under anaerobic conditions.

LAB consisting of several diverse genera are grouped as homofermentative or heterofermentative based upon the products produced from the fermentation of glucose (Table 3 and Figure 11 in Section 2.5.3.2) (Fox et al., 2000; Parente and Cogan, 2004). Homolactic fermentation produces almost exclusively lactic acid as the end-product, whereas heterolactic fermentation produces significant amounts of other end-products such as ethanol, acetate, and CO_2, in addition to lactic acid.

Table 3. End-products of lactose fermentation by various LAB (Hoier et al., 2011; Fox et al., 2000).

Organism	Transport system	Pathway	Main fermentation products[a]	Lactate isomer
Lactococcus spp.	PEP-PTS	Homofermentative	4 lactate	L
Streptococcus spp.	Permease	Homofermentative	2 or 4 lactate[b]	L
Group I *Lactobacillus* spp.	Permease	Homofermentative	2 or 4 lactate[b]	D or DL
Group II and Group III *Lactobacillus* spp.	Permease	Heterofermentative	lactate[c] + ethanol + acetate + 2 CO_2	D and/or L
Leuconostoc spp.	Permease	Heterofermentative	2 lactate + 2 ethanol + 2 CO_2	D

[a]Moles^{-1} mole lactose fermented.
[b]2 moles of lactate if galactose is stoichiometrically secreted, and 4 moles of lactate if galactose is fully metabolised.
[c]The stoichiometry of the fermentation products depends on the species and growth conditions.

2.3.2.2 Lactic starters important for Camembert cheese production

Lactic starter cultures used in cheese production may be classified into two groups based on their optimum temperature of growth (Parente and Cogan, 2004). Mesophilic starters have a growth optimum of about 30°C and thermophiles with optimum growth temperature of about 37°C or higher. Lactic starters are essential for producing large varieties of cheese (Table 4), irrespective of the type of milk. Generally, mesophilic starters are used in all cheese varieties in which temperature of the curd during the early stage of acid production does not exceed 40°C; whereas thermophilic starters are employed for cheeses which require high cooking temperatures of 38-40°C (Fox and McSweeney, 2004; Parente and Cogan, 2004).

Table 4. LAB species, of mesophilic and thermophilic, are employed in the manufacture of a broad array of cheese types and typical cheese products, irrespective of the type of milk (Law, 1997; Fox et al., 2000; Josephsen and Jespersen, 2004; Parente and Cogan, 2004; Hoier et al., 2011).

Culture types		Species names	Cheese application
Mesophilic	O-type	*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris;*	Cheddar, Feta, Cottage, Mould ripened soft cheeses (i.e., Camembert), Limburger and Brie
	L-type	*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *Leuc. lactis*, *Leuc. mesenteroides* subsp. *cremoris;*	Feta, Cheddar
	D-type	*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis;*	Cheddar, Caerphilly, Camembert, Limburger and Brie
	LD-type	*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuc. lactis*, *Leuc. mesenteroides* subsp. *cremoris;*	Gouda, Edam, Tilsitter and Mould ripened soft cheeses (i.e., Camembert, Roquefort and Blue vein), Limburger and Brie, Lactic butter, Cultured buttermilk, Continental cheese (with eyes)
Thermophilic	Streptococcus	*S. thermophilus;*	Mozzarella, stabilized Brie and Swiss
	Yoghurt	*S. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus;*	Mozzarella and Pizza
	Lactobacillus	*Lb. helveticus*, *Lb. delbrueckii* subsp. *lactis;*	Swiss and Grana
Mixed types (Mesophilic + Thermophilic)	RST type	*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *S. thermophilus;*	Chedder
	FRC type	*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *S. thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus;*	Feta and white brined

Grouping depends on the composition of cultures; lactic starters are classified into undefined-strains (natural and mixed) and defined-strain cultures (Parente and Cogan, 2004). Natural (artisanal) starter cultures and mixed-strain starters (MSS) contain a mixture of an unknown number of lactic acid bacteria, which are derived from artisanal production practice. Natural cultures are produced daily in cheese factories by some form of back-slopping. However, for many cheese manufacturers, they have been replaced by commercial MSS, derived from the 'best' natural starters and reproduced under controlled laboratory conditions. While the composition of MSS may be undefined, their reproduction under controlled conditions reduces the intrinsic variability associated with use of natural

starters (Parente and Cogan, 2004). Defined strain starters (DSS) are pure cultures, with known physiological characteristics and identifiable. Since the strain in DSS is defined, their technological performance is extremely reproducible (Parente and Cogan, 2004). Based on the composition of starters, MSS are generally used by small-scale producers, while DSS are used by the large-scale (Parente and Cogan, 2004).

In Camembert manufacture, undefined-MSS or defined-mixed lactic starters are used extensively, which may consist of single strains, combined mixed strains or multiple-species, composed of mesophilic acid-forming lactococci (mainly *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*), together with mesophilic citrate-utilizing strains, *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* species (mainly *Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*) (Table 4) (Law, 1997; Fox et al., 2000; Josephsen and Jespersen, 2004; Spinnler and Gripon, 2004; Hoier et al., 2011).

Lactococcus species are used extensively as starters for Camembert production (Law, 1997; Spinnler and Gripon, 2004). *Lactococcus* (formerly group N streptococci) are spherical or ovoid Gram-positive cocci, of 0.5-1.5 μm in size, usually non-motile and occur singly, in pairs, or elongated chains (Fox et al., 2000; Wright and Axelsson, 2012). They are mesophilic, ferment hexoses homofermentatively with more than 95% of their end-product as L (+) lactic acid only (Table 3). Of the species under the genus *Lactococcus*, *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* are of major significance (Hoier et al., 2011). The main difference between the subspecies is the salt tolerance and ability to hydrolyze arginine, both typical for subsp. *lactis* but absent in *cremoris*. It is generally believed that *L. lactis* subsp. *cremoris* gives better flavored cheese than *L. lactis* subsp. *lactis* (Fox et al., 2000). The diacetyl-producing variants of *L. lactis* subsp. *lactis* with the ability to ferment citrate are often referred as biovariance (biovar.) *diacetylactis* (Hugenholtz, 1993; Beresford and Williams, 2004; IDF, 2006).

Leuconostoc can also be part of mesophilic lactic starters used for Camembert, when in associative growth with lactococci (Table 4) (Fox et al., 2000; Parente and Cogan, 2004; Josephsen and Jespersen, 2004; Spinnler and Gripon, 2004). *Leuconostoc* are Gram-positive, spherical or lenticular cells that occur in pairs and chains; the microorganisms are catalase negative, arginine negative, produce gas from glucose and produce primarily the D (-) lactate isomers (Law, 1997). In contrast to the lactococci, leuconostocs are not competitive growers or important producers of lactic acid in cheese milk, but are important for eye and flavor development (Hugenholtz, 1993; Parente and Cogan, 2004). *Leuconostoc* spp. ferment sugars heterofermentatively rather than homofermentatively, producing lactate, ethanol and CO_2 in associative growth with the *Lactococcus* spp. (Table 3), as well as diacetyl, CO_2, and acetoin from citrate (Figure 13, Section 2.5.3.2). Currently, only two species of *Leuconostoc* have been associated with Camembert starter cultures, *Leuc. lactis* and *L. mesenteroides* subsp. *cremoris* (Spinnler and Gripon, 2004).

Depending on their metabolism and composition, mesophilic lactic starter cultures are further differentiated as O-, L-, D- and LD- type cultures (Table 5) (Parente and Cogan, 2004). O-cultures contain homofermentative acid-producing citrate$^-$ lactococci (*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*), that produce exclusively lactic acid without any gas. Heterofermentative citrate$^+$ organisms are found in L-, D- and LD-cultures, which produce lactic acid and characteristic aroma compounds (ethanol, acetaldehyde, diacetyl and acetate) and CO_2 from citrate during cheese fermentation and ripening. L-cultures contain *Leuconostoc* species (*Leuc. lactis* and *Leuc. mesenteroides* subsp*. cremoris*), while D-cultures are *L. lactis* subsp*. lactis* biovar. *diacetylactis*. LD cultures contain citrate-fermenting lactic bacteria consisting of L-and D-cultures.

Mesophilic O-type lactic cultures have traditionally been associated with production of Camembert cheese production, with *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* as dominant species used in acidification during milk fermentation (Table 5) (Law, 1997; Spinnler and Gripon, 2004). Traditionally, with the exception of certain fermented milk products, heterofermentative LAB are rarely used as dairy starter cultures, although they are not uncommon in milk and dairy products. However, use of LD-type for Camembert production has been widely reported due to their desirable role in flavor formation (Table 5) (Law, 1997; Spinnler and Gripon, 2004). Cit$^+$ *L. lactis* subsp*. lactis* biovar. *diacetylactis* vigorously ferments lactose to lactic acid, producing high amount of CO_2; whereas *Leuconostoc* spp. produce CO_2 more slowly and grows poorly in milk (Bjokroth and Holzapfel, 2006; Teuber and Geis, 2006; Walstra et al., 2006). Thus, the leuconostocs are always used in combination with the Cit$^-$fermenting *L. lactis* cultures. *Leuconostoc* spp*.* can metabolise citric acid in milk into diacetyl at pH 4.5-4.6, a pleasant buttery aroma compound, but must be combined with an acidifying *lactococci* strains in order to produce a lactic curd (Monnet et al., 1994). Their ability to produce diacetyl has therefore led to their frequent incorporation into O-type starter cultures of Camembert cheese. According to the Codex Standard for Camembert (2010), gas holes are generally absent in this cheese, but few openings and splits are acceptable. If allowed to grow to significant numbers, Cit$^+$ lactococci and *Leuconostoc* can cause openings (defects) due to excessive amount of CO_2 production, which is undesirable in Camembert (Fox et al., 2000; Fox and McSweeney, 2004; Hoier et al., 2011). Therefore, practically, Cit$^-$ lactococci must dominate in the formulated culture mixture and generally make up about 60-90% of the microorganisms present, whereas the Cit$^+$ lactococci and *Leuconostoc* should make up the rest (Fox et al., 2000; Josephsen and Jespersen, 2004). Cogan et al. (1997) studied 4379 isolates from 35 artisanal dairy products, including 24 artisanal cheeses, and identified 10% of the LAB strains as *Leuconostoc* spp. In general, L and D cultures are used to only a minor degree in the cheese industry including Camembert (Fox et al., 2000; Parente and Cogan, 2004; Hoier et al., 2011).

Table 5. Composition of different types (O-, L-, D- and LD type) of mesophilic starter cultures for cheese types with few or small eyes (Fox et al., 2000; Josephsen and Jespersen, 2004; Bjokroth and Holzapfel, 2006).

Type	Homofermentative acid-producing citrate$^-$ lactococci		Composition
	O-type	*L. lactis* subsp. *lactis,*	2-10%
		L. lactis subsp. *cremoris;*	90-98%
	Heterofermentative acid-producing citrate$^+$ organisms		
	L-type	*L. lactis* subsp. *lactis,*	5-10%
		L. lactis subsp. *cremoris,*	80-90%
		Leuconostoc. spp (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*);	5-10%
	D-type	*L. lactis* subsp. *lactis,*	5-10%
		L. lactis subsp. *cremoris,*	70-85%
		L. lactis subsp. *lactis* biovar. *diacetylactis;*	10-20%
	LD-type	*L. lactis* subsp. *lactis,*	5-10%
		L. lactis subsp. *cremoris,*	60-80%
		L. lactis subsp. *lactis* biovar. *diacetylactis,*	10-20%
		Leuconostoc. spp (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*);	5-10%

2.3.3 Secondary ripening microflora

When raw milk is used, Camembert cheese contains diverse secondary microorganisms that may include yeasts (*K. lactis, S. cerevisiae, D. hansenii*), moulds (*P. camemberti*, *G. candidum*), and bacteria (Corynebacterium and *B. linens*). The surface flora of Camembert cheeses made from pasteurized milk is however less diverse containing moulds (*P. camemberti* as the most important) together with added LAB as starters (Law, 1997; Kosikowski and Mistry, 1997; Fox et al., 2000; Spinnler and Gripon, 2004; Bockelmann, 2010; Hoier et al., 2011).

The genus *Penicillium* (*P.*) belongs to the group of Ascomycetes (Frisvad and Samson, 2004); and the organism has 'floccose' colonies with white aerial and fluffy mycelium (Figure 5 a) (Samson et al., 1977; Frisvad and Samson, 2004). For many years, *P. camemberti*, also known as *P. album*, was traditionally accepted as white mould for Camembert cheese. However, *P. camemberti* changes to a grayish appearance after several days of ripening, although its mycelia are white but the spores are gray. Therefore, since 1910 (Bockelmann, 2010), *P. caseicolum* has become widely used in Camembert production. *P. caseicolum* is a white variant of *P. camemberti* that remains a perfect snow-white colour during ripening of Camembert where permanent white surface is desired. Commercially, *P. caseicolum* is sold under the name *P. candidum* (Kosikowski and Mistry, 1997; Bockelmann, 2010).

Table 6. Different forms of white variants of *P. camemberti* (*P. caseicolum* or *P. candidum*) sold commercially.

(a)	A form with a fluffy mycelium, white at first becoming grey green;
(b)	A form with 'short hair', rapid growth, white, close-napped mycelium
(c)	A form with 'long hair', rapid growth, white, loose, tall mycelium;
(d)	'Neuchatel form', which vigorous, rapid growth, giving a thick white-yellow mycelium;

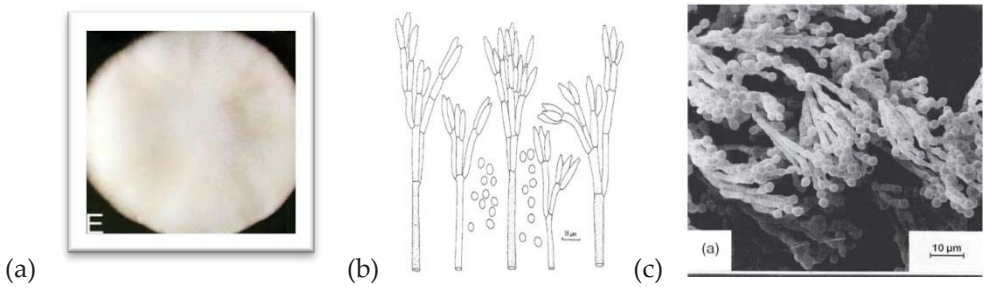


Figure 5. Morphology of *P. camemberti* species. (a) Floccose colony in *P. camemberti* (Frisvad and Samson, 2004); (b) Conidiophores and conidia of *P. camemberti* (Bockelmann, 2010); (c) Scanning electron micrographs of conidiophores of *P. camemberti* grown in submerged culture (Bockelmann, 2010).

Commercial strains of *P. caseicolum* (*P. candidum*, the white variant of *P. camemberti*) differ mainly in their rapid growth rates on cheese, the density and height of their mycelia on cheese surface with white fluffy appearance (Table 6) (Bockelmann, 2010). *P. camemberti* develops positive interactions with lactic starter bacteria when growing under ideal temperature, humidity, moisture and salt concentration. *P. candidum* will quickly flourish within 6-7 d, dominating the microflora at the cheese surface and inhibiting contamination from pathogenic bacteria (*L. monocytogenes*) or undesirable foreign moulds. In addition to the strong impact on the appearance, the technology characteristics of this white variant of *P. camemberti* such as glycolysis (breakdown of lactate), proteolytic and lipolytic enhance curd de-acidification (Section 2.5), flavor development (Section 2.6) as well as promotes a softening of body texture (Section 2.7). Furthermore, de-acidification activity of *P. camemberti* promotes acid-sensitive smear bacteria such as *B. linens* to grow, which is essential for the ripening of the cheese due to the development of typical sulphur aroma (Leclercq-Perlat et al., 2004a; Spinnler and Gripon, 2004; Bockelmann, 2010).

G. candidum is another secondary mould culture that is frequently added with *P. camemberti* and lactic starters in the production of bloomy-rind Camembert cheeses. The organism is classified as a mould but possesses yeast-like characteristics. *G. candidum* is sensitive to salt and a slight reduction in its growth occurs in the presence of 1% NaCl, and it is completely inhibited at about 6%. *G. candidium* colonises the cheese surface before the white *P. camemberti* growth commences and contributes to flavor development (Sections 2.6.2.2 and 2.6.3.2). The growth of the organism stimulates proliferation of *P. camemberti* on the cheese surface, but prevents it from being over proteolytic, which could lead to the bitterness defect.

The growth of *G. candidium* also causes a significant inhibition of fungal contaminations and undesirable microorganisms (Kosikowski and Mistry, 1997; Fox et al., 2000; Bockelmann, 2010).

Yeasts are also encountered in Camembert cheeses, with pre-dominant strains of *K. lactis*, *S. cerevisiae,* and *D. hansenii*. When pH of cheese surface is increased to 5.8 due to the action of *P. camemberti*, acid-sensitive smear bacteria flora such as *B. linens* may also be present on cheese surface. As a typical orange-red pigment smear bacteria, *B. linens* gives Camembert characteristic orange-red colour in appearance; the organism is also responsible for formation of aromatic sulphur-containing compounds due to their high proteolytic activity (Sections 2.6.2.2 and 2.6.3.2). However, as reported by Leclercq-Perlat et al. (2004a), the orange colour of cheese is commonly considered as a flaw for Camembert.

The microbiology of Camembert has been well-documented (Karahadian et al., 1985; Leclercq-Perlat et al., 2004a; McSweeney, 2004a; McSweeney, 2004b; Spinnler and Gripon, 2004; Lessard et al., 2012). The succession of microbial flora in Camembert and positive interactions with secondary ripening microorganisms and lactic starter bacteria are further discussed in detail in Section 2.5.2.

2.4 Cheese starter and ripening culture

2.4.1 Types of commercial preserved cheese cultures

'Back-slopping', the old tradition of taking the best of the previous day's fermented product and using it as inoculum or starter for the following day's production, is the earliest attempt at controlling the quality of cheese production. However, large variations are still common (Hoier et al., 2011). In modern cheese manufacturing, commercial starter bacteria have been traditionally applied as 'stock cultures' for preparation of 'bulk cultures' for the production of safe, and consistently high quality products (Fox et al., 2000; Parente and Cogan, 2004). Starters have been preserved in liquid form, in air-dried form (spray-dried and vacuum-dried), as frozen cultures and freeze-dried (lyophilized) cultures. Generally, classical liquid starter cultures has about 10^9 cfu/ml, while traditional frozen and freeze-dried (lyophilized) cultures contain $10^{10\text{-}11}$ cfu/ml, both of which must be further propagated in the factory (Parente and Cogan, 2004; Hoier et al., 2011). Bulk inocula are built up progressively from small volumes of 'stock culture' to larger volumes over several days until the desired volume is obtained. Although all these preparations are suitable as 'stock cultures' for inoculation of bulk cultures, frozen and freeze-drying preservation techniques are used most widely in the starter industry today (Tamime and Robinson, 1999; Parente and Cogan, 2004; Hoier et al., 2011).

Recently, direct-vat set cultures (DVS), also called direct-vat inoculation (DVI) cultures, have

placed greater emphasis on starter production, due to their ultimate convenience (Kindsedt, 2005; Hoier et al., 2011). DVS cultures are added directly to the cheese vat, therefore eliminate the need for bulk starter preparation and lower the risk of phage contamination in factory. DVS cultures are produced in either liquid frozen concentrate packed in aluminium cans, or as frozen concentrate available as pallet, or as powdered freeze-dried form packed in aluminium foil pouches (Fox et al., 2000; Kindsedt, 2005). DVS cultures differ from traditional bulk starters in two important ways (Kindsedt, 2005). Firstly, they are extremely concentrated and active. Frozen and freeze-dried DVS lactic starters typically contain sufficient cells of 10^{10}-10^{11} cfu/g and 10^{11}-10^{12} cfu/g, respectively, therefore the amount of DVS culture needed for acidification is noticeably smaller than that of traditional bulk culture. Secondly, in comparison to bulk starter as regard to acidification, time required for rehydrated DVS culture has generally been perceived as being a longer lag phase (Kindsedt, 2005; Hoier et al., 2011). The activity of DVS cultures are, however, normally higher than traditional bulk starter and the initial pH difference is overcome within a few hours (Figure 6). At initial cheese production, milk inoculated with DVS cultures typically show little or no change in acidity through renneting (the fall in pH by 0.05-0.1 pH units does not occur in the first h), and the resulting coagulum tends to be weaker due to the unchanged pH (Kindsedt, 2005). However, once acid production commences, DVS cultures produce acid more rapidly than traditional bulk cultures and the initial pH difference is overcome within a few hours (Figure 6) (Kindsedt, 2005; Hoier et al., 2011). Traditional cheese-making recipes assume that use of traditional bulk starter therefore often needs to be modified when DVS bulk starter is used. According to Kindsedt (2005), the manufacturer's recommendations concerning the amount of DVS starter to use are based on a quantity called a 'unit' (*U*). One *U* is the amount of starter that will turn a specific quantity of milk into lactic curd (without the addition of rennet) at a specific temperature in a certain amount of time (h).

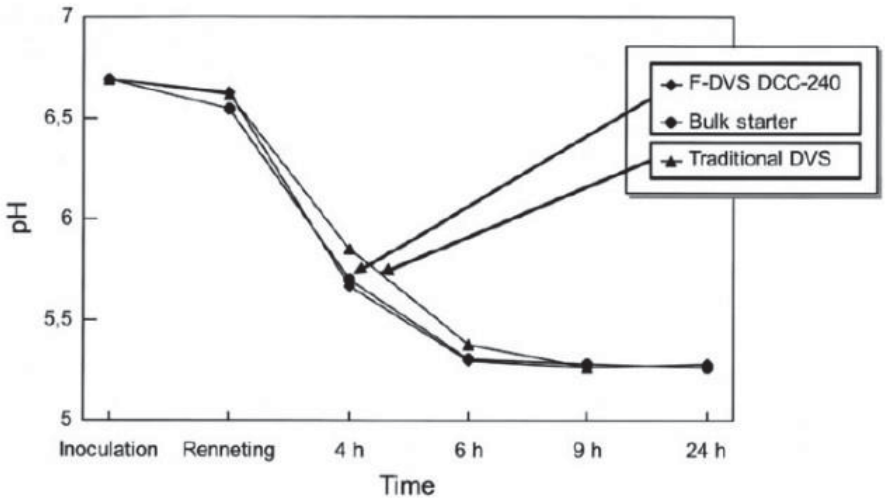


Figure 6. pH development in Gouda-type cheese manufactured using 2% bulk starter or 0.02% frozen direct-vat-set (DVS) cultures (Hoier et al., 2011).

2.4.2 Selection criteria for cheese cultures

The main criterion for selecting lactic starter bacteria is based on their viable cell counts and capability for rapid acid production (Parente and Cogan, 2004).

Generally, the population of classical liquid starter culture with about 10^9 cfu/ml and traditional frozen and freeze-dried cultures containing about 10^{8-9} cfu/g are desirable. However, the latter two types of cultures require further propagation in the factory prior to use for production. Concentrated frozen and freeze-dried starters, which may be used as DVS, must contain about 10^{11-12} cfu/g to assure the occurrence of sufficient dairy fermentation (Parente and Cogan, 2004; Hoier et al., 2011). In practice, the initial population of starters in cheese milk is around 10^6 cfu/ml at inoculation and reaches 10^9 cfu/ml when curd is transferred to the mould containers, typically after 5-6 h (Parente and Cogan, 2004). Cell inactivation in previous studies was a measurement of the number of colony forming units as a function of time (Ananta et al., 2005; Higl et al., 2007; Abe et al., 2009; Kurtmann et al., 2009c; Passot et al., 2012).

Closely linked to viability of the cell, lactic starters must be also screened for superior bacteriophage resistance (phage insensitivity), and rapid, constant acidification which gives optimal yield of lactate and flavor in the shortest possible time (Hoier et al., 2011). Acidification of lactic starter bacteria is commonly determined by measuring lactic acid production or the decrease in the pH of the culture grown under standardized conditions at a simulated cheese-making temperature profile (30°C for mesophiles or 40°C for thermophiles) such as in 10% (w/v) heat-treated reconstituted skim milk using a standardized inoculum (1%, v/v) (Nollet and Toldra, 2012). The acidification activity can be measured as (1) the time the starters takes to start acidifying milk from the inoculation time, or (2) the pH after a certain time (4, 6, or 24 h) of milk acidification at 30°C, 37°C or 43°C, depending on the characteristics of the starter culture, and (3) the time needed to acidify the milk to certain acidity level (pH≃5.3, the gelation point of cheese curd). Under the aforementioned conditions, milk pH usually reduces from 6.6 to less than 5.3 (equivalent to approximately 0.5%, w/v, lactic acid) (Fox et al., 2000; Nollet and Toldra, 2012). Cells which are not fully active or are sub-lethally stressed at inoculation usually show slower growth and consequently slower acid production.

Other technological properties of LAB strains should be considered based on their relevant metabolic capabilities in fermentation, as well as on the activity for the desired technological performance (Table 7) (Hoier et al., 2011). In regards to LD-type lactic starters, balance in culture composition must also be considered (Section 2.3.2). The type (form) of cultures and their stability during storage are also suitable selection criteria for the starters.

Table 7. Starter strains are selected with fulfils the selection criteria (Hoier et al., 2011).

Basic screening criteria	Specific selected criteria	Production
Acidification rate	Acidification rate in selected cheese-making profiles	Ease of production with high cell densities and activity
Reduced lag phase	Sugar (e.g. lactose) fermentation profile	Ease of concentration
Phage insensitivity	Controlled proteolysis of casein (proteinase and peptides activity)	Stability during freezing and drying
DNA and plasmid profiles	Controlled lipolysis of fat (lipases activity)	Storage stability
Species identification	Textural properties	Ease of production with high cell densities and activity
Flavour and off-flavour production in milk	Production of flavour compounds (taste and aroma)	
Antibiotic resistance	Strain interactions	
	Flavour screening in model cheese system	

The selected *P. camemberti* strains used in Camembert cheese production should possess appropriate density of growth and height of white mycelium on cheese after 6-7 d of ripening; and remains a perfect snow-white colour during ripening and storage at < 4°C for 10-35 d (Kosikowski and Mistry, 1997; Bockelmann, 2010). In addition to its impact on cheese appearance, glycolytic, proteolytic and lipolytic properties should also be determined. High initial cell numbers of the mould in the initial stage of cheese milk fermentation is not considered important due to its dominant role in cheese ripening. Therefore, the cell count of 10^5 cfu/ml is adequate for the production of good quality Camembert cheese (Fox and McSweeney, 2004; Parente and Cogan, 2004).

2.4.3 Stability of freeze-dried cultures during long-term storage

The preservation of LAB starter cultures by freeze-drying is of increased interested due to lower costs in storage, transportation and easy handling (Morgan et al., 2006). The various manufacturing steps impose environmental stress on bacterial cells, such as freezing, drying, and rehydration, thereby negatively affecting their viability and activity (Morgan et al., 2006; Santivarangkna et al., 2007; Santivarangkna et al., 2008). Stability of freeze-dried cells can however be improved by application of protective compounds (mono-sugars and polymers) to the suspending medium in which the cells are to be frozen and freeze-dried.

Cell preservation is also required for storage. To maintain good cell viability, freeze-dried cultures should be stored at -18°C or lower under low vacuum (Tamime and Robinson, 1999; Parente and Cogan, 2004; Hoier et al., 2011). Suitable packages that prevent oxygen, moisture and light during storage of cultures are also suggested (Tamime and Robinson, 1999). The most popular types of packaging material for freeze-dried cells are glass vial, followed by laminated, and aluminum foil sachet.

2.4.3.1 Effect of storage temperature and moisture content on viability of freeze-dried cultures during long-term storage

Several studies have reported the adverse effects of high temperature during storage on viability of freeze-dried culture cells and recommended that a temperature of -18°C or lower is ideal for long-term storage of the cultures. Bozhana Gyosheva (1995) demonstrated that *S. thermophilus* strains kept in freeze-dried state at 6°C were viable for 10 years, with no significant change in morphological, biochemical and technological characteristics. Viability of freeze-dried *Lb. bulgarius* during storage at refrigeration (5°C) and room (20°C) temperatures were compared under controlled humidity; a higher survival rate was reported at 5°C (Castro et al., 1995). The results of Casta et al. (2002), and Bruno and Shan (2003) were in agreement with these observations. Viability of *Pantoea agglomerans* decreased by 0.5 logs after 90 d at 4°C, compared to a decrease of 3 logs after 28 d at 25°C (Casta et al., 2002). Viability of bifidobacteria (*B. longum*) in freeze-dried probiotic products at -18, 4 and 20°C during prolonged storage was assessed by Bruno et al. (2003), and storage at 20°C showed the greatest decline in cell viability than at -18°C. Storage at elevated temperatures (2, 22 and 35°C) also correlated with a higher decline of viable bacteria cells of freeze-dried *Enterococcus* (*E.*) *faecium* and *Lb. plantarum* (Strasser et al., 2009).

Generally, temperature has important influence on water activity (a_w). According to many previous studies (Castro et al., 1995; Schebor et al., 2000; Vasiljevic and Jelen, 2003; Wang et al., 2003; Higl et al., 2007; Abe et al., 2009; Aschenbrenner et al., 2011), there is a strong temperature dependency for freeze-dried cultures exposed to high a_w when stored at high temperatures. For example, the initial a_w of *Lb. paracasei* ssp. *paracasei* in a lactose matrix after freeze-drying process was 0.12; after storage at 20°C for 25 d, the a_w of samples increased to 0.23 (Higl et al., 2007). Although moisture is retained in cells after freeze-drying, the increased a_w of cells during storage is largely attributed to the RH of the storage environments, and this is especially true when the dried starter cultures are filled in moisture permeable packages. It was shown that survival of freeze-dried *Lb. paracasei* ssp. *paracasei* embedded in lactose matrices decreases faster with increasing a_w and storage temperature (Higl et al., 2007). The combined adverse effects of storage temperature and moisture content on bacterial survival is also in agreement with many other studies which investigated different strains, including bifidobacteria (Abe et al., 2009), *Lb. bulgarius* (Castro et al., 1995), *Lb. acidophilus* (King et al., 1998), *S. thermophilus* (Andersen et al., 1999), *Saccharomyces* (*S.*) *cerevisiae* (Lodato et al., 1999), *Uromyces phaseoli* (Schein and Rotem, 1965) and biological materials such as soybean seeds (Sun and Leopold, 1994).

2.4.3.2 Possible inactivation mechanisms of freeze-dried starter and ripening cultures during storage

Microbial inactivation is highly complex. Viability loss of dehydrated bacterial cells during storage have been reported due to inter-related deteriorative processes, which involve both oxidation and browning reactions, as well as the physical instability of the bacteria membrane/cell wall which largely relate to glass transition reactions (Garcia, 2011; Zamora and Hidalgo, 2011).

Oxidative damage by membrane lipid oxidation reactions

Decrease in viability of freeze dried lactic acid bacteria in freeze-dried form has been reported during storage period at refrigeration (5°C) and room (20°C) temperatures under controlled humidity condition (11%) (Castro et al., 1997). The inactivation of cells was attributed to changes in the fatty acid profile of the membrane (increase in the proportion of saturated fatty acids to un-saturated fatty acids), for which lipid oxidation is an important cause. Oxidative damage has been linked to cell death due to loss of membrane structure and functionality (Santivarangkna et al., 2008). A leakage of DNA and RNA from bacterial cell could be also due to the damage of cell membrane. Moreover, the formation of free radicals is the consequence of oxidation reactions. The formed free radicals could initiate high amounts of oxidation reactions by attacking fatty acid moieties of the membrane (Santivarangkna et al., 2008; Garcia, 2011).

Browning discoloration of bacteria cells due to browning reactions

A correlation between browning discoloration of freeze-dried bacterial and viability loss during storage has been reported by Kurtmann et al. (2009c), Higl et al. (2007) and Carvalho et al. (2007), and the browning formulation was suggested to relate to non-enzymatic browning reactions, known as Maillard reaction. *Lb. acidophilus* (La-5) and *Lb. paracasei* ssp. *paracasei* freeze-dried in lactose matrix showed visible brown discoloration after a short storage at relatively mild conditions (a_w=0.22 at 30°C, a_w=0.17 at 37°C and a_w=0.29 at 20°C, respectively), and the browning formulation coincided with bacteria inactivation (Kurtmann et al., 2009c). Such a relationship is in agreement with the plant seed science study of Murthy et al. (2003), where an interaction between the formation of Maillard reaction products during storage and a decreased enzymatic activity were demonstrated. The death of bacteria was not a factor for the browning processes (Kurtmann et al., 2009c).

Under oxidizing conditions, Maillard reactions are likely to occur by condensation (in presence of moisture) between free amino groups and free carbonyl groups (Nollet and Toldra, 2012). Such free carbonyl groups involved in the reactions are mainly lactose in concentrated bacterial suspensions (Carvalho et al., 2007; Kurtmann et al., 2009c); while free

amino groups are nucleophilic groups of DNA/RNA in cells with damaged membrane. The peptidoglycan layer of the Gram-positive *Lb. acidophilus*, was also suggested to partly degrade and hydrolyze during freeze-drying and storage, forming reducing carbonyl groups, which may slowly undergo condensation reactions with available amino groups, resulting in browning of freeze-dried cells (Kurtmann et al., 2009c). Lipid oxidation and Maillard reaction are inter-related as products of lipid oxidation are able to promote Maillard reaction (Kurtmann et al., 2009c; Zamora and Hidalgo, 2011). Lipid carbonyl, formed as secondary oxidation products from lipid oxidation, was suggested to participate in browning reactions through reactions with amino groups in Maillard-like reactions.

Inactivation of β-galactosidase enzyme

Reduction in viability of LAB starters is also possibly attributed to the inactivation of β-galactosidase within in the cell membrane (Champagne et al., 1996; Mazzobre et al., 1997; Vasiljevic and Jelen, 2003). In lactococci and Leuconostoc species, once lactose is driven to inside of cell membrane, β-galactosidase is responsible for converting it to glucose and galactose-6-phosphate, and both hexoses are then metabolised concurrently to pyruvate and subsequently to lactic acid (Marshall, 1992). In a study of Mazzobre (1997) where the thermal stability of β-galactosidase stored at various temperatures were studied, the loss of enzyme activity was observed during storage even when stored at low frozen condition. The β-galactosidase activity in *B. longum* lost during storage at 20°C was about twice that observed at 4 and -20°C, while viable cell count losses at 20°C were approximately a hundred times greater than those at 4°C (Champagne et al., 1996). The decreased activity of β-galactosidase therefore may be a limiting factor for cell viability at higher storage temperature.

Glass phase transition and the role of glassy state on bacterial storage stability

Cell viability is also closely related to the glass properties of amorphous cell matrices (Santivarangkna et al., 2008). Loss of cell viability were largely reported due to occurrence of glass transition, which is attributed to a combined effect of elevated storage temperature and high relative humidity of environment above its glass temperature (Figure 7) (Lodato et al., 1999; Bhandari and Howes, 1999; Cerrutti et al., 2000; Fonseca et al., 2001; Ananta et al., 2005; Higl et al., 2007; Santivarangkna et al., 2008; Kurtmann et al., 2009a; Kurtmann et al., 2009b; Santivarangkna et al., 2011).

According to Potts (1994), the glass state may not exist on bacteria that does not formulate with carbohydrate. Fonseca et al. (2001) also showed that glass transition temperature (T_g) of washed cells of *Lb. bulgarius* cannot be detected. The glassy state of dried cells was however ascribed to the incorporation of carbohydrate solutions before freeze-drying (Bhandari and Howes, 1999; Cerrutti et al., 2000; Ananta et al., 2005; Thomsen et al., 2005; Kurtmann et al.,

2009a; Kurtmann et al., 2009b).

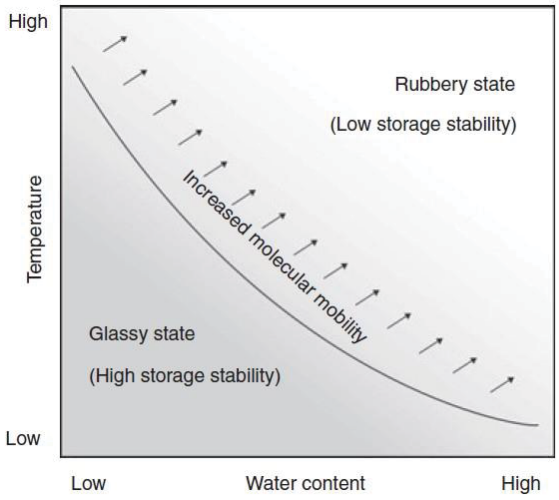


Figure 7. A simplified state diagram shows the glass transition curve which relates the glass transition to storage temperature and cell moisture content. Molecular mobility and deleterious reaction rates in the glassy state are extremely low, while they increase at the storage condition above the curve (rubbery state) (Santivarangkna et al., 2008).

Entrapment of a living cellular system by forming an amorphous glassy matrix, where individual bacterial cells are embedded, and membrane of cells is protected has been suggested as being responsible to their long-term stability (Sun et al., 1996; Lodato et al., 1999; Higl et al., 2007; Miao et al., 2008; Kurtmann et al., 2009a; Kurtmann et al., 2009b; Kurtmann et al., 2009c). Cells in dehydrated states form supersaturated, super-cooled, amorphous structures, typically referred to as rubbery (amorphous liquid) and glassy (amorphous solid) states (Figure 8) (Roos, 2002; 2004). The glassy state is referred to as amorphous metastable state that resembles a solid characteristic/appearance but with a molecular arrangement that is more typical for liquids. A glass exhibits a high molecular packing that has an extremely high viscosity (typically $\geq 10^{12}$ Pa.s) and shows low molecular mobility transition. Upon heating above T_g, glass transition (the transformation of solid-like to liquid state) occurs, where molecules gain translational mobility and enter a liquid-like state (rubbery, amorphous liquid) with a typical viscosity ranging from 10^6-10^8 Pa.s (Figure 8). Viscosity of mono- and di-saccharides decreased 4-6 orders of magnitude when increasing the temperature above T_g (Higl et al., 2007).

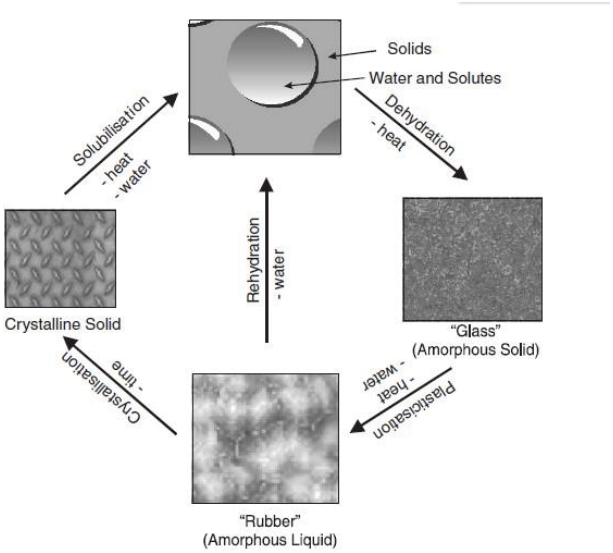


Figure 8. Formation of amorphous structure and the relationship between equilibrium (solution, crystalline solid) and non-equilibrium (amorphous solid and liquid) states (Roos, 2002; 2004).

The glassy structure of amorphous matrix is a highly effective environmental barrier with an extremely low molecular mobility. With respect to the preservation of bacteria, this condition leads to a suppression of unexpected deteriorating events on bacterial membranes, which constitute the interface to the surroundings and are predominantly exposed to various environmental abuses (Figure 9). As previously mentioned, lipid oxidation of membrane fatty acid is responsible for cell death during storage. Since translational diffusion is drastically restricted in the glassy state, the diffusion of oxygen, which preceded oxidative damage, could be limited. Other diffusion-controlled chemical reactions, such as time-dependent structure changes (collapse and sugar crystallization) and Maillard reactions, are also significantly prevented (Roos, 2002; 2004; Higl et al., 2007; Miao et al., 2008; Santivarangkna et al., 2011). Other deterioration events such as fusion of membranes, leakage of freeze-dried cells, and protein unfolding may be also reduced (Santivarangkna et al., 2011).

To reduce the occurrence of glass transition, freeze-dried culture cells should be stored at temperature below its T_g. Although T_g is an important term to understand the stability of microorganisms, it cannot be used by itself to characterize a material as it is also water-dependent (Thomsen et al., 2005; Abe et al., 2009). Aforementioned, freeze-dried cultures are prone to exposed to high a_w when they are stored at high temperatures. The moisture uptake decreases the T_g of the system (Figure 10), and rates of deteriorative reactions may be accelerated at high a_w level. For example, dry pure lactose has T_g of 101°C corresponding to a_w of 0. The T_g was found to be equal to the storage temperature of 25°C at a_w 0.395; at a_w of 0.30, T_g was found at 38 °C (Thomsen et al., 2005). According to Thomsen et al. (2005), the a_w limit for a glassy state of freeze-dried *Lb. paracasei* ssp. *paracasei* in a lactose matrix for a storage temperature of 37°C was $a_w \approx 0.17$, and for 20°C it was $a_w \approx 0.29$. Castro

(1995) demonstrated the effect of humidity on the lipid oxidation profile that at high relative humidity level, lipid oxidation occurred more rapidly and cell viability was also decreased to a higher degree. For pure amorphous lactose, crystallization was observed at a_w>0.40 at 20°C, a_w>0.32 at 30°C and a_w>0.31 at 38°C (Roos, 2002; Thomsen et al., 2005). Increased a_w of the cellular system could also accelerate the Maillard reaction (Kurtmann et al., 2009c). The lactose hydrolysing activity of β-galactosidase was also reported to be critically dependent on the a_w at which the lyophilized bacteria were kept (Vasiljevic and Jelen, 2003). High a_w level correlated with significant β-galactosidase activity loss. In addition, higher cell viability loss has been also reported by ABE et al. (2009) and Wang et al. (2003) and at increasing a_w environment after high temperature storage. The above discussion highlights the needs to take consider the storage condition of freeze-dried culture samples with regards to temperature and a_w/moisture content.

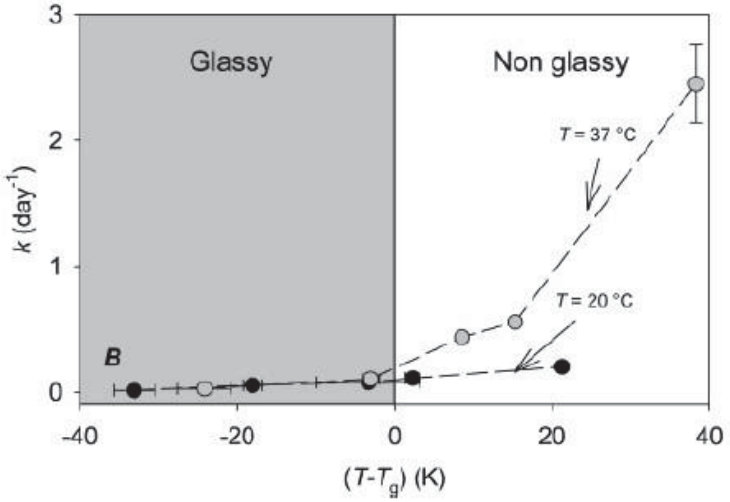


Figure 9. Inactivation rate of freeze-dried *Lb. paracasei* subsp. *paracasei* in a lactose matrix (Higl et al., 2007).

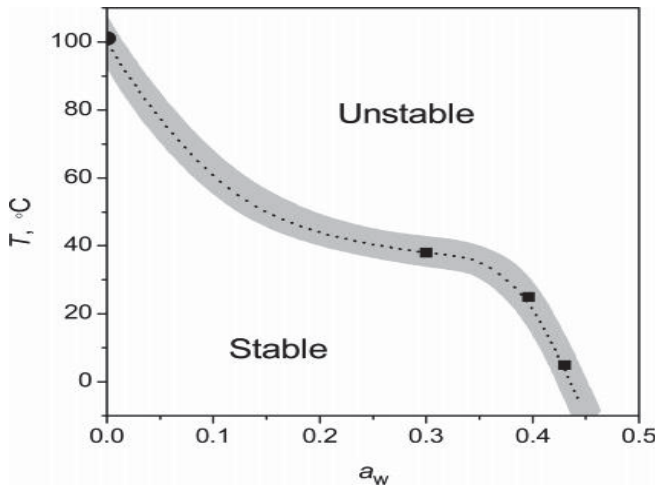


Figure 10. Water activity-temperature state diagram of amorphous lactose (Thomsen et al., 2005). The gray area indicates a transition zone separating stable amorphous lactose from unstable non-glassy lactose. The dotted line marks the borderline, where the storage temperature equals the glass transition temperature.

As reviewed by Santivarangkna et al. (2011), although glasses are often required for stabilization, various reports revealed that glassy state does not fully explain the complex bacteria inactivation. Therefore, T_g cannot be regarded as an absolute threshold of freeze-dried bacterial stability during storage because not every inactivation process is diffusion-limited (Tan et al., 1995; Lodato et al., 1999). The aging of 'glassy materials' has also been widely reported indicating that molecular motions do occur below T_g. In a study by Schebor (2000), significant loss of fermentative activity was observed in heated (45 and 55°C) freeze-dried *S. cerevisiae* kept below its T_g (64°C), which indicated that the relationship between biological stability (as measured by loss of fermentative activity) of yeast and the glass transition was not verified. Mazzobre et al. (1997) and Schebor et al. (2000) speculated the glassy state as a 'hurdle' effect rather than the sole factor determining enzyme stability. Loss of enzyme activity was observed during storage in glassy conditions, suggesting that, although molecular mobility may be significantly decreased in the glassy state, the protein molecules are still mobile enough to lead to enzyme inactivation. Works on dehydrated cells also indicated that the glass state does not play major protective role against membrane lipid oxidation. The free radical reactions are not diffusion-controlled, and therefore oxidation process may be not completely eliminated in the glassy state, although the diffusion of oxygen into the dry matrices is slow, which decreases the production rate of the free radicals (Lievense and Riet, 1994; Andersen et al., 1999; Buitink and Leprince, 2004; Kurtmann et al., 2009a; Santivarangkna et al., 2011). Non-enzymatic browning reaction were also shown to proceed at a slow rate even at well below the T_g (Karmas et al., 1992; Bell and Hageman, 1996). Since certain 'mobility' persists at below T_g, inactivation rate of freeze-dried bacteria stored frozen may result in significant changes if the time scale of observation is long enough.

The protection capacity of carbohydrate-amorphous matrix in conferring storage stability is dependent on their the glass forming capability of the constituents, correlating to the molecular weight of the compound (Bhandari and Howes, 1999; Santivarangkna et al., 2011). In comparison to low molecular weight monomers (e.g. fructose, glucose) in their pure form which have low T_g, disaccharides (e.g., sucrose, lactose, maltose) and oligomer sugars (e.g., maltodextrins, starch) provide higher storage stability due to higher T_g (Table 8). Ananta et al. (2005) investigated the capability of *Lb. rhamnosus* GG in different sugar medium to form glassy state. Although all evaluated carbohydrate-carriers were in the glassy state, a lactose matrix conferred a better protection on the probiotic bacteria than a sucrose matrix at elevated temperature. In addition to the physical stability of amorphous cell matrix, the protection ability of carbohydrates may further be explained by the water replacement hypothesis (Crowe et al., 1998; Higl et al., 2007). Carbohydrates form hydrogen bonds with membrane proteins and phospholipids when water is removed, thereby preventing protein denaturation and maintaining phospholipids bilayers which in turn preserve the structure of the membrane.

Table 8. Glass transition temperature of anhydrous sugars and carbohydrate polymers (Bhandari and Howes, 1999).

Food materials	Molecular weight	T_g (°C)
Fructose	180	5
Glucose	180	31
Galactose	180	32
Sucrose	342	62
Maltose	342	87
Raffinose	504	108
Lactose	342	101
Trehalose	342	110
Maltodextrins[a]		
DE 36	500	100
DE 25	720	121
DE 20	900	141
DE 10	1800	160
DE 5	3600	188
Starch		243 [b]

Notes: [a] Dextrose equivalent; [b] Predicted.

2.5 Microbiology and biochemistry of Camembert during manufacture and ripening

2.5.1 Introduction

Camembert cheeses are ripened for periods ranging from about 21 to 50 d (Kosikowski and Mistry, 1997). The ripening processing of cheese is very complex which involves microbiological and enzyme-catalysed biochemical changes of the curd resulting in changes to appearance, flavor and texture characteristics that affect the final quality of the cheese.

Microbiological changes during cheese ripening include the death and lysis of starter cells and the growth of secondary ripening cultures, in particular *P. camemberti*. The biochemical changes occurring during Camembert ripening may be grouped into primary events that include the metabolism of residual lactose, and of lactate and citrate by mesophilic LAB starters during glycolysis (Section 2.5.3.2). Proteolysis of milk caseins (Section 2.5.3.3) and lipolysis of fats (Section 2.5.3.4) by secondary ripening cultures, in particular *P. camemberti* also takes place. Secondary biochemical events are therefore very important for the development of flavor compounds.

Considering that flavor is the primary index of Camembert cheese acceptance by consumers, an overview of Camembert ripening is discussed in conjunction with characteristic flavor notes of the cheese. Camembert cheese texture softens during ripening as a consequence of

hydrolysis of casein micelle during proteolysis, changes to water-binding ability of the curd and pH are also reviewed in Section 2.7.

2.5.2 Microbiological changes of cheese and their interactions

The composition and evolution of the flora of Camembert cheeses is complex, particularly when raw milk is used which has very diverse secondary ripening cultures. The microbiology of Camembert is well-documented (Karahadian et al., 1985; Leclercq-Perlat et al., 2004a; McSweeney, 2004a; McSweeney, 2004b; Spinnler and Gripon, 2004; Lessard et al., 2012); and the succession of microbial flora in Camembert is determined by the changes in the manufacturing environment from the physico-chemical treatment (salt level, water activity, pH, etc).

The initial 24 h after the beginning of cheese-making is essentially dominated by mesophilic lactic starter bacteria (*L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*). However, *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* spp. (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*) may be also included in the starter cultures (Fox et al., 2000; Parente and Cogan, 2004; Spinnler and Gripon, 2004). The growth of lactic starters break down lactose and citrate in milk producing a young acid cheese curd containing high amount of lactic acid (lactate) (Section 2.5.3.2). When incubated under high humidity (85-98% RH) and at moderately low temperatures (10-15°C) for ripening, the acidic and high salt conditions of the young curd allow selective growth of yeast and filamentous *G. candidum* in early stages of ripening. The most common yeast flora found on the Camembert during ripening are *K. lactis, S. cerevisiae* and *D. hansenii* (Leclercq-Perlat et al., 2004a; Spinnler and Gripon, 2004; Lessard et al., 2012). While the salt tolerance yeasts are hydrolysed proteins and fat on curd surface, the environment becomes favorable for the growth of *P. camemberti*; the growth of *G. candidum* may be retarded due to its high salt sensitivity (McSweeney, 2004b).

The growth of *P. camemberti* is rapid and the proliferation of mycelia can be usually observed after 6-7 d of ripening due to germination of *P. camemberti* spores (Karahadian et al., 1985; Leclercq-Perlat et al., 2004a; McSweeney, 2004a; McSweeney, 2004b; Lessard et al., 2012). A dense layer of the mould covers the entire cheese surface with a typical felt-like coating of white mycelia within 8-10 d of ripening. Lessard et al. (2012) confirmed that the mycelia development of *G. candidum* and *P. camemberti* are responsible for the bloomy aspect of Camembert, with *G. candidum* and *P. camemberti* quickly dominaing the ecosystem (especially in the early ripening stage before day 10), while *K. lactis* remained less abundant.

Stimulated by the available oxygen, the filamentous fungi (*G. candidum* and *P. camemberti*), in particular the *P. camemberti*, rapidly metabolise lactate oxidatively to CO_2 and H_2O (Karahadian et al., 1985; Leclercq-Perlat et al., 2004a; McSweeney, 2004a; McSweeney, 2004b; Spinnler and Gripon, 2004; Lessard et al., 2012). Accompanied by the consumption of lactate

and the formation of alkaline metabolites (Section 2.5.3.2) during cheese ripening, the growth of *P. camemberti* de-acidify cheese at surface thereby increasing pH. After 15-20 d ripening with a high level of O_2 available, the increase in pH at cheese surface to 5.8 enables the appearance of an acid-sensitive surface bacteria flora consisting of coryneform bacteria (*B. linens, Arthrobacter, Micrococcus, Corynebacterium* and *Brachybacterium*) whose colonies are yellow or orange-colored (Karahadian et al., 1985; Law, 1997; Leclercq-Perlat et al., 2004a; McSweeney, 2004a; McSweeney, 2004b; Spinnler and Gripon, 2004; Lessard et al., 2012). The orange color of cheese is commonly considered as a defect in Camembert (Leclercq-Perlat et al., 2004a).

Camembert cheese made from pasteurized milk has a simpler flora and is initially only prepared by seeding mesophilic lactic starters and spores of *P. camemberti*. The population of other secondary ripening microorganisms is much reduced due to controlled manufacturing environment, and the cheese obtained has a neutral aroma that is perhaps not appealing. In order to diversify the organoleptic qualities and obtain products which are more typical and closer to traditional products, selected strains of *G. candidum* and coryneform bacteria (such as *B. linens*) may added to pasteurized cheese milk in addition to lactic starter bacteria and spores of *P. camemberti* (Beresford and Williams, 2004).

2.5.3 Biochemical reactions of cheese and their effects on cheese quality

2.5.3.1 An introduction

Numerous flavor compounds of Camembert involved in the cheese aroma are mainly derived from three major primary metabolic pathways: (1) glycolysis (metabolism of residual lactose, lactate and citrate (Section 2.5.3.2), (2) proteolysis (metabolism of milk casein) (Section 2.5.3.3), and (3) lipolysis (metabolism of milk fat) (Section 2.5.3.4). These primary changes are followed and overlapped by a host of secondary catabolic changes, such as free amino acid catabolism (Section 2.6.2.2) and free fatty acid catabolism (Section 2.6.3.2), which involve deamination, decarboxylation, and desulphurylation of amino acids, β-oxidation of fatty acids and esterification. The primary reactions are mainly responsible for the basic textural changes that occur in cheese curd during ripening, and are also largely responsible for basic flavor of cheese. Whereas the secondary transformations are mainly responsible for the delicate cheese flavor and modify cheese texture. The catalysts of these pathways are attributed collectively to (1) residual coagulant (chymosin and pepsin), (2) indigenous enzymes from milk (proteinase, lipase and phosphatases), (3) ripening enzymes from lactic starter bacteria and secondary micro-flora dominated by *P. camemberti*.

Lactose metabolism of curd by lactic starters, yielding lactic acid, largely influences the flavor of Camembert cheese. The metabolites of citric acid in milk, such as diacetyl (butan-2,3-dione) and possibly acetaldehyde, play major roles in cheese flavor (Hugenholtz,

1993; Urbach, 1995; McSweeney, 2004a; Spinnler and Gripon, 2004).

In addition to flavor compounds derived from lactose and citrate metabolism, lipids serves as other sources of cheese flavor. Lipolysis and oxidation cause hydrolytic and oxidative rancidity, respectively. Small amounts of γ- and δ-hydroxy acids present in milk lipids can be readily converted to γ-and δ-lactones. Unsaturated fatty acids may also hydrate to hydroxyl acids, and then via oxidation produce γ- and δ-lactones. β-oxidation, followed by decarboxylation, produces methyl ketones. Oxidation at double bonds can generate straight-chain aldehydes and ketones, which under reducing conditions may be converted to the corresponding alcohols (Molimard and Spinnler, 1996; Urbach, 1997; Sable and Cottenceau, 1999; McSweeney, 2004a; Spinnler and Gripon, 2004; Singh and Cadwallader, 2008).

Moreover, protein-derived flavors, which are derived from proteolysis and amino acid catabolism, also comprise a large amount of compounds in the volatile fraction of Camembert (Molimard and Spinnler, 1996; Urbach, 1997; Sable and Cottenceau, 1999; Yvon and Rijene, 2001; Spinnler and Gripon, 2004). Large peptides do not contribute to cheese taste, but can be hydrolysed by proteinases to shorter peptides that may be involved in undesirable bitter taste when accumulated to concentrations above their threshold. Products of proteolysis (small peptides and free amino acids) are significant in cheese taste as 'background' savoury, non-specific cheesy flavor, but are unlikely to contribute much to aroma as they are non-volatile. Of the produced amino acids, branched chain amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tyrosine and tryptophan), and methionine are the major precursors of flavoring compounds. Aldehydes can be synthesised via amino acids, and in turn may produce their corresponding alcohols. Meanwhile, sulphur-containing compounds can be derived from methionine metabolism.

Flavor profiles of Camembert have been reviewed (Molimard and Spinnler, 1996; Urbach, 1997; Sable and Cottenceau, 1999; Spinnler and Gripon, 2004). A matured Camembert cheese was characteristic mushroom, musty, and fruity notes associated with buttery notes. The flavor of Camembert cheese is the result of a complex mixture of numerous volatile compounds (Section 2.6). Among the large number of compounds present in cheese, oct-1-en-3-ol, in combination with its corresponding methyl ketone (oct-1-en-3-one), produces a characteristic mushroom-like sensation of Camembert cheese. Their presence, however, might partially mask the odorous effect of other methyl ketones, also present in the cheese. 2-phenylethanol and its corresponding ester, 2-phenylethanol acetate, are also quantitatively important as they contribute flora and fruity note of cheeses. Sulphur-containing compounds, such as methanethiol and dimethyl disulfide, are also present, and for ripe Camembert, contribute a significant garlic note in Camembert; while daicetyl gives the buttery note. The pungent and sweaty character is mainly caused by butyric acids.

2.5.3.2 Glycolysis (metabolism of residual lactose, and of lactate and citrate)

Metabolism of residual lactose

Although 96-98% of lactose is removed in the whey as lactose or lactate, the fresh cheese curd usually contains a considerable amount of lactose (0.8-1.55%) at the end of manufacture (Fox et al., 2000). Major acidification occurs in the curd of Camembert during draining, and within a week after manufacture, lactose would have been metabolised to lactic acid. Lactic starter bacteria responsible for lactic fermentation are *Lactococcus* (mainly *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*) and *Leuconostoc* species (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*).

Through the glycolytic (GLY) or phosphoketolase (PK) pathway (Figure 11), lactose is mainly converted to lactate (lactic acid), predominantly the L (+) isomer. *Leuconostoc* spp. produce exclusively the D-isomer while *Lactococcus* spp. Produce only the L-form (Mcsweeney and Fox, 2004). Lactate is the main end-product of GLY pathway by homofermentative *Lactococcus* species, while acetate, ethanol and CO_2 may additionally be produced in the PK pathway by heterofermentative *Leuconostoc* spp. (Mcsweeney and Fox, 2004). Lactic acid has a direct effect on the taste of cheese, especially young Camembert cheese, which lacks other flavor compounds. Perhaps most importantly, lactic acid reduces the pH of milk (6.6-6.7) to 4.2-4.7, hence controls microbial growth of cheese flora and prevents or retards growth of spoilage bacteria.

At post-manufacture (early stage of cheese ripening), lactose fermentation in cheese continues and allows further reduction in pH, even though starter bacteria usually ceases to grow due to the addition of salt to the cheese (Fox et al., 2000). In cases where glycolysis has not been completed by starter, the growth of adventitious flora, non-starter lactic acid bacteria (NSLAB) such as pediococci and mesophilic lactobacilli, from the environment may contribute (Spinnler and Gripon, 2004).

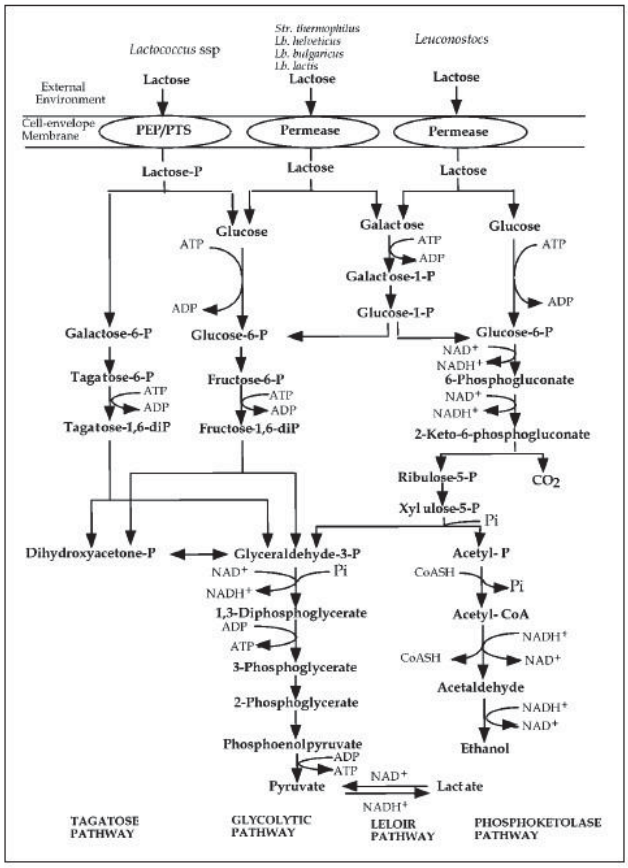


Figure 11. Probable pathways for the metabolism of lactose by mesophilic and thermophilic lactic acid bacteria (Singh et al., 2003; Singh and Cadwallader, 2008).

Modification and catabolism of lactate

Metabolism of lactate occurs extensively in Camembert (Graet et al., 1983; Graet and Brule, 1988; Schlesser et al., 1992; Law, 1997; Leclercq-Perlat et al., 2004a; Leclercq-Perlat et al., 2004b; Sullivan et al., 2005; Guizani et al., 2007; Lessard et al., 2012). During early ripening, the surface flora quickly colonize and dominate the surface of the cheeses, initially *G. candidum* and yeasts, followed by a dense growth of *P. camemberti*; and the surface of cheeses do not fully colonise until the pH has increased to 5.8. *G. candidum* and *P. camemberti* rapidly metabolise lactate to CO_2 and H_2O, increasing pH of cheese surface and diffusion of lactate from center towards the outer part of the cheese (Law, 1997; Sullivan et al., 2005) (Figure 12). When the lactate has been exhausted, *P. camemberti* metabolizes proteins, producing NH_3 which diffuse inwards, further increasing pH. Leclercq-Perlat et al. (2004b) confirmed that the fast increase of pH at cheese surface was related to NH_3 formation and fungi growth, while increase in pH of inner part of cheese was attributed to the diffusion of NH_3 from surface towards interior of cheese. The consequence of high pH causes the concentration of calcium phosphate ($Ca_3(PO_4)_2$) at the surface of cheese to exceed its solubility and precipitate as a layer of $Ca_3(PO_4)_2$, which in turn, causes the migration of calcium and phosphate to the cheese surface (Graet et al., 1983; Graet and Brule, 1988; McSweeney, 2004a) (Figure 12). According to Graet el al. (1983) and (1988), content of calcium and inorganic phosphate at

the rind of Camembert reaches to as high as 17 and 9 g/kg, respectively; whereas these contents are much lower in center of the cheese.

Results of Leclercq-Perlat et al. (2004a) confirmed the fundamental role of *P. camemberti* in the development of pH gradient already claimed by several authors (Law, 1997; Vitova et al., 2006; Vitova et al., 2007; Lessard et al., 2012). The fast increase in pH has also been well observed by several authors, including Sullivan et al. (2005), Leclercq-Perlat et al. (2004a), Schlesser et al. (1992), Lessard et al. (2012) and Guizani et al. (2007). Schlesser et al. (1992) studied pH of Camembert cheeses with respect to ripening time; and the pH of cheese body increased from 4.4 to 6.4 by 50 d. Lessard et al. (2012) studied the alkalinisation of the rind from day 0 to 14. The initial rind pH of 4.54 increased to 8.0 at d14 and remained stable until the end of ripening at day 31. Guizani et al. (2007) noted that de-acidificaiton was more pronounced on the surface than in the center of the cheese at each stage of ripening. The pH increased by 1.57 units on the surface whereas the overall pH increase was 0.84 units in the center. A ripened cheese at 31 d, a pH gradient appears between the surface and the center of the cheese to reach values of about 7.0-7.5 in surface and 5.5-6.0 in the center at the end of ripening (Law, 1997; Leclercq-Perlat et al., 2004a).

The combined action of increased pH and reduction of the concentration of $Ca_3(PO_4)_2$ in the interior leads to the characteristic softening of the body of the camembert cheese, which when mature, have an almost liquid-like consistency (Section 2.7). In addition to the texture development, the elevated pH also stimulated the action of numerous ripening enzymes (plasmin), as well as the growth of acid sensitive bacteria (micrococci and coryneform bacteria) to colonise the surface of cheese, which are responsible for proteolysis and lipolysis in cheese and contribute to the flavor quality (Sections 2.6.2 and 2.6.3). The optimum action pH of major milk enzymes (e.g. plasmin) and cheese ripening enzymes (e.g. lipase and exo-cellular peptidases of *P. camemberti* or *Lactococcus*) is often closer to neutrality (Grappin et al., 1985; Rank et al., 1985; Yvon and Rijene, 2001).

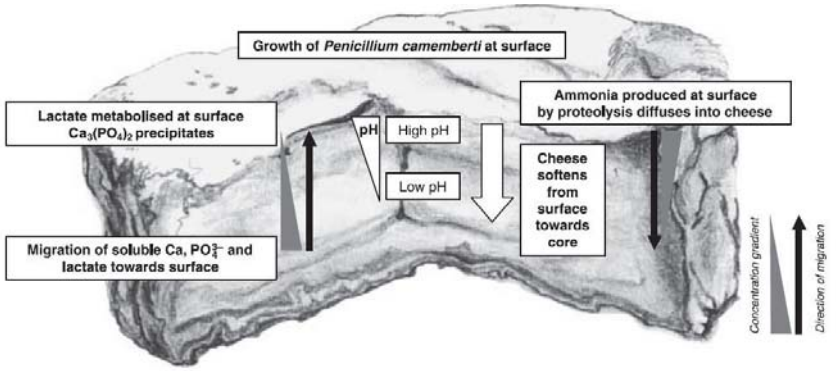


Figure 12. Schematic representation of changes which occur in Camembert-cheese during ripening as a consequence of the growth of *P. camemberti* at the surface (McSweeney, 2004b; Mcsweeney and Fox, 2004).

In addition to lactate catabolism, modification (racemizatio) of L (+) lactate to D (-) lactate and, oxidation of lactate to acetate and CO_2 may also occur during ripening in Camembert, unless the cheese is made from raw milk with a population of NSLAB. Racemization of L (+) lactate converts approximately half the L (+) lactate to D (-) lactate by NSLAB (Mcsweeney and Fox, 2004). The racemization of L (+) lactate is probably not significant from a flavor prospective, but imposes undesirable appearance on product cut-surface (Fox and McSweeney, 2004; Mcsweeney and Fox, 2004; McSweeney, 2004b; McSweeney, 2004a). Insoluble D (-) lactate can result in the formation of calcium lactate crystals and appear as white specks on cut-surface of cheese. The crystals may be mistaken by consumers as spoilage, and such crystal formation is generally considered negative. During the oxidation of lactate in Camembert cheeses, lactate may be converted to acetate and CO_2 dependent on NSLAB population and the availability of O_2, although this oxidative pathway is relatively minor in cheese due to its low oxidation-reduction (redox) potential and is limited by the availability of O_2. Production of acetate in Camembert contributes to cheese flavor, although a high concentration may cause off-flavor.

Citrate metabolism

Approximately 94% of citrate, a minor component of milk (≈2 ppm) is soluble and mostly lost in the whey (Mcsweeney and Fox, 2004). During Camembert manufacture and early ripening, citrate is metabolized by citrate-fermenting (Cit^+) strains of *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* spp. (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*) in the presence of lactose, producing acetaldehyde, ethanol, butan-1,2-diol, diacetyl (butan-2,3-dione), acetate, acetoin and CO_2 (Hugenholtz, 1993; Mcsweeney and Fox, 2004). Citrate (Figure 13) is initially cleaved to oxaloacetate and acetate by citrate lyase. In cultures with Cit^+ strains, *Lactococcus* and *Leuconostoc* spp., oxaloacetate is de-carboxylated to pyruvate. Two molecules of pyruvate are then condensed by α-acetolactate synthase after the formation of an acetaldehyde-thiaminepyrophosphate complex, forming α-acetolactate and carbon dioxide (CO_2). Alpha-acetolactate (α-acetolactic acid) (ALA) is an unstable compound that spontaneously decarboxylates to acetoin (3-hydroxybutan-2-one) and is also transformed to diacetyl in oxidizing conditions (McSweeney, 2004a; Mcsweeney and Fox, 2004) (Figure 13). Diacetyl can also be reduced to acetoin by diacetyl reductase, the latter can be further reduced to butane-2,3-diol (Figure 13).

Many studies have reported citrate metabolism in Camembert cheeses (Fryer et al., 1970; Hugenholtz, 1993; Monnet et al., 1994; Urbach, 1995; Molimard and Spinnler, 1996; Urbach, 1997; Sable and Cottenceau, 1999; Skeie et al., 2001; Singh and Cadwallader, 2008; Skeie et al., 2008). In cheeses with a controlled microflora containing non-citrate fermenting *L. lactis* subsp. *cremoris*, citrate remained constant at 0.2% up to 3 months, but decreased to 0.1% at 6 months. Meanwhile, cheeses made using *L. lactis* subsp. *cremoris* plus Cit^+ strains of *L. lactis* subsp. *lactis* biovar. *diacetylactis* contained no citrate at 3 months (Fryer et al., 1970).

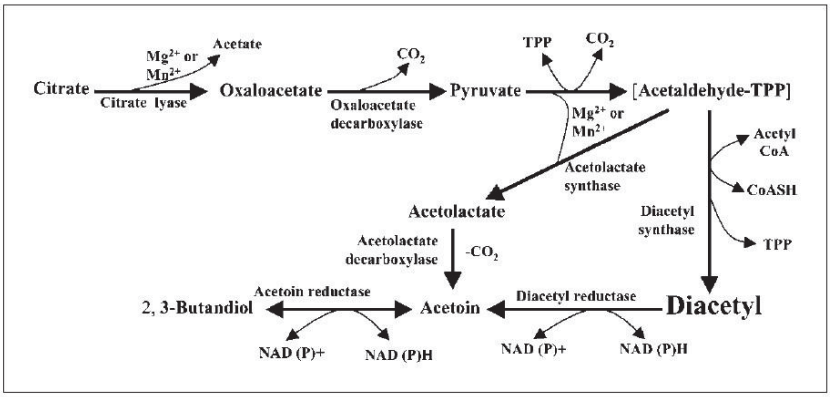


Figure 13. Metabolism of citrate in Camembert cheese (in the presence of lactose) in citrate-fermenting (Cit^+) strains of *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris* (Singh and Cadwallader, 2008).

2.5.3.3 Proteolysis in Camembert cheese

Proteolysis is a sequential process hydrolyzing water-insoluble caseins through water-soluble high molecular weight (large and intermediate) peptides, into low molecular weight peptides (small) peptides (McSweeney, 2004a). In a typical Camembert cheese, very extensive proteolysis occurs that is governed by a combined action of proteinase and peptidases enzymes from different sources (Table 9), involving coagulant agent, indigenous milk enzymes, the lactic starter and non-starter lactic cultures (*Lactococcus and Leuconostoc* spp.) and secondary ripening microorganisms (*G. candidum*, *P. camemberti*, *Corynebacterium* and *B. linens*). The hydrolysis of casein to high molecular weight peptides primarily consists of the results of chymosin and plasmin, while the subsequent hydrolysis of high molecular weight peptides are produced by proteolytic enzymes of LAB and secondary ripening microorganisms.

The extent and pattern of proteolysis in Camembert during ripening has been extensively studied due to its vital role in the development of texture, flavor or off-flavor (Sousa et al., 2001; Sousa and McSweeney, 2001; Leclercq-Perlat et al., 2004a; Sullivan et al., 2005; Guizani et al., 2007). Proteolysis contributes directly to flavor or off-flavor (e.g. bitterness) of cheese through the formation of peptides and free amino acids as well as metabolism of some free amino acids which, in turn, act as substrates for secondary catabolic changes (McSweeney, 2004a). Proteolysis also contributes to textural changes of the cheese matrix due to breakdown of the protein network and decrease in a_w through water binding by liberated carbonyl and amino groups (Section 2.7).

Table 9. Proteolytic agents in Camembert cheeses (Law, 1997).

Coagulant	Indigenous milk enzymes	Enzymes from starter and non-starter LAB	Enzymes from secondary ripening cultures
Chymosin Pepsin	Plasmin Cathepsin D	Cell envelope-associated proteinases (called CEP, PrtP, or Lactocepin); Peptidases (e.g. endopeptidases, aminopeptidases, di-/tri-peptidases and proline-specific peptidases);	Extra-cellular proteinases, amino-peptidases and carboxy-peptidases

Initial proteolysis

The initial proteolysis is largely due to proteinases from residual coagulant (mainly chymosin) in the curd as well as from indigenous milk (particularly plasmin). Proteinases from residual coagulant (particularly chymosin) in the curd rapidly hydrolyze α_{s1}-casein (α_{s1}-CN) at the Phe_{23}-Phe_{24} bond, and possibly Phe_{23}-Val_{25}, producing a large α_{s1}-CN f24-199 and small α_{s1}-CN f1-23 peptides. Chymosin however has little action on hydrolysis of β-casein and probably not on α_{s2}-casein and κ-casein (Law, 1997; Grappin et al., 1985; Fox et al., 2000; McSweeney, 2004a; McSweeney, 2004b; Spinnler and Gripon, 2004). Although chymosin has limited action on β-casein, plasmin, the major endogenous proteinase in milk, is mainly responsible for the initial hydrolysis of this protein (Grappin et al., 1985; Law, 1997; Fox et al., 2000; McSweeney, 2004a; McSweeney, 2004b; Spinnler and Gripon, 2004). Plasmin hydrolysis of β-casein results in the formation of three γ-caseins [γ_1-(β-CN f29-209), γ_2-(β-CN f106-209), and γ_3-(β-CN f108-209) caseins], and 5 proteose-peptones [β-CN f1-28, β-CN f1-105/107, and β-CN f29-105/107]. As an alkaline proteinase, plasmin is extremely thermo-stable and its activity increases after heat treatment/pasteurization either by inactivation of natural inhibitors or because it exists as plasminogen in milk and is activated by heating (Grappin et al., 1985; Sousa et al., 2001).

Electrophoretic studies of Sousa and McSweeney (2001) and Hayaloglu et al. (2007) in Camembert cheese showed that chymosin had a strong and early action on α_{s1}-casein in the whole sample, whereas β-casein was highly degraded in the outer part, but clearly in the center. The degradation product of α_{s1}-casein was detected in Camembert after 6 h of draining and the concentration of this peptide increased continuously throughout early phase of ripening. However, as the pH of the outer part of Camembert increased quickly, reaching pH 6.0 or more after 2 weeks (or pH 7.0 after 3-4 weeks), the activity of chymosin decreased because the pH optimum of chymosin is about 5.5. In comparison to other cheese varieties, activity of plasmin is most active in Camembert where the surface pH is high during late ripening phase that is not far from the optimum for plasmin (pH=8.5). Accumulation of γ-caseins in Camembert cheeses was observed at end of ripening, whereas in other cheeses, relatively large amount of β-caseins remain un-attacked.

LAB also have a role in degradation of caseins (Grappin et al., 1985; Rank et al., 1985; Fox et al., 2000; Upadhyay et al., 2004). Proteolytic system of *Lactococcus* spp. is well-known. The principle proteinase is lactocepin (also known as CEP, cell envelope associated proteinases, or Prtp) (Figure 14), which is classified into two groups: P_I- and P_{III}-type proteinases. The former act rapidly on β-casein but only slowly on α_{s1}-casein and κ-caseins, whereas P_{III}-type enzymes act rapidly on α_{s1}- and κ-caseins. Although the production of soluble nitrogen (SN) in aseptic rennet-free Gouda cheeses were much lower than in normal aseptic cheese (with rennet added), the sufficient amount of SN produced in aseptic rennet-free cheeses suggested that starter bacteria are capable of attacking κ-casein in cheese and converting it to soluble peptide products, independently of rennet action (Visser, 1977). α-caseins (α_{-s1}-, α_{-s2}-casein) in cheese are also hydrolyzed by extra-cellular proteinases synthesized from *P. camemberti*, following the sporulation of the mould at 6-7 d of ripening. Peptides with intermediate size are produced as a result of their microbial proteolytic activity (Spinnler and Gripon, 2004).

Secondary proteolysis

In Camembert, secondary proteolysis (further breakdown of large peptides to small peptides and amino acids) is largely attributed to the proteolytic systems of diverse microflora present, particularly proteinases and peptides of lactic acid starters, and the secondary ripening cultures.

The proteolytic system of *Lactococcus* spp. also includes a range of intracellular proteinases and, more importantly, peptidases (Figure 14). During the growth of LAB in cheese, lactocepin degrades intermediate-sized peptides produced from the caseins by the action of chymosin or plasmin, and the short oligo-peptides are further taken up by the cell via peptide transport system, such as oligo-peptide transport system (OPP system), and di- and tri-peptide transporters. Following the uptake, the peptides are degraded intracellularly to small peptides and free amino acids, by a variety of peptidases of *Lactococcus* spp., which can be divided into endo-peptidases, amino-peptidases, di- and tri-peptidases and proline-specific peptidases (Fox et al., 2000; Sousa et al., 2001; Upadhyay et al., 2004; Spinnler and Gripon, 2004). Proteases and peptides of all *Lactococcus* spp. have an optimum pH close to neutrality, thus the pH increase in the outer area of Camembert cheese enhances their activity (Leclercq-Perlat et al., 2004a; Spinnler and Gripon, 2004).

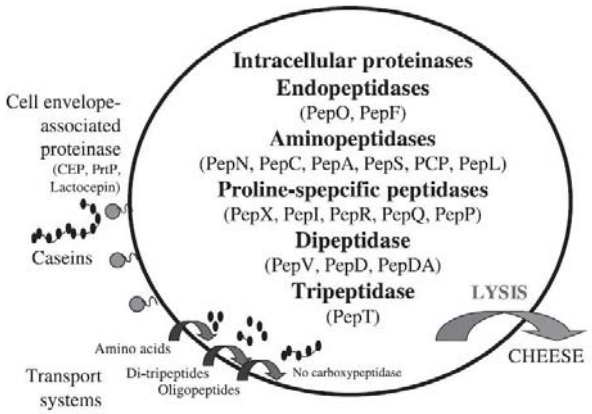


Figure 14. Summary of the proteolysis system of *Lactococcus*. The proteolysis systems of other lactic acid bacteria are generally similar (Upadhyay et al., 2004).

P. camemberti has much greater enzymatic potential for proteolysis than LAB (Spinnler and Gripon, 2004). When grown in the outer region of the cheese after 6-7 d of ripening, enzymes of *P. camemberti* actively hydrolyse β-caseins. Studies on aseptic curds in which *P. camemberti* was grown alone, showed extensive production of high and low molecular weights peptides, as well as free amino acids (Sousa et al., 2001; Spinnler and Gripon, 2004). The high proteolytic activity was due to their production of extracellular and intraceullar proteinases and peptideases. *P. camemberti* produces two extracellular endo- and exo-peptidases: metalloproteinase and aspartate proteinase in appreciable quantities, which are optimally active at pH 5.5-6.0 and 4.0, respectively. In comparison, the ability of *P. camemberti* intracellular proteinases play a more limited role than its extracellular proteinases (Fox et al., 2000; Leclercq-Perlat et al., 2004a; McSweeney, 2004a; Spinnler and Gripon, 2004; Upadhyay et al., 2004; Sullivan et al., 2005). The activity of proteinase is only detectable in the external area of Camembert cheeses which indicates that the migration of extracellular proteinases and peptideases of *P. camemberti* towards the interior of the curd remains very limited (Spinnler and Gripon, 2004).

Generally, *G. candidum* plays a much lesser role in proteolysis than *P. camemberti*, as Camembert cheeses seeded with *G. candidum* usually show lower proteolysis than those seeded with *P. camemberti* (Spinnler and Gripon, 2004). Similar result was shown by Lenoir (1984). Proteolytic activity of outer region of Camembert did not increase during the growth of *G. candidum* but increased only when *P. camemberti* was grown. The proteolytic activities of yeast and *B. linens* are also considered to be low, although their extra- and intra-cellular enzymes could participate in cheese proteolysis during ripening (Spinnler and Gripon, 2004).

The proteolysis observed on the surface of Camembert cheeses is the most significant compared to the center of the cheese due to the surface action of *P. camemberti*. Higher concentration of SN in the outer than in the inner part of the cheese as a consequence of higher proteolysis have been reported (Sousa et al., 2001; Sousa and McSweeney, 2001). In

the study, SN at a pH of 4.6 in the outer area of raw-milk Camembert represented approximately 35% of total nitrogen and only 25% in the center. The variation of proteolysis during ripening of the surface and center of Camembert cheeses were also reported by Guizani et al. (2007). Within 6 d, content of SN increased from 6.14% to 9.63%, whereas the degree of proteolysis remained constant in the center. From days 6 to 23, nitrogen solubilisation occurred rapidly, both on the surface (from 9.63 to 24.74%) and the center (from 7.12 to 13.72%). At the end of ripening, surface proteolysis slowed but it continued in the center at a slightly higher rate. Law (1997) reported very low proteolytic activity in the center of Camembert cheese which virtually does not vary during ripening. On the surface, it however suddenly increases after 6-7 d of ripening, i.e., when *Penicillium* starters develop and their enzymes are both synthesized; and the proteolytic activity is maximum after about 15 d when *Penicillium* vigorously develops. The activity then slowly diminishes although enzymes remain relatively stable in cheese. The increase of SN during late ripening in the rind was also due to actions of plasmin and proteinases of *P. camemberti* on degradation of β-caseins.

2.5.3.4 Lipolysis in Camembert cheese

Cheese is a high-fat food, with fresh Camembert containing 30 % or more fat (Codex Standard, 2010). Lipids in foods, with a high fat content, may undergo hydrolytic (enzymatic hydrolysis by lipases and esterases) and oxidative (chemical) degradation. In cheeses, oxidative changes are very limited due to the low oxidation/reduction potential (-250 mV) (Singh et al., 2003; Singh and Cadwallader, 2008). However, the hydrolysis of triglycerides is the principle biochemical transformation of fat during cheese ripening. Available lipolytic enzymes are able to cleave the ester linkage between a fatty acid and the glycerol core of the triacylglyceride (TAG), producing free fatty acids (FFAs) (include long-, intermediate- and short-free fatty acids), and di- and mono-glycerides and possibly glycerol. Compared to other cheese varieties where relatively little lipolysis occurs during ripening, extensive level of lipolysis were observed in Camembert (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999). In ripened raw-milk Camembert, FFAs reached 6-10% of total fatty acids (FAs); among which intermediate- and short-chain acids are the most important quantitatively (Spinnler and Gripon, 2004). Lower values ranging from 3-5% were also observed in products which were probably less ripened (Molimard and Spinnler, 1996). Lipolysis in Camembert is largely due to lipolytic ability of secondary flora growing on the surface of cheeses, in particular *P. camemberti*. Activity of milk lipases (in the case of cheese made from raw milk) and other microbial lipases (such as starter and NSLAB) are involved to a lesser extent (Karahadian et al., 1985; Sable and Cottenceau, 1999).

Milk contains potent indigenous lipase, lipoprotein lipase (LPL), as well as several esterases. Milk lipases are more active than starter lipases in Camembert and they hydrolyse the fat selectively and exhibits a preference for hydrolysis of triglycerides containing medium-chain

fatty acids (C_6-C_{12}) (Karahadian et al., 1985; Singh and Cadwallader, 2008). LPL activity is the most significant in raw milk cheeses as the enzyme is largely inactivated by pasteurization, although heat treatment >78°C for 10 sec is required to inactive the enzyme completely (McSweeney, 2004a; McSweeney, 2004b). Nonetheless, although Camembert cheese made from pasteurized milk does not have a strongly lipolytic agent, its lipolysis still progresses during ripening due to the action of microbial lipolytic enzymes from the LAB and secondary ripening micro-organisms (Law, 1997; McSweeney, 2004a; Spinnler and Gripon, 2004). In general, commercial rennet is not responsible for lipolysis in Camembert as it is free from lipase activity (Karahadian et al., 1985; McSweeney, 2004a; McSweeney, 2004b).

LAB present in starter cultures are weakly lipolytic and contribute relatively little to lipolysis, but the added secondary cultures, particularly *P. camemberti* exert high lipolytic activity, generating FFAs with C_4-C_{20} (Singh and Cadwallader, 2008). Lipolytic activity of *P. camemberti* appears after 10 d of ripening, during or shortly after mycelium growth, reaches maximum at 16 d and then decreases slightly until the 30th d when it increases again probably due to lysis of the mycelia (Karahadian et al., 1985). Law et al. (1997) noted that *P. camemberti* possesses only one extra-cellular lipase with mild alkaline pH (6.0) and remains very active between 0 and 20°C, which is produced together with mycelia growth after several days of ripening. The extra-cellular lipase is also reported to be more active when calcium ions are present. Furthermore, although not studied in detail, lipases from *B. lines* and *G. candidum*, which grow on the surface of the cheeses, have been reported (McSweeney, 2004a; Spinnler and Gripon, 2004). Since surface microorganisms have a much more active lipolytic role, lipolysis in Camembert is always higher in the outer area than the inner part of the cheese. According to Spinnler and Gripson (2004) and Karahadian et al. (1985), abundant amounts of short-chain and intermediate-chain even numbered free fatty acids [(C_4-C_{12}) FFAs] have been detected in the crust than in the interior of the cheese, due to the abundant growth of *P. camemberti* on the surface of the cheese; among which, acetic-, butyric-, 3-methylbutanoic-, propionic- and isobutyric-acids are the most important quantitatively.

2.6 Aroma development in Camembert and their relative perception notes

Camembert, a typical example of surface mould-ripened soft cheese, has a characteristic aroma which distinguishes it from other types of cheeses. More than 100 different flavor compounds were detected, which essentially result from the biochemical transformations occurring in the ripening of cheese (glycolysis, proteolysis and catabolism of FAAs, lipolysis and catabolism of FFAs) through the actions of the coagulant, milk enzymes, microbial flora and their enzymes. In Table 10, key aromatic compounds that contribute typical flavor for a well-ripened Camembert cheese are presented, with their associated flavor notes and perception threshold.

Table 10. Flavour notes, perception thresholds, and quantities of key volatile compounds identified in Camembert cheese.

Compounds	Flavour notes	Perception threshold (ppm)	Quantity in cheese (ppm)
Compounds derived from glycolysis (i.e., metabolism of residual lactose and of lactate and citrate)			
Ketones			
Acetoin	Buttery	0.9-1.4	++++
Diacetyl (butane-2,3-dione)	Buttery	0.007-5	0.074-0.11
Alcohol			
Ethanol	Mild, ether	0.18-100	++++
Compounds derived from proteolysis and free amino acid catabolism			
Aldehydes			
Hexanal	Green, grassy, penetrating, powerful	0.0045-0.8	Trace/0.124-0.144
Heptanal	Oily, heavy, woody, sweet penetrating	0.002-0.9	Trace
Nonanal	Floral, citrus, orange, rose, fatty, waxy	0.001-1	Trace
2-methylbutanal, 3-methylbutanal	Green, malty	0.013	0.094-0.142
Benzaldehyde	Bitter almond, aromatic, sweet	0.003-0.35	Trace
Primary alcohols			
3-methylbutan-1-ol	Fruity, alcohol	0.3-4.75	++++
2-phenylethanol	Rose, flora	0.24-9.1	1-1.15
Ester			
Ethylpropanoate	Pineapple, sweet, solvent	0.0099	Trace
Ethylbutanoate	Pineapple, sweet, banana, fragrant	0.00013-0.45	Trace to +
Ethyloctanoate	Apricot, wine, floral	--	+ to +++
2-phenylethyl acetate	Flora, rose	0.137-19.85	0.25-4.6
Sulphur-containing compounds			
Methanethiol	Cooked cabbage	2	0.26-0.542
Dimethyldisulfide (DMDS)	Cauliflower, garlic, very-ripened cheese	12-120	0.011/+++++/NQ
Dimethyltrisulfide (DMTS)	Boiled cabbage, sulphurous, penetrating, over-ripened cheese	1.2-170	0.25-0.14
Amine			
Dimethylamine	Fruity, alcoholic, varnish-like aroma	1.5-6.7	0.811-1.623
Ammonia	Ammonia, pungent	0.9-3.3	NQ
Compounds derived from lipolysis and free fatty acid catabolism			
Short- and intermediate chain free fatty acids			
Acetic acid	Vinegar, pungent	22-54	13-66
Propionic acid	Vinegar, pungent	40.3	3-137
Butyric acid	Rancid, cheesy, putrid, sweaty	0.3-7	35-206
Isobutyric acid	Sweet, mild, rotten apple	5.3	3-137
Methyl ketones and ketones			
Heptan-2-one	Blue cheese, spicy,	0.7-15	1-5.58

	Roquefort cheese, musty		
Nonan-2-one	Fruity, musty, floral	1.7-7.7	20-48
Undecan-2-one	Floral, rose, iris, herbaceous	54	0.18-0.7
Octan-3-one	Mushroom fruity, spicy	0.05	Trace
Oct-1-en-3-one	Mushroom	0.0001-0.0009	0.0022
Secondary alcohols			
Heptan-2-ol	Earthy, oily, sweetish	0.45-3.8	1.26 - ++++
Nonan-2-ol	Fatty, melon, mild green	1.2-6.6	1.82 - ++++
Oct-1-en-3-ol	Mushroom	0.001-0.048	0.075-0.130
Hydrocarbons			
Heptane	limited contribution to cheese aroma		Trace
Lactone (γ- and δ-lactone)			
γ-decalactone	Peach, apricot	0.09-1	--
δ-decalactone	Peach, coconut milk	0.14-1.4	0.91-1.08
γ-dodecalactone	Peach, buttery, musty	1	--
δ-dodecalactone	Fresh fruit, peach, pear, plum, coconut, buttery	0.1-9.8	Trace

Source: Volatile compounds belonging to different chemical classes are identified and quantified (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Yvon and Rijene, 2001; Sousa et al., 2001; Smit et al., 2005; Singh and Cadwallader, 2008). + = minor amount, ++++ = abundant amount; Trace = trace amount; NQ = Not Quantified; -- = Information has not been given.

