Pediococci in South African Cheddar and Gouda Cheese

BY

REGINAH NKI KAU
(9617151)

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DECLARATION

I, the undersigned, hereby declare that this dissertation is my original work and has never been submitted at any other university for a degree.

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ABSTRACT

PEDIOCOCCI IN SOUTH AFRICAN CHEDDAR AND GOUDA CHEESE

By

Reginah Nki Kau

Study Leader: Prof. B. H. Bester

Department: Food Science

Degree: M Inst Agrar Food Processing

The objective of this study was to survey the occurrence of pediococci in South African Cheddar and Gouda cheese and to correlate their numbers with the proximate chemical composition of the cheese. Cheese samples (11 Cheddar, 13 Gouda) were collected from the cheeses presented for the judging for the AVI–Africa Expo at the 2000 Rand Show. Ten cheese samples were also bought from retail outlets (5 Cheddar, 5 Gouda). Fresh cheeses (Cheddar and Gouda) were manufactured in the Department of Food Science at the University of Pretoria (UP) and ripened for 56 days.

Microbiological analysis was done in duplicate on each cheese sample, and chemical analyses (except pH measurements) were done in quadruplicate on the collected (commercial) samples and in sixfold on the manufactured (UP) cheese samples. Samples of the manufactured cheeses were taken every two weeks (during the ripening period of 56 days) beginning the second day after the cheese was manufactured.

According to presumptive tests on 1320 isolates, only 24 (1.82%) isolates tested positive for presumptive pediococci. Total microbiological counts reached numbers in the order of 10^6 cfu/g and the occurrence of presumptive *Pediococcus* spp. was recorded mostly in Gouda cheese and to a lesser extent in Cheddar cheese.

Statistically there were no significant differences ($p \geq 0.05$) in the salt content, fat content and FFA content **within** the UP Cheddar cheese samples, whilst the fat content and moisture content **within** the UP Gouda cheese samples showed significant differences ($p \leq 0.05$). On the other hand, the fat content and salt content varied significantly ($p \leq 0.05$) **between** the UP Cheddar and Gouda cheese samples.

Results showed that there was no significant difference ($p \geq 0.05$) in fat content, moisture content, salt content, pH and free fatty acid content within Cheddar and Gouda cheeses collected from the retail outlets and AVI-Africa Expo.

No correlation was found between the number of pediococci isolates and proximate chemical composition of the cheeses investigated.

UITTREKSEL

PEDIOKOKKE IN SUID-AFRIKAANSE CHEDDAR- EN GOUDAKAAS

Deur

Reginah Nki Kau

Studieleier: Prof. B. H. Bester

Departement: Voedselwetenskap

Graad: M Inst Agrar (Voedselprosessering)

Die doelwit van die studie was om die voorkoms van pediokokke in Suid-Afrikaanse Cheddar- en Goudakaas te ondersoek en om hulle getalle te korreleer met die chemiese kort analise van die kaas. Kaasmonsters (11 Cheddar, 13 Gouda) is versamel van die kaas aangebied vir beoordeling tydens die AVI-Africa Expo by die 2000 Randse skou. Tien kaasmonsters (5 Cheddar, 5 Gouda) is ook by kleinhandelaars gekoop. Vars kaas (Cheddar en Gouda) is in die Departement Voedselwetenskap, Universiteit van Pretoria (UP) vervaardig en vir 56 dae rypgemaak.

Mikrobiologiese analise is in duplikaat op elke kaasmonster gedoen, en chemiese analises (behalwe pH-meting) is in viervoud op die kommersiële monsters en in sesvoud op die vervaardigde kaasmonsters (UP) gedoen. Monsters van die vervaardigde kaas (UP) is elke twee weke (gedurende die rypingstyd van 56 dae) geneem vanaf die tweede dag na vervaardiging.

Volgens aanwysende toetse op 1320 isolate het net 24 (1.82%) isolate positief getoets vir waarskynlike pediokokke. Totale mikrobetellings het getalle in die orde van 10^6 kve/g bereik en waarskynlike *Pediococcus* spp. is meestal in Goudakaas gevind en in minder mate in Cheddarkaas.

Statisties was daar geen betekenisvolle verskille ($p \geq 0.05$) in die soutgehalte, vetgehalte en die vryvetsuurgehalte **binne** die UP Cheddarkaasmonsters nie, terwyl

die vetgehalte en voggehalte **binne** die UP Goudakaasmonsters betekenisvol verskil het ($p \leq 0.05$). Daarenteen het die vetgehalte en soutgehalte **tussen** UP Cheddar- en Goudakaasmonsters betekenisvol ($p \leq 0.05$) verskil.

Daar was geen betekenisvolle verskille ($p \leq 0.05$) in die vetgehalte, voggehalte, soutgehalte, pH en vryvetsuurgehalte van die Cheddar- en Goudakaas versamel van die kleinhandel en AVI-Africa Expo nie.

Geen korrelasie is tussen die aantal pediokokke-isolate en die chemiese kort analasie van die kaas gevind nie.

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CHAPTER 1

INTRODUCTION

Cheese is a fresh or matured product, obtained by the drainage of a liquid (whey) after the coagulation of milk, cream, skimmed milk, partly skimmed milk or a combination thereof (Russell, 1993). Cheese and fermented milks are among nature's most valuable contributors to civilization (Kosikowski, 1978). They provide healthful nutritional elements (Boubekri & Ohta, 1996). The evolution of fermented milk products began many centuries before Christ, probably in the warm climate of the Mediterannean Sea Basin (Kosikowski, 1978). The art of cheese-making has been developed with recipes handed down from mother to daughter, using the milk of domesticated animals (Russell, 1993).

Earthenware pots of the type still in use today to separate the milk for making the soft Egyptian cheese, Mish, were found in the tomb of King Horaha dating from around 3200BC, while both reed mats (employed to drain whey from the soft curd) and storage jars for finished cheese have been located in the tombs built during Roman occupation of the Nile Basin (Robinson, 1995). By 332BC, Domiati had become established, and like Mish it was well suited to the conditions pertaining to the Middle East. Thus, the high ambient temperatures and poor hygienic conditions meant that products with high acidities and salt contents were the most amenable to preservation, and many varieties popular with present day consumers, such as Feta, are little more than refinements of these early products (Robinson, 1995). Variants of these products increased and as different tribes intermingled as the result of war or migration, so the art of cheese-making spread. Distinctive local varieties emerged, due to conditions such as environment, animal type, etc. that were unsuitable for the production of a particular existing cheese type.

More than 400 cheese types are produced world-wide, although most of these are only regional varieties of some well-known types. In reality there are only about 18 cheese types that differ fundamentally from one another (Robinson, 1995). The two most popular cheese types manufactured and consumed in South Africa, are Gouda

and Cheddar. Gouda cheese is named after the place in Holland where it was first produced (Robinson, 1995). It is traditionally made in a form of small wheels weighing 3.5 – 25 kg and is a sweet-curd renneted cheese, made with partly skimmed milk (Chapman & Sharpe, 1981). The cheese has a pH of 5.1 and contains no lactose.

Cheddar cheese originated several decades ago in the little village of Cheddar, England (Kosikowski, 1978). Originally made in farmhouse dairies in the county of Somerset by methods handed down by word of mouth over the centuries, the general method of manufacture was standardised by Joseph Harding in 1875 and accepted into commercial practice (Robinson, 1995). It is made in the form of rindless blocks or rinded cylinders, and when mature, the body is firm and the texture is close.

Microorganisms responsible for the acid production in cheese-making are lactic acid bacteria. They are of great industrial and commercial importance and widely used as starter cultures for dairy, meats and vegetable fermentation (Cogan according to Boubekri & Ohta, 1996). The lactic acid bacteria also play an important role in food preservation (Daba, Lacroix, Huang, Simard & Lemiex, 1994), and have a potential to inhibit growth of the pathogenic and most spoilage bacteria, thereby improving the hygienic quality and extending the shelf life of different food products (Henderson, Chopko & Van Wassenaar, 1992). Lactic-acid bacteria comprise of the genera *Lactococcus, Lactobacillus, Streptococcus, Pediococcus, Leuconostoc, Vagococcus, Carnobacterium, Enterococcus, Lactosphenera* and *Oenococcus* (Jay, 1998).

Pediococci are a group of microorganisms that occur in milk and cheese, sometimes forming a high percentage of the lactic acid bacteria (Tzanetakis & Litopoulou-Tzanetaki, 1989). The role of pediococci in cheese fermentation is not clearly understood but they may survive pasteurisation of the milk and grow in Cheddar cheese, where counts may reach 10^6 to 10^7/g (Litopoulou-Tzanetaki, Graham & Beyatli, 1989).

1.1 Statement of the problem

Cheese-ripening is a long, complicated and costly process (Bhowmik, Riesterer, Van Boekel & Marthe, 1990). Several chemical changes occur, the primary reactions being degradation of protein, fat and carbohydrates followed by secondary reactions, and as a result typical body, texture and flavour characteristics are developed in the cheese (Bhowmik *et al.*, 1990).

Occurrence of pediococci in raw milk and cheese as part of its secondary flora has been observed (Bhowmik *et al.*, 1990). Their role in cheese-ripening is not clearly understood (Litopoulou-Tzanetaki *et al.*, 1989).

Early investigations suggested that pediococci enhanced cheese flavours, but Law *et al.* (according to Tzanetakis & Litopoulou-Tzanetaki, 1989) claimed that they do not have an individual effect on the flavour of the cheese, but rather are part of a complex microflora with synergistic influences. Franklin & Sharpe (1963) found that the flavour of cheese improved with increasing numbers of non-starter lactic acid bacteria that consisted of *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus brevis* and *Pediococcus* spp. Most reports available are on pediococci in Cheddar cheese and its effect on the flavour. There are no such reports for pediococci in Gouda cheese.

1.2 Objectives

- The primary objective of this project was to survey the occurrence of *Pediococcus* spp. in South African Cheddar and Gouda cheese.
- A secondary objective was to correlate the numbers of pediococci with the proximate chemical composition of the cheese.

CHAPTER 2

LITERATURE REVIEW

2.1 *Classification of cheeses*

Interactions between humans and their environment had resulted, by the Middle Ages, in a range of cheeses with quite distinctive characteristics, and many of the names referred to in Table 2.1 are still in use today (Robinson, 1995).

Table 2.1 Some of the cheese names for which early records exists (Robinson, 1995)

Name	Date (AD)	Name	Date (AD)
Gorgonzola	879	Roquefort	1070
Cheshire	1085	Maroilles	1174
Schwangenkäse	1178	Grana	1200
Taleggio	1282	Gruyere	1288
Cheddar	1500	Parmesan	1579
Emmental	1622	Dunlop	1688
Gouda	1697	Gloucester	1783
Stilton	1785	Camembert	1791
Limburger	1800	St Paulin	1816

Human activity increasingly played a dominant role in the expansion of cheese production and the changes in human life-styles (for example, moving from farms to cities) was perhaps the most important of all. Moving of people from farms to cities affected sales market for some cheese varieties (e.g. soft cheeses). Cheesemakers decided that, if the consumer could no longer reach the village markets, the cheeses had to be brought to the cities with slow and unreliable transport. Consequently, harder, drier cheeses like Cheddar cheese became the norm, because the producers

realised that even if these cheeses were transported over a long period (three weeks), the quality of hard cheese was not impaired, whereas soft cheese would be rendered totally inedible, even in winter. Although some countries retained a major interest in soft cheese, the firmer varieties like Cheddar, Gouda and Emmental became the major varieties of commerce (Robinson, 1995).

There are over 400 varieties of cheese representing fewer than 20 distinct types, that are classified according to texture or moisture content, whether ripened or unripened, and if ripened, whether by bacteria or moulds (Jay, 1998). The three textural classes of cheese are very hard, hard or soft cheese. A number of basic cheese types are outlined in Table 2.2.

In South Africa and world-wide Cheddar cheese is the best known and probably consumed in greatest quantities. Cheddar cheese varies in flavour from milky and creamy (mild Cheddar) to strong and biting (mature Cheddar), reflecting the age of the cheese (Robinson, 1995). The flavour of a mild Cheddar has developed sufficiently in 3 – 4 months, a medium cheese after 6 months and mature cheese in 8 – 12 months (Russell, 1993).

The term semi-soft is applied to the very popular continental cheeses, such as Gouda and Edam, which are eaten in large quantities in Northern Europe and in South Africa. In many respects, Gouda is much like Edam, but while Edam is usually round, Gouda is wheel-shaped, even when produced as small cheese (Robinson, 1995). The curd is lightly scalded so as to retain a higher percentage of moisture than in hard cheeses, and in addition, some of the whey is replaced by water during scalding, thereby diluting residual lactose and restricting the production of acid (Russell, 1993). A more pronounced flavour is achieved by extending the maturation time to 6 months.

Table 2.2 Some major cheese types that can be recognised on the basis of moisture content and/or method of maturation (Farkye, 1993; Robinson, 1995)

Cheese Category	Examples
Very hard	Parmesan
	Romano
Hard	Cheddar
	Emmental
	Gruyere
	Limburger
Semi-soft	Gouda
	Edam
Blue-vein mould	Blue
	Roquefort
	Gorgonzola
	Stilton
Soft	Brie
	Camembert
	Cottage
Unripened soft	Cream
	Fromage
Italian-style	Cotronese
	Mozzarella
	Provolone
White-brined	Feta

From a comparison of various cheese products produced in the world, it may be concluded that in spite of various numerous names, all products can be classified in a few groups. The principal differences between these groups depend on:

- The type of milk used
- The type of fermenting organisms
- The manufacturing process

2.1.1 Milk for cheese-making

Cheese can be made from any milk. Milk for cheese-making should be of a good bacteriological quality to avoid undesirable fermentation and enzymic reactions, and without any substances that inhibit the growth of starter bacteria (Chapman & Sharpe according to Rukure & Bester, 2001). The milk varies in composition from animal to animal and from species to species, depending also on the climate, environment, diet, etc (Russell, 1993). The composition of the milk affects the nature and quality of the cheese produced. Most cheeses are made from cow milk but goat and ewe milk is also widely used. The composition of different milks used for cheese production is shown in Table 2.3.

Gouda and Cheddar cheeses are both made from cow milk. According to Patten & Kristensen (1968), pasteurised full-cream milk of good bacteriological properties is standardised to a fat-to-casein ratio of 1:0.8 – 0.82 for the production of Gouda cheese. To ensure the correct properties for maturation of Cheddar cheese, the fat-to-casein ratio is standardised to 1:0.67 – 0.72 (Lawrence & Gilles according to Robinson, 1995).

Table 2.3 Average percentage composition of milk of various species (Russell, 1993)

	Cow	Ewe	Goat
Protein	3.81	5.85	2.63
Fat	3.80	6.45	3.5
Lactose	4.54	4.47	4.15
Vitamins and Minerals	0.75	0.83	0.79
Water	87.10	82.40	88.30

2.1.2 Type of fermenting organisms

Cheese manufacture and ripening involves the action of enzymes (rennet) and selected microorganisms (Fox, McSweeny & Lynch, 1998). Microorganisms mainly provide the enzymes that metabolise carbohydrates, proteins and lipids causing improved flavour and texture with age (Haque, Kucukoner & Aryana, 1997). Microorganisms responsible for the acid production in cheese-making are lactic acid bacteria.

Lactic acid bacteria describe a group of Gram-positive, non-sporing rods and cocci, usually non-motile, that utilise carbohydrates fermentatively and form lactic acid as a major end product (Aguirre & Collins, 1993). Morphologically members of the group are either cocci, coccobacilli or rods and divide in one plane only, with the exception of *Pediococcus* spp., so that chain formation is common (Narvhus, 1993). They are wide-spread in nature, their distribution related to wherever high concentrations of soluble carbohydrates, protein breakdown products, vitamins and a low oxygen tension occurs (Aguirre & Collins, 1993). Consequently they are common in milk and dairy products, fermented foods, intact and rotting vegetable materials.

According to Stilton (1993), cheeses in regions with a cold and temperate climate, like Northern Europe, contain mostly mesophilic bacteria (optimum temperature of 15 –

30°C), while in regions with warmer temperatures, like the Mediterranean countries, they contain mostly thermophilic bacteria (optimum temperature of 30–50°C) (Table 2.4).

Mesophilic organisms such as *Lactococcus lactis* ssp. *cremoris* and *Leuconostoc mesenteroides* ssp. *cremoris* are used for the production of Gouda cheese, while *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* are used for the production of Cheddar cheese. For a more complex flavour profile, low levels of *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* ssp. *cremoris* may be included as well (Robinson, 1995).

Table 2.4 Mesophilic and thermophilic organisms used in cheese-making (Stilton, 1993)

Mesophilic organisms	Thermophilic organisms
Homofermentative	Homofermentative
Lactococcus lactis ssp. *lactis*	*Streptococcus salivarius* ssp. *thermophilus*
Lactococcus lactis ssp. *cremoris*	*Lactobacillus delbrueckii* ssp. b*ulgaricus*
Lactococcus lactis ssp. *lactis* biovar. *diacetylactis*	*Lactobacillus helveticus*
Heterofermentative	
Leuconostoc mesenteroides ssp. *cremoris*	

2.1.3 The manufacturing process

2.1.3.1 Gouda cheese

Gouda cheese is manufactured by coagulating the pasteurised milk with rennet at 30°C. Calcium chloride (0.02%) is added to the milk to enhance coagulation. Anatto colouring is also added to give a nice, rich, yellow colour to the cheese. After about 30 min the coagulum is cut into small pieces. The pieces are lightly scalded (heated), traditionally by removing some of the whey and replacing it with water at 50 – 60°C, but in modern commercial practice the temperature is regulated by introducing steam into the jacket of double-walled cheese vats. However, the final temperature should not exceed 37°C since the lactic acid bacteria (LAB) may be inhibited or inactivated. These are needed throughout the manufacturing process to transform lactose to lactic acid. The lactic acid enhances the coagulation process and whey expulsion and also acts as a natural preservative in cheese by inhibiting unwanted bacteria.

An amount of water equivalent to 25% of the original milk is added to reduce the lactose content of the curd and control acid production by the starter bacteria. The temperature of the mixture of curds and whey is gradually increased to 36°C and then maintained for another hour while the mixture is stirred continuously (cooking or scalding). The whey is drained off and the high moisture curds, pH 5.3 – 5.4, are dipped (filled) into moulds and pressed lightly to remove more moisture, to knit the rind and mould the cheese into a neat shape. The cheese is then salted in a brine bath (20% NaCl, 10°C) for 1 – 3 d, dried to form a rind and ripened for at least 4 weeks (8 – 10°C, 80% R.H.).

A more pronounced flavour may be achieved by extending the maturation time to 6 months and some "mature" brands may be held for up to 2 years before sale (Robinson, 1995). The texture of the young cheese tends to be quite soft, as it ages the body becomes firmer and drier, but still easy to cut with a knife. A few small regular openings are visible on the cut surface.