2.6.1 Development of aromatic compounds during glycolysis

Unsaturated ketones and alcohols produced from glycolysis were detected in significant quantities in Camembert cheeses (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Leclercq-Perlat et al., 2004a; Spinnler and Gripon, 2004; Vitova et al., 2006; Vitova et al., 2007).

Diacetyl (butan-2,3-dione) is one of the most important unsaturated ketones of Camembert, produced from pyruvate during citrate metabolism. Imparting strong sweet buttery and nutty vanilla aroma, diacetyl is a significant flavor compound of Camembert, especially the in un-ripened young cheeses (Monnet et al., 1994; Molimard and Spinnler, 1996; Sable and Cottenceau, 1999). In citrate metabolism, diacetyl, can be reduced to acetoin, with a weak buttery note which can be reduced to butane-2,3-diol, a neutral compound has no flavor impact (Sable and Cottenceau, 1999). Diacetyl is produced in small amounts (<0.11 mmol L^{-1}) in cheese and acetoin production is usually 10-50 times higher (Spinnler and Gripon, 2004).

Alcohols are produced in Camembert through lactose and citrate metabolism. As mentioned earlier, butane-2,3-diol does not impart any flavor in Camembert (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Spinnler and Gripon, 2004). In young cheese, ethanol has been detected at very high concentration, but decreases sharply during ripening. Ethanol plays a limited role in cheese aroma despite its high levels, but is a precursor of aroma compounds that contribute to the formation of esters (Vitova et al., 2007).

The excessive amount of CO_2 produced by citrate metabolism may induce unwanted floating of the curd that causes openness defects. Gaseous holes are generally absent in Camembert cheese, but few openings and splits are acceptable (Codex Standard, 2010).

2.6.2 Aromatic compounds developed from casein metabolism

2.6.2.1 Aromatic compounds developed from proteolysis

Proteolysis is mainly responsible for the 'basic' flavor of cheese. Large peptides do not contribute to cheese taste, but may contribute to undesirable bitter taste when unbalanced proteolysis occurs (Fox et al., 2000; Sousa et al., 2001; Curtin and Mcsweeney, 2004; Upadhyay et al., 2004; Singh and Cadwallader, 2008). Bitterness is associated with high levels of hydrophobic peptides from the C-terminal region of β-casein (Singh and Cadwallader, 2008). Proteolysis of *P. camemberti* plays vital roles in bitterness development (Law, 1997). The presence of bitterness is higher when the pH of curd is very low after 24 h, probably because the environment enhances the growth and production of proteinase by *P. camemberti*. The overall level of bitterness in cheese depends on the relative rates at which bitter peptides are formed and degraded to non-bitter products by intracellular peptidases of starter cultures (Upadhyay et al., 2004).

Products of proteolysis (small peptides and FAAs) are significant in cheese taste as 'background' savoury, non-specific cheesy flavour. Tzanetaki et al. (1993) studied the effect of proteolysis on flavor intensity of cheeses; and reported that high levels of trichloroacetic acid and phosphotungstic acid SN, representing small peptides, are significantly correlated with flavour intensity of cheese. The results of Tzanetaki et al. (1993) were also confirmed by Smit et al. (Smit et al., 2005), Sable and Cottenceau (1999) and Leclercq-Perlat et al. (2004b). Products of proteolysis have limited contribution to aroma, but rather, the secondary transformation (catabolism of amino acids) is largely responsible for the delicate cheese aroma.

2.6.2.2 Aromatic compounds developed from catabolism of free amino acids

Of the amino acids released from cheese proteolysis, branched chain amino acids (BcAAs) (leucine (Leu), isoleucine (Ile) and valine (Val)), aromatic amino acids (ArAAs) (phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp)), and methionine (Met) are the major precursors of aroma compounds. Aldehydes are synthesised via amino acids, and in turn may produce corresponding alcohols. Meanwhile, sulphur-containing compounds can be derived from Met metabolism.

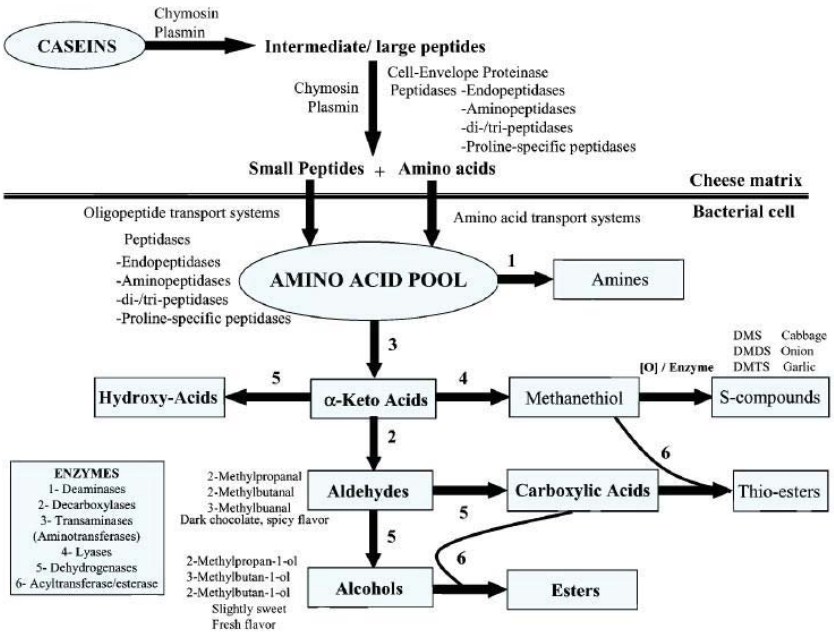


Figure 15. Generation of flavour compounds from milk protein degradation (Singh and Cadwallader, 2008). DMS-dimethyl sulfide; DMDS-dimethyl disulfide; DMTS-dimethyl trisulfide.

Liberation of free amino acids, precursors for metabolic reactions, play vital roles in flavor development of Camembert cheese. Pathways of amino acid metabolism during ripening produce many flavor compounds shown in Figure 15**.** Generally, amino acid conversion to aroma compounds proceeds by 2 different pathways. ArAAs (Trp, Tyr and Phe), BcAAs (Leu, Ile and Val), and Met in cheese appear to be catabolised by one of two major pathways initiated by the action of an aminotransferase or lyase, although other catabolic chemical pathways (deamination or decarboxylation) also occur.

Development of aroma compounds from pathway initiated by a transamination reaction

The major pathway for the conversion of amino acids to aroma compounds by cheese micro-organisms is a multi-step pathway that is initiated by transamination reaction (Figure 15). In Camembert, metabolism of ArAAs, BcAAs and Met is initiated by the action of aminotransferases, which convert an amino acid to corresponding α-keto acid. Aminotransferase activities from LAB such as *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis*, enzymes from *Propionibacterium* spp. and *B. lines* and other smear organisms have been extensively studied (Yvon and Rijene, 2001; Curtin and Mcsweeney, 2004).

As central intermediates, α-keto acids resulting from ArAA, BcAAs and Met transamination are further converted to a range of compounds: hydroxyacids, aldehydes, carboxylic acids, aldehydes and esters, by enzymatic reactions (Figure 15) (Yvon and Rijene, 2001). With the exception of hydroxyacids, all these products impart strong flavors. α-keto acids, particularly those from aromatic amino acids, may be also degraded chemically to produce important volatile flavor compounds by either dehydrogenases or oxidases.

Aldehydes

During oxidative deamination or transamination, amino acids can be transformed to α-keto acids which can be either decarboxylated or chemically degraded to aldehydes (Yvon and Rijene, 2001). Aldehydes in Camembert cheese can be also produced from β-oxidation of unsaturated fatty acids (Section 2.6.3.2). Aldehydes are transitory compounds in cheese that are usually not present in large quantities, because they are rapidly reduced to primary alcohols or oxidised to their corresponding acids (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999).

Aldehydes are characterized by green-grass or herbaceous aroma and can be very unpleasant when their concentration exceed thresholds (Sable and Cottenceau, 1999). In Camembert, aldehydes are usually present only in trace levels in cheese after week one of ripening, because aldehydes are transitory compounds in cheeses as they are rapidly transformed to corresponding alcohols or acids (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Spinnler and Gripon, 2004). The main aldehydes found in Camembert cheese are hexanal, heptanal and nonanal, 2-methylbutanal, 3-methylbutanal, and benzaldehyde. Hexanal and hex-2-enal impart the green note of immature fruit while octanal, nonanal, decanal and dodecanal have aromatic notes, resembling orange. Benzaldehyde has an aromatic note of bitter almond, while 3-methylbutanal has a green and malty odour (Table 10).

Primary alcohols

Primary alcohols are formed by the reduction of the corresponding aldehydes. They impart a fruity, nutty note to the cheese flavor, but at high levels, they could be responsible for flavour defects (Sable and Cottenceau, 1999). Of the primary alcohols, 3-methylbutan-1-ol and 2-phenylethanol are the two major compounds that are present in relatively large quantities in Camembert, giving an alcoholic, floral note. Phenyl-2-ethanol and its corresponding ester, phenylethylacetate, are mainly produced by yeast metabolism on cheese surface during early ripening, while *P. camemberti* metabolises Val to 2-methylpropanol and Leu to 3-methylbutanol (Spinnler and Gripon, 2004). Phenyl-2-ethanol and its corresponding ester have cumulative effects giving the perceptible floral note (Table 10). Phenyl-2-ethanol and phenylethylacetate reaches maximum concentration after 7 d of ripening, then decreases and stabilizes at approximately 1 ppm (mg/kg) at the end of ripening (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Leclercq-Perlat et al., 2004b; Vitova et al., 2007).

Amines

For some micro-organisms, the breakdown of amino acids starts by decarboxylation, producing amines and CO_2. Decarboxylation of Leu gives isobutylamine, while phenylethylamine and tyramine are produced by phenylalanine and tyrosine, respectively. Decarboxylase activity of the surface flora leads to release of amines. The organoleptic role of amines is not well known, it is probably not worth investigating as they are intermediate compounds which undergo oxidative deamination to form aldehydes. They can be also the initial compounds like *N*-isobutylacetamide encountered in Camembert, presumably produced by a reaction with acetic acid (Yvon and Rijene, 2001). Numerous volatile amines have been identified in Camembert cheese, including methylamine, dimethylamine, and are described as having fruity, alcoholic or varnish-like aroma notes (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Yvon and Rijene, 2001; Leclercq-Perlat et al., 2004b; Smit et al., 2005; Vitova et al., 2007; Singh and Cadwallader, 2008).

Ammonia produced by the de-amination of amino acids largely contributes to the typical aroma of Camembert cheeses (Table 10) (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Yvon and Rijene, 2001; Leclercq-Perlat et al., 2004b; Smit et al., 2005; Vitova et al., 2007; Singh and Cadwallader, 2008) and the increase in pH (Mcsweeney and Fox, 2004). De-amination activity of *P. camemberti* is responsible for the production of ammonia. Although metabolism of *P. camemberti* on the cheese is generally associated with the production of ammonia, *B. linens* also plays a major role, because most of the ammonia occurs late in ripening when *P. camemberti* is being overgrown by *B. linens* and related coryneform bacteria (Molimard and Spinnler, 1996).

Development of aroma compounds from pathway imitated by elimination reactions

A single-step pathway initiated by elimination reactions catalysed by amino acid lyases have been observed for ArAAs and Met (Figure 15). However, this is not a major pathway used by cheese micro-organisms for the metabolism of ArAAs and Met because such activities have rarely been detected in lactic acid bacteria (with exception of *L. lactis*), but yeast, micrococci and *B. linens* (Yvon and Rijene, 2001). The lyases cleave the side chain of tyrosine and tryptophan, releasing phenol and indol, respectively; while *L. lactis* is capable of cleaving side-chain of methionine, producing methanethiol directly (Yvon and Rijene, 2001; Singh and Cadwallader, 2008).

Sulphur-containing compounds

Several sulphur compounds identified in the volatile fraction of Camembert were described as having strong ‘garlic’, ‘cabbage’ or ‘higher ripening cheese’ (Table 10) (Molimard and Spinnler, 1996; Law, 1997; Sable and Cottenceau, 1999). Sulphur-containing compounds origin from the degradation of Met to methanethiol, which is very reactive and is a

precursor of other sulphur compounds. Although methanethiol can be produced by the oxidation of Met, large amounts of the compound are produced enzymatically through elimination reaction pathway by cleavage of the carbon-sulphur bond under the action of a demethanethiol via metabolism of coryneform bacteria, especially *B. linens* (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Singh et al., 2003). When coryneform bacteria grow abundantly on the surface of cheese, methanethiol is one of the characteristic flavor compounds in Camembert, and reaches 0.54 mg/kg after 3 weeks' ripening (Spinnler and Gripon, 2004). Other sulphur-containing compounds, such as dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS) and thioesters, have been also identified in significant quantities in Camembert. Sulphur compounds may quantitatively decrease and even disappear in late ripening due to their volatility (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Yvon and Rijene, 2001; Vitova et al., 2007). However, despite their low perception threshold, they can still significantly impart the final aroma of cheese.

Esters

A large diversity of esters has been detected in the volatile fraction of Camembert, with ethyl esters dominating. As a metabolic product of yeast and *G. candidum*, esters are produced when alcohols react with short- and medium-chain fatty acids. Though alcohols encountered in Camembert only have limited aromatic roles in cheese, they are the precursors of esters. The alcohol compounds may be produced from lactose fermentation (ethanol), or from catabolism of amino acids (primary alcohols) or from catabolism of fatty acids (secondary alcohols) (Law, 1997; Sousa et al., 2001; Curtin and Mcsweeney, 2004; Spinnler and Gripon, 2004).

Most esters encountered in the cheese have fruit-like aromatic note; and may contribute to the aroma by minimizing the sharpness and bitterness imparted by fatty acids and amines, respectively (Sable and Cottenceau, 1999). 2-phenylethyl acetate that imparts a floral note of cheese is the most important (Table 10) (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Spinnler and Gripon, 2004). After only 7 d of ripening, 2-phenylethyl acetate was the principle quantitative compound (4.6 mg/kg) in the aromatic profile of Camembert followed by 2-phenylethyl alcohol (Sable and Cottenceau, 1999). After 30 d of ripening, the compound was still present in large amounts (≈1 mg/kg), but was no longer the dominant volatile compound.

2.6.3 Production of aromatic compounds from fat metabolism

2.6.3.1 Development of aromatic compounds from lipolysis

Short- and intermediate-chain FFAs directly contribute to flavor of Camembert cheese, but at elevated pH, the flavor may suppressed (Singh and Cadwallader, 2008). Acetic and

propionic acids have typical vinegar odor, while butyric acid has a rancid, cheesy odor. Isobutyric acids have a mild odor, suggestive of sweat or rotten fruit (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Spinnler and Gripon, 2004) (Table 10). While the above-mentioned short- and intermediate-chain FFAs contribute directly to cheese flavor, FFAs also contribute indirectly to cheese flavor by acting as important precursors for the production of volatile compounds through a series metabolic reactions of FFAs (Figures 16 and 17). By contrast, long chain FFAs (> C_{12}) play a minor role in cheese flavor, despite their high perception thresholds (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Singh and Cadwallader, 2008). It is noteworthy to mention that major products of lipolysis in Camembert do not lead to rancid taste (even at high concentration with low perception thresholds), probably because the fatty acids are neutralized by a pH increase of the curd during ripening (Collins et al., 2004). Low pH reduces ionisation thereby increasing volatility of the acids; only protonated forms (un-dissociated form) of the fatty acids are odor-active and contribute to the ripened cheese flavor (Urbach, 1997; Sable and Cottenceau, 1999).

2.6.3.2 Aromatic compounds produced during metabolism of fatty acids

The importance of FFAs in Camembert is not only for their aromatic notes (Table 10), but also as precursors of methyl ketones, alcohols, lactones, and esters (Figures 16 and 17).

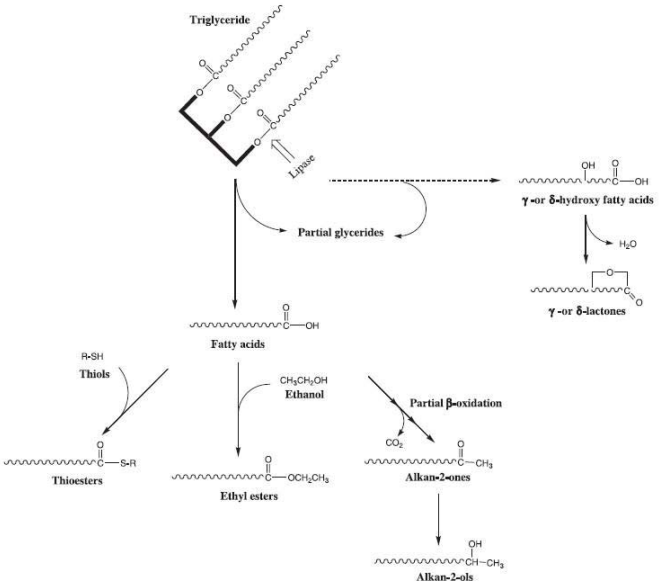


Figure 16. Pathways for the production of flavour compounds from fatty acids during cheese ripening (Fox et al., 2000; Collins et al., 2004).

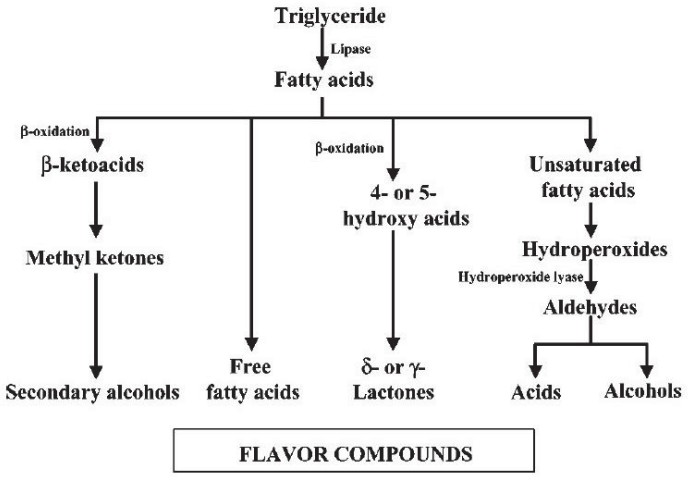


Figure 17. General pathways for the metabolism of milk triglycerides and fatty acids (Fox et al., 2000; Collins et al., 2004).

Methyl ketones produced from β-oxidation of fat

In Camembert cheese, production of saturated methyl ketones is very significant via β-oxidation. FFAs are oxidized to β-keto-acids, which are then rapidly de-carboxylated to corresponding $C_{(n-1)}$ methyl ketones (with one less carbon than the initial fatty acids), mainly from C_6-C_{12} fatty acids (Urbach, 1997; Fox et al., 2000; Collins et al., 2004; Spinnler and Gripon, 2004; Singh and Cadwallader, 2008). As noted by Wouters et al. (2002) and Spinnler and Gripon (2004), there is a positive correlation between FFAs level and the amount of methyl ketones formed in Camembert cheese. The synthesis of methyl ketones in Camembert cheese is associated with enzymatic lipase activity of secondary micro-flora, due to activity of *P. camemberti* and *G. candidum*, and perhaps yeasts (Karahadian et al., 1985; Spinnler and Gripon, 2004). The rate of production is maximum between pH 5 and 7; and this range of pH values encompasses most Camembert cheeses (Spinnler and Gripon, 2004).

Eleven methyl ketones have been identified in Camembert cheese with the highest values observed for nonan-2-one, heptan-2-one and undecan-2-one (Karahadian et al., 1985; Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Vitova et al., 2007). The longer chain ketones, probably, are much less important than the intermediate ones because of their lipophilicity, which probably limit their volatility in a fatty matrix, like cheese. Most of the methyl ketones increase steadily throughout ripening process and present from the 7th ripening day (Sable and Cottenceau, 1999). An increase in the concentration of nonan-2-one was observed during cheese ripening while the amount of heptan-2-one remained stable. However, Monias et al. (1973) reported butan-2-one and pentan-2-one in young Camembert only which seemed to disappear during ripening. Nonan-2-one and undecan-2-one have fruity, floral and musty notes while heptan-2-one has a Blue cheese note. Oct-1-en-3-one has a typical mushroom note in aqueous media and a metallic note in lipid media (Table 10). Although methyl ketones have been noted for their significance in Camembert cheese flavor,

Monias et al. (1973) believed that the concentration and type of methyl ketones in Camembert cheese were not as important due to the occurrence of 1-octen-3-ol, which imparted a masking effect on flavor notes of the methyl ketones.

Ketones produced from intra-chain oxidation of unsaturated fatty acids

Linoleic and linolenic acids are polyunsaturated FAs of cheese, and oct-1-en-3-one is derived from these via intra-chain oxidation. The principle enzymes involved are lipoxygenases and hydroperoxide lyases, active in *P. camemberti* and *G. candidum* (Spinnler and Gripon, 2004). Oct-1-en-3-one is a key compound in the overall aroma of the cheese, giving a characteristic mushroom note at low perception threshold (0.01 mg/kg) (Table 10) (Karahadian et al., 1985; Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Spinnler and Gripon, 2004; Vitova et al., 2007).

Secondary alcohols

There are many metabolic pathways implicated in the alcohol synthesis encountered in Camembert cheese. As mentioned previously, primary alcohols arise from the reduction of aldehydes via amino acid metabolism, ethanol and butane-2,3-diol are formed from lactose and citrate metabolism. In addition, secondary alcohol compounds are produced via methyl ketone degradation.

Secondary alcohol compounds of Camembert are produced by *P. camemberti* through the action of a reductase on the corresponding methyl ketones. Secondary alcohol compounds contribute significantly to Camembert flavors, with similar but heavier flavor notes than the corresponding methyl ketones. Heptan-2-ol and nonan-2-ol are principle secondary alcohols encountered in Camembert and were abundant in well-ripened cheeses (Sable and Cottenceau, 1999). The two alcohols represent, together with their corresponding methyl ketones, 10-20% and 5-10%, respectively, of all aroma compounds in Camembert. Significant quantities of pentan-2-ol were also detected in ripe Camembert (Dumont et al., 1974). However, Monias et al. (1973) did not report this alcohol in mature Camembert but the compound was present in young cheeses only and its concentration decreased to non-detectable levels during ripening. Oct-1-en-3-ol provides the characterizing mushroom-like flavor to Camembert cheese. Its production is due to the degradation of oct-1-en-3-one, which is produced by *P. camemberti* metabolism of linoleic and linolenic acids.

Hydrocarbon

Hydrocarbons are secondary products of lipid auto-oxidation and are frequently reported in cheeses, but at low concentrations (Spinnler and Gripon, 2004). One major hydrocarbon

identified in Camembert is heptane. Hydrocarbons do not contribute to aroma, but may serve as precursors for the formation of other aroma compounds.

Lactones

In Camembert, diverse γ- and δ-lactones were detected, mostly comprising γ-decalactone, δ-decalactone, γ-dodecalactone and δ-dodecalactone (Spinnler and Gripon, 2004). Lactones occur naturally in milk, and also in cheeses. These compounds are characterized by very pronounced fruity notes (peach, apricot, coconut), but they are not relevant to the flavor of cheese (Molimard and Spinnler, 1996). Hydroxylated fatty acids, naturally present in triglycerides in milk, are direct precursors of lactones which are produced from the intra-molecular esterification of hydroxyacids through the loss of water to form a ring structure (Spinnler and Gripon, 2004). The mechanism of lactone formation in cheese is a one-step, non-enzymatic reaction, where hydroxyacids esterified in triglyceride undergoes *trans*-esterification to release the lactone directly due to microbial metabolism (Spinnler and Gripon, 2004; Alewijn, 2006).

2.7 Textural development during cheese manufacture and ripening

Texture is a primary quality attribute of Camembert cheese inextricably linked to biochemical and physicochemical changes that occur during ripening. Major structure-forming constituent in cheese is casein matrix composed of overlapping and cross-linked strands of partially fused para-casein aggregates. The matrix also occludes, within its pores, fat globules, moisture, and dissolved substances (minerals, lactic acid, peptides and amino acids), microbial cells, and enzymes (Law, 1997; Banks, 1998; Fox et al., 2000; O'Callaghan and Guinee, 2004).

In general, Camembert cheese undergoes considerable modification of texture during manufacture and ripening, which may be occur in three phases (Adda, 1982; Lawrence et al., 1987; Law, 1997). According to Law (1997), three conditions for the softening of Camembert cheese are: (a) high water content of approximately 50%; (b) pH higher than 5.2; and (c) degradation of casein by coagulant.

During cheese manufacture, application of coagulant and acidification by LAB lead to high aggregation rate of curd. As the pH of curd decreases, there is concomitant loss of colloidal calcium phosphate from the casein sub-micelles and, below pH 5.5, a progressive dissociation of the sub-micelles into smaller casein aggregates. As the pH of the cheese curd approaches the isoelectric point of casein (pH=5.2-5.3), the protein assumes a more compact conformation and the cheese becomes shorter in texture. At pH below 4.8, the formation of a coarse casein network is observed, which resembles a firm, brittle and crumbly gel of cheese.

Proteolysis modifies the texture properties of the curd during ripening. In early ripening, texture modification occurs due to primary proteolysis when about 20% of the α_{s1}-casein is hydrolyzed, and the casein network is greatly weakened; the crumbly/rubbery texture of young cheese curd is rapidly converted into a smoother, more homogeneous product (Lawrence et al., 1987). The slow change in texture is largely controlled by the concentration of residual coagulant and plasmin in cheese, as well as the proteolytic activity of LAB and *P. camemberti*. The relationship between development of texture and proteolysis of Camembert cheese has been reported by many studies (Lawrence et al., 1987; Schlesser et al., 1992; Sousa and McSweeney, 2001; Leclercq-Perlat et al., 2004a; Sullivan et al., 2005; Guizani et al., 2007; Hayaloglu et al., 2008). However, the role of proteolysis is probably minor compared with pH modification of cheese (Law, 1997).

The effect of pH on Camembert cheese texture is well-documented (Adda, 1982; Schlesser et al., 1992; Sousa and McSweeney, 2001; Spinnler and Gripon, 2004). The surface flora, especially *G. candidum* and *P. camemberti*, metabolize lactic acid resulting in a pH gradient between the rind and the core. The pH gradient is amplified by the production of ammonia resulting from deaminating action of surface microorganisms. At the end of ripening, the surface pH of Camembert reaches about 7.0, while the average core pH would be about 5.5. Increasing the pH leads to an increase in the net charge of caseins and their water absorption capacity and the solubility of the caseins, thus the curd becomes smoother and soft; at higher pH levels, it may become liquid-like. Softening of cheese occurs in the outer part and then progresses towards the center as ripening progresses.

Water content of the cheese curd is another determining factor for texture. The higher pH level of curd at draining can lead to higher syneresis level. High acidification in cheese thus produces brittle curds, while insufficient acidification leaves high moisture content and a very soft texture that flows when the ripe cheese is cut. High moisture content of cheese may retain higher concentration of coagulant enzyme in the curd and effectively stimulating the growth of *P. camemberti*, thus allowing proteolysis to occur more readily (Guinee and Wilkinson, 1992). The moisture content of cheese curd reduces during ripening (Lawrence et al., 1987; Schlesser et al., 1992). The initial decrease of moisture content of curd during early ripening may be due to surface evaporation of the water (Schlesser et al., 1992). After 12 d (ripening), the surface evaporation decreases and further decrease in moisture content throughout ripening is largely attributed to proteolysis. Proteolysis causes water uptake during casein breakdown. Formation of ionic groups (-COO^- and -NH_3^+) compete for available water in the system; and water previously available for solvation of protein chains becomes held up (immobilized) with the new ionic groups (Adda, 1982; Lawrence et al., 1987).

2.8 Analytical methods used for monitoring Camembert cheese ripening

2.8.1 Partitioning Camembert cheeses and sampling

Compounds of dairy products are generally distributed in a heterogeneous way, and therefore representative samples should be analysed to produce valid results. When sampling cheeses such as Camembert with gradients from centre to surface, the cheese should be cut (Law, 1997; Fox et al., 2000; Spinnler and Gripon, 2004). A commonly used sampling technique for Camembert cheese is freezing followed by grating of the sample at low temperature. The powder can then be dispersed in water using high speed homogeniser to obtain representative samples prior to taking an aliquot (Mariaca and Bosset, 1997; Katoka et al., 2000).

Normally, Camembert cheese is divided into three portions, rind, interior and centre, respectively (Graet et al., 1983; Karahadian et al., 1985; Graet and Brule, 1988; Leclercq-Perlat et al., 2004a; Leclercq-Perlat et al., 2004b). The sampling procedure is illustrated in Figure 18 (Graet et al., 1983; Graet and Brule, 1988). Samples with lower thickness (Leclercq-Perlat et al., 2004a; Leclercq-Perlat et al., 2004b), rind region (1-3 mm) is cut from the body of the cheese.

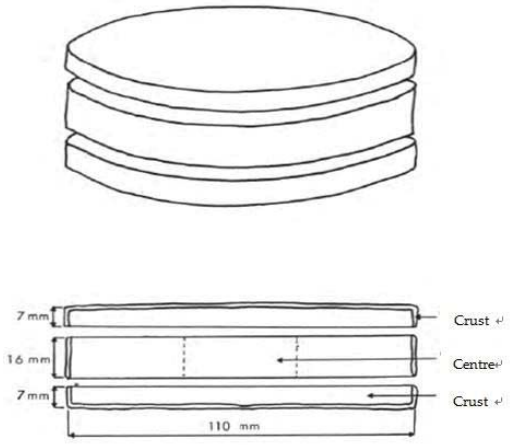


Figure 18. Partition and sampling of Camembert cheese (Graet et al., 1983; Graet and Brule, 1988).

2.8.2 Biochemical assessment of Camembert cheese ripening

Flavour and texture of cheese are results of complex microbiological, physiochemical and biochemical reactions. Thus, knowledge of the physicochemical, biochemical and microbiological characteristics during ripening is important. Most work has been done on hard cheeses, and very little on soft cheeses like Camembert (Karahadian et al., 1985; Sousa and McSweeney, 2001; Sousa et al., 2001; Leclercq-Perlat et al., 2004b; Sullivan et al., 2005;

Vitova et al., 2006; Guizani et al., 2007; Vitova et al., 2007; Hayaloglu et al., 2008). Products of proteolysis (peptides and amino acids) are the most commonly used maturation parameters for predicting ripening stage of Camembert. Since cheese maturation is inter-related, better knowledge on the aroma compound production, textural changes, and behaviour of cheese culture activity/association regards to ripening time may also help to understand the maturation process of Camembert.

2.8.2.1 Analysis of nitrogen (N) content of cheese fractions

Introduction

Cheese maturation index is an important criterion for starter bacteria selection. Proteolysis is closely linked to the maturity of cheese (Grappin et al., 1985; Rank et al., 1985; Schlesser et al., 1992; Tzanetaki et al., 1993; Ardö, 1999; Fox et al., 2000; Sousa et al., 2001; Sousa and McSweeney, 2001; Upadhyay et al., 2004; Sullivan et al., 2005; Guizani et al., 2007; Hayaloglu et al., 2008; Delgado et al., 2010). Monitoring proteolysis through peptide degradation and free amino acid formation during cheese ripening is a reasonable approach to control cheese maturation process.

A range of analytical techniques has been developed to assess the nature and extent of proteolysis (Folkertsma and Fox, 1992; McSweeney and Fox, 1997; Upadhyay et al., 2004). Methods for assessing complexity of proteolysis are classified into two classes: specific- and non-specific techniques. Non-specific techniques include quantification of SN in or extractable by solvents or precipitants and, the liberation of reactive groups. Specific techniques analyze individual peptides or free amino acids by chromatography and electrophoresis.

Evaluating proteolysis in cheeses mainly uses non-specific methods which partition cheese into different N fractions (Grappin et al., 1985; Rank et al., 1985; Schlesser et al., 1992; Tzanetaki et al., 1993; Ardö, 1999; Fox et al., 2000; Sousa et al., 2001; Sousa and McSweeney, 2001; Upadhyay et al., 2004; Sullivan et al., 2005; Guizani et al., 2007; Hayaloglu et al., 2008; Delgado et al., 2010). Different aspects of maturation of cheese and the extent of proteolysis are determined by analyzing peptide fractions using macro-Kjeldahl and free amino acids using cadmium chloride-ninhydrin reagent. One advantage of evaluating proteolysis by analyzing N content of cheese fraction is that SN is correlated with cheese age and to a lesser extent with quality (McSweeney and Fox, 1997; Fox et al., 2000).

Fractionation of N content

The principle of fractionation of N content for assessing proteolysis in cheeses is that milk caseins are insoluble in various solvents, but peptides, free amino acids and N compounds

produced during maturation of cheeses may be precipitated during solvent extraction. Proteolysis relies on changes in protein and peptide solubility of cheese as soluble peptides increase during cheese ripening (Grappin et al., 1985; Rank et al., 1985; Schlesser et al., 1992; Tzanetaki et al., 1993; Sousa et al., 2001; Roseiro et al., 2003; Sullivan et al., 2005; Hayaloglu, 2007; Guizani et al., 2007; Delgado et al., 2010).

Use of two fractionation schemes (Figures 19 and 20), containing fractions listed in Tables 11 and 12 are recommended (Grappin et al., 1985; Rank et al., 1985; Ardö, 1999; Sousa et al., 2001). The N fractions schemes are either extracted with water or in citrate solution. Therefore, cheese variety and its characteristics (e.g. pH) should be considered carefully when choosing fractionation method. Although water extraction of cheese can separate small peptides from the caseins and large peptides in mature Cheddar, it however has one major limitation in Camembert as it relies on pH. For cheeses characterized by significant increase in pH during ripening, such as Camembert, a scheme for the fractionation of a citrate dispersion of cheese and measurements of pH 4.6-soluble nitrogen (pH 4.6-SN) is preferred over water soluble nitrogen (WSN) (Ardö, 1999; Sousa et al., 2001; Sousa and McSweeney, 2001). Caseins are dissolved in the citrate solution when the pH is decreased below 4.6 with constant addition of acid to obtain a pH of 4.4-4.6. Fractionating a citrate dispersion of cheese under controlled pH (4.4-4.6) makes it possible to compare proteolysis in most cheese varieties of different pH values based on age or cheese-making procedure. Another advantage of using citrate dispersion of cheese as a preliminary step in the analysis of N fractions is that both total nitrogen (TN), pH 4.6-SN, trichloroacetic acid soluble nitrogen (TCA-SN) and phosphotungstic acid soluble nitrogen (PTA-SN) are determined from the same citrate suspension, leading to more accurate results and analysis of dry matter is therefore not necessary (Roseiro et al., 2003).

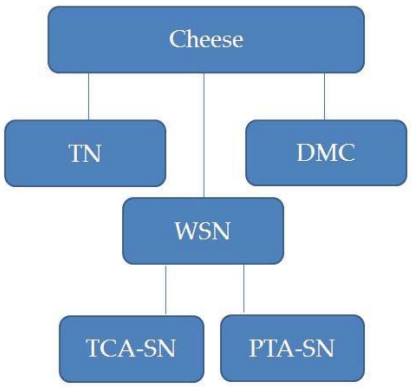


Figure 19. A scheme of cheese fractionation of water (soluble water content) to determine proteolysis comprising Kjeldahl analysis of cheese of water soluble nitrogen (WSN), trichloroacetic acid soluble nitrogen (TCA-SN) and phosphotungstic acid soluble nitrogen (PTA-SN) as % of total nitrogen (TN), respectively, and analysis of dry matter content (DMC) of cheese, which is required for the calculations (Ardö, 1999; Sousa et al., 2001).

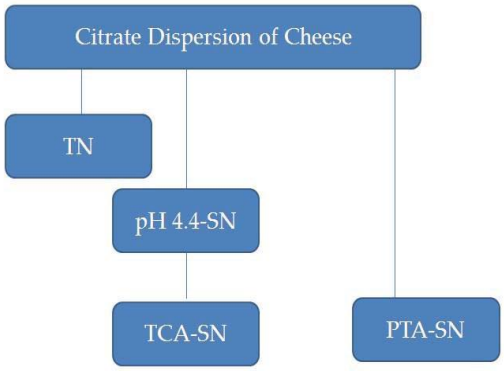


Figure 20. A scheme for the fractionation of a citrate suspension of cheese to determine proteolysis comprise Kjeldahl analysis of cheese of pH 4.6-SN, TCA-SN and PTA-SN as % TN, respectively. Analysis of dry matter is not necessary (Ardö, 1999; Sousa et al., 2001)

Table 11. Solvents used for fractionation of N components in cheese (Folkertsma and Fox, 1992; McSweeney and Fox, 1997; Ardö, 1999; Sousa et al., 2001; Roseiro et al., 2003; Guizani et al., 2007; Delgado et al., 2010).

N components	Fractionation solvents	Content	Activator
TN	--	Total proteins including most caseins	--
WSN	Water	Proteins (excluding most caseins), all peptides, amino acids and smaller N compounds, such as amines, urea and ammonium	Coagulant (e.g. chymoin) and milk indigenous enzymes (e.g. plasmin)
[1]pH 4.6-SN (ASN)	Acid buffer to pH 4.4-4.6	Proteins (excluding all caseins), all peptides, amino acids and smaller N compounds, such as amines, urea and ammonium	Coagulant (e.g. chymosin) and milk indigenous enzymes (e.g. plasmin)
[2]TCA-SN (NPN)	12%-TCA (trichloroacetic acid)	Medium-sized to small peptides, amino acids and smaller N compounds, such as amines, urea and ammonium	Largely contributed from proteinases and peptidases of starter (mesophilic LAB) and secondary bacteria (in particular *P. camemberti*), and coagulant (e.g. chyomin) to a lesser extent
PTA-SN	2.5%-PTA (phosphotungstic acid)	Very small peptides, amino acids and small N compounds except dibasic amino acids and ammonia	Peptidases of starter (mesophilic LAB) and secondary bacteria (in particular *P. camemberti*)

[1]pH 4.6-SN is also known as acid soluble nitrogen (ASN); [2]TCA-SN is also known as non-protein nitrogen (NPN).

Table 12. Fractions of N components in a citrate dispersion of cheese (McSweeney and Fox, 1997; Guizani et al., 2007; Delgado et al., 2010)

Group of N compounds	Calculation
Casein N	[TN]-[ASN]
Larger peptide N	[ASN]-[NPN]
Smaller peptides N	[NPN]-[PTA-SN]
Amino acid N	[PTA-SN]

pH 4.6-SN, TCA-SN and PTA-SN (Tables 11 and 12) are generally used as maturation parameters in the proteolysis of Camembert (Schlesser et al., 1992; Sousa and McSweeney, 2001; Leclercq-Perlat et al., 2004a; Sullivan et al., 2005; Guizani et al., 2007). pH 4.6-SN fraction in cheese, also known as acid soluble nitrogen (ASN), includes peptides and small amounts of amino acids (> 15 000 Da), produced by proteolysis through actions of residual coagulant and enzymes of secondary cultures

(particularly *P. camemberti*), and to a lesser extent, indigenous milk proteinases (plasmin) (Grappin et al., 1985; Rank et al., 1985; Roseiro et al., 2003; Sullivan et al., 2005; Guizani et al., 2007). Trichloroacetic acid (TCA) is a protein precipitant used widely for N fractionation at pH 4.6. Typically 2-12% TCA is used, depending on the degree of fractionation required (larger peptides being soluble at lower TCA concentrations) (McSweeney and Fox, 1997). TCA-SN fraction is also known as non-protein nitrogen (NPN) of cheese, generally contains smaller residues of peptides (2-20) and amino acids with molecular weights of 600 Da<MW<15000 Da. PTA-SN fraction is used as an index of free amino acids in cheese as only free amino acids and very small peptides (<600 Da) soluble in solvent. Ammonia and other organic basic N compounds may also be precipitated. Proteinases and peptidases of starter and secondary bacteria (particularly *P. camemberti*) make substantial contribution for production of NPN and PTA-SN from N fraction at pH 4.6 (Table 11) (Upadhyay et al., 2004).

Quantification of nitrogen content by Macro-Kjeldahl analysis

The Macro-Kjeldahl method is subsequently used to determine the N content in each fraction, which involves digestion, distillation and titration (Lynch and Barbano, 1999; AOAC, 2005a). Folkertsma and Fox (1992) recommended the use of Cadmium chloride-ninhydrin assay to monitor the liberation of free amino groups during ripening and the method has been widely used (Tzanetaki et al., 1993; McSweeney and Fox, 1997; Ardö, 1999; Sousa et al., 2001; Roseiro et al., 2003; Sullivan et al., 2005; Hayaloglu, 2007; Delgado et al., 2010). Cd-ninhydrin reagent can be performed on citrate-fractions, pH 4.6-SN, WSN or PTA-SN fractions of cheese but not on the TCA-SN fraction, as the latter interferes with color development.

2.8.2.2 Analysis of aroma compounds of cheese using HS-SPME/GC-MS

Volatile aroma compounds are important contributors to the quality of cheese as they impact on the flavour. Volatile components of Camembert cheese comprise of over 200 compounds in cheese matrices and usually present at extremely low concentration (< 10 μg/kg) (Imhof et al., 1995; Mariaca and Bosset, 1997; Katoka et al., 2000). Headspace solid phase microextraction combined with gas chromatography and mass spectrometer (HS-SPME/GC-MS) is commonly used to analyse the compounds (Mariaca and Bosset, 1997; Reineccius, 2006; Taylor and Linforth, 2010). SPME, an advanced sorptive technique used for sample extraction, provides representative solvent-free aroma extracts that allow capture of volatile compounds

by direct adsorption onto a solid phase of fibre; the method is simple and reproducible (Mariaca and Bosset, 1997). GC/MS is most widely used for identification and quantification of compounds (Qian et al., 2010). Identified key flavour compounds were also used to predict some sensory attributes of cheese products (Karahadian et al., 1985; Leclercq-Perlat et al., 2004b; Vitova et al., 2006; Vitova et al., 2007).

When characterizing aroma compounds in cheese samples using SPME technique, both qualitative and quantitative information is desired. However, obtaining a complete aroma profile of cheese samples is difficult to accomplish (Mariaca and Bosset, 1997; Reineccius, 2006; Taylor and Linforth, 2010).

Sampling process and description of the HS-SPME

The HS-SPME uses a fused-silica fibre that is coated on the outside with appropriate stationary phase. The device consists of fibre holder and fibre assembly with built-in fibre inside the needle which looks like a modified syringe (Figure 22). The fibre holder consists of a spring-loaded plunger, a stainless-steel barrel and an adjustable depth gauge with needle, and is designed to be used with re-usable and replaceable fibre assemblies. The fine fused-silica fibre needle (with the size of a 0.5 μl phase) is coated with a relatively thin film of several polymeric stationary phases (with several choices available depending on the polarity of analytes). The coated fibre is a modified syringe in which the needle is retractable, the film of stationary phases act like a 'sponge', concentrating the organic analytes on its surface during absorption or adsorption from the sample matrix (Mariaca and Bosset, 1997; Katoka et al., 2000; Reineccius, 2006; Taylor and Linforth, 2010).

The process of HS-SPME is illustrated in Figure 21. Cheese sample is placed in a vial, and then sealed with a septum-type cap. When sampling, the HS-SPME needle pierces the septum and the fibre is extended through the needle and is exposed in the headspace (vapour phase) above a cheese sample; the target analyte partitions from the sample matrix into the stationary phase at the equilibrium. When it reaches an equilibrium, the fibre is withdrawn into the needle; the needle is removed from the septum and is then inserted directly into the injection port of the GC-MS, where the adsorbed volatiles of analytes are thermally desorbed from the fibre coating and then transferred directly to the GC column for analysis (Mariaca and Bosset, 1997; Reineccius, 2006; Katoka et al., 2000; Taylor and Linforth, 2010).

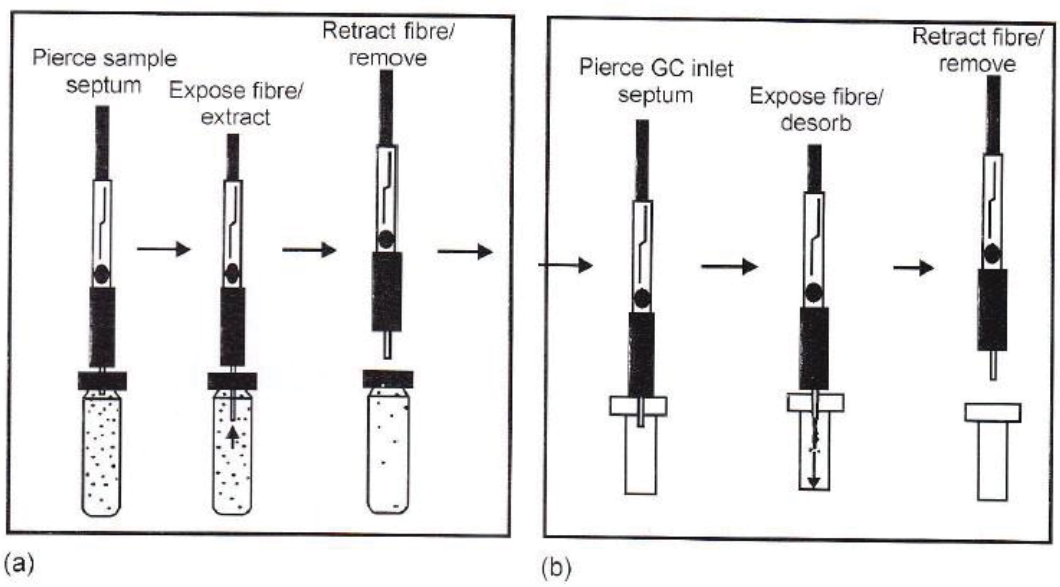


Figure 21. A schematic of the HS-SPME sampling process (Katoka et al., 2000).

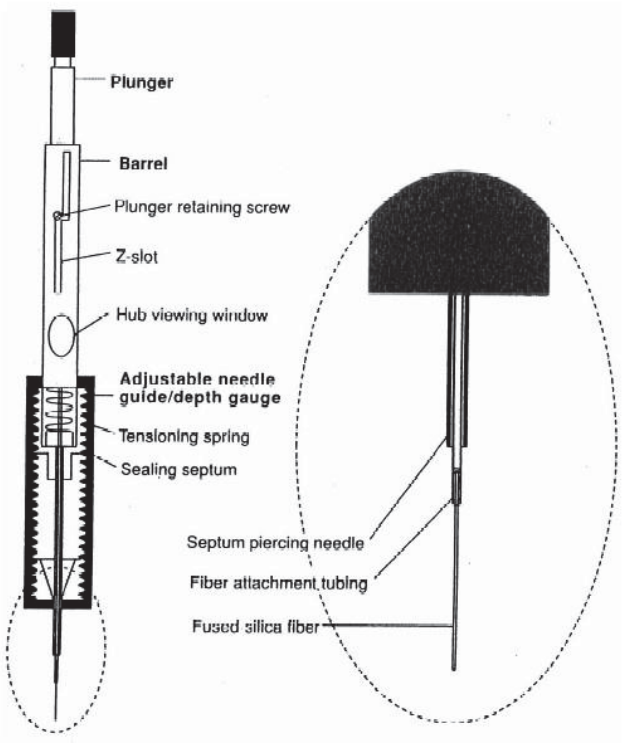


Figure 22. Commercial HS-SPME (Katoka et al., 2000).

Set of SPME parameters in previous cheese volatile studies are summarized in Table 13. Selection of appropriate fibre type for volatile compound collection is vital. Fibres of Carboxen/Polydimethylsiloxane (CAR-PDMS) and Polyacrylate (PA) are mostly used for analysis of volatile compounds in dairy products. PA and PDMS fibres were compared by Pinho et al. (2001) for the analysis of Ewe cheese. Both fibres retained

large amounts of volatile compounds from headspace of cheese, with different selectivity for different compounds; the efficiency of PA was better than PDMS at analysing fatty acids from C_4-C_{11}. Chin et al. (1996) obtained similar results in compounds of Swiss and Cheddar cheeses by HS-SPME using PDMS and PA fibres, with the latter giving better efficiency. PDMS fibre basically retained most volatile components of the headspace, in very high proportions; while PA fibre retained components of a broader volatility range (Guillen et al., 2004). SPME is an equilibrium technique, the efficiency of SPME fibre depends not only on the polarity of the stationary phase, but also on the extraction temperature and time, and adsorption temperature and time (Karahadian et al., 1985; Mariaca and Bosset, 1997; Leclercq-Perlat et al., 2004b; Vitova et al., 2006; Vitova et al., 2007; Kaminarides et al., 2007). In order to increase the rate of extraction, samples are usually heated follows agitation (Table 13). Efficient thermal desorption of an analyte in the GC injection port also requires adequate injector temperature and high linear flow around the fibre (Table 13).

Table 13. HS-SPME[1]/GC-MS methods used for the analysis of volatile compounds in cheese samples.

Cheese type	Micro-extraction				Gas-chromatography					Mass spectrometer		Reference
	Fibre type	Sample extraction	Fibre absorption	Fibre adsorption	Helium gas (ml/min)	Oven (°C /min)	Column	Injector	Injector temperature	Detector temperature	Electron energy set	
Mould-ripened	CAR/PDMS[2]	40 °C /30 min	40°C /30 min	250 °C /2 min	Linear, 1.0	40°C/ 2 min; at 5°C /min to 70°C, 1 min; at 10 °C /min to 240°C	Agilent FFAP (polar)	Splitless	250 °C	/	33-450 amu, threshold 1000, at a sampling rate of 1.11 scans/s	Hayaloglu et al. (2008), Hayaloglu (2009)
Feta	CAR/PDMS	80 °C /30 min	/	/	Linear, 1.5	35 °C/3 min; at 5 °C/min to 110 °C at 10 °C/min to 240 °C;	CO-WAX (polar)	Splitless	280 °C	250 °C	70eV	Kourkoutas et al. (2006)
Camembert	CAR/PDMS	35 °C /30 min	35 °C /20 min	250 °C /5 min	Linear, 0.9	40°C /1 min, at 2 °C/min to 120 °C at 5 °C/min to 200 °C, 5 min;	BD-WAX (polar)	Splitless	250 °C	220 °C	70eV	Vitova et al. (2007)
9 Cheese types	CAR/PDMS	35°C/30 min	35 °C /20 min	250 °C /5 min	Linear, 0.9	40°C /1 min, at 5 °C/min to 200°C, 7 min;	BD-WAX (polar)	Splitless	250 °C	220 °C	70eV	Vitova et al. (2006)
Hard-type	CAR/PDMS	80 °C /30 min	/	/	Linear, 1.5	35°C/ 3 min; at 5°C /min to	CO-WAX (polar)	Splitless	280 °C	250 °C	70eV	Eleftheria et al. (2008)

						110°C, 1 min; at 10 °C /min to 240°C, 10 min						
Smoked fresh cheese	PA[3]	50 °C /15 min	50 °C /60 min	220 °C/ 10 min	/	45°C/ 0.5 min; at 4°C /min to 250°C, 20 min;	Non-polar	Splitless	220 °C	280 °C	70eV	Guillen (2004)
Ewe cheese	PA	60°C/40 min	60 °C /20 min	220 °C/ 10 min	/	40°C/ 2 min; at 5°C /min to 230°C, 10 min;	BD-WAX (polar)	Splitless	220 °C	250 °C	70eV	Pinho et al. (2001)
Whey protein concentrates	PA	40 °C /30 min	40 °C /30 min	250 °C/ 10 min	Linear, 1.0	38 to 80 °C at 2 °C/min, to 160 °C at 6 °C/min, to 220 °C at 10 °C/min, 3 min	Wax (polar)	Splitless	/	/	/	Yang et al. (1998)
Swiss cheese	PA	40°C/30 min	/	/	/	40-80°C at 8°C /min, 2 min; at 8°C /min to 160°C, 2 min, at 8°C /min to 220°C, 10 min,	Agilent FFAP (polar)	Splitless	/	/	/	Jou and Harper (1998)

[1] Headspace sampling mode; [2]CAR/PDMS: Carboxen/polydimethylsiloxane; [3]PA: Polyacrylate;

Identification of aroma compounds by GC

Chromatography is a separation process that distributes the substances to be separated between mobile and stationary phases. GC utilizes a mobile gas phase (nitrogen or helium) to carry the gaseous mixture of sample onto a column, coated with a stationary phase which could be either solid or liquid. Since the stationary phase has selective affinity to components in the mixture, each component is thus eluted at a different time and then separation takes place (Reineccius, 2006; Taylor and Linforth, 2010; Qian et al., 2010).

The sample must be vaporized in the injection port in order to pass through the column for separation. In cheese flavour, volatiles are normally introduced onto the head of GC column for chromatographic separation directly from SPME fibre through thermal desorption, and then carried through a purge gas (mobile phase), together with a split-less injection mode to increase the sensitivity (Karahadian et al., 1985; Mariaca and Bosset, 1997; Leclercq-Perlat et al., 2004b; Vitova et al., 2006; Vitova et al., 2007; Kaminarides et al., 2007). An injection port serves the purpose of providing a place for sample introduction and its vaporization; in split-less injection, the split vent is closed and all of the analytes enter the column. For volatile compound analysis of milk and dairy products, polar columns (DB-WAX and CO-WAX (polyethylene glycol) or HP-FFAP (polyethylene glycol treated with nitroterephthalic acid) are used (Table 13). Since compound elution time and resolution are dependent upon temperature, temperature-programmed analyses are most common in cheese flavour research (Karahadian et al., 1985; Mariaca and Bosset, 1997; Leclercq-Perlat et al., 2004b; Vitova et al., 2006; Vitova et al., 2007; Kaminarides et al., 2007) (Table 13). The column is often at a lower oven temperature (e.g., 35^{o}C) and is then programmed to elevated temperatures (e.g., 250^{o}C). High temperature will allow the sample to elute faster, but may affect resolution.

Compound identification in GC is based primarily on relative retention time of test compounds compared to standards. The relative retention time of published data also provide good references for compound identification. Although, retention time obtained from the GC has been widely used for compound identification, MS is however recommended for precise results.

Quantification of aromatic compounds by MS

MS involves the interaction of electromagnetic radiation or some form of energy with molecules. In MS, electrons in a molecule become ionised when they absorb radiation

energy (a process called electron ionization). The generated ions are then resolved according to their mass-to-charge ratio (*m*/*z*) by subjecting them to electrostatic fields (mass analyser) (Smith and Thakur, 2010) and finally detected. A stage of ion fragmentation is also included before detection to elicit structure information of a molecule which is commonly known as tandem MS. The result of ion generation, separation, fragmentation and detection is manifested as mass spectrum, which is used to identify compound. It is a graphic representation of mass distribution of ions, providing information about their molecular weight and structure (Smith and Thakur, 2010). Comprehensive spectral MS libraries and efficient computerized spectrum-searching and matching system are also available which make identification of compounds simpler.

Compound quantification in MS relies on critical peak area in the chromatogram and the constructed calibration curve. The peak area is proportional to the amount of component in the sample; both internal and external standard methods can be used for generating calibration curves. In the internal standard technique, a compound is utilized that is structurally relative to, but is eluted independently from test compounds. The procedure involves analysing a test sample containing unknown amounts of each component with a predetermined amount of internal standards. The amount of each component in the sample is determined by comparing height and area of that compound peak to internal standard peak. External standard applies a set of standard mixtures containing known concentrations of test samples which are analysed and their peak areas are recorded. A standard curve is generated by plotting concentration against the amount of analyte determined.

2.8.3 Microbiological analysis of Camembert cheese

Microbiological analysis of the cheese is essential for the quality of cheese. Cheese LAB and ripening cultures play important roles in Camembert manufacture and ripening. The biochemical evolution of cheese during ripening is directly and indirectly attributed collectively to microbial association of residual starter bacteria and ripening cultures. It is therefore important to correlate the activity of cheese cultures to predict product quality (Karahadian et al., 1985; Leclercq-Perlat et al., 2004a; Guizani et al., 2007; Lessard et al., 2012).

2.8.3.1 Enumeration of lactic acid bacteria from Camembert cheese

Enumeration of total mesophilic LAB

The most reliable method for enumerating mesophilic LAB is plating on selective media, such as the de Man, Rogosa, and Sharpe (MRS) agar with pH adjusted to 6.5. With anaerobic incubation at 30°C for 72 h, the medium isolates mesophilic lactococci spp., and also supports the growth of *Leuconostoc* spp., without allowing differentiation between the two groups (Thage et al., 2005; Rehn et al., 2011; Karimi et al., 2012).

***Enumeration of Lactococcus* spp.**

M17 medium has now become the standard for enumeration and isolation of lactococci spp. (IDF, 2003; IDF, 2010). The medium contains sufficient amounts of all the nutrients necessary to support the growth of lactococci and a high concentration of β-glycerophosphate (19 g/L) as buffer (Downes and Ito, 2001). By using the medium (pH=7.2) aerobically at 30°C for 72 h, lactococci strains (*L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, and *L. lactis* subsp. *lactis* biovar. *diacetylactis)* can be isolated as well as those lacking the ability to ferment (Fox et al., 2000; Downes and Ito, 2001).

Enumeration of aromatic Cit⁺ lactic acid bacteria

Aromatic citrate (Cit^+) fermenters, containing *Leuconostoc* spp. and *L. lactis* subsp. *lactis* biovar. *diacetylactis*, are components of many mesophilic dairy LAB starters (LD-type). They are widely used in the manufacture of Camembert cheeses due to their ability to ferment citrate with concomitant production of CO_2 and diacetyl. To control gas and aroma production during Camembert fermentation, it is important to know the quantitative composition of the starter cultures used.

For the enumeration of Cit^+ fermenters in aromatic LD-type starters, the modified Nickels and Lessment medium is used (IDF, 2006; IDF, 2010). The medium allows direct differentiation on agar between the Cit^+ fermenters (*Leuc. lactis*, *Leuc. mesenteroides* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis*) and non-Cit^+ fermenters (*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*) in aromatic starters (IDF, 2006; Rehn et al., 2011). The bacteria utilize suspended insoluble calcium citrate when incubated for 3 d under aerobic conditions at 25°C. Cit^+ fermenters exhibit clear zones around the colonies. Differentiation between the leuconostoc and lactoccoci is aided by use of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). After 24 h incubation at ambient temperature, the *Leuconostoc* species are able to split the lactose

analogue X-gal, thus liberating an indigo-blue color. The *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis* are unable to split X-gal (Vogensen et al., 1987; IDF, 2006). When performing the plate count, *Leuconostoc* species are blue, with or without clear zones, while *L. lactis* subsp. *lactis* biovar. *diacetylactis* colonies are white with clear zones. *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* appear white without a clear zone (IDF, 2006).

2.8.3.2 Enumeration of *Penicillium* spp.

Selective medium used for the isolation of *P. camemberti* in Camembert cheese is limited. However, general enumeration procedures suitable for foodborne moulds are effective for enumerating all common *Penicillium* species (Downes and Ito, 2001; Lessard et al., 2012). One limitation of enumerating yeast and moulds on traditional agar media is that hyphae are multi-cellular structures, and colonies on a Petri dish rarely develop from single cells. In addition, fungi tend to rapidly invade agar surfaces, overlapping or merging with other colonies, resulting in under-estimation of their numbers (Lessard et al., 2012). Media for enumeration of *Penicillium* contain antibacterial compounds as well as compounds that inhibit mould colony spreading. An efficient selective medium for enumeration of *Penicillium* spp. is Potato Dextrose Agar (PDA); chloramphenicol is frequently added because of its broad antibacterial spectra and heat stability (Downes and Ito, 2001).

2.8.4 Instrumental analysis of cheese texture

Texture is an important quality attribute that determines the identity of a cheese and its acceptance by consumers. Texture measurement instruments have now been designed to mechanically mimic the sensory evaluation of human assessors.

The TA.XT2 texture analyser is commonly used to analyse Camembert cheese. The equipment is based on force-compression tests measuring firmness or hardness property of cheese (Lawrence et al., 1987; Schlesser et al., 1992; Sullivan et al., 2005; Guizani et al., 2007). Testing with cylinder or cone probes on cheese samples larger than the probe itself gives good indication of cheese maturity. Practically, a cylindrical or cubical cheese sample of fixed dimensions and at a fixed temperature is placed between the two parallel plates of the instrument. One plate, denoted the base plate, is fixed; the other, denoted the cross-head, is programmed to move at a fixed rate and compresses the cheese sample to a pre-determined level. The measurement of the force required to deform a sample to a certain percentage of its original height,

is denoted as the firmness or hardness of the sample.

3 MATERIALS AND METHODS

In cheese production, both lactic starters and ripening cultures are involved in biochemical activities that are vital to the development of important sensory properties of Camembert such as appearance, texture, taste, mouth-feel and aroma. These biochemical activities are largely determined by the viability of the cultures, which in turn are affected by the storage and handling conditions (Fox et al., 2000; Beresford and Williams, 2004; Fox and McSweeney, 2004; McSweeney, 2004b; Parente and Cogan, 2004). In the current study, the experiments were conducted in two integrated phases. The first phase investigated the effect of storage temperatures on the stability of 14 different cheese LAB starters and ripening cultures (intended for Camembert cheese production) for cell viability, acid production, color and composition. The characterized cultures were then screened to select the most stable cultures with good potential for prototype Camembert cheese production. The prepared cheeses were characterized for acidity, viable cell counts, texture, aromatic compounds and degree of maturation. All analyses were done in either duplicate or triplicate. All chemicals and reagents used in this study were of at least reagent grade or higher.

3.1 Screening and selection of cheese starter and ripening cultures cultures

3.1.1 Experimental Design

A 2^2 randomized complete block design (RCBD) was used to investigate the effect of storage temperatures on activity of lactic starters and ripening (mould) freeze-dried cultures, intended for Camembert cheese production. In this design, two types of treatments were investigated, 14 different commercial freeze dried DVS cheese starters and ripening cultures were stored at selected temperatures for five months. Four storage temperatures were used: -18°C (conventional freezing (as control)), 4°C (refrigeration), 20°C (ambient), and 37°C (elevated ambient temperature). The cultures were stored for five months with monthly analyses, including at baseline (zero storage time).

During storage, the cultures were analysed for cell viability, acid production, colour and composition. The length of the storage time of cultures did not allow

experiments to be repeated within the allocated study time. However, two independent samples were conducted for each analysis. At the end of the storage experiment (five months), the remaining cultures were recovered and stored at -18 °C until required for further use.

Based on preliminary tests (Section 3.1.4), the most promising cheese cultures with good stability and potential to produce Camembert cheese were selected for cheese production as described in Section 3.2.

3.1.2 Cheese starter and ripening cultures

The commercial cheese starters and ripening (mould) cultures were obtained from various manufactures: Chr. Hansen®, DSM®, Maysa®, Danisco® and Sacco®. Based on the composition of the cultures and information given by suppliers, they were divided into three separate groups; namely, O-type mesophilic lactic starter (n=5), LD-type mesophilic lactic starter (n=6), and mould ripening culture (n=3). The O-type mesophilic lactic cultures intended for acid production included *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*. The LD-type lactic cultures comprised *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis,* as well as *Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*, for acid and flavor production. The ripening culture included *P. candidum*, also known as *P. caseicolum*, which is a white variant of *P. camemberti*. For purpose of this study, the species name *P. camemberti* is used in this report. The cultures are summarized in Tables 14, 15, 16.

The cultures were obtained in freeze-dried (lyophilised) form, packed in sterilized aluminum foil sachet impervious to oxygen, moisture and light during storage (Constantia, 2012). The lyophilisate (1 g) contained pure bacteria or mould concentrate protected by sterile lactose, as supplied by the manufacturer (Table 14, 15, 16). The cultures were stored at -18°C according to manufacturers' recommendations until required for use (information on the storage history of the cultures was however not given).

Table 14. Description of mesophilic lactic starter cultures (O type*).

No.	Culture code	[1]Taxonomy	[2]Weight (g) of culture and lactose	Supplier
1	R704	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris*	culture: 0.06; lactose: 0.94	Chr. Hansen
2	Delvo-Tec LL-50A DSL	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris*	culture: 1; lactose: none	DSM
3	Choozit MA 11LYO	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris*	culture: 0.07; lactose: 0.93	Danisco
4	MO032	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris*	culture: 0.06; lactose: 0.94	SACCO
5	CM11	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris*	culture: 0.03; lactose: 0.97	Maysa

Note: *O-type starter cultures contained species of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*. [1,2]As given by the manufacturer/supplier; all culture samples were in fine powder form (white in colour) except samples from DSM supplied in granular form (yellow in colour).

Table 15. Description of mesophilic lactic starter cultures (LD type*).

No.	Culture code	[1]Taxonomy	[2]Weight of culture and lactose (g)	Supplier
6	Flora Danica	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris* *L. lactis* subsp. *lactis* biovar. *diacetylactis* *Leuconostoc.* spp.	culture: 0.13; lactose: 0.87	Chr. Hansen
7	CHN-19	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris* *L. lactis* subsp. *lactis* biovar. *diacetylactis* *Leuconostoc.* spp.	culture: 0.23; lactose: 0.77	Chr. Hansen
8	Delvo-Tec DX-33A DSL	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris* *L. lactis* subsp. *lactis* biovar. *diacetylactis* *Leuconostoc.* spp.	culture: 1.0; lactose: none	DSM
9	CM51	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris* *L. lactis* subsp. *lactis* biovar. *diacetylactis* *Leuc. mesenteroides* subsp. *cremoris*	culture: 0.04; lactose: 0.96	Maysa
10	Probat 222 LYO	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris* *L. lactis* subsp. *lactis* biovar. *diacetylactis* *Leuc. mesenteroides* subsp. *cremoris*	culture: 0.02; lactose: 0.97	Danisco
11	MO 036R	*L. lactis* subsp. *lactis* *L. lactis* subsp. *cremoris* *L. lactis* subsp. *lactis* biovar. *diacetylactis* *Leuconostoc.* spp.	culture: 0.04; lactose: 0.96	SACCO

Note: *LD-type starter cultures contained species of *L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis,* as well as *Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*. [1,2]As given by the manufacturer/supplier; all culture samples were in fine powder form (white in colour) except samples from DSM supplied in granular form (yellow in colour).

Table 16. Description of *P. candidum*[1] ripening cultures.

No.	Culture Code	[1]Taxonomy	[2]Weight of culture and lactose (g)	Supplier
12	Camemberti Swing PCTT033	*P. candidum*	culture: 0.01; lactose: 0.99	Chr. Hansen
13	PC neige	*P. candidum*	culture: 0.008; lactose: 0.992	Danisco
14	PCV5	*P. candidum*	culture: 0.06; lactose: 0.94	SACCO

Note: [1,2]As given by the manufacturer/supplier; [1]*P. candidum*, also known as *P. caseicolum*, which is a white variant of *P. camemberti*. All culture samples were in fine powder form (white in colour).

3.1.3 Storage of cultures at various temperatures

The selected cheese cultures were stored at various temperatures: -18˚C (frozen); 4˚C (refrigerated); 20˚C (ambient) and 37°C (elevated ambient), for 5 months. Temperatures of the storage areas were recorded twice per week to ensure correct temperatures were maintained.

Samples of cultures were analyzed at baseline, and then every four weeks during storage. Two independent random samples were withdrawn from each storage treatment at each sampling interval and analyzed for total cell counts (Section 3.1.4.2) and fermentation capacity (Section 3.1.4.3 for lactic starters only). In the LD cultures, viable cell counts of the citrate-fermenting bacteria were determined at the end of the storage period (Section 3.1.4.2). The color of the cultures was also measured at the end of storage (Section 3.1.4.4) as the presence of lactose is expected to lead to browning due to Maillard reactions (Kurtmann et al., 2009c). All analyses were conducted in two independent samples.

3.1.4 Characterization of cultures

3.1.4.1 Culture preparation

The methods of Higl et al. (2007), Kurtmann et al. (2009c; 2009b) and Nollet and Toldra (2012) were referred for culture preparation. For analysis of cell viability and fermentation (acidification) capability of freeze-dried lactic starter samples (O- and LD-types), 0.4 g (1% inoculum) of culture were rehydrated in 40 ml of 10% sterilized reconstituted skim milk (RSM) (w/v) [Appendix 1.1 A]. A sample (1 ml) was

immediately withdrawn for enumeration of viable cells and the remainder was incubated for 6 h at 30°C. Before sampling for analysis, the prepared culture suspensions were resuscitated by allowing to stand for 20 min for maximum cell recovery and shaken thoroughly before sampling (Kurtmann et al., 2009c). Meanwhile, 0.4 g (0.1% inoculation) freeze-dried *P. camemberti* were measured into 40 ml sterile 0.1% peptone water [Appendix 1.1 B]. Since *P. camemberti* is not expected to produce acid, analysis of acidity was therefore not performed.

3.1.4.2 Cell viability

In this part of the experiment, samples of cultures were enumerated to obtain viable cell counts. One ml sample was aseptically transferred from each prepared culture suspension into sterile Petri-dishes, giving 10^{-2} dilution for lactic starters and 10^{-3} dilution for *P. camemberti.* Suitable serial dilutions were prepared using 9 ml peptone water (0.1%) [Appendix 1.1 B] and plated by pour plating (Downes and Ito, 2001). For enumeration of LAB, MRS agar (pH 6.5), M17 agar and modified Nickels and Leesment medium were used, while *P. camemberti* was plated on YGC agar. The inoculated molten media were allowed to set and then incubated (Table 17). Control plates were included for all microbiological analyses.

Enumeration of O-type mesophilic lactic starter

Population of O-type mesophilic lactic starters comprising *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* were enumerated on M17 agar (Table 17) [Appendix 1.1 C] with incubation at 30°C for 72 h (IDF, 2003; 2010).

Enumeration of LD-type mesophilic lactic starter

Population of LD-type mesophilic lactic starters comprising citrate-fermenting *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* species (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*) and non-citrate-fermenting *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, were enumerated on MRS agar (Table 17) [Appendix 1.1 D]. Incubation was done anaerobically using Aerocult oxygen absorber (Oxoid, UK) at 30°C for 72 h (Thage et al., 2005; Rehn et al., 2011; Karimi et al., 2012).

Enumeration of P. camemberti ripening culture

P. camemberti was enumerated on YGC agar (Table 17) [Appendix 1.1 E] with aerobic

incubation at 25°C for 5 d (IDF, 2004).

Enumeration of citrate-fermenting LD-lactic acid bacteria

Citrate-fermenting (Cit^+) LD-type mesophilic lactic starters were enumerated on modified Nickels and Leesment medium (Table 17) [Appendix 1.1 F] (IDF, 2006). Plates with an overlay were incubated aerobically at 25 °C for 72 h. Citrate fermenters (Cit^+) were differentiated from non-citrate fermenters (Cit^-) based on their formation of clear zones around colonies. Citrate fermenters grown on the modified Nickels and Leesment medium were further differentiated by the addition of X-gal solution [Appendix 1.1 F]. Plates were then incubated aerobically for 24 h at ambient temperature to allow color development due to the hydrolysation of X-gal.

Table 17. Differential media[a] used to enumerate mesophilic lactic starters (O- and LD-type) and *P. camemberti* used for Camembert production.

Medium	Cheese starters and ripening cultures	Incubation conditions	References
M17 agar	O-type mesophilic lactic starters (*L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*)	aerobic incubation at 30°C for 72 h	IDF (2003) method 117, IDF (2010) method 194
MRS[1] agar	LD-type mesophilic lactic starters (*L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris, L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* species (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*))	anaerobic incubation at 30°C for 72 h	Thage et al. (2005), Rehn et al. (2011), Karimi et al. (2012)
Nickels and Leesment & X-gal medium	LD-type mesophilic lactic starters (*L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris, L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* species (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*))	25 °C for 72 h with an overlay; followed by aerobic incubation for 24 h at room temperature with addition of X-gal solution on the surface of agar	IDF (2006) method 180, IDF (2010) method 194
YGC agar	*P. camemberti*	aerobic incubation at 25°C for 5 d	IDF (2004) method 94, IDF (2010) method 194

[1]MRS agar = Molten de Man Rogosa Sharpe Agar, with pH adjusted to 6.5;

[a] Composition and preparation steps for the culture media are shown in Appendix 1.1.

Petri dishes with between 30 and 300 colonies were enumerated using the 3M® (New Zealand) colony counter. Presumptive *Lactococcus* and *Leuconostoc* species on M17 and MRS agar plates had regular shapes with smooth edges. Colonies of *P.*

camemberti on YGC agar were raised 'floccose' with white aerial and vigorous fluffy, close-napped mycelium, with 'short hair' up to 1 cm high. Colonies with clear zones on Nickels and Leesment medium with X-gal represented citrate-fermenting cocci *(L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc.* spp. (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris)*; whereas colonies without clear zones represented non-citrate fermenting cocci (*L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis*). After aerobic incubation for 24 h at ambient temperature with the addition of X-gal, *Leuconostoc* species were blue, with or without clear zones. *L. lactis* subsp. *lactis* biovar. *diacetylactis* colonies were white with a clear zone. Non-citrate fermenters (*L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*) were white without clear zones (IDF, 2006). Since differential medium was used, non-targeted bacteria could not grow. The results were expressed as mean cfu/g (colony forming unit per gram of freeze-dried bacteria culture) from two analyses of two independent samples. The coefficient of variation (CV %) were always below 15%.

To obtain the survival rate of culture after storage, the percentage of survival was calculated on cell count data using Equation 1.

$$\% \text{ Survival} = \frac{\log 10\ N}{1 \log 10\ No} \times 100 \qquad \text{Equation 1}$$

where N_o represents the initial number of cells after freeze-drying; and N is the number of cells after 5 months of storage. Both N and N_o were expressed as cfu/g.

Colony morphology

The morphology of grown colonies was confirmed by Gram staining technique. The Gram stains were examined under high magnification and oil immersion of a Carl Zeiss (model HBO 50/AC, Germany) light transmission microscope (Figure 23).

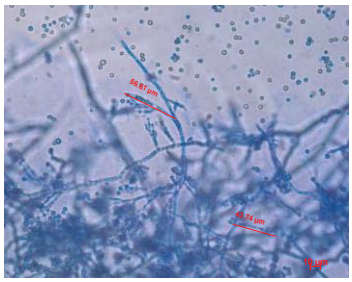


Figure 23. Gram staining of *P. camemberti* grown on YGC agar under oil immersion (100×magnification) of Carl Zeis (model HBO 50/AC, Germany) transmission light microscope.

3.1.4.3 Acidification Profile

Acidification of mesophilic lactic starters (O- and LD-type) was determined by measuring lactic acid produced after 6 h of milk incubation at 30°C. Production of lactic acid was demonstrated by decrease in pH and increase in titratable acidity (T.A.) by the culture growing in milk.

pH measurement

The pH was obtained by direct measurement of incubated milk samples using a digital pH meter (Sartorius, Japan) equipped with glass electrode (AOAC, 2005b). Prior to each measurement, the pH meter was calibrated using standard buffer solutions at pH 4.0, 7.0 and 10.0. The pH electrode was rinsed with distilled water after each measurement.

Titratable acidity

Titratable acidity was estimated following manual titration (AOAC, 2005b). The reagents used for titration were standard sodium hydroxide (0.1 M) solution, phenolphthalein indicator solution (1%) and potassium hydrogen phthalate solution (KHP) [Appendix 1.2]. The sodium hydroxide (NaOH) solution was standardized using KHP before conducting each titration. The test titrations were repeated until quadruple results were concordant, and the concentration of prepared NaOH solution was calculated using Equations 2 and 3:

$$\text{C NaOH} = \frac{\text{M KHP}}{\text{V NaOH}} \qquad \text{Equation 2}$$

$$\text{M KHP} = \frac{\text{m KHP}}{\text{MW KHP}} \qquad \text{Equation 3}$$

Where C NaOH is the concentration of NaOH, M KHP is the molarity of KHP (mol/L); V NaOH is the volume used in the titration, m KHP is the molecular weight of KHP (g); MW KHP =204.23 (g/mol)

Acidified milk (10 g) were measured into a clean conical flask and mixed thoroughly with 20 ml CO_2 free distilled water. During titration, 3-4 drops (1 ml) phenophthalein solution were added to the mixed solution and then thoroughly mixed by swirling. Standardized NaOH solution was titrateed against the test portion to a first persistent light pink color. Percentage of titratable acidity (% T.A.) was expressed as grams of lactic acid per 100 grams of sample, using equations 4, 5 and 6:

$$\text{M lactic acid} = \text{C NaOH} \times \text{V NaOH} \quad \text{Equation 4}$$

$$\text{m lactic acid} = \text{M lactic acid} * 90.08\ \text{g/mol} \quad \text{Equation 5}$$

$$\%\ \text{T. A.} = \frac{\text{m lactic acid}}{\text{m sample}} \times 100 \quad \text{Equation 6}$$

Where M lactic acid is the molarity of the lactic acid (mol/L), C NaOH is the concentration of NaOH solution (mol/ml); V NaOH is the volume (ml); m lactic acid is the mass of lactic acid; 90.08 g/ mol is the molar mass of lactic acid; % T.A. = Percentage of titratable acidity = g of lactic acid / 100 g of the test sample; m sample = weight (g) of the test sample.

3.1.4.4 Color measurement

Colour of the freeze-dried cultures was measured using the Hunter L^*, a^*, b^* colour system (Kurtmann et al., 2009c) with a Minolta colorimeter (CR-300, Japan with 2° observer, calibration plate Y=92.40, X=0.3138, y=0.3192). In this study, colour change was expressed by a^* value, which measures redness (+) or greenness (-); an increase in a^* value represents a change in colour towards redness. b^* value measures yellowness (+) or blueness (-); an increase in the b^* value reflects a change in colour towards yellow and brown. L^* value measures white (+) or black (-); a decrease in L^* value represents a change in colour toward darkness. The Hunter values were determined as the mean of three measurements at three different places on the surface of the freeze-dried culture samples.

3.1.5 Selection criteria of cultures

Table 18. Selection criteria of freeze-dried cheese starter and ripening cultures.

Culture Group*	Selection Criteria			
	high cell viability during storage	good acid production		balanced species composition
		Low pH value	high % T.A.	
O-type lactic starters	✔	✔	✔	
LD-type lactic starters	✔	✔	✔	✔
P. camemberti	✔			

* Freeze-dried cultures were stored at four storage temperatures (-18°C, 4°C, 20°C and 37°C) for 5 months

In this study, the main criterion for selecting cheese cultures was based on their cell counts (viability) at end of the storage period (Nollet and Toldra, 2012). Thus, the selected starters were expected to have minimal cell inactivation (viable cell reduction) during storage (Table 18). Acid-producing activity, colour and composition of culture were also considered when selection as additional factors

(Table 18). Under the fermentation conditions used (Section 3.1.4.3), selected starter cultures should have good acid producing ability, which at least reduce milk pH from 6.6-6.8 to less than 5.3 (equivalent to produce 0.5 %T.A.) (Fox et al., 2000; Nollet and Toldra, 2012). For LD-type starters, citrate fermenting and non-citrate fermenting species should be both present in formulated cultures, with non-citrate fermenting species dominating.

3.2 Production, ripening and characterization of Camembert cheese

3.2.1 Experimental Design

In this experiment, a 2^3 randomized complete block design (RCBD) was used to study the potential of cheese starter and ripening cultures that had been stored (Section 3.1.3) for five months to produce Camembert cheese using I-Make® Cheese domestic kits. In this design (Table 19), the treatments were: type of starter culture (1), temperature of cultures that have been stored (2), the different stages of cheese maturation (3). The type of starter culture used for Camembert production was comprised of 2 levels (O-type and LD-type); storage temperatures of cultures were at 3 levels (-18, 4 and 20°C) (Section 3.1.3). High ambient temperature of 37°C was not included because all cultures stored at this condition after 5 months showed poor acidication ability, low cell viability and were associated with undesirable high degree of discoloration (Sections 4.1.1 and 4.1.2). There were 4 stages of cheese maturation, days 3, 10, 14 and 21. The type of starter cultures used for cheese making in turn defined the type of Camembert produced; cheeses prepared with O-type starters, designated as 'acid-only cheese', and cheeses prepared with LD-type starters, designed as 'aroma-enriched cheese'. The microbiological, biochemical and texture of the cheese samples were measured during ripening (maturation).

Table 19. Randomised complete block design (2 factors) of six unique treatments for the preparation of Camembert cheeses.

Cheese-type	Storage temperature of freeze-dried cultures (°C)	Production	Sample code	Description of cheese cultures
'acid-only' cheese	-18	P1	S1	O-type starters: *L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris* Mould ripening cultures: *P. camemberti*
			S2	
		P2	S3	
			S4	
	4	P1	S1	O-type starters: *L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris* Mould ripening cultures: *P. camemberti*
			S2	
		P2	S3	
			S4	
	20	P1	S1	O-type starters: *L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris* Mould ripening cultures: *P. camemberti*
			S2	
		P2	S3	
			S4	
'aromatic-enriched 'cheese	-18	P1	S1	LD-type starters: *L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris, Leuconostoc* spp. and *L. lactis* subsp. *lactis* biovar. *diacetylactis* Mould ripening cultures: *P. camemberti*
			S2	
		P2	S3	
			S4	
	4	P1	S1	LD-type starters: *L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris, Leuconostoc* spp. and *L. lactis* subsp. *lactis* biovar. *diacetylactis* Mould ripening cultures: *P. camemberti*
			S2	
		P2	S3	
			S4	
	20	P1	S1	LD-type starters: *L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris, Leuconostoc* spp. and *L. lactis* subsp. *lactis* biovar. *diacetylactis* Mould ripening cultures: *P. camemberti*
			S2	
		P2	S3	
			S4	

3.2.2 Camembert Cheese Preparation

Two independent cheese productions were conducted using the Mad Millie™ domestic cheese-making kits (Table 19). For each production, six pilot-scale

Camembert cheese making-trials were performed, with four wheels of cheese prepared for each trial (one for each sampling point; there were four sampling points during cheese maturation) (Table 19). Using the selected cheese cultures from Section 5.0, 'Acid only O-type' cheeses were manufactured with the addition of mesophilic O-type lactic starter culture consisting of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (R704, Chr. Hansen, NZ) and *P. camemberti* ripening cultures (PCTT033, Chr. Hansen, NZ). 'Aromatic-LD type' cheeses were prepared through acidification by mesophilic LD-type lactic starter culture (Flora Danica, Chr. Hansen, NZ) consisting of acid producing *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; and aromatic producing *Leuconostoc* spp. (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris)* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*, and *P. camemberti* ripening cultures (PCTT033, Chr. Hansen, NZ).

Camembert cheeses were produced using Mad Millie™ domestic cheese-making kits. Detailed procedures used for cheese preparation are shown in Figure 24 (Shaw, 1981; Law, 1997; Kosikowski and Mistry, 1997; Banks, 1998; Fox et al., 2000; Leclercq-Perlat et al., 2004a; Leclercq-Perlat et al., 2004b; Spinnler and Gripon, 2004; Sullivan et al., 2005; Guizani et al., 2007; Law, 2010; Mccormack, 2011), which were slightly modified to mimic preparation at household level. Cell counts of the selected 'R704' and 'Flora Danica' lactic starters and 'PCTT033' mould ripening culture contained less than 10^{10} cfu/g (Sections 4.1.1 and 4.1.2); they were therefore not selected for DVS for cheese-making in this study. However, at this concentration, the cultures can be propagated to boost cell numbers prior to use in cheese-making (Fox et al., 2000; Parente and Cogan, 2004; Hoier et al., 2011). In this study, propagation of cultures was not a suitable option as the aim of the project was to use DVS cultures and Mad Millie™ cheese-making kits. Nonetheless, inoculation of about 10^6 cfu/g in milk can produce sufficient acid to reduce pH to <5.3 within 5-6 h at 30°C, reaching 10^9 cfu/ml after drainage (Parente and Cogan, 2004). For each trial, eight Camembert cheeses were prepared in sterile cheese kits (Mad Millie™, New Zealand). The cheese kits were sterilized in 2% idophor water solution (w/w) (Mad Millie™, New Zealand) for at least 30 min. For the preparation of Camembert cheese, 8 L pasteurized, non-homogenized standardised full-fat bovine milk (Meadow Fresh™, Farmhouse, NZ) was purchased from Park and Save Supermarket, Albany, Auckland, NZ. Prior to use, the milk was heated to 32±2°C and then incubated with lactic starters (0.5%) and mould ripening cultures (0.02%) [Appendix 1.3]. The milk was allowed to ferment at 32±2°C for 3 h in an incubator, after which rennet (coagulant) was added. The microbial rennet made from the enzyme of *Mucor miehei*, contained at least 60 International Milk Clotting Units (IMCU) per tablet. Coagulation occurred at 32±2°C

within approximately 45 min. The coagulum was then left undisturbed for 2 h to allow syneresis (whey expulsion) to occur. The coagulum was cut into 2-2.5 cm cubes using a sterile knife, and then curds were stirred for 10 min, and then left for 15 min to enhance syneresis. The curds were then aseptically transferred into a Camembert moulding container (Figure 25), which were placed on a drying mat to allow drainage at ambient temperature (20°C) and form a cheese block. The formed cheese block was flipped (turned) hourly for 5 h and then left overnight (16 h) for further whey drainage. By the end of the draining period, cheese blocks (pH = 4.6-4.9) with Ø≈130 mm and 60 mm height, weighing 200-250g were produced and denoted as 'day one' samples.

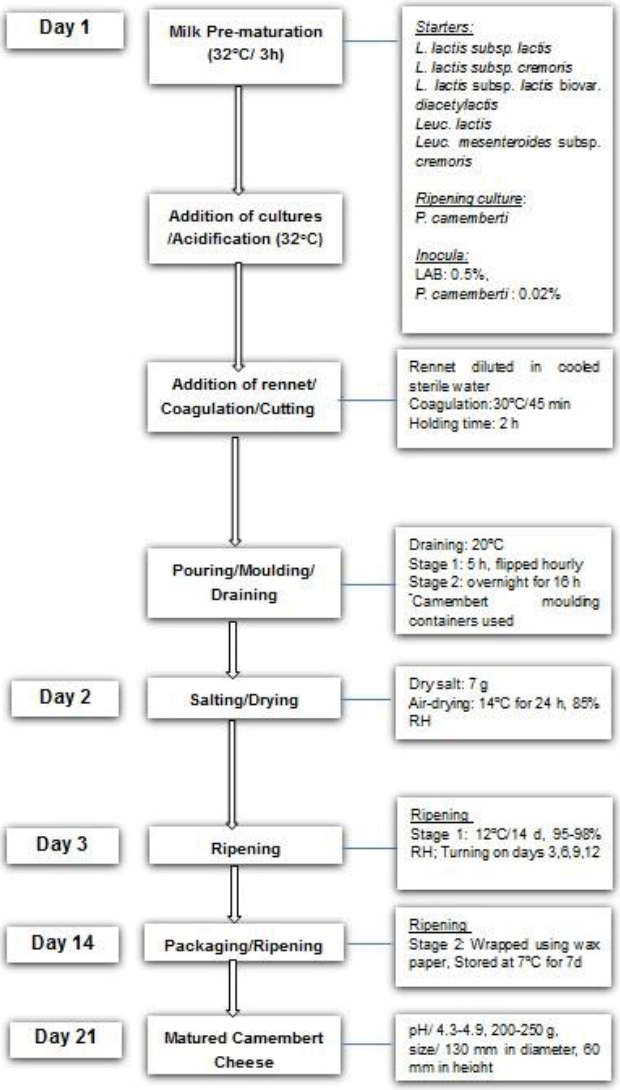


Figure 24. Preparation of Camembert cheese using Mad Millie™ cheese kits (Kosikowski and Mistry, 1997; Leclercq-Perlat et al., 2004a; Spinnler and Gripon, 2004).

Figure 25. Whey-draining of cheese curds at ambient temperature.

On day 2 (Figure 24), the surface of the cheese samples were rubbed with 7 g of table non-iodised salt (Mad Millie™). The salted cheeses were transferred into a maturation (ripening) box (Mad Millie™) and allowed to air-dry for 24 h at 14°C under 85% RH; the cheeses were denoted as 'day three' samples. Cheese ripening was carried out at 12°C under 95-97% RH for the first 14 d and then at 7°C for 7 d, giving a total ripening time of 21 d. The relative humidity environment in the maturation box was controlled by using saturated salt solutions of potassium chloride (KCl) for 85% RH and potassium sulphate (K_2SO_4) for 95-97% RH, respectively. The RH was monitored by using wet bulb and dry bulb thermometers (Bell and Labuza, 2000). The RH values were obtained by using relative humidity chart.

Throughout the first 14 d of ripening, blocks of cheese samples were turned every 2-3 d and then wrapped using waxed paper on day 14 when *P. camemberti* was present on the cheese surface. During the turning of the cheese blocks, the maturation box was opened for 5-10 s to ensure the cheeses received sufficient air (oxygen) for growth of *P. camemberti*. The matured and wrapped Camembert cheeses (Figure 26) were subsequently stored in cardboard boxes at 7°C for 7 d until required for analysis.