2.1.3.2 Cheddar cheese

The process of curd preparation is identical to that of Gouda cheese-making, except that water is not added and a slightly higher (38 – 39°C) cooking temperature is used. The main difference between the manufacture of the two cheese types starts after final drainage of the whey. In the production of cheddar cheese, the curds are collected and allowed to mat in the form of slabs with a furrow in between to allow for more drainage of whey from the curds. The slabs of curds are then cut into blocks (30 cm x 30 cm x 10 cm). These blocks are turned and inverted every 20 min during a period of 2 – 3 h (the "cheddaring process"). After completion of the cheddaring process, the blocks of curd are milled into pieces of about 2 – 5 cm long and dry salt is added. After salting, the curds are put into moulds, pressed and ripened (cured) for at least 8 weeks.

2.1.3.3 The main processing steps

A. Coagulation

Pasteurised milk is inoculated with a starter culture and rennet is added to form a curd. The proportion of rennet added should be the minimum necessary to give a firm coagulum in 30 to 40 min (Fox, 1987; Brusgaard, 1996). Calcium chloride is also added to the milk and the amount may vary depending on the desired rate of coagulation and the mineral content of the milk (Johnson, Steele, Broadbent & Weimer, 1998).

Coagulation takes place in three separate but overlapping phases: enzymic proteolysis, aggregation and syneresis (McMahon & Brown, 1984).

1. An enzymic, destabilising phase where the colloidal nature of κ-casein is destroyed and p-κ-casein is formed within the casein micelles. This phase can occur at refrigeration temperatures (Tamime, 1981).

2. Once the degree of proteolysis of the κ-casein has reached 80%, the micelles aggregate to form a curd. The curd is stabilised by electrostatic forces and hydrophobic effects.
3. The curd then shrinks and whey is lost by syneresis. This process is important in that it lowers the moisture content of the curd and the casein, fat and colloidal salts of the milk are concentrated. The process is similar for Cheddar and Gouda cheese.

B. Cooking (scalding) and whey removal

The curd is cut so as to increase the surface area and hence facilitate loss of whey. During scalding, the curd is heated to facilitate syneresis. Lactic acid is produced, the pH drops and the acidity assists the expulsion of more whey. In Gouda processing, part of the whey is replaced by water. Addition of water raises the vat temperature and also dilutes the lactose content of the curd thereby controlling acid production (Johnson *et al.*, 1998).

For Cheddar processing, the curds are collected and allowed to mat in the form of slabs with a furrow in between to allow for whey drainage. Higher cooking temperatures are used. High temperature scalds produce a drier, firmer curd and a slower maturing cheese (Bines, 1993).

C. Cheddaring

Piling and repiling of the curd blocks over a prolonged period is called cheddaring (Kosikowski, 1978). During the cheddaring process, the starter bacteria continue to multiply and the pH falls. The curd changes from being fragile and crumbly to having a smooth texture which exhibit the characteristic 'chicken breast' texture (Figure 2.1).

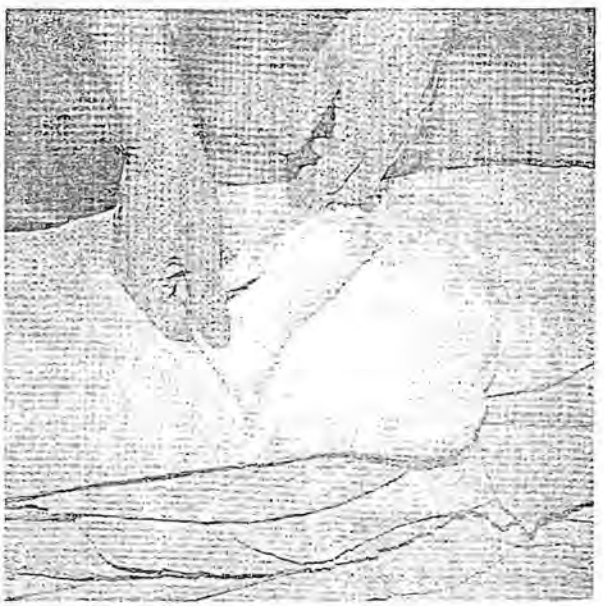

Figure 2.1 The characteristic chicken breast texture of Cheddar cheese curd (Bines, 1993)

D. Milling

This process occurs only during the manufacture of Cheddar cheese. Milling of Cheddar curd aerates and cools the curd, allows further drainage of whey and ensures uniform distribution of salt over the increased surface area (Fox, 1987; Bines, 1993).

E. Salting

Salt plays an important role in preservation and improving the organoleptic properties of cheese. It lowers the water activity of the cheese and hence negatively affects growth of undesired microorganisms and it also influences the ripening of the cheese. Schroeder, Bodyfelt, Wyatt & McDaniel (1988) showed that cheese with the least salt

showed an increase in proteolysis and water activity and supported higher lactic acid bacteria populations.

Gouda cheese is salted in a brine bath whilst Cheddar cheese is dry-salted. Sufficient time must be allowed after salting to ensure adsorption onto the curd surfaces (Bines, 1993). According to Johnson *et al.* (1998) brining of Gouda cheese serves not only as a means to get salt into the cheese, but also to cool the cheese and delay gas formation. After salting Cheddar cheese is pressed, packaged and ripened.

F. Cheese-ripening

Most cheese varieties are not ready for consumption at the end of manufacture. They undergo a period of ripening which varies from 4 weeks to 2 years, the duration of ripening being inversely related to the moisture content of the cheese (Fox, 1987). Many cheese varieties may be consumed at several stages of maturity depending on the consumer preferences.

The unique characteristics of individual cheeses develop during ripening, although in most cases the biochemical changes that occur during cheese-ripening, and hence flavour, aroma and texture development of the mature cheese, are predetermined largely by the manufacturing process, i.e. by composition, especially moisture, salt, pH, type of starter and in many cases secondary inocula added to the cheese milk or curd (Fox, 1987).

The chemical changes responsible for cheese-ripening are:

- Fermentation of lactose to lactic acid, small amounts of acetic acid and propionic acid, CO_2 and diacetyl, resulting in a decrease in pH (Luyten & van Vliet, 1996).
- Proteolysis and lipolysis, which are brought about by enzymes from the milk, the rennet, the lactic-acid bacteria and other microorganisms of the cheese.

2.2 Importance of cheese

Cheese-making is the oldest way of preserving the nutrients of milk and cheese is essentially a concentrated form of milk (Buttriss, 1993). Table 2.5 shows the nutrient composition of Cheddar, soft and Cottage cheeses.

Table 2.5 Nutrients per 100 g of cheese (Scott, 1981)

Food	Cheddar	Soft cheese	Cottage cheese
Protein (g)	26	22.8	13.6
Fat (g)	33.5	25.5	4.0
Calcium (mg)	800	150	60
Iron (mg)	0.5	0.4	0.1
Thiamine (mg)	0.04	0.06	0.07
Retinol (Vit A) (µg)	310	155	32
Riboflavin (mg)	0.5	0.4	0.3
Ascorbic acid (Vit C)	0	0	0
Nicotinic acid (mg)	0.5	0.4	0.3
Energy (Kcal)	406	285	96

Cheese is a rich source of protein, calcium and vitamin A, B2 (riboflavin) and B12. Cheddar cheese contains about 25% protein compared to 20% in meat and 8% in bread (Scott, 1981). The major protein in cheese is casein, which is a high quality protein containing all the essential amino acids in roughly the proportions required by the body for health. The fermentation process has little effect on the mineral content of cheese. Minerals in cheese, such as calcium and zinc, are particularly well absorbed and utilised (Buttriss, 1993). Rao *et al.* (according to Beukes, 1999), mentioned that some species of *Lc. lactis, Lc. cremoris, S. thermophilus* and *Lb. acidophilus* are able to produce folic acid and vitamin B2 during fermentation.

Cheese can be used by pregnant woman and vegetarians as an excellent source of calcium (Buttriss, 1993). According to a study done on nutrient intakes of South Africans (Food Industries of South Africa, 1996) it was concluded that adult women from black, coloured and Indian populations had very low calcium intakes (39 – 46% of RDA). According to the guidelines of the Department of Health, a balanced, healthy diet for adult South Africans requires daily consumption of at least 400 ml of milk equivalent (including all kinds of dairy products) in order to meet calcium needs (Langenhoven *et al.* according to Beukes, 1999).

Hard cheese contains little lactose since most of the lactose is lost during whey drainage and the rest is converted to lactic acid. Cheese can be a useful source of calcium and other essential nutrients for people who are lactose intolerant. Most of the world's population lose their ability to digest lactose in adult age in the same way as other mammals do. Lactose maldigestion is common all over the world but its frequency varies considerably between different races and ethnic groups (Beukes, 1999).

Reports on the frequency of lactose maldigestion in Africa are confusing since some tribes consume milk in considerable amounts without ill effects, whereas their fairly near neighbours may have a high incidence of intolerance (Abbot according to Beukes, 1999). Segal (1983) found that South African Blacks are lactase deficient, despite the fact that two of the largest groups (Zulu and Xhosa) traditionally were cattle herders and milk drinkers. The reason for lactase deficiency in South African Blacks was probably due to migration from West and Central Africa where milk was not produced, which led to those people having taken up milk and dairy products fairly recently, not having had enough time for genetic selection for lactase availability through life.

Cheese and other fermented dairy products may help to alleviate the problem of lactose intolerance since their lactose content is lowered during fermentation. Cheese is also an excellent energy food, tasty, highly digestible and suitable for almost all age groups. Only heat-sterilised cheeses are recommended for infant feeding (Kosikowski, 1978).

2.3 Some characteristics of Gouda and Cheddar cheeses

2.3.1 Selected chemical characteristics

Uniformity of composition of cheese is best achieved by a grading system based on compositional analysis. The average chemical composition of Cheddar and Gouda cheese is outlined in Table 2.6.

Table 2.6 The average chemical composition of Cheddar and Gouda cheese

	Cheddar	Reference	Gouda	Reference
Fat (%)	33 – 34.4	Grandison (1993)	29.2	Lolkema & Blaauw (1974)
Moisture (%)	37 – 39		41	
Salt (%)	1.5 – 2.0		1.5 – 2.2	
PH	4.9 – 5.3	Fox (1987)	5.1 – 5.2	
ADV	1.2 – 1.8	Deeth & Fitz-Gerald (1976)	nd	nd
SM (%)	4.5 – 5.5	Fox (1987)	nd	nd
FIDM (%)	50 – 57	Fox (1987)	49.5	Lolkema & Blaauw (1974)

ADV = Acid Degree Value

SM = Salt in Moisture

FIDM = Fat in Dry Matter

nd = No data

2.3.1.1 pH

The pH value is important in that it provides an indication of the extent of acid production throughout the cheese-making process (Fox, 1987). Acidity of cheese and fermented milk foods is measured by both titratable acidity and pH, but it is well to know which method is more suitable for a given product or circumstances (Kosikowski, 1978). For hard, ripened cheese, such as Cheddar titratable acidity and pH measurements are used independently at different points in its manufacture and subsequent ripening.

In Cheddar cheese manufacture, during the first separation of curd and whey, until the curd milling stage, the acidity of the free whey is measured by titratable acidity. Thereafter, free whey becomes unavailable and the fermentation rate is observed by changes in pH. Cheddar cheese curds are milled at 0.6 – 0.7% acidity, and salted curds are pressed at a pH of 5.2 – 5.3 (Kosikowski, 1978). Gouda cheese normally has a pH of 5.1.

Given reliable starter activity, the pH reached in dry-salted cheeses like Cheddar, is determined by the salt in moisture (SM) value, since this controls the extent of starter activity after salting, the rate of lactose utilisation in salted curd and thus the pH reached (Fox, 1987). In cheese with a SM of 4.5%, the starter is not completely inhibited and lactose is rapidly metabolised. This explains why the pH values of one day old Cheddar cheese may range from 5.3 down to 4.9 (Fox, 1987).

2.3.1.2 Salt in moisture (SM)

In young Cheddar cheese the SM is the major parameter controlling water activity. This in turn determines the rate of bacterial growth and enzyme activity in cheese (Fox, 1987). SM is calculated using the percentage moisture and salt of the cheese.

Cheddar cheese is made to about 37% moisture content to insure better keeping quality (Kosikowski, 1978). Mistry & Anderson (according to Mistry & Kasperson,

1998) mentioned that the moisture content of Cheddar cheese may vary from <39% in full fat cheese to >50%, depending on the fat content. The moisture content in low fat cheese must be increased to levels substantially greater than those normally employed in normal Cheddar manufacture in order to improve the textural properties of the cheese (Banks, Brechany & Christie, 1989).

The salt or NaCl content of the cheese and fermented milk foods is variable. Cheddar cheese and Gouda cheese contain 1.5 – 2% salt (Kosikowski, 1978) and 1.5 – 2.2% salt (Lolkema & Blaauw, 1974) respectively. Salt has a very wide consumer tolerance depending upon the type of food with which it is mixed. Salt in ripened cheese has several important functions, including the proper development of flavour and body, improved keeping quality, and bacteriological safety (Mistry & Kasperson, 1998). As salt content increases, proteolysis and the general rate of ripening decrease (Bechaz, Hickey, Limsowtin & Morga, 1998; Mistry & Kasperson, 1998).

Cheesemakers normally aim for a SM value in Cheddar cheese between 4.5 and 5.5% (Fox, 1987). A lowering of the SM to that of lower fat cheeses allows excessive bacterial growth and proteolysis during cheese ripening (Fox, 1987; Kelly *et al.* according to Mistry & Kasperson, 1998), which may be responsible for the poor flavour development and flavour defects of these cheeses. SM is an important parameter, for example Mistry & Kasperson (1998) observed that in low-fat cheddar cheese the increased moisture content lowered the SM and, consequently, altered the ripening characteristics of the cheese. Increased SM in the cheese reduces moisture and consequently increases the hardness and firmness of the cheese.

2.3.1.3 Fat in dry matter (FIDM)

Milk fat exists as spherical globules in milk, their interaction with the protein matrix in a clotted milk gel depends on the surface characteristics of the fat globules. Shrinkage of the gel (curd particles) and increased temperature during cheese manufacturing distort the fat globules and rupture the surface membrane (Green *et al.* according to Olson & Johnson, 1990). The final cheese consists of islands of fat entrapped in the curd matrix. Recommended fat content for Gouda cheese is 29.2 %

(Lolkema & Blaauw, 1974), and that of Cheddar cheese is 33 – 34.4 % (Grandison, 1993).

The fat content of cheese plays a major role in the overall quality or acceptability of the product (McGregor & White, 1990). Reduction of fat level in cheese is generally accompanied by texture defects, which include increased firmness, elasticity, dryness and graininess (Jameson, 1990; Fenelon, Guinee & Reville, 1999) and low flavour level (McGregor & White, 1990). Legal limits for fat are usually specified in terms of FIDM (Fox, 1987). An advantage of using FIDM is that it can be controlled directly by milk standardisation whereas the fat content of cheese cannot. Recommended FIDM for Gouda cheese is 49.2 % (Lolkema & Blaauw, 1974), and that of Cheddar cheese ranges from 50 to 57 % (Fox, 1987).

2.3.1.4 Free fatty acids (FFA) in cheese

The free fatty acids in cheese can be derived either from the breakdown of fat by lipolysis or the metabolism of carbohydrates and amino acids by bacteria (Georgala, Kandarakis, Kaminarides & Anifatakis, 1999). McSweeny & Fox according to Georgala *et al.* (1999) stated that the level of lipolysis varies among cheese varieties from slight (Cheddar, Emmental and Gouda-type cheese) to extensive (Blue-mould ripened and hard Italian type cheeses). It is considered that free fatty acids are mainly produced from fat hydrolysis by the lactic starters and secondary flora during the ripening of cheese (Georgala *et al.*, 1999).

Typical Cheddar cheese flavour is due to a balance between fatty acids in low amounts during aging and other flavour constituents (Deeth & Fitz-Gerald, 1976). Excessive levels of fatty acids impart unclean, butyric or rancid flavours to the cheese. The acid degree value (ADV) is a measure of the amount of fatty acids which have been freed by hydrolysis from their glycerides through the action of water, temperature and lipolytic enzymes and not as a normal component of vegetable or animal lipids (Deeth & Fitz-Gerald, 1976). The ADV of good quality Cheddar cheese is around 1.2 – 1.8, whereas rancid flavours are evident at ADV over 3.0 (Deeth & Fitz-Gerald, 1976).

2.3.2 Selected microbiological characteristics

Traditionally, the microflora of cheese consisted of adventitious microorganisms from the environment that contaminated the milk or cheese during manufacture or ripening (Fox *et al.*, 1998). Many factors such as temperature, salt, pH and nutrient requirements affect the microbial population. In the case of Cheddar and Gouda cheeses, the microflora is dominated by mesophilic lactobacilli, although *Pediococcus* species have been reported by some authors (Franklin & Sharpe, 1963; Fox *et al.*, 1998). Some bacterial groups may develop sequentially with one group often enhancing the growth of another, before eventually having its growth inhibited by its own metabolic by-products (Haque *et al.*, 1997).

Cheddar cheese undergoes a sequence of changes in which other bacteria, the lactobacilli in particular, replace the initial flora of streptococci (Farkye, 1993; Haque *et al.*, 1997). Microorganisms multiply from 10^6 to 10^7 cfu/ml in milk to ~10^9 cfu/g of fresh cheese. In Cheddar and Gouda cheeses, the starter population declines to 10^3 cfu/g within the first few weeks (Farkye, 1993). The time until pediococci appear in Cheddar cheese is about the same as for the lactobacilli, they sometimes attain numbers as high as 10^7 to 10^8 /g (Franklin & Sharpe, 1963; Farkye, 1993).

2.3.2.1 Pediococcus: some general characteristics

Pediococcus belong to a group of homo-fermentative lactic acid bacteria. They are the only lactic acid bacteria dividing in two planes (Garvie, 1986). The cells are spherical and never elongated. Single cells are rare and chains of cells are not formed. Pediococci are Gram positive, catalase-negative, non-motile facultative anaerobes and do not form spores (Günther & White, 1961). Tolerance to oxygen varies in different species (Garvie, 1986). Colonies vary in size from 1.0 to 2.5 mm in diameter and are smooth, round and grayish white (Günther & White, 1961).

In stab culture, growth is along the stab with little surface growth. Broth cultures usually have uniform turbidity. No strains are pathogenic to plants and animals and they are widely used in commercial scale fermentation processes, particularly in meat

and vegetable processing (Daeschel, 1989; Skytta, Haikara & Matila-Sandholm, 1993). *Pediococcus* is known to produce a range of bacteriocins and other secondary metabolites with a broad-spectrum antibacterial activity.

All species grow at 30°C but optimum temperature range from 25 to 40°C. Their growth is dependent on the presence of fermentable carbohydrates and glucose is fermented by the Embden-Meyerhof pathway to DL or L (+) lactate (Jay, 1998). According to Garvie (1986) species can be separated by tolerance to temperature, pH and NaCl (Table 2.7). The acid tolerant *P. damnosus* and *P. parvulus*, require the most anaerobic conditions. Cysteine hydrochloride (0.05 to 0.1%) added to broth improves their growth (Garvie, 1986). *P. pentosaceus* and *P. acidilactici* grow rapidly in suitable broth media and they grow equally well aerobically on an agar surface. *P. halophilus* and *P. urinaequi* are more aerobic than other species and no special incubation conditions are required.