Figure 26. Camembert cheese at day 14 before wrapping showing the white fluffy *P. camemberti.*

3.2.3 Camembert Cheese Characterization

3.2.3.1 pH and microbiological composition of cheese curd during milk fermentation

Growth of starter and ripening cultures during fermentation of cheese milk for 24 h was monitored by enumeration of viable cell counts and pH measurement (Leclercq-Perlat et al., 2004a). Duplicate samples from each treatment (one from each production) and duplicate samples from each cheese were obtained and analyzed (Note: four samples were obtained).

One mL of milk sample or twenty-five grams of cheese samples was aseptically obtained at various intervals [at initial inoculation (0 h), after milk pre-maturation (3 h), initial draining (6 h), second draining (11 h), and overnight draining (24 h)], and diluted with sterile 0.1% peptone water to make serial dilutions of up to 10^9 cfu/g or cfu/ml. The diluted samples were plated on appropriate molten agar. Lactic starter bacteria were enumerated on MRS and M17 agar, respectively, while *P. camemberti* was enumerated on YGC agar (Table 17).

The pH of milk was obtained by direct measurement using a calibrated pH meter (Sartorius, Japan) equipped with a glass electrode (AOAC, 2005b). The pH of the cheese curd was measured using a Sartorious pH meter (PB-20) with cheese electrode (model L8880, Schott). Ten g of cheese curd were mixed with 20 ml of CO_2-free distilled water prior to pH measurement (AOAC, 2005b). The pH electrode was rinsed with distilled water after each measurement.

3.2.3.2 Evaluation of cheese during maturation

Sampling

Duplicate cheese blocks from each treatment (one from each production) and duplicate samples from each cheese were obtained (Table 19) and analyzed on days 3, 10, 14 and 21 (Note: four samples were obtained). Using a modified method, 2 mm of the rind (exterior of the cheese), and the inner layer of each cheese including the centre were separated (Graet et al., 1983; Karahadian et al., 1985; Graet and Brule, 1988; Leclercq-Perlat et al., 2004a; Leclercq-Perlat et al., 2004b). The cheese rind was discarded while the body was kept at -80°C overnight, and then grated to produce homogeneous frozen powders (Figure 27). The cheese samples (body, without rind)

were then analysed for nitrogen fractions [total nitrogen (TN), acid-soluble nitrogen (pH 4.6-SN), non-protein nitrogen (NPN)], free amino acid content (FAA) and pH (Figure 27). Meanwhile, samples (body and rind) were used to determine levels of starter microorganisms and *P. camemberti* as well as textural firmness of cheese (Figure 27). Aroma compounds in samples were analysed from cheese body (without rind). For respective treatments, one cheese wheel from each of the two independent productions (n=2) was analysed at each sampling interval.

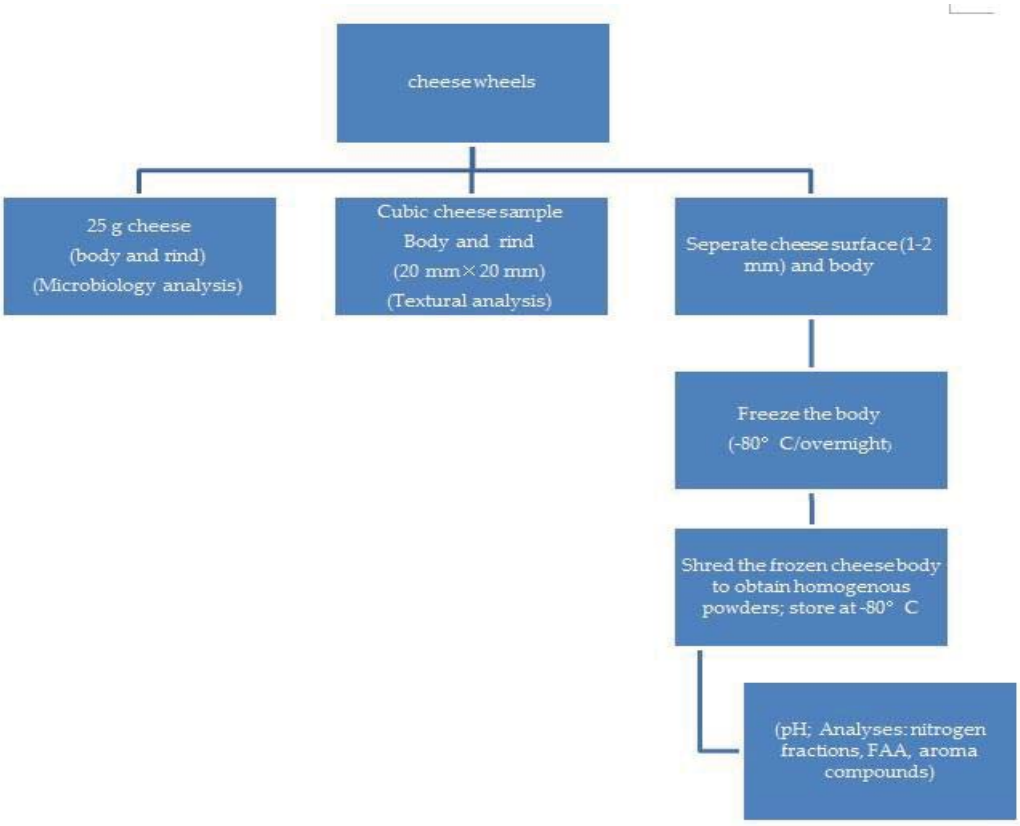


Figure 27. Cheese sampling plan.

pH

Ten g cheese powders were mixed with 20 ml of CO_2-free distilled water. The pH of the mixture was measured using a calibrated pH meter (PB-20, Sartorious) equipped with cheese electrode (model L8880, Schott) (AOAC, 2005b). The pH electrode was rinsed with distilled water after each measurement.

Analysis of nitrogen content of cheese

Two types of cheese analyses were used to monitor the extent of proteolysis during maturation of Camembert cheese (McSweeney and Fox, 1997; Ardö, 1999). The

nitrogen content of three fractions of cheese samples was analyzed using the Micro-Kjeldahl method (AOAC, 2005a); these included TN, pH 4.6-SN, and NPN. FAA content of cheese was determined by the Cd-ninhydrin method (Folkertsma and Fox, 1992).

Fractionation of nitrogen content (Sample extraction)

The IDF 337 method (Ardö, 1999) was used for analysing various nitrogen fractions in cheeses. About 10 g grated cheese were dispersed in 50 ml warm 0.5 M tri-sodium citrate (AR, LabServ Pronalys) solution and stirred for 60 min using a magnetic stirrer on a hotplate at 50±2°C. The suspension was cooled to room temperature (about 20°C) and then made up to 200 ml with distilled water. Of the suspension, 10 ml were analyzed by the Macro-Kjeldahl method for TN analysis. SN was fractionated by adjusting 80 ml of the citrate suspension to pH 4.6 using 1.0 M hydrochloric acid (HCl) (AR, LabServ Pronalys). The suspension was shaken vigorously while the pH was being adjusted to 4.35-4.55, and the pH was constantly monitored by a pH meter (PB-20, Sartorious). The volume was then made up to 100 ml by the addition of distilled water. Of the mixture, 25 ml was filtered using quantitative filter paper (model LBS0040.110, LabServ Filtration) and used for pH 4.6-SN analysis. To analyse for NPN, 12% TCA (AR, Normapur) solution was used for extraction. Fifty ml pH 4.6-SN sample were added to 50 ml of 24% TCA. The solution was kept overnight at 4°C and 50 ml filtered samples were then analyzed by Macro-Kjeldahl method.

Nitrogen analysis of cheese samples (Digest of the extract)

The Macro-Kjeldahl method was used to determine the nitrogen content of TN, pH 4.6-SN and NPN using the 'block digestion/steam distillation system' analyzer (FOSS™, Tecator Kjeltec system, Denmark) (Lynch and Barbano, 1999; AOAC, 2005a; AOAC, 2005c). Samples were measured into Kjeldahl tubes (20 tubes per batch). Two Kjeldahl digestion tablets (Kjeltabs, FOSS™) and 20 ml concentrated sulphuric acid (95-98% pure) (LabServ Pronalys, Reagent Grade) were added into each digestion tube. Digestion was carried out on a block digester (model No. 2020, FOSS™, Tecator Kjeltec system, Denmark) at 420°C for 4 h until the solution became clear. When the digestion was complete, sample tubes were removed from the digester and allowed to cool overnight. The cooled digest was liquid or liquid with only a few small crystals; a solid cake was not accepted for testing.

Steam-distillation of digested samples

The digestion tubes with samples were placed in an automatic distillation unit (model No. 1026, FOSS™, Tecator Kjeltec system, Denmark), while 80 ml distilled water and 80 ml of 40% (w/v) sodium hydroxide solution (NaOH) (LabServ, Reagent Grade) were transferred into a water and alkali tank of the distillation unit, respectively. Samples were then distilled against 40 ml of 4% (w/v) boric acid (LabServ, Reagent Grade) solution containing 1% bromocresol green (w/v in 95% ethanol) (LabServ, Reagent Grade) and 0.7% methyl red (w/v in 95% ethanol) (LabServ, Reagent Grade) in a receiving flask (the condenser tip leading into the receiving flask extended below the surface of the boric acid collecting solution) (AOAC, 2005a; AOAC, 2005c). A steam-distillation was set at 5 min (with 100% steam efficiency) and at least 150 ml distillates were collected. During distillation, the presence of nitrogen in sample induced a color change from bright pink/red to green in the boric acid. The nitrogen content of samples was then determined by titrating the samples against standardized 0.1 M HCl solution to a grey-mauve-pink color. The first 'persistent' pink color with no hint of grey was taken as the end-point; and the progression of color was from green to grey-green, to grey-mauve color with pink overtones (AOAC, 2005a). The flask was lightly stirred to mix for accurate end-point determination and the volume of HCl used was recorded to the nearest 0.05 ml.

Efficiency of analysis and calculation of proteolysis parameters of cheese

Efficiency of analysis of nitrogen fractions was determined by using 0.12 g ammonium sulfate standard (>99% purity) (LabServ, Reagent Grade) and 0.85 g sucrose (LabServ, Reagent Grade) per tube. The sample was digested and distilled under the same conditions used for the test portion; average recoveries were at least 99%. Digestion efficiency was also evaluated by using 0.18 g tryptophan (LabServ, Reagent Grade) standard with 0.67 g sucrose per tube, with recovery rates above 98%. Analyses of blank samples for TN, SN-pH 4.6 and NPN were conducted for each respective nitrogen fraction analysed.

Results were calculated as follows in Equation 7:

$$\boldsymbol{Nitrogen}\ \% = \frac{\mathbf{1.4007 \times (Vs - Vb) \times M}}{\mathbf{W}} \qquad \text{Equation 7}$$

Where Vs and Vb = volumes of HCl (titre) used for test portion and blank (mL), respectively; M = molarity of HCl solution (g/L); and W = weight of test portion (g)

Degree of proteolysis in cheese samples was determined by the 'ripening index' as pH 4.6-SN and NPN against TN (Leclercq-Perlat et al., 2004a; Sullivan et al., 2005;

Guizani et al., 2007) through calculation using Equations 8 and 9:

$$\text{SN (as \% TN)} = \frac{\text{pH 4.6} - \text{SN}}{\text{TN}} \times 100 \quad \text{Equation 8}$$

$$\text{NPN (as \% TN)} = \frac{\text{NPN}}{\text{TN}} \times 100 \quad \text{Equation 9}$$

In addition to the calculated 'ripening index', cheese proteolysis was also evaluated based on the estimated values of protein nitrogen, casein nitrogen and peptide nitrogen content (Delgado et al., 2010; Delgado et al., 2011) through calculation using Equations 10, 11 and 12:

Protein Nitrogen = TN - NPN Equation 10
Casein Nitrogen = TN – pH 4.6-SN Equation 11
Peptide Nitrogen = pH 4.6-SN - NPN Equation 12

Determination of total FAA in cheese samples

Total FAA level was determined in pH 4.6-SN fractions of the cheeses using the Cd-ninhydrin method (Folkertsma and Fox, 1992; Hayaloglu, 2007; Hayaloglu et al., 2008; Hayaloglu, 2009). A 100-µl sample was diluted to 1 ml with distilled water and 2 ml Cd-ninhydrin reagent was added [0.8 g ninhydrin (AR, LabServ, Pronalys) dissolved in a mixture of 80 ml of 99.5% ethanol (LabServ, Reagent Grade) and 10 ml acetic acid (LabServ, Reagent Grade), followed by the addition of 1 g Cadmium chloride (CdCl2) (AR, LabServ, Pronalys) dissolved in 1 ml of distilled water]. The mixture was heated at 84±2°C for 5 min, cooled and the absorbance was measured in a spectrophotometer (UV-VIS model No. 1800, SHIMADZU) at 507 nm. A calibration curve of L-leucine (AR, LabServ, Pronalys) for concentrations of 0.001-0.1 mg/ml was used. The results were expressed as mg Leucine per 100 g cheese.

Key aromatic compounds analysis

SPME

Since obtaining complete aroma profiles of cheese samples is difficult to accomplish (Mariaca and Bosset, 1997; Reineccius, 2006; Taylor and Linforth, 2010), key volatile compounds responsible for characteristic sensory properties of cheese during ripening were analysed by the SPME/GC-MS. The key volatile compounds are good indicators of the main metabolic reactions responsible for flavour synthesis

(Delahunty and Drake, 2004; Quere, 2004). The methods of Kourkoutas et al. (2006), Katechaki et al. (2008), Rehn et al. (2011), Vitova et al. (2006; 2007), Kaminarides et al. (2007), Hayaloglu et al. (2008), and Leclercq-Perlat et al. (2004b) were adapted, with some minor modifications. For each shredded cheese sample (approximately 4 g) and 0.4 ml internal standard (IS) solution (0.008μg/ml) were transferred into a 20 ml headspace vial (fitted with a Teflon-lined septum sealed with an aluminum crimp seal) (SHIMADZU). The SPME programme used in this study is shown in Appendix 3.5. Volatiles were equilibrated by keeping the sample vial at 60°C (thermostatically controlled) for 30 min, with agitation at 500 rpm. In this experiment, sampling was done by using an auto-sampler (AOC-5000, AUTO-INJECTOR, SHIMADZU); volatile compounds extraction was achieved by inserting a 2-cm SPME fiber coated with 85 μm CAR/PDMS fused silica film (divinylbenzene-carboxen on polydimethylsiloxane, pH 2-11, working temperature 250-310°C, maximum heating time 2 h) (SUPELCO, Bellefonte, PA) into the vial through the septum, exposing it to the headspace for 20 min at 60°C. The fiber was positioned at 22.0 cm in each run. Both PA and PDMS fibers have been widely used for sampling volatiles from dairy samples (Pinho et al., 2001). In this experiment, PDMS was preferred due to its greater extraction efficiency (Chin et al., 1996; Guillen et al., 2004).

GC-MS

GC-MS programmes used in this study were set up as shown in Appendix 3.5. Thermal desorption of the extracted volatiles was performed in the GC injection port at 250°C for 5 min in splitless mode, with helium as the carrier gas at a flow rate of 1.0 mL min^{-1}. Gas chromatography (GC-2010, SHIMADZU) was performed with a mass spectrometer detector (GC-MS-QP 2010 PLUS, SHIMADZU). The injection port was equipped with a narrow-bore (0.75 mm. i.d.) glass liner (SUPELCO, Bellefonte, PA) to minimize peak broadening. The split valve was opened 5 min after injection. The mass spectrometer was programmed as follows: the ion source temperature set at 220°C, the interface temperature set at 200°C, detector voltage set at 0.8 kV, mass scan range from 50 m/z to 300 m/z and scan speed of 526 ms. For volatile compound analysis of milk and dairy products, a polar column is desirable for separation (Mariaca and Bosset, 1997). Other workers have recommended use of DB-Wax and Co-Wax columns (Pinho et al., 2001; Kourkoutas et al., 2006; Vitova et al., 2007; Katechaki et al., 2008) but these were not available in our study. Therefore, a low polarity column was used in this study; temperature-program of column was optimized to obtain higher resolution and eliminate peak co-elution and broadening. Volatile compounds were separated on a fused silica RESTEK X-5 column (low

polarity phase, crossbound, diphenyl dimethyl polysiloxane (60 m, 0.25 mm i.d., 0.25 μm thickness, and temperature range of 20°C-230°C). The column temperature was held at 30°C for 3 min (during desorption), then increased at 0.25°C/ min to 46°C, after which it ramped at 5°C/min to 115°C. The temperature was then raised at 20°C/min to 230°C. A final extension was applied at 230°C for 20 min for column clean up, giving a total run time of 121.55 min.

Compound identification and quantification

In this study, dimethyl sulfide, 1-octen-3-one, 1-octen-3-ol, 2-heptanone, 2-nonanone, 3-methyl-butanol, butyric acid (or butanoic acid), 3-methylbutanal (or isovaleraldehyde) and butane-2,3-dione (or diacetyl) were used as external aromatic standards and methyl octanoate was used as internal standard (Leclercq-Perlat et al., 2004b; Kourkoutas et al., 2006; Katechaki et al., 2008; Vitova et al., 2007). For each standard (internal and external), single stock solution was prepared separately using ethanol. Mixed standard solutions (2 ml) were made at various concentrations (0.0008-0.32 μg/ml), using prepared external standard stock solutions, IS solution and Millipore water [Table 34, Appendix 3.5]. In each mixed standard solution, the same amount (0.4 ml) of IS solution (0.008μg/ml) was used. The prepared mixed standard solutions were transferred into 15 ml headspace vials and sealed for analysis. All the above standard compounds were pure GC grade or reagent grade (>95%) and purchased from Fluka and Sigma-Aldrich Pty Ltd (NZ). Millipore water was used for carrying standard solutions instead of organic solvents, although all standards were only slightly soluble in the water. Millipore water was used because most of the organic solvents have low boiling points. When organic solvents are used for headspace analysis, high concentration of solvent would appear in vapour phase which could easily cause the detector being over saturated making mass scan difficult.

Identification of unknown compounds was achieved by comparing retention time of standard compounds and data from database library (Real Time Analysis Database Library, SHIMADZU). However, the use of retention time alone for compound identification is considered weak (Quere, 2004). Therefore, mass spectra of the compounds provided by MS were also used to provide additional data supporting identification beyond GC data. For quantification of volatile compounds, peak areas of external and internal standard (IS) at various concentrations were used to generate calibration curves. Calibration curves were constructed for each standard by plotting concentration of volatile compound against the peak area ratio of analytes and

internal standard determined (Equation 13). Calculation of the volatile compounds in test samples was determined using Equation 14.

$$\frac{\text{peak area STD}}{\text{peak area ISSTD}} = \text{a} \times \text{con. STD} * + \text{b} \qquad \text{Equation 13}$$

Where STD=external standard, ISSTD=internal standard, con.STD=concentration of external standard (μl/ml), 'a' is a factor, 'b' is a constant. *since concentration of internal standard (0.008 μl/ml) was applied constantly in both standard solutions and samples, standard curve was not determined using concentration ratio of STD/ISSTD (IOFI, 2011; Biedermann et al., 2007). In each standard, a linear relationship between response and concentration was obtained over the desired range for the analysis of cheese samples used in this study.

$$\text{Concentration of volatile compound} = \frac{\left(\frac{\text{peak area of compound}}{\text{peak area ISSTD}} - \text{b}\right)}{a \times \text{cheese weight}} \times 10^6 \qquad \text{Equation 14}$$

Where the concentration of volatile compounds in cheese samples is calculated in μg/kg, cheese weight (g).

Microbiological analysis

Samples (body and rind) were analyzed on days 3, 10, 14 and 21 to evaluate microbial changes of cheese during maturation. Duplicate cheese from each treatment (one from each production) and duplicate samples from each cheese were used, giving four samples. Description of microbial analyse of cheese fermentation profiles can be found in Section 3.2.3.1. Twenty-five grams of each of cheese sample were aseptically withdrawn at various intervals for analysis. Lactic starter bacteria were enumerated on MRS and M17 agar (IDF, 2003; Thage et al., 2005; IDF, 2010), while *P. camemberti* was enumerated on YGC agar (IDF, 2004).

Instrumental texture analysis

Textural profile of cheese samples (with rind) during ripening was assessed on days 3, 10, 14 and 21; duplicate cheese from each treatment (one from each production) and duplicate samples from each cheese were used, giving four samples.

Texture analysis of Camembert cheese samples were performed using a TA-TX2 Texture Analyzer (SMS Stable Micro System Ltd, United Kingdom), operating in the compression mode (Guamisa et al., 1997; Antoniou et al., 2000; Saldo et al., 2000). Cubic cheese samples with rind (20 mm diameter × 20 mm high) were obtained using a knife lubricated with food grade Canola™ oil (vegetable oil) to prevent fracture

during cutting. Prepared samples were kept at room temperature (20°C) for 2 h prior to analysis. Texture analysis of samples was done in a temperature-controlled room set at 20°C. During texture analysis, test pieces of cheeses were compressed between 50 mm diameter flat upper plate probe and a square stainless steel bottom plate to 50% of the original height. A pre-test speed of 1.0 mm/sec, test speed of 2.0 mm/sec, post-test speed of 10.0 mm/sec, and an automatic trigger force of 5.0 g were used [Appendix 3.4]. Both sample surfaces in contact with the plates were lubricated with Canola™ oil to prevent friction.

The TX.TA2 equipment was connected to a computer with data transfer rate for force, displacement and time data. The test measurements were analyzed using the Texture Analyzer TE32 software. Data were collected in the form of force/time/displacement curves, using the software Texture Expert (Stable Micro System). Hardness of the cheese samples was determined as the end force required to compress samples (point of maximum deflection of the curve during the first pressing cycle).

3.4 Statistical Analysis

Data were analysed by statistical tests using procedures of Minitab 16 Statistical Software (Minitab Inc., State College, PA, USA). Data for normality and homogeneity of variance were tested using the Shapiro-Wilk and Levene tests, respectively at 95% confidence levels (α=0.05) to determine whether data were parameteric. Non-parametric data were $\log_{10}$ transformed to ensure normal distribution. Since cell counts in the present study exhibited trends of microbial growth, normal distribution could not be easily obtained. Therefore, cell counts were log-transformed to allow for statistical data analysis. Raw data of various analyses are in shown in Appendix 2-3 and all statistical outputs are shown in Appendix 4.

A. Screening and selection of cheese starter and ripening cultures

The data (cell counts, acidity and colour) were subjected to Analysis of Variance (ANOVA) using General Linear Model (GLM) to determine the effects of storage temperature, time and the brand of cultures ($P<0.05$). The ANOVA test was performed separately with respective culture type. Significant differences ($P<0.05$) between the means were separated using Tukey's multiple comparison test. Descriptive statistics were also used on parametric data to calculate mean values and standard deviations for each treatment.

To obtain the rate of bacteria inactivation during storage, the regression line was determined from the plot of the normal logarithm of the residual cell count (log_{10} cfu/g) versus storage period (month) for respective culture samples under each temperature. The absolute value of the slope (regression coefficient) determined from the regression line was used as the inactivation rate constant at that specific condition only when the Pearson's correlation coefficient (r^2) of the regression line was greater than 0.90.

Data (cell counts, acidity and culture composition) were also used to generate curves to show trends or changes for specific parameters against time. The curves were generated using Orign Pro (Version 8, OrignLab, Corporation, Northampton, MA, USA). Each point on the line graph represents the mean of two/four samples at each time interval. The standard deviation was also depicted at each mean value.

B. Production, ripening and characterization of Camembert cheese

ANOVA was performed using GLM to establish the effect of culture storage temperature, starter type and ripening time. Tukey's test was applied to compare the mean values of the investigated parameters. The level of significance difference between treatments was determined ($P<0.05$). Descriptive statistics were used on paramedic data to calculate mean values and standard deviations for each treatment.

Correlation between microbiological, physico-chemical and textural properties of cheese was determined by Pearson's correlation analysis ($P<0.05$).

A multivariate analysis was also performed on principle components analysis (PCA) using data from SPME-GC/MS. The purpose of PCA analysis was to determine any relationships between the volatile compounds (VC) and to establish any correlation between cheese samples (Juric et al., 2002; Sullivan et al., 2005; Hayaloglu et al., 2008). The analysis was performed by using volatile compound concentrations as variables. PCA was conducted using covariance matrix and varimax rotation on standardised values, weighted by the inverse of the standard deviation (1/SD)) (Juric et al., 2002). Multivariable analyses of data were analysed using procedures of SPSS Version 19 (SPSS Inc; Chicago, IL, 2013).

4 STABILITY OF CHEESE STARTER AND RIPENING CULTURES

4.1 Cell counts of samples during storage

4.1.1 Lactic starter cultures

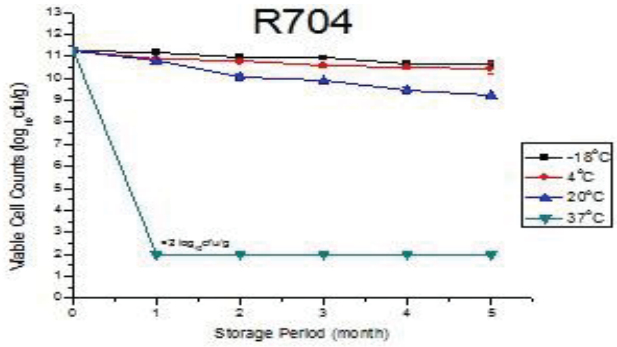
Fourteen different freeze-dried culture samples (O-type and LD-type starters and *P. camemberti*) were stored for 5 months at different temperatures (Section 3.1.3). The trends of viable cell counts obtained are illustrated in Figures 28 and 30. For the viability of freeze-dried starter bacteria stored at -18°C, 4°C and 20°C, regression curves were fitted and the corresponding first order rate constants (k) representing the inactivation kinetic rate of cells are presented in Table 22. Cell counts and survival rates of O-type and LD-type starters and *P. camemberti*, after storage for 5 months, are also shown in Tables 20, 21 and Figures 29, 31.

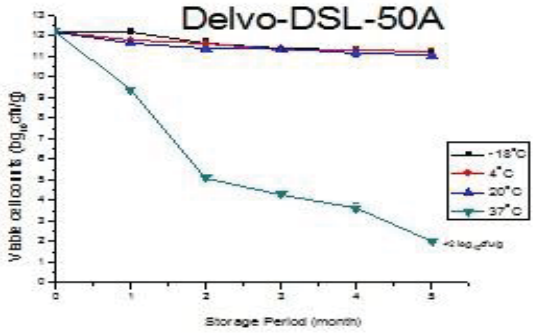
Considerable high cell counts (10^{9-12} cfu/g for O-type starters; 10^{9-11} cfu/g for LD-type starters) were detected at initial sampling time in all lactic starter cultures. At the four temperature conditions (-18, 4, 20, 37°C), viability of all starters (O- and LD-types) decreased ($P<0.05$) to different levels throughout storage. Considerable reduction in cell counts was observed in both starter types, with LD-type being more thermal resistant than O-type starters at three temperatures except 37 °C. Survival of starter bacteria was also inversely ($P<0.05$) affected by the period of storage.

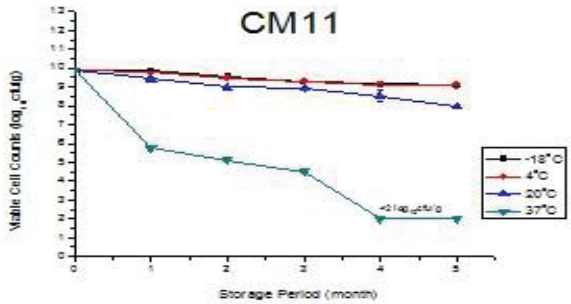
For both starter types (O- and LD-types), Figure 28 shows that the viability of cells remained fairly stable at -18, 4 and 20°C for 2-3 months, then steadily decreased during the remaining storage period. Cultures stored at -18°C were less affected than cultures stored at 4 and 20°C (Tables 24, 25). However, final cell counts of culturs remained above 10^7 cfu/g at all three temperatures at the end of the storage period. Similar levels of cell viability degradation were observed for samples stored at refrigeration temperature, with survival rate ranging from 91.07-92.70% for O-type and 91.07-92.70% for LD-type starters at end of storage time (Figure 28; Table 20). Cell counts of O-type starter cultures stored at -18°C and 4°C were comparable ($P=0.8190$). However, cell counts of the cultures decreased by 10-20 % during storage at ambient temperature (Figure 28; Table 20). Although lower cell counts of bacteria were obtained, the remaining cells were still significantly high (10^{7-10} cfu/g) in the two

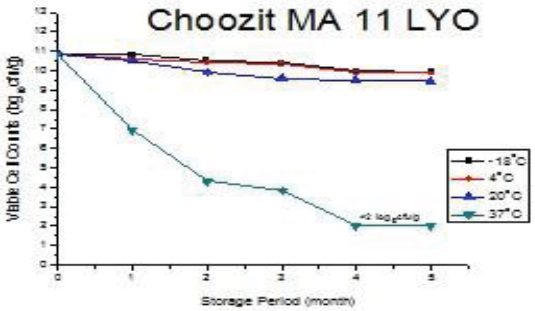
types of cultures at end of storage period that above the recommended level of 10^7 cfu/g. Storage at 37°C had the highest effect on loss viable cells; there was a rapid loss of cells at this temperature. O-type cultures lost between 16.39-64.06% of the initial cell concentration, while LD experienced losses of 20-73% after 30 d of storage (data not shown). There were <100 cfu/g cells in the cultures after 5 months of storage.

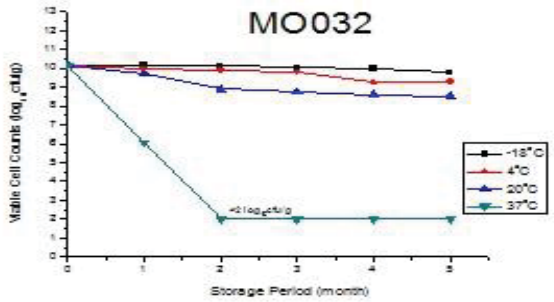
After storage for 5 months at various temperatures, significant differences in cell counts were observed between samples ($P<0.05$) for both starter types. Generally, Delvo-tec LL-50 DSL (O-type) and Delvo-tec-33A (LD-type) samples from DSM had the highest cell viability at three temperatures (-18, 4 and 20°C) (Table 20 and Figure 29). This was followed by good cell survival in R704 (O-type), CHN-19 (LD-type) and Flora Danica (LD-type) supplied by Chr. Hansen rated second. Samples from Danisco (11 LYO and Probat 222), SACCO (MO032 and MO036) and Maysa (CM11 and CM51) showed relatively lower cell viability at all three temperatures (Table 20 and Figure 29). Maysa samples (CM11 and CM51) exhibited the poorest cell survival of all the starter cultures studied.


R704
Viable Cell Counts (log10 cfu/g)
Storage Period (month)
-18°C
4°C
20°C
37°C

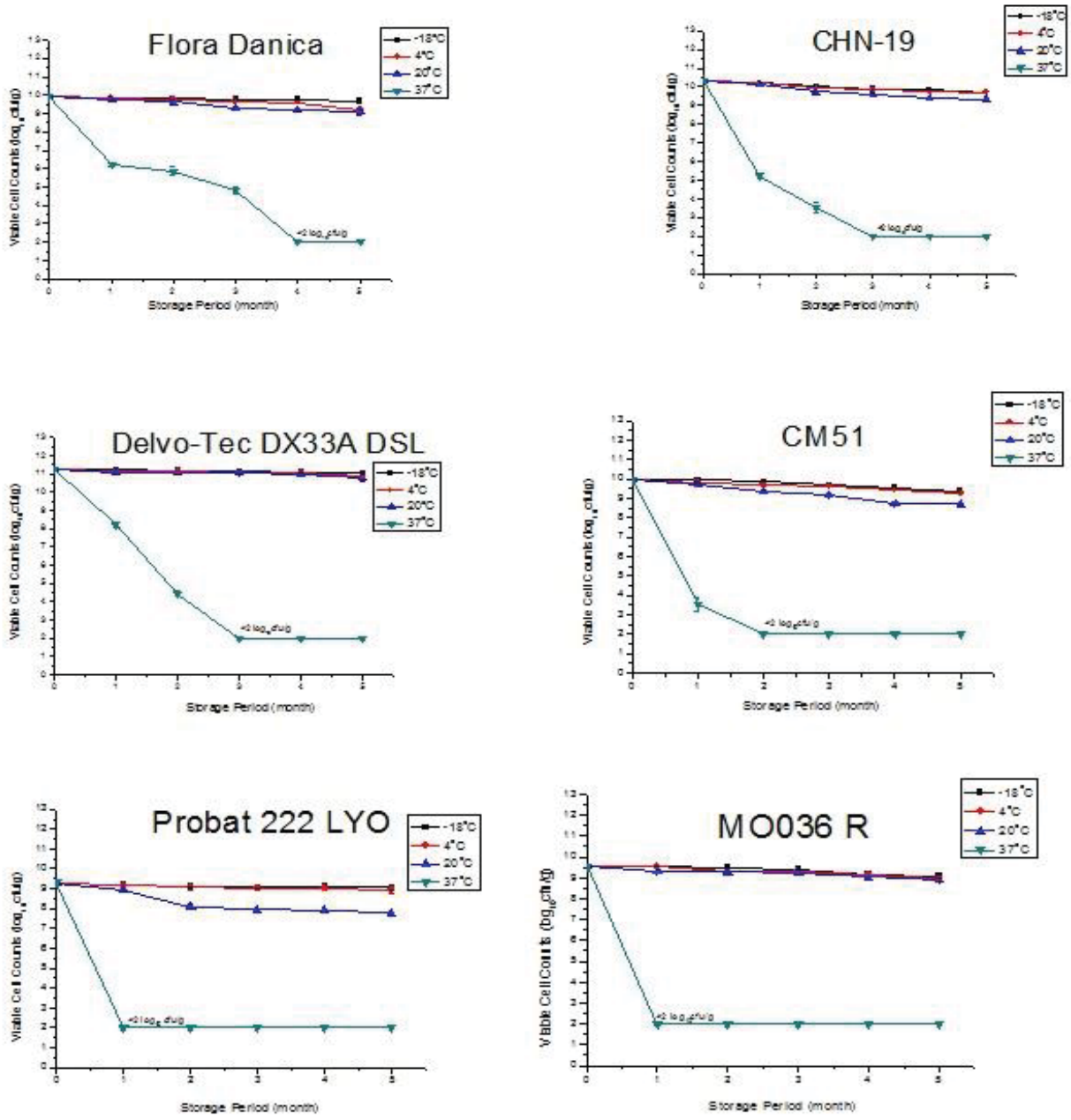

Delvo-DSL-50A
Storage Period (month)
-18°C
4°C
20°C
37°C


CM11
Storage Period (month)
-18°C
4°C
20°C
37°C


Choozit MA 11 LYO
Storage Period (month)
-18°C
4°C
20°C
37°C


MO032
Storage Period (month)
-18°C
4°C
20°C
37°C

(a)



(b)

Figure 28. Mean (± SD) viable cell counts (n=2) of (a) O-type- and (b) LD-type starter cultures (freeze-dried), at each temperature level during storage for 5 months. O-type LAB starters: R704 (Chr. Hansen), DSL (DSM), CM11 (Maysa), 11 LYO (Danisco) and MO032 (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; LD-type LAB starters: Flora Danica and CHN-19 (Chr. Hansen), DX-33A DSL (DSM), CM51 (Maysa), Probat 222 LYO (Danisco) and M 036R (SACCO), containing strains of *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis, Leuc. lactis, Leuc. mesenteroides* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*. Storage temperature: -18°C, 4°C, 20°C and 37°C.

Table 20. [1]Mean viable cell counts (log cfu/g) and survival rates (%) of mesophilic O-type[2] and LD-type[3] starter LAB samples after 5 months of storage time at various temperatures[4].

Starter type	Codes of cultures	Treatment Temperatures (°C)			
		-18	4	20	37
O-type	R704	^{z}10.62±0.00^{a}	^{z}10.45±0.24^{a}	^{z}9.22±0.01^{b}	<2.00±0.00^{c}
		94.36	92.70	81.81	0
	DSL 50A	^{w}11.25±0.02^{a}	^{w}11.23±0.16^{a}	^{w}10.04±0.12^{a}	<2.00±0.00^{b}
		92.22	92.07	90.47	0
	MA 11 LYO	^{y}9.96±0.01^{a}	^{zy}9.88±0.04^{a}	^{z}9.47±0.07^{b}	<2.00±0.00^{c}
		91.78	91.07	87.24	0
	MO032	^{xy}9.49±0.33^{a}	^{y}9.50±0.25^{a}	^{y}8.52±0.08^{b}	<2.00±0.00^{c}
		95.91	91.23	83.69	0
	CM11	^{x}9.09±0.09^{a}	^{x}9.09±0.15^{a}	^{x}7.96±0.03^{b}	<2.00±0.00^{c}
		91.84	91.90	80.48	0
LD-type	Flora Danica	^{xy}9.68±0.14^{a}	^{xy}9.23±0.10^{ab}	^{yz}9.10±0.15^{b}	<2.00±0.00^{c}
		97.65	93.12	91.85	0
	CHN-19	^{y}9.71±0.07^{a}	^{z}9.71±0.03^{a}	^{z}9.34±0.06^{b}	<2.00±0.00^{c}
		94.20	94.18	90.59	0
	DSL 33A	^{z}11.02±0.04^{a}	^{w}10.85±0.03^{a}	^{w}10.79±0.16^{a}	<2.00±0.00^{b}
		98.05	96.55	95.96	0
	CM51	^{xy}9.38±0.03^{a}	^{y}9.30±0.01^{a}	^{y}8.72±0.05^{b}	<2.00±0.00^{c}
		94.03	93.22	87.40	0
	Probat 222 LYO	^{x}9.06±0.12^{a}	^{x}8.91±0.16^{a}	^{x}7.78±0.06^{b}	<2.00±0.00^{c}
		97.29	95.74	83.56	0
	MO036	^{x}9.09±0.08^{a}	^{xy}8.96±0.09^{a}	^{y}8.88±0.11^{a}	<2.00±0.00^{b}
		95.18	93.80	93.02	0

[1]mean (± SD), (n=2). [2]O-type LAB starters: R704 (Chr. Hansen), DSL (DSM), CM11 (Maysa), 11 LYO (Danisco) and MO032 (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; [3]LD-type LAB starters: Flora Danica and CHN-19 (Chr. Hansen), DX-33A DSL (DSM), CM51 (Maysa), Probat 222 LYO (Danisco) and M 036R (SACCO), containing strains of *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *Leuc. lactis, Leuc. mesenteroides* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*. [4]Storage temperature: -18°C, 4°C, 20°C and 37°C.
Within rows, mean values followed by different superscripts are significantly different (Tukey's test, $P<0.05$). Within columns, mean values preceeded by different superscripts are significantly different (Tukey's test, $P<0.05$), with respect to one type of culture.

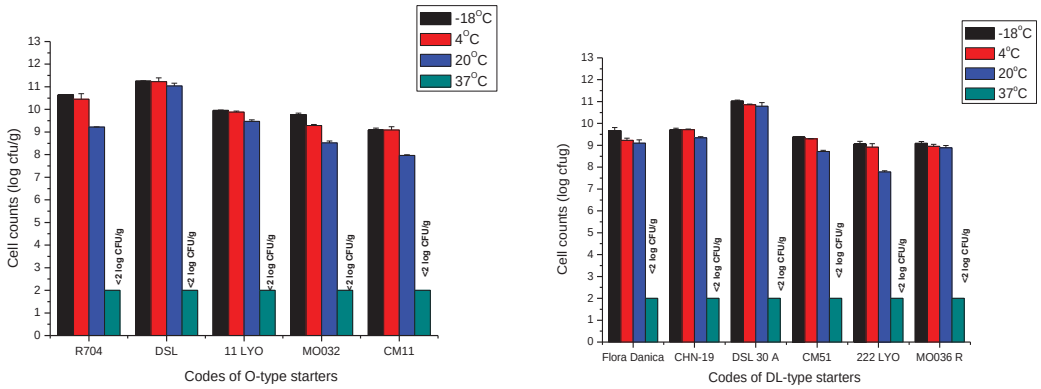


Figure 29. Mean (± SD) viable cell counts (n=2) of O-type and LD-type lactic starter samples after storage for 5 months at four different temperatures. O-type LAB starters: R704 (Chr. Hansen), DSL (DSM), CM11 (Maysa), 11 LYO (Danisco) and MO032 (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; LD-type LAB starters: Flora Danica and CHN-19 (Chr. Hansen), DX-33A DSL (DSM), CM51 (Maysa), Probat 222 LYO (Danisco) and M 036R (SACCO), containing strains of *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *Leuc. lactis, Leuc. mesenteroides* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*. Storage temperature: -18°C, 4°C, 20°C, 37°C.

4.1.2 Ripening (*P. camemberti*) cultures

Initial cell concentration of mould cultures were fairly high, ranging from 10^7-10^8 cfu/g. Moulds stored at higher temperatures had lower cell counts ($P<0.05$). High numbers of viable *P. camemberti* cells were recorded after storage at conventional frozen, refrigeration and ambient temperatures, but not at the high ambient temperature (37°C). Cell concentrations were similar ($P>0.05$) between samples stored at refrigeration and ambient temperatures. A significant decrease ($P<0.05$) in cell viability was also observed during storage at all temperatures.

During storage, viable cells of mould cultures stored at frozen temperature were stable that above 10^7 cfu/g (Table 21 and Figure 30). Cell counts of samples stored at refrigeration and room temperatures declined to almost the same levels (10^6-10^7 cfu/g), maintaining between 82.13-94.83% and 79.75-89.95% of survival rates, respectively. Although cell viability declined faster in samples stored at lower temperatures compared to the those under frozen conditions, high cell concentrations were generally above the recommended level of 10^5 cfu/g (Parente and Cogan, 2004). Poor viability mould cells were especially observed following storage at high ambient temperature. Residual viability of samples was generally reduced sharply in the first 30 d of storage losing about 50% of initial viability (data not shown), and then decreased to $<10^3$ cfu/g at the end of storage period (Table 21 and Figure 30).

After storage for 5 months at various temperatures, significant differences were detected between cell counts of samples (P <0.05). PCTT033 (Chr. Hansen) and PCV5 (SACCO), generally exhibited higher viability compared to PC Neige (Daniso) at all temperatures, except 37 °C (Table 21 and Figure 31). Although cell concentrations between samples were different (P<0.05), viability of samples were comparable and above the recommended level.

Table 21.[1]Mean viable cell counts (log cfu/g) and survival rates (%) of *P. camemberti* samples[2] after 5 months of storage at various temperatures[3].

Codes of cultures	Treatment Temperatures (°C)			
	-18	4	20	37
PCTT033	7.75±0.03^{a}	^{y}7.50±0.05^{b}	^{y}7.07±0.02^{c}	<3.00±0.00^{d}
	98.68	94.83	89.95	0
PCV5	7.68±0.05^{a}	^{xy}7.20±0.12^{b}	^{y}7.03±0.10^{b}	<3.00±0.00^{c}
	91.56	85.79	83.86	0
PC NEIGE	7.48±0.25^{a}	^{x}6.46±0.06^{b}	^{x}6.27±0.22^{b}	<3.00±0.00^{c}
	95.06	82.13	79.75	0

[1] mean ± SD, (n=2). [2]*P. camemberti*: PCTT033 (Chr. Hansen), PCV5 (SACCO), and PC NEIGE (Danisco), [3]Storage temperature: -18°C, 4°C, 20°C and 37°C.
Within rows, mean values followed by different superscripts are significantly different (Tukey's test *P*<0.05). Within columns, mean values preceeded by different superscripts are significantly different (Tukey's test *P*<0.05).

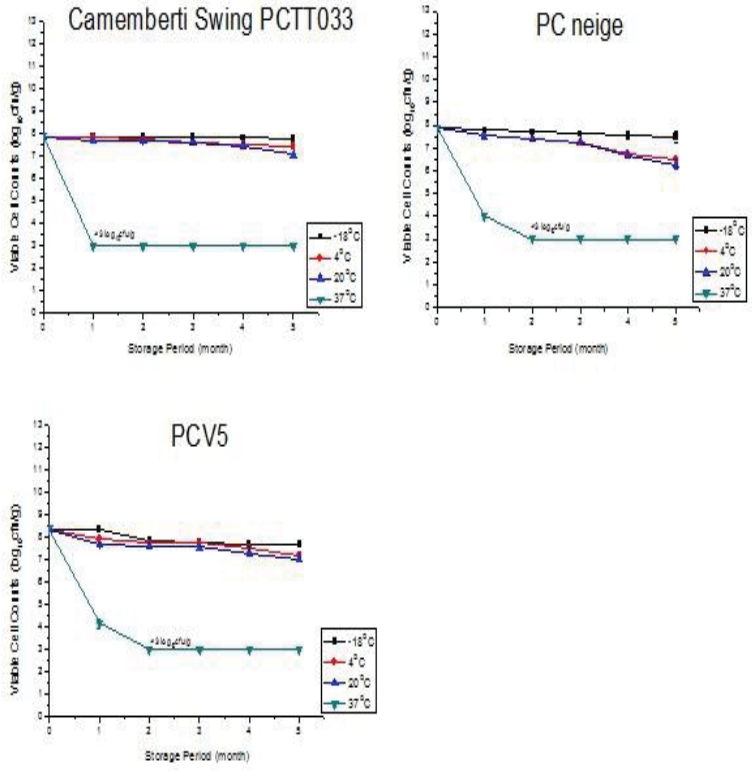


Figure 30. Mean (±SD) viable cell counts (n=2) of *P. camemberti* ripening cultures (freeze-dried), at each temperature during storage for 5 months. *P. camemberti*: PCTT033 (Chr. Hansen), PCV5 (SACCO) and PC Neige (Danisco). Storage temperature: -18, 4, 20 and 37°C.

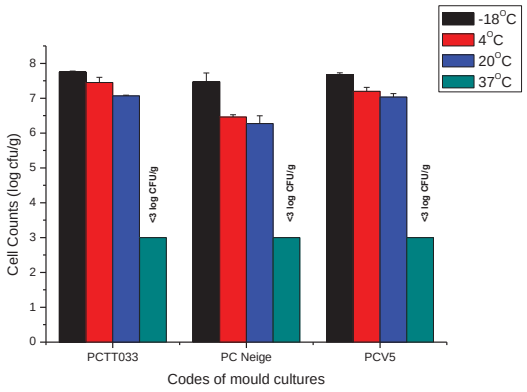


Figure 31. Mean (±SD) viable cell counts (n=2) of *P. camemberti* culture samples after stored for 5 months at four different temperatures. *P. camemberti*: PCTT033 (Chr. Hansen), PCV5 (SACCO) and PC Neige (Danisco). Storage temperature: -18, 4, 20 and 37°C.

4.1.3 Rate of viability loss

Table 22. First order rate constant (k, month^{-1})* of viability loss of freeze-dried starter bacteria stored at -18, 4 and 20°C.

Starter type	Codes of cultures	Storage temperatures (°C)		
		-18	4	20
O-type	R704	0.1385	0.1583	0.4130
	DSL 50A	0.1864	0.2063	0.2180
	MA 11 LYO	0.1986	0.2044	0.2957
	MO 032	0.0775	0.1939	0.3362
	CM11	0.1785	0.1866	0.3554
LD-type	Flora Danica	0.0406	0.1209	0.1754
	CHN-19	0.1198	0.1279	0.2061
	DSL 33A	0.0462	0.0645	0.0793
	CM51	0.1257	0.1317	0.2740
	Probat 222 LYO	0.047	0.0712	0.3119
	MO036	0.099	0.1196	0.1213

*With high correlation coefficients (R^2 >0.73); First order rate constant describes the inactivation kinetic rates of the microbial cell.

Table 23. First order rate constants (k, month^{-1})* of viability loss of freeze-dried *P. camemberti* ripening cultures stored at -18, 4 and 20°C.

Codes of cultures	Storage temperatures (°C)		
	-18	4	20
PCTT033	0.0180	0.0929	0.1365
PR4	0.0797	0.1492	0.1585
PCV5	0.1601	0.2035	0.2281
PC NEIGE	0.0786	0.2696	0.3055

*With high correlation coefficients R^2>0.84; First order rate constant describes the inactivation kinetic rates of the microbial cell.

Tables 22 and 23 summarise the inactivation rate values (k, month^{-1}) of starter and *P. camemberti* samples for each storage temperature. The inactivation rate of cultures at -18, 4 and 20°C followed first-order kinetic reaction, which agreed with other reports (Mazzobre et al., 1997; Andersen et al., 1999; Higl et al., 2007; Abe et al., 2009; Cerrutti et al., 2000; Schebor et al., 2000). The inactivation rate of cell cultures stored at 20°C was the highest compared to 4 °C and -18 °C which were lower (Tables 22 and 23).

When the inactivation rate constants (k) obtained in Tables 22 and 23 and the storage temperatures were analysed, the natural logarithm of the inactivation rate constant (ln k) showed a negative linear relationship with the temperature of storage (1/T) [Figures 1-3, Appendix 2.2]. The results confirmed previous findings which indicated that storage temperature had significant effect on the stability of culture (King et al., 1998; Andersen et al., 1999; Schebor et al., 2000). Since Arrhenius equations can model the effect of temperature on inactivation rate of cells, the equations obtained from this study [Figures 1-3, Appendix 2.2] can be used as quick methods to predict stability of cultures during storage at different temperatures.

4.2 Acidification

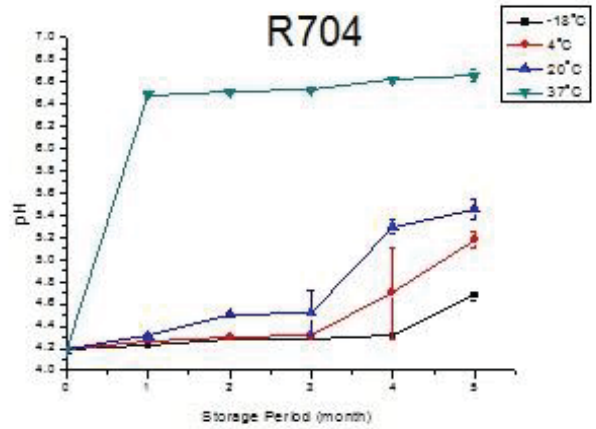
One of the most important characteristics of starter strains is the ability to acidify milk rapidly. To evaluate the ability of starters to recover their acidification activity after dehydration, the pH and titratable acidity were measured on milk fermented for 6 hr. Changes in pH and titratable acidity of the milk are shown in Tables 24, 25 and Figures 32, 35.

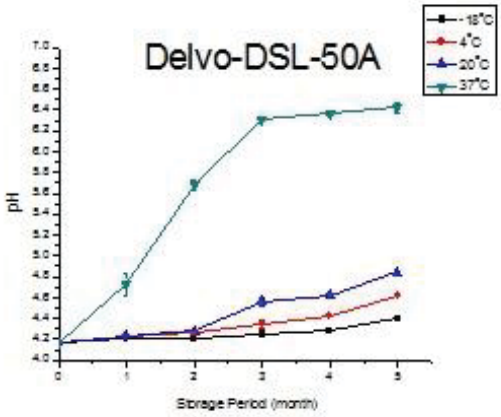
Maximum acidification activity of cultures was observed at the initial storage time with pH ranging from 4.17-4.52 and T.A. from 0.74-1.05 for O-type, the pH of LD starters ranged from 4.45-5.04 and T.A. 0.53-0.81 (data not shown). Compared to LD-type starters, O-type cultures produced acid faster. The observation was as expected due to the differences of strain composition. *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* in O-type starter cultures are rapid acid producers compared to *Leuconostoc* spp. in LD-type samples. The *Leuconostoc* are mainly included in dairy starter cultures for aroma production (diacetyl) as they are capable of metabolising citrate (Parente and Cogan, 2004; Mcsweeney and Fox, 2004). Acid-producing ability of starter samples reduced decreased during storage ($P<0.05$). For both starter types, significant differences ($P<0.05$) in acidification activity were also observed between samples stored at the four temperatures.

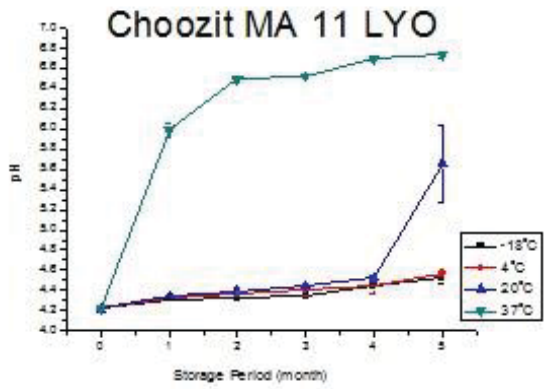
Acidification capability was less pronounced in samples stored at higher storage temperatures. Storage of starters at high temperatures significantly diminishes the

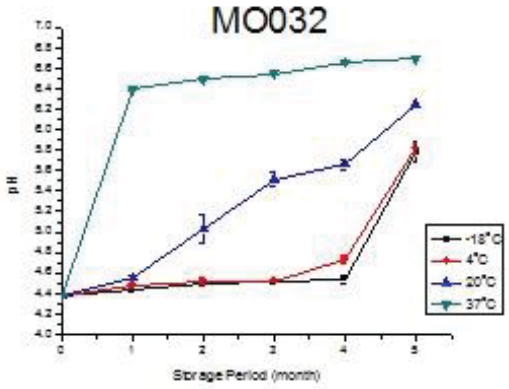
acidification activity of cultures, especially with samples stored at 37°C, where pH decreased by only 0.02-0.3 after 6 h of fermentation in 3 months old cultures (Tables 24, 25 and Figures 32, 33). Cultures stored at -18°C were less affected than cultures stored at 4 and 20°C (Tables 24, 25). Although a relatively higher degree of activity loss was observed in samples stored at ambient temperature, low pH and high T.A. values were still noted after fermentation (Tables 24, 25). Viability and acidification of samples were correlated ($P<0.05$) for both starter types. The decrease in acid-producing activity can be caused by cell death and/or cell membrane damage under high storage temperature leading to higher latent phase for acidification (Higl et al., 2007; Kurtmann et al., 2009c).

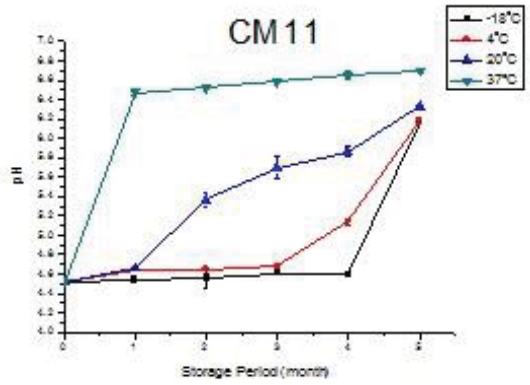
There were differences ($P <0.05$) between acidification activity of the cultures (Table 24, 25 and Figure 34, 35) which consistent with the cell viability results reported in Section 4.1.1. Among all starter samples (n=11), Delvo-tec LL-50 DSL and Delvo-tec-33A produced the highest amount of acid for cultures stored at lower temperatures other than 37°C. High acidification activity of R704 (Chr. Hansen) and Flora Dancia (Chr. Hansen) was also observed; and R704 showed insignificant difference to Delvo-tec LL-50 DSL ($P=0.1275$ for pH; $P=0.4701$ for T.A.). Compared to these samples, Danisco cultures (11 LYO and Probat 222), SACCO (MO032 and MO036) and Maysa (CM11 and CM51) showed relatively lower acid-producing ability (Table 24, 25 and Figure 34, 35). Samples CM11 and CM51 from Maysa exhibited the poorest acidification activity of the starter cultures studied.


R704
-18°C
4°C
20°C
37°C
pH
Storage Period (month)

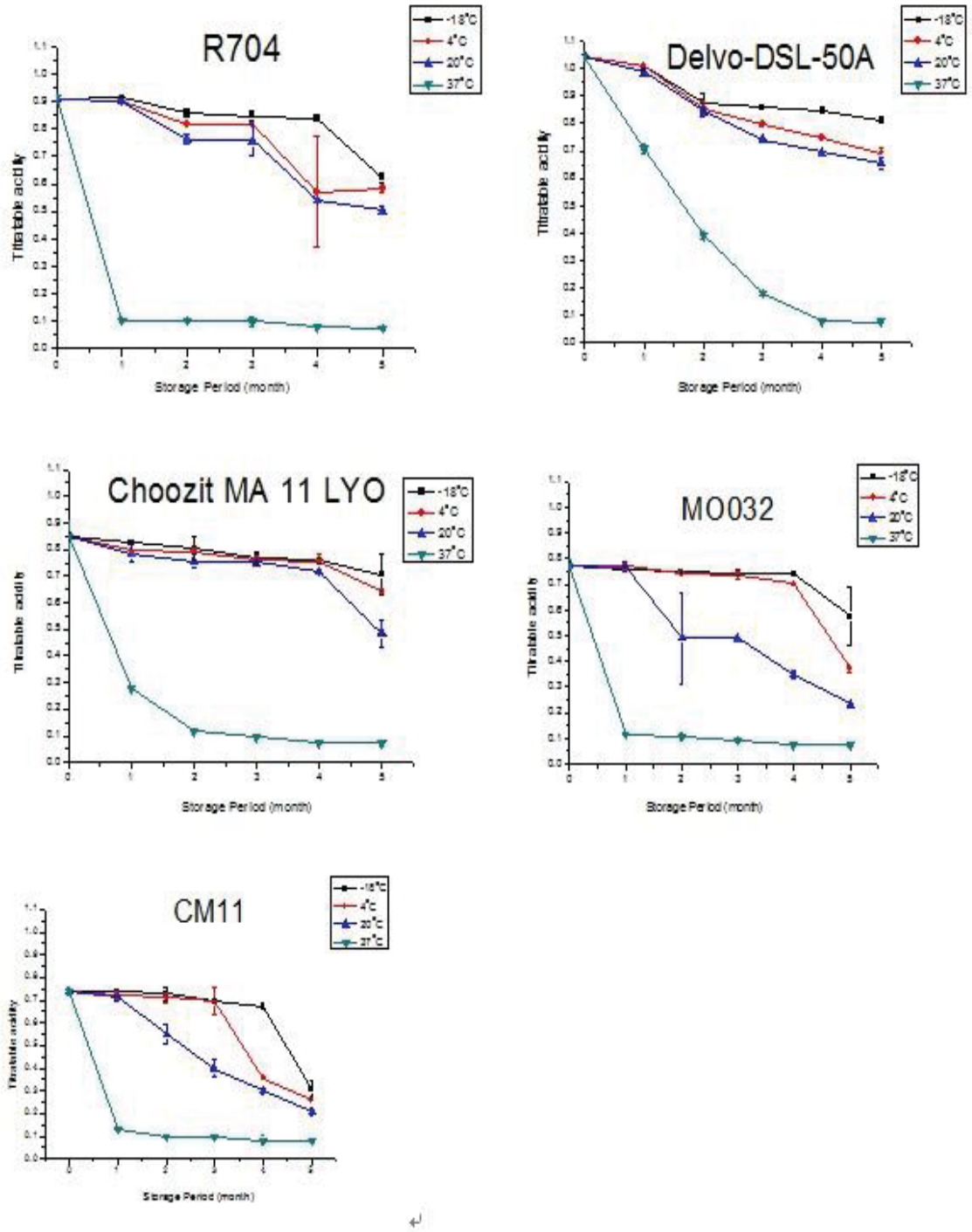

Delvo-DSL-50A
-18°C
4°C
20°C
37°C
pH
Storage Period (month)


Choozit MA 11 LYO
-18°C
4°C
20°C
37°C
pH
Storage Period (month)


MO032
-18°C
4°C
20°C
37°C
pH
Storage Period (month)

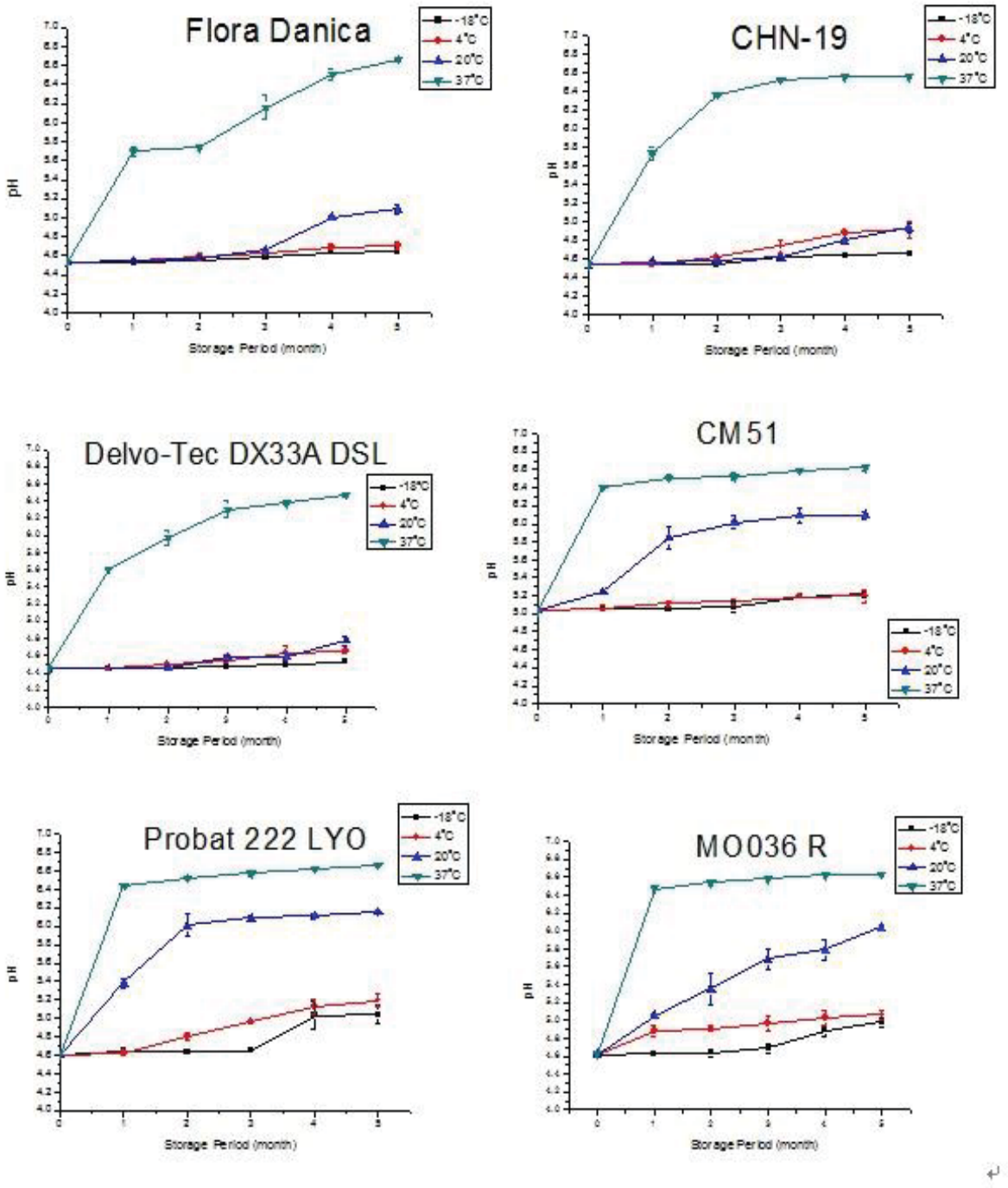

CM11
-18°C
4°C
20°C
37°C
pH
Storage Period (month)

(a)

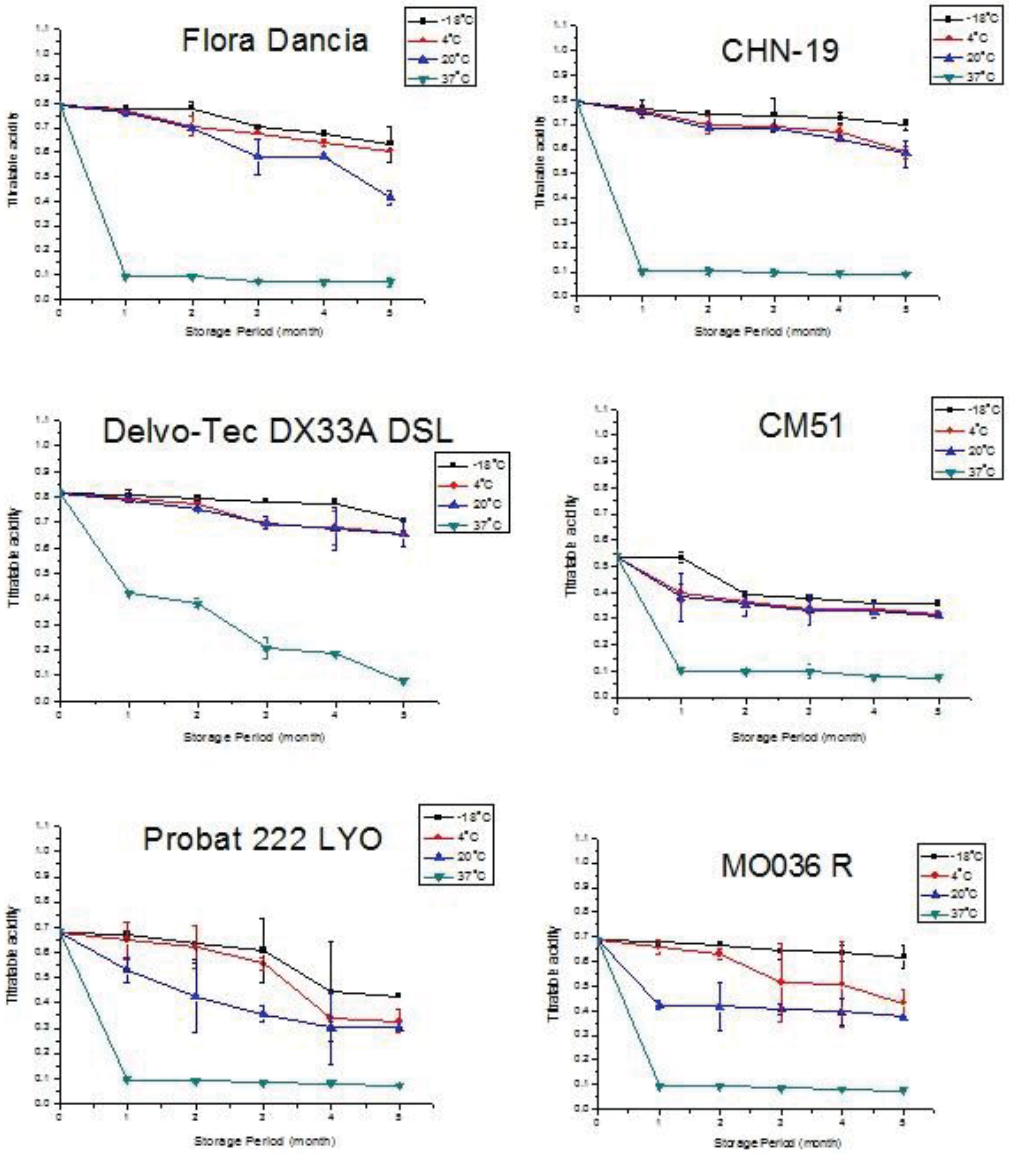


(b)

Figure 32. Mean (±SD) (a) pH and (b) titratable acidity values (n=2) of O-type starter cultures, at each temperature level during storage of 5 months. O-type LAB starters: R704 (Chr. Hansen), DSL (DSM), CM11 (Maysa), 11 LYO (Danisco) and MO032 (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; Storage temperature: -18°C , 4°C, 20°C, 37°C.


Flora Danica
CHN-19
Delvo-Tec DX33A DSL
CM51
Probat 222 LYO
MO036 R
-18°C
4°C
20°C
37°C
pH
Storage Period (month)

(a)



(b)

Figure 33. Mean (a) pH and (b) titratable acidity values (n=2) of LD-type starter cultures, at each temperature level during storage of 5 months. LD-type LAB starters: Flora Danica and CHN-19 (Chr. Hansen), DX-33A DSL (DSM), CM51 (Maysa), Probat 222 LYO (Danisco) and M 036R (SACCO), containing strains of *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *Leuc. lactis*, *Leuc. mesenteroides* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*. Storage temperature: -18°C, 4°C, 20°C, 37°C.

Table 24. [1]Mean pH values of mesophilic O-type[2] and LD-type[3] starter LAB samples (freeze-dried) after 5 months of storage at various temperatures[4].

Starter type	Codes of cultures	Storage temperatures (°C)			
		-18	4	20	37
O-type	R704	$^{y}4.68\pm0.04^{c}$	$^{y}5.18\pm0.08^{b}$	$^{xy}5.46\pm0.09^{b}$	$^{y}6.66\pm0.06^{a}$
	DSL 50A	$^{x}4.40\pm0.02^{d}$	$^{x}4.62\pm0.01^{c}$	$^{x}4.84\pm0.01^{b}$	$^{x}6.42\pm0.04^{a}$
	MA 11 LYO	$^{xy}4.53\pm0.06^{c}$	$^{x}4.57\pm0.01^{c}$	$^{yz}5.66\pm0.38^{b}$	$^{y}6.73\pm0.03^{a}$
	MO 032	$^{z}5.79\pm0.11^{c}$	$^{z}5.81\pm0.05^{c}$	$^{z}6.25\pm0.01^{b}$	$^{y}6.70\pm0.01^{a}$
	CM11	$^{w}6.18\pm0.03^{c}$	$^{w}6.19\pm0.04^{c}$	$^{z}6.33\pm0.01^{b}$	$^{y}6.70\pm0.01^{a}$
LD-type	Flora Danica	$^{y}4.66\pm0.02^{c}$	$^{xy}4.71\pm0.04^{c}$	$^{y}5.09\pm0.06^{b}$	$^{z}6.67\pm0.02^{a}$
	CHN-19	$^{y}4.66\pm0.01^{c}$	$^{yz}4.92\pm0.08^{b}$	$^{y}4.94\pm0.04^{b}$	$^{xy}6.57\pm0.02^{a}$
	DSL 33A	$^{x}4.53\pm0.01^{c}$	$^{x}4.66\pm0.02^{bc}$	$^{x}4.78\pm0.05^{b}$	$^{x}6.47\pm0.03^{a}$
	CM51	$^{y}5.22\pm0.04^{c}$	$^{w}5.20\pm0.08^{c}$	$^{z}6.10\pm0.04^{b}$	$^{yz}6.62\pm0.03^{a}$
	Probat 222 LYO	$^{y}5.05\pm0.11^{c}$	$^{w}5.20\pm0.06^{c}$	$^{z}6.15\pm0.01^{b}$	$^{yz}6.66\pm0.01^{a}$
	MO036	$^{y}4.98\pm0.06^{c}$	$^{zw}5.06\pm0.06^{c}$	$^{z}6.04\pm0.03^{b}$	$^{yz}6.63\pm0.03^{a}$

[1] mean (±SD), (n=2). [2]O-type LAB starters: R704 (Chr. Hansen), DSL (DSM), CM11 (Maysa), 11 LYO (Danisco) and MO032 (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; [3]LD-type LAB starters: Flora Danica and CHN-19 (Chr. Hansen), DX-33A DSL (DSM), CM51 (Maysa), Probat 222 LYO (Danisco) and M 036R (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, *Leuc. lactis, Leuc. mesenteroides* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*; [4]Storage temperature: -18°C , 4°C, 20°C and 37°C. Within rows, mean values followed by different superscripts are significantly different (Tukey's test $P<0.05$). Within columns, mean values proceeded by different superscripts are significantly different (Tukey's test, $P<0.05$), with respect to one type of culture.

Table 25. [1]Mean T.A. values of mesophilic O-type[2] and LD-type[3] starter LAB samples (freeze-dried) after 5 months of storage at various temperatures[4].

Starter type	Codes of cultures	Treatment Temperatures (°C)			
		-18	4	20	37
O-type	R704	$^{y}0.62\pm0.02^{c}$	$^{z}0.58\pm0.02^{b}$	$^{y}0.50\pm0.02^{a}$	0.08 ± 0.00^{a}
	DSL 50A	$^{y}0.81\pm0.01^{a}$	$^{w}0.69\pm0.03^{b}$	$^{z}0.66\pm0.02^{b}$	0.08 ± 0.00^{c}
	MA 11 LYO	$^{y}0.70\pm0.08^{a}$	$^{zw}0.64\pm0.02^{ab}$	$^{y}0.48\pm0.05^{b}$	0.07 ± 0.00^{c}
	MO032	$^{y}0.58\pm0.11^{a}$	$^{y}0.37\pm0.02^{ab}$	$^{z}0.23\pm0.01^{bc}$	0.07 ± 0.00^{c}
	CM11	$^{x}0.30\pm0.04^{c}$	$^{x}0.26\pm0.00^{b}$	$^{z}0.21\pm0.02^{ab}$	0.08 ± 0.01^{a}
LD-type	Flora Danica	$^{y}0.63\pm0.07^{a}$	$^{y}0.61\pm0.00^{a}$	$^{x}0.42\pm0.03^{b}$	0.07 ± 0.02^{c}
	CHN-19	$^{y}0.70\pm0.02^{a}$	$^{y}0.59\pm0.03^{a}$	$^{y}0.58\pm0.05^{a}$	0.09 ± 0.00^{b}
	DSL 33A	$^{y}0.71\pm0.00^{a}$	$^{y}0.66\pm0.00^{a}$	$^{y}0.66\pm0.05^{a}$	0.08 ± 0.01^{b}
	CM51	$^{x}0.36\pm0.00^{a}$	$^{x}0.32\pm0.01^{b}$	$^{x}0.31\pm0.00^{b}$	0.08 ± 0.00^{c}
	Probat 222 LYO	$^{x}0.43\pm0.00^{a}$	$^{x}0.33\pm0.05^{ab}$	$^{x}0.30\pm0.00^{b}$	0.07 ± 0.00^{c}
	MO036R	$^{y}0.62\pm0.05^{a}$	$^{x}0.43\pm0.06^{b}$	$^{x}0.37\pm0.01^{b}$	0.08 ± 0.00^{c}

[1]mean (±SD), (n=2). [2]O-type LAB starters: R704 (Chr. Hansen), DSL (DSM), CM11 (Maysa), 11 LYO (Danisco) and MO032 (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; [3]LD-type LAB starters: Flora Danica and CHN-19 (Chr. Hansen), DX-33A DSL (DSM), CM51 (Maysa), Probat 222 LYO (Danisco) and M 036R (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, *Leuc. lactis, Leuc. mesenteroides* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*; [4]Storage temperature: -18°C , 4°C, 20°C and 37°C. Within rows, mean values followed by different superscripts are significantly different (Tukey's test $P<0.05$). Within columns, mean values proceeded by different superscripts are significantly different (Tukey's test, $P<0.05$), with respect to one type of culture.

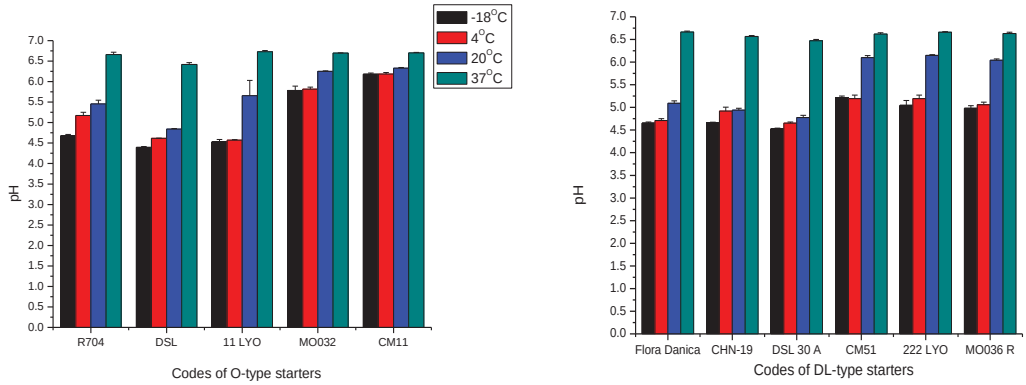


Figure 34. Mean (±SD) pH values (n=2) of O-type and LD-type LAB starter samples after 5 months storage at four different temperatures. O-type LAB starters: R704 (Chr. Hansen), DSL (DSM), CM11 (Maysa), 11 LYO (Danisco) and MO032 (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; LD-type LAB starters: Flora Danica and CHN-19 (Chr. Hansen), DX-33A DSL (DSM), CM51 (Maysa), Probat 222 LYO (Danisco) and M 036R (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, *Leuc. lactis*, *Leuc. mesenteroides* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*; Storage temperature: -18°C , 4°C, 20°C and 37°C.

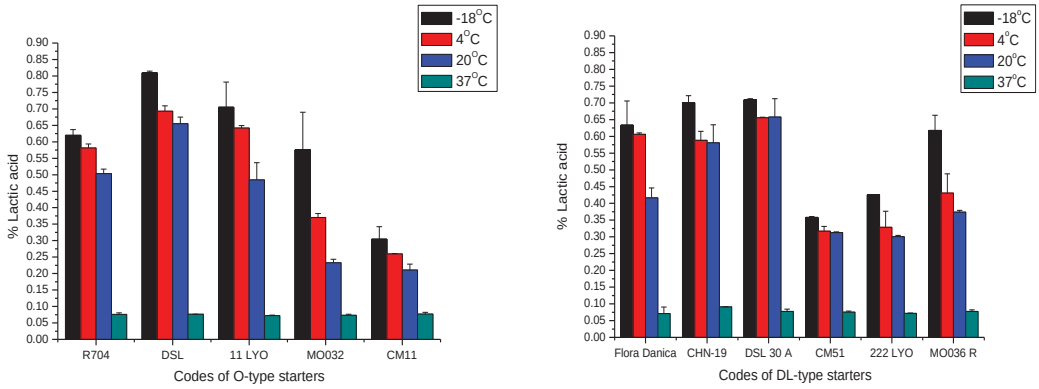


Figure 35. Mean±(SD) T.A. values (n=2) of O-type and LD-type LAB starter samples after 5 months storage at four different temperatures. O-type LAB starter: R704 (Chr. Hansen), DSL (DSM), CM11 (Maysa), 11 LYO (Danisco) and MO032 (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; LD-type LAB starters: Flora Danica and CHN-19 (Chr. Hansen), DX-33A DSL (DSM), CM51 (Maysa), Probat 222 LYO (Danisco) and M 036R (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, *Leuc. lactis*, *Leuc. mesenteroides* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*; Storage temperature: -18°C , 4°C, 20°C and 37°C.

4.3 Colour of samples

At end of storage for 5 months, colour of freeze-dried lactic starters and *P. camemberti* cultures at four temperatures were measured using the Hunter L^*, a^*, b^* system. Colour of samples are presented in Tables 26-27 as L^*, a^*, b^* values.

Post-storage, all freeze-dried samples stored at higher temperature, especially at 37°C had higher ($P<0.05$) a^* and b^* values and lower L^* values, indicating the brown colouring of samples. Browning was not observed for frozen samples. There was minor browning of samples stored at refrigeration temperature, but the colour difference was not distinguishable to the naked eyes. The brown colour was very pronounced with samples at 37°C. The colour change of samples was clearly visible to the naked eye even after a short period of storage for two months. The results confirmed the objective results [Figures 4-10, Appendix 2.4].

Varying magnitude of discoloration was observed on samples (Table 26-27). The most substantial browning was observed with DSM cultures (DSL-50A and 33A), particularly samples stored at 37°C.

Table 26. [1]Changes in colour (Hunter's L*, a*, b*) of mesophilic O-type[2] and LD-type[3] LAB starter cultures (freeze-dried) after 5 months storage at various temperatures[4].

Parameter	Starter type	Codes of cultures	Treatment Temperatures (°C)			
			-18	4	20	37
L values*	O-type	R704	98.53±0.01[a]	97.64±0.01[b]	95.50±0.06[c]	85.60±0.00[d]
		DSL 50A	73.63±0.02[a]	72.60±0.08[b]	71.46±0.14[c]	29.51±0.03[d]
		MA 11 LYO	98.11±0.03[a]	98.02±0.00[a]	97.74±0.03[b]	94.82±0.03[c]
		MO032	97.96±0.04[a]	97.95±0.01[a]	97.87±0.04[a]	90.28±0.01[b]
		CM11	98.01±0.00[a]	98.00±0.02[a]	97.42±0.01[b]	91.15±0.01[c]
	LD-type	Flora Danica	96.52±0.03[a]	96.25±0.04[b]	95.04±0.01[c]	78.75±0.00[d]
		CHN-19	95.98±0.01[a]	95.10±0.05[b]	94.31±0.03[c]	63.21±0.16[d]
		DSL 33A	73.91±0.02[a]	53.20±0.01[b]	50.18±0.01[c]	14.72±0.08[d]
		CM51	98.08±0.02[a]	97.82±0.00[b]	97.37±0.01[c]	91.50±0.01[d]
		Probat 222 LYO	98.21±0.08[a]	97.97±0.02[b]	97.25±0.01[c]	85.17±0.02[d]
		MO032	97.77±0.04[a]	97.67±0.01[a]	97.49±0.01[b]	89.51±0.05[c]
a values*	O-type	R704	-0.42±0.00[d]	-0.22±0.00[c]	0.36±0.01[b]	2.54±0.01[a]
		DSL 50A	3.13±0.01[d]	3.25±0.01[c]	3.90±0.01[b]	14.02±0.01[a]
		MA 11 LYO	-0.64±0.00[d]	-0.18±0.00[c]	0.06±0.01[b]	0.60±0.00[a]
		MO032	-0.36±0.01[d]	-0.025±0.01[c]	0.22±0.00[b]	1.35±0.01[a]
		CM11	-0.14±0.00[d]	0.05±0.00[c]	0.21±0.00[b]	0.95±0.00[a]
	LD-type	Flora Danica	0.51±0.00[d]	0.83±0.00[c]	1.56±0.01[b]	4.26±0.02[a]
		CHN-19	0.13±0.00[d]	0.64±0.01[c]	1.50±0.01[b]	6.70±0.00[a]
		DSL 33A	3.66±0.01[d]	4.00±0.00[c]	4.26±0.01[b]	15.36±0.01[a]
		CM51	-0.13±0.00[d]	0.30±0.00[c]	0.53±0.01[b]	1.15±0.01[a]
		Probat 222 LYO	-0.60±0.00[d]	0.07±0.01[c]	0.11±0.01[b]	2.25±0.00[a]
		MO032	0.14±0.00[d]	0.15±0.00[c]	0.16±0.00[b]	1.13±0.00[a]
b values*	O-type	R704	4.55±0.01[b]	5.14±0.67[b]	5.82±0.00[b]	11.42±0.02[a]
		DSL 50A	25.33±0.00[c]	25.40±0.00[b]	26.72±0.00[a]	13.71±0.00[d]
		MA 11 LYO	3.45±0.01[b]	3.48±0.01[b]	3.49±0.01[b]	6.16±0.01[a]
		MO032	3.44±0.01[c]	3.46±0.03[c]	3.89±0.00[b]	6.74±0.00[a]
		CM11	3.19±0.01[d]	3.42±0.01[c]	4.00±0.01[b]	6.17±0.00[a]
	LD-type	Flora Danica	5.19±0.01[c]	5.20±0.01[c]	6.28±0.01[b]	13.52±0.01[a]
		CHN-19	6.16±0.00[d]	6.35±0.01[c]	8.55±0.00[b]	16.44±0.04[a]
		DSL 33A	24.48±0.04[c]	26.01±0.72[b]	27.75±0.02[a]	17.14±0.01[d]
		CM51	3.34±0.04[d]	3.62±0.08[c]	4.04±0.02[b]	6.14±0.01[a]
		Probat 222 LYO	3.43±0.01[d]	3.57±0.00[c]	4.48±0.00[b]	10.03±0.01[a]
		MO032	3.07±0.00[d]	3.20±0.00[c]	3.85±0.00[b]	6.64±0.01[a]

[1]mean (±SD), (n=3). [2]O-type LAB starters: R704 (Chr. Hansen), DSL (DSM), CM11 (Maysa), 11 LYO (Danisco) and MO032 (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; [3]LD-type LAB starters: Flora Danica and CHN-19 (Chr. Hansen), DX-33A DSL (DSM), CM51 (Maysa), Probat 222 LYO (Danisco) and M 036R (SACCO), containing strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, *Leuc. lactis*, *Leuc. mesenteroides* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*; [4]Storage temperature: -18°C , 4°C, 20°C and 37°C.

Within rows, mean values followed by different superscripts are significantly different (Tukey's test $P<0.05$).

Table 27. [1]Changes in colour (Hunter's L*, a*, b*) of *P. camemberti* samples[2] (freeze-dried) after 5 months of storage at various temperatures[3].

Parameter	Codes of cultures	Treatment Temperatures (°C)			
		-18	4	20	37
L values*	PCTT033	98.21±0.01^{a}	97.95±0.00^{b}	97.92±0.02^{b}	96.63±0.01^{c}
	PC Neige	98.29±0.03^{a}	98.26±0.02^{a}	97.90±0.00^{b}	97.02±0.03^{c}
	PCV5	98.51±0.00^{a}	98.36±0.00^{b}	98.25±0.03^{c}	96.04±0.02^{d}
a values*	PCTT033	-0.22±0.00^{d}	-0.02±0.00^{c}	0.30±0.00^{b}	0.43±0.00^{a}
	PC Neige	-0.10±0.00^{d}	-0.09±0.00^{c}	0.06±0.00^{b}	0.29±0.00^{a}
	PCV5	-0.48±0.01^{d}	-0.17±0.01^{c}	-0.08±0.00^{b}	0.40±0.01^{a}
b values*	PCTT033	2.47±0.01^{d}	2.56±0.01^{c}	2.75±0.01^{b}	3.77±0.03^{a}
	PC Neige	2.25±0.00^{c}	2.28±0.01^{c}	2.75±0.01^{b}	3.58±0.02^{a}
	PCV5	2.93±0.01^{d}	2.99±0.01^{c}	3.16±0.00^{b}	4.32±0.00^{a}

[1]mean (±SD), (n=3). [2]*P. camemberti*: PCTT033 (Chr. Hansen), PCV5 (SACCO), and PC NEIGE (Danisco); [3]Storage temperature: -18°C , 4°C, 20°C and 37°C.
Within rows, mean values followed by different superscripts are significantly different (Tukey's test $P<0.05$).

Cultures with loss of bacterial viability and low viable cells (Tables 20-23) were characterised by higher degree of browning (Tables 26, 27). Samples stored at 37°C had the highest discolouration than those stored at 4°C and 20°C. High correlation ($P<0.05$) was also observed between cell counts and their colour for both starter types. Our findings agree with previous studies. A correlation between discoloration and loss of bacteria viability in freeze-dried *Lb. acidophilus* cultures (La-5) has been reported (Carvalho et al., 2007; Kurtmann et al., 2009a; Kurtmann et al., 2009c). In plant seed science, an interaction between the formation of brown reaction products during storage and a decreased enzymatic activity has also been demonstrated (Murthy et al., 2003). Carvalho et al. (2007) and Kurtmann et al. (2009c) however suggested that death of freeze-dried bacteria may be not a prerequisite for their participation in browning reactions, most probably, by making protein side chains more available for reaction with lactose in Maillard reactions. In this respect, a brown colour of dried cultures can be used, as least under certain conditions, as indicator for decreased viability and consequently acidification activity of samples.

In many previous studies (Carvalho et al., 2007; Kurtmann et al., 2009a; Kurtmann et al., 2009c), brown colour formation was suggested to relate to non-enzymatic browning reactions (known as Maillard reaction). Maillard reaction occurs by condensation (in the present of moisture) between amino group of proteins on cell membrane and lactose in the concentrated bacterial suspension. Lactose can also be available through the addition of skim milk (Passot et al., 2012). Maillard reaction may be also induced by lactose reacting with milk proteins. If Maillard reaction was

the cause of the observed browning, a large difference would have been expected between samples with either high, low, or no sugar present. It is interesting to note that, among all samples, DSL and DXT330 which contained no lactose in formulation exhibited the most degree of browning at 37°C. The observation could be explained by cell inactivation due to the damage from the cell wall component. The peptidoglycan layer, which constitutes the cell wall of the Gram-positive *L. acidophilus*, was suggested to partially degrade and hydrolyse during freeze-drying and storage, particularly at high temperature in the presence of high a_w, forming reducing carbonyl groups (Kurtmann et al., 2009c). Such carbonyl groups may undergo condensation reactions with amino group of protein of cell membrane, resulting in browning of the freeze-dried cells

4.4 Composition of starter cultures (LD-type)

The use of LD-type starter cultures for Camembert production has been widely reported. Their role in flavour formation is attributed to the presence of Cit^+ strains in culture composition (Parente and Cogan, 2004). In this regard, the effect of storage temperature on LD-type starters was investigated in this study. The constituent species of the cultures at the end of the 5 months storage is shown in Table 28. Figure 36 shows an image of Cit^+ and Cit^- bacteria growing on modified Nickels and Leesment medium with X-gal (Vogensen et al., 1987; IDF, 2006).

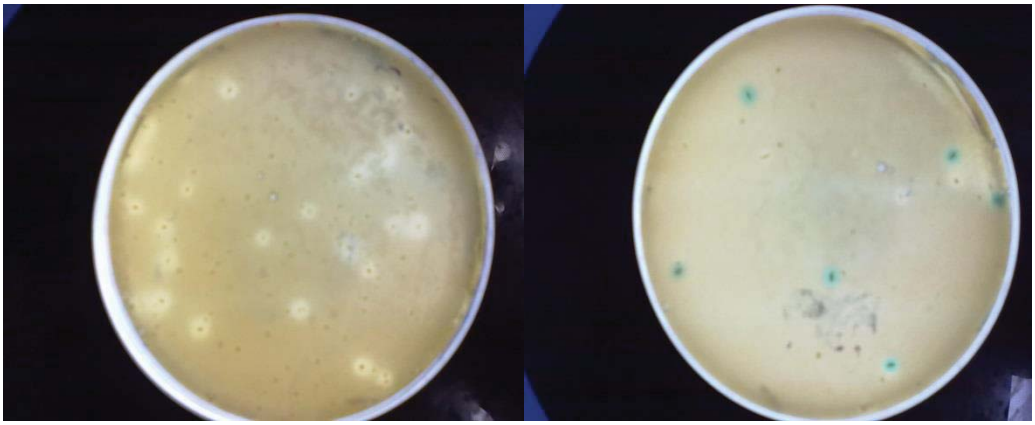


Figure 36. Cit^+ and Cit^- bacteria growing on Nickels and Leesment medium with X-gal. Cit^- bacteria: *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* are white without a clear zone (left). Cit^+ bacteria: *L. lactis* subsp. *lactis* biovar. *diacetylactis* colonies are white with a clear zone (left) and *Leuconostoc* species are blue, with or without a clear zone (right).

Table 28. Mean cell counts of constituent species (log cfu/g) in LD-type starter cultures after 5 months of storage at various temperature[a]

Starter type	Treatment Temperature (°C)	Total LAB[1]	Cit$^-$ LAB[2]	Cit$^+$ LAB[3]	*L. lactis* subsp. *lactis* biovar. *diacetylactis*	*Leuc.* spp.
Flora Danica	-18	9.70±0.05	9.50±0.02	9.25±0.10	9.04±0.06	8.83±0.18
	4	9.42±0.20	9.17±0.14	9.04±0.27	8.84±0.34	8.59±0.16
	20	9.04±0.18	8.78±0.02	8.66±0.40	8.50±0.38	8.15±0.43
	37	<2±0.00	<2±0.00	<2±0.00	<2±0.00	<2±0.00
CHN-19	-18	9.54±0.07	9.45±0.10	8.82±0.05	8.69±0.12	8.15±0.21
	4	9.51±0.10	9.41±0.12	8.82±0.05	8.76±0.03	7.84±0.20
	20	9.34±0.12	9.23±0.19	8.65±0.12	8.58±0.21	<6±0.00
	37	<2±0.00	<2±0.00	<2±0.00	<2±0.00	<2±0.00
DSL	-18	11.08±0.10	11.00±0.14	10.30±0.09	10.29±0.12	<6±0.00
	4	10.88±0.07	10.80±0.08	10.10±0.02	10.10±0.02	<6±0.00
	20	10.83±0.13	10.73±0.12	10.14±0.13	10.14±0.13	<6±0.00
	37	<2±0.00	<2±0.00	<2±0.00	<2±0.00	<2±0.00
CM51	-18	9.36±0.12	9.17±0.24	8.87±0.12	8.83±0.12	7.80±0.14
	4	9.25±0.10	8.99±0.12	8.90±0.08	8.86±0.08	7.81±0.05
	20	8.67±0.21	8.19±0.26	8.49±0.18	8.45±0.18	7.39±0.12
	37	<2±0.00	<2±0.00	<2±0.00	<2±0.00	<2±0.00
Probat 222 LYO	-18	9.07±0.03	9.06±0.02	7.42±0.60	6.93±0.46	7.25±0.66
	4	8.90±0.21	8.88±0.22	7.52±0.07	7.02±0.09	7.34±0.15
	20	7.92±0.08	7.90±0.08	6.54±0.09	6.19±0.06	6.26±0.21
	37	<2±0.00	<2±0.00	<2±0.00	<2±0.00	<2±0.00
MO032	-18	9.01±0.15	8.70±0.43	8.62±0.18	8.19±0.21	8.42±0.16
	4	8.94±0.10	8.66±0.07	8.58±0.30	8.18±0.26	8.35±0.34
	20	8.93±0.13	8.46±0.58	8.64±0.17	8.27±0.10	8.33±0.41
	37	<2±0.00	<2±0.00	<2±0.00	<2±0.00	<2±0.00

[a] mean (±SD), n=2. Storage temperatures: -18, 4 and 20 °C; [1] Total LAB include cell counts of cit$^+$ LAB and cit$^-$ LAB; [2] Cit$^-$ LAB include *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*;[3] Cit$^+$ LAB include *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc.* spp..

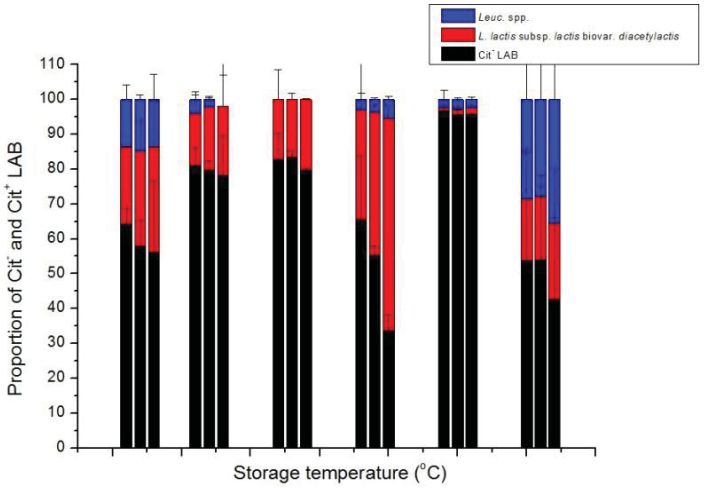


Figure 37. LD-type starter samples, consisting of species of *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*, *Leuc. lactis, Leuc. mesenteroides* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis*, were stored at three temperatures (-18°C, 4°C, 20°C from left to right). Each bar set represents a culture sample, Flora Danica, CHN-19 (Chr. Hansen), DSL 30A (DSM), CM51 (Maysa), Probat 222 LYO (Danisco) and MO032 (SACCO), from left to right.

Culture composition varied in the culture samples (Table 28, Figure 37). In all the samples, both cit$^+$ (*L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc*) and cit$^-$ strains (*L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*) were present in different proportions; the composition was generally dominated by the cit$^-$ bacteria (60-98%) [Table 29, Appendix 2.5]. Probat 222 (96.73%) had the highest cit$^-$ species in the cultures, followed by CHN-19 (81.02%), DSL (83.40%) and Flora Danica (64.25%). A relatively lower portion cit$^-$ strains was obtained in samples CM51 (65.60%) and MO032 (54.07%). All samples, with the exception of DSL-30A, contained both *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc*. spp., however, the ratio varied between samples. In DSL-30A, only the citrate-fermenting *L. lactis* subsp. *lactis* biovar. *diacetylactis* was present. In samples of Flora Danica, CHN-19 and CM51, *L. lactis* subsp. *lactis* biovar. *diacetylactis* dominated in composition as cit$^+$ bacteria, whereas *Leuconostoc* dominated in Probat 222 and MO032 (Figure 37). The species composition of samples agrees with Parente and Cogan (2004), who reported that the Leuconostocs are usually used in combination with the non-citrate fermenting *L. lactis* cultures, which must dominant in the culture formulation, making up about 65-90% of the microorganisms present.

It is well-known that *L. lactis* grows faster in milk than *Leuconostoc* species (Parente and Cogan, 2004). Kihal et al. (2009) showed that *Leuconostoc* strains grow synergistically with acid-producing *Lactococcus* (cit$^+$ and cit$^-$ lactococci) strains. In spite of their (*Leuconostoc*) ability to produce flavour, the species has limited potential to carry out fermentation. According to Kihal et al. (2009), the pH of pure culture of *Leuc. mesenteriodes* decreased from 6.7 to 5.6 after 10 h, whereas in mixed culture of *L.*

lactis and *L. mesenteriodes*, the pH progressively dropped to 4.4 after the same incubation time. Therefore, sample containing a higher proportion of *Leuc.* spp., such as MO032, is expected to produce acid in milk fermentation slowly. The speculation is confirmed by acidification results (Section 4.2) obtained in this study as previously reported. Culture MO032 is however expected to exhibited higher activity in citrate metabolism and gas production (Hugenholtz, 1993; Mcsweeney and Fox, 2004).

Table 28 shows decrease in total numbers of LAB cells, cit^- and cit^+ LAB strains; the declining proportion of cit^- LAB bacteria (Figure 37) was clearly observed in all samples with increase of storage temperature. These confirm a detrimental effect of temperature on strains of LAB. The finding agrees with Rehn et al. (2011) and also supports the cell enumeration results of the study as previously reported (Section 4.1.1). The decrease in the cell counts and the proportional differences in constituent species of cultures may be explained by variations in the composition of the starters and their thermal tolerance. The negative relationship between the species of LD-type starters and storage temperature needs further investigation.

4.5 Discussion

Considerable high cell concentration of cell samples at initial storage period demonstrated the ability of the strains used to withstand high temperature during freeze-drying. Thus, this preservation method is suitable for preparing dairy-based culture powders containing high numbers of viable cells of bacteria. Higher initial cell concentration was observed for O-type culture samples than LD-type samples, but was not significantly variable. Our results agree with Yao (2009), who reported low survival rates after freeze-drying of *Leuconostoc* strains compared with *Lactococcus* or *Lactobacillus* strains under the same conditions. Furthermore, variations in cell viability of each product at initial sampling time were assumed independent of the freeze-drying protocol used, especially the drying temperature.

Throughout storage, higher cell counts and acidification of freeze-dried lactic starters and *P. camemberti* cultures were observed at lower storage temperature, with storage conditions at 37°C showing the least stability. The loss of bacterial viability and higher inactivation of cells were associated with higher degree of discoloration, with brown colour being most pronounced among samples stored at 37°C for 5 months. As regards culture composition, significant decrease in cit^+ and cit^- bacteria occurred in all samples with increase of storage temperature. Findings of this study are in agreement with previous studies. Gyosheva et al. (1995) reported that *S. thermophilus* strains kept in freeze-dried state at 6°C were viable for 10 years, with no significant change in morphological, biochemical and technological characteristics. According to

Bullimore (1983), most starters of lactic cultures can be kept for 6 months at 4°C. Castro et al. (1995) reported a higher decrease in viability of *Lb. bulgarius* under 20°C than 5°C during storage. Wang et al. (2003) showed a difference of 20% in the survival rate of freeze-dried *S. thermophilus* stored at 4°C and 25°C. Viability of *Pantoea agglomerans* decreased by 0.5 logs after 90 d at 4 °C, compared to a decrease of 3 logs after 28 d at 25 °C (Casta et al., 2002). Bruno and Shan (2003) demonstrated that temperature maintained at -18°C was ideal for the long-term storage of probiotic capsules to maximize viability of bifidobacteria. Storage at 20°C showed the highest decline in the viability of cells, whereas that at -18°C showed the least decrease. Higher storage temperature conditions also result in lower survival rates of bifidobacteria (Abe et al., 2009; Wirjantoro and Phianmongkhol, 2009) and *E. faecium* and *Lb. plantarum* (Strasser et al., 2009) in freeze-dried powder.

It is apparent from the results of this study and other studies that although frozen and refrigeration storage may be impractical from commercial point of view, it is however necessary for optimal culture viability of stored freeze-dried powders. The results of the high-temperature storage test also lead us to consider the effect of transportation temperature on the viability of product samples. Although there was accelerated decrease in viability of cultures stored at room temperature compared with their storage at frozen and refrigeration temperatures, it may also be feasible to store cells at ambient temperature due to the remaining high cell counts at end of storage above the recommended level. Storage at ambient temperatures can also remove the more costly requirements of refrigeration, energy and suitable package. However, the application of dairy cultures stored at high ambient temperature above 20°C should be limited and further work is also needed in this area.

The results showed that single cultures of *L.* subsp. *lactis* and mixed cultures of *L.* subsp. *lactis* and *Leuconostoc* exhibited tolerance at high temperatures. Compared to O-type starters, LD-type starters produce lactic acid at a slower rate, probably because *Leuconostoc* species are slow-acid producers. *Leuconostoc* spp. probably had longer lag time than *L.* subsp. *lactis* for repairing reversible cellular injuries prior to growth and acid production (Parente and Cogan, 2004). The shorter lag time needed for *L.* subsp. *lactis* was probably due to the better survival of the bacteria under stress of dehydration during freeze-drying and storage (Tamime and Robinson, 1999).

Microbial inactivation is highly complex. The behaviour of lyophilized cultures during storage is speculated in here in order to explain the reason for loss of viability. Studies by Castro et al. (1995; 1996; 1997) reported the adverse effect of oxygen on survival of freeze-dried LAB during storage at high temperature, during which polyunsaturated fatty acids of cellular membrane were oxidised and showed a

decrease in ratio of unsaturated and saturated fatty acids of cells. The change in lipid ratio can consequently lead to a disruption of membrane structure and fluidity. Under oxidizing conditions, Maillard reactions between nucleophilic groups in oxidized DNA and free carbonyl groups also susceptible to occur in presence of water, eventually lead to browning of cultures (Higl et al., 2007; Kurtmann et al., 2009a; Kurtmann et al., 2009c). Such carbonyl groups involved in reactions are mainly reducing fermented medium containing sugars in the concentrated bacterial suspension. As mentioned earlier, the peptidoglycan layer, a cell wall component of LAB, could also be hydrolysed during storage forming reducing carbonyl groups, which undergo condensation reactions with amino group of cell membrane protein, resulting in browning of freeze-dried cells. According to Garcia (2011), oxidation and browning process in freeze-dried bacteria may be inter-related processes. Reduction in cell viability at high storage temperature may also be ascribed to inactivation of β-galactosidase within in the LAB cell membrane (Champagne et al., 1996; Mazzobre et al., 1997; Vasiljevic and Jelen, 2003). The lactose hydrolysing activity of β-galactosidase in *B. longum* lost during storage at 20°C was about twice that observed at 4 and -20°C, while losses of viable cell counts at 20°C were approximately a hundred times greater than those at 4°C (Champagne et al., 1996).

Freeze-dried samples, with the exception of DSL-LL50A and DSL-30A, contained in each laminated sachet were made to 1 g from pure culture and lactose. Since lactose was the only compound incorporated in pure bacterial species of samples except for DSL-LL50A and DSL-30A, discrepancy of results in cell viability may be attributed to differences in physical state of lactose in samples. Several authors have reported on the stabilization effect of lactose in cultures due to their ability to form glassy states (Schebor et al., 1997; Schebor et al., 2000; Thomsen et al., 2005; Higl et al., 2007). As mentioned previously, microbial inactivation is derived from complex physico-chemical reactions. Principally, being a glassy state below T_g, the sugar-bacteria matrix has very high viscosity and low mobility (Roos, 2002; 2004), thus rates of both oxidative and Maillard reactions are limited. Crystallization of amorphous lactose is a spontaneous process, which is also very slow under conditions of high viscosity. A low reduction in β-galactosidase activity within cell membrane was also reported when cells were stored under glassy state (Champagne et al., 1996).

In this study, the addition of lactose in freeze-dried samples probably had low pronounced effect on bacterial stability; this aspect was not investigated. During storage, high cell inactivation was observed in all samples irrespective of the presence of lactose. Samples containing lactose as a major part of the freeze-drying matrix exhibited higher losses of viability in comparison to samples containing no

lactose. Higher viability loss was also observed in samples with higher concentration of lactose in their formulation. Considering that samples containing lower amounts of lactose had higher constituent species content in the formulation, it may reasonable to attribute higher stability of cells due to the higher concentration of bacteria. The results may be also associated with changes in T_g of culture-lactose matrix due to impact of a_w during storage, especially when stored at high temperatures (Higl et al., 2007).

Although T_g is an important in understanding the stability of microorganisms, it cannot be used in isolation to characterize material as it is also water-dependent (Thomsen et al., 2005; Abe et al., 2009). Several previous studies demonstrated that T_g for freeze-dried cultures in lactose is strongly dependent on both a_w and storage temperature (Thomsen et al., 2005; Kurtmann et al., 2009b) (Figure 38). For each of the investigated storage tempeatures, -18, 4, 20 and 37°C, a certain a_w exists where the T_g equals the stroage tempeature, thus a_w creates a border between the two physical states (glassy and non-glassy) of the sample at a specific storage temperature (Higl et al., 2007; Kurtmann et al., 2009b). Dry pure lactose has T_g of 101°C corresponding to a_w of 0. The T_g was reported to be equal to the storage temperature (25°C) with a_w of 0.395; at a_w of 0.30, the storage temperature was 38°C (Thomsen et al., 2005). According to Thomsen et al. (2005), the a_w limit for a glassy state of freeze-dried *L. paracasei* in a lactose matrix stored at 37°C was ≈0.17 a_w, while for 20°C it was ≈0.29 a_w.

Water activity has a higher deteriorative effect/impact on cell inactivation at higher storage temperature (Higl et al., 2007; Kurtmann et al., 2009b; Passot et al., 2012). The effect of a_w on culture viability loss and their contribution to accelerate rates of deteriorative reactions has been widely reported (Wang et al., 2003; Thomsen et al., 2005; Higl et al., 2007; Abe et al., 2009; Kurtmann et al., 2009a; Kurtmann et al., 2009b; Kurtmann et al., 2009c; Passot et al., 2012). Castro et al. (1995) reported the effect of RH on lipid oxidation profile; at high RH, lipid oxidation occurred more rapidly while cell viability markedly decreased. High a_w of the cellular system could also accelerate Maillard reactions (Kurtmann et al., 2009a; Kurtmann et al., 2009c). This reflects faster browning of freeze-dried samples and loss of viability observed in this study as a consequence of Maillard reaction after high storage temperature. Moreover, water is produced by Maillard reaction (Zamora and Hidalgo, 2011). Thus, lactose crystallization may also be triggered due to an increase of released water into the matrix; this probably explains the occasional caking observed on some cultures by end of storage at high temperature [Figures 4 and 8, Appendix 2.4]. For pure amorphous lactose, crystallization was observed at a_w>0.40 at 20°C, a_w>0.32 at 30°C and a_w>0.31 at 38°C (Roos, 2002; Thomsen et al., 2005). The lactose hydrolysing

activity of β-galactosidase was critically dependent on the a_w at which the lyophilized bacteria was kept (Vasiljevic and Jelen, 2003). High a_w was correlated to significant β-galactosidase activity loss.

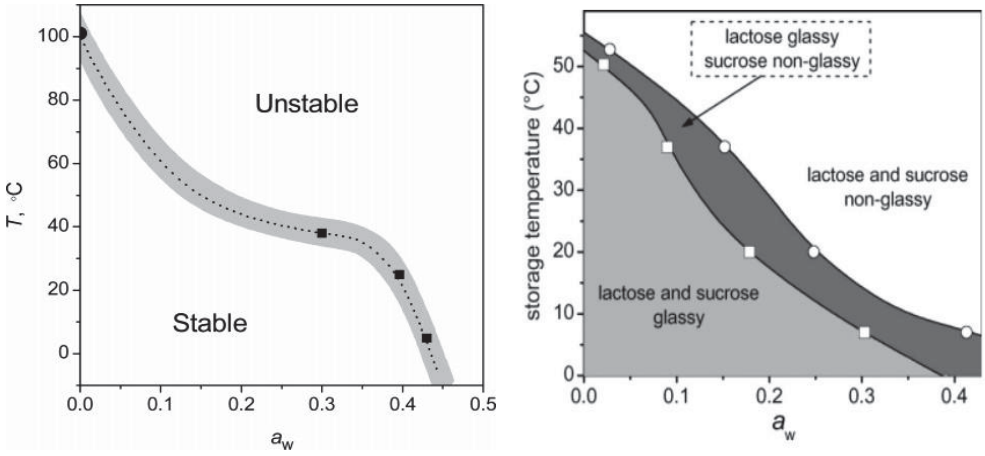


Figure 38. (a) a_w-temperature state diagram of amorphous lactose (Thomsen et al., 2005). The gray area indicates a transition zone separating stable amorphous lactose from unstable non-glassy lactose. The dotted line shows the borderline, where the storage temperature is identical to the T_g. (b) a_w-temperature state diagrams of *Lb. acidophilus* freeze-dried in sucrose or lactose matrix (Kurtmann et al., 2009b). (○) lactose matrix, (□) sucrose matrix.

The preceeding discussion highlights the need to consider the storage condition of freeze-dried culture samples with regards to temperature and a_w. Stability of dried LAB during storage has been shown to be optimal at 0.1-0.2 a_w zone with 4% moisture content (Champagne et al., 1996; Simpson et al., 2005). In this study, a_w of samples was not standardized throughout storage and the actual atmosphere in sample bags was also not measured. Initial a_w of cultures were found to have a significant impact on the storage stability of cultures (Andersen et al., 1999). The initial a_w may be different between samples as conditions for freeze-drying procedure may not be similar as they are obtained from different suppliers. The high storage temperature (37°C) used in this experiment may have amplified the response to a_w in samples (Kurtmann et al., 2009b; Higl et al., 2007); and the real a_w of samples in laminated package at each temperature may be different. Although cultures were packed in laminated sachets with low oxygen transmission and flushed with nitrogen. However, a_w of cultures may still increase during storage due to the uptake of moisture from storage environment which influence by permeability of packaging material (Wirjantoro and Phianmongkhol, 2009). The initial a_w of *Lb. paracasei* ssp. *paracasei* in a lactose matrix after freeze-drying process was 0.12; after storage at 20°C for 25 d, the a_w of samples increased to 0.23 (Higl et al., 2007). In order to achieve the T_g level required for prolonged storage life and stability of cultures, low temperatures have to be compensated for the increased water activity throughout storage.

In this study, bacterial inactivation below T_g was observed, even with -18°C cultures.

It was probably because T_g could not be regarded as an absolute threshold of bacterial stability during storage, although the rate of deterioration reactions were markedly reduced (Schebor et al., 2000; Higl et al., 2007). The glassy state, as reported in various studies (Higl et al., 2007; Kurtmann et al., 2009b; Passot et al., 2012), does not prevent bacterial inactivation. However, formation of a glassy state during storage may be of practical importance to the producer/user of freeze-dried bacteria cultures. The inactivation of bacteria below T_g also suggests that good survival of bacterial cells at conventional frozen temperature may not solely depend on a sugar protectant.

5 SCREENING AND SELECTION OF STARTER AND RIPENING CULTURES

In this study, the potential of test samples as suitable freeze-dried starter and ripening cultures for Camembert cheese production was evaluated. The main criterion for selecting cheese cultures was based on their viable cell counts at the end of storage period. Acid-producing activity, colour and composition of cultures were also considered during selection as additional factors.

Based on high cell counts and acidification before and after storage at various temperatures except 37°C, all studied freeze-dried lactic starters and *P. camemberti* cultures generally had commercial potential after 5 months storage, although a clear difference was observed between samples. All tested *P. camemberti* strains also possessed appropriate density of growth and height of white mycelium on YGC agar after 4 d of incubation time, and remained a perfect snow-white colour during subsequent incubation and storage at <4°C.

Among all studied samples, R704 (O-type, Chr. Hansen) and Flora Danica (LD-type, Chr. Hansen) were selected as promising lactic starters and PCTT033 (Chr. Hansen) as mould ripening culture for Camembert production in Phase II of the study. These samples had high stability during storage at both frozen and refrigeration temperatures for 5 months. When stored at room temperature, despite higher inactivation rate, good survival rates were found with considerable high counts above recommended level (Parente and Cogan, 2004; Hoier et al., 2011). These samples also generally had reasonably good survival rate during storage at 37 °C for 1-2 months. At all storage temperatures except 37°C, these cultures also had small colour changes throughout storage that was not visible to the naked eye. Additionally, Flora Danica (LD-type starter), exhibited balanced composition, in respect to cit^+ and cit^- ratio, at end of storage period. Considering that the final cell concentration for the selected starters and mould cultures post-storage were $<10^{11\text{-}12}$ cfu/g, they were therefore not selected for DVS for cheese-making due to the insufficient cell counts. However, at this concentration, the cultures may be propagated to boost cell numbers prior to use in cheese-making (Hoier et al., 2011). As aforementioned, propagation of cultures in this study was not a suitable option as the aim of the product was to use DVS cultures and Mad Millie™ cheese-making kits. Nonetheless, inoculation of about 10^6 cfu/g in milk can produce sufficient acid to reduce pH to less than 5.3 within 5-6 h at 30°C, reaching 10^9 cfu/ml after drainage (Parente and Cogan, 2004). High cell numbers of the mould in the initial stage of cheese milk fermentation is not considered important due to its dominant role in cheese ripening.

In spite of the highest cell viability and a substantially higher rate of acid production, cultures from DSM (DSL-LL50A and 30A) were not selected for two main reasons. The formation of browning during storage was undesirable since the ultimate goal is to market the samples for domestic Camembert production using commercial kits. The browning may not appeal to the consumer. Secondly, Maillard reactions that occur can reduce viability of cells, thus making it difficult to predict functional activity after prolonged storage. CHN-19 (LD-type, Chr. Hansen) was also not included in further study, although its activity was as good as 'R704' and additionally had a more balanced composition. CHN-19 is not currently used by I-Make™ Ltd and the culture has no immediate commercial plans at the company. Furthermore, CHN-19 is relatively expensive cultures.

6 PRODUCTION, RIPENING AND CHARACTERIZATION OF CAMEMBERT CHEESE

The effects of three variables, (1) type of starter culture, (2) culture storage temperature and (3) time, on fermentation and ripening of Camembert cheese were investigated in this study. The prepared cheese samples (treatments) were denoted by type of starter cultures and respective storage temperature used follows: O-LAB 18°C, O-LAB 4°C, O-LAB 20°C, LD-LAB 18°C, LD-LAB 4°C and LD-LAB 20°C.

6.1 Fermentation profiles of Camembert cheese samples

Table 29 summarises results of the concentrations of LAB starter cultures and *P. camemberti* involved in the fermentation of Camembert cheese and the pH of the samples, while the other characteristics of the samples during fermentation are shown in Figures 39-41.

Table 29. Levels of (log cfu/g) LAB starters and *P. camemberti* and pH values (mean ±SD) (n=4) in cheese samples during fermentation for 24 h.

Parameter	Cheese type	Culture storage temperature (°C)	Fermentation time				
			at inoculation (0 h)	at pre-maturation of milk (3 h)	at initial draining (6 h)	at secondary draining (11 h)	after overnight draining (24 h)
LAB	O-type[1]	-18	6.66±0.03	8.06±0.15	8.51±0.08	9.45±0.08	9.84±0.06
		4	6.43±0.057	7.76±0.07	8.04±0.04	8.76±0.19	9.74±0.08
		20	5.79±0.10	7.29±0.25	7.60±0.08	8.72±0.51	9.62±0.12
	LD-type[2]	-18	5.42±0.07	6.12±0.17	7.49±0.13	8.54±0.12	9.28±0.17
		4	5.25±0.07	5.87±0.09	7.25±0.47	8.31±0.26	9.08±0.05
		20	5.08±0.08	5.48±0.15	7.10±0.13	8.13±0.19	9.02±0.10
P.camemberti	O-type	-18	3.54±0.04	3.68±0.06	3.76±0.04	3.90±0.03	4.51±0.08
		4	3.35±0.05	3.48±0.04	3.68±0.05	3.82±0.03	4.17±0.05
		20	3.13±0.06	3.38±0.04	3.55±0.05	3.59±0.06	3.81±0.06
	LD-type	-18	3.36±0.07	3.46±0.10	3.72±0.02	3.94±0.05	4.36±0.19
		4	3.28±0.06	3.39±0.12	3.63±0.03	3.79±0.04	4.23±0.20
		20	3.12±0.08	3.25±0.15	3.51±0.05	3.64±0.05	3.89±0.07
pH	O-type	-18	6.7±0.01	6.64±0.01	6.29±0.08	5.46±0.08	4.63±0.06
		4	6.7±0.01	6.66±0.01	6.38±0.09	5.59±0.14	4.75±0.08
		20	6.71±0.01	6.68±0.01	6.44±0.05	5.96±0.09	4.89±0.04
	LD-type	-18	6.70±0.01	6.63±0.04	6.48±0.02	5.66±0.06	4.88±0.02
		4	6.71±0.03	6.66±0.02	6.5±0.03	5.85±0.12	4.92±0.01
		20	6.73±0.03	6.69±0.01	6.59±0.01	6.08±0.03	4.94±0.04

[1]O-type: 'acid-only' cheese samples produced using O-type LAB starters consisted of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (R704, Chr. Hansen) and *P. camemberti* (PCTT033, Chr. Hansen) as ripening culture; [2]LD-type: 'aroma-enriched' cheese samples produced using LD-type LAB starters consisted of *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuc. lactis* and *Leuc. mesenteriodes* subsp. *cremoris* (Flora Danica, Chr. Hansen) and *P. camemberti* as ripening cultures (PCTT033, Chr. Hansen).

6.1.1 Growth of lactic starters and increase of pH

Increase in levels of LAB cells and the concomitant decrease in pH (Table 29 and Figures 39, 40) were observed during fermentation. Growth of all LAB starters showed a typical sigmoidal curve divided into lag, logarithmic and stationary phases. All of the lactic cultures was less pronounced at the beginning of the lag phase, but increased when it entered the logarithmic phase, which occurred after milk 3 h of fermentation. In the exponential growth phase, the growth of LAB starters was more pronounced during drainage, associated with strong acidification giving pH values ranging from 4.63-4.94 in the curd after 24 h. The LAB bacteria continued to grow until the stationary phase which was observed between 16 h and day 3 (Figure 39). Increase in LAB cell counts gradually slowed down after fermentation as ripening progressed (Figure 39). At day 3, pH values had decreased from 6.70 to 4.67-4.81 in O-type cheese and 4.62-4.80 in the LD-type cheeses (Figure 40). The acidification of

cheese during fermentation is mainly attributed to the metabolism of lactose to lactic by LAB (Beresford and Williams, 2004; Fox and McSweeney, 2004; McSweeney, 2004b; Mcsweeney and Fox, 2004). However, other acids, although in smaller concentrations are also produced during milk fermentation.

Growth and acidification patterns of LAB starters in all samples were generally similar, however significant differences in cell counts of the LAB species and pH values were obtained between the types of cultures ($P<0.05$) and the storage temperatures ($P<0.05$). O-type cultures had better growth and acidification activity than LD-starters during fermentation (Table 29 and Figure 39, 40), which may be attributed to differences in strain composition. LAB cultures stored at higher temperature had poor growth and low acidification. Lactic cultures stored at -18°C showed higher viability and better acidification than those stored at higher temperatures (4 and 20°C) (Table 29 and Figures 39, 40). As previously discussed, cells exposed to higher temperatures were probably inactivated during storage.

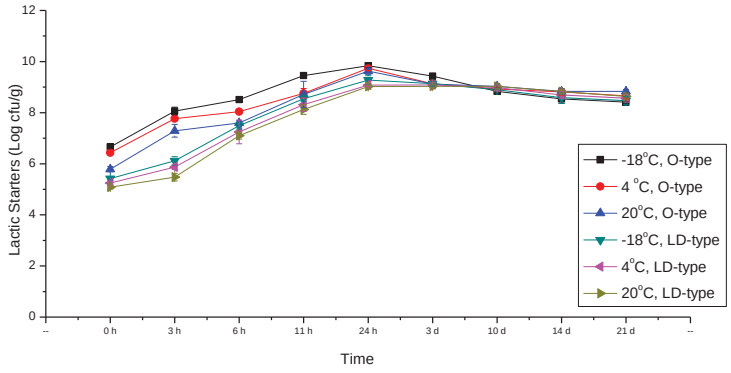


Figure 39. Growth of lactic acid starters in Camembert cheese samples during 24 h of fermentation and 21 d of ripening. Each point represents mean log cfu/g of four independent samples with error bars representing ±SD. Cheese samples with rind.

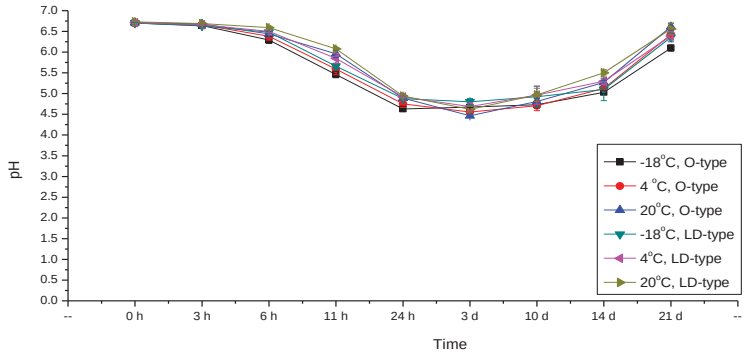


Figure 40. Changes in pH in Camembert cheese samples during 24 h of fermentation and 21 d of ripening. Each point represents mean pH values of four independent samples with error bars represented as ±SD. Cheese samples without rind.

In this study, 0.5% inocula were used for cheese milk fermentation. During fermentation, cell counts of LAB starters increased from 10^5-10^6 to 10^9 cfu/g (Table 29 and Figure 39). The final concentrations of LAB starters (10^9 cfu/g) in samples fermented with 4 and 20°C cultures were comparable to samples inoculated with -18°C cultures and levels recommended by industry (Parente and Cogan, 2004). Therefore, there is good potential to store cultures at these temperatures and use them for Camembert cheese at domestic level (Fox et al., 2000; Fox and McSweeney, 2004; McSweeney, 2004b; Mcsweeney and Fox, 2004). This is an important outcome as the cultures could be stored at either ambient or refrigeration temperatures without affecting their activities. Furthermore, low inoculation rate (0.5%) can be used, thus bringing economy to the production of the cheese. Dave and Shah (1997) also showed that LAB cultures grew faster accompanied with faster acidification in yogurt at lower levels of inocula than at higher levels. Kosasih (2011) reported that inocula rates of 0.5%, 1%, 2% and 3% had similar cell counts during fermentation irrespective the type of bacteria used.

In agreement with Fox et al. (2000), the viability of lactic starters used in this study was well-correlated with their acidification capability. Thus, low pH was accompanied by low cell counts ($P<0.05$, r^2=-0.736). The lag phase for growth of LAB was relatively short, but only small amounts of acid were produced during 3 h of 'pre-maturation of milk' with pH values only decreasing by 0.03-0.06 units for O-type cheese samples and 0.04-0.07 units for LD-type samples. With similar inoculation levels and incubation conditions, LAB used as starters by other studies decreased milk pH by 0.3-0.4 units (Schlesser et al., 1992; Wium et al., 1998; Josephsen and Jespersen, 2004; Leclercq-Perlat et al., 2004a; Leclercq-Perlat et al., 2004b; Kourkoutas et al., 2006; Guizani et al., 2007; Hayaloglu et al., 2008). This suggests that the strains used in our study may not be classified as 'rapid-acidifier' starter bacteria. The observation was not surprising because previous studies used bulk starters, while our study used freeze-dried DVS. Generally, DVS cultures require longer lag phase to rehydrate cells compared to traditional bulk liquid starters (Hoier et al., 2011). The DVS freeze-dried starters used in this study may be also partially sub-lethally stressed during manufacture, handling and storage (Tamime and Robinson, 1999; Parente and Cogan, 2004; Santivarangkna et al., 2008), thereby affecting their activities. However, once acid production commences, DVS cultures normally produce acid more rapidly than bulk starters, the initial pH difference is overcome within a few hours and a higher amount of acid is produced at end of fermentation (Kindsedt, 2005; Hoier et al., 2011). It is noteworthy to mention that the lactic strains used in the current study may be different from the strains reported in the literature. As the characteristic of each bacterium is strain specific, the difference in acidification

pattern between the same species is not uncommon. A recent study by Grzeskowiak et al. (2011a) showed that even the properties of the same strain of *L. rhamnosus GG* differed significantly due to different manufacturing processes involved in the production of the bacterium.

Higher cell counts accompanied with higher acidification rate were consistently observed in cheese samples with O-type starters than samples with LD-type starters. The finding of the results did not reveal the synergistic effects between starters lactococci and *Leuconostoc*; The results are not in line with the study of Tzanetaki et al. (1993), which suggested that the presence of the *Leuconostoc* in starter composition stimulates the growth of lactococci in cheese matrix. Further studies on this subject may be useful to confirm the behavior of our strains used in the cultures. Such differences in cell viability and acidification activity between O-type and LD-type starters during fermentation may be attributed to the differences in culture composition. O-type starters comprised mesophilic acid-forming lactococci only (*L. lactis* subsp. *Lactis*, and *L. lactis* subsp. *cremoris*). The LD-type starters not only included the acid-producing lactococci strains, but also contained citrate-utilizing LAB (*L. lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuc. lactis* and *Leuc. mesenteriodes* subsp. *cremoris*). Such differences in composition may contribute to the difference in metabolic pattern. The importance of strain specificity in dairy fermentation and metabolism is well-documented (Fox et al., 2000; Beresford and Williams, 2004; Fox and McSweeney, 2004; Parente and Cogan, 2004). Genus *Lactococcus*, including the diacetyl-producing variants of *L. lactis* subsp. *lactis*. biovar. *diacetylactis*, are well-known for their strong acid production ability. Strains of *Leuconostoc* spp. (*Leuc. lactis* and *Leuc. mesenteriodes* subsp. *cremoris*) can metabolize citrate to CO_2, diacetyl and acetoin at acid pH; their ability for producing the flavor compounds has led to their frequent incorporation as starters for Camembert cheese production. In contrast to *Lactococcus*, *Leuconostoc* spp. are not competitive growers in milk and are poor acid producers (Hugenholtz, 1993; Monnet et al., 1994; Mcsweeney and Fox, 2004; Parente and Cogan, 2004; IDF, 2006). Therefore, to produce lactic curd, it is recommended to use a mixture with the Cit^--fermenting *L. lactis* cultures.

6.1.2 Growth of *P. camemberti*

Although the growth of *P. camemberti* during fermentation was not as rapid as LAB starter, the cell counts increased significantly ($P<0.05$) from 10^3 to 10^4 in all samples (Table 29 and Figure 41). The sigmoidal growth of *P. camemberti* showed prolonged lag phase before exponential growth, but as the environment gradually became more acidic due to LAB activity, the growth rate of the mould started to increase noticeably.

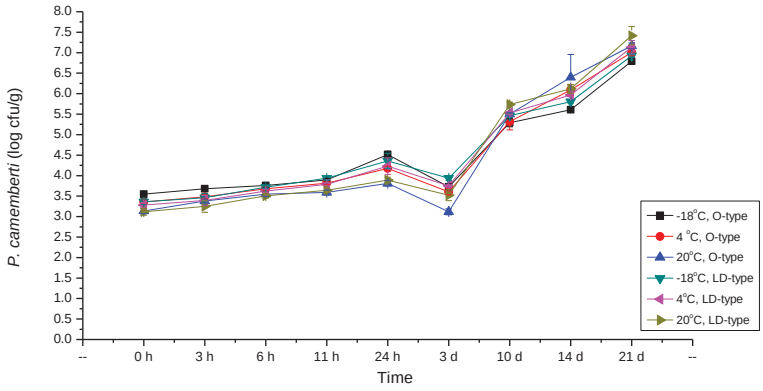


Figure 41. Growth of *P. camemberti* in Camembert cheese samples during 24 h of fermentation and 21 d of ripening. Each point represents mean log cfu/g of four independent samples with error bars representing ±SD. Cheese samples with rind.

Throughout fermentation, the pattern of growth by the mould strain was fairly similar in all samples (Figure 41). As expected, the fermentation profiles of *P. camemberti* in cheese milk/curd were similar ($P>0.05$) in both O-type and LD-type cheese samples, irrespective the type of lactic starters present; and this was probably because identical mould strain was used in cheese production. In comparison to samples containing -18°C cultures, samples with 4 and 20°C cultures had consistently relative lower cell counts ($P<0.05$) of *P. camemberti* throughout fermentation, suggesting that the temperature of storage affected their growth during milk fermentation. This was probably due to the reduced activity of strains after storage at higher temperatures for prolonged time. Nonetheless, the final concentrations of *P. camemberti* in samples (10^4 cfu/g) with 4 and 20°C cultures obtained at end of fermentation were comparable to samples with -18°C cultures and levels recommended by industry (Parente and Cogan, 2004). Therefore, the potential of using cultures previously stored at higher storage temperatures for prolonged time for Camembert cheese may be feasible.

It is well known that *P. camemberti* is acid-tolerant (Spinnler and Gripon, 2004). Although the interaction between *P. camemberti* and the lactic starters during fermentation is beyond the scope of this study, we observed steady growth of the mould during fermentation when co-inoculated with LAB. The observation suggests compatibility between *P. camemberti* and lactic acid bacteria. It was further observed that growth of *P. camemberti* was not affected by the amount of acid produced by LAB activity.

6.2 Ripening profile of Camembert cheese samples

6.2.1 Microbial and pH changes during cheese ripening

The development of the main microbial groups (LAB starters and *P. camemberti*) involved in the ripening of Camembert cheese and the pH of samples are shown in Table 30. The cell counts (log cfu/g) of each microorganism and pH values during ripening are shown in Figures 39-41.

Table 30. LAB[1] and *P. camemberti*[2] cell counts (log cfu/g) and pH[3] values (mean±SD) (n=4) in cheese samples at 21 d of ripening.

Parameter	Sampling time during ripening (d)	Acid-only cheese/O-type[4]			Aroma-enriched cheese/LD-type[5]		
		-18°C	4°C	20°C	-18°C	4°C	20°C
LAB	3	^{z}9.43±0.08^{a}	^{y}9.13±0.15^{a}	^{x}9.12±0.04^{b}	9.13±0.05^{a}	9.09±0.09^{a}	9.03±0.05^{a}
	10	^{x}8.83±0.04^{b}	^{x}8.93±0.05^{ab}	^{y}9.08±0.08^{a}	8.90±0.03^{a}	8.98±0.10^{a}	9.03±0.09^{a}
	14	^{x}8.54±0.17^{c}	^{y}8.81±0.13^{bc}	^{y}8.83±0.12^{a}	8.59±0.23^{b}	8.69±0.14^{b}	8.83±0.10^{b}
	21	^{x}8.41±0.03^{d}	^{y}8.65±0.10^{c}	^{z}8.83±0.08^{b}	8.47±0.16^{b}	0.57±0.15^{b}	8.64±0.10^{c}
P. camembert	3	^{y}3.73±0.07^{d}	^{y}3.61±0.07^{d}	^{x}3.12±0.07^{d}	^{z}3.94±0.05^{d}	^{y}3.75±0.03^{d}	^{x}3.52±0.13^{d}
	10	5.29±0.10^{c}	5.32±0.20^{c}	5.50±0.04^{c}	^{x}5.46±0.05^{c}	^{x}5.53±0.03^{c}	^{y}5.73±0.06^{c}
	14	^{x}5.61±0.06^{b}	^{xy}6.09±0.13^{b}	^{y}6.40±0.55^{b}	^{x}5.81±0.03^{b}	^{y}5.97±0.02^{b}	^{z}6.12±0.11^{b}
	21	^{x}6.79±0.08^{a}	^{y}7.01±0.08^{a}	^{y}7.17±0.07^{a}	^{x}6.92±0.04^{a}	^{xy}7.14±0.16^{a}	^{y}7.42±0.22^{a}
pH	3	^{z}4.68±0.01^{c}	^{y}4.56±0.03^{d}	^{x}4.47±0.04^{d}	^{y}4.80±0.08^{b}	^{xy}4.69±0.09^{c}	^{x}4.62±0.06^{d}
	10	4.73±0.08^{c}	4.71±0.12^{c}	4.81±0.07^{c}	4.93±0.25^{b}	4.96±0.22^{c}	4.97±0.16^{c}
	14	^{x}5.03±0.04^{b}	^{y}5.13±0.02^{b}	^{z}5.27±0.05^{b}	^{x}5.10±0.27^{b}	^{xy}5.30±0.08^{b}	^{y}5.50±0.08^{b}
	21	^{x}6.10±0.07^{a}	^{y}6.39±0.10^{a}	^{z}6.56±0.14^{a}	6.36±0.10^{a}	6.42±0.03^{a}	6.61±0.08^{a}

[1,2] Cheese samples with rind; [3]cheese samples without rind. [4]Acid-only cheese/O-type: cheese samples produced using O-type LAB starters (stored at -18, 4, 20 °C) consisted of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (R704, Chr. Hansen) and *P. camemberti* (PCTT033, Chr. Hansen) as ripening culture; [5]Aroma-enriched'cheese/LD-type: cheese samples produced using LD-type LAB starters (stored at -18, 4, 20 °C) consisted of *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuc. lactis* and *Leuc. mesenteriodes* subsp. *cremoris* (Flora Danica, Chr. Hansen) and *P. camemberti* as ripening cultures (PCTT033, Chr. Hansen).
Within rows, mean values preceded by different superscripts are significantly different at P<0.05, with respect to one type of cheese. Within columns, mean values followed by different letters are significantly superscripts at P<0.05. Tukey test was used to separate significantly different mean values.

Growth of LAB during cheese ripening

Cell counts of LAB varied insignificantly (P=0.966) between two different types of

cheese. However, they reduced significantly ($P<0.05$) during ripening, irrespective of the type of starter cultures used. A similar degradation trend during ripening was observed for all cheese samples, with LAB cell counts decreasing to different levels at day 21 (Table 30 and Figure 39). Although the decrease of LAB cell population was significant during ripening, high levels (10^{7-8} cfu/g) of the bacteria were still present in all cheese samples by the end of ripening. At day 21 of ripening, samples containing 20°C cultures had notably higher ($P<0.05$) cell counts compared to samples of the other treatments (Table 30).

The higher initial LAB counts in cheese samples with O-type starters than samples with LD-type starters were probably attributed to their higher growth during fermentation. During the initial 3 d of ripening, the LAB cell counts of samples with LD-type starters were relatively stable, whereas cell reductions occurred in samples with O-type starters. The depletion of lactose and high acidity environment of cheese samples can considerably slow down the growth of cells of cultures (McSweeney, 2004b). Reduction in cell population was also largely attributed to the presence of added salt to the curd during cheese manufacture. High salt concentration can inhibit LAB to some extent (Fox and McSweeney, 2004; McSweeney, 2004b). The only difference of O-type and LD-type starters was the species composition. The relatively smaller reduction of cell counts in LD-cheese samples during the initial 3 d of ripening was probably due to the presence of the *Leuconostoc* species in the culture, which is more tolerance to high salt concentration than other lactic acid bacteria (Bjokroth and Holzapfel, 2006). Although lactic starter bacteria are stimulated by low levels of NaCl, they are strongly inhibited at >2.5% NaCl (Parente and Cogan, 2004; Spinnler and Gripon, 2004). Compared to *L. lactis* subsp. *lactis, L. lactis* subsp. *cremoris* shows higher salt sensitivity. LAB is proteolytic in cheese. When the lactose concentration in cheese curd has been depleted to very low levels, the survival of LAB cells at high numbers (10^8 cfu/g) during ripening may be attributed to the proteolytic activity of bacteria (Beresford and Williams, 2004; McSweeney, 2004b; Parente and Cogan, 2004).

Growth of P. camemberti

Cell counts of *P. camemberti* increased ($P<0.05$) during cheese ripening. The growth of *P. camemberti* in all Camembert samples increased exponentially during the entire ripening process (Figure 41), which is consistent with published data (Leclercq-Perlat et al., 2004a; Guizani et al., 2007; Lessard et al., 2012). Between days 3 to 10, microbial growth rate of *P. camemberti* was significant, but the cell counts increased at a slower rate between days 10-21, reaching the highest value at day 21. The reduction in the rate of cell increase of *P. camemberti* towards the end of ripening may be due to the

rind becoming drier, thus contributing to the inhibition of the growth of the mould (Law, 1997). In agreement with Leclercq-Perlat et al. (2004a) and Lessard et al. (2012), the study showed that the growth of *P. camemberti* mycelium began to develop from day 10. There was heavy growth of the mould between days 10-14. The white mycelia evenly covered the entire cheese surface by day 14 before packaging and remained relatively constant until the end of ripening (day 21). Cell counts of *P. camemberti* were however not correlated to the intense growth of the mycelia. This observation was also reported by Lessard et al. (2012) where the concentration of *P. camemberti* mycelia was not correlated with viable cell counts. This anomaly has been attributed to the sampling method, during which mycelia are destroyed resulting in low viable cell counts of *P. camemberti* (Lessard et al., 2012).

Throughout the ripening period, cell counts of *P. camemberti* were different ($P<0.05$) between the two types of cheeses. Storage temperatures for cultures also played a significant effect ($P<0.05$) on the growth of *P. camemberti*. Cheeses produced with LD-starters and cultures stored at ambient and refrigeration temperatures contained significantly higher mould counts than samples of alternative treatments that were ripened under the same conditions (Table 30 and Figure 41). Samples produced from LD-starters and cultures stored at 4 and 20°C had relatively higher initial pH after fermentation (Table 20 and Figure 40). Our results contradict results of previous studies. The growth of *P. camemberti* is expected to be higher when the pH of curd is low as the high acidity enhances the growth of the mould and the production of protease (Law, 1997; Fox et al., 2000; Spinnler and Gripon, 2004). This phenomenon cannot be explained easily. The difference observed in our study may be attributed to strain differences. It is however, well-known that higher moisture is retained in higher pH curds after drainage due to lower syneresis of the curds (Guinee and Wilkinson, 1992). The higher amount of moisture may effectively stimulate the growth of mould cultures and this indicates a possible relationship between the syneresis level of the cheese curd and the growth of *P. camemberti*.

Changes in pH during cheese ripening

Throughout maturation period, the variations in pH were significantly between three storage temperature of cultures ($P<0.05$) and two cheese types ($P<0.05$). Significant changes of cheese pH with ripening time were also recorded ($P<0.05$), with a similar pattern for both cheese types. Changes in pH levels of cheese samples for each treatment were shown in Table 30 and Figure 40.

From the end of fermentation to day 3 of ripening, pH of all samples generally remained stable (Figure 40), suggesting that 'post-acidification' was not significant.

The pH was lower than 4.80 for all cheeses as expected for young Camembert cheeses (Schlesser et al., 1992; Sousa and McSweeney, 2001; Perko, 2002; Leclercq-Perlat et al., 2004a; Sullivan et al., 2005; Guizani et al., 2007). The mean initial pH levels were considerably lower in O-type cheeses and in samples which contained -18°C cultures. The higher acidity values were probably due to a higher extent of acidification during fermentation.

There were important changes in the pH of the cheese samples (without rind) from days 3 to 21. Slow rate of pH increase was observed from days 3 to 10, whereas the pH of cheese steadily increased from day 10 up to day 21 during ripening. At day 21, the highest pH values were attained for all cheese samples, ranging from 6.10-6.61 for O-type cheeses and 6.36-6.56 for LD type cheeses (Figure 40). Similar results were obtained by Sullivan et al. (2005), who reported that surface pH of matured Camembert could be as high as 7.0 while the centre increased from 4.8 to 6.7. Lawrence et al. (1987) reported that the centre pH of cheese was only 4.8 after 17 d of ripening; thereafter, the pH increased to about 5.2 at 25 d ripening. In the study of Leclercq-Perlat et al. (2004a), the initial core pH of curd remained stable at 4.7 from days 2 to 13, suggesting that 'post-acidification' was not pronounced. After day 13, the rate of pH increased rapidly reaching 7.0 at 50 d of ripening. According to work done by Sousa and McSweeney (2001), the core pH of Cooleeney, an Irish farmhouse Camembert-type cheese made from cow's milk, increased from 4.5 to 5.0 over 50 d of ripening. Similarity, Schlesser et al. (1992) reported that the core pH in raw-milk-made Camembert increased from 4.4 at day 1, to 6.4 by day 50. Compared to samples reported in previous studies, the pH content of cheese samples (without rind) produced in this study were found to be within acceptable levels of good Camembert cheese. pH of Camembert samples of this study increased at relatively faster rate, suggesting a faster rate and higher degree of proteolysis producing a softer texture (Spinnler and Gripon, 2004; Upadhyay et al., 2004).

The significant increase of cheese surface pH of this study may be attributed to the metabolism of lactate by *P. camemberti* by forming alkaline metabolites. This speculation agrees with reports of several studies (Fox et al., 2000; Leclercq-Perlat et al., 2004a; McSweeney, 2004b; Spinnler and Gripon, 2004). According to Leclercq-Perlat et al. (2004a), lactate concentration in cheese, particularly on the rind, is balanced by activities of fungal flora (which consume lactate) and the LAB (which produce lactate). Initially, *P. camemberti* metabolizes lactic acid; the assimilation of lactate reduces cheese curd acidity, and residual lactose diffuses from inside of cheese towards the surface. Consequently, ammonia is produced at cheese surface due to the de-amination activity of *P. camemberti*. The increase of pH is further attributed to the diffusion of ammonia from the surface towards the core.

Lactose and lactate concentrations in cheese samples during ripening were not determined in this work. However, Leclercq-Perlat et al. (2004a) reported correlation of viable LAB to carbon substrate concentration in cheese samples, while changes in lactate quantities were related to the development of fungal flora. Based on the current results of changes in viability of LAB and *P. camemberti* as well as the increase of pH values in samples, depletion of lactose and assimilation of lactate were linked. The lactose concentration was speculated to be rapidly consumed during the first 3-10 ripening days after which it may reach to a very low level. This period corresponded to remarkable increase in pH from 4.5 to 6.5, which was also linked to vigorous mycelium development by *P. camemberti*. The diffusion of lactate from cheese core to rind was highly suspected after 10 d of ripening due to the vigorous growth of mould. Further, based on observations that proteolysis was highly correlated with the cell counts of *P. camemberti* (Section 6.2.2), the growth of the mould in this work was highly related to nitrogen evolution of cheeses, thus de-acidifying activity of surface mould is therefore confirmed.

At day 21, mean pH values were generally higher ($P<0.05$) in samples with LD-type starters and cheeses containing cultures stored at 20°C (Table 30 and Figure 40). The higher pH in samples was probably attributed to the lower acid production during fermentation, which leads to higher syneresis in the cheese curd (Guinee and Wilkinson, 1992). As mentioned earlier, higher amount of whey retained in the curd may stimulate faster growth of mould, with de-acidifying activity occurring more readily.

6.2.2 Proteolysis changes in cheese during ripening cheeses

Variation of proteolysis of surface and centre of Camembert cheese has been widely reported (Law, 1997; Leclercq-Perlat et al., 2004a; Guizani et al., 2007). In the present study, the magnitude of proteolysis of Camembert cheese was investigated on samples without rind. The degree of proteolysis in cheese samples was determined by the 'ripening index' as ASN and NPN against TN. Analysis of FAA concentration was also used (Schlesser et al., 1992; Leclercq-Perlat et al., 2004a; Sullivan et al., 2005; Guizani et al., 2007). In addition to the measured 'ripening index' and FAA concentration, cheese proteolysis of this study was evaluated based on the estimated values of protein nitrogen, casein nitrogen and peptide nitrogen content (Delgado et al., 2010; Delgado et al., 2011).

Changes in ripening indices, ASN (as % TN) and NPN (as % TN) during ripening are shown in Figures 42-43, while various cheese nitrogen fractions (protein-N, casein-N

and peptide-N) of the samples are illustrated in Figures 44-46. The levels of free amino acid concentration in cheese samples are shown in Figure 47. Mean values and standard deviations of proteolysis data on cheese ripening at each stage of maturation are presented in Table 31. Correlation matrices of nitrogen fractions and the cultures are presented in Table 32.

Table 31.Nitrogen fractions[a,c] and FAA[c] in Camembert cheese samples[1] during ripening for 21 d.

Nitrogen fractions	Sampling time during ripening (d)	Acid-only cheese/O-type[2]			Aroma-enriched cheese/LD-type[3]		
		-18°C	4°C	20°C	-18°C	4°C	20°C
ASN (as % TN)	3	7.49±1.39^{b}	9.49±0.92^{c}	8.17±1.53^{c}	9.35±1.21^{d}	9.62±1.37^{c}	9.70±2.20^{c}
	10	9.97±1.16^{b}	12.21±1.45^{bc}	10.87±1.35^{c}	12.56±2.22^{c}	13.03±1.39^{c}	12.06±1.66^{bc}
	14	16.64±3.53^{a}	14.50±1.38^{b}	16.35±2.53^{b}	17.73±1.01^{b}	20.30±0.97^{b}	18.00±2.43^{b}
	21	20.19±3.10^{a}	22.52±1.85^{a}	23.75±3.62^{a}	28.85±1.14^{a}	29.20±2.61^{a}	30.57±4.44^{a}
NPN (as %TN)	3	5.82±0.76^{bc}	4.89±0.59^{c}	4.81±0.57^{d}	4.50±0.74^{d}	4.47±0.45^{d}	4.99±1.24^{c}
	10	6.85±1.45^{c}	5.85±0.85^{bc}	7.39±0.67^{c}	7.56±1.38^{c}	8.37±1.48^{c}	7.22±1.09^{c}
	14	9.92±1.20^{b}	8.55±2.01^{b}	11.21±0.99^{b}	11.76±0.54^{b}	14.07±1.20^{b}	11.67±1.35^{b}
	21	14.65±3.33^{a}	14.51±2.14^{a}	16.17±1.52^{a}	20.15±1.08^{a}	20.58±1.17^{a}	20.96±4.15^{a}
Protein Nitrogen (%, w/w)	3	2.44±0.28^{a}	2.35±0.08^{a}	2.28±0.22	2.38±0.03^{c}	2.33±0.06^{a}	2.38±0.04^{a}
	10	2.27±0.11^{ab}	2.24±0.08^{ab}	2.14±0.24	2.23±0.10^{b}	2.23±0.02^{a}	2.28±0.12^{ab}
	14	2.18±0.01^{ab}	2.13±0.07^{b}	2.16±0.21	2.16±0.03^{b}	2.07±0.11^{b}	2.15±0.31^{ab}
	21	2.11±0.05^{b}	2.10±0.10^{b}	2.12±0.04	2.02±0.02^{a}	1.93±0.08^{b}	1.96±0.03^{b}
Casein Nitrogen (%, w/w)	3	2.34±0.17^{a}	2.39±0.07^{a}	2.35±0.02^{a}	^{y}2.34±0.06^{a}	^{xy}2.20±0.13^{a}	^{x}2.06±0.11^{a}
	10	2.23±0.03^{ab}	2.21±0.07^{b}	2.29±0.07^{a}	^{y}2.03±0.10^{b}	^{x}2.01±0.06^{b}	^{x}1.98±0.04^{a}
	14	2.06±0.02^{bc}	2.12±0.07^{bc}	2.07±0.04^{b}	^{y}2.01±0.02^{b}	^{x}1.83±0.06^{c}	^{x}1.80±0.09^{b}
	21	1.94±0.08^{c}	2.00±0.10^{c}	1.90±0.06^{c}	^{y}1.79±0.05^{c}	^{xy}1.71±0.06^{c}	^{x}1.66±0.06^{b}
Peptide nitrogen (%, w/w)	3	0.05±0.02^{c}	0.08±0.01^{c}	0.07±0.01^{c}	0.08±0.00^{c}	0.10±0.01^{c}	0.10±0.01^{c}
	10	^{x}0.10±0.01^{b}	^{y}0.14±0.03^{b}	^{z}0.19±0.01^{a}	0.11±0.03^{bc}	0.12±0.03^{c}	0.12±0.01^{c}
	14	0.12±0.02^{b}	0.15±0.02^{b}	0.15±0.02^{b}	0.15±0.03^{b}	0.18±0.02^{b}	0.18±0.02^{b}
	21	0.21±0.00^{a}	0.20±0.01^{a}	0.22±0.02^{a}	0.27±0.02^{a}	0.27±0.02^{a}	0.31±0.03^{a}
FAA (mg Leu/g of cheese)	3	0.47±0.10^{b}	0.48±0.11^{c}	0.51±0.12^{b}	0.39±0.05^{c}	0.38±0.08^{d}	0.42±0.13^{c}
	10	^{x}0.60±0.04^{b}	^{x}0.57±0.07^{c}	^{y}0.70±0.03^{b}	0.61±0.16^{bc}	0.70±0.13^{c}	0.74±0.10^{bc}
	14	^{x}0.88±0.23^{a}	^{y}1.08±0.12^{b}	^{y}1.09±0.39^{a}	1.44±0.27^{b}	1.09±0.15^{b}	1.63±0.10^{ab}
	21	1.21±0.06^{a}	1.37±0.11^{a}	1.47±0.21^{a}	1.53±0.06^{a}	1.70±0.37^{a}	1.75±0.33^{a}

[a]Nitrogen fraction of cheeses includes: TN, ASN, NPN; [b]mean±SD, n=4; [1]cheese samples without rind; [2]Acid-only cheese/O-type: cheese samples produced using O-type LAB starters (stored at -18, 4, 20°C) consistent of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (R704, Chr. Hansen) and *P. camemberti* (PCTT033, Chr. Hansen) as ripening culture; [3]Aroma-enriched'cheese/LD-type: cheese samples produced using LD-type starters (stored at -18, 4, 20°C) consistent of *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuc. lactis* and *Leuc. mesenteriodes* subsp. *cremoris* (Flora Danica, Chr. Hansen) and *P. camemberti* as ripening cultures (PCTT033, Chr. Hansen).

Within rows, mean values preceded by different superscripts are significantly different at $P<0.05$, with respect to one type of cheese. Within columns, mean values followed by different superscripts are significantly different at $P<0.05$.

Proteolysis indices during cheese ripening

In all the cheeses (O- and DL-types), levels of ASN (as % TN) and NPN (as % TN) increased ($P<0.05$) during ripening due to proteolysis, indicating that ripening time was the main factor affected the ripening indices. It suggests that proteolysis was closely linked to the maturity of cheese, which agrees with several reports (Schlesser et al., 1992; Leclercq-Perlat et al., 2004a; Sullivan et al., 2005; Guizani et al., 2007). During ripening, both ASN and NPN indices followed a similar pattern (Figures 42, 43). The indices increased slowly during early stages of ripening between days 3-10 (from 0.07-0.09% to 0.10-0.13% for ASN, and from 0.05-0.06% to 0.07-0.08% for NPN); from day 10, the rate of increase was rapid with all samples reaching peak values at 21 d (0.20-0.31% for ASN and 0.15-0.21% for NPN). The phenomenon depicted a quadratic/polynomial relationship between the levels of ASN and NPN and ripening time.

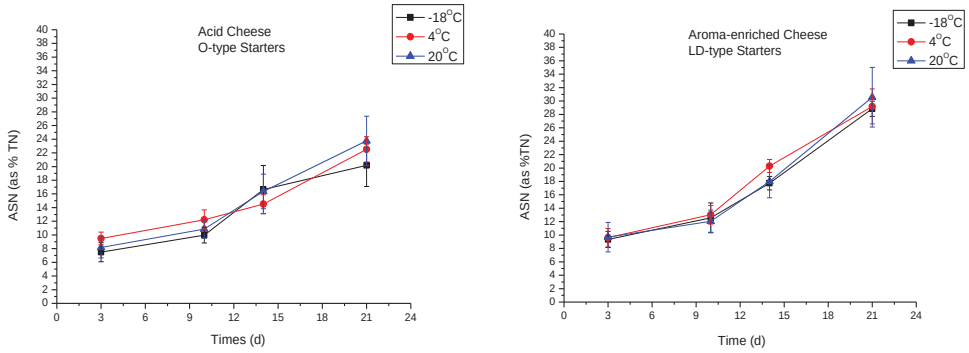


Figure 42. Changes in acid-soluble nitrogen (ASN as %TN) in Camembert cheese samples (without rind) during 21 d of ripening. Each point represents mean of four independent analyses (n=4). Error bars are ±SD.

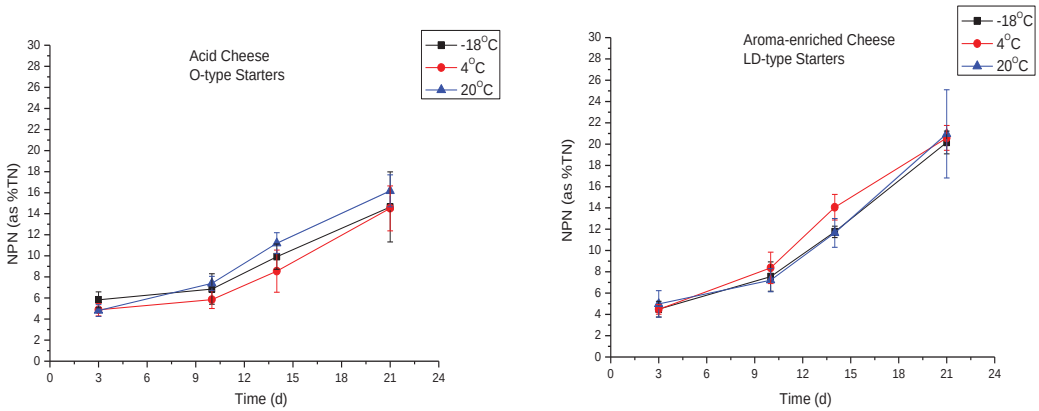


Figure 43. Changes in non-protein nitrogen (NPN as %TN) in Camembert cheese samples (without rind) during 21 d of ripening. Each point represents mean of four independent analyses (n=4). Error bars are ±SD.

Cheese proteolysis was also affected (P<0.05) by starter type during ripening. ASN (as %TN) and NPN (as %TN) were generally higher in samples fermented by LD-type starters suggesting that they had a more extensive proteolysis (Table 31 and Figures 42, 43). The difference in magnitude of proteolysis may be explained by variations in the composition of the starters and their metabolism (Rehn et al., 2011; Parente and Cogan, 2004; Upadhyay et al., 2004).

For both types of cheeses, it is interesting to note that samples with 20°C cultures generally had higher ASN and NPN levels than their alternative treatments (Table 31 and Figures 42, 43). Since the differences were not significant (P=0.219 and P=0.541 for ASN and NPN, respectively), therefore culture storage temperature was probably not a significant factor influencing cheese proteolysis. This finding was however unexpected as cultures stored at lower storage temperatures for prolonged times should have higher activity and viability, allowing proteolysis to occur more readily.

Concentration of nitrogen fractions

At the beginning of cheese ripening, most (90.5-98.0%) of the protein nitrogen belonged to the caseins. All cheese samples had similar content protein nitrogen (2.28-2.38 g/100g) (Table 31), which allowed proteolysis to be compared.

Age of cheese was the main significant factor (P<0.05) influencing the levels of N-fractions (protein-, casein- and peptide-N). During cheese ripening, increase in the degree of proteolysis was reflected by the decrease of protein and casein nitrogen, with simultaneous increase in peptide nitrogen content of cheese (Table 31; Figures 44-46). The increase of peptide nitrogen followed similar patterns with respect to the increase of ripening index. At the beginning of cheese ripening (day 3), samples had relatively low peptide content but with high levels of casein. The hydrolysis of casein became activated after 10 d of ripening and the level of peptide concentration in all samples also started to increase (Figure 46). Hydrolysis of casein in the cheese samples was most intense between 14-21 d of ripening, producing high amounts of peptides. Meanwhile, the peptides produced were also in turn hydrolysed extensively resulting in an increase of FAA level (Table 31 and Figure 47).

The type of starter culture played a significant role (P<0.05) in the levels of casein and peptide N-fractions of samples. Higher (P<0.05) peptide nitrogen content was observed in LD-type cheese samples than in O-type cheeses (Table 31, Figure 46). This result suggests the higher proteolysis activity of the LD cultures. Culture storage temperature also exhibited significant (P<0.05) impact on casein and peptide content

of samples, in particular where LD-starters were used. Higher ($P<0.05$) peptide content accompanied by lower protein and casein levels were generally obtained in samples with 20°C cultures than their alternative treatments of cultures stored at -18°C and 4°C (Table 31 and Figures 45, 46). However, neither type of starters and culture storage temperature were significant factors ($P=0.185$ and $P=0.241$ respectively) affecting the protein nitrogen level of samples throughout ripening (Table 31; Figure 44).

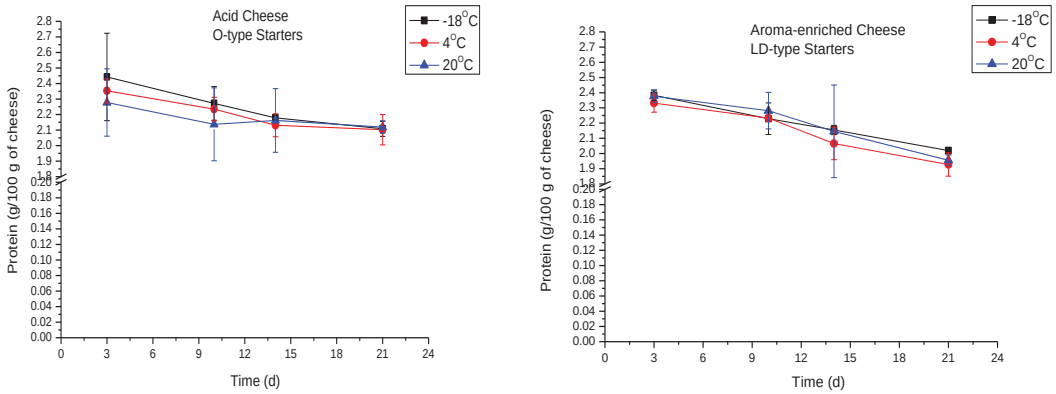


Figure 44. Changes in protein content in Camembert cheese samples (without rind) during 21 d of ripening. Each point represents a mean of four independent analyses (n=4). Error bars are ±SD.

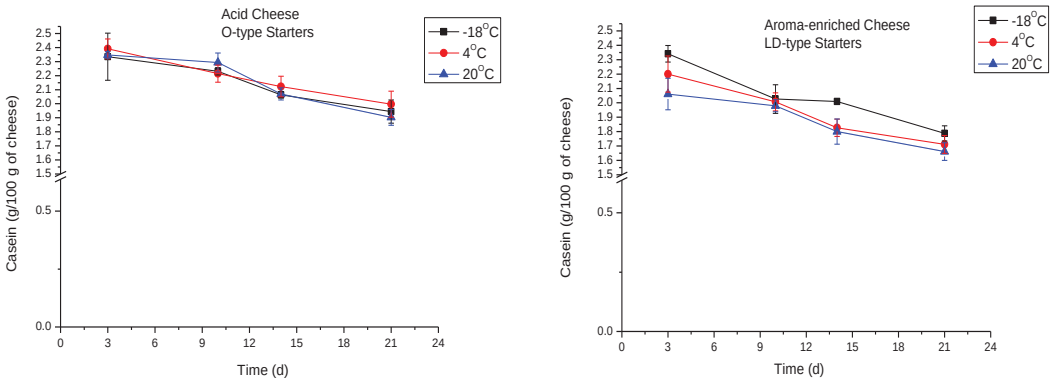


Figure 45. Changes in casein content in Camembert cheese samples (without rind) during 21 d of ripening. Each point represents a mean of four independent analyses (n=4). Error bars are ±SD.

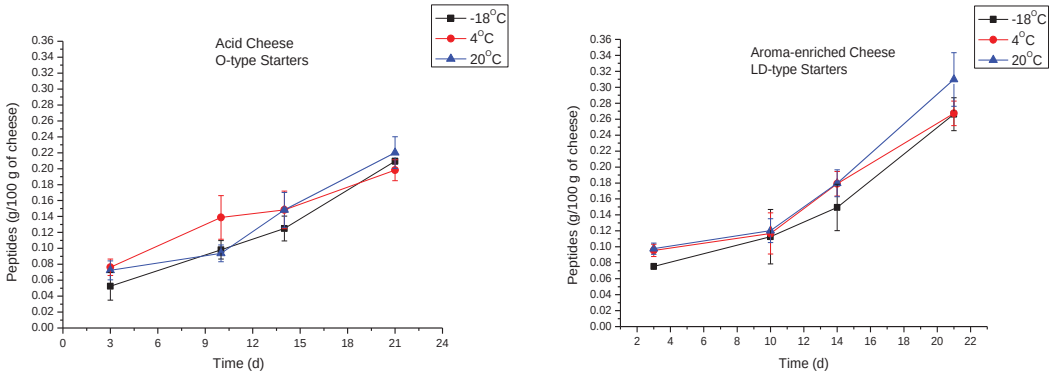


Figure 46. Changes in peptide concentration in Camembert cheese samples (without rind) during 21 d of ripening. Each point represents a mean of four independent analyses (n=4). Error bars are ±SD.

Free amino acid content

Concentration of FAA showed large deviations during ripening in the samples tested and were largely affected by the starter type and ripening time ($P<0.05$). The effect of culture storage temperature on the maturation of cheese was also significant ($P<0.05$).

A similar trend on FAA levels during ripening was observed in both cheese types (Table 31 and Figure 47). FAA concentration increased from 0.47-0.51 (mg/g of cheese) to 1.53-1.75 (mg/g of cheese) for LD-type cheeses, and 0.38-0.42 (mg/g of cheese) to 1.21-1.47 (mg/g of cheese) for O-type cheeses. FAA content slowly increased between day 3 and 10, with significant increases from day 10 to day 21 ($P< 0.05$). These results agreed with the trends detected by two cheese ripening indices (ASN (as %TN) and NPN (as %TN)) and peptide-nitrogen analysis.

Cheese fermented by LD-cultures showed higher ($P<0.05$) FAA content than samples produced with O-cultures (Table 31; Figure 47). This observation suggested the more active role of LD cultures in peptidolytic activity. Significant differences ($P<0.05$) were also observed in FAA development between LD-type cheese samples with different culture storage temperatures. Although the differences were significant, they were more pronounced in 10- and 14-d old samples than 21-d old samples (Table 31), suggesting that the final level of proteolysis was not affected by the storage temperature of the culture.

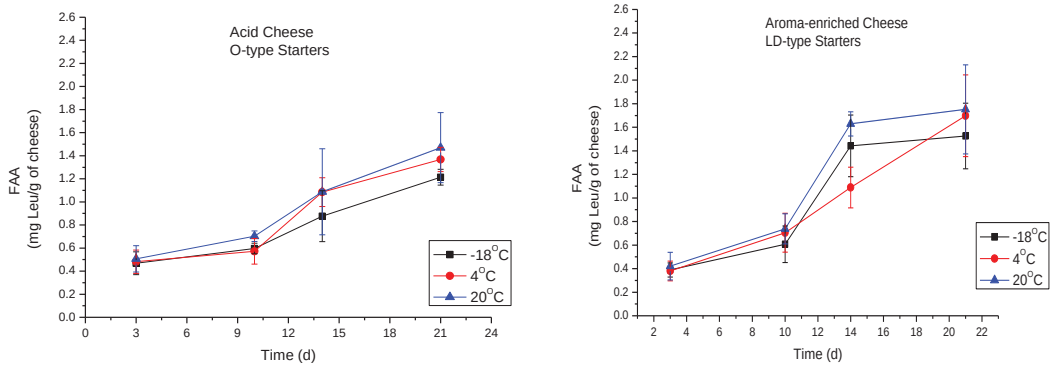


Figure 47. Changes in FAA in Camembert cheese samples (without rind) during 21 d of ripening. Each point represents a mean of four independent analyses (n=4); Error bars are ±SD.

Correlation of variables involved in proteolysis of Camembert cheese

Table 32 shows significant correlations between variables involved in proteolysis of Camembert cheese during ripening.

ASN (as %TN) showed good correlations with NPN (as %TN). The ripening indices were also highly correlated with their corresponding peptide content and FAA concentration of samples, but negatively related to the protein and casein levels (Table 32). The pH of sample was also highly correlated with the ripening index (ASN and NPN).

Ripening indices (ASN and NPN) of cheeses were negatively correlated with the growth of LAB but positively with the growth of *P. camemberti* (Table 32). High correlations were also observed between viable cell counts of *P. camemberti* and proteolytic products present in the cheese. Cell counts of *P. camemberti* were positively correlated with peptide content and free amino acid concentration of cheese samples but negatively correlated with casein fractions (Table 32). The negative correlation between cell counts of *P. camemberti* and protein content of sample was also observed, but to a lesser extent. *P. camemberti* also showed strong positive correlation with the pH of the cheese samples during ripening.

Meanwhile, cell counts of LAB were negatively correlated with the degree of proteolysis, as indicated by the increased values of ripening indices (ASN and NPN), peptide and FAA, but with lower values of protein and casein (Table 32). Negative relationship was also shown between the cell counts of LAB and the pH of samples (Table 32).

Table 32. Correlation matrices[1] between viable cell counts of LAB and *P. camemberti*, pH and proteolytic variables measured during ripening of Camembert cheese.

Parameters[2]	LAB	*P. camemberti*	FAA	Peptides N	Protein N	Casein N	NPN	SN
P. camemberti	-0.577							
FAA	-0.668	0.785						
Peptides N	-0.533	0.844	0.659					
Protein N	0.481	-0.674	-0.530	-0.653				
Casein N	0.525	-0.765	-0.553	-0.763	0.589			
NPN	-0.606	0.833	0.718	0.872	-0.676	-0.820		
SN	-0.607	0.832	0.713	0.875	-0.715	-0.825	0.951	
pH	-0.736	0.810	0.794	0.835	-0.604	-0.727	0.882	0.888

[1]Correlations were statistically significant at $P<0.05$; [2]LAB and *P. camemberti* (log cfu/g); FAA (mg Leu g^{-1}); Peptides N (%, w/w); Protein N (%, w/w); Casein N (%, w/w); NPN (% TN); ASN (% TN).

Overall, the results of proteolysis showed that the insoluble caseins during ripening were gradually converted into high molecular weight peptides soluble at pH 4.6. The peptides were further degraded into various small-sized peptides and amino acids soluble in TCA solution (McSweeney and Fox, 1997; Upadhyay et al., 2004; McSweeney, 2004b). The significant development in FAA concentration suggested gradual degradation of small peptides present in samples (Upadhyay et al., 2004). Considerable proteolysis in Camembert cheese samples during ripening was supported by significant increases of ripening indices (ASN and NPN), polypeptide and FAA content with simultaneous decrease of protein and casein.

In this study, increase in ripening indices and peptides was accompanied by decrease in casein of cheese samples during ripening. The results demonstrate the occurrence of primary proteolysis during early cheese ripening, which may be attributed to proteolytic enzymes of rennet and plasmin retained in cheese curd, and to a lesser extent, proteases from *P. camemberti* (Spinnler and Gripon, 2004; Upadhyay et al., 2004). Secondary proteolysis occurs during late ripening (Rank et al., 1985) when peptides are hydrolysed to FAA. In this study, this was demonstrated by increase in FAA concentration coupled with decrease of peptide content of samples. These metabolic activities may be attributed to plasmin activity, as well as proteases and peptidase enzymes of lactic starters and *P. camemberti* (Fox et al., 2000; Spinnler and Gripon, 2004; Upadhyay et al., 2004). The production of ammonia is regarded as the final step of proteolysis when free amino acids are released (Spinnler and Gripon, 2004; Upadhyay et al., 2004; Leclercq-Perlat et al., 2004a). The concentration of ammonia in cheese samples was not however measured in this study. Since high FAA concentration was detected in cheese samples with ''liquid-like' texture (Figure 49) and recognisable 'ammonia-like' smell, this therefore supports the presence ammonia on cheese rind at 21 d. Further research on this subject may provide better

understanding of proteolysis of Camembert cheese.

A similar trend of proteolysis during cheese ripening was observed in all cheese samples. The results showed that proteolysis in the interior of cheese samples was relatively low at the beginning of ripening (day 3), but became activated after 10 d of ripening as the concentration of *P. camemberti* increased, reaching maximum at 21 d. By the end of ripening, values of proteolystic indicators (ripening index, peptide nitrogen and FAA) had increased by at least three times of starting value (Table 3). As discussed earlier, ripening indices of ASN (as %TN) and NPN (as %TN) increased during ripening as a consequence of proteolysis, in all tested cheese samples. The measurements did not, however, differ between cheeses containing cultures stored -18°C, 4°C and 20°C, suggesting that the storage temperature of the culture did not affect proteolysis of cheese. However, the storage temperature of culture, generally, affected changes in casein, polypeptides and FAA levels. This result was not surprising since protein-, casein- and peptide-nitrogen levels of samples were estimated. Although estimation of the nitrogen fractions provided quick information, it is however important to obtain empirical data. Results obtained in this study support the 'ripening index results', suggesting that the use of different temperatures for culture storage had no apparent impact on cheese proteolysis ($P>0.05$).

Trend and the extent of proteolysis observed in this study were comparable to previous studies on Camembert cheese (Schlesser et al., 1992; Sousa and McSweeney, 2001; Leclercq-Perlat et al., 2004a; Sullivan et al., 2005; Guizani et al., 2007) and many other cheese varieties (Tzanetaki et al., 1993; Pavia et al., 2000; Hayaloglu, 2009). In the study of Guizani et al. (2007), ASN (as %TN) remained stable in the centre of Camembert for 6 d, but increased rapidly from 7.12 to 21.29% by day 23. At day 30, the increase of ASN slowed and reaches to 22.62%. According to Leclercq-Perlat et al. (2004a), proteolysis activity in the centre of Camembert cheese was very low compared to surface samples which remained stable (6-8%) during the first week of ripening. Faster rate (30%) of proteolysis has been observed at day 15, when growth of *P. camemberti* commenced and their enzymes were synthesized. When *P. camemberti* vigorously developed at the end of ripening, the ASN reached about 45% of total nitrogen. At the same time, the NPN (as %TN) remained stable at 5% during the first 4 d, reaching about 35% by day 45. Proteolysis then slowly diminished although enzymes remained relatively stable in the cheese. Sullivan et al. (2005) also reported that the level of ASN (as %TN) in Camembert cheeses using calf rennet increased from 8.1% to 18.2% within 50 d of ripening. The free amino acid concentration of samples also increased to 6.3 mg/g from 1.8 mg/g within 21 d and reached 21.0 mg/g by the end of ripening at 50 d. Similarity, Sousa and McSweeney (2001) found that the ASN at core of Cooleeney, an Irish farmhouse Camembert-type cheese made from

cow's milk increased from 6.8% (day 1) to 14.5% (day 13), and 25% at day 30 of ripening. Small peptides were abundant in the ASN representing 20% of total nitrogen. Schlesser et al. (1992) also reported that casein was hydrolysed to ASN increasing its value from 8.4% (day 1) to 48.8% (day 29).

In other cheese varieties, the ASN (as %TN) in Manchego-type cheeses increased from about 6-7% to 18-19% within 90 d of ripening, while the NPN (as %TN) increased from about 3% to about 16% (Pavia et al., 2000). Hayaloglu et al. (2009) obtained similar results when evaluating 12 different samples of raw milk mature Kashar cheese. Indices of proteolysis varied from 10.72% to 23.75% and 7.09% to 12.26% for SN- and NPN-nitrogen fractions, respectively; total FAA concentrations ranged from 6.36 to 36.03 mg Leu g^{-1} of cheese. In the study of Tzanetaki et al. (1993), ASN (as %TN) in Feta cheese increased from about 15 to 33% within 240 d of ripening, while the level of NPN (as %TN) increased from about 5 to 20%.

Overall, the results of cheese proteolysis were within acceptable levels of Camembert cheese reported in previous studies. Variation in the levels of proteolysis indicators could be attributed to differences in production methods of Camembert cheese and the age the products. It is also noteworthy to mention that the LAB starter strain used in the current study may be different from the strains reported in published data. As the characteristics of each bacterium are strain specific, the difference in metabolic pattern between the same species is not uncommon. A recent study by Grezskowiak et al. (2011b) showed that even the properties of the same strain of *Lb. rhamnosus* GG may differ significantly due to different manufacturing process involved in the production of the bacterium.

Cheese samples fermented by LD-type starters in this study showed more proteolysis than samples produced from O-type starters during ripening for 21 d. This suggested a higher degree of cheese ripening, which was probably attributed to more active role of LD-starters in proteolytic and peptidolytic activity (Upadhyay et al., 2004). The only difference between the two types of cheese samples was the starter composition; the presence of *Leuconostoc* spp. in the composition may contribute to different metabolic patterns. Our results are in agreement with Tzanetaki et al. (1993), who reported high activity of peptidases when *Leuc. mesenteriodes* subsp. *cremoris* with a more active peptidolytic system was used in mixed cultures with *L. lactis* in Feta production. The presence of *Leuc. mesenteriodes* subsp. *cremoris* was also suggested to enhance the growth of lactococci spp. in cheese matrix.

The coagulating enzymes specifically cleaves the Phe_{105}-Met_{106} bond of kappa

(κ)-casein during milk clotting, while some of the enzymes become adsorbed onto para-κ-casein, becoming part of the cheese (Law, 1997; Fox et al., 2000; Fox and McSweeney, 2004; McSweeney, 2004b). During cheese draining, the coagulating enzymes are retained, which subsequently promote proteolysis during early cheese ripening. It is well-known that primary proteolysis of Camembert is attributed to the coagulant enzyme activity (Grappin et al., 1985; Law, 1997; Upadhyay et al., 2004). The distribution of coagulating enzyme between curd and whey was pH-dependent. It was reported that more rennet is retained in the cheese when the milk is more acidic at setting/cutting/whey-draining (Holmes et al., 1997). Reducing the pH of curd at cutting/draining results in a curvilinear increase in the quantity of gastric proteinase rennet enzymes (e.g. chymosin) (Guinee and Wilkinson, 1992).

In this study, cheeses with LD-type cultures acidified to 4.88-4.94 had higher proteolysis during ripening than samples with O-type cultures acidified to 4.63-4.89. In view of the preceding discussion, lower degree of proteolysis in cheeses with O-type cultures was not expected. The cheeses were expected to retain more coagulating enzymes because of their lower pH at cheese-draining stage, which meant that the rate of proteolysis would be higher than in the LD-type cheeses (Grappin et al., 1985; Guinee and Wilkinson, 1992). This however did not agree with our observations because there was higher proteolysis in LD-type cheese with higher (curd) pH. In the present study, *Mucor* (*M.*) *miehei* aspartic proteinase was used as substitute for chymosin as milk coagulant (Section 3.2.2). The results may be explained by the fact that *M. miehei* is not pH-sensitive, thus the amount of microbial proteases retained in curd is not influenced by pH at curd-cutting or whey-draining (Guinee and Wilkinson, 1992; Holmes et al., 1997; Ward et al., 2009). The amount of rennet retained at whey-drainage increases with moisture content of cheese (Guinee and Wilkinson, 1992). Increased proteolysis in cheeses with LD-cultures was probably associated with higher pH level of cheese curd at draining, which can lead to higher syneresis (Schlesser et al., 1992). High whey content of cheese curd may retain higher concentration of coagulating enzyme in the curd (Guinee and Wilkinson, 1992), thus probably allowing primary proteolysis to occur more readily (Grappin et al., 1985; Guinee and Wilkinson, 1992). The increased moisture level in cheese samples may also enhance the growth of *P. camemberti* and its proteolytic system, resulting in higher final pH by end of ripening, thereby contributing to a greater degree of proteolysis (Spinnler and Gripon, 2004).

Slightly higher degree of proteolysis was also observed in samples fermented with 20°C than with -18°C and 4°C cultures, although the differences were not significant. After manufacture, cheeses made from 20°C cultures had higher pH (4.89-4.90) than samples fermented with -18°C and 4°C cultures (4.63-4.88 and 4.75-4.92, respectively),

irrespective of the type of culture used. As discussed earlier, the increased proteolysis in cheeses with 20°C-stored cultures may be due to the relatively higher pH of samples, which may increase microbial protease availability in curd, thus contributing to a greater degree of primary proteolysis.

LAB and their proteolytic enzymes play important roles in cheese proteolysis. Parente and Cogan (2004) and Law (1997) postulated that when growth of starters reaches a point where growth ceases and autolysis occurs, intracellular peptides may be released after bacterial death and accumulate in the cheese during ripening leading to increased proteolysis. This report agrees with Tzanetaki et al. (1993), who indicated that peak growth for proteolytic LAB did not coincide with peak formation of proteolytic products in the cheeses. Our results agree with the speculation that proteolysis in the cheese was negatively related to viable cell counts of LAB present in the samples (Table 32). LAB are proteolytic in cheeses, especially when lactose concentration has been depleted to a very low level (Beresford and Williams, 2004; Parente and Cogan, 2004). This phenomenon partially explains the presence of higher levels of proteolytic products in cheese samples towards the end of ripening (Table 32, Figures 42-47). During late phase of ripening when pH of cheese was high, the less acidity in cheese may permit the development of adjunct lactobacilli and aerophilic acid-sensitive bacterial flora, referred as 'wild flora', on the cheese surface (Beresford and Williams, 2004). The likely presence of 'wild flora', at either low or high concentration may increase the formation of FAA in samples, but this ability is species- and strain-dependent (Fox et al., 2000).