P. halophilus fails to grow in the absence of 5% NaCl, but other species have no requirement for salt. MRS broth and agar can be used for growing all pediococci. Amino acid requirement is not known for all species except for *P. pentosaceus* and *P. acidilactici*, which require amino acids for growth (Jensen & Seely according to Garvie, 1986).). Vitamin requirements are similar to other species. Some strains of *P. pentosaceus* require folic acid for growth but this requirement is not shared by other species (Nakegewa & Kithara according to Garvie, 1986).

Table 2.7 Differential conditions of growth of the genus *Pediococcus* (Garvie, 1986)

Characteristic	*P. damnosus*	*P. parvulus*	*P. inopinatus*	*P. dextriinictus*	*P. pentosaceus*	*P. acidilactici*	*P. halophilus*	*P. urinaeequi*
Growth at								
35°C	-	+	+	+	+	+	+	+
40°C	-	-	-	+	+	+	-	+
50°C	-	-	-	-	-	+	-	-
Growth at								
pH 4.2	+	+	-	-	+	+	-	-
pH 7.2	-	+	d	+	+	+	+	+
pH 8.5	-	-	-	-	d	+	+	+
Growth in								
4 % NaCl	-	+	+	+	+	+	d	+
6.5 % NaCl	-	+	d	-	+	+	+	+
18 % NaCl	-	-	-	-	-	d	+	-

+ = 90 % or more strains are positive

- = 90 % or more of strains are negative

d = 11-89 % of strains are positive

2.3.2.2 Pediococci in cheese

Pediococci are rarely used as starters for the production of cheese (Tzanetakis, Litopoulou-Tzanetaki & Vafopoulou-mastrojiannaki, 1991). Nevertheless, pediococci are considered important to some food fermentation industries (Smith & Pulumbo according to Tzanetakis *et al.*, 1991). It has been reported that cheese ripening can be accelerated and cheese flavour enhanced by adding pediococci concurrently with the starter culture (Tzanetakis *et al.* according to Vafopoulou-Mastrojiannaki, Litopoulou-Tzanetaki & Tzanetakis, 1994).

The only bacteria that grow in Cheddar cheese during ripening are mesophilic lactobacilli (Peterson & Marshall, 1990) and perhaps pediococci, collectively referred to as non-starter lactic acid bacteria (NSLAB) (Shakeel-ur-Rehman, McSweeney & Fox, 1999). Dacre (1958) identified *Pediococcus* strains that were found to constitute about a quarter of the normal lactic acid flora during the ripening of New Zealand Cheddar cheese. According to Günther & White (1961) the presence of pediococci in milk and cheese is understandable since they are also present in silage, the rumen and saliva of the cow. The appearance and development of the flavour followed more closely with the numbers of pediococci than that of the lactobacilli (Dacre, 1958). Dacre (1958) noticed that lactobacilli and pediococci appeared within 18 d from the time of manufacture of the cheese and always before the starter bacteria disappeared.

Bhowmik *et al.* (1990) found that the maximum number of pediococci appeared in 1–month old low-fat Cheddar cheese, and then the number remained nearly constant throughout the ripening process. Bouton, Guyot & Grappin (1998) isolated pediococci strains after 22 weeks in Comté cheese, whilst Fryer & Sharpe (1967) isolated 59 strains of pediococci (*Pedicoccus cerevisiae*) from 16 Cheddar cheese made in the pilot plant.

Litopoulou-Tzanetaki, Graham & Beyatli (1989) found that the Gram-positive streptococci were the dominant flora early in the fermentation, and the Gram-positive rods became the dominant flora about the fourth week of fermentation. Very few pediococci (1%) were isolated from the cheeses, and those were found late in the ripening stage (Litopoulou-Tzanetaki *et al.*, 1989). Elliot & Mulligan (according to Litopoulou-Tzanetaki *et al.*, 1989), reported 20 pediococci among 2000 cultures isolated from 20 different cheeses that were at the same ripening stage.

Tzanetakis *et al.* (1991) found that the composition of Teleme cheese was satisfactory after the addition of *P. pentosaceus* strain as a starter culture. It was also discovered that the pH of the cheese was lower than that of the control cheese due to enhanced acid production when *P. pentosaceus* grew together with commercial starter bacteria (Tzanetakis *et al.*, 1991). The acid degree value increased significantly and it seemed

possible that fat hydrolysis in the cheese could be partly attributed to starter enzymes and mainly lipase of other organisms present in milk (Tzanetakis *et al.*, 1991).

According to Shakeel-ur-Rehman *et al.* (1999) the composition (i.e. fat, protein, moisture, NaCl and SM) of the cheese in which antibiotics were added, was within the normal ranges for Cheddar cheese even if the number of NSLAB decreased. There were only small differences in pH between the antibiotic treated cheeses and their respective controls. In a study conducted by Fenelon, Ryan, Rea, Guinee, Ross, Hill & Harrington (1999), it was found that differences in the rate of growth and the final population of NSLAB in the cheeses had no significant effect on proteolysis, flavour, aroma, and texture, and on the gross composition of the cheese.

2.4 Starter function

Starter cultures were introduced at the end of the 19th century with the objective of standardising the rate of acid production and hence improving the quality of the cheese (Fox *et al.*, 1998). Starter cultures always include lactic-acid bacteria that are responsible for the conversion of lactose to lactic acid (lactate) in cheese (Boubekri & Ohta, 1996; Jay, 1998). Starter cultures have a significant impact on the body and texture of the cheese (Oberg, Broadbent & McMahon, 1998). In 1919 three independent workers in Holland, Denmark and the United States established that lactic starters that showed good flavour development were in fact a mixture of two different types of lactic-acid bacteria (Beukes, 1999). One of these bacterial groups is responsible for acid production (*Lactococcus lactis* and *Lactococcus lactis* ssp. *cremoris*) and the other for flavour production (*Leuconostoc* spp. and *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis*).

In most milk fermentations lactococci, or the closely related lactobacilli, are responsible for the production of lactic acid. The transformation of lactose to lactic acid is the primary function of the starter culture (Schaack & Marth, 1988; Stilton, 1993; Jay, 1998; Beukes, 1999). Starter bacteria also contribute to the final organoleptic qualities of cheese and fermented milks by producing substances that contribute to the flavour of the product (Schaack & Marth, 1988; Stilton, 1993).

According to Dias & Weimer (1998) lactobacilli, micrococci and pediococci have been used as adjunct bacteria to aid in flavour development.

4.1 Lactose fermentation

The fermentation of milk by lactic acid bacteria is an essential step in the manufacture of many dairy products (van Hooydonk, Hagedoorn & Boerrigter, 1986). As mentioned above, production of lactic acid from lactose is the primary function of the starter culture. Lactose is essential for the early stages of cheese-making, for it is the sugar that is readily metabolised by the starter bacteria. Lactic acid bacteria is divided into two groups based on the end products of lactose fermentation. Those that produce lactic acid as the major product of glucose (from lactose) fermentation are called homofermentative lactic acid bacteria (homolactics), whereas those that produce equal amounts of lactic acid (lactate), CO_2 and ethanol from hexoses are called heterofermentative lactic acid bacteria (heterolactics) (Schaack & Marth, 1988; Jay, 1998). Metabolic pathways for lactose fermentation are outlined in Figure 2.2.

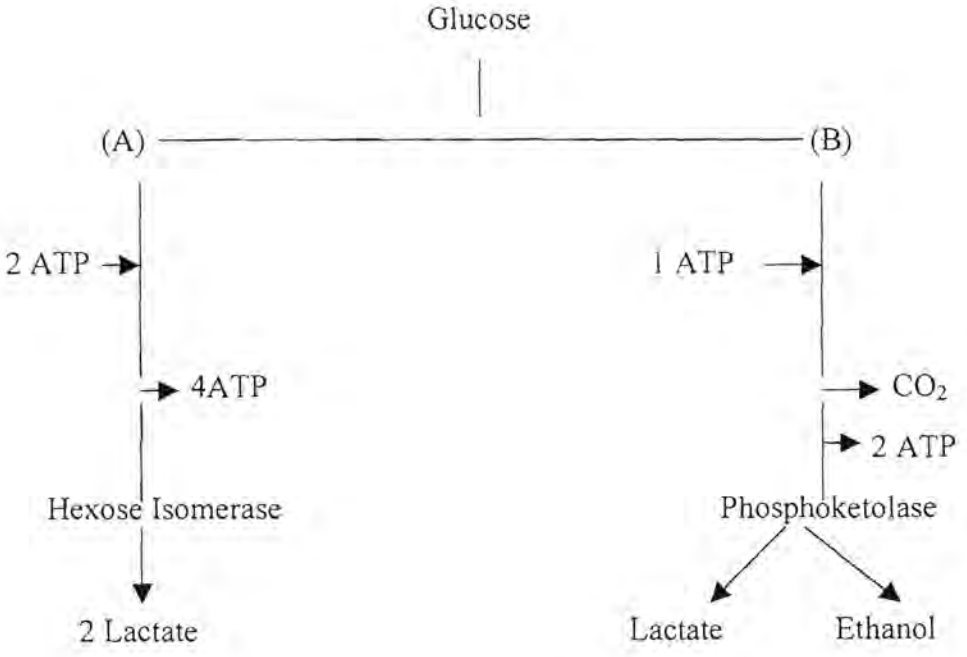

Figure 2.2: Generalised pathways for the production of some fermentation products from glucose by lactic acid bacteria. A: homofermentative lactics; B: heterofermentative lactics (Jay, 1998)

The homolactics possess the enzymes aldolase and hexose isomerase but lack phosphoketolase. They use the Embden-Meyerhof–Parnas (EMP) pathway towards their production of the two lactate molecules. The heterolactics, on the other hand, have phosphoketolase but do not possess aldolase and hexose isomerase, and instead of the EMP pathway for glucose degradation these organisms use the hexose monophosphate or pentose pathway for the production of lactic acid and ethanol (Jay, 1998).

Lactic acid is an odourless, non-volatile acid that creates the typical acid sensation of fermented dairy products (Margalith according to Beukes, 1999). The pH is reduced by fermentation of milk by lactic acid bacteria (Law, 1996; Renault, Gastaldi, Cuq & Torado de la Fuente, 2000). This is important in cheese-making because it leads to proper coagulation of the curd and proper synerisis of the whey (Schaack & Marth, 1988; Zoon, van Vleit & Walstra, 1989; Renault *et al.*, 2000). The rate at which the pH is lowered and the minimum pH reached are the main criteria for the selection of the right culture.

Holler & Steele (according to Beukes, 1999) mentioned that the ideal starter culture rapidly and dependably produces lactic acid in milk. Cultures that produce acid rapidly are desired for Cheddar cheese manufacture, whereas those that produce acid slowly are favoured for the production of Gouda, Edam and other low-acid cheeses. Starting from the milk pH of 6.6, the pH is lowered to 5.25 in 4.5 h for Cheddar cheese, and pH 5.1 in 20 h for Gouda cheese (Stilton, 1993).

2.4.2 *Production of flavour compounds*

The heterofermentative lactics produce volatile flavour compounds, such as diacetyl and CO_2 from citrate (Stilton, 1993). Cogan (according to Beukes, 1999) reported that CO_2 may as well be involved in flavour perception when one considers the dramatic effect it has on the taste of carbonated drinks.

In certain products a relatively high concentration of microbiologically generated CO_2 is desirable for creating the open texture as in Cheshire cheese and for hole formation as in Gouda and Edam cheeses. The starter for Gouda cheese is a mixture of *Lc. lactis* ssp. *cremoris*, and the citrate using *Lc. lactis* ssp. *lactis* and *Leuconostoc mesenteroides* ssp. cremoris. The gas formers make up 15 – 25 % of the total culture, with the leuconostoc making up 60 – 75% of the gas formers (Johnson *et al.*, 1998).

Broadbent, Brennand, Johnson, Steele, Strickland & Weimer (1997) demonstrated that starter cultures play a role in the development of desirable and undesirable flavours in cheese. Tzanetakis & Litopoulou-Tzanetaki (1989) reported that pediococci do not have an individual effect on the flavour of Cheddar cheese, but rather are part of a complex microflora with synergistic influences.

The starter organisms contribute a major source of both proteinase and peptidase enzymes (Kang, Vézinz, Laberge & Simard, 1998), whose activity is of great importance in assisting to produce the flavour of mature cheese Stilton (1993). The release of small peptides and amino acids from casein during cheese-ripening due to starter culture proteolytic enzymes have either a direct bearing on cheese flavour or are precursors in the formation of flavour compounds (Matínez-Cuesta, Peláez, Juárez & Requena, 1997).

Lactococcus spp. have 95% of their proteinase activity associated with the cleavage of the milk protein into peptides (detected by bitter, harsh flavour notes), while the peptidase enzymes reduce the harsh, bitter flavour by degrading the peptides to amino acids (Stilton, 1993; Engels & Visser, 1996; Kang *et al.* 1998). The starter should therefore provide a balanced proteolytic system if well-rounded mature flavours are desired.

CHAPTER 3

MATERIALS AND METHODS

3.1 Cheese manufacture

3.1.1 Manufacture of Gouda cheese

Gouda cheese was manufactured in the pilot plant of the Department of Food Science, University of Pretoria, using a method described by Kosikowski (1978). The following procedure was followed:

- The milk (not standardised), obtained from the experimental farm of the University of Pretoria, was pasteurised in a batch pasteuriser at 63°C for 30 min and cooled down to 30°C.
- The milk was then inoculated with 0.75% of the starter culture CH-N22 (a mixture of *Lactococcus lactis* and *Lactococcus lactis* ssp. *cremoris).* The stock cultures were supplied in freeze-dried form by CHR Hansen's Laboratories (Denmark).
- The milk was renneted, using (2ml/100L milk) fromase rennet (Wallenstein Company, Denmark)
- After 30 – 45 min at 30°C, the coagulated milk was cut and after 10 min resting of the curd, part of the whey (10% of the volume of milk) was removed and replaced by the same amount of water.
- The temperature of the curd-whey mixture was gradually raised from 30°C to 36°C (cooking temperature) over a period of 30 – 45 min. On reaching the cooking temperature, whey was drained to 50% of the original volume of milk (half way). The curd and whey mixture was held for 1,0 – 1,25 h at the cooking temperature.
- The whey was then drained, the curd put into moulds and pressed for 3 – 4 h.
- The green cheese was brine salted for 24 h (small cheese i.e.< 3 kg) or 3 d (large cheese i.e. 9 kg), allowed to dry off for 2 d, vacuum packed in plastic bags and cured for 56 d at 10 – 12°C.

3.1.2 Manufacture of Cheddar cheese

Cheddar cheese was manufactured in the pilot plant of the Department of Food Science, University of Pretoria, using a method described by Kosikowski (1978). The procedure outlined in Figure 3.1 was followed. Rennilase type T rennet (Novo Industries, Denmark) was used to coagulate the milk.

3.2 Cheese samples

3.2.1 Origin of the samples

Cheese samples (24) were collected from the cheese presented for judging for the AVI-Africa Expo at the 2000 Rand Show. Of these samples 11 were Cheddar cheese and 13 Gouda cheese. The samples collected from the Expo were manufactured at different cheese factories in South Africa (Table 3.1). After collection, the samples were kept on ice and transported to the Department of Food Science, University of Pretoria where they were stored at 4°C in the cold rooms.

Ten cheese samples were bought from retail outlets in Pretoria of which five were Gouda and five were Cheddar. Fresh cheeses (Gouda and Cheddar) were manufactured in the Department of Food Science, University of Pretoria. The fresh cheese were matured for 56 d in the hard cheese ripening rooms of the Department of Food science and examined every two weeks.

3.2.2 Sampling

Approximately 100 g cheese samples were collected aseptically (IDF Standard 50B, 1985) from each of the cheeses presented for judging for the AVI-Africa Expo at the 2000 Rand Show, placed in sterile plastic sample bags (Whirl Paks, Nasco Sampling Equipment) and stored at 4°C in the cold rooms at the Department of Food Science, University of Pretoria. For analysis packaged cheese samples (five per day) were

picked randomly from the samples collected from the AVI-Africa Expo and from the retail outlets.

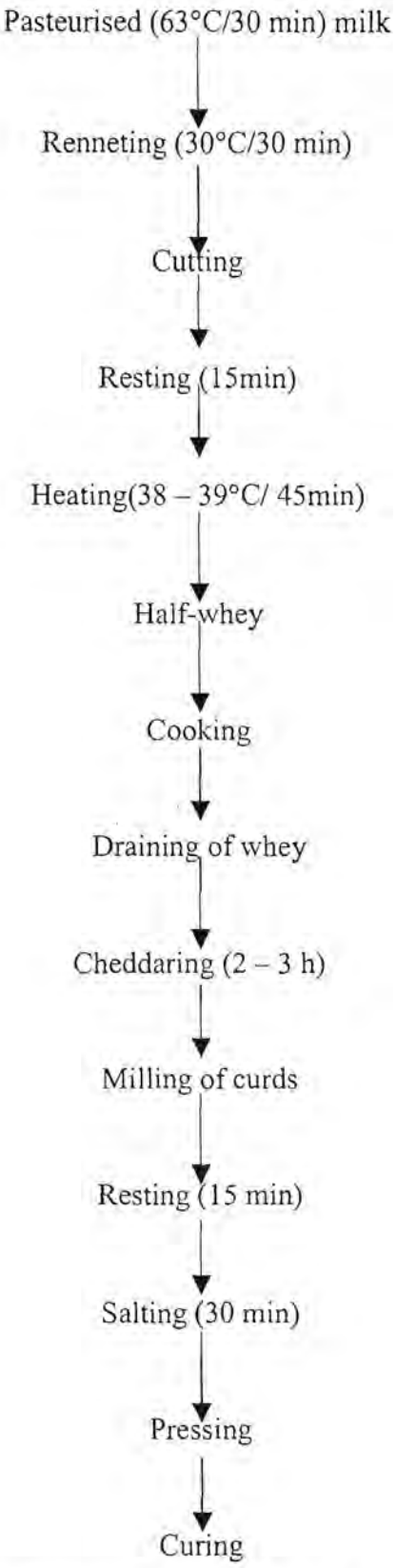

Figure 3.1 Manufacture of Cheddar cheese (Kosikowski, 1978)

The packages were opened and each sample grated aseptically using a grater that had been sterilised in the autoclave at 121°C for 15 min. After proper mixing of the grated sample, the sample for microbiological analyses was taken asceptically, before the samples for chemical analyses were taken. Microbiological analysis was done in duplicate on each sample, and chemical analyses (except pH measurements) were done in quadruplicate on the collected samples and in six-fold on the cheese samples manufactured in the Department of Food Science, University of Pretoria. Samples of manufactured cheeses were taken every two weeks (for 56 d) beginning from the second day after the cheese was manufactured.