In addition to LAB activity, the growth of *P. camembert* is also associated with proteolysis (Fox et al., 2000; Spinnler and Gripon, 2004). The species has a very strong proteolytic system and release several amino-peptidases and carboxypeptidases that rapidly degrade cheese proteins to low molecular peptides and amino acids. During the late phase of ripening when lactate has been exhausted at the cheese surface, *P. camemberti* may use protein and peptide fractions as substrates (Spinnler and Gripon, 2004; Bockelmann, 2010). The uptake of proteins and peptides may increase proteolysis due to production of proteolytic and peptidase enzymes by the mould. As mentioned earlier, results of this work are in agreement with many studies and confirmed that the growth of *P. camemberti* was highly associated with cheese proteolysis. The growth of *P. camemberti* was considerable between days 10-21 (Table 30, Figure 41), while the increase in the ripening indices of samples was negligible at days 3 and 10, but increased significantly from day 14 onwards (Table 31, Figure 42,43). The rate of increase in the proteolytic products of samples was also observed towards the end of the ripening period. Fungal proteinases diffuse only for a very small distance from the surface (Noomen, 1983; Leclercq-Perlat et al., 2004a), and

therefore, their action is probably limited to the rind region. Thus, higher proteolysis at cheese surface is expected as reported by other workers (Leclercq-Perlat et al., 2004a; Schlesser et al., 1992; Sousa and McSweeney, 2001; Sullivan et al., 2005; Guizani et al., 2007). However, the proteolytic products of cheese diffuse slowly from the surface to the centre during ripening, resulting in a pH gradient (Spinnler and Gripon, 2004). As the rind dries up towards the end of the ripening period, the growth of mould and their enzyme activity may be inhibited, resulting in a slower rate of increase of proteolysis (Law, 1997).

pH is another important factor of cheese proteolysis. As discussed earlier, curd pH at whey-drainage can affect the retention of clotting enzyme in curd, which contributes to the degree of proteolysis. High acidity (low pH) of samples at early ripening also adversely affects the proteolysis of cheese (Leclercq-Perlat et al., 2004a; Tzanetaki et al., 1993). In this study, proteolysis in all samples was lower in the early stages of ripening, which had higher acidity levels. The rates of change for the ripening indices, nitrogen fractions (protein, casein, peptides) and FAA were slow and insignificant at the beginning of ripening (days 3-10), but rapidly increased from day 10 until the end of ripening at day 21. Our results agree with the study by Leclercq-Perlat el al. (2004a), who speculated that post-acidification of cheese fermented by LAB may slow down the ripening process. Tzanetaki et al. (1993) also suggested that the high acidity environment at early ripening decreases activity of cell-envolope proteases produced by LAB, although the growth of starters was high that allows high production of the enzyme. This speculation may help to explain the relatively lower degree of initial proteolysis in samples fermented by -18°C-stored cultures, which had higher acid levels.

During late phase of ripening, higher proteolysis was observed in cheese samples with higher pH and changes in peptide-N and FAA levels were positively correlated with pH increase. At day 21, pH values >6.10 were observed for all samples. The pH levels mentioned here were close to the optimum pH (8.5) for plasmin (Grappin et al., 1985; Rank et al., 1985). The increase of plasmin activity was therefore highly suspected. It is thus assumed that the presence of plasmin had an impact on cheese proteolysis during the last 7 d of ripening. Coagulant present in cheese curd is however supposed to have limited action on α-caseins because the pH optimum of the enzyme on caseins is about 5.5. The increase of pH in the Camembert after 10 d of ripening also could favour the activity of a range of extracellular and intracellular proteinases as well as peptides from lactic acid starters and *P. camemberti* in hydrolysing peptides and β-caseins. The pH range (5.5-6.0) of the samples was more favourable for the activity of the cultures (Grappin et al., 1985). However, the migration of extra-cellular proteinase and peptidases of *P. camemberti* towards the

interior of the curd is limited (Spinnler and Gripon, 2004).

6.2.3 Texture changes in ripening cheeses

Rheological data summarised in Table 33 and Figure 48 show changes in deformation force values of cheese samples during ripening. The firmness of the samples was determined from the highest peak of the texture analyser graph as shown in Figure 11, Appendix 3.4. A higher deformation force indicated that the cheese sample was relatively firmer and a lower deformation force was associated with a softer sample. The cheese firmness profiles were similar in all samples, irrespective of the type of cultures used.

Table 33. Mean[1] firmness (N) of Camembert cheese samples (with rind) during ripening for 21 d.

Sampling time during ripening (d)	Acid-only cheese/O-type[2]			Aroma-enriched cheese/LD-type[3]		
	-18°C	4°C	20°C	-18°C	4°C	20°C
3	15.10±0.56^{ab}	14.23±1.32^{a}	13.75±1.67^{b}	11.16±0.96^{b}	10.67±0.68^{ab}	10.14±0.86^{ab}
10	16.20±1.78^{a}	16.32±1.72^{a}	17.13±0.81^{a}	12.85±1.77^{ab}	12.12±1.28^{a}	12.64±1.36^{a}
14	^{z}12.35±1.54^{b}	^{xy}10.25±1.27^{b}	^{x}9.89±0.61^{c}	^{z}12.24±0.81^{a}	^{y}9.74±1.34^{b}	^{x}9.12±0.66^{b}
21	6.28±0.32^{c}	5.62±0.60^{c}	5.19±0.94^{c}	^{z}5.45±1.17^{c}	^{xy}5.16±0.62^{c}	^{x}3.60±0.38^{d}

[1]Mean±SD, n=6 [2]Acid-only cheese/O-type: cheese samples produced using O-type LAB starters (stored at -18, 4, 20°C) consisted of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (R704, Chr. Hansen) and *P. camemberti* (PCTT033, Chr. Hansen) as ripening culture; [3]Aroma-enriched' cheese/LD-type: cheese samples produced using LD-type LAB starters (stored at -18, 4, 20°C) consisted of *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuc. lactis* and *Leuc. mesenteriodes* subsp. *cremoris* (Flora Danica, Chr. Hansen) and *P. camemberti* as ripening cultures (PCTT033, Chr. Hansen);
Within rows, mean values preceded by different superscripts are significantly different at $P<0.05$, with respect to one type of cheese. Within columns, mean values followed by different superscripts are significantly different at $P<0.05$.

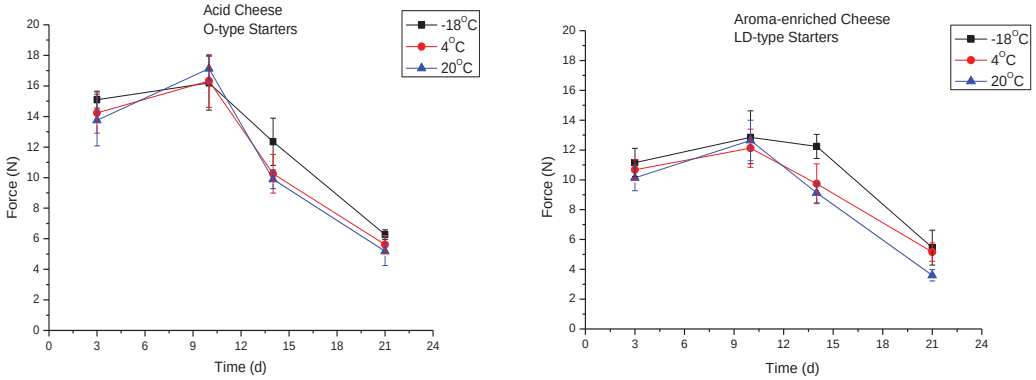


Figure 48. Changes in force (N) at 50% deformation (firmness) for Camembert cheese samples (with rind) during ripening for 21 d. Each point represents the mean of gel firmness of 6 independent analyses (n=6 for each treatment); Error bars are ±SD.

Throughout ripening (Table 33; Figure 48), ripening time was the main factor that affected ($P<0.05$) the firmness of cheese, suggesting that texture was closely linked to the maturity of cheese, which agrees with other studies (Sullivan et al., 2005; Guizani et al., 2007; Delgado et al., 2011). Consistently higher ($P<0.05$) softness was observed for LD-type cheeses than O-type samples. Cheeses containing 20°C-stored cultures exhibited higher softness than their alternative treatments, although the differences were not significant ($P=0.212$). This suggests that the storage temperature of the culture did not affect the texture of cheese.

At day 3, the texture of young cheese curd was rubbery at lower pH level ($pH<4.8$) (subjective inspection, data not shown). Cheeses containing LD-starters and 20°C cultures generally had higher softness than their alternative treatments (Table 33, Figure 48). These samples exhibited a slight higher acidity and it is well-known that higher moisture is retained in higher pH curds (Schlesser et al., 1992; Tzanetaki et al., 1993), therefore producing more soften curds. At day 10, the rubbery texture of young cheese curd was converted into a smoother, more homogenous product (subjective inspection, data not shown), although their texture did not soften. It is interesting to note that the firmness of all samples increased between day 3 and day 10 (Table 33, Figure 48). The observation was expected due to the dehydration of the cheese (the moisture loss occurred as water evaporates from cheese surface to the surrounding atmosphere) (Schlesser et al., 1992). Major textural changes in cheese were observed from day 10 (Figure 48). The cheeses became softer reaching a desirable level at day 21, when the samples were spreadable, attaining an almost liquid-like consistency in the inner portion (Figure 49a). Cheese ripening formed a gradient (Spinnler and Gripon, 2004). The softening of cheese first occurred in the outer part and then progressed towards the centre during ripening. Upon examination of the cross-section of the cheese, the external part appeared more

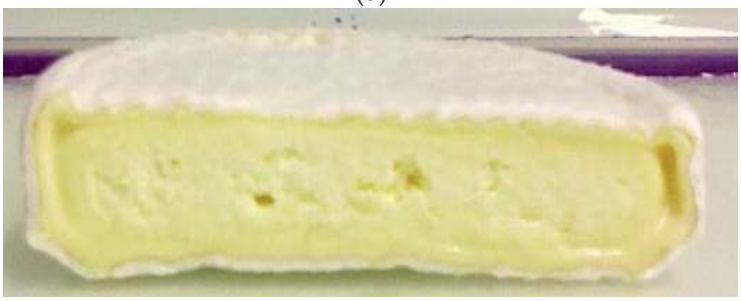
homogenous, smoother with greater softness than the interior. Texture development of cheese samples of this study generally followed a similar pattern that showed decrease in firmness during ripening which is characteristic of Camembert. The firmness of cheese samples at day 21 were also comparable to expected levels of firmness suggested by previous studies (Schlesser et al., 1992; Sousa and McSweeney, 2001; Sullivan et al., 2005; Saldo et al., 2000; Delgado et al., 2010; Delgado et al., 2011).

Correlations between proteolytic variables, the cultures and texture of cheese samples are shown in Appendix 4.2. Cheese firmness was shown to be positively correlated with the growth of LAB ($P<0.05$, $r^2=0.599$), but negatively with cell counts of mould ($P<0.05$, $r^2=-0.599$). Proteolytic parameters and the pH of samples were also showed high correlations with corresponding texture data. Cheese firmness was negatively correlated with ripening indices ($P<0.05$, $r^2=-0.732$ for ASN and $P<0.05$, $r^2=-0.736$ for NPN) and free amino acids ($P<0.05$, $r^2=-0.670$) of samples, as well as estimated values of peptide contents ($P<0.05$, $r^2=-0.683$). A negative correlation was also found between the pH and firmness value of samples ($P<0.05$, $r^2=-0.806$). Protein and casein content of samples were correlated with cheese firmness, but to a lesser extent ($P<0.05$, $r^2=0.475$ for protein, $P<0.05$, $r^2=0.446$ for casein).

At 21 d of ripening, samples of all treatments produced continuous, flowy inner body of cheeses (Figure 49a). Some LD-type cheese samples made by cultures stored at 20°C were characterised by very soft liquid-like texture and had lost their structural integrity (Figure 49 b). This observation suggests that the samples might have been over-ripened. Over-ripening of cheese is considered a defect (Law, 1997) as the product loses its characteristic attributes. Occurrence of unevenness of the core of the cheese (Figure 49c) was also observed in some O-type starter-containing samples cultures (-18^{o}C), suggesting that the level of proteolysis had not attained full potential within the centre of the cheese.

(a)

(b)



(c)

Figure 49. (a) Softening texture of Camembert cheese at 21 d of ripening. As cheese ages, the degraded portion of the cheese becomes apparent, and this was shown by the 'flowy texture' of the cheese paste in the outer portion and a core with a spreadable, almost liquid-like consistency; (b) LD-type cheese fermented with 20°C cultures showing very soft texture which is about to lose its structural integrity; (c) Core of O-type cheese (produced by -18°C cultures) with 'uneven' texture with soft texture in the outer portion, and a firm core. Images captured by a Nokia phone, N9 (Model Nokia, Finland) with 8 mega pixels camera.

At 21 d of ripening, higher degree of softness observed in LD-type cheeses and samples with 20°C cultures may be explained by their higher proteolytic activity, as shown by high ripening indices [ASN (as %TN) and NPN (as %TN)] and FAA, as well as estimated values of protein, casein and peptide contents. Correlations ($P<0.05$) between proteolytic variables and texture of the cheese samples were also observed. The result reveals that decrease in firmness of chees samples during ripening may be attributed to progressive proteolytic activity that takes place in the matrix of cheese (Adda, 1982; Law, 1997; Spinnler and Gripon, 2004). The speculation is consistent with studies on Camembert cheeses (Schlesser et al., 1992; Sousa and McSweeney, 2001; Sullivan et al., 2005; Guizani et al., 2007) and other cheese varieties (Wium et al., 1998; Saldo et al., 2000; O'Mahony et al., 2005; Delgado et al., 2010; Delgado et al.,

2011). As discussed earlier, proteolysis in Camembert was largely related to the amount of coagulant retained in the curd during draining and the activity of the cultures (LAB and *P. camemberti*) used.

In this study, negative relationship ($P<0.05$) was found between firmness of samples and pH. Cheese firmness was negatively correlated ($P<0.05$) with the growth of the mould. The results help to confirm that softening of cheese may be caused by the increase of pH due to the growth of *P. camemberti* as reported in other studies (Leclercq-Perlat et al., 2004a; Spinnler and Gripon, 2004; Guizani et al., 2007). Lactate assimilation and proteolytic activity of the mould create a pH gradient in cheese from surface to interior with lower pH values in the outer parts than the interior. At increased pH level, activity of proteases and peptidases from LAB, *P. camemberti* and plasmin are more activated, which contributes to a greater degree of proteolysis (Spinnler and Gripon, 2004).

In addition to cheese proteolysis and pH modification, migration of calcium to the crust plays a role in the softening of the texture (Lawrence et al., 1987). The mould establishes a pH gradient but, in addition, a mineral gradient is also set up. The calcium and phosphorus content of Camembert is initially uniform in the cheese, but as cheese ripens, a large part of the minerals migrate rapidly to the surface. A crust of mould and calcium phosphate forms on the surface since the latter is low solubility in high pH environment. According to Lawrence et al. (1987), about 75% of the calcium and 33% of the phosphate migrate from the centre of Camembert cheese to the surface in 17 d. Migration of calcium results in greater solubility of casein and contributes to softer texture (Spinnler and Gripon, 2004). This subject was outside the scope of the present study, future investigation would provide useful information to Camembert production.

At 21 d of ripening, samples of all treatments produced a continuous, flowy inner body of cheese. Some samples produced from LD-type starters and 20°C cultures were very soft with liquid-like texture, having lost their structure integrity. Meanwhile, core unevenness was observed in some samples produced from O-type starters containing -18°C cultures. Samples exhibited a very soft liquid-like texture, attributed to extensive proteolysis. The incidence of core unevenness may be attributed to lower proteolysis. As discussed earlier, the extent of proteolysis was largely related to the amount of rennet retained in the curd during draining and the activity of the cultures (LAB and *P. camemberti*) used. The storage temperature of the culture did not affect the texture of cheese, and both cheese types showed characteristic textural changes of Camembert. However, since the degree of softening in cheeses with LD-starter and 20°C cultures occurred rapidly compared to samples

with alternative treatments, LD-type starters may be preferred over O-type and 20°C may be preferred over -18°C and 4°C as culture storage temperature for Camembert production. This is because they promote faster proteolysis and higher degree of cheese softening; thereby effectively reduce cheese ripening time. However, the optimum ripening time and/or processing variables should be studied further to find ways of without encouraging too much structural degradation.

Moisture loss in cheese samples were also expected during ripening. During early ripening, loss of moisture content in curd was probably due to evaporation of the water at cheese surface (Schlesser et al., 1992). Whereas, further decrease in moisture content during ripening was probably attributed to proteolysis. Proteolysis causes the uptake of water due to the breakdown casein fractions which are water-soluble and cannot contribute to protein matrix. Formation of ionic groups ($-COO^-$ and $-NH_3^+$) compete for available water in the system; water previously available for solvation of protein chains become held together with new ionic groups (Adda, 1982; Lawrence et al., 1987). As discussed earlier, cheeses produced from LD-starters and 20°C cultures showed higher degree of proteolysis than their alternative treatments. With higher degree of proteolysis, the samples were expected to have lower moisture to casein ratio at the end of ripening while the texture was markedly softened.

6.2.4 Aroma changes in ripening cheeses

Quantitative analysis of aroma compounds in cheese samples

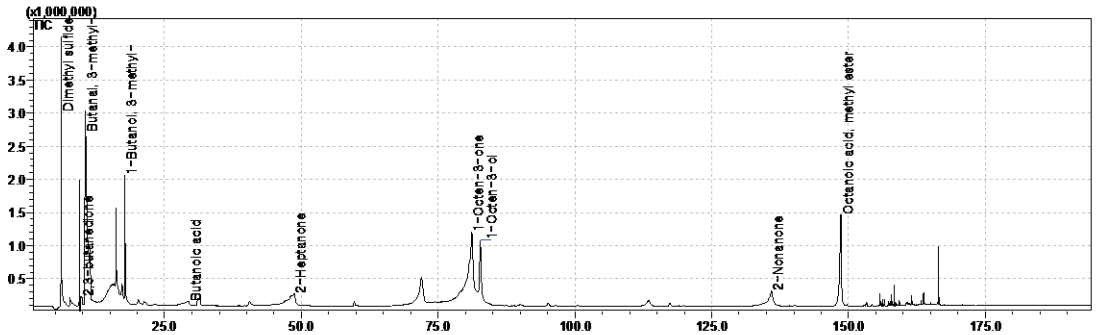
1. Method optimization and validation

In this study, key volatile aroma compounds of Camembert analysed were 3-methylbutanal, 3-methylbutanol, 2-heptanone, 2-nonanone and butyric acid. The compounds were extracted by SPME and separated by GC. Identification and quantitation of compounds were carried out by GC/MS analysis and confirmed by comparing retention times (RT) with respective pure standards. Mass spectra of all the compounds were compared with mass spectra of pure standards and standard mass spectra in the database supplied with the equipment by SHIMADZU.

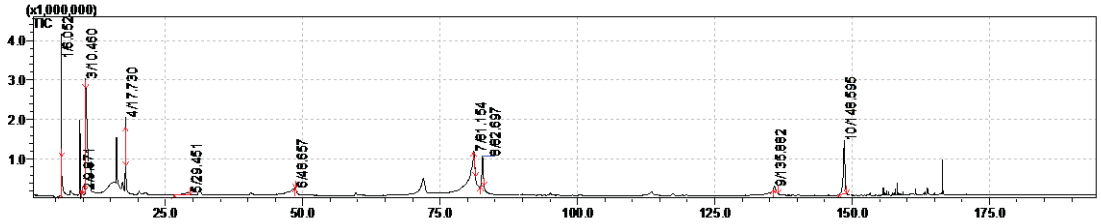
At the onset, the equipment was conditioned following GC/MS scientific instrument guide (SHIMADZU, 2010). Blank samples were analysed to determine any background noise by using air in the lab without adding samples. Sources of impurities or ghost peaks in GC chromatogram were chemical residues at the injection port or on the inlet septum, between the glass liner and the GC column. The peaks disappeared after replacing injection port septum, inlet glass liner and baking the GC column at 300°C. An example of chromatogram of blank sample is shown in

Figure 12, Appendix 3.5. CAR/PDMS and PA fibres were also compared for selective and sensitivity (data not shown). PDMS fibre can retain the most volatile component of the headspace in relatively high proportions. Based on its greater extraction efficiency, PDMS fibre was chosen to analyse volatile compounds of cheese samples, as suggested by other reports (Chin et al., 1996; Guillen et al., 2004). The repeatability of the compound's retention time (RT) was good, with individual CV% lower than 0.5% within-daily injections (3-samples), which ranged from 0.1-0.9% for injections on different days (Figure 21 and Table 43, Appendix 3.5). The peak area response repeatability was acceptable with CV% ranging from 0.5-33%, which was determined by three replicated cheese samples (Table 44, Appendix 3.5).

By using internal standard, peak area of compounds were normalised. Calibration curves of the compounds were then constructed using external standards (Figures 13-20, Appendix 3.5). The linearity of the curves was within the range of 0.0008–0.32 μg/ml, the correlation coefficients were >0.95%. Typical GC chromatograms of the standards obtained in the analysis are shown in Figure 50. Although 2,3-butanedione was present in the cheese samples in appreciable amounts, the compound could not be quantified. An unknown peak with RT of 7.84 min overlapped with 2,3-butanedione standard. Poor separation of the 2,3-butanedione can be caused by several factors including equipment settings (Monnet et al., 1994; Katoka et al., 2000; Pinho et al., 2001; Qian et al., 2010). In the study, temperature-programmed analysis and slow flow rate were used. The poor separation of the 2,3-butanedione may be caused by low separation efficiency of the column used.



(a)



(b)

Figure 50. Chromatogrammes of a mixed standard solution. The mixed standard solution contained: 1-octen-3-one: 0.0016 μg/ml; 2-heptanone, 1-octen-3-ol, 2-nonanone: 0.004 μg/ml; dimethyl sulphide, 3-methylbutanal, 3-methylbutanol: 0.005 μg/ml; butyric acid 0.01μg/ml. (a) Peaks of standard compounds; (b) Retention time of standard compounds.

2. Concentration of volatile aroma compounds during cheese ripening

Concentrations of 3-methylbutanal, 3-methylbutanol, 2-heptanone, 2-nonanone and butyric acid in cheese samples are presented in Figures 51-55. Volatile analysis of the aroma compounds (Figures 51-55; Table 34) showed that they had similar patterns during cheese ripening but with different concentrations. The volatile compounds increased ($P<0.05$) during cheese ripening in all samples.

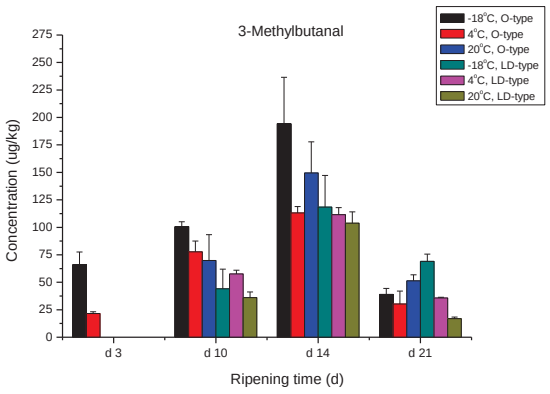


Figure 51. Concentration of 3-methylbutanal in cheese samples (without rind) during 21 d of ripening. Each data point represents mean±SD of two independent analyses (n=2). Error bars represent SD of the mean.

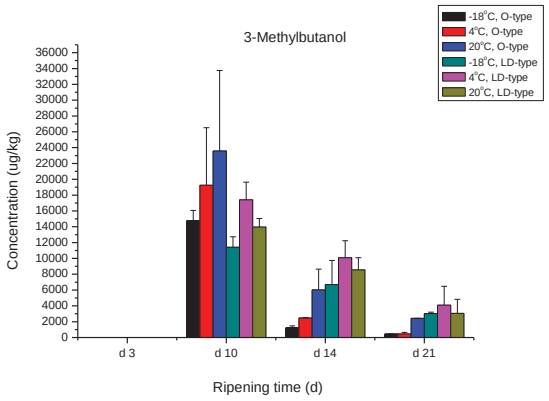


Figure 52. Concentrations of 3-methylbutanol in cheese samples (without rind) during 21 d of ripening. Each point represents mean±SD of two independent analyses (n=2). Error bars represent SD of the mean.

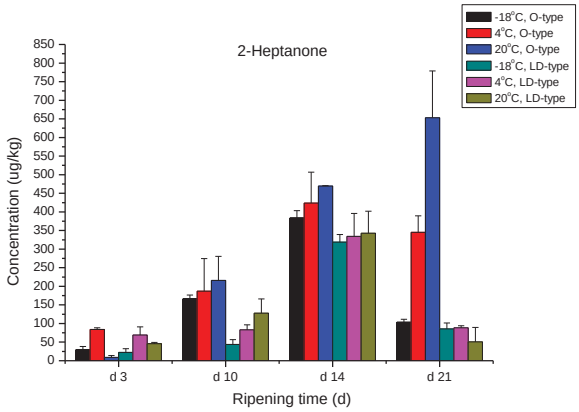


Figure 53. Concentration of 2-heptanone in cheese samples (without rind) during 21 d of ripening. Each data point represents mean±SD of two independent analyses (n=2). Error bars represent SD of the mean.

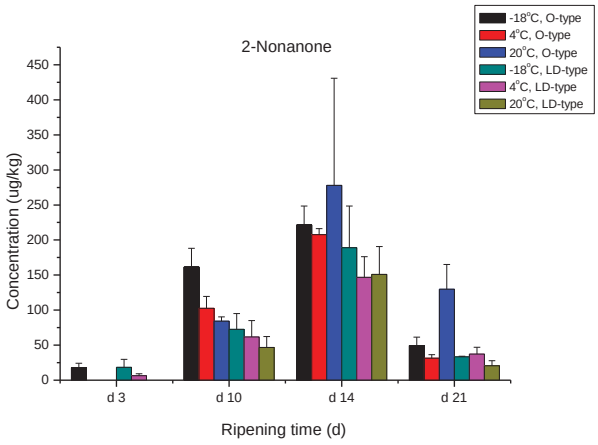


Figure 54. Concentrations of 2-nonanone in cheese samples (without rind) during 21 d of ripening. Each data point represents mean±SD of two independent analyses (n=2). Error bars represent SD of the mean.

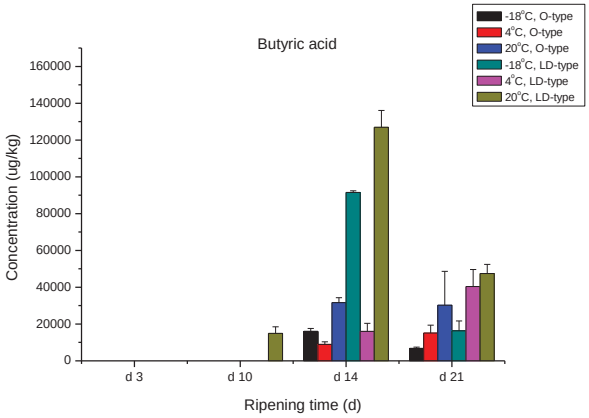


Figure 55. Concentrations of butyric acid in cheese samples (without rind) during 21 d of ripening. Each data point represents mean±SD of two independent analyses (n=2). Error bars represent SD of the mean.

Table 34. Concentration (μg/kg)[1] of volatile compounds in Camembert cheese samples (without rind) at different stages of ripening.

Culture storage temperature (°C)	Sampling time during ripening (d)	Acid-only cheese/O-type[2]			Aroma-enriched cheese/LD-type[3]		
Volatile compounds		-18	4	20	-18	4	20
3-Methylbutanal	3	^{y}66.05±11.54^{b}	^{x}21.51±1.67^{c}	^{x}nd^{b}	nd^{b}	nd^{d}	nd^{c}
	10	100.75±4.33^{b}	77.74±9.73^{b}	69.80±23.45^{b}	44.14±17.80^{b}	57.59±3.44^{b}	36.11±5.12^{b}
	14	194.46±42.06^{a}	113.18±5.80^{a}	149.44±28.31^{a}	118.43±28.80^{a}	111.50±6.45^{a}	103.73±10.29^{a}
	21	38.88±5.52^{b}	30.33±11.62^{c}	51.30±5.51^{b}	^{z}69.09±6.61^{ab}	^{y}35.70±0.62^{c}	^{x}16.88±1.50^{bc}
3-Methylbutanol	3	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{c}	nd^{c}
	10	14735.±1337.45^{a}	19256.68±7260.80^{a}	23572.70±10170.31^{a}	11414.2±1310.48^{a}	17412.85±2230.45^{a}	13954.39±1085.52^{a}
	14	1210.45±260.79^{b}	2476.48±27.55^{b}	6028.61±2615.35^{ab}	6696.09±3044.61^{ab}	10078.86±2149.37^{ab}	8556.45±1526.89^{b}
	21	^{x}422.30±38.81^{b}	^{x}454.36±200.28^{b}	^{y}2423.12±2.36^{ab}	3021.64±186.07^{b}	4091.32±2392.32^{bc}	3053.96±1770.46^{c}
2-Heptanone	3	^{x}29.22±8.94^{d}	^{y}83.90±4.85^{b}	^{x}9.08±4.64^{c}	22.33±10.05^{c}	69.15±21.90^{b}	45.61±3.57^{b}
	10	166.15±10.25^{b}	186.78±87.80^{ab}	215.71±64.88^{bc}	43.97±12.70^{bc}	83.10±13.15^{b}	127.75±38.61^{b}
	14	383.49±20.11^{a}	423.71±83.08^{a}	469.46±1.26^{ab}	318.96±20.21^{a}	334.38±61.56^{a}	342.44±59.41^{a}
	21	^{x}103.26±8.39^{c}	^{xy}345.04±44.35^{ab}	^{y}652.81±126.21^{a}	85.75±15.88^{b}	88.51±5.49^{b}	50.75±28.73^{b}
2-Nonanone	3	^{y}17.86±6.24^{b}	^{x}nd^{c}	^{x}nd	18.41±11.42^{b}	6.32±2.76^{b}	nd^{b}
	10	^{y}161.61±26.50^{a}	^{xy}102.65±16.78^{b}	^{x}84.14±6.20	72.44±22.76^{ab}	61.73±23.78^{b}	46.80±15.59^{b}
	14	221.59±26.88^{a}	207.72±8.55^{a}	277.81±152.86	188.88±59.59^{a}	146.71±29.44^{a}	150.81±39.78^{a}
	21	^{xy}49.48±11.81^{b}	^{x}31.48±4.95^{c}	^{y}129.88±35.35	33.48±6.74^{b}	37.31±9.73^{b}	20.50±7.27^{b}
Butyric acid	3	nd^{c}	nd^{b}	nd^{b}	nd^{c}	nd^{b}	nd^{c}
	10	nd^{c}	nd^{b}	nd^{b}	^{x}nd^{c}	^{x}nd^{b}	^{y}14897.05±3575.20^{b}
	14	^{x}16063.35±1529.25^{a}	^{x}8928.20±1394.70^{ab}	^{y}31662.87±2631.66^{a}	91549.64±816.60^{a}	16062.45±4354.46^{a}	126969.74±9124.54^{a}
	21	6728.50±762.72^{b}	15146..40±4226.90^{a}	30224.53±18466.76^{a}	^{x}16275.29±5413.59^{b}	^{xy}40412.76±9253.16^{a}	^{y}47409.13±4966.54^{b}

[1]Mean±SD of two production trials (n=2). [2]Acid-only cheese/O-type: cheese samples produced using O-type LAB consisting of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* as starter cultures (R704, Chr. Hansen) and *P. camemberti* (PCTT033, Chr. Hansen) as ripening culture; [3]Aroma-enriched' cheese/LD-type: cheese samples produced using LD-type lactic acid cultures consisting of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; *Leuc. lactis*, *Leuc. mesenteriodes* subsp.

cremoris and *L. lactis* subsp. *lactis* biovar. *diacetylactis* as starters (Flora Danica, Chr. Hansen) and *P. camemberti* as ripening cultures (PCTT033, Chr. Hansen); nd: not detected.
Within rows, mean values preceded by different letters are significantly different at $P<0.05$, with respect to one type of cheese. Within columns, mean values followed by different letters are significantly different at $P<0.05$. Tukey test was used to separate significantly different mean values.

3-methylbutanal increased ($P<0.05$) during ripening in all cheese samples, with lowest concentration at 3 d (no detection–66.05 μg/kg) and highest at 14 d (103.73-194.46 μg/kg). At day 21, the compound had decreased to between 16.88-69.09 μg/kg (Table 34, Figure 51). The development pattern of 3-methylbutanal in Camembert cheese during ripening was similar to results reported by other researchers (Sable and Cottenceau, 1999; Leclercq-Perlat et al., 2004b; Bachmann et al., 2009). Leclercq-Perlat et al. (2004b), detected 3-methylbutanal during the entire ripening period of Camembert, with maximum concentrations at 15 d and then decreasing. The decrease of 3-methylbutanal after day 14 may be explained by the transitory properties of aldehydes. The compound can be reduced rapidly to primary alcohols or oxidised to their corresponding acids (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Curioni and Bosset, 2002; Garcia, 1996).

Concentrations of 3-methylbutanol during ripening increased ($P<0.05$) at variable rates in all samples (Figure 52). The alcohol peaked at about 10 d (11414.2-23572.7 μg/kg) and only trace amounts were present at d 3 (Table 34). 3-methylbutanol was synthesised from its corresponding aldehyde (3-methylbutanal) (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Curioni and Bosset, 2002). After day 10, the concentrations of 3-methylbutanol decreased upto days 21, probably due to the degradation of the compound to other metabolites such as 3-methylbutanoic acid (Molimard and Spinnler, 1996). The concentration pattern of 3-methylbutanol obtained in the study agrees with other previous studies (Sable and Cottenceau, 1999; Vitova et al., 2007; Hayaloglu et al., 2008; Hayaloglu, 2009).

During ripening, steady increase ($P<0.05$) in concentrations of ketones were observed in all samples and the pattern of change was similar (Figures 53, 54). The two compounds had lower concentrations at 3 d of cheese ripening and highest at day 14 (Table 34). At day 21, compound concentrations were decreased to between 50.75-652.81 μg/kg for heptanone; and 20.50-129.88 μg/kg for nonanone (Table 34). Steady increase of 2-heptanone and 2-nonanone concentration during cheese ripening has been widely reported (Dumont et al., 1974; Garcia, 1996; Sable and Cottenceau, 1999; Vitova et al., 2007). Sable and Cottenceau (1999) detected 2-heptanone and 2-nonanone in first week of young Camembert (1-5% of total methyl ketones) and their concentrations increased to 20-40% in ripe cheese. However, the compounds were absent in fully matured cheeses at days 45. Dumont et al. (1974) also reported steady increases of 2-nonanone and 2-heptanone levels during ripening of Camembert, with decreases observed after 30 d. The decrease in 2-heptanone and 2-nonanone concentration after day 14 were probably due to the

reduction of the ketones to corresponding secondary alcohols (2-heptanol and 2-nonanol) (Table 36) by reductase activity of *P. camemberti* (Molimard and Spinnler, 1996; Curioni and Bosset, 2002). The detection of heptan-2-ol and nonan-2-ol in cheese samples were expected (Table 36) as they are principle secondary alcohols in Camembert (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999).

During ripening, butyric acid in all samples increased ($P<0.05$), and the pattern of change in concentration was similar (Figure 55). Butyric acid is mainly formed through lipolysis of Camembert by *P. camemberti*, although the compound can also be derived from oxidation of ketones, esters and aldehydes (Molimard and Spinnler, 1996; Curioni and Bosset, 2002). Butyric acid was present in trace amounts during early Camembert ripening but reached maximum (8928.20-126969.74 μg/kg) at about 14 d. Significant decrease in butyric acid was subsequently observed at day 21 (6728.50-47409.13 μg/kg) (Table 34). Qualitative analysis of samples at day 21 showed the presence of the ethyl ester butanoic acid in cheese (Table 36). The detection of the ester suggest the esterification between butyric acid and ethanol (Molimard and Spinnler, 1996). The development and concentration of butyric acid in Camembert during ripening agrees with other reports (Garcia, 1996; Vitova et al., 2007).

SPME/GC-MS analyses of Camembert cheese samples indicate the activities of LAB and *P. camemberti* cultures in several metabolic pathways involved in the biosynthesis of volatile organic compounds (Yvon and Rijene, 2001; Spinnler and Gripon, 2004).

3-methylbutanal, a branch-chain aldehyde, is mainly originates from amino acid (leucine (Leu)) degradation due to LAB activity (Yvon and Rijene, 2001; Curioni and Bosset, 2002; Curtin and Mcsweeney, 2004). During cheese ripening, amino acids are liberated during proteolysis. The degradation of aromatic Leu can occur readily once the amino acids are present in free form. During oxidative deamination or transamination, Leu can be transformed to α-keto acids by aminotransferases, which can be either decarboxylated by decarboxylases or chemically degraded to aldehydes (Yvon and Rijene, 2001). Aminotransferases and decarboxylase have been identified from many LAB, including *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (Yvon and Rijene, 2001). Aldehydes in Camembert cheese can be also produced from β-oxidation of unsaturated fatty acids (Sable and Cottenceau, 1999). The synthesised 3-methylbutanal can then be reduced to 3-methylbutanal through *P. camemberti* activity (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999). Thus in the study, changes in concentrations of 3-methylbutanal and 3-methylbutanol profiles reflected the catabolism of Leu, formation of the aldehyde and its degradation to branched

alcohols. The increase of 3-methylbutanal concentration in all samples suggested the catabolism of Leu by LAB due to enhanced peptidolytic activity of LAB. 3-methylbutanal was detected since 3 d of ripening, while 3-methylbutanol was observed in 10-21 d old samples (Figures 51, 52). This suggests that 3-methylbutanal was synthesised before the alcohol; it is reasonable to presume that 3-methylbutanal was rapidly reduced to alcohol by enzymatic activity of *P. camemberti*. At days 14 and 21, decreasing concentrations of 3-methylbutanal and 3-methylbutanol (Figures 51, 52) with subsequent detection of 3-methybutanic acid (Table 36) may indicate the degradation of the aldehyde and alcohol as substrates leading to synthesis of the corresponding acid (Sable and Cottenceau, 1999).

As discussed previously, formation of butyric acid, 2-heptanone and 2-nonanone in samples during ripening is linked to lipolysis (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Curioni and Bosset, 2002). Butyric acids are short-chain FFAs produced through lipolysis. In cheese, FFAs are produced during lipolysis of milk fat, which is mainly attributed to the action of microbial lipases, particularly those of *P. camemberti*, and to a lesser extent, the action of milk lipases (Curioni and Bosset, 2002). LAB present in starter cultures are generally weakly lipolytic (Collins et al., 2004). In Camembert cheese, production of saturated methyl ketones is very significant through β-oxidation of FFA. FFA are oxidised to β-keto-acids, which are then rapidly de-carboxylated to corresponding methyl ketones (Urbach, 1997; Fox et al., 2000; Collins et al., 2004; Spinnler and Gripon, 2004; Singh and Cadwallader, 2008). In Camembert cheese metabolites, there is positive correlation between FFAs level and the amount of methyl ketones formed (Wouters et al., 2002; Spinnler and Gripon, 2004). The synthesis of methyl ketones in Camembert cheeses is associated with enzymatic lipase activity of secondary micro-flora, due to activity of *P. camemberti* and *G. candidum*, and perhaps yeasts (Karahadian et al., 1985; Spinnler and Gripon, 2004). In this study, high concentrations of 2-heptanone, 2-nonanone and butyric acid in cheese samples during ripening suggest lipolytic activity in all samples. The presence of methyl ketones further suggests the connection of metabolic pathways to β-oxidation. Since these compounds were produced in sufficiently high concentrations, our results also demonstrated the strong activity of *P. camembert* involved in the pathway. According to Leclercq-Perlat et al. (2004b), *Penicillium* spores metabolise free fatty acids to produce methylketones even though its mycelium seems to be inhibited by the FFAs.

During manufacture and early ripening of cheese, butan-2,3-dione can be produced through citrate metabolism by citrate-fermenting strains of *L. lactis* subsp. *lactis*

biovar. *diacetylactis* and *Leuc.* spp. (*Leuc. lactis* and *Leuc. mesenteroides* subsp. *cremoris*) (Hugenholtz, 1993; Mcsweeney and Fox, 2004). Although 2,3-butanedione was not quantified in this study, the ketone was however detected at 3-10 d in unripe 'LD-type' cheese samples. As expected, 2,3-butanedione was not identified in ripened samples, but acetoin (3-hydroxybutan-2-one) and butane-2,3-diol were found in some samples at days 14 and 21 (Table 36). The presence of butan-2,3-dione in samples supports the culture composition analysis (Section 4.4), which revealed an active role of *Leuc.* spp. and *L. lactis* subsp. *lactis* biovar. *diacetylactis* in metabolizing citrate. The results also show the reduction of butan-2,3-dione to acetoin and 2,3-butanediol by citrate-fermenting LAB starters (Mcsweeney and Fox, 2004). 2-butanol and 2-butanone were occasionally observed in some cheese samples (17%) (data not shown). These two compounds can be produced from 2,3-butanediol by starter LAB (Curioni and Bosset, 2002; Mcsweeney and Fox, 2004).

Table 35. Concentration (μg/Kg)[1] of volatile compounds identified in Camembert cheese samples (without rind) after 21 d of ripening and their corresponding flavour notes and perception thresholds levels.

Compound	**Flavor notes[2,3]**	**Concentration in cheese (μg/Kg)**	**Concentration[4,5,6,7] in Camembert cheese of previous studies (μg/Kg)**	**Perception threshold (μg/Kg)[2,3]**
3-methylbutanal	Green, malty	16.88-69.09	54-142[4]	13
3-methylbutanol	Fruity, alcohol	422.30-4091.32	400[5] 4000[6]	0.300-4750
Heptan-2-one	Blue cheese, spicy, Roquefort cheese, musty	50.75-652.81	5580[4] 6800-17400[7]	700-1500
Nonan-2-one	Fruity, musty, floral	20.50-129.88	20000-48000[4] 19600-47800[7]	1700-7700
Butyric acid	Rancid, cheesy, putrid, sweaty	6728.50-47409.13	35000-206000[4]	300-7000

[1]Mean±SD of two production trials (n=2); [2,3]Reference: (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Yvon and Rijene, 2001; Sousa et al., 2001; Smit et al., 2005; Vitova et al., 2006; Singh and Cadwallader, 2008). [4](Sable and Cottenceau, 1999); [5](Leclercq-Perlat et al., 2004b); [6](Vitova et al., 2007); [7](Molimard and Spinnler, 1996).

At day 21, concentrations of 3-methylbutanal, 3-methylbutanol and butyric acid in samples were generally comparable to Camembert reported in studies (Table 35) (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Leclercq-Perlat et al., 2004b; Vitova et al., 2007). However, notably lower concentrations of 2-heptanone and 2-nonanone were found in cheese samples (Table 35) (Molimard and Spinnler,

1996; Sable and Cottenceau, 1999; Leclercq-Perlat et al., 2004b; Vitova et al., 2007). This probably suggests that the compounds were quickly converted to their corresponding secondary alcohols, acids and esters by activity of *P. camemberti* (Molimard and Spinnler, 1996; Curioni and Bosset, 2002). The relatively lower 2-heptanone and 2-nonanone contents observed in the cheeses may also attributed to cheese samples (body, without rind) collected for analysis. As mentioned earlier, formation of 2-heptanone and 2-nonanone in cheese during ripening is linked to lipolysis (Molimard and Spinnler, 1996). Due to a preferential lipolysis by *P. camemberti* at cheese surface than at the centre of cheese, lower concentrations of 2-heptanone and 2-nonanone may be observed in the cheese body. The diffusion of lipase of *P. camemberti* and synthesised methyl ketones from cheese surface into the interior of cheese can occur (Leclercq-Perlat et al., 2004b), although the amount is limited (Karahadian et al., 1985; Sable and Cottenceau, 1999). The difference in volatile concentration of samples may also be reflected by differences in the technology of cheese production. Therefore, cheeses produced in this study may be different to cheeses from other studies as regards to microbial properties and chemical composition. The discrepancies between our results and other studies may also originate from the use of different extraction techniques and analysis of volatile cheese compounds (Chin et al., 1996; Guillen et al., 2004). Although several methods for the determination of milk volatile compounds are available, the problem of accurate measurements still remains, particularly low molecular weight compounds (Chin et al., 1996).

1-octen-3-ol and 1-octen-3-one are produced by polyunsaturared fatty acids of cheese, particularly linoleic and linolenic acids, via intra-chain oxidation. The principle enzymes involved are lipoxygenases and hydroperoxide-lyases, active in *P. camemberti*. In this study, 1-octen-3-one and 1-octen-3-ol were not detected throughout all stages of ripening, even at day 21. The result was unexpected as these compounds are abundant in Camembert and their mushroom note contributes to authenticity of the cheese (Spinnler and Gripon, 2004). The absence of the two ketones may be attributed to the type of samples (body of cheese without rind) used for analysis. As mentioned earlier, lipolysis by *P. camemberti* in cheese centre is notably less than it at surface. Lower concentrations of 1-octen-3-ol and 1-octen-3-one may be therefore observed in cheese body. According to Molimard and Spinnler (1996), healthy mould mats of Camembert provided a burst of mushroom-like flavours that showed two-fold increase of 1-octen-3-ol and 1-octen-3-one than that of a similar analysis for the body of cheese. Although these compounds can migrate into the body of the cheese during ripening, their concentration from a cheese body

was substantially different from the cheese surface (Leclercq-Perlat et al., 2004b).

Dimethyl sulphide was also not found in samples at all stages of ripening. The observation was unexpected as the compound is recognized as key volatile of Camembert contributing garlic flavour note (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999). Dimethyl sulphide generally develops from the degradation of Met. Methanethiol, produced by the oxidation of Met, is very reactive and is a precursor of many sulphur-containing compounds, including dimethyl sulphide. Dimethyl sulphide is produced enzymatically through elimination reaction pathway by cleavage of the carbon-sulphur bond under the action of demethanethiol through metabolism of coryneform bacteria, especially *B. linens* (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Singh et al., 2003). Absence of dimethyl sulphide suggests that the occurrence of methionine degradation did not take place, probably due to the absence of *B. linens* and coryneform spp. activity in the cheeses. Coryneform bacteria were not added to cheese milk as starter cultures during manufacture. The microorganisms may be present on the surface of cheese as the 'wild flora', but probably do not grow abundantly. The cheeses used in this study were prepared in a very clean environment in the Product Development Laboratory at Massey University, Albany Campus. The failure in detecting sulphur-containing compounds in cheese samples may be also explained by the type of sample (body of cheese without rind) used for analysis.

Of the quantified volatile compounds, 3-methylbutanol and butyric acid were present in relatively high quantities in cheese samples at 21 d of ripening (Table 35). Comparing to published threshold values (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Leclercq-Perlat et al., 2004b; Vitova et al., 2007), concentrations of 3-methylbutanol and butyric acid obtained in our study were higher, while concentrations of 3-methylbutanal were almost similar to their odour threshold levels. These compounds have a larger impact on the cheese flavour. The concentration of 3-methylbutanal obtained in this study provides unique enhancing contribution to the 'green, malty' flavour of cheese, while high concentration of 3-methylbutanol gives a strong alcoholic, floral note (Sable and Cottenceau, 1999). The high concentration of butyric acid, which was well-above its perception threshold, could contribute to a significant flavour of rancidity, cheesy, putrid, and sweaty. However, elevated pH (6.10-6.61) of well-ripened cheese at day 21 can markedly suppress the flavour (Molimard and Spinnler, 1996).

As 2-heptanone and 2-nonanone were found in concentrations below the odour

threshold levels (Table 35), the compounds are not expected to play a major role in cheese flavour. However, the two ketones can contribute to the overall cheese flavour, but without giving the cheese a detectable 'blue cheese and musty' note. Their metabolic products, 2-heptanol and 2-nonanol, were detected in 14% of samples after 21 d of ripening (Table 36). 2-heptanol and 2-nonanol are characterized as having 'earth, oily and sweetish' and 'fatty, melon and mild green' flavour notes, respectively. The synthesised secondary alcohols have similar but heavier flavour notes than their corresponding methyl ketones.

3. Comparison of aroma compounds between cheeses

Aroma compounds (3-methylbutanal, 3-methylbutanol, 2-heptanone, 2-nonanone and butyric acid) of cheese samples were compared between the cheeses.

The amount of 3-methylbutanal varied ($P<0.05$) between two different types of cheese, which reflect the differences in type of starter cultures used. The effect of culture storage temperature on the formation of 3-methylbutanal in cheeses was also significant ($P<0.05$). As previously discussed, the concentration of 3-methylbutanal in cheese increased with ripening time ($P<0.05$). The amount of 3-methylbutanol in cheese was not influenced by the type starter culture used ($P=0.938$) and culture storage temperature ($P=0.365$), but only affected by ripening time ($P<0.05$). Comparing the maximum concentration of 3-methylbutanal at day 14, samples containing -18°C cultures and cheeses with O-type starters were notably higher than other treatments (Figure 51). At day 10 when 3-methylbutanol had reached maximum level, higher content were found in cheeses with O-type than LD-type starters. However, at end of cheese ripening, relatively lower concentration of 3-methylbutanol was observed in O-type samples.

Relatively higher concentrations of 3-methylbutanal and its corresponding 3-methylbutanol were found in O-type cheeses than LD-type cheeses. The discrepancy may be attributed to different type of starter cultures used. LAB of O-type starters may exhibit higher activity in degrading Leu to 3-methylbutanal than LD-type starters (Yvon and Rijene, 2001). Higher growth of LAB in cheeses produced with O-type starters at early ripening time (Section 6.2.1) may be also responsible for differences in the amount of 3-methylbutanal. As 3-methylbutanal can be reduced to 3-methylbutanol by enzymes of *P. camemberti*, higher amount of 3-methylbutanol found in O-type samples may be correlated with the high 3-methylbutanal concentration in cheese. Lower concentration of 3-methylbutanol at day 21 suggested

the degradation of the compound to corresponding metabolites. It seems that 3-methylbutanol in O-type cheeses degrade faster than in LD-type cheeses.

Although 3-methylbutanol content in samples produced with cultures stored at -18, 4°C and 20°C were similar ($P>0.05$), cheeses produced with 4 and 20°C cultures generally had higher concentration than cheeses with -18°C cultures. Since 3-methylbutanol is synthesised from 3-methylbutanal by enzymes of *P. camemberti* (Karahadian et al., 1985), the high growth of the mould during ripening (Section 6.2.1) may responsible for high concentration of 3-methylbutnol in cheese.

The relatively lower production of 3-methylbutanal in cheese samples containing 4 and 20°C cultures was probably attributed to the reduced activity of LAB starters after storage at higher temperature for prolonged time. Less active aminotransferases from lactococci release lower amounts of α-keto acids from Leu thereby producing reduced concentration of 3-methylbutanal. Mariaca and Bosset (1997) suggested that good flavour producing starters lyse more rapidly than those which do not produce intense flavour. During ripening, viability of LAB in cheeses with -18°C cultures decreased faster than samples containing 4 and 20°C (Section 6.2.1). Enzymes which were released by lysis from -18°C cheeses may metabolise Leu faster leading to higher production of 3-methylbutanal than samples with 4 and 20°C cultures containing more slowly lysing bacteria.

Significant differences were observed in concentrations of 2-heptanone, 2-nonanone and butyric acid between samples containing different starters ($P<0.05$). Higher 2-heptanone and 2-nonanone levels were generally found in cheeses with O-type starters, while relatively higher butyric acid concentrations were present in LD-type cheeses. The differences in levels of the compounds observed in the cheeses were not expected and there is no easy explanation for the outcome. Formation of the two ketones and butyric acids is linked to lipolysis of cheese and β-oxidation of fatty acid, which are enzymatically derived from *P. camemberti* (Spinnler and Gripon, 2004). Starter cultures have however minor role in producing these compounds. Perhaps the strains of LAB used in the starter cultures of have special traits with higher ability to metabolise fats in cheese, although this aspect is not been published before.

Marginal differences in 2-heptanone content were also detected between samples produced with cultures stored at different temperatures ($P=0.045$). Since enzyme systems of *P. camemberti* have a great effect on the biosynthesis of methyl ketones, the slightly higher concentration of 2-heptanone in samples with 20°C cultures was

probably due to higher growth of the mould in cheese (Section 6.2.1). Higher viability of *P. camemberti* on cheese surface can release increased amount of lipase to produce FFAs from triglycerides which are then synthesised into methyl ketones in cheese (Spinnler and Gripon, 2004). In cheese samples containing 20°C cultures, higher concentrations of 2-nonanone and butyric acid were also observed, although the content of compounds were not affected by storage temperature of cultures ($P= 0.331$ and $P=0.130$, respectively).

The results reveal that culture storage temperature generally did not affect production of volatile compounds in cheese (with the exception of 3-methylbutanal). As discussed earlier, these compounds are mainly synthesised through *P. camemberti* activity. This therefore suggests that the ability of the mould to reduced aldehyde and producing FFAs through lipolysis, as well as production of methyl ketones via β-oxidation pathway was not affected (Spinnler and Gripon, 2004). The finding was however unexpected as the growth of *P. camemberti* during ripening was largely ($P<0.05$) influenced by temperature storage of culture (Section 6.2.1), therefore, production of volatile compounds between cheeses fermented with cultures stored at different temperatures thus may be different. The result may be attributed to the type of cheese sample analysed (body without rind), where there was less contact with enzymes of *P. camemberti*. Although these compounds can diffuse into the cheese body from cheese surface during ripening, the amount is rather limited (Karahadian et al., 1985). In future studies, two parts of cheese, which are the body and the surface should be both analysed and the results compared.

PCA was performed on GC/MS data using concentrations of 3-methylbutanal, 3-methylbutanol, 2-heptanone, 2-nonanone and butyric acid as variables. The purpose of this analysis was to determine any relationships between the volatile compounds and to establish any correlation between cheese samples.

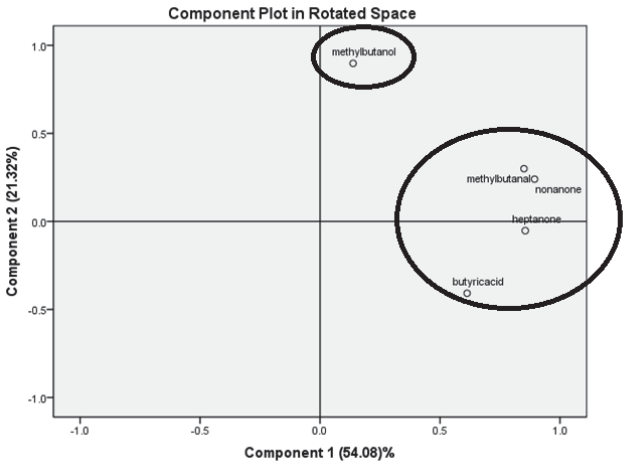


Figure 56. PCA score plot of volatile compounds in 21 d old Camembert samples.

PC1 and PC2 accounted for 54.08% and 21.32% of variance, respectively, as shown in Figure 56. The total variability explained by the 2 functions is 75.4%. In the score-plot, the distance between compounds can be interpreted as the degree of difference between compounds. The PCA analysis indicates that there is a tendency to group 3-methylbutanal, 2-nonanone, 2-heptanone and butyric acid, while 3-methylbutanol was distinguished from the other compounds. Figure 56 suggests that 3-methylbutanol was slightly influenced by PC1, the highest loading was attributed onto PC2. PC1 may separate compounds based on the effect of starter cultures in changing concentration between samples, while PC2 may separate compounds on the basis of the effect of culture storage temperature. ANOVA result reported earlier confirms our interpretation, the difference between 3-methylbutanol and other compounds was probably due to its concentration in samples which was not influenced by the type starter used and culture storage temperature. 3-Methylbutanal, 2-nonanone, 2-heptanone and butyric acid were grouped together may be because their concentration differed significantly ($P<0.05$) in cheeses containing different starters. Higher butyric acid was generally present in cheeses containing LD-type starters, whereas the concentrations of other compounds were higher in cheeses with O-type starters. This may contribute to the slight differences between butyric acid with other compounds in the same group.

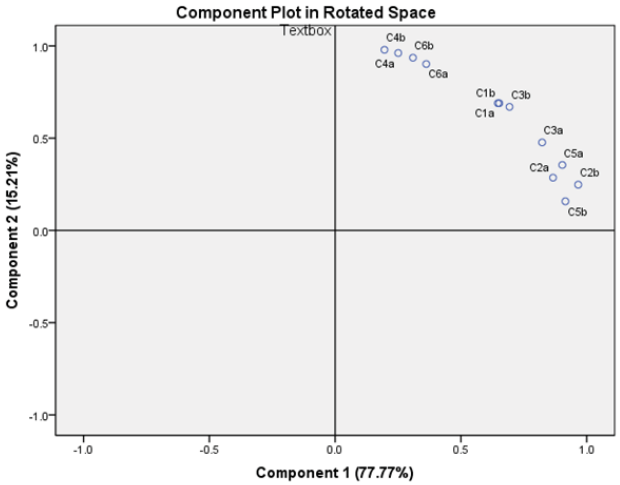


Figure 57. PCA score plot of cheese samples. C1: O-type cheese, -18°C culture; C2: O-type cheese, 4°C culture; C3: O-type cheese, 20°C culture; C4: LD-type cheese, -18°C culture; C5: LD-type cheese, 4°C culture; C6: LD-type cheese, 20°C culture; Two scores from each sample indicate duplicate analysis of each cheese.

Score-plot of cheese samples are presented in Figure 57. Samples were similar as they all located on the positive sides of PC1 and PC2, and no obvious trend was observed between samples with respect to effect of starters used and culture storage temperature. It may be therefore concluded that all the investigated cheese samples had similar aroma levels. The plot (Figure 57) also shows that deviations between duplicate cheese samples were generally small.

Qualitative analysis (identification) of aroma compounds in cheese and potential flavor notes of samples

To further understand flavour profile of cheese samples, levels of volatile compounds were determined qualitatively at day 21 of ripening. Although quantitative analysis was not conducted, similar peaks were identified from chromatographs and grouped according to their chemical classes as listed in Table 36. A typical chromatogram of most significant aroma compounds identified in cheese is shown in Figure 58. A range of volatile compounds (aldehydes, ketones, alcohols, acids, esters) were identified in cheese samples (Table 36, Figure 58); they have been previously reported and recognized as typical to Camembert flavour compounds (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Curioni and Bosset, 2002).

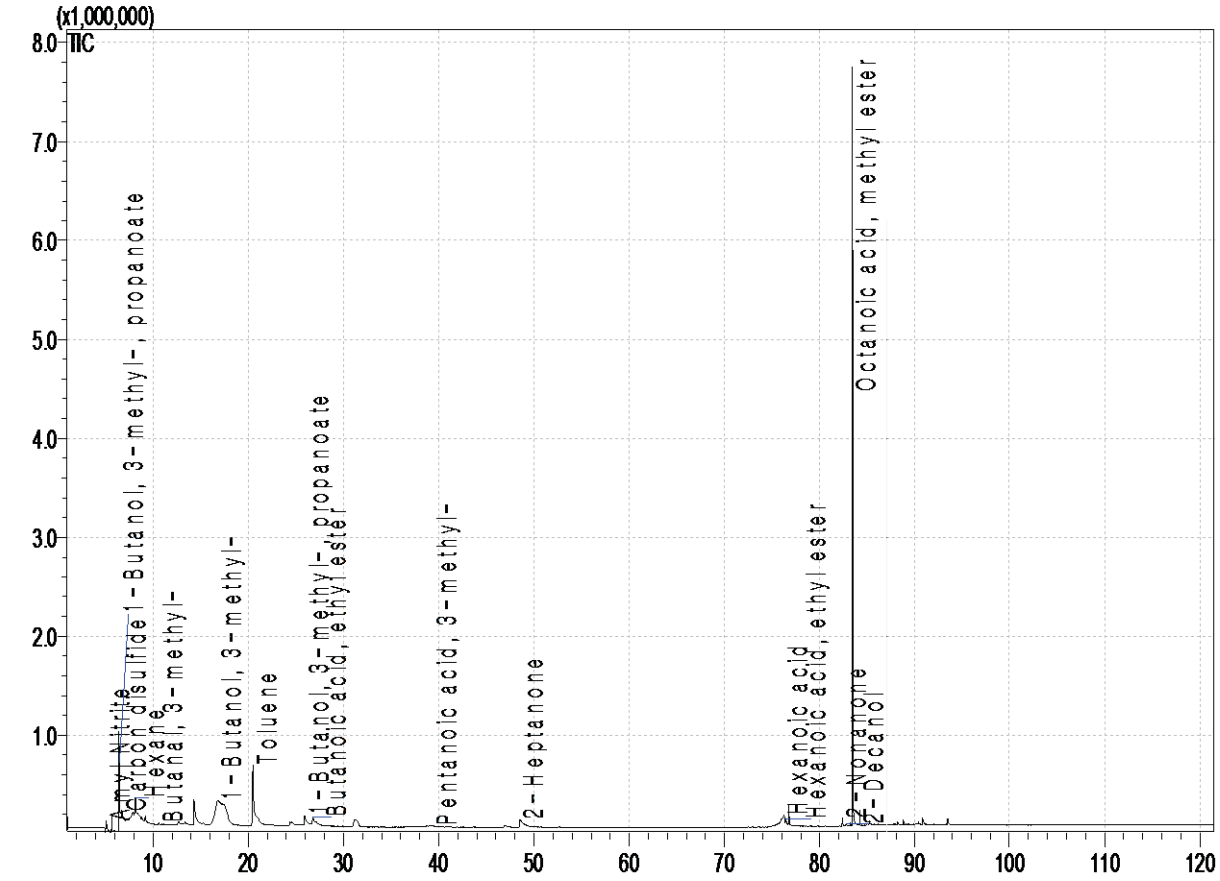


Figure 58. Chromatogrammes of an 'O-type' cheese sample (3.413 g) at day 21.

Table 36. Common volatile compounds in Camembert cheese samples (without rind) at 21 d of ripening.

Compounds		Retention time (min)	Flavor notes[1]	Identification methods[2,3,4]
Ketones	Acetone	5.813	Ethereal, powerful, fruity	RT
	Acetoin (3-hydroxybutanone)	7.678	Buttery	RT
	Heptan-2-one	48.194	Blue cheese, spicy, Roquefort cheese, musty	RT/MS
	Nonan-2-one	135.726	Fruity, musty, floral	RT/MS
Alcohols	1-Pentanol	17.227	Mild green, fusel oil	RT
	1-Heptanol	10.934	Fragrant, oily, heavy, woody	RT
	Heptan-2-ol	22.692	Earthy, oily, sweetish	RT
	Nonan-2-ol	19.643	Fatty, melon, mild green	RT
	2-methylbutan-1-ol	17.783	Fruity, alcohol	RT
	2-methylpropanol	19.335	Alcohol, penetrating	RT
	3-methylbutan-1-ol	17.332	Fruity, alcohol	RT/MS
	2,3-butanediol (diacetyl)	9.045	Limited contribution to cheese aroma	RT
Aldehydes	Hexanal	26.270	Green, grassy, penetrating, powerful	RT
	Nonanal	76.941	Floral, citrus, orange, rose, fatty, waxy	RT
	2-methyl propanal	11.050	Green, malty	RT
	2-methylbutanal	10.933	Green, malty	RT
	3-methylbutanal	10.386	Green, malty	RT/MS
Sulphur containing compounds	Carbon disulfide	6.803	Sulphury	RT
Fatty acids	Acetic acid	9.835	Vinegar, pungent	RT
	Butyric acid	28.353	Rancid, cheesy, putrid, sweaty	RT/MS
	Isobutyric acid	29.201	Sweet, mild, rotten apple	RT
	Pentanoic acid	6.725	Cheeseylike, sweaty, rancid, waxy	RT
	Hexanoic acid	8.133	Pungent, blue cheese, sour	RT
	2-methylpropanoic acid,	13.785	Sweet, applelike, rancid butter	RT

	3-methylbutanoic acid	11.245	Rotten fruit, mild, sweaty	RT
Ester	3-methyl-1-butanol propanoate	18.563	Apricot, pineapple	RT
	Hexanoic acid, ethyl ester	92.197	Pineapple, banana, apple, powerful	RT
	Butanoic acid, ethyl ester,	26.675	Pineapple, sweet, banana, fragrant	RT
Miscellaneous compounds	Isobutylamine	4.476	Fruity, alcoholic, varnish aromatic	RT
	Heptane	15.673	limited contribution to cheese aroma	RT
	Ammonia	----	Ammonia-like, pungent smell	Visual inspection and smell

[1]References: (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999). [2]RT: identification by retention time of standard compounds generated in the laboratory; [3]MS: identification by mass spectra obtained from GC-MS SHIMADZ database library.

Ketones

In this study, 2,3-butanedione (diacetyl), a diketone, was present only in 'un-ripened' cheese samples produced with LD-type starters. The presence of the compound was expected from citrate metabolism and its production is mainly due to the activity of citrate-fermenting LAB (*L. lactis* ssp. *lactis* biovar. *diacetylactis* and *Leuconostoc* spp.) (Hugenholtz, 1993; Mcsweeney and Fox, 2004). As expected at day 21, the diketone was not detected in all samples, but its metabolic by-products, acetoin (3-hydroxy-2-butanone) and 2,3-butanediol were identified in some cheese samples (Table 36). As mentioned earlier, 2,3-butandione is reduced to acetoin and 2,3-butanediol by citrate-fermenting LAB starters (Mcsweeney and Fox, 2004). 2,3-butanedione imparts strong sweet buttery and nutty vanilla aroma and acetoin with buttery aroma, whereas 2,3-butanediol does not have a flavour impact (Table 36). In 68% of ripened cheese samples, traces of acetone were also found at day 21 (Table 36). Acetone is produced via lactose and citrate metabolism (Mcsweeney and Fox, 2004); and may provide 'ethereal, powerful, fruity' flavour to cheese. As reported in other studies (Garcia, 1996; Curioni and Bosset, 2002; Vitova et al., 2007; Hayaloglu et al., 2008; Hayaloglu, 2009), 2-heptanone and 2-nonanone were the most important methyl ketones identified in cheese samples. The compounds may contribute 'blue cheese, spicy, musty' and 'fruity, musty and floral' flavour notes, respectively (Table 36).

Alcohols

Primary and secondary alcohols as well as branched-chain primary alcohols were identified in cheese samples (Table 36). Primary alcohols, 1-pentanol and 1-heptanol, were detected in about 9% cheese samples and they impact an alcoholic, winery, sweet fruity notes in cheese. Branched-chain primary alcohols containing methyl groups found in cheese samples were 2-methylbutanol, 2-methylpropanol and 3-methylbuanol. These compounds were probably generated from their corresponding aldehydes (2-methylbutanal and 3-methylbutanal) produced from respective aromatic branched amino acids (Ile, Val and Leu) (Yvon and Rijene, 2001). Formation of these compounds also indicate the active metabolism system of LAB in reducing aldehydes produced from catabolism of amino acids (Yvon and Rijene, 2001). The detected branched-chain primary alcohols usually impart a green, malty note to cheese flavour (Table 36).

Secondary alcohols, 2-heptanol and 2-nonanol, were also found in 37% of cheese samples at day 21 (Table 36). The presence of heptan-2-ol and nonan-2-ol in samples are expected as they are principle secondary alcohols in Camembert (Curioni and Bosset, 2002). These alcohols were probably produced enzymatically by *P. camemberti* from their corresponding methyl ketones (2-heptone and 2-nonanone) (Curioni and Bosset, 2002). In cheeses, 2-heptanol and 2-nonanol have similar but heavier flavour notes than their corresponding methyl ketones, and contributing 'earthy, oily, sweetish' and 'fatty, melon, mild green' flavours to cheese, respectively (Table 36).

Ethanol was detected in 'un-ripened' 'LD-type' cheese samples. Formation of ethanol probably resulted from lactose fermentation by heterofermentative LAB in starters via the pentose phosphate pathway. Ethanol can also come from acetaldehyde reduction by an alcohol dehydrogenase (Mcsweeney and Fox, 2004). Traces of ethanol were present in 21 d old ripened cheeses. The limited amount of ethanol in ripened cheeses was expected as the compound serves as a precursor for the formation of other compounds such as ethyl esters (Molimard and Spinnler, 1996). The presence of hexanoic acid and butyric acid in cheese samples supports the esterification reactions that occur between ethanol and short- to medium-chain FAs (Molimard and Spinnler, 1996). In addition, ethanol has a limited aromatic role in cheeses (Curioni and Bosset, 2002).

Alcohols were among the main volatile compounds in the prototype Camembert

cheeses and the compounds with the highest concentrations were 2-methylbutanol, 3-methylbutanol, 2-heptanol and 2-nonanol, which is consistent with other reports (Molimard and Spinnler, 1996).

Aldehydes

Major aldehydes detected in Camembert samples were hexanal, nonanal, 2-methyl propanal, 2-methylbutanal and 3-methylbutanal which are characterised by green, grassy, malty or herbaceous aroma (Table 36). Straight-chain aldehydes such as hexanal and nonanal were probably produced from β-oxidation of unsaturated FA or from amino acids by the Strecker degradation reaction (Curioni and Bosset, 2002). These are simple reactions and can occur without enzymatic catalysis during ripening. Branched-chain aldehydes such as 2-methylpropanal, 2-methylbutanal and 3-methylbutanal, probably originate from amino acid degradation via enzymatic as well as non-enzymatic of Strecker degradation process (Yvon and Rijene, 2001). According to Yvon and Rijene (2001), 2-methylpropanal, 2-methylbutanal and 3-methylbutanal are produced through catabolism of aromatic branched amino acids from Ile, Val and Leu, respectively. In tested samples, higher peak area of 3-methylbutanal was generally observed than 2-methylbutanal and 2-methylpropanal (data not shown). The observation is consistent with findings of Thage et al. (2005), which reported that 5-10 times more aroma compounds were formed from Leu than from Val and Ile. In the study of Thage et al. (2005), the dominance of aroma compounds from catabolism of Leu was explained by a much higher content of this amino acid in all the cheeses (2 or 5 times higher amounts than Val or Ile, respectively). The preference for Leu by transporter enzymes of lactococci was also suspected.

Fatty acids

A range of fatty acids were also identified in cheeses tested, namely acetic acid, butyric acid, hexanoic acid, isobutyric acid, pentanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid (Table 36). Butyric acid, hexanoic acid, isobutyric acid and pentanoic acid may originate from lipolysis, while acetic acid was probably degraded from lactose and amino acids (Molimard and Spinnler, 1996; Curioni and Bosset, 2002). 2-methylpropanoic acid and 3-methylbutanoic acid are derived from ketones, esters and aldehydes by oxidation (Molimard and Spinnler, 1996). At day 21, these fatty acids can contribute 'rancid, cheesy, acid, pungent, sweaty' flavours to cheeses (Table 36), although elevated pH (6.10-6.61) of cheeses can significantly suppress the

flavours (Sable and Cottenceau, 1999). According to several reports (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Curioni and Bosset, 2002), the detected fatty acids do not only serve as aromatic compounds, but they are also precursors of methyl ketones, alcohols, esters and lactones.

Esters

Esters are common cheese volatiles (Molimard and Spinnler, 1996). In tested samples, limited amount of esters were however identified, involving hexanoic acid ethyl ester, butanoic acid ethyl ester, 3-methyl-1-butanol propanoate (Table 36). As mentioned earlier, the presence of hexanoic acid ethyl ester and butanoic acid ethyl ester in samples supports the esterification reactions occurring between ethanol and fatty acids. Leclercq-Perlat et al. (2004b) described the evolution of esters in Camembert cheese and reported that their formation was slow, attaining maximum level after 45 days. Three esters identified in Niva cheese (mould-ripened cheese) reached maximum concentrations in about 40 d of ripening (Vitova et al., 2006). The low detection of ester compounds in the tested samples may be a result of insufficient ripening time (cheeses were ripened for only 21 d in present study). Absence of ester compounds in cheese samples was also probably attributed to either lack of sufficient activity of yeast and *G. candidium* or their absence (Molimard and Spinnler, 1996). In the present study, yeast and *G. candidium* were not part of the mixed cultures, hence the absence of ester compounds produced by the low metabolic activities of yeasts and *G. candidium*. The microorganisms (yeasts and *G. candidium*) can also find their way into cheese as part of the wild flora (Bockelmann, 2010). The cheeses used in this study were prepared in a very clean environment in the product development laboratory.

Miscellaneous compounds

Isobutylamine was occasionally detected in 3% of cheese samples, which suggests the decarboxylation of Leu (Molimard and Spinnler, 1996). Isobutylamine is described as having fruity, alcoholic and varnish aromatic notes (Table 36). Heptane, an aromatic hydrocarbon, was also commonly identified in 74% of cheese samples. Ammonia is also an important element of Camembert cheese aroma (Molimard and Spinnler, 1996). Ammonia is derived from amino acid deamination; *P. camemberti*, *G. candidum* and *B. linens* play major roles in ammonia production (Molimard and Spinnler, 1996; Curioni and Bosset, 2002). In the present study, ammonia could not be identified in samples due to the settings of equipment. The compound was however recognised at

day 21 by sensory evaluation. No formal sensory of the cheeses was conducted, however, the cheese samples were evaluated by experienced consumers who are familiar and consume the products. The presence of ammonia in samples is consistent with many previous studies (Molimard and Spinnler, 1996; Ardö, 1999; Upadhyay et al., 2004). In this study, since *G. candidum* and *B. linens* were not actively involved in cheese ripening, the extensive degradation of protein is therefore attributed to high proteolytic activity of *P. camemberti* (Bockelmann, 2010).

In summary, the results presented in this section confirmed dominant roles of fatty acids, alcohols, aldehydes and ketones in volatile fractions of cheese samples, which is in agreement with several previous studies (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999; Curioni and Bosset, 2002). It is however not known which compounds determine the organoleptic quality of cheeses, because the aroma of cheese is, in fact, the result of equilibrium among all the numerous volatile compounds present (Urbach, 1997). Cheese samples produced in this study had pronounced and characteristic Camembert flavour notes and their aroma profiles were generally similar. Differences in patterns of aroma compounds may be attributed to variations in cheese production, mainly the type of culture used.

Molimard and Spinnler (1996) suggested that oct-1-en-3-ol, oct-1-en-3-one, 2-phenylethanol and 2-phenylethyl acetate, as well as sulphur-containing compounds are important volatile compounds of Camembert-type cheeses. These compounds however were not detected in samples of this study. Carbon disulphide was the only sulphur-containing compound identified in cheese samples of the study, although it is not recognized as the key sulphur compound of Camembert (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999). Carbon disulphide was present in 3-d-old cheeses and it increased during ripening. Sulphur-containing compounds are synthesised during the degradation of Met by coryneform bacteria (particularly *B. linens*), while 2-phenylethanol and 2-phenylethyl acetate are produced from Phe which is essentially produced by yeasts (Molimard and Spinnler, 1996). The lack of detection of sulphite-containing compounds and 2-phenylethanol and 2-phenylethyl acetate indicates that the catabolism of Met and Phe did not take place. This may be explained by the lack of abundant growth of coryneform related-microorganisms (particularly *B. linens*) and yeast in cheese. However, since increased concentration of carbon disulphide was observed in some cheese samples and the compound is not usually detected in cow's milk (Majcher et al., 2011), the catabolism of Met may possibly occur but to a limited extent. Linoleic and linolenic acids are precursors of 1-octen-3-ol and 1-octen-3-one and production of these compounds is attributed to *P.*

camemberti metabolism. As mentioned earlier, the absence of 1-octen-3-ol and 1-octen-3-one in samples may be largely attributed to the body part of cheese samples (without rind) analysed in the study. Although these compounds can migrate into the body of the cheese during ripening, the amount is rather limited (Karahadian et al., 1985).

Sulphur compounds are key odorants of sulphurous, garlic note in the Camembert, while 2-phenylethanol and 2-phenylethyl acetate provide a rose-like note to the cheese. A characteristic mushroom-like sensation is attributed to oct-1-en-3-ol and oct-1-en-3-one (Molimard and Spinnler, 1996; Sable and Cottenceau, 1999). Although these compounds were not detected in cheese samples at day 21, this does not suggest that the prototype Camembert cheese samples of this study were not typical. Volatile concentration from a cheese body was demonstrated substantially different from the cheese surface (Leclercq-Perlat et al., 2004b). Therefore, the analysis of cheese samples from two parts, the body and the surface, is important when comparing cheese flavour profile with published studies.

7 OVERALL SUMMARY AND CONCLUSION

Reductions in viable cell counts and acidification during storage of cultures increased with storage time and temperature with cultures stored at 37°C being affected most. The proportion of citrate-fermenting LAB bacteria in LD-type starters also decreased in a similar pattern. Cell inactivation at high temperature was probably attributed to higher levels of oxidation, browning reactions, lactose crystallization, changes in T_g of culture-lactose matrix and loss of β-galactosidase enzyme activity, which was also possibly affected by a_w of the cultures during storage. Nonetheless, final cell concentrations of 4 and 20 °C cultures after 5 months' storage were comparable to the -18°C cultures as well as levels recommended in commercial productions. It is therefore feasible to use cultures stored at 4 and 20°C for cheese production.

During Camembert cheese fermentation, similar patterns of microbial growth and acidification were observed in both (O- and LD-type) cheeses, with samples fermented with O-type starters showing better growth and acidification activity. This was probably attributed to differences in composition of culture, which may contribute to different metabolic patterns. In comparison to samples containing -18°C cultures, cheeses with 4 and 20°C cultures had consistently lower cell counts of LAB and *P. camemberti* with higher pH throughout fermentation, irrespective of the type of starters used. The results suggested that the temperature of storage affected their growth during milk fermentation and this was probably due to reduced activity of species after prolonged storage at higher temperatures.

During Camembert cheese ripening, changes in microbial counts, acidity, proteolysis, texture and aroma compound, were similar in all samples, and changed significantly with ripening time. Cell counts of LAB were reduced irrespective of the type of starter culture used, whereas pH and growth of *P. camemberti* were increased. The increase of pH probably resulted from the metabolism of lactate by *P. camemberti* forming alkaline environment in the cheese due to its deamination activity. Proteolysis of samples correlated with activities of LAB and *P. camemberti* and pH of samples. Softening of cheese was associated with increased proteolysis and pH due to intense growth of *P. camemberti* in cheese as reported in several studies.

A range of volatile compounds, dominated by fatty acids, alcohols and aldehyde were identified in cheese samples. Of the aldehydes, the increase of 3-methylbutanal concentration in the cheeses suggested the synthesis of aldehyde from metabolism of Leu by peptidolytic activity of LAB. Reduction of 3-methylbutanal and concomitant

increase of 3-methylbutanol suggested the degradation of the aldehyded to produce the alcohol by *P. camemberti* activity. The subsequent reduction of 3-methylbutanol level suggests its degradation to other metabolic compounds. Increased concentration of 2-heptanone, 2-nonanone and butyric acid in cheese samples during ripening is indicative of lipolytic activity in the products. Strong activity of *P. camembert* involved in β-oxidation pathway for producing methyl ketones was also demonstrated. The absence of 1-octen-3-one and 1-octen-3-ol in cheese samples suggested that the metabolism of linoleic and linolenic acids and methionine did not occur. The preferential lipolysis by *P. camemberti* at cheese surface than in the centre may be explained by the type of sample (body of cheese without rind) used for analysis. Small quantities of 1-octen-3-ol and 1-octen-3-one present in samples at the surface may migrate into the interior of cheese body, but the amount is rather limited. Absence of dimethyl sulphide in samples suggested that the absence of methionine degradation, probably due to the absence of *B. linens* and coryneform related-microorganisms activity in the cheeses.

Cheeses produced from LD-type starters contained similar viable cell counts of LAB, with higher *P. camemberti* growth and pH than samples with O-type lactic starters. Higher proteolysis and softness were observed in LD-cheeses than O-type, suggesting a higher degree of cheese ripening, which is probably attributed to more active role of LD-starters in proteolytic and peptidolytic activity. Higher proteolysis could be also related to higher pH level of cheese curd at draining, which can lead to higher syneresis level. High whey content of cheese curd may retain higher concentration of coagulant enzyme in the curd and effectively stimulate the growth of mould, thus probably allowing proteolysis to occur more readily. Relatively higher concentration of 3-methylbutanal were present in O-type than in LD-type cheeses, suggesting that LAB in O-type starters exhibited higher activity in degrading Leu to 3-methylbutanal than LD-type starters. 2,3-butandione was detected in LD-type cheeses but not in O-type samples. This suggested the active of role of mixed cultures of *Leuconostoc* spp. and *L. lactis* subsp. *lactis* biovar. *diacetylactis* during citrate metabolism. Higher 2-heptanone and 2-nonanone levels were found in cheeses with O-type starters, while relatively higher butyric acid concentration was present in 'LD-type' cheeses.

Storage temperature of cultures played a significant role in the changes of cell counts and pH of cheese samples, but generally did not affect proteolysis, texture modification of cheese, as well as production of volatile compounds (with an exception of 3-methylbutanal). Cheese samples containing 4 and 20°C cultures had

similar LAB levels but higher *P. camemberti* and pH compared to samples with -18°C cultures. Although there were no differences between Camembert cheeses, 4 and 20°C cultures in cheese may enhance the ripening process than -18°C cultures, as indicated by relatively higher proteolysis and degree of softening of the respective products. The higher proteolysis in cheeses containing 20°C cultures may be attributed to the higher retention of coagulant enzymes and growth of *P. camemberti*, which are related to the lower acidity of curd at whey-draining. Relatively lower production of 3-methylbutanal in samples containing 4 and 20°C cultures was probably due to reduced aminotransferases activity of LAB strains after storage at higher temperature for prolonged time. The slightly higher concentrations of 2-heptanone, 2-nonanone and butyric acids in cheese samples with 4 and 20°C cultures was probably attributed to increased lipolytic activity due to higher growth of the mould in cheese.

In conclusion, samples of this study were regarded as typical Camembert as their fermentation and ripening characteristics as well as flavour notes were comparable to levels of this type of cheeses reported in previous studies. In addition to the production of 2,3-butandione, cheese fermented with LD-type starters showed better potential for producing accelerated-ripened Camembert, as demonstrated by extensive proteolysis and higher degree of softening. This finding is of significant importance to commercial Camembert cheese production, as it can be bring cost savings. LAB starter and *P. camemberti* cultures stored for 5 months at either ambient or refrigeration temperatures did not affect microbial activities, as well as the quality of the produced prototype Camembert cheeses. Thus, there is good potential to use the cultures for Camembert production at household level. The use of these cultures may also help to enhance cheese ripening process. However, when using mixed LD-type lactic starters and *P. camemberti* that have had stored at ambient temperatures for Camembert production, reducing the ripening time by 1-2 d is suggested to compensate for rapid structural degradation of texture. Cultures stored at 37°C for 5 months were characterised by poor viable cells and capability to the produce acid, therefore, they were not suitable for Camembert cheese production.

8 RECOMMENDATIONS FOR FURTHER INVESTIGATIONS

Screening and selection of freeze-dried cheese starter and ripening cultures

(1) The stability of freeze-dried cheese starter and ripening cultures in this study was solely based on temperature. Factors such as O_2, and a_w can also affect cultures (Santivarangkna et al., 2008), which were however not studied in this work. Future work should indicate the potential impact of the two factors on stability of freeze-dried cultures when stored at high temperatures. Careful selection of the packaging material to control gas exchanges and prevent the loss or gain of moisture, is also useful to reduce cell inactivation (Tamime and Robinson, 1999).

(2) In this study, stability of freeze-dried LAB cultures is generally considered with respect to survival and acid production. It is also noteworthy that the role of storage temperature is correlated to biological functional properties of cells (Higl et al., 2007; Santivarangkna et al., 2011). Alternative criteria include metabolic activity, membrane potential, membrane integrity, bacteriocin or antimicrobial production may also be applied (Nollet and Toldra, 2012).

Production, ripening and characterization of Camembert cheese

(1) Results obtained in the study demonstrated that cheese starter and ripening cultures can be stored for 5 months at either ambient or refrigeration temperatures without affecting their activities, as well as the quality of the produced prototype Camembert cheeses. Although the laboratory-scale experiments (small-scale prototypes at domestic level) conducted in this study provide some good indications, future studies could also be conducted and their performance evaluated for industrial pilot-scale production. This is particularly important for industry cheese production as many problems can be incurred when scaling up and the characteristics of cultures and cheeses may be also affected in large scale operations.

(2) Further work on microbial, physicochemical and sensory changes occurring in produced prototype cheeses during shelf life is therefore suggested. For commercial application, it is also recommended to compare the property of the produced prototype cheese with commercial products.

(3) Levels of lactose, lactate and ammonia in cheese samples during ripening were not investigated in this study. Further research is needed in order to better understand the effect of these changes on characteristics (biochemical and sensory) of cheeses. In future studies, analysis of casein fractions of cheese samples is suggested to provide more information on proteolysis.

(4) By increasing proteolysis and degree of softening, cheeses produced from LD-type starters and ripening cultures stored at 20°C generally ripened faster. However, extensive level of proteolysis can weaken cheese texture (Spinnler and Gripon, 2004). When using mixed LD-type starters and cultures stored at 20°C for Camembert production, shortening ripening time by 1-2 d is suggested to compensate for rapid structural degradation of texture.

(5) In the study, *Mucor miehei* aspartic proteinase was used to coagulate milk. Compared to other coagulation enzymes like chymosin, microbial proteinase exhibits prolonged proteolytic activity resulting in potential production of bitter peptides and undesirable product softening (Guinee and Wilkinson, 1992; Ward et al., 2009). In future studies, chymosin is suggested as a substitute to reduce defects of cheese.

(6) To improve sensory quality of cheese, the use of *G. candidum* along with *P. camemberti* as secondary ripening microorganism is suggested. *G. candidum* can effectively control the extensive growth of mould and reduce the occurrence of bitterness defect in cheese (Bockelmann, 2010). *G. candidum* may also introduce a flavour profile to the blandness of the cheese (Bockelmann, 2010).

(7) In this study, samples of all treatments produced a continuous, flowy inner body of cheese at 21 d of ripening. This created a problem during the measurement of cheese firmness, as the sample pieces could no longer support their own weight after the sample had attained ambient temperature. Although the purpose of instrumental analysis of texture was to mimic the behaviour of human sensory evaluation, modifications should be therefore made depending on the conditions of the samples. Thus instrumental analysis of texture of cheese with extensive softness should be conducted at lower temperatures such as 10-12°C. This would allow more accurate comparison between samples of different treatments and eliminate variations caused by inconsistent cheese texture at high temperatures.

(8) In this study, body part of cheese (without rind) was obtained for analysing aroma compounds of samples. Volatile compound concentration from a cheese body was demonstrated to be substantially different from the cheese surface (Leclercq-Perlat et al., 2004b; Molimard and Spinnler, 1996). Although these compounds can migrate into the body of the cheese during ripening, the amount is rather limited (Karahadian et al., 1985). In future studies, two parts of cheese, the body and the surface, should both be studied.

(9) Sensory evulation of the produced prototype Camembert cheeses was not include in this study due to time, personal and financial constraints involved (Appendix 3.6). In future studies, descriptive sensory analysis and consumer acceptability test are both recommended. Descriptive sensory analysis (e.g., flavour profile method or quantitative descriptive method) will involve profiling (discrimination and description) of prototype cheese samples on the perceived sensory characteristics, by trained and experienced panelists. Meanwhile, consumer acceptability test will evaluate product/market acceptability in a general aspect, by potential consumers. Both sensory analysis results will be used to to investigate the effects of starter type and culture storage temperature on sensory properties of samples. Additionally, data from descriptive sensory analysis and instrumental (SPME/GC-MS, textural, proteolysis) will be correlated and possible relationships will be determined. For commercial application, sensory data of prototype cheeses may also be used for comparison with commercial products to explore differences in sensory properties.

9 REFERENCES

Abe F, Miyauchi H, Uchijima A, et al. (2009) Effects of storage temperture and water activity on the survival of bifidobacteria in powder form. *International Journal of Dairy Technology* 62: 234-239.

Adda J. (1982) The chemistry of flavour and texture generation in cheese. *Food Chemistry* 9: 115-129.

Alewijn M. (2006) The formation of fat-derived flavour compounds during the ripening of Gouda-type cheese. *Wageningen University.* The Netherlands: Wageningen University, 1-136.

Ananta E, Volkert M and Knorr D. (2005) Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. *International Dairy Journal* 15: 399-409.

Andersen AB, Fog-Peterson MS, Larsen H, et al. (1999) Storage stability of freeze-dried starter cultures (*Streptococcus thermophilus*) as related to physical state of freezing matrix. *Food Science Technology* 32: 540-547.

Antoniou KD, Petridis D, Raphaelides S, et al. (2000) Texture Assessment of French Cheeses. *Journal of Food Science* 65: 168-172.

AOAC. (2005a) AOAC Offical Method 920.123: Nitrogen in cheese-Kjeldahl methods. *AOAC International*.

AOAC. (2005b) AOAC Offical Method 947.05: Acidity of milk, titrimetric method. American Organization of Analytical Chemists International.

AOAC. (2005c) AOAC Offical Method 991.20: Nitrogen (total) in milk-Kjeldahl methods. *AOAC International*.

Ardö Y. (1999) Chemical methods for evaluating proteplysis in cheese maturation/Part 2: Evaluating proteolysis by analysing the N content of cheese fractions. *International Dairy Federation* Bulletin of the IDF 337: 4-9.

Aschenbrenner M, Kulozik U and Forst P. (2011) The role of glassy state in production and storage of freeze-dried starter cultures (11th International Congress on Engineering and Food (ICEF11)). *Procedia Food Science* 1: 347-354.

Bachmann H, Kruijswijk Z, Molenaar D, et al. (2009) A high-throughout cheese manufacturing model for effective cheese starter culture screening. *Journal of Dairy Science* 92: 5868-5882.

Banks JM. (1998) Cheese. In: Early R (ed) *The technology of dairy products.* 2nd ed. London: Blackie Academic & Professional.

Bell LN and Hageman MJ. (1996) Glass transition explanation for the effect of polyhydroxy compounds on protein denaturation in dehydrated solids. *Journal of Food Science* 61: 372-378.

Bell LN and Labuza TP. (2000) Moisture sorption: practical aspects of isotherm measurement and use. American Association of Cereal Chemists.

Beresford T and Williams A. (2004) The microbiology of cheese ripening. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

Bhandari BR and Howes T. (1999) Implication of glass transition for the drying and stability of dried foods. *Journal of Food Engineering* 40: 71-79.

Biedermann M, Biedermann-Brem S, Noti A, et al. (2007) Mitteilungen aus Lebensmitteluntersuchung und Hygiene.

Bjokroth J and Holzapfel W. (2006) Genera *Leuconostoc*, *Oenococcus* and *Weissella*. *Prokaryotes.* 267-319.

Bockelmann W. (2010) Secondary cheese starter cultures. In: Law BA and Tamime AY (eds) *Technology of cheesemaking*. 2nd ed. New York: Blackwell Publishing Ltd.

Bruno FA and Shan NP. (2003) Viability of two freeze-dried strains of *Bifidobacterium* and of commercial preparations at various tempertures during prolonged storage. *Journal of Food Science* 68: 2336-2339.

Buitink J and Leprince O. (2004) Glass formation in plant anhydrobiotes: survival in the dry state. *Cryobiology* 48: 215-228.

Bullimore BK. (1983) Dairy starters reviewed. *Dairy Industries International* 48: 19-23.

Carvalho AS, Silva J, Ho P, et al. (2007) Relationship between solubility of freeze-dried skim milk and death of freeze-dried *Lactobacillus delbrueckii* subsp. *bulgaricus* during storage. *Milchwissenschaft* 62: 148-150.

Casta E, Usall J, Teixido N, et al. (2002) Effect of package and storage conditions on viability and efficacy of the freeze-dried biocontrol agent *Pantoea agglomerans* strain CPA-2. *Journal of Applied Microbiology* 92: 873-878.

Castro HP, Teixeira PM and Kirby R. (1995) Storage of lyophilized cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* under different relative humidites and atmospheres. *Applied Microbiology and Biotechnology* 44: 172-176.

Castro HP, Teixeira PM and Kirby R. (1996) Changes in the cell membrane of *Lactobacillus delbrueckii* subsp. *bulgaricus* during storage following freeze-drying. *Biotechnology Letters* 18: 99-104.

Castro HP, Teixeira PM and Kirby R. (1997) Evidence of membrane damage in *Lactobacillus delbrueckii* subsp. *bulgaricus* following freeze drying. *Journal of Applied Microbiology* 82: 87-94.

Cerrutti P, Segovia HM, Galvagno M, et al. (2000) Commerical baker's yeast stability as affected by intracellular content of trehalose, dehydration procedure and the physical properties of external matrices. *Applied Microbiology and Biotechnology* 54: 575-580.

Champagne CP, Mondou F, Raymond Y, et al. (1996) Effect of polymers and storage temperture on the stability of freeze-dried lactic acid bacteria. *Food Research International* 29: 555-562.

Chin HW, Bernhard RA and Rosenberg M. (1996) Solid phase microextraction for cheese volatile compound analysis. *Journal of Food Science* 61: 1118-1123.

Codex Standard. (2010) Codex standard for Camembert. *Camembert (CODEX STAN 276-1973).*

Cogan TM, Barbosa M, Beuvier E, et al. (1997) Characterization of the lactic acid bacteria in artisanal dairy products. *Journal of Dairy Research*: 409-421.

Collins YF, Mcseeney PLH and Wilkinson MG. (2004) Lipolysis and catabolism of fatty acids in cheese. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds)

Cheese: chemsitry, physics and microbiology. Volume 1: General aspects. 3rd ed. London: Elsevier Applied Science.

Constantia. (2012) Regulatory compliance statement for food contact materials: Flexible packaging laminate. 1-2.

Crowe JH, Carpenter JF and Crowe LM. (1998) The role of vitrification in anhydrobiosis. *Annual Review of Physiology* 60: 73-103.

Curioni PMG and Bosset JO. (2002) Key odorants in various cheese types as determined by gas chromatography-olfactometry. *International Dairy Journal* 12: 959-984.

Curtin AC and Mcsweeney PLH. (2004) Catabolism of amino acids in cheese during ripening. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

Dalgleish DG and Corredig M. (2012) The structure of the casein micelle of milk and its changes during processing. *Annual Review of Food Science and Technology* 3: 449-467.

Dave RI and Shah NP. (1997) Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal* 7: 31-41.

Delahunty CM and Drake MA. (2004) Sensory character of cheese and its evaluation. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

Delgado FJ, Gonzalez-Crespo J, Cava R, et al. (2010) Proteolysis and texture changes of a Spanish soft cheese ('Torta del Casar') manufactured with raw ewe milk and vegetable rennet during ripening. *International Journal of Food Science and Technology* 45: 512-519.

Delgado FJ, Gonzalez-Crespo J, Cava R, et al. (2011) Proteolysis, texture and colour of a raw goat milk cheese throughout the maturation. *European Food Research Technology* 233: 483-488.

Downes FP and Ito K. (2001) Compendium of Methods for the Microbiological Examination of Foods. 4 th ed. United States of America: American Public Health Association.

Dumont JP, Roger S, Cerf P, et al. (1974) Neutral volatiles in Camembert cheese. *Lait* 54: 501-516.

FAO. (2008) Dairy Summit. Rome, Italy: Food and Agricultural Organisation of the United Nations.

Folkertsma B and Fox PF. (1992) Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. *Journal of Dairy Research* 59: 217-224.

Fonseca F, Obert JP, Beal C, et al. (2001) State diagrams and sorption isotherms of bacterial suspensions and fermented medium. *Thermochim Acta* 366: 167-182.

Fox PF and Cogan TM. (2004) Factors that affect the quality of cheese. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

Fox PF, Guinee TP, Cogan TM, et al. (2000) Fundamentals of cheese science. 1st ed. United States of America: ASPEN Publication.

Fox PF and McSweeney PLH. (2004) Cheese: an overview. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

Frisvad JC and Samson RA. (2004) Polyphasic taxonomy of *Pencillium* subgenus *Penicillium*: A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. *Studies in Mycology* 49: 1-174.

Fryer TF, Sharp ME and Reiter B. (1970) Utilization f milk citrate by lactic acid bacteria and 'blowing of film wrapped cheese'. *Journal of Dairy Research* 37 17-28.

FSANZ. (2008) Fermented milk products: Standard 2.5.4 Cheese. Melbourne: Australia New Zealand Food Stanard Code.

Garcia AH. (2011) Anhydrobiosis in bacteria: From physiology to applications. *Journal of Biological Science* 36: 1-12.

Garcia EF. (1996) Use of headspace sampling in the quantitative analysis of artisanal spanish cheese aroma. *Journal of Agricultural and Food Chemistry* 44: 1833-1839.

Graet YL and Brule G. (1988) Mineral migration of soft cheeses during ripening. *Le Lait* 68: 219-234.

Graet YL, Lepienne A, Brule G, et al. (1983) Migration of calcium and inorganic phosphate in Camembert-type cheeses during ripening. *Le Lait* 63: 317-332.

Grappin R, Rank TC and Olson NF. (1985) Primary proteolysis of cheese proteins during ripening: A review. *Journal of Dairy Science* 68: 531-540.

Grzeskowiak L, Isolauri E, Salminen S, et al. (2011a) Manufacturing process influences properties of probiotic bacteria. *British Journal of Nutrition* 105: 887-894.

Grzeskowiak L, Isolauri E, Salminen S, et al. (2011b) Manufacturing process influences properties of probiotic bacteria. *British Journal of Nutrition*: 1-8.

Guamisa B, Trujilloa AJ, Ferraguta V, et al. (1997) Ripening control of Manchego type cheese salted by brine vacuum impregnation. *International Dairy Journal* 7: 185-192.

Guillen MD, Ibargoitia ML, Sopelana P, et al. (2004) Components detected by headspace-solid phase microextraction in artisanal fresh goat's cheese smoked using dry prickly pear (*Opuntia ficus indica*). *Lait* 84: 385-397.

Guinee TP and Wilkinson MG. (1992) Rennet coagulation and coagulants in cheese manufacture. *Jounral of Dairy Technology* 45: 94-104.

Guizani N, Kasapis S, Al-Attabi ZH, et al. (2007) Microbiological, physicochemical, and biochemical changes during ripening of Camembert cheese made of pasteurized cow's milk. *International Journal of Food Properties* 5: 483-494.

Gyosheva B, Petrova L and Mutafchieva M. (1995) Preservation of *Streptococcus thermophilus* strains after long-term storage in lyophilized state. *Journal of Culture Collections* 1: 34-37.

Hayaloglu AA. (2007) Comparisons of different single-strain starter cultures for their effects on ripening and grading of Beyaz cheese. *International Journal of Food*

Science and Technology 42: 930-938.

Hayaloglu AA. (2009) Volatile composition and proteolysis in traditionally produced mature Kashar cheese. *International Journal of Food Science and Technology* 44: 1388-1394.

Hayaloglu AA, Brechany EY, Deegan KC, et al. (2008) Characterization of the chemistry, biochemistry and volatile profile of Kuflu cheese, a mould-ripened variety. *Lebenson Wiss Technol* 41 1323-1334.

Higl B, Kurtmann L, Carlsen CU, et al. (2007) Impact of water activity, temperture, and physical state on the storage stability of *Lactobacillus paracasei* subsp. *paracasein* freeze-dried in a lactose matrix. *Biotechnology Progress* 23: 794-800.

Hoier E, Janzen T, Rattray F, et al. (2011) The production, application and action of lactic cheese starter cultures. In: Law BA and Tamime AY (eds) *Technology of Cheesemaking.* 2nd ed.: Wiley-Blackwell, 166-192.

Holmes DG, Duersch JW and Ernstrom CA. (1997) Distribution of milk clotting enzymes between curd and whey and their survival during cheddar cheese making. *Journal of Dairy Science* 60: 862-868.

Hugenholtz J. (1993) Citrate metabolism in lactic acid bacteria. *Fems Microbiology Reviews* 12: 165-178.

IDF. (2003) Yogurt-Enumeration of characteristic microorganisms, colony count technique at 37°C (IDF 117). Brussel: International Dairy Federation.

IDF. (2004) Milk and milk products-Enumeration of colony-forming units of yeasts and/or moulds, colony count technique at 25°C (IDF 94). Brussels: International Dairy Federation.

IDF. (2006) Milk, milk products and mesophilic starter cultures-Enumeration of citrate-fermenting lactic acid bacteria, colony count technique at 25°C (ISO 17792 / IDF 180). Brussels: International Dairy Federation.

IDF. (2010) Fermented milk products-Bacterial starter cultures, standard of identity (IDF 149). Brussels: International Dairy Federation.

Imhof R, Glattli H and Bosset JO. (1995) Volatile organic compounds produced by thermophilic and mesophilic single strain dairy starter cultures. *Lebenson Wiss Technol* 28: 78-86.

IOFI. (2011) Guidelines for the quantitative gas chromatography of volatile flavouring substances from the Working Group on Method Analysis of the International Organisation of the Flavour Industry (IOFI). *Flavour and Fragrance Journal* 26: 297-299.

Johnson M and Law BA. (2010) The origins, development and basic operations of cheesemaking technology. In: Law BA and Tamime AY (eds) *Technology of cheesemaking.* 2nd ed.: Blackwell Publishing Ltd.

Josephsen J and Jespersen L. (2004) Starter cultures and fermented products. In: Hui YH, Goddik LM, Hansen OS, et al. (eds) *Handbook of Food and Beverage Fermentation Technology.* United States of America: CRC Press 27-59.

Jou KD and Harper WJ. (1998) Pattern recognition of swiss cheese aroma compounds by SPME/GC and electronic nose. *Milchwissenschaft* 53: 259-263.

Juric M, Bertelsen G, Mortensen G, et al. (2002) Light-induced colour and aroma

changes in sliced, modified atmosphere packaged semi-hard cheeses. *International Dairy Journal* 13: 239-249.

Kaminarides S, Stamou P and Massouras T. (2007) Changes of organic acids, volatile aroma compounds and sensory characteristics of Halloumi cheese kept in the brine. *Food Chemistry* 100: 219-225.

Karahadian C, Josephson DB and Lindsay RC. (1985) Contribution of *Penicillium* spp. to the flavours of Brie and Camembert cheese. *Journal of Dairy Science* 68: 1865-1877.

Karimi R, Mortazavian AM and Rigi AA. (2012) Selective enumeration of probiotic microorganisms in cheese. *Food Microbiology* 29: 1-9.

Karmas R, Buera MP and Karel M. (1992) Effect of glass transition on rates of non-enzymatic browning in food systems. *Journal of Agricultural and Food Chemistry* 40: 873-879.

Katechaki E, Panas P, Rapti K, et al. (2008) Production of hard-type cheese using free or immobilized freeze-dried kefir cells as a starter culture. *Journal of Agricultural and Food Chemistry* 56: 5316-5323.

Katoka H, Lord HL and Pawliszyn J. (2000) Review: Applications of solid-phase microextraction in food analysis. *Journal of Chromatography A* 880: 35-62.

Kihal M, Prevost H, Henni DE, et al. (2009) Carbon dioxide production by *Leuconostoc mesenteroides* grown in single and mixed culture with *Lactococcus lactis* in skim milk. *Scientific Research and Essay* 4: 1348-1353.

Kindsedt P. (2005) American farmstead cheese: The complete guide to making and selling artisan cheeses. United States of America: Chelsea Green Publishing.

King VAE, Lin HJ and Liu CF. (1998) Accelerated storage testing of freeze-dried and controlled low-temperture vacuum dehydrated *Lactobacillus acidophilus*. *Journal of General and Applied Microbiology* 44: 161-165.

Kosasih MF. (2011) Development of a probiotics rich yogurt dry mix. *Institute of Human Nutrition and Food Technology.* Auckland, New Zealand: Massey University.

Kosikowski FV and Mistry VV. (1997) Cheese and fermented milk foods. Volume I: Origins and principles. 3rd ed. New York: Edwards Brothers, Inc.

Kourkoutas Y, Kandylis P, Panas P, et al. (2006) Evaluation of freeze-dried kefir coculture as starter in feta-type cheese production. *Applied and Environmental Microbiology* 72: 6124-6135.

Kurtmann L, Carlsen CU, Risbo J, et al. (2009a) Storage stability of freeze-dried *Lactobacillus acidophilus* (La-5) in relation to water activity and presence of oxygen and ascorbate. *Cryobiology* 58: 175-180.

Kurtmann L, Carlsen CU, Skibsted LH, et al. (2009b) Water-activity-temperature state diagrams of freeze-dried *Lactobacillus acidophilus* (La-5): Influence of physical state on bacterial survival during storage. *Biotechnology Progress* 25: 265-270.

Kurtmann L, Skibsted LH and Carlsen CU. (2009c) Browning of freeze-dried probiotic bacteria cultures in relations to loss of viability during storage. *Journal of Agricultural and Food Chemistry* 57: 6736-6741.

Law BA. (1997) Microbiology and biochemistry of cheese and fermented milk. 2nd ed.

United Kingdom: Blackie Academic & Professional.

Law MHE. (2010) Ultra filtration (UF) process development for the production of camembert cheese. *College of Sciences.* New Zealand: Massey University.

Lawrence RC, Creamer LK and Gilles J. (1987) Cheese ripening technology: Texture development during cheese ripening. *Jouranl of Dairy Science* 70: 1748-1760.

Leclercq-Perlat MN, Buono F, Lambert D, et al. (2004a) Controlled production of Camembert-type cheeses. Part I: Microbiological and physicochemical evolutions. *Journal of Dairy Research* 71: 346-354.

Leclercq-Perlat MN, Latrille E, Corrieu G, et al. (2004b) Controlled production of Camembert-type cheese. Part II: Changes in the composition of the more volatile compounds. *Journal of Dairy Research* 71: 355-366.

Lenoir J. (1984) The surface flora and its role in the ripening of cheese. *Bulletin 171, International Dairy Federation.* Brussels, 3-20.

Lessard MH, Belanger G, Gelais DS, et al. (2012) The composition of Camembert cheese-ripening cultures modulates both mycelial growth and appearance. *Applied and Environmental Microbiology* 78: 1813-1819.

Lievense LC and Riet KV. (1994) Convective drying of bacteria. Part II: Factors influencing survival. *Advances in Biochemical Engineering/Biotechnology* 51: 71-89.

Lodato P, Huergo MS and Buera MP. (1999) Viability and thermal stability of a strain of *Saccharomyces cerevisiae* freeze-dried in different sugar and polymer matrices. *Applied Microbiology Biotechnology* 52: 215-220.

Lynch JM and Barbano DM. (1999) Kjeldahl nitrogen analysis as a reference method for protein determination in dairy products. *Journal of AOAC International* 82: 1389-1398.

Majcher MA, Goderska K, Pikul J, et al. (2011) Changes in volatile, sensory and microbial profiles during preparation of smoked ewe cheese. *Journal of Science in Food Agriculture* 91: 1416-1423.

Mariaca R and Bosset JO. (1997) Instrumental analysis of volatile (flavour) compounds in milk and dairy products. *Lait* 77: 13-40.

Marshall VM. (1992) Starter cultures for milk fermentation and their characteristics. *Journal of the Society of Dairy Technology* 46: 49-56.

Mazzobre MF, Buera MdP and Chirife J. (1997) Glass transition and thermal stability of lactase in low-moisture amorphous polymeric matrices. *Biotechnology Progress* 13: 195-199.

Mccormack E. (2011) Shelf life study of starter cultures used to make Camembert and Quark cheeses. *College of Sciences.* New Zealand: Massey University.

McSweeney PLH. (2004a) Biochemistry of cheese ripening. *International Journal of Dairy Technology* 57: 127-144.

McSweeney PLH. (2004b) Biochemistry of cheese ripening: Introduction and overview. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

McSweeney PLH and Fox PF. (1997) Chemical methods for the characterization of

proteolysis in cheese during ripening. *Lait* 77: 41-76.

Mcsweeney PLH and Fox PF. (2004) Metabolism of residual lactose and of lactate and citrate. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

Meilaard MC, Civille GV and Carr BT. (1999) Sensory evaluation techniques. 3rd edition ed. United States of America: CRC Press.

Miao S, Mills S, Stanton C, et al. (2008) Effect of disaccharides on survival during storage of freeze-dried probiotics. *Dairy Science Technology* 88: 19-30.

Molimard P and Spinnler HE. (1996) Compounds involved in the flavor of surface mold-ripened cheeses: Origins and properties. *Journal of Dairy Science* 79: 169-184.

Monias M, Groux M and Horman I. (1973) Flavour of cheese: New method for isolation of volatile constituents, application to Roquefort and Camembert. *Lait* 53: 601-609.

Monnet C, Schmitt P and Divies C. (1994) Method for assaying volatile compounds by headspace gas chromatography and application to growing starter cultures. *Journal of Dairy Science* 77: 1809-1815.

Morgan CA, Herman N, White PA, et al. (2006) Preservation of micro-organisms by drying: A review. *Journal of Microbiol Methods* 66: 183-193.

Murthy UM, Kumar PP and Sun WQ. (2003) Mechanisms of seed ageing under different storage conditions for *Vigna radiate* (L.) Wilczek: lipid peroxidation, sugar hydrolysis, maillard reactions and their relationship to glass state transition. *Journal of Experimental Botany* 54: 1057-1067.

Nollet LML and Toldra F. (2012) Handbook of dairy foods analysis. 1st ed. London: CRC Press.

Noomen A. (1983) The role of the surface flora in the softening of cheeses with a low initial pH. *Journal of Netherlands Milk and Dairy* 37: 229-232.

O'Callaghan DJ and Guinee TP. (2004) Rheology and texture of cheese. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

O'Mahony JA, Lucey JA and McSweeney PLH. (2005) Chymosin-mediated proteolysis, calcium solubilization, and texture development during the ripening of Cheddar cheese. *Journal of Dairy Science* 88: 3101-3114.

Parente E and Cogan TM. (2004) Starter cultures: General aspects. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

Passot S, Cenard S, Douania I, et al. (2012) Critical water activity and amorphous state for optimal preservation of lyophilized lactic acid bacteria. *Food Chemistry* 132: 1699-1705.

Pavia M, Trujillo AJ, Guamis B, et al. (2000) Ripening control of salt-reduced Manchego-type cheese obtained by brine vacuum-impregnation. *Food*

Chemistry 70: 155-162.

Perko B. (2002) Lactose fermentation at Camembert, made by classic and stabilised technology. *Mljekarstvo* 52: 5-18.

Pinho O, Ferreira IMPLVO, Casal S, et al. (2001) Method optimization for analysis of the volatile fraction of ewe cheese by solid-phase microextraction. *Chromatographia Supplement* 53: 390-393.

Potts M. (1994) Desiccation tolerance of prokaryotes. *Microbiological Reviews* 58: 755-805.

Qian MC, Peterson DG and Reineccius GA. (2010) Gas Chromatography. In: Nielsen SS (ed) *Food analysis.* 4th ed. United States of America: Springer Science and Bussiness Media.

Quere JLL. (2004) Cheese flavour: Instrumental techniques. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

Rank TC, Grappin R and Olson NF. (1985) Seconday proteolysis of cheese during ripening: A review. *Journal of Dairy Science* 68: 801-805.

Rehn U, Vogensen FK, Persson SE, et al. (2011) Influence of microflora on texture and contents of amino acids, organic acids, and volatiles in semi-hard cheese made with DL-starter and propionibacteria. *American Dairy Science Association* 94: 1098-1111.

Reineccius G. (2006) Flavour chemistry and technology. Part II: Flavour chemistry. *Flavour analysis.* 2nd ed. United State of Ameria: Taylor & Francis Group.

Roos YH. (2002) Importance of glass transition and water activity to spray drying and stability of dairy powders. *Lait* 82: 475-484.

Roos YH. (2004) Phase and state transitions in dehydration of biomaterials and foods. In: Mujumdar AS (ed) *Dehydration of products of biological origin.* United States of America: Science Publishers, 3-22.

Roseiro LB, Risco MG, Barbosa M, et al. (2003) Evaluation of Serpa cheese proteolysis by nitrogen content and capillary zone electrophoresis. *International Journal of Dairy Technology* 56: 99-104.

Sable S and Cottenceau G. (1999) Current knowledge of soft cheeses flavor and related compounds. *Journal of Agricultural and Food Chemistry* 47: 4825-4836.

Saldo J, Sendra E and Guamis B. (2000) High hydrostatic pressure for accelerating ripening of goat's milk cheese: proteolysis and texture. *Journal of Food Science* 65: 636-640.

Samson RA, Eckardt C and Orth R. (1977) The taxonomy of *Pencillium* species from fermented cheeses. *Antonie van Leeuwenhoek* 43: 341-350.

Santivarangkna C, Aschenbrenner M, Kulozik U, et al. (2011) Role of glassy state on stabilities of freeze-dried probiotics. *Journal of Food Science* 76: 152-156.

Santivarangkna C, Kulozik U and Foerst P. (2007) Alternative drying process for the industrial preservation of lactic acid starter cultures. *Biotechnology Progress* 23: 302-315.

Santivarangkna C, Kulozik U and Foerst P. (2008) Inactivation mechanisms of lactic acid starter cultures preserved by drying processes. *Journal of Applied*

Microbiology 105: 1-13.

Schebor C, Buera MP and Chirife J. (1997) Glassy state in relation to the thermal inactivation of the enzyme invertase in amorphous dried matrices of trehalose, maltodextrin and PVP. *Journal of Food Engineering* 30: 269-273.

Schebor C, Galvogno M, Buera MP, et al. (2000) Glass transition temperatures and fermentative activity of heat-treated commercial active dry yeasts. *Biotechnology Progress* 16: 163-168.

Schein RD and Rotem J. (1965) Temperture and humidity effects on uredospore viability. *Mycologia* 57: 397-403.

Schlesser JE, Schmidt SJ and Speckman R. (1992) Characterization of chemical and physical changes in Camembert cheese during ripening. *Journal of Dairy Science* 75: 1753-1760.

Shaw MB. (1981) The manufacture of soft, surface mould, ripened cheese in France with particular reference to Camembert. *Journal of Society of Dairy Technology* 34: 131-138.

SHIMADZU. (2010) GCMS-QP2010SE Gas chromatograph mass spectrometer scientific instrument guide. SHIMADZU.

Simpson PJ, Stanton C, Fitzgerald GF, et al. (2005) Intrinsic tolerance of Bifidobacterium species to heat and oxygen and survival following spray drying and storage. *Journal of Applied Microbiology* 99: 493-501.

Singh TK and Cadwallader KR. (2008) Cheese. In: Chandan RC (ed) *Dairy processing & quality assurance.* John Wiley & Sons, Inc, 273-299.

Singh TK, Drake MA and Cadwallader KR. (2003) Flavor of Cheddar cheese: a chemical and sensory perspective. *Comprehensive Reviews in Food Science and Food Safety* 2: 139-162.

Skeie S, Kieronczyk A, Naess RM, et al. (2008) *Lactobacillus* adjuncts in cheese: their influence on the degradation of citrate and serine during ripening of a washed curd cheese. *International Dairy Journal* 18: 158-168.

Skeie S, Lindberg C and Narvhus J. (2001) Development of amino acids and organic acids in Norvegia, influence of milk treatment and adjunct *Lactobacillus*. *International Dairy Journal* 11: 399-411.

Smit G, Smit BA and Engels WJM. (2005) Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *Fems Microbiology Reviews* 29: 591-610.

Smith JS and Thakur RA. (2010) Mass spectrometry. In: Nielsen SS (ed) *Food analysis.* 4th ed. United States of America: Springer Science and Bussiness Media.

Sousa MJ, Ardo Y and McSweeney PLH. (2001) Advances in the study of proteolysis during cheese ripening. *International Dairy Journal* 11: 327-345.

Sousa MJ and McSweeney PLH. (2001) Studies on the ripening of Cooleeney, an Irish farmhouse Camembert-type cheese. *Irish Journal of Agriculture and Food Research* 40: 83-95.

Spinnler HE and Gripon JC. (2004) Surface mould-ripened cheeses. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 2: Major cheese groups.* 3rd ed. London: Elsevier Applied

Science.

Strasser S, Neureiter M, Geppl M, et al. (2009) Influence of lyophilization, fluidized bed drying, addition of protectants, and storage on the viability of lactic acid bacteria. *Journal of Applied Microbiology* 107: 167-177.

Sullivan NAO, Sousa MJ, Walsh DOG, et al. (2005) Ripening of Camembert-type cheese made from caprine milk using calf rennet or kid rennet as coagulant. *International Journal of Dairy Technology* 58: 13-18.

Sun WQ, Leopold C, Crowe L, et al. (1996) Stability of dry liposomes in sugar glasses. *Biophysical Journal* 70: 1769-1776.

Sun WQ and Leopold CA. (1994) Glassy state and seed storage stability: A viability equation analysis. *Annals of Botany* 74: 601-604.

Tamime AY and Robinson RK. (1999) Preservation and production of starter cultures. In: Tamime AY and Robinson RK (eds) *Yoghurt science and technology.* New York: Woodhead Publishing in Food Science and Technology.

Tan SS, Van-Ingen CW, Talsma H, et al. (1995) Freeze-drying of fungi: influence of composition and glass transition temperture of the protectant. *Cryobiology* 32: 60-67.

Taylor AJ and Linforth RST. (2010) Food flavour technology. In: Reineccius G (ed) *Instrumental method of analysis.* 2nd ed. United Kingdom: Wiley-Blackwell Publishing Ltd.

Teuber M and Geis A. (2006) The genus *Lactococcus*. In: Dworkin M and Falkow S (eds) *The prokaryotes. Vol. 4: Bacteria: firmicutes, cyanobacteria.* United States of America: Springer US, 205-228.

Thage BV, Broe ML, Petersen MH, et al. (2005) Aroma development in semi-hard reduced-fat cheese inoculated with *Lactobacillus paracasei* strains with different aminotransferase profiles. *International Dairy Journal* 15: 795-805.

Thomsen MK, Jespersen L, Sjostrom K, et al. (2005) Water activity-temperature state diagram of amorphous lactose. *Journal of Agricultural and Food Chemistry* 53: 9182-9185.

Tzanetaki EL, Tzanetakis N and Mastrojiannaki AV. (1993) Effect of the type of lactic starter on microbiological, chemical and sensory characteristics of Feta cheese. *Food Microbiology* 10: 31-41.

Upadhyay VK, Mcsweeney PLH, Magboul AAA, et al. (2004) Proteolysis in cheese during ripening. In: Fox PF, Mcsweeney PLH, Cogan TM, et al. (eds) *Cheese: chemsitry, physics and microbiology. Volume 1: General aspects.* 3rd ed. London: Elsevier Applied Science.

Urbach G. (1995) Contribution of lactic acid bacteria to flavour compound formation in dairy products. *International Dairy Journal* 5: 877-903.

Urbach G. (1997) The flavour of milk and dairy products. Part II: Cheese: contribution of volatile compounds. *International Journal of Dairy Technology* 50: 79-89.

Vasiljevic T and Jelen P. (2003) Drying and storage of crude β-galactosidase extracts from *Lactobacillus delbrueckii* subsp. *bulgaricus* 11842. *Innovative Food Science and Emerging Technologies* 4: 319-329.

Visser FMW. (1977) Contribution of enzymes from rennet, starter bacteria and milk to proteolysis and flavour development in Gouda cheese. 3. Protein breakdown: analysis of the soluble nitrogen and amino acid fractions. *Neth Milk Dairy Journal* 31: 210-239.

Vitova E, Loupancova B, Stoudkova H, et al. (2007) Application of SPME-GC method for analysis of the aroma of white surface mould cheeses. *Journal of Food and Nutrition Research* 46: 84-90.

Vitova E, Loupancova B, Zemanova J, et al. (2006) Solid phase microextraction for analysis of mould cheese aroma. *Czech Journal of Food Science* 24: 268-274.

Vogensen FK, Karst T, Larsen JJ, et al. (1987) Improved direct differentiation between *Leuconostoc cremoris*, *Streptococcus lactis* subsp. *diacetylactis* and *Streptococcus cremoris*/*Streptococcus lactis* on agar. *Milchwissenschaft* 42: 646-648.

Walstra P, Wouters JTM and Geurts TJ. (2006) Dairy Science and Technology. 2nd ed. New York: Marcel Dekker.

Wang YC, Yu RC, Yang HY, et al. (2003) Sugar and acid contents in soymilk fermented with lactic acid bacteria alone or simultaneously with bifidobacteria. *Food Microbiology* 20: 333-338.

Ward OP, Rao MB and Kulkarni A. (2009) Proteases, production. *Applied Microbiology: Industrial* Elsevier Inc.

Wirjantoro TI and Phianmongkhol A. (2009) The viability of lactic acid bacteria and *Bifidobacterium bifidum* in yoghurt powder during storage. *Journal of National Science* 8: 95-104.

Wium H, Kristiansen KR and Qvist KB. (1998) Proteolysis and its role in relation to texture of Feta cheese made from ultrafiltered milk with different amounts of rennet. *Journal of Dairy Research* 65: 665-674.

Wouters JTM, Ayad EHE, Hugenholtz J, et al. (2002) Microbes from raw milk for fermented dairy products. *International Dairy Journal* 12: 91-109.

Wright AV and Axelsson L. (2012) Lactic acid bacteria: An introduciton. In: Lahtinen S, Ouwehand AC, Salminen S, et al. (eds) *Lactic acid bacteria: microbiological and functional aspects.* London: CRC Press, 1-16.

Yang J, Li WL and Harper WJ. (1998) Use of solid phase microextraction of volatile compounds in whey protein concentrates. *Milchwissenschaft* 53: 209-212.

Yao AA, Dortu C, Egounlety M, et al. (2009) Production of freeze-dried lactic acid stater culture for Cassava fermentation into gari. *African Journal of Biotechnology* 8: 4996-5004.

Yvon M and Rijene L. (2001) Cheese flavour formation by amino acid catabolism. *International Dairy Journal* 11: 185-201.

Zamora R and Hidalgo FJ. (2011) The Maillard reaction and lipid oxidation. *Lipid Technology* 23: 59-62.

Appendix 1 Material and Method

1.1 Microbiological Media Preparation for Analysis of Cheese Cultures

Referenced from International Dairy Federation documents (IDF, 2003; IDF, 2004; IDF, 2006; IDF, 2010)

A. 10% Reconstituted skim milk solution

10% (w/w) Reconstituted skim milk is prepared by mixing 4 g skim milk powder (Anchor skim milk powder, < 0.1% fat) with 20 ml of distilled water and topped to 40 ml. Sterilize by autoclave at 115°C for 10 minutes.

B. 0.1% Peptone solution *(Supplier: Oxoid, UK)*

Directions: Complete dissolve 1 g of peptone in 1000 ml of distilled water. Using disperser, disperse into appropriate volume depends on the tests. Sterilize by autoclave at 121°C for 15 minutes. Cool to below 50°C before in use.

C.* M17 agar *(Supplier: Oxoid, UK)

Composition:

Tryptone	5.0 g
Soy peptone	5.0 g
Yeast extract (dried)	2.5 g
Meat digest	5.0 g
Ascorbic acid	0.5 g
Di-sodium ß-Glycerophosphate	19.0 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.25 g
Lactose monohydrate	5.0 g
Agar	11 g
Water up to	950 ml
Final pH (at 25°C)	7.1 ±0.2

Directions: Suspend 48.25 g in 950 ml of distilled water and bring gently to the boil. Sterilize by autoclave at 121°C for 15 minutes. Cool to 50°C and add 50 ml of sterile lactose solution (10%, w/v).

Lactose Solution *(Supplier: BDH, USA)*

Dissolve 10 g of lactose in 100ml of distilled water. Sterilizing by autoclaving at 121°C for 15 minutes.

D.* MRS agar (adjusted to 6.5)(Molten de Man Rogosa Sharpe Agar)*(Supplier: Oxoid, UK)

Composition:

Peptone 1 (tryptic digest of casein)	10.0 g
'Lab-Lemco' powder	8.0 g
Yeast extract (dried)	4.0 g
Glucose	20.0 g
Tween 80 (sorbitan mono-oleate)	1.0 ml
Di-potassium hydrogen phosphate (K_2HPO_4)	2.0 g
Sodium acetate trihydrate ($Na\ CH_3CO_2.3H_2O$)	5.0 g
Tri-ammonium citrate ($(NH_4)_2\ HC_6H_5O_7$)	2.0 g
Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)	0.2 g
Manganese sulphate tetrahydrate ($MnSO_4.4H_2O$)	0.05 g

Agar	10 g
Water up to	1000 ml
Final pH (at 25°C)	6.6 ±0.1

Directions: Suspend 62 g in 1 L of distilled water and bring gently to the boil. Sterilize by autoclave at 121°C for 15 minutes.

E. YGC agar (Yeast Extract Glucose Chloramphenicol agar)*(Supplier: Merk, UK)*

Composition:

Yeast extract powder	5.0 g
Dextrose/Glucose ($C_6H_{12}O_6$)	20.0 g
Chloramphenicol ($C_{11}H_{12}Cl_2N_5O_5$)	0.1 g
Agar	12-15 g
Water up to	1000 ml
Final pH (at 25°C)	6.6 ±0.2

Directions: Suspend 40 g in 1 L of distilled water and bring gently to the boil. Sterilize by autoclave at 121°C for 15 minutes.

F. Nickels and Leesment medium + X-gal

Basic medium

Composition:

Tryptic digest of casein	20.0 g
Yeast extract	5.0g
Gelatine	2.5g
Dextrose/Glucose ($C_6H_{12}O_6$)	5.0 g
Lactose ($C_{12}H_{22}O_{11}$)	5.0 g
Sodium chloride (NaCl)	4.0g
Trisodium citrate dehydrate ($C_6H_5Na_3O_7.2H_2O$)	2.0g
Agar	12-15 g
Water up to	1000 ml
Final pH (at 25°C)	6.6-6.7

Directions: Suspend separately each of the above-mentioned components in 1 L of distilled water and bring gently to the boil for complete dissolve. Sterilize by autoclave at 121°C for 15 minutes.

Calcium lactate solution

Composition

Calcium lactate pentahydrate ($C_6H_{10}CaO_6.5H_2O$) (*Thermofisher, NZ)*	8.0g
Distilled water up to	100 ml

Dissolve the calcium lactate pentrahydrate in the water by heating. Sterilize in an autoclave set at 121°C for 15 minutes.

Calcium citrate suspension

Composition

Tricalcium dicitrate tetrahydrate ($C_{12}H_{10}Ca_3O_{14}.4H_2O$) (*Thermofisher, NZ)*	13.3g
Carboxymethylcellulose (CMC)	0.8g
Distilled water up to	100 ml

Grind the calcium citrate tetrahydrate, which has previously been sifted through a sieve of 0.8 mm nominal aperture size, and the CMC together in a mortar.

Slowly add pre-warmed water at approximately 45°C to a final volume of 100 ml. Blend the obtained mixture for 10 min and vacuum filter through cotton cloth. Sterilize the filtered suspension in an autoclave set at 121°C for 15 minutes. Note: Approximately 30% of the calcium citrate is lost during filtration.

Starter culture serum

Prepare the starter culture medium by growing a L-, D-, or DL-starter culture in autoclaved recombined skimmed milk at 25 ± 1 °C for 24 h. Filter through filter paper and sterilize the filtrate at 115 ± 1 °C for 15 min. Remove any sediment by decantation and sterilize 200 ml of filtrate once again.

X-gal solution

Composition

X-gal (5-Bromo-4-chloro-3-indolyl- ß-D-galactopyranoside)	400mg
N-Methyl-2-pyrrolidone (NMP)	100ml

X-gal and NMP are toxic and were handled in a fume cupboard. Dissolve the X-gal in NMP and sterilize by filtration through a 0.45 μm Durapore filter (from Milipore). Store the solution at 20 °C.

Complete medium

Composition

Basic medium	900 ml
Calcium lactate solution	100ml
Calcium citrate suspension	50ml
Starter culture serum	100ml

Before use, melt the basic medium and cool it to between 48 and 50 °C. Pre-warm the calcium lactate solution, the calcium citrate suspension and the starter culture serum in a water bath set at between 48 and 50 °C. Aseptically, add each of them to the melted basic medium and mix.

General preparation of culture media

Each reconstituted milk solution, 0.1% peptone solution, MRS, M17 and Nickels and Leesment agar medium were prepared from various dehydrated medium following the manufactures instructions. In all cases, dehydrated powder mix was suspended in the cold distilled water and heated to boiling point with frequent agitation until the complete dissolve. The medium were distributed in portions of 200 ml into bottles of 250 ml capacity or in portions of 400 ml into bottles of 500 ml capacity, and in subsequent were sterilized in autoclave at 121°C for 15 minutes for agars and peptone solution, and at 115°C for 10 minutes for reconstituted milk. The prepared mediums were preferred to be used immediately. Before in use, they were cooled in a water bath to 45 ± 1°C without exposure to direct sunlight.

1.2 Titratable acidity Media preparation

Referenced from International American Organization of Analytical Chemists International (2005b) standard method 947.05

Preparation of reagents

1% phenolphthalein indicator solution: Dissolve 1 g phenolphthalein powder into 100 ml ethanol (95%) and mix thoroughly. Store in bottle.

0.1 M sodium hydroxide solution (NaOH): Weight out 4 g NaOH pellets and dissolves in 1 L CO_2-free distilled water in a 1 L volumetric flask. Once cool, make up to volume an invert carefully to thoroughly mix solution. Always standardise solution to confirm concentration.

Potassium hydrogen phthalate-as standard solution: Dry a small quantity of Potassium hydrogen phthalate at 120 °C for 2 h and then cool in air-tight desiccators with fresh desiccant for at least 60 minutes. Transfer quickly to a clean, dry air-tight jar and tighten lid firmly. To prepare the solution, hot dissolve about 0.2042 g oven dried potassium phthalate (record weight to 0.0001 g) into 25 ml of distilled water using hotplate. Allow to cool.

1.3 Calculation for Direct-Vat-Set (DVS) purpose

Accoding to Kindsedt (2005), the manufacturer's recommendations concerning the amount of DVS starter to use are based on a quantity called a unit (The U on the package stands for 'units'), which usually refer as 1% innoculation. One unit is the amount of starter that will turn a specific quantity of milk into lactic curd (without the addition of rennet) at a specific temperature in a certain amount of time.

Calculations:

DVS R704 culture with bulk bag weighted 5.12 g

$$1\% = \frac{50\text{U}}{500\text{ L}} = \frac{5.12\text{ g}}{500\text{ L}} = \frac{0.01024\text{ g of pure concentrate culture}}{\text{L}}$$

Since R704 packed sachet contained 0.06 g of concentrate culture and 0.96 g of lactose, to obtain 0.01024 g of pure conentrate culture from the packed R704 sachet, 0.17067 g of culture samples is needed for innoculating 1 L of milk at 1% innoculation level. To inoculate at 0.5% level in the expeirment, 0.0853 g of culture samples were weighted to treat 1 L of milk;

DVS Flora Dancia culture with bulk bag weighted 11.11 g

$$1\% = \frac{50\text{U}}{500\text{ L}} = \frac{11.11\text{g}}{500\text{ L}} = \frac{0.0222\text{ g of pure concentrate culture}}{\text{L}}$$

Since Flora Dancia packed sachet contained 0.13 g of concentrate culture and 0.87 g of lactose, to obtain 0.0222 g of pure conentrate culture from the packed Flora Danica sachet, 0.1708 g of culture samples is needed for innoculating 1 L of milk at 1% innoculation level. To inoculate at 0.5% level in the expeirment, 0.0853 g of culture samples were weighted to treat 1 L of milk;

DVS PCTT033 culture with bulk bag weighted 2.36 g

$$1\% = \frac{10\text{U}}{1000\text{ L}} = \frac{2.36\text{g}}{1000\text{ L}} = \frac{0.00236\text{ g of pure concentrate culture}}{\text{L}}$$

Since PCTT packed sachet contained 0.01 g of concentrate culture and 0.99 g of lactose, to obtain 0.00236 g of pure conentrate culture from packed the PCTT033 sachet, 0.236 g of culture samples is needed for innoculating 1 L of milk at 1% innoculation level. To inoculate at 0.02% level in the expeirment, 0.00472 g of culture samples were weighted to treat 1 L of milk;

Appendix 2 Screen and selection of cheese starter and ripening cultures

2.1 Enumeration of cell counts

Group 1: LD-type LAB starters

Table 1. Flora Danica

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	8.60×10^{9}	6.70×10^{9}	$8.10\times10^{9}\pm7.07\times10^{8}$	9.93	9.88	9.91±0.04
	4	7.60×10^{9}	6.20×10^{9}	$6.90\times10^{9}\pm9.99\times10^{8}$	9.88	9.79	9.84±0.06
	8	6.10×10^{9}	6.80×10^{9}	$6.45\times10^{9}\pm4.95\times10^{8}$	9.79	9.83	9.81±0.03
	12	5.70×10^{9}	6.10×10^{9}	$5.90\times10^{9}\pm2.83\times10^{8}$	9.76	9.79	9.77±0.02
	16	5.40×10^{9}	6.20×10^{9}	$5.80\times10^{9}\pm5.66\times10^{8}$	9.73	9.79	9.76±0.04
	20	5.90×10^{9}	3.80×10^{9}	$4.85\times10^{9}\pm1.48\times10^{9}$	9.77	9.58	9.68±0.14
4 °C	0	8.60×10^{9}	6.70×10^{9}	$8.10\times10^{9}\pm7.07\times10^{8}$	9.93	9.88	9.91±0.04
	4	6.50×10^{9}	7.10×10^{9}	$6.80\times10^{9}\pm4.24\times10^{8}$	9.81	9.85	9.83±0.03
	8	6.20×10^{9}	5.40×10^{9}	$5.80\times10^{9}\pm5.66\times10^{8}$	9.79	9.73	9.76±0.04
	12	4.70×10^{9}	4.60×10^{9}	$4.65\times10^{9}\pm7.07\times10^{7}$	9.67	9.66	9.67±0.01
	16	3.70×10^{9}	4.10×10^{9}	$3.90\times10^{9}\pm2.83\times10^{8}$	9.57	9.61	9.59±0.03
	20	1.42×10^{9}	1.99×10^{9}	$1.71\times10^{9}\pm4.03\times10^{8}$	9.15	9.30	9.23±0.10
20 °C	0	8.60×10^{9}	6.70×10^{9}	$8.10\times10^{9}\pm7.07\times10^{8}$	9.93	9.88	9.91±0.04
	4	5.50×10^{9}	5.90×10^{9}	$5.70\times10^{9}\pm2.83\times10^{8}$	9.74	9.77	9.76±0.02
	8	5.10×10^{9}	3.20×10^{9}	$4.15\times10^{9}\pm1.34\times10^{9}$	9.71	9.51	9.61±0.14
	12	1.76×10^{9}	1.98×10^{9}	$1.87\times10^{9}\pm1.56\times10^{8}$	9.25	9.30	9.27±0.04
	16	1.28×10^{9}	1.69×10^{9}	$1.49\times10^{9}\pm2.90\times10^{8}$	9.11	9.23	9.17±0.09
	20	9.90×10^{8}	1.60×10^{9}	$1.30\times10^{9}\pm4.31\times10^{8}$	9.00	9.20	9.10±0.15
37 °C	0	8.60×10^{9}	6.70×10^{9}	$8.10\times10^{9}\pm7.07\times10^{8}$	9.93	9.88	9.91±0.04
	4	1.87×10^{6}	1.39×10^{6}	$1.63\times10^{6}\pm3.39\times10^{5}$	6.27	6.14	6.21±0.09
	8	4.80×10^{5}	1.02×10^{6}	$7.50\times10^{5}\pm3.82\times10^{5}$	5.68	6.01	5.84±0.23
	12	9.10×10^{4}	4.90×10^{4}	$7.00\times10^{4}\pm2.97\times10^{4}$	4.96	4.70	4.82±0.19
	16	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00
	20	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00

Table 2. CHN-19

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	1.70×10^{10}	1.27×10^{10}	$1.49\times10^{10}\pm3.04\times10^{9}$	10.23	10.10	10.17±0.09
	4	1.80×10^{10}	1.31×10^{10}	$1.56\times10^{10}\pm3.46\times10^{9}$	10.26	10.12	10.19±0.10
	8	9.80×10^{9}	1.06×10^{10}	$1.02\times10^{10}\pm5.06\times10^{8}$	9.99	10.03	10.01±0.02
	12	9.30×10^{9}	6.70×10^{9}	$8.00\times10^{9}\pm1.84\times10^{9}$	9.97	9.83	9.90±0.10
	16	7.10×10^{9}	6.20×10^{9}	$6.65\times10^{9}\pm6.36\times10^{8}$	9.85	9.79	9.82±0.04
	20	5.80×10^{9}	4.60×10^{9}	$5.20\times10^{9}\pm8.49\times10^{8}$	9.76	9.66	9.71±0.07
	0	1.70×10^{10}	1.27×10^{10}	$1.49\times10^{10}\pm3.04\times10^{9}$	10.23	10.10	10.17±0.09

4 °C	4	1.49×10^{10}	1.61×10^{10}	$1.55\times10^{10}\pm8.49\times10^{8}$	10.17	10.21	10.19±0.02
	8	9.50×10^{9}	8.70×10^{9}	$9.10\times10^{9}\pm5.67\times10^{8}$	9.98	9.94	9.96±0.03
	12	7.80×10^{9}	6.20×10^{9}	$7.00\times10^{9}\pm1.13\times10^{9}$	9.89	9.79	9.84±0.07
	16	5.60×10^{9}	5.30×10^{9}	$5.45\times10^{9}\pm2.12\times10^{8}$	9.75	9.72	9.74±0.02
	20	5.40×10^{9}	4.90×10^{9}	$5.15\times10^{9}\pm3.54\times10^{8}$	9.73	9.69	9.71±0.03
20 °C	0	1.70×10^{10}	1.27×10^{10}	$1.49\times10^{10}\pm3.04\times10^{9}$	10.23	10.10	10.17±0.09
	4	1.37×10^{10}	1.40×10^{10}	$1.39\times10^{10}\pm2.12\times10^{8}$	10.14	10.15	10.14±0.01
	8	5.60×10^{9}	5.20×10^{9}	$5.40\times10^{9}\pm2.83\times10^{8}$	9.75	9.72	9.73±0.02
	12	4.27×10^{9}	3.93×10^{9}	$4.10\times10^{9}\pm2.40\times10^{8}$	9.63	9.59	9.61±0.03
	16	2.62×10^{9}	2.35×10^{9}	$2.49\times10^{9}\pm1.91\times10^{8}$	9.42	9.37	9.39±0.03
	20	2.00×10^{9}	2.40×10^{9}	$2.20\times10^{9}\pm2.83\times10^{8}$	9.30	9.38	9.34±0.06
37 °C	0	1.70×10^{10}	1.27×10^{10}	$1.49\times10^{10}\pm3.04\times10^{9}$	10.23	10.10	10.17±0.09
	4	1.50×10^{5}	1.90×10^{5}	$1.70\times10^{5}\pm2.83\times10^{4}$	5.18	5.28	5.23±0.07
	8	5.10×10^{3}	2.20×10^{3}	$3.65\times10^{3}\pm2.05\times10^{3}$	3.71	3.34	3.52±0.26
	12	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00
	16	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00
	20	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00

Table 3. Delvo-Tec DX-33A DSL

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	1.90×10^{11}	1.60×10^{11}	$1.75\times10^{11}\pm2.12\times10^{10}$	11.28	11.21	11.21±0.05
	4	1.67×10^{11}	1.69×10^{11}	$1.68\times10^{11}\pm1.41\times10^{9}$	11.22	11.23	11.23±0.00
	8	1.61×10^{11}	1.58×10^{11}	$1.60\times10^{11}\pm2.12\times10^{9}$	11.21	11.20	11.20±0.01
	12	1.48×10^{11}	1.31×10^{11}	$1.40\times10^{11}\pm1.20\times10^{10}$	11.17	11.12	11.14±0.04
	16	1.20×10^{11}	1.16×10^{11}	$1.18\times10^{11}\pm2.83\times10^{9}$	11.08	11.06	11.07±0.01
	20	1.13×10^{11}	9.80×10^{10}	$1.06\times10^{11}\pm1.06\times10^{10}$	11.05	10.99	11.02±0.04
4 °C	0	1.90×10^{11}	1.60×10^{11}	$1.75\times10^{11}\pm2.12\times10^{10}$	11.28	11.21	11.21±0.05
	4	1.29×10^{11}	1.71×10^{11}	$1.50\times10^{11}\pm2.97\times10^{10}$	11.11	11.23	11.17±0.09
	8	1.22×10^{11}	1.67×10^{11}	$1.45\times10^{11}\pm3.18\times10^{10}$	11.09	11.22	11.15±0.10
	12	1.12×10^{11}	1.42×10^{11}	$1.27\times10^{11}\pm2.12\times10^{10}$	11.05	11.15	11.10±0.07
	16	1.41×10^{11}	1.04×10^{11}	$1.23\times10^{11}\pm2.62\times10^{10}$	11.15	11.02	11.08±0.09
	20	6.80×10^{10}	7.50×10^{10}	$7.15\times10^{10}\pm4.95\times10^{9}$	10.83	10.88	10.85±0.03
20 °C	0	1.90×10^{11}	1.60×10^{11}	$1.75\times10^{11}\pm2.12\times10^{10}$	11.28	11.21	11.21±0.05
	4	1.39×10^{11}	1.21×10^{11}	$1.30\times10^{11}\pm1.27\times10^{10}$	11.14	11.08	11.11±0.04
	8	1.29×10^{11}	1.04×10^{11}	$1.17\times10^{11}\pm1.77\times10^{10}$	11.11	11.02	11.06±0.07
	12	1.06×10^{11}	1.11×10^{11}	$1.09\times10^{11}\pm3.54\times10^{9}$	11.03	11.05	11.04±0.01
	16	9.20×10^{10}	8.80×10^{10}	$9.00\times10^{10}\pm2.83\times10^{9}$	10.96	10.94	10.95±0.01
	20	8.00×10^{10}	4.70×10^{10}	$6.35\times10^{10}\pm2.33\times10^{10}$	10.90	10.67	10.79±0.16
	0	1.90×10^{11}	1.60×10^{11}	$1.75\times10^{11}\pm2.12\times10^{10}$	11.28	11.21	11.21±0.05
	4	1.34×10^{8}	2.03×10^{8}	$1.69\times10^{8}\pm4.88\times10^{7}$	8.13	8.31	8.22±0.13
	8	2.15×10^{4}	3.32×10^{4}	$2.74\times10^{4}\pm8.27\times10^{3}$	4.33	4.52	4.43±0.13
	12	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00

37 °C	16	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00
	20	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00

Table 4. CM51

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	9.70×10^9	9.20×10^9	$9.45\times10^9\pm3.54\times10^8$	9.99	9.96	9.98±0.02
	4	9.30×10^9	8.70×10^9	$9.00\times10^9\pm4.24\times10^8$	9.97	9.94	9.95±0.02
	8	8.10×10^9	7.40×10^9	$7.75\times10^9\pm4.95\times10^8$	9.91	9.87	9.89±0.03
	12	4.70×10^9	5.30×10^9	$5.00\times10^9\pm4.24\times10^8$	9.67	9.72	9.70±0.04
	16	3.30×10^9	3.70×10^9	$3.50\times10^9\pm2.83\times10^8$	9.52	9.57	9.54±0.04
	20	2.50×10^9	2.30×10^9	$2.40\times10^9\pm1.41\times10^8$	9.40	9.36	9.38±0.03
4 °C	0	9.70×10^9	9.20×10^9	$9.45\times10^9\pm3.54\times10^8$	9.99	9.96	9.98±0.02
	4	5.20×10^9	8.90×10^9	$7.05\times10^9\pm2.62\times10^9$	9.72	9.95	9.83±0.17
	8	6.30×10^9	4.60×10^9	$5.45\times10^9\pm1.20\times10^9$	9.80	9.66	9.73±0.10
	12	4.20×10^9	4.80×10^9	$4.50\times10^9\pm4.24\times10^8$	9.62	9.68	9.65±0.04
	16	2.72×10^9	2.92×10^9	$2.82\times10^9\pm1.41\times10^8$	9.43	9.47	9.45±0.02
	20	1.97×10^9	2.01×10^9	$1.99\times10^9\pm2.83\times10^7$	9.29	9.30	9.30±0.01
20 °C	0	9.70×10^9	9.20×10^9	$9.45\times10^9\pm3.54\times10^8$	9.99	9.96	9.98±0.02
	4	5.10×10^9	6.40×10^9	$5.75\times10^9\pm9.19\times10^8$	9.71	9.81	9.76±0.07
	8	2.12×10^9	2.61×10^9	$2.37\times10^9\pm3.46\times10^8$	9.33	9.42	9.37±0.06
	12	1.57×10^9	1.35×10^9	$1.46\times10^9\pm1.56\times10^8$	9.20	9.13	9.16±0.05
	16	6.10×10^8	4.60×10^8	$5.35\times10^8\pm1.06\times10^8$	8.79	8.66	8.72±0.09
	20	5.70×10^8	4.80×10^8	$5.25\times10^8\pm6.36\times10^7$	8.76	8.68	8.72±0.05
37 °C	0	9.70×10^9	9.20×10^9	$9.45\times10^9\pm3.54\times10^8$	9.99	9.96	9.98±0.02
	4	6.00×10^3	2.00×10^3	$4.00\times10^3\pm2.83\times10^3$	3.78	3.30	3.54±0.34
	8	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00
	12	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00
	16	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00
	20	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00

Table 5. Probat 222 LYO

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	2.20×10^9	1.90×10^9	$2.05\times10^9\pm2.12\times10^8$	9.34	9.28	9.31±0.05
	4	1.84×10^9	1.37×10^9	$1.61\times10^9\pm3.32\times10^8$	9.26	9.14	9.20±0.09
	8	1.27×10^9	1.23×10^9	$1.25\times10^9\pm2.83\times10^7$	9.10	9.09	9.10±0.01
	12	1.09×10^9	1.32×10^9	$1.21\times10^9\pm1.63\times10^8$	9.04	9.12	9.08±0.06
	16	9.50×10^8	1.52×10^9	$1.24\times10^9\pm4.03\times10^8$	8.98	9.18	9.08±0.14
	20	1.39×10^9	9.40×10^8	$1.17\times10^9\pm3.18\times10^8$	9.14	8.97	9.06±0.12
	0	2.20×10^9	1.90×10^9	$2.05\times10^9\pm2.12\times10^8$	9.34	9.28	9.31±0.05
	4	1.24×10^9	1.72×10^9	$1.48\times10^9\pm3.39\times10^8$	9.09	9.24	9.16±0.10

4 °C	8	1.48×10^{9}	9.6×10^{8}	$1.22\times10^{9}\pm3.68\times10^{8}$	9.17	8.98	9.08±0.13
	12	9.10×10^{8}	1.24×10^{9}	$1.08\times10^{9}\pm2.33\times10^{8}$	8.95	9.09	9.03±0.10
	16	9.40×10^{8}	1.12×10^{9}	$1.03\times10^{9}\pm1.37\times10^{8}$	8.97	9.05	9.01±0.05
	20	6.30×10^{8}	1.07×10^{9}	$8.50\times10^{8}\pm3.11\times10^{8}$	8.80	9.03	8.91±0.16
20 °C	0	2.20×10^{9}	1.90×10^{9}	$2.05\times10^{9}\pm2.12\times10^{8}$	9.34	9.28	9.31±0.05
	4	8.20×10^{8}	9.70×10^{8}	$8.95\times10^{8}\pm1.06\times10^{8}$	8.91	8.99	8.95±0.05
	8	1.08×10^{8}	1.28×10^{8}	$1.18\times10^{8}\pm1.41\times10^{7}$	8.03	8.11	8.07±0.05
	12	9.60×10^{7}	8.50×10^{7}	$9.05\times10^{7}\pm7.78\times10^{6}$	7.98	7.93	7.96±0.04
	16	8.20×10^{7}	7.70×10^{7}	$7.95\times10^{7}\pm3.54\times10^{6}$	7.91	7.89	7.90±0.02
	20	6.60×10^{7}	5.50×10^{7}	$6.05\times10^{7}\pm7.78\times10^{6}$	7.82	7.74	7.78±0.06
37 °C	0	2.20×10^{9}	1.90×10^{9}	$2.05\times10^{9}\pm2.12\times10^{8}$	9.34	9.28	9.31±0.05
	4	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00
	8	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00
	12	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00
	16	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00
	20	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00

Table 6. M036R

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	3.30×10^{9}	3.80×10^{9}	$3.55\times10^{9}\pm3.54\times10^{8}$	9.52	9.58	9.55±0.04
	4	3.20×10^{9}	3.70×10^{9}	$3.45\times10^{9}\pm3.54\times10^{8}$	9.51	9.57	9.54±0.04
	8	2.91×10^{9}	3.08×10^{9}	$3.00\times10^{9}\pm1.20\times10^{8}$	9.46	9.49	9.48±0.02
	12	2.56×10^{9}	2.34×10^{9}	$2.45\times10^{9}\pm1.56\times10^{8}$	9.41	9.40	9.39±0.03
	16	1.63×10^{9}	1.39×10^{9}	$1.51\times10^{9}\pm1.70\times10^{8}$	9.21	9.14	9.18±0.45
	20	1.41×10^{9}	1.07×10^{9}	$1.24\times10^{9}\pm2.40\times10^{8}$	9.15	9.03	9.09±0.08
4 °C	0	3.30×10^{9}	3.80×10^{9}	$3.55\times10^{9}\pm3.54\times10^{8}$	9.52	9.58	9.55±0.04
	4	3.20×10^{9}	4.10×10^{9}	$3.65\times10^{9}\pm6.36\times10^{8}$	9.51	9.61	9.56±0.08
	8	2.15×10^{9}	2.35×10^{9}	$2.25\times10^{9}\pm1.41\times10^{8}$	9.33	9.37	9.35±0.03
	12	1.99×10^{9}	2.00×10^{9}	$2.00\times10^{9}\pm7.07\times10^{6}$	9.30	9.30	9.30±0.00
	16	1.51×10^{9}	1.31×10^{9}	$1.41\times10^{9}\pm1.41\times10^{8}$	9.18	9.12	9.15±0.04
	20	1.05×10^{9}	7.80×10^{8}	$9.15\times10^{8}\pm1.91\times10^{8}$	9.02	8.89	8.96±0.09
20 °C	0	3.30×10^{9}	3.80×10^{9}	$3.55\times10^{9}\pm3.54\times10^{8}$	9.52	9.58	9.55±0.04
	4	1.87×10^{9}	2.12×10^{9}	$2.00\times10^{9}\pm1.77\times10^{8}$	9.27	9.33	9.30±0.04
	8	1.85×10^{9}	2.04×10^{9}	$1.95\times10^{9}\pm1.34\times10^{8}$	9.27	9.31	9.29±0.03
	12	1.48×10^{9}	1.91×10^{9}	$1.70\times10^{9}\pm3.04\times10^{8}$	9.17	9.28	9.23±0.08
	16	1.05×10^{9}	1.12×10^{9}	$1.09\times10^{9}\pm4.95\times10^{7}$	9.02	9.05	9.04±0.02
	20	6.40×10^{8}	9.10×10^{8}	$7.75\times10^{8}\pm1.91\times10^{8}$	8.81	8.96	8.88±0.11
37 °C	0	3.30×10^{9}	3.80×10^{9}	$3.55\times10^{9}\pm3.54\times10^{8}$	9.52	9.58	9.55±0.04
	4	<1000	<1000	<1000±0.00	<3	<3	<3±0.00
	8	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00
	12	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00

	16	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00
	20	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00

Group 2: O-type LAB starters

Table 7. Mesophilic R704

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	1.81×10^{11}	1.95×10^{11}	$1.88\times10^{11}\pm9.90\times10^{9}$	11.26	11.29	11.27±0.02
	4	1.42×10^{11}	1.72×10^{11}	$1.57\times10^{11}\pm2.12\times10^{10}$	11.15	11.24	11.19±0.06
	8	8.70×10^{10}	9.20×10^{10}	$8.95\times10^{10}\pm3.54\times10^{9}$	10.94	10.96	10.95±0.02
	12	8.80×10^{10}	8.30×10^{10}	$8.55\times10^{10}\pm3.54\times10^{9}$	10.94	10.92	10.93±0.02
	16	3.40×10^{10}	5.70×10^{10}	$4.55\times10^{10}\pm1.63\times10^{10}$	10.53	10.76	10.64±0.16
	20	4.20×10^{10}	4.50×10^{10}	$4.35\times10^{10}\pm2.12\times10^{9}$	10.62	10.65	10.64±0.02
4 °C	0	1.81×10^{11}	1.95×10^{11}	$1.88\times10^{11}\pm9.90\times10^{9}$	11.26	11.29	11.27±0.02
	4	8.90×10^{10}	7.30×10^{10}	$8.10\times10^{10}\pm1.13\times10^{10}$	10.94	10.86	10.91±0.06
	8	5.30×10^{10}	6.80×10^{10}	$6.05\times10^{10}\pm1.06\times10^{10}$	10.72	10.83	10.78±0.08
	12	3.60×10^{10}	4.40×10^{10}	$4.00\times10^{10}\pm5.66\times10^{9}$	10.56	10.64	10.60±0.06
	16	3.30×10^{10}	2.90×10^{10}	$3.10\times10^{10}\pm2.83\times10^{9}$	10.52	10.46	10.49±0.04
	20	1.90×10^{10}	4.20×10^{10}	$3.05\times10^{10}\pm1.63\times10^{10}$	10.28	10.62	10.45±0.24
20 °C	0	1.81×10^{11}	1.95×10^{11}	$1.88\times10^{11}\pm9.90\times10^{9}$	11.26	11.29	11.27±0.02
	4	6.60×10^{10}	5.90×10^{10}	$6.25\times10^{10}\pm4.95\times10^{9}$	10.82	10.77	10.80±0.03
	8	9.30×10^{9}	1.52×10^{10}	$1.23\times10^{10}\pm4.17\times10^{9}$	9.97	10.18	10.08±0.15
	12	7.60×10^{9}	8.20×10^{9}	$7.90\times10^{9}\pm4.24\times10^{8}$	9.88	9.91	9.90±0.02
	16	2.20×10^{9}	3.70×10^{9}	$2.95\times10^{9}\pm1.06\times10^{9}$	9.34	9.57	9.46±0.16
	20	1.65×10^{9}	1.69×10^{9}	$1.67\times10^{9}\pm2.83\times10^{7}$	9.22	9.23	9.22±0.01
37 °C	0	1.81×10^{11}	1.95×10^{11}	$1.88\times10^{11}\pm9.90\times10^{9}$	11.26	11.29	11.27±0.02
	4	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00
	8	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00
	12	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00
	16	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00
	20	$<10^2$	$<10^2$	$<10^2$±0.00	<2	<2	<2±0.00

Table 8. Delvo-Tec LL-50A DSL

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	1.90×10^{12}	1.31×10^{12}	$1.61\times10^{12}\pm4.17\times10^{11}$	12.28	12.12	12.20±0.11
	4	1.78×10^{12}	1.34×10^{12}	$1.56\times10^{12}\pm3.11\times10^{11}$	12.25	12.13	12.19±0.09
	8	3.10×10^{11}	5.90×10^{11}	$4.50\times10^{11}\pm1.98\times10^{11}$	11.49	11.77	11.63±0.20
	12	2.18×10^{11}	2.27×10^{11}	$2.23\times10^{11}\pm6.36\times10^{9}$	11.34	11.36	11.35±0.01
	16	2.09×10^{11}	2.10×10^{11}	$2.10\times10^{11}\pm7.07\times10^{8}$	11.32	11.32	11.32±0.00
	20	1.73×10^{11}	1.82×10^{11}	$1.78\times10^{11}\pm6.36\times10^{9}$	11.24	11.26	11.25±0.02
	0	1.90×10^{12}	1.31×10^{12}	$1.61\times10^{12}\pm4.17\times10^{11}$	12.28	12.12	12.20±0.11
	4	5.20×10^{11}	6.60×10^{11}	$5.90\times10^{11}\pm9.90\times10^{10}$	11.72	11.82	11.77±0.07

4 °C	8	3.90×10^{11}	3.80×10^{11}	$3.85\times10^{11}\pm7.07\times10^{9}$	11.59	11.58	11.59±0.01
	12	2.12×10^{11}	1.91×10^{11}	$2.02\times10^{11}\pm1.49\times10^{10}$	11.33	11.28	11.30±0.03
	16	1.92×10^{11}	2.08×10^{11}	$2.00\times10^{11}\pm1.13\times10^{10}$	11.28	11.32	11.30±0.02
	20	1.30×10^{11}	2.22×10^{11}	$1.76\times10^{11}\pm6.51\times10^{10}$	11.11	11.34	11.23±0.16
20 °C	0	1.90×10^{12}	1.31×10^{12}	$1.61\times10^{12}\pm4.17\times10^{11}$	12.28	12.12	12.20±0.11
	4	3.70×10^{11}	4.90×10^{11}	$4.30\times10^{11}\pm8.49\times10^{10}$	11.57	11.69	11.63±0.09
	8	2.80×10^{11}	1.90×10^{11}	$2.35\times10^{11}\pm6.36\times10^{10}$	11.45	11.28	11.36±0.12
	12	2.68×10^{11}	1.97×10^{11}	$2.33\times10^{11}\pm5.02\times10^{10}$	11.43	11.29	11.36±0.09
	16	1.09×10^{11}	1.93×10^{11}	$1.51\times10^{11}\pm5.94\times10^{10}$	11.04	11.29	11.16±0.18
	20	8.90×10^{10}	1.32×10^{11}	$1.11\times10^{11}\pm3.04\times10^{10}$	10.95	11.12	11.03±0.12
37 °C	0	1.90×10^{12}	1.31×10^{12}	$1.61\times10^{12}\pm4.17\times10^{11}$	12.28	12.12	12.20±0.11
	4	2.60×10^{9}	2.13×10^{9}	$2.37\times10^{9}\pm3.32\times10^{8}$	9.41	9.33	9.37±0.06
	8	1.04×10^{5}	1.54×10^{5}	$1.29\times10^{5}\pm3.54\times10^{4}$	5.02	5.19	5.10±0.12
	12	2.40×10^{5}	1.72×10^{4}	$2.06\times10^{4}\pm4.81\times10^{3}$	4.38	4.24	4.31±0.10
	16	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00
	20	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00

Table 9. Choozit MA 11LYO

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	7.53×10^{10}	6.70×10^{10}	$7.12\times10^{10}\pm5.87\times10^{9}$	10.88	10.83	10.85±0.04
	4	7.40×10^{10}	6.20×10^{10}	$6.80\times10^{10}\pm8.49\times10^{9}$	10.87	10.79	10.83±0.05
	8	3.29×10^{10}	3.17×10^{10}	$3.23\times10^{10}\pm8.49\times10^{8}$	10.52	10.50	10.51±0.01
	12	2.21×10^{10}	2.74×10^{10}	$2.48\times10^{10}\pm3.75\times10^{9}$	10.34	10.44	10.39±0.07
	16	8.10×10^{9}	1.09×10^{10}	$9.50\times10^{9}\pm1.98\times10^{9}$	9.91	10.04	9.97±0.09
	20	9.30×10^{9}	8.90×10^{9}	$9.10\times10^{9}\pm2.83\times10^{8}$	9.97	9.95	9.96±0.01
4 °C	0	7.53×10^{10}	6.70×10^{10}	$7.12\times10^{10}\pm5.87\times10^{9}$	10.88	10.83	10.85±0.04
	4	3.70×10^{10}	4.50×10^{10}	$4.10\times10^{10}\pm5.65\times10^{9}$	10.57	10.65	10.61±0.06
	8	2.85×10^{10}	2.61×10^{10}	$2.73\times10^{10}\pm1.70\times10^{9}$	10.45	10.42	10.44±0.03
	12	2.58×10^{10}	2.07×10^{10}	$2.33\times10^{10}\pm3.61\times10^{9}$	10.41	10.32	10.36±0.07
	16	1.02×10^{10}	7.20×10^{9}	$8.70\times10^{9}\pm2.12\times10^{9}$	10.01	9.86	9.93±0.11
	20	7.10×10^{9}	8.20×10^{9}	$7.65\times10^{9}\pm7.78\times10^{8}$	9.85	9.91	9.88±0.04
20 °C	0	7.53×10^{10}	6.70×10^{10}	$7.12\times10^{10}\pm5.87\times10^{9}$	10.88	10.83	10.85±0.04
	4	3.20×10^{10}	3.80×10^{10}	$3.50\times10^{10}\pm4.24\times10^{9}$	10.51	10.58	10.54±0.05
	8	9.40×10^{9}	7.10×10^{9}	$8.25\times10^{9}\pm1.63\times10^{9}$	9.97	9.85	9.91±0.09
	12	3.70×10^{9}	4.40×10^{9}	$4.05\times10^{9}\pm4.95\times10^{8}$	9.57	9.64	9.61±0.05
	16	3.20×10^{9}	3.16×10^{9}	$3.18\times10^{9}\pm2.83\times10^{7}$	9.51	9.50	9.50±0.00
	20	3.30×10^{9}	2.60×10^{9}	$2.95\times10^{9}\pm4.95\times10^{8}$	9.52	9.41	9.47±0.07
37 °C	0	7.53×10^{10}	6.70×10^{10}	$7.12\times10^{10}\pm5.87\times10^{9}$	10.88	10.83	10.85±0.04
	4	1.00×10^{7}	8.00×10^{6}	$9.00\times10^{6}\pm1.41\times10^{6}$	7.00	6.90	6.95±0.07
	8	2.30×10^{4}	1.90×10^{4}	$2.10\times10^{4}\pm2.83\times10^{3}$	4.36	4.28	4.32±0.06
	12	6.40×10^{3}	7.20×10^{3}	$6.80\times10^{3}\pm5.66\times10^{2}$	3.81	3.86	3.83±0.04

	16	$<10^2$	$<10^2$	$<10^2 \pm 0.00$	<2	<2	<2±0.00
	20	$<10^2$	$<10^2$	$<10^2 \pm 0.00$	<2	<2	<2±0.00

Table 10. MO 032

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	1.59×10^{10}	1.45×10^{10}	$1.52\times10^{10}\pm9.9\times10^{8}$	10.20	10.16	10.18±0.03
	4	1.50×10^{10}	1.39×10^{10}	$1.45\times10^{10}\pm7.7\times10^{8}$	10.18	10.14	10.16±0.02
	8	1.37×10^{10}	1.44×10^{10}	$1.41\times10^{10}\pm4.95\times10^{8}$	10.14	10.16	10.15±0.02
	12	1.27×10^{10}	1.04×10^{10}	$1.16\times10^{10}\pm1.63\times10^{9}$	10.10	10.02	10.06±0.06
	16	9.90×10^{9}	9.20×10^{9}	$9.55\times10^{9}\pm4.95\times10^{8}$	9.99	9.96	9.98±0.02
	20	5.20×10^{9}	6.50×10^{9}	$5.85\times10^{9}\pm9.19\times10^{8}$	9.72	9.81	9.76±0.07
4 °C	0	1.59×10^{10}	1.45×10^{10}	$1.52\times10^{10}\pm9.9\times10^{8}$	10.20	10.16	10.18±0.03
	4	1.03×10^{10}	9.60×10^{9}	$9.95\times10^{9}\pm4.95\times10^{8}$	10.01	9.98	10.00±0.10
	8	7.30×10^{9}	8.07×10^{9}	$7.69\times10^{9}\pm5.44\times10^{8}$	9.86	9.91	9.89±0.03
	12	6.70×10^{9}	6.20×10^{9}	$6.45\times10^{9}\pm3.54\times10^{8}$	9.83	9.79	9.81±0.02
	16	1.71×10^{9}	1.84×10^{9}	$1.78\times10^{9}\pm9.19\times10^{7}$	9.23	9.26	9.25±0.02
	20	2.10×10^{9}	1.80×10^{9}	$1.95\times10^{9}\pm2.12\times10^{8}$	9.32	9.26	9.29±0.05
20 °C	0	1.59×10^{10}	1.45×10^{10}	$1.52\times10^{10}\pm9.9\times10^{8}$	10.20	10.16	10.18±0.03
	4	5.30×10^{9}	4.80×10^{9}	$5.05\times10^{9}\pm3.54\times10^{8}$	9.72	9.68	9.70±0.03
	8	9.90×10^{8}	6.70×10^{8}	$8.30\times10^{8}\pm2.26\times10^{8}$	9.00	8.83	8.91±0.12
	12	6.60×10^{8}	4.80×10^{8}	$5.70\times10^{8}\pm1.27\times10^{8}$	8.82	8.68	8.75±0.10
	16	4.30×10^{8}	3.70×10^{8}	$4.00\times10^{8}\pm4.24\times10^{7}$	8.63	8.57	8.60±0.05
	20	2.90×10^{8}	3.80×10^{8}	$3.35\times10^{8}\pm6.36\times10^{7}$	8.46	8.58	8.52±0.08
37 °C	0	1.59×10^{10}	1.45×10^{10}	$1.52\times10^{10}\pm9.9\times10^{8}$	10.20	10.16	10.18±0.03
	4	1.37×10^{6}	1.24×10^{6}	$1.16\times10^{6}\pm1.20\times10^{5}$	6.03	6.09	6.06±0.05
	8	$<10^2$	$<10^2$	$<10^2 \pm 0.00$	<2	<2	<2±0.00
	12	$<10^2$	$<10^2$	$<10^2 \pm 0.00$	<2	<2	<2±0.00
	16	$<10^2$	$<10^2$	$<10^2 \pm 0.00$	<2	<2	<2±0.00
	20	$<10^2$	$<10^2$	$<10^2 \pm 0.00$	<2	<2	<2±0.00

Table 11. CM11

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	8.00×10^{9}	7.70×10^{9}	$7.85\times10^{9}\pm2.12\times10^{8}$	9.90	9.89	9.89±0.01
	4	8.10×10^{9}	7.20×10^{9}	$7.65\times10^{9}\pm6.40\times10^{8}$	9.91	9.86	9.89±0.03
	8	4.90×10^{9}	3.00×10^{9}	$3.95\times10^{9}\pm1.34\times10^{9}$	9.69	9.48	9.58±0.15
	12	1.71×10^{9}	1.84×10^{9}	$1.78\times10^{9}\pm9.19\times10^{7}$	9.23	9.26	9.25±0.02
	16	1.62×10^{9}	1.31×10^{9}	$1.47\times10^{9}\pm2.19\times10^{8}$	9.21	9.12	9.16±0.07
	20	1.06×10^{9}	1.41×10^{9}	$1.24\times10^{9}\pm2.47\times10^{8}$	9.03	9.15	9.09±0.09
	0	8.00×10^{9}	7.70×10^{9}	$7.85\times10^{9}\pm2.12\times10^{8}$	9.90	9.89	9.89±0.01
	4	5.60×10^{9}	6.40×10^{9}	$6.00\times10^{9}\pm5.66\times10^{8}$	9.75	9.81	9.78±0.04
	8	2.90×10^{9}	3.10×10^{9}	$3.00\times10^{9}\pm1.41\times10^{8}$	9.46	9.49	9.48±0.02

4 °C	12	1.92×10^{9}	1.78×10^{9}	$1.85\times10^{9}\pm9.90\times10^{7}$	9.28	9.25	9.27±0.02
	16	1.37×10^{9}	1.16×10^{9}	$1.27\times10^{9}\pm1.48\times10^{8}$	9.14	9.06	9.10±0.05
	20	1.57×10^{9}	9.80×10^{8}	$1.28\times10^{9}\pm4.17\times10^{8}$	9.20	9.00	9.10±0.14
20 °C	0	8.00×10^{9}	7.70×10^{9}	$7.85\times10^{9}\pm2.12\times10^{8}$	9.90	9.89	9.89±0.01
	4	3.10×10^{9}	2.40×10^{9}	$2.75\times10^{9}\pm4.95\times10^{8}$	9.49	9.38	9.44±0.08
	8	1.00×10^{9}	9.10×10^{8}	$9.55\times10^{8}\pm6.37\times10^{7}$	9.00	8.96	8.98±0.03
	12	8.60×10^{8}	7.80×10^{8}	$8.20\times10^{8}\pm5.66\times10^{7}$	8.93	8.89	8.91±0.03
	16	5.50×10^{8}	2.10×10^{8}	$3.80\times10^{8}\pm2.40\times10^{8}$	8.74	8.32	8.53±0.30
	20	8.70×10^{7}	9.70×10^{7}	$9.20\times10^{7}\pm7.07\times10^{6}$	7.94	7.99	7.96±0.03
37 °C	0	8.00×10^{9}	7.70×10^{9}	$7.85\times10^{9}\pm2.12\times10^{8}$	9.90	9.89	9.89±0.01
	4	7.00×10^{5}	5.00×10^{5}	$6.00\times10^{5}\pm1.41\times10^{5}$	5.85	5.70	5.77±0.10
	8	1.25×10^{5}	1.39×10^{5}	$1.32\times10^{5}\pm9.90\times10^{3}$	5.10	5.14	5.12±0.03
	12	3.18×10^{4}	3.41×10^{4}	$3.30\times10^{4}\pm1.63\times10^{3}$	4.50	4.53	4.52±0.02
	16	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00
	20	$<10^{2}$	$<10^{2}$	$<10^{2}\pm0.00$	<2	<2	<2±0.00

Group 3: Penicillium (ripening) cultures

Table 12. Camemberti Swing PCTT033

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	7.90×10^{7}	6.50×10^{7}	$7.20\times10^{7}\pm9.90\times10^{6}$	7.90	7.81	7.86±0.06
	4	6.60×10^{7}	7.10×10^{7}	$6.85\times10^{7}\pm3.54\times10^{6}$	7.82	7.85	9.84±0.02
	8	5.80×10^{7}	8.20×10^{7}	$7.00\times10^{7}\pm1.70\times10^{7}$	7.76	7.91	7.84±0.11
	12	6.20×10^{7}	7.80×10^{7}	$7.00\times10^{7}\pm1.13\times10^{7}$	7.79	7.89	7.84±0.07
	16	6.20×10^{7}	6.30×10^{7}	$6.25\times10^{7}\pm7.07\times10^{5}$	7.79	7.80	7.80±0.00
	20	5.90×10^{7}	5.40×10^{7}	$5.65\times10^{7}\pm3.54\times10^{6}$	7.77	7.73	7.75±0.03
4 °C	0	7.90×10^{7}	6.50×10^{7}	$7.20\times10^{7}\pm9.90\times10^{6}$	7.90	7.81	7.86±0.06
	4	4.90×10^{7}	5.20×10^{7}	$5.05\times10^{7}\pm2.12\times10^{6}$	7.69	7.72	7.70±0.02
	8	5.30×10^{7}	3.90×10^{7}	$4.60\times10^{7}\pm9.90\times10^{6}$	7.72	7.59	7.66±0.09
	12	4.00×10^{7}	3.90×10^{7}	$3.95\times10^{7}\pm7.07\times10^{5}$	7.60	7.59	7.60±0.01
	16	2.20×10^{7}	3.80×10^{7}	$3.00\times10^{7}\pm1.13\times10^{7}$	7.34	7.58	7.46±0.17
	20	3.40×10^{7}	2.90×10^{7}	$3.15\times10^{7}\pm3.54\times10^{6}$	7.53	7.46	7.50±0.05
20 °C	0	7.90×10^{7}	6.50×10^{7}	$7.20\times10^{7}\pm9.90\times10^{6}$	7.90	7.81	7.86±0.06
	4	6.50×10^{7}	6.80×10^{7}	$6.65\times10^{7}\pm2.12\times10^{6}$	7.81	7.83	7.82±0.01
	8	5.70×10^{7}	5.80×10^{7}	$5.75\times10^{7}\pm7.07\times10^{5}$	7.76	7.76	7.76±0.01
	12	4.30×10^{7}	4.10×10^{7}	$4.20\times10^{7}\pm1.41\times10^{6}$	7.63	7.61	7.62±0.01
	16	3.40×10^{7}	2.30×10^{7}	$2.85\times10^{7}\pm7.78\times10^{6}$	7.53	7.36	7.45±0.12
	20	1.21×10^{7}	1.12×10^{7}	$1.17\times10^{7}\pm6.36\times10^{5}$	7.08	7.05	7.07±0.02
37 °C	0	7.90×10^{7}	6.50×10^{7}	$7.20\times10^{7}\pm9.90\times10^{6}$	7.90	7.81	7.86±0.06
	4	$<10^{3}$	$<10^{3}$	$<10^{3}\pm0.00$	<3	<3	<3±0.00
	8	$<10^{3}$	$<10^{3}$	$<10^{3}\pm0.00$	<3	<3	<3±0.00
	12	$<10^{3}$	$<10^{3}$	$<10^{3}\pm0.00$	<3	<3	<3±0.00
	16	$<10^{3}$	$<10^{3}$	$<10^{3}\pm0.00$	<3	<3	<3±0.00

	20	$<10^3$	$<10^3$	$<10^3$±0.00	<3	<3	<3±0.00

Table 13. PC neige

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	7.80×10^7	6.90×10^7	$7.35\times10^7\pm6.36\times10^6$	7.89	7.84	7.87±0.04
	4	5.70×10^7	6.10×10^7	$5.90\times10^7\pm2.83\times10^6$	7.76	7.79	7.77±0.02
	8	4.80×10^7	5.30×10^7	$5.05\times10^7\pm3.54\times10^6$	7.68	7.72	7.70±0.05
	12	3.80×10^7	4.50×10^7	$4.15\times10^7\pm4.95\times10^6$	7.58	7.65	7.62±0.05
	16	4.40×10^7	2.60×10^7	$3.50\times10^7\pm1.27\times10^7$	7.64	7.41	7.53±0.16
	20	4.50×10^7	2.00×10^7	$3.25\times10^7\pm1.77\times10^7$	7.65	7.30	7.48±0.25
4 °C	0	7.80×10^7	6.90×10^7	$7.35\times10^7\pm6.36\times10^6$	7.89	7.84	7.87±0.04
	4	3.30×10^7	3.60×10^7	$3.45\times10^7\pm2.12\times10^6$	7.52	7.56	7.54±0.03
	8	2.20×10^7	2.30×10^7	$2.25\times10^7\pm7.07\times10^5$	7.34	7.36	7.35±0.01
	12	2.10×10^7	1.60×10^7	$1.85\times10^7\pm3.54\times10^6$	7.32	7.20	7.26±0.08
	16	6.60×10^6	5.10×10^6	$5.85\times10^6\pm1.06\times10^6$	6.82	6.71	6.76±0.08
	20	2.60×10^6	3.20×10^6	$2.90\times10^6\pm4.24\times10^5$	6.41	6.51	6.46±0.06
20 °C	0	7.80×10^7	6.90×10^7	$7.35\times10^7\pm6.36\times10^6$	7.89	7.84	7.87±0.04
	4	3.10×10^7	3.70×10^7	$3.40\times10^7\pm4.24\times10^6$	7.49	7.57	7.53±0.05
	8	3.10×10^7	2.10×10^7	$2.60\times10^7\pm7.07\times10^6$	7.49	7.32	7.41±0.12
	12	1.80×10^7	1.80×10^7	$1.80\times10^7\pm0.00$	7.26	7.26	7.26±0.00
	16	5.50×10^6	4.00×10^6	$4.75\times10^6\pm1.06\times10^6$	6.74	6.60	6.67±0.10
	20	1.30×10^6	2.70×10^6	$2.00\times10^6\pm9.90\times10^5$	6.11	6.43	6.27±0.22
37 °C	0	7.80×10^7	6.90×10^7	$7.35\times10^7\pm6.36\times10^6$	7.89	7.84	7.87±0.04
	4	1.00×10^4	1.00×10^4	$1.00\times10^4\pm0.00$	4.00	4.00	4.00±0.00
	8	$<10^3$	$<10^3$	$<10^3\pm0.00$	<3	<3	<3±0.00
	12	$<10^3$	$<10^3$	$<10^3\pm0.00$	<3	<3	<3±0.00
	16	$<10^3$	$<10^3$	$<10^3\pm0.00$	<3	<3	<3±0.00
	20	$<10^3$	$<10^3$	$<10^3\pm0.00$	<3	<3	<3±0.00

Table 14. PCV 5

Storage Temp (°C)	Storage Time (weeks)	Viable cells (cfu/g)			Log Viable cell (log cfu/g)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	2.94×10^8	2.03×10^8	$2.49\times10^8\pm6.43\times10^7$	8.47	8.31	8.39±0.11
	4	2.30×10^8	2.30×10^8	$2.30\times10^8\pm0.00$	8.36	8.36	8.36±0.00
	8	6.70×10^7	7.80×10^7	$7.25\times10^7\pm7.78\times10^6$	7.83	7.89	7.86±0.05
	12	6.30×10^7	6.30×10^7	$6.30\times10^7\pm0.00$	7.80	7.80	7.80±0.00
	16	5.70×10^7	4.30×10^7	$5.00\times10^7\pm9.90\times10^6$	7.76	7.63	7.69±0.09
	20	5.20×10^7	4.40×10^7	$4.80\times10^7\pm5.66\times10^6$	7.72	7.64	7.68±0.05
4 °C	0	2.94×10^8	2.03×10^8	$2.49\times10^8\pm6.43\times10^7$	8.47	8.31	8.39±0.11
	4	7.60×10^7	8.40×10^7	$8.00\times10^7\pm5.66\times10^6$	7.88	7.92	7.90±0.03
	8	5.40×10^7	6.80×10^7	$6.10\times10^7\pm9.90\times10^6$	7.73	7.83	7.78±0.07
	12	5.80×10^7	5.50×10^7	$5.65\times10^7\pm2.12\times10^6$	7.76	7.74	7.75±0.02

	16	4.30×10^7	2.60×10^7	$3.45\times10^7\pm1.20\times10^7$	7.63	7.41	7.52±0.15
	20	1.90×10^7	1.30×10^7	$1.60\times10^7\pm4.24\times10^6$	7.28	7.11	7.20±0.12
20 °C	0	2.94×10^8	2.03×10^8	$2.49\times10^8\pm6.43\times10^7$	8.47	8.31	8.39±0.11
	4	4.50×10^7	4.90×10^7	$4.70\times10^7\pm2.83\times10^6$	7.65	7.69	7.67±0.03
	8	4.80×10^7	3.90×10^7	$4.35\times10^7\pm6.36\times10^6$	7.68	7.59	7.64±0.06
	12	4.80×10^7	3.00×10^7	$3.90\times10^7\pm1.27\times10^7$	7.68	7.48	7.58±0.14
	16	2.20×10^7	1.70×10^7	$1.95\times10^7\pm3.54\times10^6$	7.34	7.23	7.29±0.08
	20	9.20×10^6	1.27×10^7	$1.10\times10^7\pm2.47\times10^6$	6.96	7.10	7.03±0.10
37 °C	0	2.94×10^8	2.03×10^8	$2.49\times10^8\pm6.43\times10^7$	8.47	8.31	8.39±0.11
	4	2.00×10^4	1.00×10^4	$1.50\times10^4\pm7.07\times10^3$	4.30	4.00	4.15±0.21
	8	$<10^3$	$<10^3$	$<10^3\pm0.00$	<3	<3	<3±0.00
	12	$<10^3$	$<10^3$	$<10^3\pm0.00$	<3	<3	<3±0.00
	16	$<10^3$	$<10^3$	$<10^3\pm0.00$	<3	<3	<3±0.00
	20	$<10^3$	$<10^3$	$<10^3\pm0.00$	<3	<3	<3±0.00

2.2 Rate of viability loss, Arrhenius equation

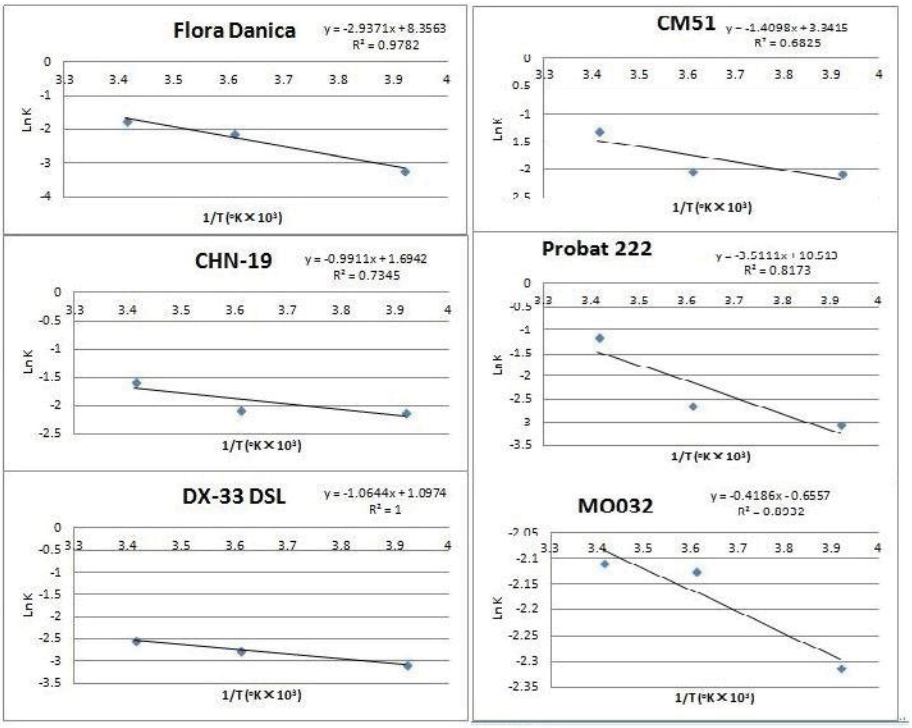


Figure 1. Arrhenius plots of LD-type LAB starter samples.