Table 3.1 Origin of Cheddar and Gouda cheese samples analysed

Cheddar sample	Collection point	Gouda Sample	Collection point
3	Retail outlet (Hyperama)	1	Retail outlet (Hyperama)
4	Retail outlet (Hyperama)	2	Retail outlet (Woolworths)
5	Retail outlet (Pick & Pay)	6	Retail outlet (Pick & Pay)
7	Retail outlet (Pick & Pay)	9	Retail outlet (Spar)
8	Retail outlet (Spar)	10	Retail outlet (Spar)
15	AVI-Expo	52	AVI-Expo
16	AVI-Expo	54	AVI-Expo
17	AVI-Expo	55	AVI-Expo
18	AVI-Expo	57	AVI-Expo
19	AVI-Expo	59	AVI-Expo
21	AVI-Expo	60	AVI-Expo
23	AVI-Expo	62	AVI-Expo
31	AVI-Expo	63	AVI-Expo
33	AVI-Expo	66	AVI-Expo
37	AVI-Expo	67	AVI-Expo
39	AVI-Expo	68	AVI-Expo
C2 to C56	Manufactured UP	69	AVI-Expo
		70	AVI-Expo
		G2 to G56	Manufactured UP

UP = University of Pretoria

C2 to C56 = Cheddar cheese at day two to day 56

G2 to G56 = Gouda cheese at day two to day 56

3.3 Microbiological analyses

3.3.1 Preparation of dilution series

The diluent (2% sodium citrate solution, IDF 100A, 1985) was dispersed in 9 ml amounts in McCartney bottles and 90 ml amounts in 250 ml wide-mouth bottles and sterilised at 121°C for 20 min. Appropriate dilutions (10^{-1} to 10^{-7}) were prepared with sterile sodium citrate solution as diluent.

3.3.2 Enumeration of micro-organisms

The standard plate count procedure (IDF 100A, 1985) was performed on each cheese sample, using MRS agar (Biolab) with 4 % salt added and pH adjusted to pH 7.5, pH 8.5 or pH 4.0, respectively. These selective conditions were chosen since they are suitable for the growth of most of *Pediococcus* ssp (Garvie, 1986). The medium was sterilized in an autoclave at 121°C for 15 min.

Dilutions (10^{-3} to 10^{-7}) were plated using the standard plate count method (IDF 100A, 1985). Incubation was conducted aerobically at 35°C for 7 days. Plates were then examined for colonies and those with pin-point size colonies (usually >300 colonies per plate) were ignored. The colonies on plates with between 10 and 300 colonies per plate were counted.

3.3.3 Isolation and cultivation of presumptive pediococci

Using the Harrison Disc method (Harrigan & McCance, 1986), the square root of the number of counted colonies were isolated from the countable plates. These colonies were then further examined by the Gram-staining method and by testing for catalase production with hydrogen peroxide (Harrigan & McCance, 1986). Catalase negative colonies containing Gram-positive cocci in tetrads were considered to be presumptive pediococci (Table.3.2) and were subcultured in MRS agar slants (pH 4, pH7.5 or pH 8.5) at 35°C for 24 h.

Table 3.2 Differential characteristics of pediococci and lactobacilli (Garvie, 1986; Litopoulou- Tzanetakis, 1989; Beukes, 1999)

Characteristic	Pediococci	Lactobacilli
Cell form	Spherical	Rods/ Coccobacilli
Cellular Arrangement	Pairs, tetrads, clusters, single cells are rare, no chains	Chain formation is common
Growth at		
10°C	±	ND
15°C	ND	±
35°C	+	ND
45°C	±	±
Growth in		
4.2 % NaCl	±	ND
6.5 % NaCl	±	ND
18 % NaCl	±	±
Gram stain	+	+
Catalase reaction	Weak +	-

+ = Growth

- = No growth

± = Responses varies between species

ND = No Data

3.4 Chemical analyses

3.4.1 Moisture content

Moisture content was determined by oven drying at 105°C overnight as described by James (1995).

3.4.2 Fat content

Fat content of the cheese was determined by the Gerber method (Kosikowski, 1978).

3.4.3 Free fatty acids content

Free fatty acids were determined by the acid degree value (ADV) method described by Triebold & Aurand (1963) and Hammond (1993).

3.4.4 Salt content

The salt content was determined according to the method described by the Australian Society of Dairy Technology (1966).

3.4.5 pH Measurement

The pH measurements were performed on grated, undiluted cheese sample as outlined by Kosikowski (1978).

3.4.6 Salt in moisture (SM) and Fat in dry matter (FIDM)

These quantities were determined by calculation as follows:

% SM= (% NaCl ÷ % Moisture) x 100

% FIDM= (% Fat ÷ 100 - % Moisture) x 100

3.5 Statistical analysis

Data for each attribute was analysed by using a SAS computer program (SAS®, 2002). Main attributes analysed were pH, moisture content, free fatty acid content (ADV), salt content, fat content, FIDM and SM. By combining values for samples collected from the AVI-Africa Expo and for samples purchased at retail outlets, the mean, standard deviation, minimum, maximum and p-value between and within Cheddar and Gouda cheeses were obtained. For further analyses, the results for UP Cheddar and Gouda cheeses were treated separately, by considering effect of ripening on the above attributes. Evaluations were based on a 5% significance level and statistical significance was accepted if the probability values were $p \leq 0.05$. No correlation analyses was performed for the number of pediococci isolates found and proximate chemical analyses, due to the low number of isolates involved.

Chapter 4

RESULTS

4.1 Microbiological analyses

4.1.1. Microbiological counts on MRS agar

All microbiological counts obtained for cheese samples are fully expanded in Table 4.1. Microbiological counts were in the order of 10^6 cfu/g. Counts for Cheddar and Gouda cheeses obtained from retail outlets were lower on average compared to Cheddar and Gouda cheeses obtained from the AVI-Africa Expo. On MRS at pH 4 counts were $<1 \times 10^1$ for most of the samples (especially samples from retail outlets). No growth was observed for UP Gouda cheese on MRS at pH 4. Counts for samples on MRS at pH 7.5 and pH 8.5 did not differ significantly. Microbial counts were on average higher at day 2, pH 7.5 for both UP Cheddar (6.0×10^6) and UP Gouda (0.5×10^6) compared to other days.

Table 4.1 Microbiological counts (cfu per g) obtained from cheese samples plated on MRS agar

Sample no.	Type of cheese	pH 4	pH 7.5	pH 8.5
1	G	$<1 \times 10^1$	0.15×10^6	0.22×10^6
2	G	$<1 \times 10^1$	0.11×10^6	$<1 \times 10^1$
3	C	$<1 \times 10^1$	0.3×10^6	0.005×10^6
4	C	0.002×10^6	0.074×10^6	0.32×10^6
5	C	$<1 \times 10^1$	0.18×10^6	0.16×10^6
6	G	$<1 \times 10^1$	1.1×10^6	$<1 \times 10^1$
7	C	0.3×10^6	1.6×10^6	$<1 \times 10^1$
8	C	$<1 \times 10^1$	0.006×10^6	$<1 \times 10^1$
9	G	0.037×10^6	0.004×10^6	$<1 \times 10^1$
10	G	$<1 \times 10^1$	0.3×10^6	0.001×10^6
15	C	1.3×10^6	25.0×10^6	21.0×10^6
16	C	1.8×10^6	3.0×10^6	17.0×10^6
17	C	4.3×10^6	26.0×10^6	28.0×10^6

C = Cheddar cheese G = Gouda cheese

Table 4.1 Microbiological counts (cfu per g) obtained from cheese samples plated on MRS agar (continued)

Sample no.	Type of cheese	pH 4	pH 7.5	pH 8.5
18	C	0.3×10^6	34.0×10^6	37.0×10^6
19	C	0.17×10^6	14.0×10^6	24.0×10^6
21	C	0.9×10^6	11.0×10^6	11.0×10^6
23	C	$<1 \times 10^1$	0.45×10^6	$<1 \times 10^1$
31	C	10×10^6	2×10^6	24.0×10^6
33	C	0.23×10^6	7.4×10^6	7.2×10^6
37	C	0.3×10^6	$<1 \times 10^1$	$<1 \times 10^1$
39	C	$<1 \times 10^1$	5.0×10^6	$<1 \times 10^1$
52	G	$<1 \times 10^1$	0.03×10^6	0.031×10^6
54	G	2.6×10^6	0.09×10^6	$<1 \times 10^1$
55	G	0.08×10^6	0.34×10^6	0.23×10^6
57	G	9.5×10^6	11.0×10^6	17.0×10^6
59	G	$<1 \times 10^1$	3.8×10^6	$<1 \times 10^1$

C = Cheddar cheese G = Gouda cheese

Table 4.1 Microbiological counts (cfu per g) obtained from cheese samples plated on MRS agar (continued)

Sample no.	**Type of cheese**	pH 4	pH 7.5	pH 8.5
60	G	6.6×10^6	4.6×10^6	6.7×10^6
62	G	0.97×10^6	2.1×10^6	0.22×10^6
63	G	6.4×10^6	1.0×10^6	24.0×10^6
66	G	0.2×10^6	1.5×10^6	1.8×10^6
67	G	1.4×10^6	3.2×10^6	2.4×10^6
68	G	0.34×10^6	24.0×10^6	24.0×10^6
69	G	4.8×10^6	0.5×10^6	79.0×10^6
70	G	0.59×10^6	63.0×10^6	73.0×10^6

C = Cheddar cheese G = Gouda cheese

Table 4.1 Microbiological counts (cfu per g) obtained from cheese samples plated on MRS agar (continued)

Sample no.	Type of cheese	pH 4	pH 7.5	pH 8.5
C2	C	0.19×10^6	6.0×10^6	0.5×10^6
C14	C	$<1 \times 10^1$	3.0×10^6	0.02×10^6
C28	C	0.28×10^6	0.3×10^6	0.1×10^6
C42	C	0.11×10^6	0.3×10^6	0.3×10^6
C56	C	0.03×10^6	3.0×10^6	0.05×10^6
G2	G	$<1 \times 10^1$	17×10^6	11×10^6
G14	G	$<1 \times 10^1$	0.02×10^6	0.028×10^6
G28	G	$<1 \times 10^1$	24×10^6	14×10^6
G42	G	$<1 \times 10^1$	0.091×10^6	0.061×10^6
G56	G	$<1 \times 10^1$	0.07×10^6	0.049×10^6

C = Cheddar cheese G = Gouda cheese

The mean microbiological counts on MRS at pH 8.5 were on average higher for Cheddar and Gouda cheese collected from the retail outlets and AVI-Africa Expo (15.7×10^6 and 31.4×10^6 cfu/g respectively), compared to counts at pH 7.5 and 4.0. For UP Cheddar and Gouda cheese the counts were higher on MRS at pH 7.5 (2.55×10^6 and 0.18×10^6 cfu/g respectively) compared to counts at pH 8.5 and 4.0. The mean microbiological counts for cheese samples at different pH values are outlined in Table 4.2.