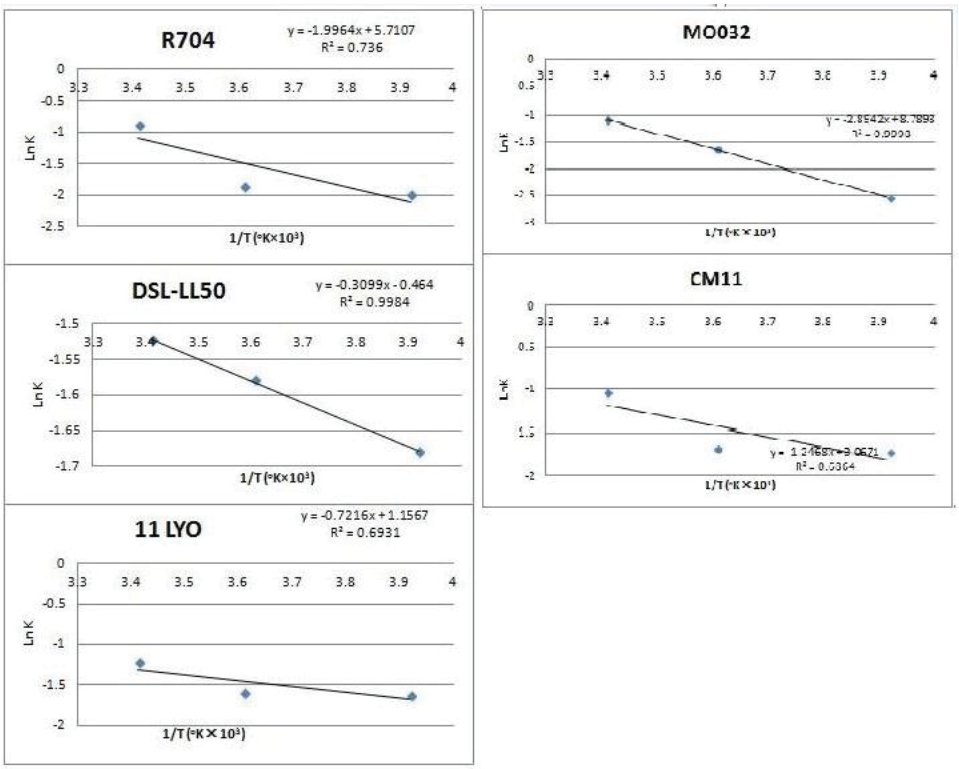


Figure 2. Arrhenius plots of O-type LAB starter samples.

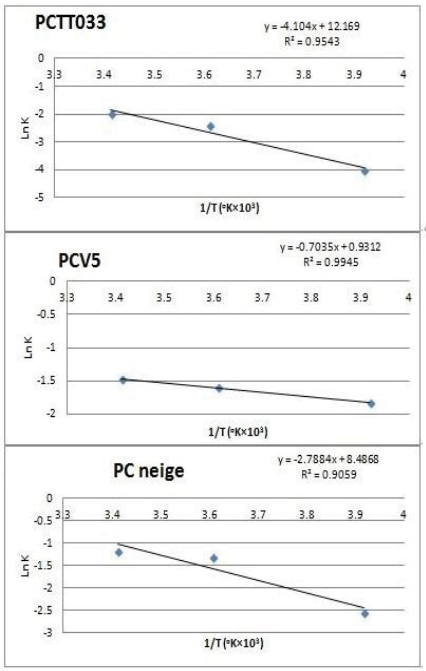


Figure 3. Arrhenius plots of *P.camemberti* culture samples.

2.3 Acidity measurements (pH and T.A.)

Group 1: LD-type LAB starters

Table 15. Flora Danica

Storage Temp (°C)	Storage Time (weeks)	pH measurements			Lactic acid (%)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	4.55	4.52	4.54±0.02	0.78	0.80	0.79±0.02
	4	4.55	4.52	4.54±0.02	0.78	0.78	0.78±0.00
	8	4.55	4.56	4.56±0.01	0.76	0.80	0.78±0.03
	12	4.59	4.58	4.59±0.01	0.70	0.71	0.70±0.01
	16	4.63	4.64	4.64±0.01	0.68	0.68	0.68±0.01
	20	4.64	4.67	4.66±0.02	0.68	0.58	0.63±0.07
4 °C	0	4.55	4.52	4.54±0.02	0.78	0.80	0.79±0.02
	4	4.55	4.52	4.54±0.02	0.78	0.80	0.79±0.02
	8	4.56	4.61	4.59±0.04	0.74	0.68	0.71±0.04
	12	4.63	4.64	4.64±0.01	0.68	0.68	0.68±0.00
	16	4.67	4.72	4.70±0.04	0.65	0.63	0.64±0.01
	20	4.68	4.74	4.71±0.04	0.61	0.60	0.61±0.00
20 °C	0	4.55	4.52	4.54±0.02	0.78	0.80	0.79±0.02
	4	4.54	4.56	4.55±0.01	0.77	0.75	0.76±0.02
	8	4.57	4.57	4.57±0.00	0.70	0.69	0.70±0.00
	12	4.65	4.67	4.66±0.01	0.53	0.64	0.58±0.07
	16	4.98	5.02	5.00±0.03	0.58	0.58	0.58±0.00
	20	5.13	5.05	5.09±0.06	0.44	0.40	0.42±0.03
37 °C	0	4.55	4.52	4.54±0.02	0.78	0.80	0.79±0.02
	4	5.73	5.66	5.70±0.05	0.09	0.09	0.09±0.00
	8	5.75	5.73	5.74±0.01	0.09	0.09	0.09±0.00
	12	6.07	6.25	6.16±0.13	0.08	0.07	0.08±0.00
	16	6.47	6.55	6.51±0.06	0.07	0.07	0.07±0.00
	20	6.65	6.68	6.67±0.02	0.08	0.06	0.07±0.02

Table 16. CHN-19

Storage Temp (°C)	Storage Time (weeks)	pH measurements			Lactic acid (%)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	4.55	4.53	4.54±0.01	0.78	0.80	0.79±0.01
	4	4.54	4.54	4.54±0.00	0.79	0.74	0.76±0.03
	8	4.54	4.55	4.55±0.01	0.75	0.74	0.74±0.01
	12	4.66	4.61	4.64±0.04	0.69	0.77	0.73±0.05
	16	4.64	4.65	4.65±0.01	0.74	0.71	0.72±0.02
	20	4.67	4.65	4.66±0.01	0.69	0.72	0.70±0.02
	0	4.55	4.53	4.54±0.01	0.78	0.80	0.79±0.01
	4	4.56	4.54	4.55±0.01	0.76	0.75	0.76±0.01
	8	4.61	4.65	4.63±0.03	0.67	0.72	0.70±0.04

4 °C	12	4.79	4.69	4.74±0.07	0.69	0.69	0.69±0.00
	16	4.91	4.87	4.89±0.03	0.69	0.65	0.67±0.03
	20	4.98	4.86	4.92±0.08	0.57	0.61	0.59±0.03
20 °C	0	4.55	4.53	4.54±0.01	0.78	0.80	0.79±0.01
	4	4.56	4.59	4.58±0.02	0.77	0.73	0.75±0.02
	8	4.58	4.59	4.59±0.01	0.68	0.69	0.68±0.00
	12	4.62	4.62	4.62±0.00	0.68	0.69	0.68±0.00
	16	4.78	4.82	4.80±0.03	0.64	0.65	0.64±0.01
	20	4.97	4.91	4.94±0.04	0.54	0.62	0.58±0.05
37 °C	0	4.55	4.53	4.54±0.01	0.78	0.80	0.79±0.01
	4	5.69	5.79	5.74±0.07	0.10	0.11	0.10±0.00
	8	6.39	6.35	6.37±0.03	0.11	0.09	0.10±0.01
	12	6.53	6.53	6.53±0.00	0.11	0.09	0.10±0.02
	16	6.57	6.56	6.57±0.01	0.09	0.09	0.09±0.00
	20	6.55	6.58	6.57±0.02	0.09	0.09	0.09±0.00

Table 17. Delvo-Tec DX-33A DSL

Storage Temp	Storage Time	pH measurements			Lactic acid (%)		
(°C)	(weeks)	Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	4.45	4.44	4.45±0.01	0.83	0.80	0.81±0.02
	4	4.45	4.45	4.45±0.00	0.82	0.79	0.81±0.03
	8	4.44	4.47	4.46±0.02	0.79	0.80	0.79±0.01
	12	4.47	4.47	4.47±0.00	0.78	0.78	0.78±0.00
	16	4.51	4.49	4.50±0.01	0.76	0.79	0.77±0.02
	20	4.52	4.54	4.53±0.01	0.71	0.71	0.71±0.00
4 °C	0	4.45	4.44	4.45±0.01	0.83	0.80	0.81±0.02
	4	4.46	4.44	4.45±0.01	0.79	0.80	0.79±0.00
	8	4.51	4.49	4.50±0.01	0.79	0.76	0.77±0.02
	12	4.52	4.56	4.54±0.03	0.68	0.70	0.69±0.01
	16	4.57	4.69	4.63±0.08	0.72	0.63	0.68±0.06
	20	4.64	4.67	4.66±0.02	0.66	0.65	0.66±0.00
20 °C	0	4.45	4.44	4.45±0.01	0.83	0.80	0.81±0.02
	4	4.46	4.46	4.46±0.00	0.78	0.80	0.79±0.01
	8	4.48	4.44	4.46±0.03	0.76	0.75	0.75±0.01
	12	4.60	4.56	4.58±0.03	0.68	0.72	0.70±0.02
	16	4.62	4.55	4.59±0.05	0.62	0.73	0.68±0.08
	20	4.74	4.81	4.78±0.05	0.70	0.62	0.66±0.05
37 °C	0	4.45	4.44	4.45±0.01	0.83	0.80	0.81±0.02
	4	5.60	5.62	5.61±0.01	0.42	0.42	0.42±0.00
	8	5.91	6.03	5.97±0.08	0.40	0.37	0.38±0.02
	12	6.38	6.23	6.31±0.11	0.24	0.18	0.21±0.04
	16	6.38	6.37	6.38±0.01	0.18	0.19	0.18±0.00
	20	6.49	6.45	6.47±0.03	0.08	0.07	0.08±0.01

Table 18. CM51

Storage Temp (°C)	Storage Time (weeks)	pH measurements			Lactic acid (%)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	5.03	5.04	5.04±0.01	0.55	0.52	0.54±0.02
	4	5.04	5.08	5.06±0.03	0.52	0.55	0.53±0.02
	8	5.06	5.05	5.06±0.01	0.40	0.39	0.39±0.00
	12	5.04	5.12	5.08±0.06	0.39	0.37	0.38±0.01
	16	5.18	5.20	5.19±0.01	0.36	0.36	0.36±0.00
	20	5.24	5.19	5.22±0.04	0.36	0.36	0.36±0.00
4 °C	0	5.03	5.04	5.04±0.01	0.55	0.52	0.54±0.02
	4	5.09	5.04	5.07±0.04	0.37	0.42	0.40±0.03
	8	5.14	5.09	5.12±0.04	0.36	0.37	0.36±0.01
	12	5.16	5.11	5.14±0.04	0.32	0.36	0.34±0.02
	16	5.18	5.20	5.19±0.01	0.33	0.34	0.33±0.00
	20	5.14	5.25	5.20±0.08	0.33	0.31	0.32±0.01
20 °C	0	5.03	5.04	5.04±0.01	0.55	0.52	0.54±0.02
	4	5.23	5.26	5.25±0.02	0.45	0.32	0.38±0.09
	8	5.75	5.93	5.84±0.13	0.38	0.33	0.36±0.04
	12	5.96	6.07	6.02±0.08	0.29	0.37	0.33±0.06
	16	6.15	6.03	6.09±0.08	0.35	0.31	0.33±0.03
	20	6.13	6.07	6.10±0.04	0.31	0.31	0.31±0.00
37 °C	0	5.03	5.04	5.04±0.01	0.55	0.52	0.54±0.02
	4	6.41	6.40	6.41±0.01	0.10	0.11	0.10±0.01
	8	6.53	6.49	6.51±0.03	0.10	0.11	0.10±0.01
	12	6.56	6.49	6.53±0.05	0.12	0.08	0.10±0.02
	16	6.57	6.61	6.59±0.03	0.08	0.08	0.08±0.00
	20	6.64	6.60	6.62±0.03	0.07	0.08	0.08±0.00

Table 19. Probat 222 LYO

Storage Temp (°C)	Storage Time (weeks)	pH measurements			Lactic acid (%)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	4.61	4.61	4.61±0.00	0.68	0.68	0.68±0.00
	4	4.65	4.65	4.65±0.00	0.68	0.66	0.67±0.02
	8	4.63	4.64	4.64±0.01	0.69	0.58	0.63±0.07
	12	4.65	4.65	4.65±0.00	0.70	0.52	0.61±0.13
	16	5.14	4.92	5.03±0.16	0.30	0.58	0.44±0.20
	20	4.97	5.12	5.05±0.11	0.43	0.43	0.43±0.00
4 °C	0	4.61	4.61	4.61±0.00	0.68	0.68	0.68±0.00
	4	4.65	4.61	4.63±0.03	0.60	0.70	0.65±0.07
	8	4.77	4.83	4.80±0.04	0.68	0.56	0.62±0.09
	12	4.97	4.95	4.96±0.01	0.54	0.57	0.55±0.02
	16	5.18	5.07	5.13±0.08	0.34	0.33	0.34±0.00

	20	5.16	5.24	5.20±0.06	0.29	0.36	0.33±0.05
20 °C	0	4.61	4.61	4.61±0.00	0.68	0.68	0.68±0.00
	4	5.34	5.42	5.38±0.06	0.49	0.56	0.53±0.05
	8	5.92	6.09	6.01±0.12	0.53	0.32	0.43±0.14
	12	6.06	6.11	6.09±0.04	0.38	0.33	0.36±0.03
	16	6.09	6.13	6.11±0.03	0.20	0.40	0.30±0.15
	20	6.16	6.14	6.15±0.01	0.29	0.30	0.30±0.00
37 °C	0	4.61	4.61	4.61±0.00	0.68	0.68	0.68±0.00
	4	6.43	6.45	6.44±0.01	0.10	0.09	0.10±0.00
	8	6.54	6.51	6.53±0.02	0.09	0.09	0.09±0.00
	12	6.55	6.59	6.57±0.03	0.09	0.09	0.09±0.00
	16	6.62	6.62	6.62±0.00	0.08	0.08	0.08±0.00
	20	6.67	6.65	6.66±0.01	0.07	0.07	0.07±0.00

Table 20. M036R

Storage Temp	Storage Time	pH measurements			Lactic acid (%)		
(°C)	(weeks)	Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	4.62	4.62	4.62±0.00	0.69	0.69	0.69±0.00
	4	4.63	4.62	4.63±0.01	0.69	0.67	0.68±0.01
	8	4.61	4.67	4.64±0.04	0.68	0.66	0.67±0.01
	12	4.73	4.65	4.69±0.06	0.62	0.66	0.64±0.03
	16	4.84	4.93	4.89±0.06	0.65	0.61	0.63±0.03
	20	4.94	5.02	4.98±0.06	0.59	0.65	0.62±0.05
4 °C	0	4.62	4.62	4.62±0.00	0.69	0.69	0.69±0.00
	4	4.84	4.93	4.89±0.06	0.68	0.64	0.66±0.03
	8	4.93	4.89	4.91±0.03	0.62	0.65	0.63±0.02
	12	4.90	5.02	4.96±0.08	0.63	0.38	0.50±0.18
	16	5.08	4.97	5.03±0.08	0.38	0.63	0.51±0.17
	20	5.02	5.10	5.06±0.06	0.39	0.47	0.43±0.06
20 °C	0	4.62	4.62	4.62±0.00	0.69	0.69	0.69±0.00
	4	5.05	5.06	5.06±0.01	0.41	0.44	0.42±0.02
	8	5.23	5.47	5.35±0.17	0.35	0.49	0.42±0.10
	12	5.60	5.77	5.69±0.12	0.42	0.39	0.41±0.02
	16	5.87	5.71	5.79±0.11	0.36	0.43	0.40±0.06
	20	6.06	6.02	6.04±0.03	0.38	0.37	0.37±0.01
37 °C	0	4.62	4.62	4.62±0.00	0.69	0.69	0.69±0.00
	4	6.47	6.47	6.47±0.00	0.09	0.09	0.09±0.00
	8	6.54	6.54	6.54±0.00	0.09	0.09	0.09±0.00
	12	6.59	6.57	6.58±0.01	0.10	0.09	0.09±0.01
	16	6.62	6.62	6.62±0.00	0.08	0.08	0.08±0.00
	20	6.65	6.61	6.63±0.03	0.07	0.08	0.08±0.00

Group 2: O-type LAB starters

Table 21. Mesophilic R704

Storage Temp (°C)	Storage Time (weeks)	pH measurements			Lactic acid (%)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	4.20	4.17	4.19±0.02	0.92	0.90	0.91±0.01
	4	4.21	4.24	4.23±0.02	0.91	0.91	0.91±0.00
	8	4.29	4.29	4.29±0.00	0.85	0.87	0.86±0.01
	12	4.29	4.29	4.29±0.00	0.86	0.84	0.85±0.01
	16	4.31	4.32	4.32±0.01	0.84	0.84	0.84±0.00
	20	4.70	4.65	4.68±0.04	0.61	0.63	0.62±0.02
4 °C	0	4.20	4.17	4.19±0.02	0.92	0.90	0.91±0.01
	4	4.27	4.27	4.27±0.00	0.90	0.90	0.90±0.00
	8	4.30	4.29	4.30±0.01	0.81	0.82	0.82±0.00
	12	4.31	4.31	4.31±0.00	0.82	0.82	0.82±0.00
	16	4.99	4.41	4.70±0.41	0.43	0.71	0.57±0.20
	20	5.23	5.12	5.18±0.08	0.60	0.56	0.58±0.01
20 °C	0	4.20	4.17	4.19±0.02	0.92	0.90	0.91±0.01
	4	4.32	4.30	4.31±0.01	0.89	0.91	0.90±0.01
	8	4.50	4.50	4.50±0.00	0.75	0.77	0.76±0.01
	12	4.38	4.66	4.52±0.20	0.72	0.80	0.76±0.06
	16	5.25	5.34	5.30±0.06	0.53	0.55	0.54±0.01
	20	5.39	5.52	5.46±0.05	0.51	0.49	0.50±0.01
37 °C	0	4.20	4.17	4.19±0.02	0.92	0.90	0.91±0.01
	4	6.48	6.48	6.48±0.00	0.10	0.11	0.10±0.01
	8	6.51	6.51	6.51±0.00	0.10	0.10	0.10±0.00
	12	6.54	6.51	6.53±0.02	0.11	0.09	0.10±0.02
	16	6.62	6.61	6.62±0.01	0.08	0.08	0.08±0.00
	20	6.62	6.67	6.66±0.06	0.08	0.08	0.08±0.00

Table 22. Delvo-Tec LL-50A DSL

Storage Temp (°C)	Storage Time (weeks)	pH measurements			Lactic acid (%)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	4.19	4.14	4.17±0.04	1.05	1.04	1.05±0.01
	4	4.20	4.21	4.21±0.01	1.01	1.00	1.01±0.01
	8	4.22	4.20	4.21±0.01	0.85	0.90	0.88±0.03
	12	4.25	4.25	4.25±0.00	0.86	0.85	0.86±0.01
	16	4.29	4.27	4.28±0.01	0.84	0.85	0.85±0.01
	20	4.41	4.38	4.40±0.02	0.81	0.81	0.81±0.01
4 °C	0	4.19	4.14	4.17±0.04	1.05	1.04	1.05±0.01
	4	4.22	4.24	4.23±0.01	1.01	1.01	1.01±0.00
	8	4.25	4.27	4.26±0.01	0.83	0.87	0.85±0.03
	12	4.32	4.37	4.35±0.04	0.79	0.80	0.79±0.01
	16	4.42	4.44	4.43±0.01	0.74	0.75	0.74±0.00

	20	4.62	4.61	4.62±0.01	0.67	0.72	0.69±0.02
20 °C	0	4.19	4.14	4.17±0.04	1.05	1.04	1.05±0.01
	4	4.24	4.23	4.24±0.01	1.00	0.98	0.99±0.01
	8	4.28	4.28	4.28±0.05	0.86	0.83	0.84±0.02
	12	4.53	4.59	4.56±0.04	0.74	0.74	0.74±0.00
	16	4.63	4.61	4.62±0.01	0.69	0.70	0.69±0.01
	20	4.83	4.85	4.84±0.01	0.64	0.67	0.66±0.02
37 °C	0	4.19	4.14	4.17±0.04	1.05	1.04	1.05±0.01
	4	4.65	4.80	4.73±0.11	0.72	0.70	0.71±0.02
	8	5.72	5.65	5.69±0.05	0.38	0.40	0.39±0.01
	12	6.32	6.29	6.31±0.02	0.18	0.19	0.18±0.01
	16	6.37	6.35	6.36±0.01	0.08	0.08	0.08±0.00
	20	6.45	6.39	6.42±0.04	0.08	0.08	0.08±0.00

Table 23. Choozit MA 11 LYO

Storage Temp (°C)	Storage Time (weeks)	pH measurements			Lactic acid (%)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	4.23	4.21	4.22±0.01	0.81	0.89	0.85±0.06
	4	4.32	4.31	4.32±0.01	0.82	0.83	0.83±0.00
	8	4.32	4.33	4.33±0.01	0.84	0.77	0.80±0.05
	12	4.37	4.33	4.35±0.03	0.79	0.75	0.77±0.02
	16	4.47	4.44	4.46±0.02	0.75	0.77	0.76±0.01
	20	4.57	4.49	4.53±0.06	0.65	0.76	0.70±0.08
4 °C	0	4.23	4.21	4.22±0.01	0.81	0.89	0.85±0.06
	4	4.33	4.33	4.33±0.00	0.80	0.79	0.79±0.01
	8	4.37	4.36	4.37±0.01	0.78	0.80	0.79±0.01
	12	4.45	4.37	4.41±0.06	0.75	0.77	0.76±0.02
	16	4.39	4.49	4.44±0.07	0.78	0.73	0.76±0.03
	20	4.56	4.58	4.57±0.01	0.65	0.63	0.64±0.01
20 °C	0	4.23	4.21	4.22±0.01	0.81	0.89	0.85±0.06
	4	4.35	4.35	4.35±0.00	0.76	0.81	0.79±0.03
	8	4.42	4.37	4.40±0.04	0.74	0.77	0.76±0.03
	12	4.43	4.46	4.45±0.02	0.76	0.74	0.75±0.01
	16	4.51	4.54	4.53±0.04	0.72	0.71	0.72±0.01
	20	5.39	5.92	5.66±0.37	0.44	0.52	0.48±0.05
37 °C	0	4.23	4.21	4.22±0.01	0.81	0.89	0.85±0.06
	4	6.03	5.94	5.99±0.06	0.28	0.28	0.28±0.00
	8	6.50	6.48	6.49±0.01	0.11	0.12	0.12±0.01
	12	6.53	6.51	6.52±0.01	0.09	0.09	0.09±0.00
	16	6.69	6.69	6.69±0.00	0.07	0.08	0.07±0.00
	20	6.75	6.71	6.73±0.03	0.07	0.07	0.07±0.00

Table 24. MO 032

Storage Temp (°C)	Storage Time (weeks)	pH measurements			Lactic acid (%)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	4.38	4.37	4.38±0.01	0.76	0.79	0.78±0.02
	4	4.42	4.46	4.44±0.03	0.77	0.75	0.76±0.01
	8	4.49	4.50	4.50±0.01	0.75	0.75	0.75±0.00
	12	4.50	4.52	4.51±0.01	0.75	0.74	0.74±0.01
	16	4.57	4.51	4.54±0.04	0.74	0.74	0.74±0.00
	20	5.86	5.71	5.79±0.11	0.50	0.66	0.58±0.11
4 °C	0	4.38	4.37	4.38±0.01	0.76	0.79	0.78±0.02
	4	4.46	4.48	4.47±0.01	0.76	0.78	0.77±0.01
	8	4.50	4.55	4.53±0.04	0.75	0.74	0.74±0.01
	12	4.52	4.53	4.53±0.01	0.75	0.73	0.74±0.01
	16	4.76	4.70	4.73±0.04	0.70	0.71	0.70±0.00
	20	5.78	5.85	5.82±0.05	0.39	0.35	0.37±0.01
20 °C	0	4.38	4.37	4.38±0.01	0.76	0.79	0.78±0.02
	4	4.57	4.54	4.56±0.02	0.78	0.75	0.77±0.02
	8	4.93	5.13	5.03±0.14	0.62	0.37	0.49±0.18
	12	5.56	5.47	5.52±0.06	0.49	0.49	0.49±0.00
	16	5.70	5.63	5.67±0.05	0.36	0.34	0.35±0.01
	20	6.24	6.26	6.25±0.01	0.23	0.24	0.23±0.01
37 °C	0	4.38	4.37	4.38±0.01	0.76	0.79	0.78±0.02
	4	6.40	6.38	6.39±0.01	0.12	0.11	0.12±0.00
	8	6.50	6.48	6.49±0.01	0.12	0.10	0.11±0.02
	12	6.54	6.55	6.55±0.01	0.09	0.09	0.09±0.00
	16	6.65	6.67	6.66±0.01	0.08	0.07	0.07±0.00
	20	6.70	6.69	6.70±0.01	0.08	0.07	0.07±0.00

Table 25. CM11

Storage Temp (°C)	Storage Time (weeks)	pH measurements			Lactic acid (%)		
		Rep 1	Rep 2	Mean ±SD	Rep 1	Rep 2	Mean ±SD
-18 °C	0	4.49	4.55	4.52±0.04	0.75	0.73	0.74±0.01
	4	4.56	4.53	4.55±0.02	0.74	0.74	0.74±0.00
	8	4.49	4.63	4.56±0.10	0.75	0.70	0.73±0.03
	12	4.60	4.61	4.61±0.01	0.69	0.70	0.70±0.01
	16	4.60	4.60	4.60±0.00	0.68	0.66	0.67±0.01
	20	6.20	6.16	6.18±0.03	0.28	0.33	0.30±0.04
4 °C	0	4.49	4.55	4.52±0.04	0.75	0.73	0.74±0.01
	4	4.64	4.66	4.65±0.01	0.74	0.71	0.72±0.02
	8	4.66	4.64	4.65±0.01	0.72	0.70	0.71±0.02
	12	4.67	4.68	4.68±0.01	0.74	0.65	0.69±0.06
	16	5.12	5.15	5.14±0.02	0.35	0.36	0.36±0.01
	20	6.16	6.21	6.19±0.04	0.26	0.26	0.26±0.00

20 °C	0	4.49	4.55	4.52±0.04	0.75	0.73	0.74±0.01
	4	4.67	4.65	4.66±0.01	0.70	0.72	0.71±0.01
	8	5.42	5.32	5.37±0.07	0.58	0.52	0.55±0.04
	12	5.62	5.78	5.70±0.11	0.37	0.43	0.40±0.04
	16	5.90	5.83	5.87±0.05	0.31	0.29	0.30±0.01
	20	6.34	6.32	6.33±0.01	0.20	0.22	0.21±0.02
37 °C	0	4.49	4.55	4.52±0.04	0.75	0.73	0.74±0.01
	4	6.45	6.50	6.48±0.04	0.13	0.13	0.13±0.00
	8	6.52	6.53	6.53±0.01	0.10	0.10	0.10±0.00
	12	6.59	6.58	6.59±0.01	0.09	0.09	0.09±0.00
	16	6.63	6.69	6.66±0.04	0.09	0.06	0.08±0.02
	20	6.69	6.71	6.70±0.01	0.07	0.08	0.08±0.01

2.4 Colour measurements of freeze-dried cultures

Codes of cultures: 1=Flora Danica; 2=CHN-19; 3=33A DSL; 4=CM51; 5=222LYO; 6=MO036

Temp: 1=-18°C, 2=4°C; 3=20°C; 4=37°C; Block: 1=replicate 1; 2=replicate 3;

Table 26. colour data; LD-type LAB starters

Codes of cultures	Temp	Block	L* values	a* values	b* values
1	1	1	96.5	0.51	5.19
1	2	1	96.22	0.83	5.2
1	3	1	95.04	1.55	6.28
1	4	1	78.75	4.24	13.53
1	1	2	96.54	0.51	5.18
1	2	2	96.27	0.83	5.19
1	3	2	95.03	1.56	6.27
1	4	2	78.75	4.27	13.51
2	1	1	95.99	0.13	6.16
2	2	1	95.06	0.64	6.34
2	3	1	94.29	1.49	8.55
2	4	1	63.32	6.7	16.46
2	1	2	95.97	0.13	6.16
2	2	2	95.13	0.63	6.36
2	3	2	94.33	1.5	8.55
2	4	2	63.09	6.7	16.41
3	1	1	73.92	3.66	24.51
3	2	1	53.21	4	26.52
3	3	1	50.19	4.25	27.76
3	4	1	14.66	15.35	17.13
3	1	2	73.89	3.65	24.45
3	2	2	53.19	4	25.5
3	3	2	50.17	4.26	27.73

3	4	2	14.78	15.37	17.14
4	1	1	98.06	-0.13	3.36
4	2	1	97.82	0.3	3.56
4	3	1	97.36	0.53	4.05
4	4	1	91.5	1.15	6.15
4	1	2	98.09	-0.13	3.31
4	2	2	97.82	0.3	3.67
4	3	2	97.37	0.52	4.02
4	4	2	91.49	1.14	6.13
5	1	1	98.15	-0.6	3.42
5	2	1	97.98	0.06	3.57
5	3	1	97.24	0.1	4.48
5	4	1	85.18	2.25	10.02
5	1	2	98.26	-0.6	3.44
5	2	2	97.95	0.08	3.57
5	3	2	97.25	0.11	4.48
5	4	2	85.15	2.25	10.04
6	1	1	97.74	0.14	3.07
6	2	1	97.66	0.15	3.2
6	3	1	97.48	0.16	3.85
6	4	1	89.54	1.13	6.65
6	1	2	97.79	0.14	3.07
6	2	2	97.68	0.15	3.2
6	3	2	97.5	0.16	3.85
6	4	2	89.47	1.13	6.63

Codes of cultures: 1=R704; 2=DSL; 3=MA11LYP; 4=MO032; 5=CM11;

Temp: 1=-18°C, 2=4°C; 3=20°C; 4=37°C; Block: 1=replicate 1; 2=replicate 3;

Table 27. Colour data; O-type LAB starters

Codes of cultures	Temp	Block	L* values	a* values	b* values
1	1	1	98.53	-0.42	4.54
1	2	1	97.64	-0.22	5.61
1	3	1	95.54	0.35	5.82
1	4	1	85.6	2.53	11.43
1	1	2	98.52	-0.42	4.55
1	2	2	97.63	-0.22	4.66
1	3	2	95.46	0.36	5.82
1	4	2	85.6	2.54	11.4
2	1	1	73.64	3.12	25.33
2	2	1	72.54	3.24	25.4
2	3	1	71.56	3.9	26.72
2	4	1	29.49	14.01	13.71

2	1	2	73.61	3.14	25.33
2	2	2	72.65	3.26	25.4
2	3	2	71.36	3.89	26.72
2	4	2	29.53	14.03	13.71
3	1	1	98.09	-0.64	3.46
3	2	1	98.02	-0.18	3.47
3	3	1	97.76	0.05	3.5
3	4	1	94.8	0.6	6.15
3	1	2	98.13	-0.64	3.44
3	2	2	98.02	-0.18	3.48
3	3	2	97.72	0.07	3.48
3	4	2	94.84	0.6	6.17
4	1	1	97.98	-0.35	3.43
4	2	1	97.94	-0.02	3.48
4	3	1	97.9	0.22	3.89
4	4	1	90.28	1.36	6.74
4	1	2	97.93	-0.36	3.44
4	2	2	97.95	-0.03	3.44
4	3	2	97.84	0.22	3.89
4	4	2	90.27	1.34	6.74
5	1	1	98.01	-0.14	3.18
5	2	1	98.01	0.05	3.43
5	3	1	97.42	0.21	4.01
5	4	1	91.15	0.95	6.17
5	1	2	98.01	-0.14	3.19
5	2	2	97.98	0.05	3.41
5	3	2	97.41	0.21	3.99
5	4	2	91.14	0.95	6.17

Codes of cultures: 1=Camemberti Swing PCTT033; 2=PC neige; 3=PCV5

Temp: 1=-18°C, 2=4°C; 3=20°C; 4=37°C; Block: 1=replicate 1; 2=replicate 3;

Table 28. Colour data; mould (ripening) cultures

Codes of cultures	Temp	Block	L^* values	a^* values	b^* values
1	1	1	98.2	-0.22	2.47
1	2	1	97.95	-0.02	2.55
1	3	1	97.93	0.3	2.75
1	4	1	96.62	0.43	3.75
1	1	2	98.21	-0.22	2.46
1	2	2	97.95	-0.02	2.56
1	3	2	97.9	0.3	2.74
1	4	2	96.64	0.43	3.79
2	1	1	91.02	-0.7	1.67

2	2	1	90.77	-0.53	1.68
2	3	1	90.66	-0.51	1.72
2	4	1	90.37	-0.19	2.06
2	1	2	91	-0.7	1.67
2	2	2	90.79	-0.53	1.69
2	3	2	90.62	-0.51	1.72
2	4	2	90.35	-0.19	2.07
3	1	1	90.6	-0.81	1.3
3	2	1	90.32	-0.55	1.25
3	3	1	90.38	-0.53	1.3
3	4	1	90.08	-0.24	2.19
3	1	2	90.89	-0.81	1.28
3	2	2	90.53	-0.55	1.33
3	3	2	90.44	-0.54	1.31
3	4	2	90.11	-0.21	2.18
4	1	1	98.31	-0.1	2.25
4	2	1	98.27	-0.09	2.27
4	3	1	97.9	0.06	2.75
4	4	1	97	0.29	3.56
4	1	2	98.27	-0.1	2.25
4	2	2	98.24	-0.09	2.28
4	3	2	97.9	0.06	2.74
4	4	2	97.04	0.29	3.59
5	1	1	98.51	-0.47	2.93
5	2	1	98.36	-0.16	2.99
5	3	1	98.27	-0.08	3.16
5	4	1	96.05	0.4	4.32
5	1	2	98.51	-0.48	2.92
5	2	2	98.36	-0.17	2.98
5	3	2	98.23	-0.08	3.16
5	4	2	96.02	0.39	4.32

Severe browning discoloration and caking were observed on freeze-dried cheese cultures that had been stored at four temperatures (-18°C, 4°C, 20°C and 37°C) for 5 months. Images captured by a Nokia phone, N9 (Model Nokia, Finland) with 8 mega pixels camera.

(a) LD-type LAB starters

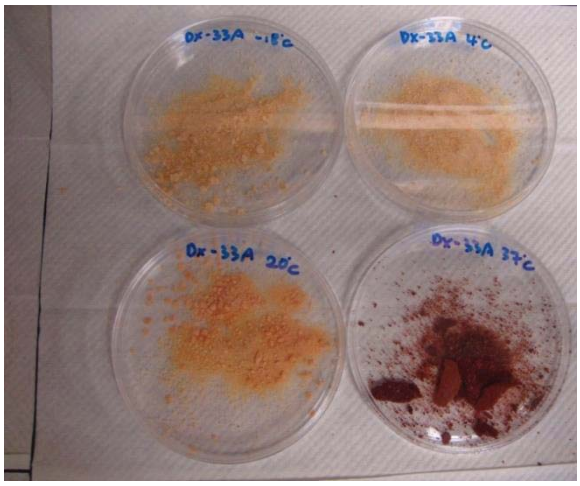


Figure 4. DX-33A, DSM

Figure 5. Flora Danica, Chr. Hansen

Figure 6. CHN-19, Chr. Hansen

Figure 7. CM51, Maysa

(b) O-type LAB starters

Figure 8. DSL-50A, DSM

Figure 9. R704, Chr. Hansen

(c) Mould cultures

Figure 10. PC neige, Danisco

2.5 Species composition of culture (LD-type)

Table 29. Strain composition (in proportion) of cheese starter bacteria (DL-type) after 5 months of storage time at various temperature[a]

Codes of cultures	Storage temperature (°C)	Cit⁻ LAB[2]/Total LAB[1]	Cit⁺ LAB[3]/Total LAB[1]	*L. lactis* subsp. *lactis* biovar. *diacetylactis*[4]/Total LAB[1]	*L. lactis* subsp. *lactis* biovar. *diacetylactis*[4]/ Cit⁺ LAB[3]	*Leuc.* spp[5]/Total LAB[1]	*Leuc.*spp[5]/ Cit⁺ LAB[3]
Flora Danica	-18	64.25±4.44	35.75±4.44	21.98±0.34	61.90±6.73	13.77±4.10	38.10±6.73
	4	57.97±7.34	42.03±7.34	27.19±8.68	63.87±9.51	14.84±1.34	36.13±9.51
	20	56.17±2.59	43.89±0.59	30.16±13.3	69.32±2.13	13.66±7.25	30.68±2.13
	37	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CHN-19	-18	81.02±5.09	18.98±5.09	14.81±6.43	76.19±13.47	4.18±1.35	23.81±13.47
	4	79.68±2.69	20.32±2.69	18.12±3.18	88.90±3.91	2.20±0.50	11.10±3.91
	20	78.15±11.52	21.85±11.52	20.00±14.14	86.49±19.11	0.00±0.00	0.00±0.00
	37	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
33A DSL	-18	82.85±7.32	17.15±7.32	17.28±8.51	99.23±7.23	0.00±0.00	0.00±0.00
	4	83.40±1.85	16.60±1.85	16.60±1.85	100±0.00	0.00±0.00	0.00±0.00
	20	79.75±0.34	20.24±0.34	20.24±0.34	100±0.00	0.00±0.00	0.00±0.00
	37	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CM51	-18	65.60±18.34	34.40±18.34	31.40±16.63	91.39±0.39	3.00±1.71	8.61±0.39
	4	55.24±2.69	44.76±2.69	41.10±2.22	91.83±0.56	3.67±0.47	8.17±0.56
	20	33.61±4.68	66.39±4.68	61.05±3.66	91.99±0.97	5.34±1.02	8.01±0.97
	37	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
222 LYO	-18	96.73±3.36	3.27±3.36	0.90±0.77	32.86±10.10	2.36±2.59	67.14±10.10
	4	95.74±1.30	4.26±1.30	1.48±0.94	32.85±12.06	2.78±0.36	67.15±12.06
	20	95.79±0.06	4.21±0.06	1.93±0.62	45.83±15.32	2.29±0.68	54.17±15.32
	37	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	-18	53.69±32.28	46.31±32.28	17.75±13.31	37.38±2.69	28.56±18.97	62.62±2.69

MO036	4	54.07±21.00	45.93±21.00	18.03±6.39	40.29±4.51	27.90±14.61	59.71±4.51
	20	42.55±37.63	57.45±37.63	21.87±1.86	47.13±27.63	35.57±35.77	52.87±27.63
	37	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

[a] Mean±SD, proportion (%); storage temperatures: -18°C, 4 °C and 20°C; [1] Total LAB includes Cit^+ LAB and Cit^- LAB;[2] Cit^- LAB: *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*; [3] Cit^+ LAB: *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuc.* spp;

Appendix 3 Production, ripening and characterization of Camembert cheese

3.1 Microbiological & pH changes during fermentation

Table 30. Raw data

Cheese type: 1=O-type cheese, 2=LD-type cheese; Culture temp: 1= -18°C, 2=4°C, 3=20°C; Fermentation time : 1= [at initial inoculation (0 h), after milk pre-maturation (3 h), initial draining (6 h), second draining (11 h), and overnight draining (24 h)].

Cheese type	production batch	Repeat	Culture temp	Fermentation (h)	Log LAB	Log Mould	pH
1	1	1	1	1	6.681241	3.491362	6.7
1	1	1	2	1	6.462398	3.278754	6.69
1	1	1	3	1	5.681241	3.041393	6.7
1	1	1	1	2	8.230449	3.755875	6.65
1	1	1	2	2	7.763428	3.531479	6.67
1	1	1	3	2	7.053078	3.39794	6.67
1	1	1	1	3	8.568202	3.812913	6.37
1	1	1	2	3	8.075547	3.70757	6.48
1	1	1	3	3	7.501059	3.612784	6.49
1	1	1	1	4	9.380211	3.924279	5.49
1	1	1	2	4	8.579784	3.845098	5.7
1	1	1	3	4	8.25042	3.633468	5.99
1	1	1	1	5	9.740363	4.591065	4.55
1	1	1	2	5	9.672098	4.230449	4.85
1	1	1	3	5	9.568202	3.838849	4.85
1	1	2	1	1	6.612784	3.568202	6.71
1	1	2	2	1	6.491362	3.380211	6.7
1	1	2	3	1	5.755875	3.146128	6.71
1	1	2	1	2	8.130334	3.681241	6.64
1	1	2	2	2	7.662758	3.491362	6.65
1	1	2	3	2	7.123852	3.431364	6.68
1	1	2	1	3	8.591065	3.770852	6.34
1	1	2	2	3	8.029384	3.724276	6.44
1	1	2	3	3	7.591065	3.568202	6.45
1	1	2	1	4	9.50515	3.908485	5.55
1	1	2	2	4	8.612784	3.799341	5.72
1	1	2	3	4	8.298853	3.623249	6.07
1	1	2	1	5	9.838849	4.531479	4.69
1	1	2	2	5	9.653213	4.113943	4.79
1	1	2	3	5	9.491362	3.724276	4.87
1	2	3	1	1	6.690196	3.556303	6.68
1	2	3	2	1	6.361728	3.39794	6.7
1	2	3	3	1	5.924279	3.176091	6.7

1	2	3	1	2	7.880814	3.612784	6.63
1	2	3	2	2	7.78533	3.462398	6.65
1	2	3	3	2	7.591065	3.342423	6.68
1	2	3	1	3	8.431364	3.740363	6.22
1	2	3	2	3	7.995635	3.612784	6.31
1	2	3	3	3	7.620136	3.491362	6.37
1	2	3	1	4	9.380211	3.857332	5.38
1	2	3	2	4	8.954243	3.838849	5.46
1	2	3	3	4	9.103804	3.591065	5.88
1	2	3	1	5	9.875061	4.39794	4.61
1	2	3	2	5	9.838849	4.20412	4.67
1	2	3	3	5	9.770852	3.812913	4.95
1	2	4	1	1	6.653213	3.579784	6.7
1	2	4	2	1	6.414973	3.361728	6.71
1	2	4	3	1	5.792392	3.176091	6.72
1	2	4	1	2	8.0086	3.672098	6.64
1	2	4	2	2	7.838849	3.447158	6.65
1	2	4	3	2	7.39794	3.342423	6.69
1	2	4	1	3	8.462398	3.716003	6.21
1	2	4	2	3	8.068186	3.672098	6.3
1	2	4	3	3	7.690196	3.518514	6.44
1	2	4	1	4	9.531479	3.897627	5.4
1	2	4	2	4	8.875061	3.78533	5.49
1	2	4	3	4	9.212188	3.50515	5.91
1	2	4	1	5	9.897627	4.531479	4.67
1	2	4	2	5	9.78533	4.146128	4.69
1	2	4	3	5	9.662758	3.869232	4.9
2	1	1	1	1	5.447158	3.431364	6.69
2	1	1	2	1	5.322219	3.342423	6.69
2	1	1	3	1	5.041393	3.041393	6.7
2	1	1	1	2	6.278754	3.462398	6.66
2	1	1	2	2	5.934498	3.230449	6.67
2	1	1	3	2	5.653213	3.041393	6.69
2	1	1	1	3	7.544068	3.70757	6.46
2	1	1	2	3	7.591065	3.612784	6.47
2	1	1	3	3	7.206826	3.50515	6.59
2	1	1	1	4	8.568202	3.944483	5.62
2	1	1	2	4	8.462398	3.763428	5.76
2	1	1	3	4	8.342423	3.70757	6.08
2	1	1	1	5	9.50515	4.20412	4.89
2	1	1	2	5	9.10721	4.113943	4.93
2	1	1	3	5	9.056905	3.826075	4.92

2	1	2	1	1	5.447158	3.30103	6.7
2	1	2	2	1	5.20412	3.230449	6.7
2	1	2	3	1	5.176091	3.079181	6.71
2	1	2	1	2	6.230449	3.322219	6.67
2	1	2	2	2	5.959041	3.380211	6.68
2	1	2	3	2	5.531479	3.255273	6.7
2	1	2	1	3	7.623249	3.690196	6.45
2	1	2	2	3	7.50515	3.672098	6.48
2	1	2	3	3	7.214844	3.579784	6.57
2	1	2	1	4	8.681241	3.986772	5.63
2	1	2	2	4	8.60206	3.826075	5.74
2	1	2	3	4	8.230449	3.633468	6.09
2	1	2	1	5	9.278754	4.255273	4.86
2	1	2	2	5	9.130334	4.113943	4.92
2	1	2	3	5	9.146128	3.857332	4.93
2	2	3	1	1	5.462398	3.414973	6.68
2	2	3	2	1	5.176091	3.322219	6.7
2	2	3	3	1	5.113943	3.113943	6.76
2	2	3	1	2	5.934498	3.568202	6.61
2	2	3	2	2	5.80618	3.447158	6.63
2	2	3	3	2	5.39794	3.380211	6.67
2	2	3	1	3	7.313867	3.724276	6.49
2	2	3	2	3	7.318063	3.591065	6.51
2	2	3	3	3	6.934498	3.477121	6.59
2	2	3	1	4	8.39794	3.863323	5.75
2	2	3	2	4	8.068186	3.80618	5.96
2	2	3	3	4	7.986772	3.653213	6.05
2	2	3	1	5	9.120574	3.934498	4.86
2	2	3	2	5	9.021189	3.716003	4.93
2	2	3	3	5	8.919078	3.716003	4.91
2	2	4	1	1	5.322219	3.30103	6.71
2	2	4	2	1	5.278754	3.230449	6.75
2	2	4	3	1	5	3.230449	6.76
2	2	4	1	2	6.017033	3.491362	6.59
2	2	4	2	2	5.770852	3.518514	6.65
2	2	4	3	2	5.322219	3.322219	6.69
2	2	4	1	3	7.480007	3.748188	6.5
2	2	4	2	3	6.568202	3.623249	6.53
2	2	4	3	3	7.053078	3.462398	6.6
2	2	4	1	4	8.531479	3.94939	5.63
2	2	4	2	4	8.113943	3.748188	5.94
2	2	4	3	4	7.94939	3.579784	6.11

2	2	4	1	5	9.201397	3.886491	4.89
2	2	4	2	5	9.045323	3.838849	4.9
2	2	4	3	5	8.959041	3.724276	5

3.2 Microbiological & pH changes during ripening

Table 31. Rata data.

Cheese type: 1=O-type cheese, 2=LD-type cheese; Culture temp: 1= -18°C, 2=4°C, 3=20°C; ripening time: 1=3 d; 2=10 d; 3=14 d; 4=21 d;

Cheese type	production batch	Repeat	Culture temp	Ripening time (d)	LAB log	Mould log	pH
1	1	1	1	1	9.342423	3.826075	4.48
1	1	1	2	1	8.977724	3.591065	4.56
1	1	1	3	1	8.78533	3.037426	4.69
1	1	1	1	2	8.78533	5.30103	4.9
1	1	1	2	2	8.869232	5.462398	4.85
1	1	1	3	2	9.033424	5.544068	4.84
1	1	1	1	3	8.214844	5.591065	5.28
1	1	1	2	3	8.732394	5.986772	5.13
1	1	1	3	3	8.913814	5.579784	4.98
1	1	1	1	4	7.94939	6.716003	6.58
1	1	1	2	4	8.681241	6.94939	6.45
1	1	1	3	4	8.913814	7.193125	6.02
1	1	2	1	1	9.431364	3.716003	4.51
1	1	2	2	1	9.017033	3.690196	4.59
1	1	2	3	1	8.886491	3.146128	4.67
1	1	2	1	2	8.826075	5.146128	4.83
1	1	2	2	2	8.90309	5.518514	4.76
1	1	2	3	2	9.068186	5.491362	4.73
1	1	2	1	3	8.346353	5.531479	5.31
1	1	2	2	3	8.70757	6.012837	5.15
1	1	2	3	3	8.94939	6.568202	5.02
1	1	2	1	4	7.875061	6.755875	6.51
1	1	2	2	4	8.531479	6.963788	6.38
1	1	2	3	4	8.857332	7.235528	6.05
1	2	3	1	1	9.414973	3.716003	4.44
1	2	3	2	1	9.255273	3.623249	4.53
1	2	3	3	1	8.819544	3.079181	4.67
1	2	3	1	2	8.892095	5.342423	4.75
1	2	3	2	2	8.94939	5.113943	4.63
1	2	3	3	2	9.198657	5.447158	4.68
1	2	3	1	3	8.612784	5.633468	5.28
1	2	3	2	3	8.819544	6.278754	5.1

1	2	3	3	3	9.158362	6.740363	5.06
1	2	3	1	4	7.913814	6.892095	6.68
1	2	3	2	4	8.763428	7.130334	6.41
1	2	3	3	4	8.80618	7.173186	6.16
1	2	4	1	1	9.544068	3.653213	4.43
1	2	4	2	1	9.255273	3.518514	4.54
1	2	4	3	1	8.838849	3.20412	4.67
1	2	4	1	2	8.819544	5.361728	4.75
1	2	4	2	2	8.982271	5.176091	4.59
1	2	4	3	2	9.170262	5.518514	4.68
1	2	4	1	3	8.462398	5.672098	5.2
1	2	4	2	3	8.986772	6.079181	5.12
1	2	4	3	3	9.089905	6.716003	5.06
1	2	4	1	4	7.929419	6.80618	6.68
1	2	4	2	4	8.643453	6.982271	6.45
1	2	4	3	4	8.735599	7.068186	6.16
2	1	1	1	1	9.033424	3.913814	4.57
2	1	1	2	1	9.017033	3.778151	4.6
2	1	1	3	1	9.004321	3.568202	4.75
2	1	1	1	2	8.934498	5.447158	4.83
2	1	1	2	2	8.959041	5.531479	4.75
2	1	1	3	2	8.982271	5.80618	4.7
2	1	1	1	3	8.354108	5.770852	5.41
2	1	1	2	3	8.556303	5.94939	5.24
2	1	1	3	3	8.819544	6.255273	4.86
2	1	1	1	4	8.30103	6.924279	6.68
2	1	1	2	4	8.471292	7.041393	6.48
2	1	1	3	4	8.570543	7.247973	6.44
2	1	2	1	1	9.075547	3.875061	4.56
2	1	2	2	1	9	3.716003	4.63
2	1	2	3	1	8.973128	3.322219	4.73
2	1	2	1	2	8.897627	5.531479	4.83
2	1	2	2	2	8.851258	5.568202	4.8
2	1	2	3	2	8.929419	5.716003	4.71
2	1	2	1	3	8.431364	5.845098	5.47
2	1	2	2	3	8.591065	5.968483	5.22
2	1	2	3	3	8.690196	6.004321	4.87
2	1	2	1	4	8.359835	6.944483	6.68
2	1	2	2	4	8.434569	7.082785	6.47
2	1	2	3	4	8.531479	7.20412	6.45
2	2	3	1	1	9.209515	3.963788	4.66
2	2	3	2	1	9.130334	3.724276	4.73

2	2	3	3	1	9.08636	3.591065	4.84
2	2	3	1	2	8.908485	5.431364	5.11
2	2	3	2	2	9.079181	5.531479	5.14
2	2	3	3	2	9.064458	5.653213	5.14
2	2	3	1	3	8.770852	5.78533	5.54
2	2	3	2	3	8.792392	5.963788	5.35
2	2	3	3	3	8.886491	6.079181	5.33
2	2	3	1	4	8.591065	6.954243	6.43
2	2	3	2	4	8.755875	7.380211	6.3
2	2	3	3	4	8.690196	7.568202	6.26
2	2	4	1	1	9.217484	3.991226	4.69
2	2	4	2	1	9.1959	3.770852	4.79
2	2	4	3	1	9.064458	3.60206	4.89
2	2	4	1	2	8.857332	5.447158	5.1
2	2	4	2	2	9.029384	5.491362	5.16
2	2	4	3	2	9.139879	5.755875	5.15
2	2	4	1	3	8.799341	5.826075	5.58
2	2	4	2	3	8.819544	5.986772	5.37
2	2	4	3	3	8.913814	6.130334	5.34
2	2	4	1	4	8.612784	6.869232	6.44
2	2	4	2	4	8.612784	7.037426	6.31
2	2	4	3	4	8.748188	7.643453	6.27

3.3 Nitrogen fractions and FAA

Table 32. Raw data.

Cheese type: 1=O-type cheese, 2=LD-type cheese; Culture temp: 1= -18°C, 2=4°C, 3=20°C; ripening time: 1=3 d; 2=10 d; 3=14 d; 4=21 d;

Cheese type	production batch	Repeat	culture temp	Ripening time (d)	SN/TN	NPN/TN	PROTEIN	CASEIN	PEPTIDES	FAA
1	1	1	1	1	0.063319	0.067686	2.786974	2.300028	0.066725	0.682651
1	1	1	2	1	0.102564	0.04359	2.457375	2.401009	0.078887	0.60555
1	1	1	3	1	0.094527	0.039801	2.46535	2.324838	0.0712	0.471785
1	1	1	1	2	0.090426	0.047872	2.364426	2.258754	0.105673	0.738792
1	1	1	2	2	0.103365	0.064904	2.292664	2.275623	0.101903	0.5705
1	1	1	3	2	0.088832	0.07197	2.459716	2.316476	0.20584	0.569285
1	1	1	1	3	0.129032	0.089862	2.185511	2.074751	0.111298	1.677644
1	1	1	2	3	0.125	0.056731	2.175818	2.1992	0.179388	1.003357
1	1	1	3	3	0.1275	0.0975	2.378446	2.089938	0.118662	1.072406
1	1	1	1	4	0.155941	0.118812	2.157218	1.957897	0.209143	2.060301
1	1	1	2	4	0.200935	0.123832	2.228115	2.087642	0.201439	2.043026
1	1	1	3	4	0.183333	0.14375	2.176722	1.863671	0.225401	1.738405
1	1	2	1	1	0.0675	0.0575	2.246269	2.526138	0.02709	0.482716

1	1	2	2	1	0.102995	0.057143	2.277396	2.41542	0.0615	0.524607
1	1	2	3	1	0.086387	0.052356	2.46502	2.356	0.088523	0.587763
1	1	2	1	2	0.09111	0.0501	2.291919	2.244	0.1011	0.7225
1	1	2	2	2	0.123215	0.0491	2.2311	2.131	0.1345	0.675
1	1	2	3	2	0.119161	0.0691	1.896092	2.369438	0.1879	0.574218
1	1	2	1	3	0.173877	0.113986	2.1911	2.081973	0.121	1.7275
1	1	2	2	3	0.15404	0.089	2.0899	2.030359	0.122	0.922
1	1	2	3	3	0.181191	0.119271	1.885302	2.09844	0.145	1.604575
1	1	2	1	4	0.223941	0.194912	2.1191	1.832097	0.211	1.775
1	1	2	2	4	0.227053	0.1356	2.008115	2.019821	0.204	1.989
1	1	2	3	4	0.254633	0.179186	2.113	1.85956	0.221	1.7825
1	2	3	1	1	0.094675	0.059666	2.553228	2.125072	0.0591	0.401933
1	2	3	2	1	0.085526	0.046053	2.357375	2.291	0.079	0.353081
1	2	3	3	1	0.059524	0.050595	2.075879	2.376497	0.069522	0.350487
1	2	3	1	2	0.102339	0.055556	2.116068	2.198754	0.104821	0.672579
1	2	3	2	2	0.138728	0.066474	2.2877	2.215623	0.161971	0.531584
1	2	3	3	2	0.113772	0.083832	2.094725	2.277163	0.199	0.65235
1	2	3	1	3	0.15099	0.10396	2.178437	2.031183	0.119958	1.486257
1	2	3	2	3	0.15404	0.093434	2.0501	2.101027	0.145459	1.169482
1	2	3	3	3	0.164179	0.116915	2.217102	2.005896	0.1663	1.422713
1	2	3	1	4	0.212446	0.135193	2.04124	1.954071	0.213	1.287826
1	2	3	2	4	0.227053	0.147343	2.128115	1.867696	0.208294	1.432217
1	2	3	3	4	0.2575	0.1675	2.084961	1.980555	0.193351	1.306054
1	2	4	1	1	0.073919	0.04928	2.181612	2.3888	0.056541	0.45661
1	2	4	2	1	0.088296	0.048715	2.319834	2.456	0.085603	0.4435
1	2	4	3	1	0.086387	0.049658	2.104136	2.334	0.060203	0.467
1	2	4	1	2	0.115042	0.079339	2.316068	2.221	0.081123	0.6765
1	2	4	2	2	0.123215	0.053471	2.129534	2.235	0.156914	0.509895
1	2	4	3	2	0.112957	0.070598	2.100436	2.21	0.182553	0.589404
1	2	4	1	3	0.211571	0.08903	2.161046	2.065553	0.147253	1.624362
1	2	4	2	3	0.147011	0.102907	2.209659	2.158041	0.146	1.259399
1	2	4	3	3	0.181191	0.114546	2.169161	2.084659	0.163265	1.668215
1	2	4	1	4	0.215147	0.136912	2.115212	2.031731	0.204752	1.885848
1	2	4	2	4	0.245909	0.17374	2.04644	2.0123	0.178744	1.328994
1	2	4	3	4	0.254633	0.156491	2.103535	1.910184	0.241065	1.276992
2	1	1	1	1	0.103938	0.042018	2.395297	2.31	0.073306	0.447389
2	1	1	2	1	0.097256	0.051	2.379348	2.33	0.08935	0.379
2	1	1	3	1	0.076401	0.053	2.343305	2.179555	0.104	0.453201
2	1	1	1	2	0.13657	0.090476	2.239257	1.933442	0.122627	0.608827
2	1	1	2	2	0.115558	0.063557	2.216977	2.093867	0.12311	0.781277
2	1	1	3	2	0.096113	0.079365	2.357589	1.992	0.110225	0.439021
2	1	1	1	3	0.176396	0.122457	2.141554	2.010874	0.130674	0.81943

2	1	1	2	3	0.196264	0.146057	1.975997	1.737463	0.158534	1.007785
2	1	1	3	3	0.191862	0.116065	1.877831	1.716808	0.161024	0.673984
2	1	1	1	4	0.282103	0.206579	2.032143	1.838709	0.257	1.36302
2	1	1	2	4	0.302436	0.215293	1.841658	1.63714	0.287	1.24416
2	1	1	3	4	0.323204	0.234	1.994638	1.600081	0.337761	1.265733
2	1	2	1	1	0.086093	0.036424	2.404962	2.387292	0.075585	0.588899
2	1	2	2	1	0.086093	0.04023	2.245452	2.045452	0.105	0.345331
2	1	2	3	1	0.122642	0.066038	2.434521	2.020792	0.093304	0.3215
2	1	2	1	2	0.105058	0.068093	2.212755	2.124984	0.087771	0.70965
2	1	2	2	2	0.124224	0.083851	2.216977	1.993961	0.080195	0.6755
2	1	2	3	2	0.126984	0.057692	2.330199	2.020792	0.112	0.565
2	1	2	1	3	0.176471	0.122	2.123015	1.992341	0.146	0.774898
2	1	2	2	3	0.19337	0.153	2.220958	1.87244	0.186	1.0715
2	1	2	3	3	0.166667	0.134	2.229779	1.858149	0.178	0.924
2	1	2	1	4	0.293182	0.195455	1.993449	1.751307	0.242142	1.22336
2	1	2	2	4	0.32093	0.213953	1.884	1.718231	0.27068	1.494141
2	1	2	3	4	0.360294	0.220994	1.91274	1.73293	0.261708	1.141569
2	2	3	1	1	0.080292	0.053701	2.333381	2.276022	0.08005	0.322187
2	2	3	2	1	0.086207	0.044	2.353487	2.155116	0.08935	0.301525
2	2	3	3	1	0.081081	0.042	2.350887	2.115	0.103304	0.387084
2	2	3	1	2	0.109195	0.060345	2.358762	2.099	0.156617	0.83655
2	2	3	2	2	0.148148	0.098765	2.239079	1.993961	0.141132	0.541535
2	2	3	3	2	0.132653	0.081633	2.102784	1.985963	0.142362	0.827159
2	2	3	1	3	0.165929	0.112832	2.161943	2.03255	0.191407	1.624362
2	2	3	2	3	0.208333	0.125	2.041678	1.847232	0.17713	0.998123
2	2	3	3	3	0.153846	0.101	2.539802	1.892	0.201978	0.718614
2	2	3	1	4	0.276537	0.214	2.04	1.827563	0.278147	1.605654
2	2	3	2	4	0.259548	0.204	1.988911	1.702	0.250847	1.4025
2	2	3	3	4	0.271983	0.235294	1.962157	1.622668	0.326947	1.199933
2	2	4	1	1	0.103566	0.047638	2.397066	2.391877	0.073086	0.324877
2	2	4	2	1	0.115079	0.043651	2.348	2.27	0.098371	0.493964
2	2	4	3	1	0.107692	0.038462	2.378588	1.92893	0.09	0.395
2	2	4	1	2	0.151515	0.083333	2.105622	1.949006	0.083536	0.796
2	2	4	2	2	0.1332	0.0887	2.257522	1.945	0.122689	0.821207
2	2	4	3	2	0.126667	0.07	2.336407	1.921	0.116821	0.5975
2	2	4	1	3	0.190476	0.113095	2.19382	2.002413	0.129393	1.130107
2	2	4	2	3	0.213855	0.138554	2.026373	1.849242	0.194446	1.260877
2	2	4	3	3	0.207692	0.115385	1.935621	1.733643	0.178	1.186002
2	2	4	1	4	0.302083	0.190104	2.011715	1.733568	0.288	1.687424
2	2	4	2	4	0.285	0.19	1.997	1.787951	0.261	1.328994
2	2	4	3	4	0.267196	0.148148	1.956	1.692	0.312659	1.246874

3.4 Texture analysis

Instrument setting:

Test mode: compression;

Pre-test speed: 1.00 mm/sec; Test speed: 2.00 mm/sec; Post-test speed: 10.00 mm/sec

Target mode: distance; distance=10 mm (50% of original height of sample)

Trigger mode: auto; trigger force: 5.00 g

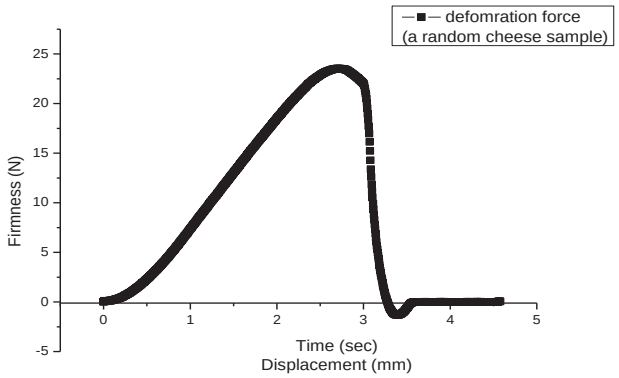


Figure 11. Texture profile of a random cheese sample ripened for 14 days. Mean firmness (N) (n=6).

Table 33. Raw data of texture analysis

Cheese type: 1=O-type cheese, 2=LD-type cheese; Culture temp: 1= -18°C, 2=4°C, 3=20°C; ripening time: 1=3 d; 2=10 d; 3=14 d; 4=21 d;

Cheese type	production batch	Repeat	culture temp	Ripening time (d)	Texture
1	1	1	1	1	10.345
1	1	1	2	1	9.765
1	1	1	3	1	9.345
1	1	1	1	2	13.563
1	1	1	2	2	13.4231
1	1	1	3	2	12.459
1	1	1	1	3	9.0456
1	1	1	2	3	10.1129
1	1	1	3	3	12.569
1	1	1	1	4	6.5113
1	1	1	2	4	6.1496
1	1	1	3	4	6.664
1	1	2	1	1	10.876
1	1	2	2	1	11.235
1	1	2	3	1	10.001
1	1	2	1	2	12.225
1	1	2	2	2	12.269
1	1	2	3	2	12.996

1	1	2	1	3	10.003
1	1	2	2	3	10.768
1	1	2	3	3	12.447
1	1	2	1	4	4.301
1	1	2	2	4	6.1137
1	1	2	3	4	6.321
1	2	3	1	1	10.8697
1	2	3	2	1	10.5214
1	2	3	3	1	11.3597
1	2	3	1	2	13.8631
1	2	3	2	2	12.441
1	2	3	3	2	12.4016
1	2	3	1	3	8.4048
1	2	3	2	3	10.2937
1	2	3	3	3	11.0828
1	2	3	1	4	4.8713
1	2	3	2	4	4.9896
1	2	3	3	4	5.896
1	2	4	1	1	12.5411
1	2	4	2	1	11.1542
1	2	4	3	1	9.8364
1	2	4	1	2	10.9028
1	2	4	2	2	10.3531
1	2	4	3	2	11.098
1	2	4	1	3	9.0364
1	2	4	2	3	7.7741
1	2	4	3	3	15.3139
1	2	4	1	4	5.0687
1	2	4	2	4	5.234
1	2	4	3	4	6.231
2	1	1	1	1	15.1829
2	1	1	2	1	15.1648
2	1	1	3	1	14.4564
2	1	1	1	2	17.5105
2	1	1	2	2	13.7763
2	1	1	3	2	15.1435
2	1	1	1	3	10.1869
2	1	1	2	3	8.5337
2	1	1	3	3	13.8609
2	1	1	1	4	3.118
2	1	1	2	4	4.7457
2	1	1	3	4	5.714

2	1	2	1	1	15.7887
2	1	2	2	1	15.3459
2	1	2	3	1	15.7539
2	1	2	1	2	17.7693
2	1	2	2	2	17.2652
2	1	2	3	2	17.7861
2	1	2	1	3	10.4064
2	1	2	2	3	11.429
2	1	2	3	3	13.4926
2	1	2	1	4	3.4855
2	1	2	2	4	6.0591
2	1	2	3	4	4.082
2	2	3	1	1	14.9915
2	2	3	2	1	12.4969
2	2	3	3	1	12.7549
2	2	3	1	2	15.9595
2	2	3	2	2	17.4774
2	2	3	3	2	17.6261
2	2	3	1	3	9.9632
2	2	3	2	3	10.923
2	2	3	3	3	10.998
2	2	3	1	4	3.789
2	2	3	2	4	4.789
2	2	3	3	4	6.89
2	2	4	1	1	14.4362
2	2	4	2	1	13.9161
2	2	4	3	1	12.045
2	2	4	1	2	17.278
2	2	4	2	2	16.7685
2	2	4	3	2	14.2327
2	2	4	1	3	9.013
2	2	4	2	3	10.123
2	2	4	3	3	11.034
2	2	4	1	4	4.001
2	2	4	2	4	5.034
2	2	4	3	4	5.124

3.5 Key aroma compounds production

(1) SPME-GC/MS programme set up

SPME programme set-up (AOC-5000, AUTO-INJECTOR, SHIMADZU)

Method syringe: fiber;
Method sequence:

Pre-incubation time: 30:00 (m:ss); incubation time: 60°C; agitator speed: 500 rpm; agitator on time: 0.05 (m:ss); agitator off time: 0.02 (m:ss); vial penetration: 22 mm; extraction time: 20:00 (m:ss);

Desorb to GC injector 1: injection penetration: 44 mm; desorption time: 5:00 (m:ss); post-fiber conditing time: 25.00 (m:ss);

GC/MS programme set up

GC (GC-2010, SHIMADZU)

1. Column type: RTX-5; 60 m in length; thickness: 0.25μm; diameter: 0.25μm; maxmim usable temp: 300°C; serial no. 867542; column oven temp: 30.00°C; column flow: 0.8 ml/min;
2. Injection temp: 250°C; injection mode: spitless; sampling time: 1:00 min;
3. Carrier gas: He, prim.preesure 500-900; purge flow: 1.0 ml/min; flow control mode: linear volecity; pressure: 84.7 kPa; total flow: 9.8 ml/min; linear velocity: 22.7 cm/sec

Column oven programme (total run time: 121.55 min)

	Rate	Final T°C	Hold time
0	---	30.00	3.00
1	0.25	46.00	0.00
2	5.00	115.00	0.00
3	20.00	230.00	35.00

MS (GC/MS-QP2010)

Ion source temp: 220°C; interface temp: 200°C; solvent cut-time: 1 min; micro scan width: 0 μ; detector volatge: relative to tunning results; 0.8 kv; threshold: 500; mode: scan; scan speed: 526; start m/z=50.00; end m/z=300.00;

(2) Blank sample

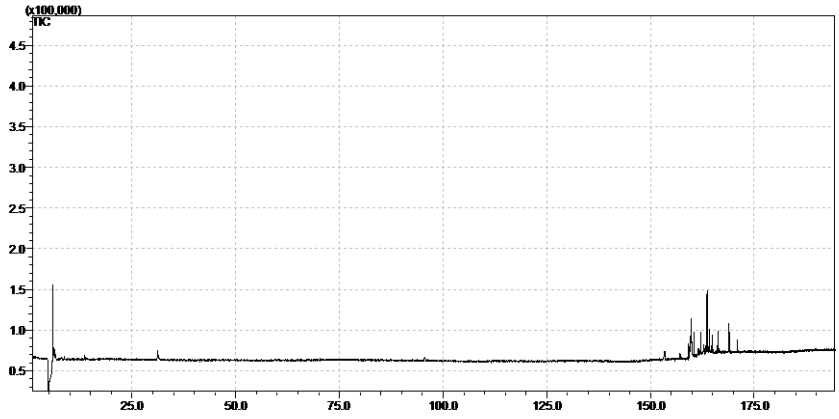


Figure 12. The chromatogram of blank samples.

Table 34. Concentrations of standard volatile compounds used for quantitative analysis

Compounds	Retention time (min)[1]	Calibration level (µg/ml)							Curve slope (*b*)	correlation coefficient (r^2)
		1	2	3	4	5	6	7		
External standard										
Dimethyl sulfide	6.118	0.001	0.005	0.01	0.04	0.08	0.16	0.24	16.576	09696
3-methylbutanal	10.386	0.001	0.005	0.01	0.08	0.16	0.32		20.859	0.9523
3-methylbutanol	17.332	0.001	0.005	0.01	0.04	0.08	0.16	0.32	17.662	0.9602
Butyric acid	28.353	0.001	0.01	0.04	0.08	0.16	0.24	0.32	0.3195	0.9873
2-Heptanone	48.194	0.001	0.004	0.008	0.016	0.024	0.04	0.08	65.254	0.9563
1-Octen-3-one	81.154	0.0008	0.0016	0.0032	0.016	0.032			176.06	0.9989
1-Octen-3-ol	82.801	0.001	0.004	0.008	0.012	0.08	0.16		11.316	0.995
2-Nonanone	135.726	0.001	0.004	0.008	0.012	0.016	0.024		65.852	0.9927
Internal standard										
Octanoic acid, methyl ester/ Methyl octanoate	148.618	0.008	0.008	0.008	0.008	0.008	0.008	0.008		

(3) Standard curves
STD: volatiles standard
ISSTD: internal standard (methyl octanoate)

Table 35. Dimethyl sulfide

STD Conc. (μl/ml)	Mean peak area ratio (STD:ISSTD)	SD
0	0	0
0.001	0.0287	0.007
0.005	0.153	0.02
0.01	0.325	0.07
0.04	0.542	0.031113
0.08	1.063333	0.056616
0.16	2.191689	0.328714
0.24	4.384803	0.233543

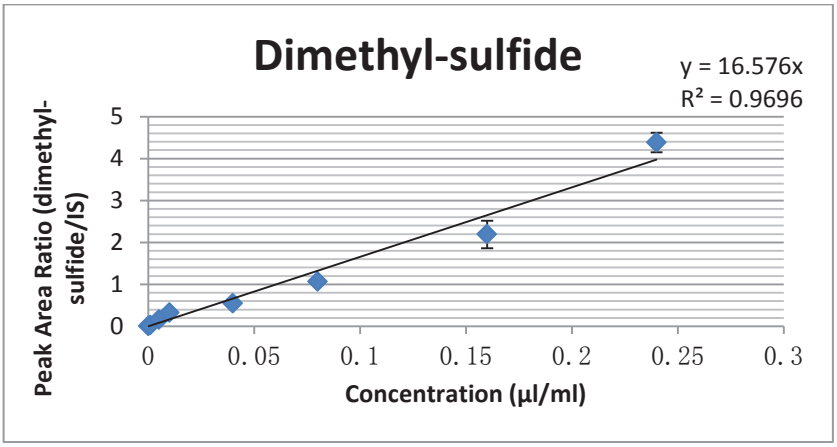


Figure 13. Dimethyl-sulfide standard curve.

Table 36. 3-methylbutanal

STD Conc. (μl/ml)	Mean peak area ratio (STD:ISSTD)	SD
0	0	0
0.001	0.0214	0.009
0.005	0.101	0.0008
0.01	0.22	0.004
0.08	1.007641	0.11
0.16	2.268334	0.142642
0.32	7.374257	0.59

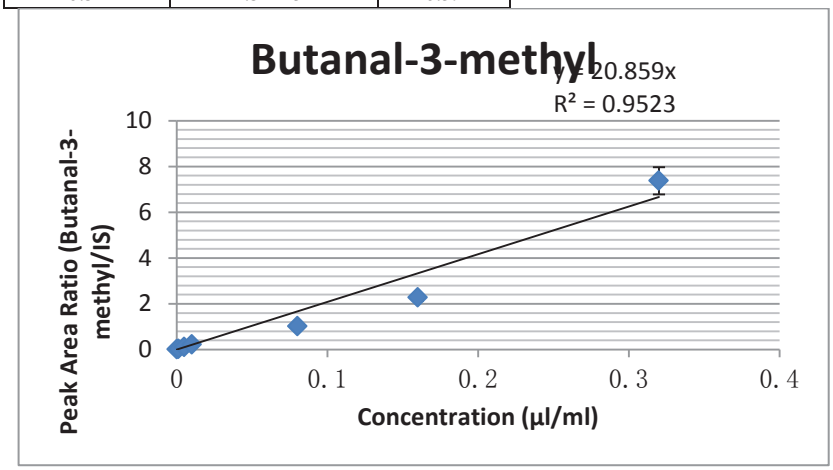


Figure 14. 3-methylbutanal standard curve.

Table 37. 3-methylbutanol

STD Conc. (µl/ml)	Mean peak area ratio (STD:ISSTD)	SD
0	0	0
0.001	0.0175	0.006
0.005	0.0876	0.024
0.01	0.171	0.06
0.08	0.972036	0
0.16	2.280206	0.306916
0.24	4.750037	0.599

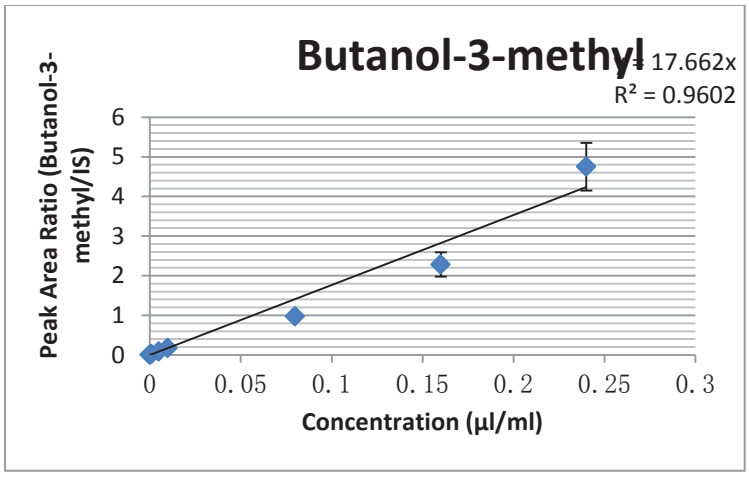


Figure 15. 3-methylbutanal standard curve.

Table 38. 2-Heptanone

STD Conc. (µl/ml)	Mean peak area ratio (STD:ISSTD)	SD
0	0	0
0.001	0.062	0.009
0.004	0.175216	0.084
0.008	0.259087	0.046739
0.016	0.757938	0.138625
0.024	1.296263	0.073761
0.04	1.833993	0.363
0.08	5.777226	0.563

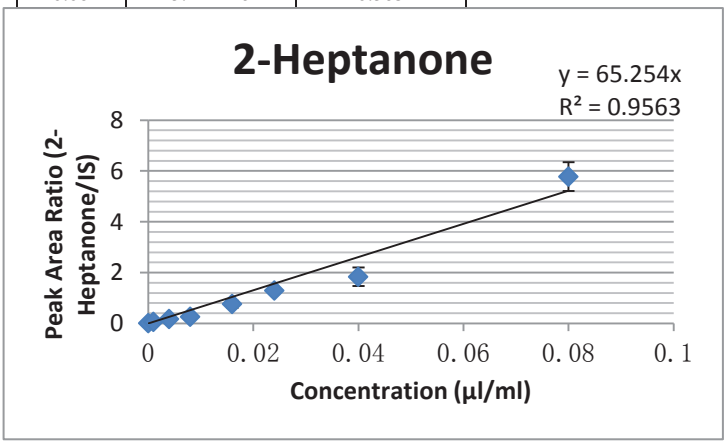


Figure 16. 2-heptanone standard curve.

Table 39. 1-Octen-3-ol

STD Conc.	Mean peak area ratio	SD

(µl/ml)	(STD:ISSTD)	
0	0	0
0.001	0.014	0.003
0.004	0.02748	0.009
0.008	0.100729	0.0314
0.012	0.203364	0.0861
0.08	0.987401	0.042833
0.16	1.764286	0.37042

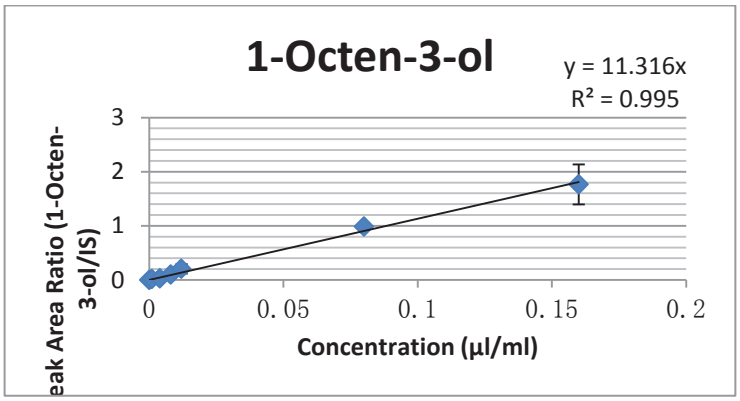


Figure 17. 1-octen-3-ol standard curve.

Table 40. 1-Octen-3-one

STD Conc. (µl/ml)	Mean peak area ratio (STD:ISSTD)	SD
0	0	0
0.0008	0.136	0.0013
0.0016	0.209636	0.034
0.0032	0.45035	0.021
0.016	2.733263	0.583318
0.032	5.69076	0.592142

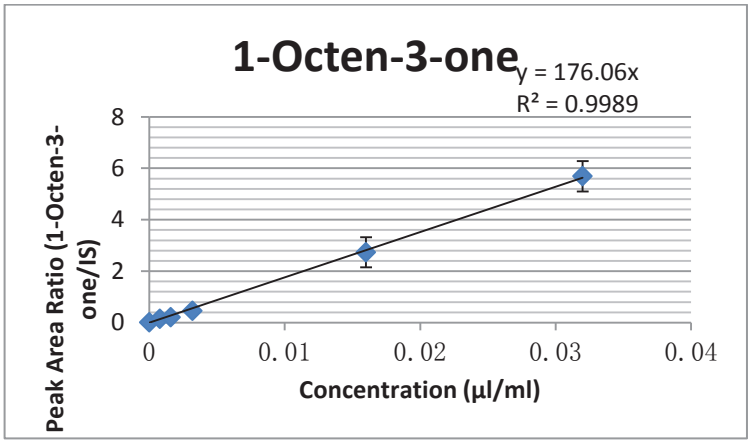


Figure 18. 1-octen-3-one standard curve.

Table 41. 2-Nonanone

STD Conc. (µl/ml)	Mean peak area ratio (STD:ISSTD)	SD
0	0	0
0.001	0.065	0.003
0.004	0.258	0.015

0.008	0.601659	0.058196
0.012	0.735181	0.044945
0.016	0.991464	0.136759
0.024	1.625404	0.136957

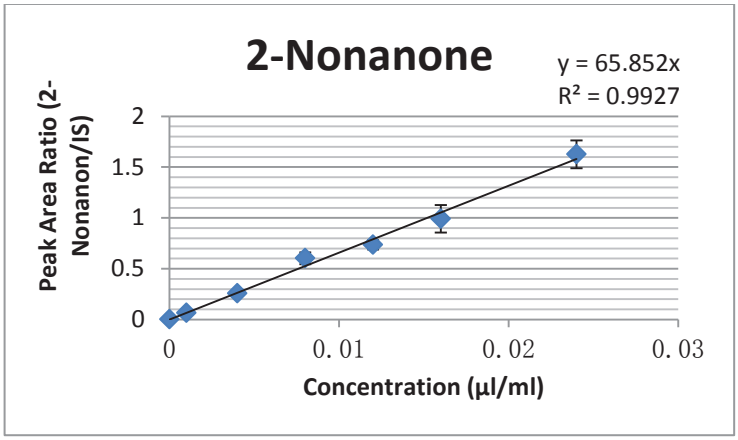


Figure 19. 2-nonanone standard curve.

Table 42. Butyric acid

STD Conc. (µl/ml)	Mean peak area ratio (STD:ISSTD)	SD
0	0	0
0.001	0.000287	0
0.01	0.003005	0
0.04	0.01265	0.004
0.08	0.016584	0.004687
0.16	0.048023	0.000173
0.24	0.073185	0.010815
0.32	0.108705	0.011705

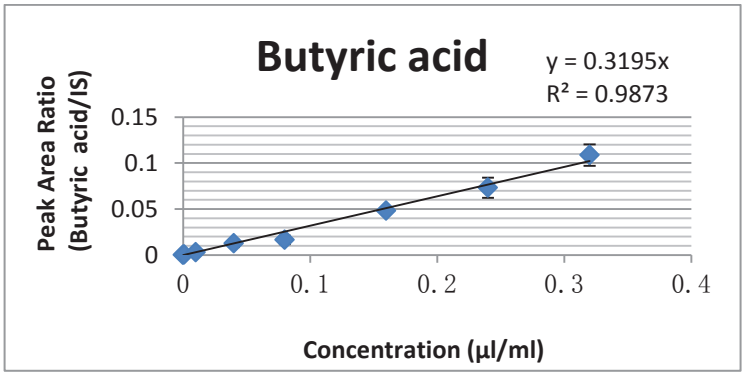


Figure 20. Butyric acid standad curve.

Qualitative Table

Print Edit View Similarity Search

Peak	Ret.Time	Start Tm	End Tm	m/z	Area	Area%	Height	Height%	A/H	Mark	Name
1	6.118	6.067	6.208	TIC	11401210	4.02	4063859	22.16	2.81		Dimethyl sulfide
2	8.428	8.408	8.442	TIC	288629	0.10	154633	0.84	1.87		2,3-butanedione
3	10.386	10.308	10.408	TIC	2771571	0.98	671152	3.66	4.13		Butanal, 3-methyl-
4	17.332	17.308	17.408	TIC	59557	0.02	23747	0.13	2.51		1-Butanol, 3-methyl-
5	28.353	25.100	28.833	TIC	4036229	1.42	46680	0.25	86.47	MI	Butanoic acid
6	48.194	46.758	49.392	TIC	40218201	14.19	752474	4.10	53.45	MI	2-Heptanone
7	81.154	81.133	81.375	TIC	4092589	1.44	311954	1.70	13.12		1-Octen-3-one
8	82.801	81.992	83.008	TIC	80962260	28.57	2900468	15.81	27.91	V	1-Octen-3-ol
9	135.726	135.025	136.008	TIC	28613223	10.10	1196728	6.53	23.91		2-Nonanone
10	148.618	148.175	148.725	TIC	110970878	39.16	8218514	44.82	13.50		Octanoic acid, methyl ester

Figure 21. An example of retention time of volatile compounds

Table 43. Repeatability of compounds' retention time (RT)

Compounds		Within day			Between day			
	1	2	3	CV%	1	2	3	CV%
Dimethyl sulfide	6.118	6.118	6.119	0.009	6.118	6.119	6.115	0.034
2,3-butanedione	8.428	8.442	8.441	0.093	8.428	8.553	8.521	0.764
3-methylbutanal	10.386	10.384	10.389	0.024	10.386	10.441	10.394	0.286
3-methylbutanol	17.332	17.352	17.467	0.419	17.332	17.465	17.679	0.887
Butyric acid	28.353	28.345	28.369	0.043	28.353	28.534	28.441	0.318
2-heptanone	48.194	48.234	48.221	0.042	48.194	48.335	48.211	0.160
1-octen-3-one	81.154	81.221	81.175	0.042	81.154	8.143	82.131	0.696
1-octen-3-ol	82.801	82.804	82.900	0.068	82.801	83.001	82.814	0.135
2-nonanone	135.726	135.884	135.792	0.031	135.726	135.834	135.736	0.044
Octanoic acid, methyl ester	148.618	148.625	148.704	0.032	148.618	148.668	148.834	0.076

Table 44. Repeatability of compounds' peak area ratio

Compounds	Mixed standard solution[1]				Cheese sample			
	1	2	3	CV%	1	2	3	CV%
Dimethyl sulfide	1.098	1.094	0.998	5.324				
3-methylbutanal	1.007	1.012	1.031	1.245	0.0346	0.0358	0.0327	4.548
3-methylbutanol	0.972	0.973	0.980	0.447	0.00669	0.00742	0.00711	5.18
Butyric acid	0.021	0.017	0.019	10.526	0.0196	0.026	0.022	14.35
2-heptanone	0.227	0.207	0.226	5.122	0.0427	0.0232	0.0279	32.55
1-octen-3-one	0.209	0.211	0.232	5.862				
1-octen-3-ol	0.123	0.104	0.114	8.362				
2-nonanone	0.561	0.642	0.612	6.769	0.0075	0.0082	0.0094	11.484

[1] Conc. 0.08 μl/ml: dimethyl sulphide, 3-methylbutanal, 3-methylbutanol, butyric acid; Conc. 0.008 μl/ml: 2-heptanone, 1-oct-3-one, 2-nonanone; Conc. 0.004μl/ml: 1-oct-3-ol;

Table 45. Rata data of volatile analysis.
Cheese type: 1=O-type cheese, 2=LD-type cheese; Culture temp: 1= -18°C, 2=4°C, 3=20°C; ripening time: 1=3 d; 2=10 d; 3=14 d; 4=21 d;

Cheese type	Ripening time	Culture temp	replicate	3-methyl butanal	3-methyl butanol	heptone	nonanone	butyric acid
1	1	1	1	57.89602	0	22.8988	13.451	0
1	1	1	2	74.20962	0	35.54153	22.27145	0
1	1	2	1	22.6941	0	87.32558	0	0
1	1	2	2	20.33213	0	80.47087	0	0
1	1	3	1	0	0	12.357	0	0
1	1	3	2	0	0	5.798	0	0
1	2	1	1	97.6829	1379.231	173.4	180.3472	0

1	2	1	2	103.8097	15681.68	158.9	142.8645	0
1	2	2	1	84.62294	17091.77	124.6944	114.52	0
1	2	2	2	70.86255	14122.51	248.8569	90.78571	0
1	2	3	1	53.21487	16381.21	169.8304	79.75146	0
1	2	3	2	86.38105	30764.2	261.5898	88.52946	0
1	3	1	1	224.2025	1026.045	369.2697	240.5993	14982
1	3	1	2	164.7228	1394.857	397.7148	202.5874	17144.69
1	3	2	1	109.0793	2495.966	364.9633	213.764	9914.4
1	3	2	2	117.2846	2457	482.46	201.678	7942
1	3	3	1	129.4236	7877.942	468.5694	385.9014	29802
1	3	3	2	169.4618	4179.274	470.3504	169.7232	33523.74
1	4	1	1	34.97488	449.7498	109.198	57.8321	6189.176
1	4	1	2	42.784	394.8574	97.33112	41.13514	7267.83
1	4	2	1	38.542	595.9757	313.6759	34.984	2157.527
1	4	2	2	22.11231	312.7436	376.401	27.97878	18135.26
1	4	3	1	55.19852	2424.793	742.0597	104.8905	43282.5
1	4	3	2	47.40158	2421.454	563.5683	154.8764	17166.56
2	1	1	1	0	0	15.22152	10.3325	0
2	1	1	2	0	0	29.43353	26.48375	0
2	1	2	1	0	0	17.893	8.273308	0
2	1	2	2	0	0	22.23362	4.3692	0
2	1	3	1	0	0	23.563	0	0
2	1	3	2	0	0	34.567	0	0
2	2	1	1	31.55778	10487.51	52.94894	56.34542	0
2	2	1	2	56.731	12340.81	34.985	88.52622	0
2	2	2	1	60.02716	18990.01	73.80282	45.20132	0
2	2	2	2	55.15534	15835.68	61.2179	78.26033	0
2	2	3	1	32.48452	18091.43	43.09219	57.821	17425.1
2	2	3	2	39.72643	14721.96	48.13527	35.77443	12369
2	3	1	1	138.793	8848.953	333.2496	146.743	90972.57
2	3	1	2	98.06023	4543.228	304.6636	231.0214	92126.71
2	3	2	1	116.0628	11598.69	290.8515	125.89	19141.52
2	3	2	2	106.9342	8559.024	377.9074	167.52	12983.39
2	3	3	1	111.0101	9636.123	300.4345	178.934	20517.71
2	3	3	2	96.45601	7476.776	384.4505	122.6803	133421.8
2	4	1	1	73.76499	2890.067	74.51716	32.95574	20103.27
2	4	1	2	64.421	3153.203	96.98082	33.99847	12447.3
2	4	2	1	36.13393	5782.952	92.39462	44.19333	33869.78
2	4	2	2	35.25691	2399.692	84.62681	30.43281	46955.73
2	4	3	1	17.93711	4305.862	23.35833	25.63775	50921
2	4	3	2	15.82265	1802.049	78.13273	15.35419	43897.26

3.6 Sensory evalution

Sensory evaluation of cheese samples was not Sensory evaluation of prototype cheeses was not included in this current research and has to be conducted in consequent study due to two major reasons listed in below:

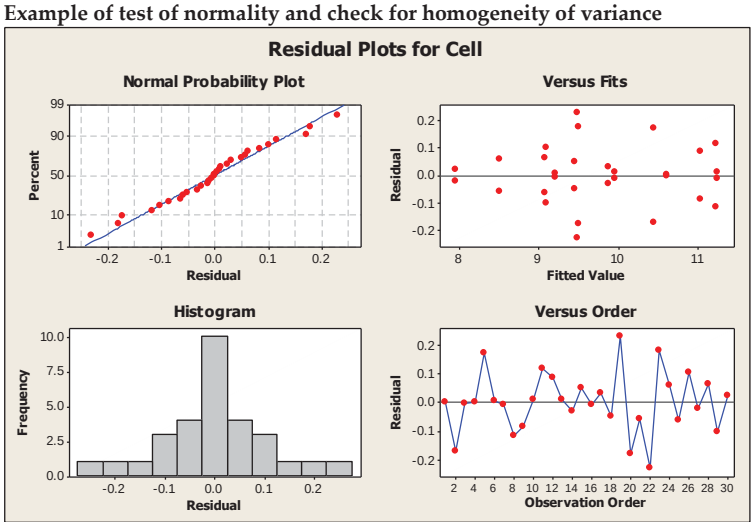
1. Consumer acceptability analysis helps with market acceptability and reveals consumer liking of product (Meilaard et al., 1999). Because the produced cheese prototypes of this study were similar in several aspects (e.g., proteolysis, texture and key aromatic compounds), run the analysis alone may not sufficient to investigate the effect of starter types and culture storage temperatures on the final sensory quality of prototype cheeses. Therefore, descriptive sensory analysis was suggested to conduct additionally.

2. Descriptive sensory analysis involves detection (discrimination) and description quantitative and qualitative sensory characteristic of product (Meilaard et al., 1999). In this current study, the analysis was not able to include due to time, personal and financial constraints involved. To conduct descriptive sensory analysis, highly trained panellists, with sufficient experiences in cheese products, are required and they have large impact on the success of the analysis (Meilaard et al., 1999). During research time, the personals were however unable to gather. The entire process of recruiting and training sensory panellists can be time-consuming and expensive. Considering the amount of work involved in culture storage trial and prototype cheese production and characterization, recruiting and training sensory panellist will not be easily achieved in this one-year master programme and therefore sensory analysis were not included as project objectives.

Appendix 4 Statistical outputs

4.1 Screening and selection of cheese starter and ripening cultures

Example of test of normality and check for homogeneity of variance



Enumeration, pH and T.A.

O-type starter

General Linear Model: Cell count_1 versus Brands_1, Temp_1

Factor	Type	Levels	Values
Brands_1	fixed	5	1, 2, 3, 4, 5
Temp_1	fixed	4	1, 2, 3, 4

Analysis of Variance for Cell count_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brands_1	4	15.954	15.954	3.989	252.43	0.000
Temp_1	3	458.957	458.957	152.986	9682.19	0.000
Brands_1*Temp_1	12	6.508	6.508	0.542	34.33	0.000
Error	20	0.316	0.316	0.016		
Total	39	481.736				

S = 0.125701 R-Sq = 99.93% R-Sq(adj) = 99.87%

General Linear Model: pH versus Brands_1, Temp_1

Factor	Type	Levels	Values
Brands_1	fixed	5	1, 2, 3, 4, 5
Temp_1	fixed	4	1, 2, 3, 4

Analysis of Variance for pH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brands_1	4	9.2916	9.2916	2.3229	254.57	0.000
Temp_1	3	14.1211	14.1211	4.7070	515.84	0.000
Brands_1*Temp_1	12	3.2364	3.2364	0.2697	29.56	0.000
Error	20	0.1825	0.1825	0.0091		
Total	39	26.8316				

S = 0.0955249 R-Sq = 99.32% R-Sq(adj) = 98.67%

General Linear Model: T.A. versus Brands_1, Temp_1

Factor	Type	Levels	Values
Brands_1	fixed	5	1, 2, 3, 4, 5
Temp_1	fixed	4	1, 2, 3, 4

Analysis of Variance for T.A., using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brands_1	4	0.60398	0.60398	0.15099	112.04	0.000
Temp_1	3	1.59037	1.59037	0.53012	393.35	0.000
Brands_1*Temp_1	12	0.24963	0.24963	0.02080	15.44	0.000
Error	20	0.02695	0.02695	0.00135		
Total	39	2.47092				

S = 0.0367110 R-Sq = 98.91% R-Sq(adj) = 97.87%

LD-Type starter

General Linear Model: cell versus Brand, Temp

Factor Type Levels Values
Factor Type Levels Values
Brand_1 fixed 6 1, 2, 3, 4, 5, 6
Temp_1 fixed 4 1, 2, 3, 4
Analysis of Variance for cell, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand	5	19.0121	19.0121	3.8024	417.29	0.000
Temp	2	1.9514	1.9514	0.9757	107.08	0.000
Brand*Temp	10	1.1823	1.1823	0.1182	12.97	0.000
Error	18	0.1640	0.1640	0.0091		
Total	35	22.3098				

S = 0.0954571 R-Sq = 99.26% R-Sq(adj) = 98.57%

General Linear Model: pH versus Brand_1, Temp_1

Factor Type Levels Values
Brand_1 fixed 6 1, 2, 3, 4, 5, 6
Temp_1 fixed 4 1, 2, 3, 4
Analysis of Variance for pH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand_1	5	3.9020	3.4501	0.6900	273.43	0.000
Temp_1	3	22.7740	21.5564	7.1855	2847.30	0.000
Brand_1*Temp_1	15	2.0193	2.0193	0.1346	53.34	0.000
Error	24	0.0606	0.0606	0.0025		
Total	47	28.7558				

S = 0.0502356 R-Sq = 99.79% R-Sq(adj) = 99.59%

General Linear Model: T.A. versus Brand_1, Temp_1

Factor Type Levels Values
Brand_1 fixed 6 1, 2, 3, 4, 5, 6
Temp_1 fixed 4 1, 2, 3, 4
Analysis of Variance for T.A., using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand_1	5	0.53607	0.44468	0.08894	97.59	0.000
Temp_1	3	1.65272	1.59239	0.53080	582.47	0.000
Brand_1*Temp_1	15	0.18740	0.18740	0.01249	13.71	0.000
Error	24	0.02187	0.02187	0.00091		
Total	47	2.39807				

S = 0.0301874 R-Sq = 99.09% R-Sq(adj) = 98.21%

Mould ripening cultures

General Linear Model: Cell_1 versus Brands_1, Temp_1

Factor Type Levels Values
Brands_1 fixed 5 1, 2, 3, 4, 5
Temp_1 fixed 4 1, 2, 3, 4
Analysis of Variance for Cell_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brands_1	4	1.5863	1.5863	0.3966	28.74	0.000
Temp_1	3	128.6229	128.6229	42.8743	3107.12	0.000
Brands_1*Temp_1	12	1.1433	1.1433	0.0953	6.90	0.000
Error	20	0.2760	0.2760	0.0138		
Total	39	131.6285				

S = 0.117468 R-Sq = 99.79% R-Sq(adj) = 99.59%

Colour measurement

O-type starter

L^*

General Linear Model: L-values_1 versus Brand_1, Temp_1

Factor Type Levels Values
Brand_1 fixed 5 1, 2, 3, 4, 5

Temp_1 fixed 4 1, 2, 3, 4
Analysis of Variance for L-values_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand_1	4	7481.05	7481.05	1870.26	1019217.20	0.000
Temp_1	3	1568.35	1568.35	522.78	284894.74	0.000
Brand_1*Temp_1	12	1598.19	1598.19	133.18	72579.08	0.000
Error	20	0.04	0.04	0.00		
Total	39	10647.63				

S = 0.0428369 R-Sq = 100.00% R-Sq(adj) = 100.00

a * measurements

General Linear Model: a-values_1 versus Brand_1, Temp_1

Factor Type Levels Values
Brand_1 fixed 5 1, 2, 3, 4, 5
Temp_1 fixed 4 1, 2, 3, 4
Analysis of Variance for a-values_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand_1	4	216.894	216.894	54.224	867577.84	0.000
Temp_1	3	82.631	82.631	27.544	440697.32	0.000
Brand_1*Temp_1	12	103.701	103.701	8.642	138267.52	0.000
Error	20	0.001	0.001	0.000		
Total	39	403.227				

S = 0.00790569 R-Sq = 100.00% R-Sq(adj) = 100.00%

b* Measurments

General Linear Model: b-values_1 versus Brand_1, Temp_1

Factor Type Levels Values
Brand_1 fixed 5 1, 2, 3, 4, 5
Temp_1 fixed 4 1, 2, 3, 4
Analysis of Variance for b-values_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand_1	4	2094.50	2094.50	523.62	23082.43	0.000
Temp_1	3	5.49	5.49	1.83	80.72	0.000
Brand_1*Temp_1	12	314.07	314.07	26.17	1153.72	0.000
Error	20	0.45	0.45	0.02		
Total	39	2414.51				

S = 0.150615 R-Sq = 99.98% R-Sq(adj) = 99.96%

LD- type starters

L* values

General Linear Model: L-values versus Brand, Temp

Factor Type Levels Values
Brand fixed 6 1, 2, 3, 4, 5, 6
Temp fixed 4 1, 2, 3, 4
Analysis of Variance for L-values, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand	5	13972.58	13972.58	2794.52	1297260.99	0.000
Temp	3	3783.92	3783.92	1261.31	585519.62	0.000
Brand*Temp	15	2213.06	2213.06	147.54	68489.39	0.000
Error	24	0.05	0.05	0.00		
Total	47	19969.62				

S = 0.0464130 R-Sq = 100.00% R-Sq(adj) = 100.00%

a* values

General Linear Model: a-values versus Brand, Temp

Factor Type Levels Values
Brand fixed 6 1, 2, 3, 4, 5, 6
Temp fixed 4 1, 2, 3, 4
Analysis of Variance for a-values, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand	5	245.102	245.102	49.020	941192.68	0.000
Temp	3	158.361	158.361	52.787	1013513.00	0.000
Brand*Temp	15	121.279	121.279	8.085	155236.65	0.000

Error 24 0.001 0.001 0.000
Total 47 524.744
S = 0.00721688 R-Sq = 100.00% R-Sq(adj) = 100.00%

b values
General Linear Model: b-values versus Brand, Temp
Factor Type Levels Values
Brand fixed 6 1, 2, 3, 4, 5, 6
Temp fixed 4 1, 2, 3, 4
Analysis of Variance for b-values, using Adjusted SS for Test

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand	5	2248.19	2248.19	449.64	20252.05	0.000
Temp	3	119.49	119.49	39.83	1794.03	0.000
Brand*Temp	15	333.43	333.43	22.23	1001.18	0.000
Error	24	0.53	0.53	0.02		
Total	47	2701.64				

S = 0.149004 R-Sq = 99.98% R-Sq(adj) = 99.96%

Mould ripening cultures

L* measurement
General Linear Model: L-values_1_1 versus Brand_1_1, Temp_1_1
Factor Type Levels Values
Brand_1_1 fixed 5 1, 2, 3, 4, 5
Temp_1_1 fixed 4 1, 2, 3, 4
Analysis of Variance for L-values_1_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand_1_1	4	500.689	500.689	125.172	34891.24	0.000
Temp_1_1	3	10.439	10.439	3.480	969.91	0.000
Brand_1_1*Temp_1_1	12	3.805	3.805	0.317	88.40	0.000
Error	20	0.072	0.072	0.004		
Total	39	515.005				

S = 0.0598957 R-Sq = 99.99% R-Sq(adj) = 99.97%

a-values
General Linear Model: b-values_1_1 versus Brand_1_1, Temp_1_1
Factor Type Levels Values
Brand_1_1 fixed 5 1, 2, 3, 4, 5
Temp_1_1 fixed 4 1, 2, 3, 4
Analysis of Variance for b-values_1_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand_1_1	4	18.9894	18.9894	4.7473	18259.02	0.000
Temp_1_1	3	7.4467	7.4467	2.4822	9547.10	0.000
Brand_1_1*Temp_1_1	12	1.0118	1.0118	0.0843	324.31	0.000
Error	20	0.0052	0.0052	0.0003		
Total	39	27.4532				

S = 0.0161245 R-Sq = 99.98% R-Sq(adj) = 99.96%

b* Measurments
General Linear Model: b-values_1 versus Brand_1, Temp_1
Factor Type Levels Values
Brand_1 fixed 5 1, 2, 3, 4, 5
Temp_1 fixed 4 1, 2, 3, 4
Analysis of Variance for b-values_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brand_1	4	2094.50	2094.50	523.62	23082.43	0.000
Temp_1	3	5.49	5.49	1.83	80.72	0.000
Brand_1*Temp_1	12	314.07	314.07	26.17	1153.72	0.000
Error	20	0.45	0.45	0.02		
Total	39	2414.51				

S = 0.150615 R-Sq = 99.98% R-Sq(adj) = 99.96%

Grouping Information for Cell Counts and Acidity measurements (Tukey's test outputs)

LD-type, -18°C vs Brands

One-way ANOVA: Cell versus Brand

Grouping Information Using Tukey Method

Brand	N	Mean	Grouping
3	2	11.0222	A
2	2	9.7131	B
1	2	9.5349	B C
4	2	9.3798	B C
6	2	9.0893	C
5	2	9.0581	C

One-way ANOVA: pH versus Brand

Grouping Information Using Tukey Method

Brand	N	Mean	Grouping
4	2	5.2150	A
5	2	5.0450	A
6	2	4.9800	A
1	2	4.6900	B
2	2	4.6600	B
3	2	4.5300	B

One-way ANOVA: T.A versus Brand

Grouping Information Using Tukey Method

Brand	N	Mean	Grouping
3	2	0.70926	A
2	2	0.70057	A
1	2	0.64412	A
6	2	0.61788	A
5	2	0.42572	B
4	2	0.35796	B

4°C vs Brands

One-way ANOVA: Cell_1 versus Brand_1

Grouping Information Using Tukey Method

Brand_1	N	Mean	Grouping
3	2	10.8538	A
2	2	9.7113	B
4	2	9.2988	C
1	2	9.2256	C D
6	2	8.9566	C D
5	2	8.9144	D

One-way ANOVA: pH_1 versus Brand_1

Grouping Information Using Tukey Method

Brand_1	N	Mean	Grouping
5	2	5.20000	A
4	2	5.19500	A
6	2	5.06000	A B
2	2	4.92000	B C
1	2	4.71000	C D
3	2	4.65500	D

One-way ANOVA: T.A_1 versus Brand_1

Grouping Information Using Tukey Method

Brand_1	N	Mean	Grouping
3	2	0.65566	A
1	2	0.60637	A
2	2	0.58805	A
6	2	0.43087	B
5	2	0.32837	B
4	2	0.31697	B

20°C vs Brands

One-way ANOVA: Cell_2 versus Brand_2

Grouping Information Using Tukey Method

Brand_2	N	Mean	Grouping
3	2	10.7876	A
2	2	9.3406	B
1	2	9.0999	B C
6	2	8.8826	C
4	2	8.7186	C
5	2	7.7800	D

One-way ANOVA: pH_2 versus Brand_2

Grouping Information Using Tukey Method

Brand_2	N	Mean	Grouping
5	2	6.1500	A
4	2	6.1000	A
6	2	6.0400	A
1	2	5.0900	B
2	2	4.9400	B
3	2	4.7750	C

One-way ANOVA: T.A_2 versus Brand_2

Grouping Information Using Tukey Method

Brand_2	N	Mean	Grouping
3	2	0.65751	A
2	2	0.58066	A
1	2	0.41654	B
6	2	0.37416	B
4	2	0.31297	B
5	2	0.30095	B

37°C vs Brands

One-way ANOVA: Cell_3 versus Brand_3

Grouping Information Using Tukey Method

Brand_3	N	Mean	Grouping
6	2	2.000000000	A
5	2	2.000000000	B
4	2	2.000000000	C
3	2	2.000000000	D
2	2	2.000000000	E
1	2	2.000000000	F

One-way ANOVA: pH_3 versus Brand_3

Grouping Information Using Tukey Method

Brand_3	N	Mean	Grouping
1	2	6.66500	A
5	2	6.66000	A B
6	2	6.63000	A B
4	2	6.62000	A B
2	2	6.56500	B C
3	2	6.47000	C

One-way ANOVA: T.A_3 versus Brand_3

Grouping Information Using Tukey Method

Brand_3	N	Mean	Grouping
2	2	0.090944	A
3	2	0.077286	A
6	2	0.077282	A
4	2	0.075550	A
5	2	0.071828	A
1	2	0.071089	A

Flora Danica:

One-way ANOVA: Cell versus Temp

Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
1	2	9.6753	A
2	2	9.2256	A B
3	2	9.0999	B
4	2	2.0000	C

One-way ANOVA: pH versus Temp

Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
4	2	6.6650	A
3	2	5.0900	B

2 2 4.7100 C
1 2 4.6550 C

One-way ANOVA: T.A versus Temp

Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
1	2	0.63335	A
2	2	0.60637	A
3	2	0.41654	B
4	2	0.07109	C

CHN-19

One-way ANOVA: Cell_1 versus Temp_1

Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
1	2	9.7131	A
2	2	9.7113	A
3	2	9.3406	B
4	2	2.0000	C

One-way ANOVA: pH_1 versus Temp_1

Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
4	2	6.5650	A
3	2	4.9400	B
2	2	4.9200	B
1	2	4.6600	C

One-way ANOVA: T.A_1 versus Temp_1

Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
1	2	0.70057	A
2	2	0.58805	A
3	2	0.58066	A
4	2	0.09094	B

Delvo-Tec DX-33A DSL

One-way ANOVA: Cell_2 versus Temp_2

Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
1	2	11.0222	A
2	2	10.8538	A
3	2	10.7876	A
4	2	2.0000	B

One-way ANOVA: pH_2 versus Temp_2

Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
4	2	6.4700	A
3	2	4.7750	B
2	2	4.6550	B C
1	2	4.5300	C

One-way ANOVA: T.A_2 versus Temp_2

Temp_2	N	Mean	Grouping
1	2	0.70926	A
3	2	0.65751	A
2	2	0.65566	A
4	2	0.07729	B

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One-way ANOVA: Cell_3 versus Temp_3

Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
1	2	9.3798	A
2	2	9.2988	A
3	2	8.7186	B
4	2	2.0000	C

One-way ANOVA: pH_3 versus Temp_3

Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
4	2	6.6200	A
3	2	6.1000	B
1	2	5.2150	C
2	2	5.1950	C

One-way ANOVA: T.A_3 versus Temp_3

Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
1	2	0.35796	A
2	2	0.31697	B
3	2	0.31297	B
4	2	0.07555	C

Probat 222 LYO

One-way ANOVA: Cell_4 versus Temp_4

Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
1	2	9.0581	A
2	2	8.9144	A
3	2	7.7800	B
4	2	2.0000	C

One-way ANOVA: pH_4 versus Temp_4

Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
4	2	6.6600	A
3	2	6.1500	B
2	2	5.2000	C
1	2	5.0450	C

One-way ANOVA: T.A_4 versus Temp_4

Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
1	2	0.42572	A
2	2	0.32837	A B
3	2	0.30095	B
4	2	0.07183	C

M036R

One-way ANOVA: Cell_5 versus Temp_5

Grouping Information Using Tukey Method

Temp_5	N	Mean	Grouping
1	2	9.0893	A
2	2	8.9566	A
3	2	8.8826	A
4	2	2.0000	B

One-way ANOVA: pH_5 versus Temp_5

Grouping Information Using Tukey Method

Temp_5	N	Mean	Grouping
4	2	6.6300	A
3	2	6.0400	B
2	2	5.0600	C
1	2	4.9800	C

One-way ANOVA: T.A_5 versus Temp_5

Grouping Information Using Tukey Method

Temp_5	N	Mean	Grouping
1	2	0.61788	A
2	2	0.43087	B
3	2	0.37416	B
4	2	0.07728	C

O-type,

-18°C vs Brands

One-way ANOVA: Cell versus Brand

Grouping Information Using Tukey Method

Brand	N	Mean	Grouping
2	2	11.2491	A
1	2	10.6232	B
3	2	9.9589	C
4	2	9.4856	C D
5	2	9.0873	D

One-way ANOVA: Ph versus Brand
Grouping Information Using Tukey Method

Brand	N	Mean	Grouping
5	2	6.1800	A
4	2	5.7850	B
1	2	4.6750	C
3	2	4.5300	C D
2	2	4.3950	D

One-way ANOVA: t.a. versus Brand
Grouping Information Using Tukey Method

Brand	N	Mean	Grouping
2	2	0.80950	A
3	2	0.70491	A
1	2	0.61981	A
4	2	0.57583	A
5	2	0.30448	B

4°C vs Brands
One-way ANOVA: Cell_1 versus Brand_1
Grouping Information Using Tukey Method

Brand_1	N	Mean	Grouping
2	2	11.2301	A
1	2	10.4510	B
3	2	9.8825	B C
4	2	9.5017	C D
5	2	9.0936	D

One-way ANOVA: Ph_1 versus Brand_1
Grouping Information Using Tukey Method

Brand_1	N	Mean	Grouping
5	2	6.1850	A
4	2	5.8150	B
1	2	5.1750	C
2	2	4.6150	D
3	2	4.5700	D

One-way ANOVA: t.a._1 versus Brand_1
Grouping Information Using Tukey Method

Brand_1	N	Mean	Grouping
2	2	0.69326	A
3	2	0.64190	A B
1	2	0.58126	B
4	2	0.37019	C
5	2	0.25957	D

20°C vs Brands
One-way ANOVA: Cell_1_1 versus Brand_1_1
Grouping Information Using Tukey Method

Brand_1_1	N	Mean	Grouping
2	2	11.0350	A
3	2	9.4667	B
1	2	9.2227	B
4	2	8.5211	C
5	2	7.9631	D

One-way ANOVA: Ph_1_1 versus Brand_1_1
Grouping Information Using Tukey Method

Brand_1_1	N	Mean	Grouping
5	2	6.3300	A
4	2	6.2500	A
3	2	5.6550	A B
1	2	5.4550	B C
2	2	4.8400	C

One-way ANOVA: t.a._1_1 versus Brand_1_1
Grouping Information Using Tukey Method

Brand_1_1	N	Mean	Grouping
2	2	0.65531	A
1	2	0.50343	B
3	2	0.48434	B
4	2	0.23280	C
5	2	0.21066	C

37°C vs Brands
One-way ANOVA: Cell_1_1_1 versus Brand_1_1_1
Grouping Information Using Tukey Method

Brand_1_1_1	N	Mean	Grouping
5	2	2.000000000	A
4	2	2.000000000	B
3	2	2.000000000	C
2	2	2.000000000	D
1	2	2.000000000	E

One-way ANOVA: Ph_1_1_1 versus Brand_1_1_1
Grouping Information Using Tukey Method

Brand_1_1_1	N	Mean	Grouping
3	2	6.73000	A
5	2	6.70000	A
4	2	6.69500	A
1	2	6.66000	A
2	2	6.42000	B

One-way ANOVA: t.a._1_1_1 versus Brand_1_1_1
Grouping Information Using Tukey Method

Brand_1_1_1	N	Mean	Grouping
5	2	0.077037	A
2	2	0.076316	A
1	2	0.075962	A
4	2	0.073247	A
3	2	0.072336	A

R704
One-way ANOVA: Cell versus Temp
Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
1	2	10.6232	A
2	2	10.4510	A
3	2	9.2227	B
4	2	2.0000	C

One-way ANOVA: Ph versus Temp
Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
4	2	6.6600	A
3	2	5.4550	B
2	2	5.1750	B
1	2	4.6750	C

One-way ANOVA: t.a. versus Temp
Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
1	2	0.61981	A
2	2	0.58126	A
3	2	0.50343	B
4	2	0.07596	C

Delvo-Tec LL-50A DSL
One-way ANOVA: Cell_1 versus Temp_1
Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
1	2	11.2491	A
2	2	11.2301	A
3	2	11.0350	A
4	2	2.0000	B

One-way ANOVA: Ph_1 versus Temp_1
Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
4	2	6.4200	A
3	2	4.8400	B
2	2	4.6150	C

1 2 4.3950 D

One-way ANOVA: t.a._1 versus Temp_1

Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
1	2	0.80950	A
2	2	0.69326	B
3	2	0.65531	B
4	2	0.07632	C

Choozit MA 11 LYO

One-way ANOVA: Cell_2 versus Temp_2

Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
1	2	9.9589	A
2	2	9.8825	A
3	2	9.4667	B
4	2	2.0000	C

One-way ANOVA: Ph_2 versus Temp_2

Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
4	2	6.7300	A
3	2	5.6550	B
2	2	4.5700	C
1	2	4.5300	C

One-way ANOVA: t.a._2 versus Temp_2

Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
1	2	0.70491	A
2	2	0.64190	A B
3	2	0.48434	B
4	2	0.07234	C

MO032

One-way ANOVA: Cell_3 versus Temp_3

Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
2	2	9.5017	A
1	2	9.4856	A
3	2	8.5211	B
4	2	2.0000	C

One-way ANOVA: Ph_3 versus Temp_3

Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
4	2	6.6950	A
3	2	6.2500	B
2	2	5.8150	C
1	2	5.7850	C

One-way ANOVA: t.a._3 versus Temp_3

Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
1	2	0.57583	A
2	2	0.37019	A B
3	2	0.23280	B C
4	2	0.07325	C

Cm11

One-way ANOVA: Cell_4 versus Temp_4

Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
2	2	9.0936	A
1	2	9.0873	A
3	2	7.9631	B
4	2	2.0000	C

One-way ANOVA: Ph_4 versus Temp_4

Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
4	2	6.70000	A
3	2	6.33000	B
2	2	6.18500	C
1	2	6.18000	C

One-way ANOVA: t.a._4 versus Temp_4

Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
1	2	0.30448	A
2	2	0.25957	A B
3	2	0.21066	B
4	2	0.07704	C

Mould, -18°C vs Brands

One-way ANOVA: Cell count versus Brand

Grouping Information Using Tukey Method

Brand	N	Mean	Grouping
1	2	7.7516	A
5	2	7.6797	A
4	2	7.4771	A
3	2	7.4621	A
2	2	7.4098	A

4°C vs Brands

One-way ANOVA: Cell count_1 versus Brand_1

Grouping Information Using Tukey Method

Brand_1	N	Mean	Grouping
1	2	7.4969	A
5	2	7.1963	A B
2	2	6.9221	B
3	2	6.5425	C
4	2	6.4601	C

20°C vs Brands

One-way ANOVA: Cell count_2 versus Brand_2

Grouping Information Using Tukey Method

Brand_2	N	Mean	Grouping
1	2	7.0660	A
5	2	7.0338	A
2	2	6.9887	A
3	2	6.5262	A B
4	2	6.2727	B

37°C vs Brands

One-way ANOVA: Cell count_3 versus Brand_3

Grouping Information Using Tukey Method

Brand_3	N	Mean	Grouping
5	2	3.000000000	A
4	2	3.000000000	B
3	2	3.000000000	C
2	2	3.000000000	D
1	2	3.000000000	E

Camemberti Swing PCTT033

One-way ANOVA: Cell count versus Temp

Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
1	2	7.7516	A
2	2	7.4969	B
3	2	7.0660	C
4	2	3.0000	D

PC neige

One-way ANOVA: Cell count_3 versus Temp_3

Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
1	2	7.4771	A
2	2	6.4601	B
3	2	6.2727	B
4	2	3.0000	C

PCV5

One-way ANOVA: Cell count_4 versus Temp_4
Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
1	2	7.6797	A
2	2	7.1963	B
3	2	7.0338	B
4	2	3.0000	C

Grouping Information for colour measurements
LD-type
L measurements
Flora Danica
One-way ANOVA: L-values versus Temp
Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
1	2	96.5200	A
2	2	96.2450	B
3	2	95.0350	C
4	2	78.7500	D

CHN-19
One-way ANOVA: L-values_1 versus Temp_1
Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
1	2	95.9800	A
2	2	95.0950	B
3	2	94.3100	C
4	2	63.2050	D

Delvo-Tec DX-33 A DSL
One-way ANOVA: L-values_2 versus Temp_2
Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
1	2	73.9050	A
2	2	53.2000	B
3	2	50.1800	C
4	2	14.7200	D

CM51
One-way ANOVA: L-values_3 versus Temp_3
Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
1	2	98.0750	A
2	2	97.8200	B
3	2	97.3650	C
4	2	91.4950	D

Probat 222 LYO
One-way ANOVA: L-values_4 versus Temp_4
Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
1	2	98.2050	A
2	2	97.9650	B
3	2	97.2450	C
4	2	85.1650	D

MO036 R
One-way ANOVA: L-values_4_1 versus Temp_4_1
Grouping Information Using Tukey Method

Temp_4_1	N	Mean	Grouping
1	2	97.7650	A
2	2	97.6700	A
3	2	97.4900	B
4	2	89.5050	C

A measurements
Flora Danica
One-way ANOVA: a-values versus Temp
Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
4	2	4.2550	A
3	2	1.5550	B
2	2	0.8300	C
1	2	0.5100	D

CHN-19
One-way ANOVA: a-values_1 versus Temp_1
Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
4	2	6.70000	A
3	2	1.49500	B
2	2	0.63500	C
1	2	0.13000	D

Delvo-Tec DX-33 A DSL
One-way ANOVA: a-values_2 versus Temp_2
Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
4	2	15.3600	A
3	2	4.2550	B
2	2	4.0000	C
1	2	3.6550	D

CM51
One-way ANOVA: a-values_3 versus Temp_3
Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
4	2	1.14500	A
3	2	0.52500	B
2	2	0.30000	C
1	2	-0.13000	D

Probat 222 LYO
One-way ANOVA: a-values_4 versus Temp_4
Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
4	2	2.25000	A
3	2	0.10500	B
2	2	0.07000	C
1	2	-0.60000	D

MO036 R
One-way ANOVA: a-values_4_1 versus Temp_4_1
Grouping Information Using Tukey Method

Temp_4_1	N	Mean	Grouping
4	2	1.130000	A
3	2	0.160000	B
2	2	0.150000	C
1	2	0.140000	D

B measurements
Flora Danica
One-way ANOVA: b-values versus Temp
Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
4	2	13.52000	A
3	2	6.27500	B
2	2	5.19500	C
1	2	5.18500	C

CHN-19
One-way ANOVA: b-values_1 versus Temp_1
Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
4	2	16.4350	A

3 2 8.5500 B
2 2 6.3500 C
1 2 6.1600 D

Delvo-Tec DX-33 A DSL

One-way ANOVA: b-values_2 versus Temp_2

Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
3	2	27.745	A
2	2	26.010	B
1	2	24.480	C
4	2	17.135	D

CM51

One-way ANOVA: b-values_3 versus Temp_3

Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
4	2	6.1400	A
3	2	4.0350	B
2	2	3.6150	C
1	2	3.3350	D

Probat 222 LYO

One-way ANOVA: b-values_4 versus Temp_4

Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
4	2	10.0300	A
3	2	4.4800	B
2	2	3.5700	C
1	2	3.4300	D

MO036 R

One-way ANOVA: b-values_4_1 versus Temp_4_1

Grouping Information Using Tukey Method

Temp_4_1	N	Mean	Grouping
4	2	6.64000	A
3	2	3.85000	B
2	2	3.20000	C
1	2	3.07000	D

O-TYPE

L measurements

R704

One-way ANOVA: L-values versus Temp

Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
1	2	98.5250	A
2	2	97.6350	B
3	2	95.5000	C
4	2	85.6000	D

Delvo-Tec LL-50A DSL

One-way ANOVA: L-values_1 versus Temp_1

Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
1	2	73.6250	A
2	2	72.5950	B
3	2	71.4600	C
4	2	29.5100	D

Choozit MA 11 LYO

One-way ANOVA: L-values_2 versus Temp_2

Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
1	2	98.1100	A
2	2	98.0200	A
3	2	97.7400	B
4	2	94.8200	C

MO032

One-way ANOVA: L-values_3 versus Temp_3

Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
1	2	97.9550	A
2	2	97.9450	A
3	2	97.8700	A
4	2	90.2750	B

CM11

One-way ANOVA: L-values_4 versus Temp_4

Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
1	2	98.0100	A
2	2	97.9950	A
3	2	97.4150	B
4	2	91.1450	C

A measurements

R704

One-way ANOVA: a-values versus Temp

Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
4	2	2.53500	A
3	2	0.35500	B
2	2	-0.22000	C
1	2	-0.42000	D

Delvo-Tec LL-50A DSL

One-way ANOVA: a-values_1 versus Temp_1

Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
4	2	14.0200	A
3	2	3.8950	B
2	2	3.2500	C
1	2	3.1300	D

Choozit MA 11 LYO

One-way ANOVA: a-values_2 versus Temp_2

Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
4	2	0.60000	A
3	2	0.06000	B
2	2	-0.18000	C
1	2	-0.64000	D

MO032

One-way ANOVA: a-values_3 versus Temp_3

Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
4	2	1.35000	A
3	2	0.22000	B
2	2	-0.02500	C
1	2	-0.35500	D

CM11

One-way ANOVA: a-values_4 versus Temp_4

Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
4	2	0.95000	A
3	2	0.21000	B
2	2	0.05000	C
1	2	-0.14000	D

B-measurements

R704

One-way ANOVA: b-values versus Temp

Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
4	2	11.4150	A
3	2	5.8200	B
2	2	5.1350	B
1	2	4.5450	B

Delvo-Tec LL-50A DSL
One-way ANOVA: b-values_1 versus Temp_1
Grouping Information Using Tukey Method

Temp_1	N	Mean	Grouping
3	2	26.7200	A
2	2	25.4000	B
1	2	25.3300	C
4	2	13.7100	D

Choozit MA 11 LYO
One-way ANOVA: b-values_2 versus Temp_2
Grouping Information Using Tukey Method

Temp_2	N	Mean	Grouping
4	2	6.1600	A
3	2	3.4900	B
2	2	3.4750	B
1	2	3.4500	B

MO032
One-way ANOVA: b-values_3 versus Temp_3
Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
4	2	6.7400	A
3	2	3.8900	B
2	2	3.4600	C
1	2	3.4350	C

CM11
One-way ANOVA: b-values_4 versus Temp_4
Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
4	2	6.1700	A
3	2	4.0000	B
2	2	3.4200	C
1	2	3.1850	D

Mould cultures
L-measurments
Camemberti Swing PCTT033
One-way ANOVA: L-values versus Temp
Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
1	2	98.2050	A
2	2	97.9500	B
3	2	97.9150	B
4	2	96.6300	C

PC Neige
One-way ANOVA: L-values_3 versus Temp_3
Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
1	2	98.2900	A
2	2	98.2550	A
3	2	97.9000	B
4	2	97.0200	C

PCV5
One-way ANOVA: L-values_4 versus Temp_4
Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
1	2	98.5100	A
2	2	98.3600	B
3	2	98.2500	C
4	2	96.0350	D

A-measurments
Camemberti Swing PCTT033
One-way ANOVA: a-values versus Temp
Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
4	2	0.430000	A
3	2	0.300000	B
2	2	-0.020000	C
1	2	-0.220000	D

PC neige
One-way ANOVA: a-values_3 versus Temp_3
Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
4	2	0.290000	A
3	2	0.060000	B
2	2	-0.090000	C
1	2	-0.100000	D

PCV5
One-way ANOVA: a-values_4 versus Temp_4
Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
4	2	0.39500	A
3	2	-0.08000	B
2	2	-0.16500	C
1	2	-0.47500	D

B-measurements
Camemberti Swing PCTT033
One-way ANOVA: b-values versus Temp
Grouping Information Using Tukey Method

Temp	N	Mean	Grouping
4	2	3.7700	A
3	2	2.7450	B
2	2	2.5550	C
1	2	2.4650	D

PC neige
One-way ANOVA: b-values_3 versus Temp_3
Grouping Information Using Tukey Method

Temp_3	N	Mean	Grouping
4	2	3.5750	A
3	2	2.7450	B
2	2	2.2750	C
1	2	2.2500	C

PCV5
One-way ANOVA: b-values_4 versus Temp_4
Grouping Information Using Tukey Method

Temp_4	N	Mean	Grouping
4	2	4.32000	A
3	2	3.16000	B
2	2	2.98500	C
1	2	2.92500	D

4.2 Production, ripening and characterization of Camembert cheese

Fermentation period data

General Linear Model: log LAB, LOG MOULD versus Cheese type, production b, ...
Analysis of Variance for log LAB, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	29.487	29.487	29.487	294.18	0.000
production batch	1	0.054	0.054	0.054	0.54	0.466
starter temp	2	6.142	6.142	3.071	30.64	0.000
Fermentation hrs	4	203.558	203.558	50.890	507.71	0.000
Error	111	11.126	11.126	0.100		
Total	119	250.367				

S = 0.316597 R-Sq = 95.56% R-Sq(adj) = 95.24%

Analysis of Variance for LOG MOULD, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	0.2970	0.2970	0.2970	27.29	0.000
production batch	1	0.0207	0.0207	0.0207	1.90	0.171
starter temp	2	2.0432	2.0432	1.0216	93.84	0.000
Fermentation hrs	4	8.2015	8.2015	2.0504	188.35	0.000
Error	111	1.2084	1.2084	0.0109		
Total	119	11.7708				

S = 0.104337 R-Sq = 89.73% R-Sq(adj) = 88.99%

Ripening period data

General Linear Model: pH versus Cheese type, starter temp, ...

Analysis of Variance for pH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	0.4620	0.4620	0.4620	21.09	0.000
starter temp	2	0.2958	0.2958	0.1479	6.75	0.002
Ripening time	3	44.9464	44.9464	14.9821	683.80	0.000
production batch	1	0.0726	0.0726	0.0726	3.31	0.072
Error	88	1.9281	1.9281	0.0219		
Total	95	47.7050				

S = 0.148021 R-Sq = 95.96% R-Sq(adj) = 95.64%

General Linear Model: SN/TN versus Cheese type, starter temp, ...

Analysis of Variance for SN/TN, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	0.025089	0.025089	0.025089	41.41	0.000
starter temp	2	0.001873	0.001873	0.000936	1.55	0.219
Ripening time	3	0.397814	0.397814	0.132605	218.85	0.000
production batch	1	0.000725	0.000725	0.000725	1.20	0.277
Error	88	0.053321	0.053321	0.000606		
Total	95	0.478822				

S = 0.0246154 R-Sq = 88.86% R-Sq(adj) = 87.98%

General Linear Model: NPN/TN versus Cheese type, starter temp, ...

Analysis of Variance for NPN/TN, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	0.011847	0.011847	0.011847	30.90	0.000
starter temp	2	0.000475	0.000475	0.000237	0.62	0.541
Ripening time	3	0.233391	0.233391	0.077797	202.92	0.000
production batch	1	0.000031	0.000031	0.000031	0.08	0.778
Error	88	0.033739	0.033739	0.000383		
Total	95	0.279483				

S = 0.0195804 R-Sq = 87.93% R-Sq(adj) = 86.97%

General Linear Model: PROTEIN versus Cheese type, starter temp, ...

Analysis of Variance for PROTEIN, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	0.02882	0.02882	0.02882	1.79	0.185
starter temp	2	0.04668	0.04668	0.02334	1.45	0.241
Ripening time	3	1.34626	1.34626	0.44875	27.83	0.000
production batch	1	0.02009	0.02009	0.02009	1.25	0.267
Error	88	1.41913	1.41913	0.01613		
Total	95	2.86098				

S = 0.126990 R-Sq = 50.40% R-Sq(adj) = 46.45%

General Linear Model: CASEIN versus Cheese type, starter temp, ...

Analysis of Variance for CASEIN, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	1.04033	1.04033	1.04033	134.63	0.000
starter temp	2	0.09691	0.09691	0.04845	6.27	0.003

Ripening time	3	2.62457	2.62457	0.87486	113.21	0.000
production batch	1	0.00551	0.00551	0.00551	0.71	0.401
Error	88	0.68002	0.68002	0.00773		
Total	95	4.44733				

S = 0.0879059 R-Sq = 84.71% R-Sq(adj) = 83.49%

General Linear Model: PEPTIDES versus Cheese type, starter temp, ...

Analysis of Variance for PEPTIDES, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	0.013927	0.013927	0.013927	17.85	0.000
starter temp	2	0.016072	0.016072	0.008036	10.30	0.000
Ripening time	3	0.350686	0.350686	0.116895	149.81	0.000
production batch	1	0.001819	0.001819	0.001819	2.33	0.130
Error	88	0.068665	0.068665	0.000780		
Total	95	0.451170				

S = 0.0279336 R-Sq = 84.78% R-Sq(adj) = 83.57%

General Linear Model: FAA versus Cheese type, starter temp, ...

Analysis of Variance for FAA, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	0.7530	0.7530	0.7530	17.17	0.000
starter temp	2	0.3893	0.3893	0.1946	4.44	0.015
Ripening time	3	17.2202	17.2202	5.7401	130.91	0.000
production batch	1	0.0042	0.0042	0.0042	0.09	0.759
Error	88	3.8586	3.8586	0.0438		
Total	95	22.2252				

S = 0.209398 R-Sq = 82.64% R-Sq(adj) = 81.26%

General Linear Model: LAB log versus Cheese type, starter temp, ...

Analysis of Variance for LAB log, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	0.00007	0.00007	0.00007	0.00	0.966
starter temp	2	0.71999	0.71999	0.36000	8.91	0.000
Ripening time	3	4.96726	4.96726	1.65575	40.99	0.000
production batch	1	0.46352	0.46352	0.46352	11.48	0.119
Error	88	3.55425	3.55425	0.04039		
Total	95	9.70509				

S = 0.200971 R-Sq = 63.38% R-Sq(adj) = 60.46%

General Linear Model: Mould log versus Cheese type, starter temp, ...

Analysis of Variance for Mould log, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	0.469	0.469	0.469	8.14	0.005
starter temp	2	0.516	0.516	0.258	4.48	0.014
Ripening time	3	151.068	151.068	50.356	874.13	0.000
production batch	1	0.067	0.067	0.067	1.16	0.284
Error	88	5.069	5.069	0.058		
Total	95	157.189				

S = 0.240014 R-Sq = 96.77% R-Sq(adj) = 96.52%

General Linear Model: Texture versus Cheese type, starter temp, ...

Analysis of Variance for Texture, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Cheese type	1	78.26	78.26	78.26	26.51	0.000
starter temp	2	9.33	9.33	4.67	1.58	0.212
Ripening time	3	1136.10	1136.10	378.70	128.28	0.000
production batch	1	5.21	5.21	5.21	1.76	0.187
Error	88	259.78	259.78	2.95		
Total	95	1488.68				

S = 1.71816 R-Sq = 82.55% R-Sq(adj) = 81.16%

Key aromatic compounds versus starter type, culture storage temperature and ripening time

Factor	Type	Levels	Values
starter type	fixed	2	1, 2
Ripening time	fixed	4	1, 2, 3, 4
temperature	fixed	3	1, 2, 3
replication	fixed	2	1, 2

General Linear Model for 3-methylbutanal, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P

starter type	1	8548	8548	8548	19.12	0.000
Ripening time	3	91073	91073	30358	67.89	0.000
temperature	2	6350	6350	3175	7.10	0.002
replication	1	29	29	29	0.07	0.800
Error	40	17886	17886	447		
Total	47	123886				

S = 21.1457 R-Sq = 85.56% R-Sq(adj) = 83.04%

General Linear Model for 3-methylbutanol, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
starter type	1	114382	114382	114382	0.01	0.938
Ripening time	3	1715676452	1715676452	571892151	31.09	0.000
temperature	2	37996180	37996180	18998090	1.03	0.365
replication	1	4336	4336	4336	0.00	0.988
Error	40	735757873	735757873	18393947		
Total	47	2489549223				

S = 4288.82 R-Sq = 70.45% R-Sq(adj) = 65.27%

General Linear Model for 2-heptanone, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
starter type	1	176644	176644	176644	16.09	0.000
Ripening time	3	729751	729751	243250	22.15	0.000
temperature	2	73390	73390	36695	3.34	0.045
replication	1	6033	6033	6033	0.55	0.463
Error	40	439239	439239	10981		
Total	47	1425058				

S = 104.790 R-Sq = 69.18% R-Sq(adj) = 63.78%

General Linear Model for 2-nonanone, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
starter type	1	20905	20905	20905	12.63	0.001
Ripening time	3	243022	243022	81007	48.95	0.000
temperature	2	3766	3766	1883	1.14	0.331
replication	1	686	686	686	0.41	0.523
Error	40	66189	66189	1655		
Total	47	334569				

S = 40.6784 R-Sq = 80.22% R-Sq(adj) = 76.75%

General Linear Model for Butyric acid, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
starter type	1	3162974357	3162974357	3162974357	7.41	0.010
Ripening time	3	13436746607	13436746607	4478915536	10.49	0.000
temperature	2	1835927727	1835927727	917963864	2.15	0.130
replication	1	154451434	154451434	154451434	0.36	0.551
Error	40	17077834448	17077834448	426945861		
Total	47	35667934574				

S = 20662.7 R-Sq = 52.12% R-Sq(adj) = 43.74%

Correlations: Texture, LAB log, mould log, FAA, PEPTIDES, CASEIN, PROTEIN, ...

	Texture	LAB log	mould log	FAA	PEPTIDES	CASEIN
LAB log	0.599					
	0.000					
mould log	-0.559	-0.577				
	0.000	0.000				
FAA	-0.670	-0.668	0.785			
	0.000	0.000	0.000			
PEPTIDES	-0.683	-0.533	0.844	0.659		
	0.000	0.000	0.000	0.000		
CASEIN	0.446	0.525	-0.765	-0.553	-0.763	
	0.000	0.000	0.000	0.000	0.000	
PROTEIN	0.475	0.481	-0.674	-0.530	-0.652	0.589
	0.000	0.000	0.000	0.000	0.000	0.000
NPN/TN	-0.736	-0.606	0.833	0.718	0.872	-0.820
	0.000	0.000	0.000	0.000	0.000	0.000
SN/TN	-0.732	-0.607	0.832	0.713	0.875	-0.825
	0.000	0.000	0.000	0.000	0.000	0.000
pH	-0.806	-0.736	0.810	0.794	0.835	-0.727

	0.000	0.000	0.000	0.000	0.000	0.000
	PROTEIN	NPN/TN	SN/TN			
NPN/TN	-0.676					
	0.000					
SN/TN	-0.715	0.951				
	0.000	0.000				
pH	-0.604	0.882	0.888			
	0.000	0.000	0.000			

Cell Contents: Pearson correlation
P-Value

Tukey's test - Grouping Information for ripening data (microbiological, nitrogen fraction, FAA and texture)

Day 3, cheese 1

One-way ANOVA: pH versus starter temp

Grouping Information Using Tukey Method

starter

temp	N	Mean	Grouping
3	4	4.67500	A
2	4	4.55500	B
1	4	4.46500	C

Means that do not share a letter are significantly different.

One-way ANOVA: SN/TN versus starter temp

Grouping Information Using Tukey Method

starter

temp	N	Mean	Grouping
2	4	0.09485	A
3	4	0.08171	A
1	4	0.07485	A

One-way ANOVA: NPN/TN versus starter temp

Grouping Information Using Tukey Method

starter

temp	N	Mean	Grouping
1	4	0.058533	A
2	4	0.048875	A
3	4	0.048102	A

One-way ANOVA: PROTEIN versus starter temp

Grouping Information Using Tukey Method

starter

temp	N	Mean	Grouping
1	4	2.4420	A
2	4	2.3530	A
3	4	2.2776	A

One-way ANOVA: CASEIN versus starter temp

Grouping Information Using Tukey Method

starter

temp	N	Mean	Grouping
2	4	2.3909	A
3	4	2.3478	A
1	4	2.3350	A

One-way ANOVA: PEPTIDES versus starter temp

Grouping Information Using Tukey Method

starter

temp	N	Mean	Grouping
2	4	0.07625	A
3	4	0.07236	A
1	4	0.05236	A

One-way ANOVA: FAA versus starter temp

Grouping Information Using Tukey Method

starter

temp	N	Mean	Grouping
1	4	0.5060	A
2	4	0.4817	A
3	4	0.4693	A

One-way ANOVA: LAB log versus starter temp

Grouping Information Using Tukey Method

starter

temp	N	Mean	Grouping
1	4	9.4332	A
2	4	9.1263	B
3	4	8.8326	C

One-way ANOVA: Mould log versus starter temp

Grouping Information Using Tukey Method

starter

temp	N	Mean	Grouping
1	4	3.72782	A
2	4	3.60576	A
3	4	3.11671	B

One-way ANOVA: 3-methylbutanal versus temperature

Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
1	2	66.05	A
2	2	21.51	B
3	2	0.00	B

One-way ANOVA: 3-methylbutanol versus temperature

Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
3	2	0.000000000	A
2	2	0.000000000	B
1	2	0.000000000	C

One-way ANOVA: 2-heptanone versus temperature

Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
2	2	83.90	A
1	2	29.22	B
3	2	9.08	B

One-way ANOVA: 2-nonanone versus temperature

Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
1	2	17.861	A
3	2	0.000	B
2	2	0.000	B

One-way ANOVA: 2-nonanone versus temperature

Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
1	2	17.861	A
3	2	0.000	B
2	2	0.000	B

One-way ANOVA: Butyric acid versus temperature
Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
3	2	0.000000000	A
2	2	0.000000000	B
1	2	0.000000000	C

Day 3, Cheese type 2

One-way ANOVA: Mould log_1 versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	3.93597	A
2	4	3.74732	B
3	4	3.52089	C

One-way ANOVA: LAB log_1 versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	9.13399	A
2	4	9.08582	A
3	4	9.03207	A

One-way ANOVA: FAA_1 versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	0.42084	A
3	4	0.38920	A
2	4	0.37996	A

One-way ANOVA: PEPTIDES_1 versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.097652	A
2	4	0.095518	A
1	4	0.075507	B

One-way ANOVA: CASEIN_1 versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	2.3413	A
2	4	2.2001	A B
3	4	2.0611	B

One-way ANOVA: PROTEIN_1 versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	2.38268	A
3	4	2.37683	A
2	4	2.33157	A

One-way ANOVA: NPN/TN_1 versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.049875	A
1	4	0.044945	A
2	4	0.044720	A

One-way ANOVA: SN/TN_1 versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.09695	A
2	4	0.09616	A
1	4	0.09347	A

One-way ANOVA: pH_1 versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	4.80250	A
2	4	4.68750	A B
1	4	4.62000	B

One-way ANOVA: 3-methylbutanal versus temperature
Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
3	2	0.000000000	A
2	2	0.000000000	B
1	2	0.000000000	C

One-way ANOVA: 3-methylbutanol versus temperature
Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
3	2	0.000000000	A
2	2	0.000000000	B
1	2	0.000000000	C

One-way ANOVA: 2-heptanone versus temperature
Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
2	2	69.15	A
3	2	45.61	A
1	2	22.33	A

One-way ANOVA: 2-nonanone versus temperature
Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
1	2	18.408	A
2	2	6.321	A
3	2	0.000	A

One-way ANOVA: Butyric acid versus temperature
Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
3	2	0.000000000	A
2	2	0.000000000	B
1	2	0.000000000	C

Day 10, cheese 1

One-way ANOVA: pH versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	4.80750	A
3	4	4.73250	A
2	4	4.70750	A

One-way ANOVA: SN/TN versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
2	4	0.12213	A
3	4	0.10868	A
1	4	0.09973	A

One-way ANOVA: NPN/TN versus starter temp
Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.07388	A
2	4	0.05849	A
1	4	0.05822	A

One-way ANOVA: PROTEIN versus starter temp
Grouping Information Using Tukey Method
starter

temp N Mean Grouping
1 4 2.2721 A
2 4 2.2352 A
3 4 2.1377 A

One-way ANOVA: CASEIN versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
3 4 2.29327 A
1 4 2.23063 A
2 4 2.21431 A

One-way ANOVA: PEPTIDES versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
3 4 0.19382 A
2 4 0.13882 B
1 4 0.09818 C

One-way ANOVA: FAA versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
1 4 0.70259 A
3 4 0.59631 B
2 4 0.57174 B

One-way ANOVA: LAB log versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
3 4 9.11763 A
2 4 8.92600 B
1 4 8.83076 B

One-way ANOVA: Mould log versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
3 4 5.5003 A
2 4 5.3177 A
1 4 5.2878 A

One-way ANOVA: 3-methylbutanal_1 versus temperature

Grouping Information Using Tukey Method
temperature N Mean Grouping
1 2 100.75 A
2 2 77.74 A
3 2 69.80 A

One-way ANOVA: 3-methylbutanol_1 versus temperature

Grouping Information Using Tukey Method
temperature N Mean Grouping
3 2 23573 A
2 2 19257 A
1 2 14736 A

One-way ANOVA: 2-heptanone_1 versus temperature

Grouping Information Using Tukey Method
temperature N Mean Grouping
3 2 215.71 A
2 2 186.78 A
1 2 166.15 A

One-way ANOVA: 2-nonanone_1 versus temperature

Grouping Information Using Tukey Method
temperature N Mean Grouping
1 2 161.61 A
2 2 102.65 A B
3 2 84.14 B

One-way ANOVA: Butyric acid_1 versus temperature

Grouping Information Using Tukey Method
temperature N Mean Grouping
3 2 0.000000000 A
2 2 0.000000000 B
1 2 0.000000000 C

Day 10, cheese type 2

One-way ANOVA: pH_1 versus starter temp

Grouping Information Using Tukey Method
Starter
temp N Mean Grouping
1 4 4.9675 A
2 4 4.9625 A
3 4 4.9250 A

One-way ANOVA: SN/TN_1 versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
2 4 0.13028 A
1 4 0.12558 A
3 4 0.12060 A

One-way ANOVA: NPN/TN_1 versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
2 4 0.08372 A
1 4 0.07556 A
3 4 0.07217 A

One-way ANOVA: PROTEIN_1 versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
3 4 2.28174 A
2 4 2.23264 A
1 4 2.22910 A

One-way ANOVA: CASEIN_1 versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
1 4 2.02661 A
2 4 2.00670 A
3 4 1.97994 A

One-way ANOVA: PEPTIDES_1 versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
3 4 0.12035 A
2 4 0.11678 A
1 4 0.11264 A

One-way ANOVA: FAA_1 versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping
1 4 0.7378 A
2 4 0.7049 A
3 4 0.6072 A

Means that do not share a letter are significantly different.

One-way ANOVA: LAB log_1 versus starter temp

Grouping Information Using Tukey Method
starter
temp N Mean Grouping

3 4 9.02901 A
2 4 8.97972 A
1 4 8.89949 A

One-way ANOVA: Mould log_1 versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	5.73282	A
2	4	5.53063	B
1	4	5.46429	B

One-way ANOVA: 3-methylbutanal_1 versus temperature_1

Grouping Information Using Tukey Method

temperature_1	N	Mean	Grouping
2	2	57.59	A
1	2	44.14	A
3	2	36.11	A

One-way ANOVA: 3-methylbutanol_1 versus temperature_1

Grouping Information Using Tukey Method

temperature_1	N	Mean	Grouping
2	2	17413	A
3	2	13954	A
1	2	11414	A

One-way ANOVA: 2-heptanone_1 versus temperature_1

Grouping Information Using Tukey Method

temperature_1	N	Mean	Grouping
3	2	127.75	A
2	2	83.10	A
1	2	43.97	A

One-way ANOVA: 2-nonanone_1 versus temperature_1

Grouping Information Using Tukey Method

temperature_1	N	Mean	Grouping
1	2	72.44	A
2	2	61.73	A
3	2	46.80	A

One-way ANOVA: Butyric acid_1 versus temperature_1

Grouping Information Using Tukey Method

temperature_1	N	Mean	Grouping
3	2	14897	A
2	2	0	B
1	2	0	B

<u>Day 14, cheese type 1</u>

One-way ANOVA: pH versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	5.26750	A
2	4	5.12500	B
3	4	5.03000	C

One-way ANOVA: SN/TN versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	0.16637	A
3	4	0.16352	A
2	4	0.14502	A

One-way ANOVA: NPN/TN versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.11206	A
1	4	0.09921	A
2	4	0.08552	A

One-way ANOVA: PROTEIN versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	2.1790	A
3	4	2.1625	A
2	4	2.1314	A

One-way ANOVA: CASEIN versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
2	4	2.12216	A
3	4	2.06973	A
1	4	2.06337	A

One-way ANOVA: PEPTIDES versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.14831	A
2	4	0.14821	A
1	4	0.12488	A

One-way ANOVA: FAA versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	1.6289	A
3	4	1.4420	A B
2	4	1.0886	B

One-way ANOVA: LAB log versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	9.0279	A
2	4	8.8116	A
1	4	8.4091	B

One-way ANOVA: Mould log versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	6.4011	A
2	4	6.0894	A B
1	4	5.6070	B

One-way ANOVA: 3-methylbutanal_2 versus temperature

Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
1	2	194.46	A
3	2	149.44	A
2	2	113.18	A

One-way ANOVA: 3-methylbutanol_2 versus temperature

Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
3	2	6029	A
2	2	2476	A
1	2	1210	A

One-way ANOVA: 2-heptanone_2 versus temperature

Grouping Information Using Tukey Method

temperature	N	Mean	Grouping
3	2	469.46	A

2 2 423.71 A
1 2 383.49 A

One-way ANOVA: 2-nonanone_2 versus temperature

Grouping Information Using Tukey Method

temperature N Mean Grouping
3 2 277.81 A
1 2 221.59 A
2 2 207.72 A

One-way ANOVA: Butyric acid_2 versus temperature_2

Grouping Information Using Tukey Method

temperature_2 N Mean Grouping
3 2 31663 A
1 2 16063 B
2 2 8928 B

Day 14, cheese type 2

One-way ANOVA: pH_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
1 4 5.5000 A
2 4 5.2950 A B
3 4 5.1000 B

One-way ANOVA: SN/TN_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
2 4 0.20296 A
3 4 0.18002 A
1 4 0.17732 A

One-way ANOVA: NPN/TN_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
2 4 0.14065 A
1 4 0.11760 B
3 4 0.11661 B

One-way ANOVA: PROTEIN_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
1 4 2.1551 A
3 4 2.1458 A
2 4 2.0663 A

One-way ANOVA: CASEIN_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
1 4 2.00954 A
2 4 1.82659 B
3 4 1.80015 B

One-way ANOVA: PEPTIDES_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
3 4 0.17975 A
2 4 0.17903 A
1 4 0.14937 A

One-way ANOVA: FAA_1 versus starter temp

Grouping Information Using Tukey Metho

starter
temp N Mean Grouping
1 4 1.0872 A
2 4 1.0846 A
3 4 0.8756 A

One-way ANOVA: LAB log_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
3 4 8.8275 A
2 4 8.6898 A
1 4 8.5889 A

One-way ANOVA: Mould log_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
3 4 6.11728 A
2 4 5.96711 B
1 4 5.80684 C

One-way ANOVA: 3-methylbutanal_2 versus temperature_2

Grouping Information Using Tukey Method

temperature_2 N Mean Grouping
1 2 118.43 A
2 2 111.50 A
3 2 103.73 A

One-way ANOVA: 3-methylbutanol_2 versus temperature_2

Grouping Information Using Tukey Method

temperature_2 N Mean Grouping
2 2 10079 A
3 2 8556 A
1 2 6696 A

One-way ANOVA: 2-heptanone_2 versus temperature_2

Grouping Information Using Tukey Method

temperature_2 N Mean Grouping
3 2 342.44 A
2 2 334.38 A
1 2 318.96 A

One-way ANOVA: 2-nonanone_2 versus temperature_2

Grouping Information Using Tukey Method

temperature_2 N Mean Grouping
1 2 188.88 A
3 2 150.81 A
2 2 146.71 A

One-way ANOVA: Butyric acid_2 versus temperature_2

Grouping Information Using Tukey Method

temperature_2 N Mean Grouping
1 2 91550 A
3 2 76970 A
2 2 16062 A

day 21, cheese 1

One-way ANOVA: pH versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
1 4 6.61250 A
2 4 6.42250 B
3 4 6.09750 C

One-way ANOVA: SN/TN versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
3 4 0.23753 A

2 4 0.22524 A
1 4 0.20187 A

One-way ANOVA: NPN/TN versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.16173	A
1	4	0.14646	A
2	4	0.14513	A

One-way ANOVA: PROTEIN versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	2.11955	A
1	4	2.10819	A
2	4	2.10270	A

One-way ANOVA: CASEIN versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
2	4	1.99686	A
1	4	1.94395	A
3	4	1.90349	A

One-way ANOVA: PEPTIDES versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.22020	A
1	4	0.20947	A
2	4	0.19812	A

One-way ANOVA: FAA versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	1.7522	A
2	4	1.6983	A
3	4	1.5260	A

One-way ANOVA: LAB log versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	8.8282	A
2	4	8.6549	B
1	4	7.9169	C

One-way ANOVA: Mould log versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	7.16751	A
2	4	7.00645	B
1	4	6.79254	C

One-way ANOVA: 3-methylbutanal_3 versus temperature_2

Grouping Information Using Tukey Method

temperature_2	N	Mean	Grouping
3	2	51.300	A
1	2	38.879	A
2	2	30.327	A

One-way ANOVA: 3-methylbutanol_3 versus temperature_2

Grouping Information Using Tukey Method

temperature_2	N	Mean	Grouping
3	2	2423.1	A
2	2	454.4	B
1	2	422.3	B

One-way ANOVA: 2-heptanone_3 versus temperature_2

Grouping Information Using Tukey Method

temperature_2	N	Mean	Grouping
3	2	652.8	A
2	2	345.0	A B
1	2	103.3	B

One-way ANOVA: 2-nonanone_3 versus temperature_2

Grouping Information Using Tukey Method

temperature_2	N	Mean	Grouping
3	2	129.88	A
1	2	49.48	A B
2	2	31.48	B

One-way ANOVA: Butyric acid_3 versus temperature_2

Grouping Information Using Tukey Method

temperature_2	N	Mean	Grouping
3	2	30225	A
2	2	15146	A
1	2	6729	A

<u>Day 21, cheese type 2</u>

One-way ANOVA: pH_1 versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	6.5575	A
2	4	6.3900	A
3	4	6.3550	A

One-way ANOVA: SN/TN_1 versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.30567	A
2	4	0.29198	A
1	4	0.28848	A

One-way ANOVA: NPN/TN_1 versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.20961	A
2	4	0.20581	A
1	4	0.20153	A

One-way ANOVA: PROTEIN_1 versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	2.01933	A
3	4	1.95638	A
2	4	1.92789	A

One-way ANOVA: CASEIN_1 versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
1	4	1.78779	A
2	4	1.71133	A B
3	4	1.66192	B

One-way ANOVA: PEPTIDES_1 versus starter temp

Grouping Information Using Tukey Method
starter

temp	N	Mean	Grouping
3	4	0.30977	A

2 4 0.26738 A
1 4 0.26632 A

One-way ANOVA: FAA_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
1 4 1.4699 A
2 4 1.3674 A
3 4 1.2135 A

One-way ANOVA: LAB log_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
3 4 8.6351 A
2 4 8.5686 A
1 4 8.4662 A

One-way ANOVA: Mould log_1 versus starter temp

Grouping Information Using Tukey Method

starter
temp N Mean Grouping
3 4 7.4159 A
2 4 7.1355 A B
1 4 6.9231 B

One-way ANOVA: 3-methylbutanal_3 versus temperature_3

Grouping Information Using Tukey Method
temperature_3 N Mean Grouping
1 2 69.093 A
2 2 35.695 B
3 2 16.880 C

One-way ANOVA: 3-methylbutanol_3 versus temperature_3

Grouping Information Using Tukey Method
temperature_3 N Mean Grouping
2 2 4091 A
3 2 3054 A
1 2 3022 A

One-way ANOVA: 2-heptanone_3 versus temperature_3

Grouping Information Using Tukey Method
temperature_3 N Mean Grouping
2 2 88.51 A
1 2 85.75 A
3 2 50.75 A

One-way ANOVA: 2-nonanone_3 versus temperature_3

Grouping Information Using Tukey Method
temperature_3 N Mean Grouping
2 2 37.313 A
1 2 33.477 A
3 2 20.496 A

One-way ANOVA: Butyric acid_3 versus temperature_3

Grouping Information Using Tukey Method
temperature_3 N Mean Grouping
3 2 47409 A
2 2 40413 A B
1 2 16275 B

Temperature 1; cheese type 1

One-way ANOVA: pH versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 6.6125 A
3 4 5.2675 B
2 4 4.8075 C
1 4 4.4650 D

One-way ANOVA: SN/TN versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 0.20187 A
3 4 0.16637 A
2 4 0.09973 B
1 4 0.07485 B

One-way ANOVA: NPN/TN versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 0.14646 A
3 4 0.09921 B
1 4 0.05853 B C
2 4 0.05822 C

One-way ANOVA: PROTEIN versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
1 4 2.4420 A
2 4 2.2721 A B
3 4 2.1790 A B
4 4 2.1082 B

One-way ANOVA: CASEIN versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
1 4 2.33501 A
2 4 2.23063 A B
3 4 2.06337 B C
4 4 1.94395 C

One-way ANOVA: PEPTIDES versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 0.20947 A
3 4 0.12488 B
2 4 0.09818 B
1 4 0.05236 C

One-way ANOVA: FAA versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 1.7522 A
3 4 1.6289 A
2 4 0.7026 B
1 4 0.5060 B

One-way ANOVA: LAB log versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
1 4 9.4332 A
2 4 8.8308 B
3 4 8.4091 C
4 4 7.9169 D

One-way ANOVA: Mould log versus Ripening time
Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 6.7925 A
3 4 5.6070 B
2 4 5.2878 C
1 4 3.7278 D

One-way ANOVA: 3-methylbutanal versus Time
Grouping Information Using Tukey Method
Time N Mean Grouping
3 2 194.46 A
2 2 100.75 B
1 2 66.05 B
4 2 38.88 B

One-way ANOVA: 3-methylbutanol versus Time
Grouping Information Using Tukey Method
Time N Mean Grouping
2 2 14736 A
3 2 1210 B
4 2 422 B
1 2 0 B

One-way ANOVA: 2-heptanone versus Time
Grouping Information Using Tukey Method
Time N Mean Grouping
3 2 383.49 A
2 2 166.15 B
4 2 103.26 C
1 2 29.22 D

One-way ANOVA: nonanone versus Time
Grouping Information Using Tukey Method
Time N Mean Grouping
3 2 221.59 A
2 2 161.61 A
4 2 49.48 B
1 2 17.86 B

One-way ANOVA: Butyric acid versus Time
Grouping Information Using Tukey Method
Time N Mean Grouping
3 2 16063 A
4 2 6729 B
2 2 0 C
1 2 0 C

Temp 1, Cheese type 2,

One-way ANOVA: pH_1 versus Ripening time
Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 6.5575 A
3 4 5.5000 B
2 4 4.9675 C
1 4 4.6200 D

One-way ANOVA: SN/TN_1 versus Ripening time
Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 0.28848 A
3 4 0.17732 B
2 4 0.12558 C
1 4 0.09347 D

One-way ANOVA: NPN/TN_1 versus Ripening time
Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 0.20153 A
3 4 0.11760 B
2 4 0.07556 C
1 4 0.04495 D

One-way ANOVA: PROTEIN_1 versus Ripening time
Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
1 4 2.38268 A
2 4 2.22910 B
3 4 2.15508 B
4 4 2.01933 C

One-way ANOVA: CASEIN_1 versus Ripening time
Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
1 4 2.34130 A
2 4 2.02661 B
3 4 2.00954 B
4 4 1.78779 C

One-way ANOVA: PEPTIDES_1 versus Ripening time
Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 0.26632 A
3 4 0.14937 B
2 4 0.11264 B C
1 4 0.07551 C

One-way ANOVA: FAA_1 versus Ripening time
Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 1.4699 A
3 4 1.0872 A B
2 4 0.7378 B C
1 4 0.4208 C

One-way ANOVA: LAB log_1 versus Ripening time
Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
1 4 9.1340 A
2 4 8.8995 A
3 4 8.5889 B
4 4 8.4662 B

One-way ANOVA: Mould log_1 versus Ripening time
Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 6.9231 A
3 4 5.8068 B
2 4 5.4643 C
1 4 3.9360 D

One-way ANOVA: 3-methylbutanal versus Time
Grouping Information Using Tukey Method
Time N Mean Grouping
3 2 118.43 A
4 2 69.09 A B
2 2 44.14 B
1 2 0.00 B

One-way ANOVA: 3-methylbutanol versus Time
Grouping Information Using Tukey Method
Time N Mean Grouping

2 2 11414 A
3 2 6696 A B
4 2 3022 B
1 2 0 B

Grouping Information Using Tukey Method

Time N Mean Grouping
3 2 318.96 A
4 2 85.75 B
2 2 43.97 B C
1 2 22.33 C

One-way ANOVA: 2-nonanone versus Time

Grouping Information Using Tukey Method
Time N Mean Grouping
3 2 188.88 A
2 2 72.44 A B
4 2 33.48 B
1 2 18.41 B

One-way ANOVA: Butyric acid versus Time

Grouping Information Using Tukey Method
Time N Mean Grouping
3 2 91550 A
4 2 16275 B
2 2 0 C
1 2 0 C

Temperature 2; Cheese type 1

One-way ANOVA: pH versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 6.4225 A
3 4 5.1250 B
2 4 4.7075 C
1 4 4.5550 D

One-way ANOVA: SN/TN versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 0.22524 A
3 4 0.14502 B
2 4 0.12213 B C
1 4 0.09485 C

One-way ANOVA: NPN/TN versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 0.14513 A
3 4 0.08552 B
2 4 0.05849 B C
1 4 0.04888 C

One-way ANOVA: PROTEIN versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
1 4 2.35300 A
2 4 2.23525 A B
3 4 2.13137 B
4 4 2.10270 B

One-way ANOVA: CASEIN versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
1 4 2.39086 A
2 4 2.21431 B
3 4 2.12216 B C
4 4 1.99686 C

One-way ANOVA: PEPTIDES versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 0.19812 A
3 4 0.14821 B
2 4 0.13882 B
1 4 0.07625 C

One-way ANOVA: FAA versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 1.6983 A
3 4 1.0886 B
2 4 0.5717 C
1 4 0.4817 C

One-way ANOVA: LAB log versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
1 4 9.1263 A
2 4 8.9260 A B
3 4 8.8116 B C
4 4 8.6549 C

One-way ANOVA: Mould log versus Ripening time

Grouping Information Using Tukey Method
Ripening
time N Mean Grouping
4 4 7.0064 A
3 4 6.0894 B
2 4 5.3177 C
1 4 3.6058 D

One-way ANOVA: 3-methylbutanal_1 versus Time_1

Grouping Information Using Tukey Method
Time_1 N Mean Grouping
3 2 113.18 A
2 2 77.74 B
4 2 30.33 C
1 2 21.51 C

One-way ANOVA: 3-methylbutanol_1 versus Time_1

Grouping Information Using Tukey Method
Time_1 N Mean Grouping
2 2 19257 A
3 2 2476 B
4 2 454 B
1 2 0 B

One-way ANOVA: 2-heptanone_1 versus Time_1

Grouping Information Using Tukey Method
Time_1 N Mean Grouping
3 2 423.71 A
4 2 345.04 A B
2 2 186.78 A B
1 2 83.90 B

One-way ANOVA:2- nonanone_1 versus Time_1

Grouping Information Using Tukey Method
Time_1 N Mean Grouping
3 2 207.72 A
2 2 102.65 B
4 2 31.48 C
1 2 0.00 C

One-way ANOVA: Butyric acid_1 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
4	2	15146	A
3	2	8928	A B
2	2	0	B
1	2	0	B

Temperature 2; Cheese type 2

One-way ANOVA: pH_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	6.3900	A
3	4	5.2950	B
2	4	4.9625	C
1	4	4.6875	C

One-way ANOVA: SN/TN_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	0.29198	A
3	4	0.20296	B
2	4	0.13028	C
1	4	0.09616	C

One-way ANOVA: NPN/TN_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	0.20581	A
3	4	0.14065	B
2	4	0.08372	C
1	4	0.04472	D

One-way ANOVA: PROTEIN_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
1	4	2.33157	A
2	4	2.23264	A
3	4	2.06625	B
4	4	1.92789	B

One-way ANOVA: CASEIN_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
1	4	2.20014	A
2	4	2.00670	B
3	4	1.82659	C
4	4	1.71133	C

One-way ANOVA: PEPTIDES_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	0.26738	A
3	4	0.17903	B
2	4	0.11678	C
1	4	0.09552	C

One-way ANOVA: FAA_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	1.3674	A
3	4	1.0846	B
2	4	0.7049	C
1	4	0.3800	D

One-way ANOVA: LAB log_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
1	4	9.0858	A
2	4	8.9797	A
3	4	8.6898	B
4	4	8.5686	B

One-way ANOVA: Mould log_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	7.1355	A
3	4	5.9671	B
2	4	5.5306	C
1	4	3.7473	D

One-way ANOVA: 3-methylbutanal_1 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
3	2	111.50	A
2	2	57.59	B
4	2	35.70	C
1	2	0.00	D

One-way ANOVA: 3-methylbutanol_1 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
2	2	17413	A
3	2	10079	A B
4	2	4091	B C
1	2	0	C

One-way ANOVA: 2-heptanone_1 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
3	2	334.38	A
4	2	88.51	B
2	2	83.10	B
1	2	69.15	B

One-way ANOVA: 2-nonanone_1 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
3	2	146.71	A
2	2	61.73	B
4	2	37.31	B
1	2	6.32	B

One-way ANOVA: Butyric acid_1 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
4	2	40413	A
3	2	16062	B
2	2	0	B
1	2	0	B

Temperature 3 ; Cheese type 1

One-way ANOVA: pH versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	6.0975	A
3	4	5.0300	B
2	4	4.7325	C
1	4	4.6750	C

One-way ANOVA: SN/TN versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	0.23753	A
3	4	0.16352	B
2	4	0.10868	C
1	4	0.08171	C

One-way ANOVA: NPN/TN versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	0.16173	A
3	4	0.11206	B
2	4	0.07388	C
1	4	0.04810	D

One-way ANOVA: PROTEIN versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
1	4	2.2776	A
3	4	2.1625	A
2	4	2.1377	A
4	4	2.1196	A

One-way ANOVA: CASEIN versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
1	4	2.34783	A
2	4	2.29327	A
3	4	2.06973	B
4	4	1.90349	C

One-way ANOVA: PEPTIDES versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	0.22020	A
2	4	0.19382	A
3	4	0.14831	B
1	4	0.07236	C

Means that do not share a letter are significantly different.

One-way ANOVA: FAA versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	1.5260	A
3	4	1.4420	A
2	4	0.5963	B
1	4	0.4693	B

One-way ANOVA: LAB log versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
2	4	9.11763	A
3	4	9.02787	A
1	4	8.83255	B
4	4	8.82823	B

One-way ANOVA: Mould log versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	7.1675	A
3	4	6.4011	B
2	4	5.5003	C
1	4	3.1167	D

One-way ANOVA: 3-methylbutanal_2 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
3	2	149.44	A
2	2	69.80	B
4	2	51.30	B
1	2	0.00	B

One-way ANOVA: 3-methylbutanol_2 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
2	2	23573	A
3	2	6029	A B
4	2	2423	A B
1	2	0	B

One-way ANOVA: 2-heptanone_2 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
4	2	652.8	A
3	2	469.5	A B
2	2	215.7	B C
1	2	9.1	C

One-way ANOVA: 2-nonanone_2 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
3	2	277.81	A
4	2	129.88	A
2	2	84.14	A
1	2	0.00	A

One-way ANOVA: Butyric acid_2 versus Time_1

Grouping Information Using Tukey Method

Time_1	N	Mean	Grouping
3	2	31663	A
4	2	30225	A
2	2	0	A
1	2	0	A

Temperature 3; Cheese type 2

One-way ANOVA: pH_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	6.3550	A
3	4	5.1000	B
2	4	4.9250	B
1	4	4.8025	B

One-way ANOVA: SN/TN_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	0.30567	A
3	4	0.18002	B
2	4	0.12060	B C
1	4	0.09695	C

One-way ANOVA: NPN/TN_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping
4	4	0.20961	A
3	4	0.11661	B
2	4	0.07217	B C
1	4	0.04987	C

One-way ANOVA: PROTEIN_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening

time	N	Mean	Grouping

1	4	2.3768	A
2	4	2.2817	A B
3	4	2.1458	A B
4	4	1.9564	B

One-way ANOVA: CASEIN_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening time	N	Mean	Grouping
1	4	2.06107	A
2	4	1.97994	A
3	4	1.80015	B
4	4	1.66192	B

One-way ANOVA: PEPTIDES_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening time	N	Mean	Grouping
4	4	0.30977	A
3	4	0.17975	B
2	4	0.12035	C
1	4	0.09765	C

One-way ANOVA: FAA_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening time	N	Mean	Grouping
4	4	1.2135	A
3	4	0.8756	B
2	4	0.6072	B C
1	4	0.3892	C

One-way ANOVA: LAB log_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening time	N	Mean	Grouping
1	4	9.03207	A
2	4	9.02901	A
3	4	8.82751	B
4	4	8.63510	C

One-way ANOVA: Mould log_1 versus Ripening time

Grouping Information Using Tukey Method

Ripening time	N	Mean	Grouping
4	4	7.4159	A
3	4	6.1173	B
2	4	5.7328	C
1	4	3.5209	D

One-way ANOVA: 3-methylbutanal_2 versus Time_2

Grouping Information Using Tukey Method

Time_2	N	Mean	Grouping
3	2	103.73	A
2	2	36.11	B
4	2	16.88	B C
1	2	0.00	C

One-way ANOVA: 3-methylbutanol_2 versus Time_2

Grouping Information Using Tukey Method

Time_2	N	Mean	Grouping
2	2	13954	A
3	2	8556	B
4	2	3054	C
1	2	0	C

One-way ANOVA: 2-heptanone_2 versus Time_2

Grouping Information Using Tukey Method

Time_2	N	Mean	Grouping
3	2	342.44	A
2	2	127.74	B
4	2	50.75	B
1	2	45.61	B

One-way ANOVA: 2-nonanone_2 versus Time_2

Grouping Information Using Tukey Method

Time_2	N	Mean	Grouping
3	2	150.81	A
2	2	46.80	B
4	2	20.50	B
1	2	0.00	B

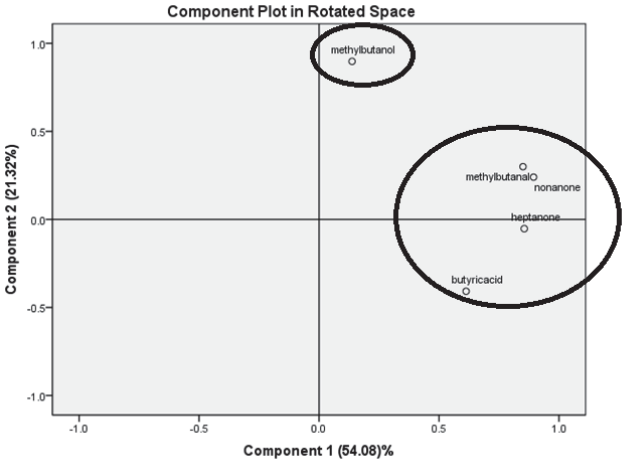
One-way ANOVA: Butyric acid_2 versus Time_2

Grouping Information Using Tukey Method

Time_2	N	Mean	Grouping
3	2	76970	A
4	2	47409	A
2	2	14897	A
1	2	0	A

Principle component analysis outputs

Output for volatile compounds



KMO and Bartlett's Test[a]

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.729
Bartlett's Test of Sphericity	Approx. Chi-Square	94.689
	df	10
	Sig.	.000

a. Based on correlations

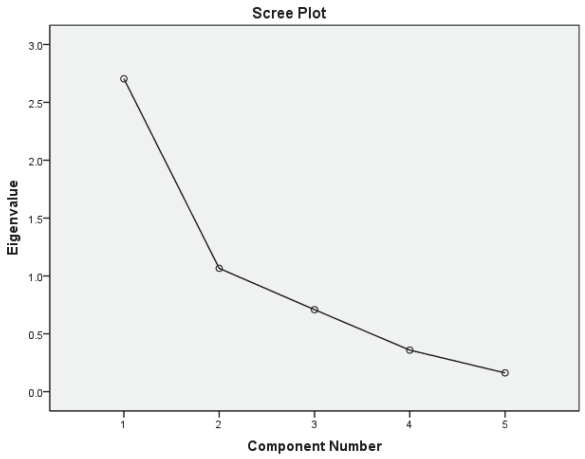
Total Variance Explained

		Initial Eigenvalues[a]			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Raw	1	2.704	54.075	54.075	2.704	54.075	54.075	2.648	52.951	52.951
	2	1.066	21.318	75.393	1.066	21.318	75.393	1.122	22.443	75.393
	3	.709	14.177	89.571						
	4	.359	7.183	96.754						
	5	.162	3.246	100.000						
Rescaled	1	2.704	54.075	54.075	2.704	54.075	54.075	2.648	52.951	52.951

	2	1.06	21.318	75.393	1.06	21.318	75.393	1.12	22.443	75.393
		6			6			2		
	3	.709	14.177	89.571						
	4	.359	7.183	96.754						
	5	.162	3.246	100.000						

Extraction Method: Principal Component Analysis.

a. When analyzing a covariance matrix, the initial eigenvalues are the same across the raw and rescaled solution.



Component Matrix[a]

	Raw		Rescaled	
	Component		Component	
	1	2	1	2
methylbutanal	.891	.137	.891	.137
methylbutanol	.302	.856	.302	.856
heptanone	.831	-.210	.831	-.210
nonanone	.923	.070	.923	.070
butyricacid	.526	-.514	.526	-.514

Extraction Method: Principal Component Analysis.

a. 2 components extracted.

Rotated Component Matrix[a]

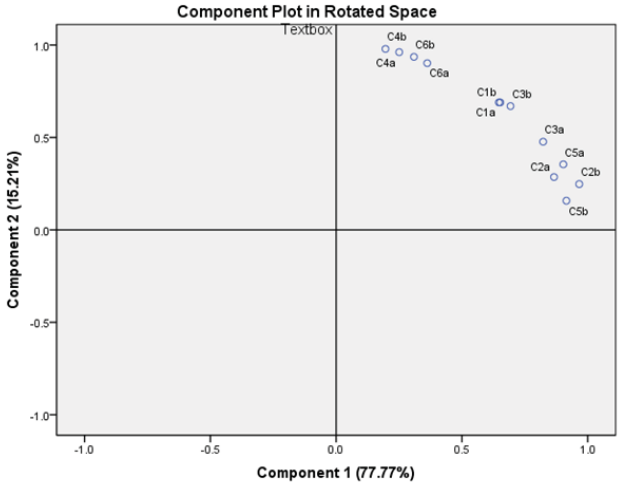
	Raw		Rescaled	
	Component		Component	
	1	2	1	2
methylbutanal	.850	.300	.850	.300
methylbutanol	.138	.898	.138	.898
heptanone	.855	-.052	.855	-.052
nonanone	.894	.240	.894	.240
butyricacid	.613	-.408	.613	-.408

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 3 iterations.

Output for cheese samples



Component Transformation Matrix

Component	1	2
1	.715	.699
2	.699	-.715

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

Rotated Component Matrix[a]

	Raw		Rescaled	
	Component		Component	
	1	2	1	2
C1a	.647	.690	.647	.690
C1b	.652	.689	.652	.689
C2a	.866	.286	.866	.286
C2b	.966	.248	.966	.248
C3a	.823	.477	.823	.477
C3b	.693	.670	.693	.670
C4a	.251	.962	.251	.962
C4b	.196	.979	.196	.979
C5a	.903	.355	.903	.355
C5b	.915	.158	.915	.158

C6a	.362	.902	.362	.902
C6b	.309	.936	.309	.936

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

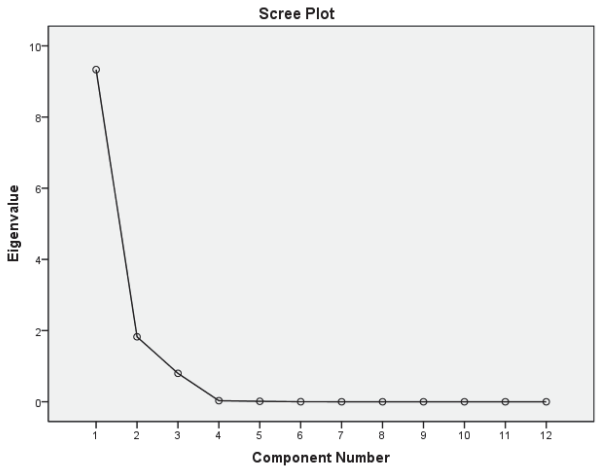
a. Rotation converged in 3 iterations.

Component Matrix[a]

	Raw		Rescaled	
	Component		Component	
	1	2	1	2
C1a	.945	-.041	.945	-.041
C1b	.948	-.037	.948	-.037
C2a	.819	.401	.819	.401
C2b	.864	.498	.864	.498
C3a	.922	.234	.922	.234
C3b	.964	.006	.964	.006
C4a	.851	-.512	.851	-.512
C4b	.825	-.563	.825	-.563
C5a	.894	.377	.894	.377
C5b	.765	.527	.765	.527
C6a	.889	-.392	.889	-.392
C6b	.875	-.453	.875	-.453

Extraction Method: Principal Component Analysis.

a. 2 components extracted.



Total Variance Explained

Component	Initial Eigenvalues[a]	Extraction Sums of Squared Loadings	Rotation Sums of Squared Loadings

		Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Raw	1	9.333	77.772	77.772	9.333	77.772	77.772	5.665	47.209	47.209
	2	1.825	15.211	92.983	1.825	15.211	92.983	5.493	45.774	92.983
	3	.798	6.648	99.631						
	4	.031	.256	99.888						
	5	.012	.104	99.992						
	6	.001	.006	99.997						
	7	.000	.002	99.999						
	8	7.453E-5	.001	100.000						
	9	2.709E-5	.000	100.000						
	10	9.084E-6	7.570E-5	100.000						
	11	2.789E-6	2.324E-5	100.000						
	12	8.891E-8	7.409E-7	100.000						
Rescaled	1	9.333	77.772	77.772	9.333	77.772	77.772	5.665	47.209	47.209
	2	1.825	15.211	92.983	1.825	15.211	92.983	5.493	45.774	92.983
	3	.798	6.648	99.631						
	4	.031	.256	99.888						
	5	.012	.104	99.992						
	6	.001	.006	99.997						
	7	.000	.002	99.999						
	8	7.453E-5	.001	100.000						
	9	2.709E-5	.000	100.000						
	10	9.084E-6	7.570E-5	100.000						
	11	2.789E-6	2.324E-5	100.000						
	12	8.891E-8	7.409E-7	100.000						

Extraction Method: Principal Component Analysis.

a. When analyzing a covariance matrix, the initial eigenvalues are the same across the raw and rescaled solution.

KMO and Bartlett's Test[a]

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.623
Bartlett's Test of Sphericity	Approx. Chi-Square	1157.082
	df	66
	Sig.	.000

a. Based on correlations