Table 4.2 Mean microbiological counts (cfu/g) for Cheddar and Gouda cheese samples when plated on MRS agar at different pH values

Cheese type	pH 4.0	pH 7.5	pH 8.5
Cheddar	1.78×10^6 (n = 11)	9.9×10^6 (n = 15)	15.7×10^6 (n = 11)
Gouda	2.79 x 106 (n = 12)	18.0×10^6 (n = 18)	31.4×10^6 (n = 13)
UP Cheddar	0.152×10^6 (n = 4)	2.55×10^6 (n = 5)	0.19×10^6 (n = 5)
UP Gouda	$<1 \times 10^1$ (n = 5)	0.18×10^6 (n = 5)	0.078×10^6 (n = 5)

Cheddar = Cheddar cheese collected from the AVI- Africa Expo and Retail outlets

Gouda = Gouda cheese collected from the AVI- Africa Expo and Retail outlets

UP Cheddar = Cheddar cheese manufactured in the Department of Food Science, University of Pretoria, ripened for 56 days

UP Gouda = Gouda cheese manufactured in the Department of Food Science, University of Pretoria, ripened for 56 days

4.1.2 Presumptive pediococci

Altogether 1320 isolates from 36 cheese samples (34 commercial and 2 manufactured at UP) were collected from petri dishes and analysed microscopically for presumptive pediococci. From those, only 41 isolates tested positive for presumptive pediococci and were further subjected to primary identification tests. All 41 isolates were Gram-positive and 13 were catalase-negative. The total number of isolates that were

identified as presumptive pediococci, grouped by morphological characteristics, were 24 (Table 4.3 and 4.4), representing 1.82 % of the total number of isolates. Of the 24 identified isolates five were from Cheddar cheese and 19 from Gouda cheese. For UP Cheddar and Gouda cheese, pediococci first appeared at day 2 and day 14 respectively. From our observations (results not shown), the Gram-positive, chain-forming cocci (streptococci) predominated and in the UP cheese Gram-positive rods became dominant in the cheese as ripening continued.

Table 4.3 Number of isolates that tested positive for presumptive pediococci in collected commercial cheese samples

Cheddar		**Gouda**	
Sample number	3 5	Sample number	1 2 6 9 67 68 69 70
Number of isolates from each sample	1 1	Number of isolates from each sample	2 1 3 1 2 2 1 2
Total no. of cheddar isolates	2	Total no. of Gouda isolates	14

Table 4.4 Number of isolates that tested positive for presumptive pediococci during ripening (UP cheese samples)

Type of cheese	Number of isolates at day					Total number of isolates
	2	14	28	42	58	
Cheddar	1	1	—	1	—	3
Gouda	—	5	—	—	—	5

4.2 Proximate Analyses

4.2.1 Proximate chemical composition of Cheddar and Gouda cheeses collected from retail outlets and AVI-AFRICA EXPO.

Table 4.5 outlines means of pH, salt content, moisture content, fat content, ADV, SM and FIDM for individual Cheddar cheese samples analysed in quadruplicate, and there were no significant differences ($p > 0.05$) within most of Cheddar samples under investigation. Some exceptions were observed where there were significant differences as in:

- The p-values for the mean moisture content for samples 4, 5, 7 and 8 were in the range $p < 0.004$ and $p > 0.013$.
- The p-values of the mean salt content for samples 8, 17, 21 and 37 were in the range $p < 0.0009$ and $p > 0.026$.
- The p-values of the mean fat content for samples 7 and 15 were in the range $p < 0.002$ and $p > 0.007$.
- The p-values of the mean free fatty acid content for samples 21, 23, 31, 37 and 39 were in the range $p < 0.0001$ and $p > 0.0009$.
- The p-value of the mean pH for sample 21 was in the range $p < 0.0025$ and $p > 0.034$.

Table 4.5 Means of pH, salt content, moisture content, fat content, ADV, SM and FIDM for individual collected Cheddar cheese samples collected from retail outlets and AVI-AFRICA EXPO and analysed in qudruplicate

Sample no.	Moisture (%)	Salt (%)	Fat (%)	FFA (ADV)	pH	SM (%)	FIDM (%)
3	33.42 (0.31)*	1.60 (0.04)	34.38 (0.23)	1.94 (0.06)	5.20 (0.00)	4.79 (0.18)	51.63 (0.51)
4	34.86 (0.19)	2.00 (0.03)	32.68 (0.25)	1.85 (0.06)	5.20 (0.00)	5.63 (0.09)	50.16 (0.35)
5	35.41 (0.26)	1.99 (0.03)	34.53 (0.17)	1.90 (0.002)	5.20 (0.00)	5.62 (0.11)	53.45 (0.32)
7	35.49 (0.21)	1.81 (0.02)	33.70 (0.18)	1.85 (0.05)	5.20 (0.00)	5.09 (0.06)	52.24 (0.39)
8	34.48 (0.37)	1.83 (0.03)	34.48 (0.17)	1.75 (0.06)	5.20 (0.00)	5.32 (0.12)	52.62 (0.31)
15	38.08 (0.07)	2.00 (0.05)	33.40 (0.14)	1.98 (0.01)	5.12 (0.03)	5.25 (0.11)	53.93 (0.24)
16	38.41 (0.43)	1.60 (0.05)	31.07 (0.15)	2.08 (0.02)	5.10 (0.00)	4.16 (0.13)	50.46 (0.34)
17	38.57 (0.68)	2.01 (0.04)	32.75 (0.10)	2.14 (0.02)	5.20 (0.00)	5.21 (0.12)	53.31 (0.69)
18	38.33 (0.47)	1.81 (0.03)	32.68 (0.15)	2.08 (0.002)	5.10 (0.00)	4.73 (0.07)	52.98 (0.27)
19	38.19 (0.13)	1.55 (0.04)	34.56 (0.25)	2.01 (0.02)	5.20 (0.00)	4.05 (0.12)	55.94 (0.48)
21	34.68 (0.60)	1.98 (0.03)	31.80 (0.20)	1.90 (0.01)	5.12 (0.01)	5.71 (0.16)	48.69 (0.51)
23	34.86 (0.04)	1.87 (0.04)	34.40 (0.20)	1.89 (0.01)	5.20 (0.00)	5.37 (0.12)	52.81 (0.32)
31	34.28 (0.09)	1.93 (0.03)	34.6 (0.20)	1.67 (0.03)	5.20 (0.00)	5.62 (0.08)	52.65 (0.32)
33	38.18 (0.48)	1.96 (0.08)	33.10 (0.71)	1.99 (0.02)	5.13 (0.03)	5.13 (0.18)	53.54 (1.29)
37	34.40 (0.09)	2.09 (0.06)	32.00 (1.54)	1.90 (0.02)	5.14 (0.02)	6.07 (0.18)	48.78 (2.39)
39	33.90 (0.07)	1.55 (0.05)	34.55 (0.06)	1.80 (0.01)	5.20 (0.00)	4.58 (0.13)	52.27 (0.13)

*Figures in brackets are standard deviations

FFA = Free Fatty Acid

ADV = Acid Degree value

SM = Salt in Moisture

FIDM = Fat in Dry Matter

Table 4.6 outlines means of pH, salt content, moisture content, fat content, ADV, SM and FIDM for individual Gouda cheese samples analysed in quadruplicate. There were no significant differences ($p > 0.05$), within most of Gouda samples under investigation. Some exceptions were observed where there were significant differences as in:

- The p-values for the mean moisture content for samples 2, 6, 69 and 70 were in the range $p < 0.003$ and $p > 0.044$.
- The p-values of the mean salt content for samples 1, 2 and 6 were in the range $p < 0.002$ and $p > 0.027$.
- The p-values of the mean fat content for samples 6, 9, 10, 52 and 55 were in the range $p < 0.001$ and $p > 0.04$
- The p-values of the mean free fatty acid content for samples 1 and 2 were in the range $p < 0.003$ and $p > 0.034$.

Table 4.6 Means of pH, salt content, moisture content, fat content, ADV, SM and FIDM for individual collected Gouda cheese samples collected from retail outlets and AVI-AFRICA EXPO and analysed in quadruplicate

Sample no.	Moisture (%)	Salt (%)	Fat (%)	FFA (ADV)	pH	SM	FIDM
1	38.69 (0.30)*	1.60 (0.02)	28.55 (0.39)	1.90 (0.08)	5.20 (0.00)	4.15 (0.05)	46.05 (0.47)
2	39.4 (0.27)	1.77 (0.02)	29.40 (0.24)	1.80 (0.003)	5.20 (0.00)	4.49 (0.06)	48.52 (0.55)
6	40.26 (0.31)	1.97 (0.03)	33.30 (0.24)	1.95 (0.10)	5.20 (0.00)	4.90 (0.07)	55.74 (0.54)
9	38.75 (0.22)	1.79 (0.03)	35.02 (1.09)	1.65 (0.06)	5.10 (0.00)	4.61 (0.09)	57.19 (1.79)
10	38.97 (0.45)	1.73 (0.05)	35.20 (0.21)	1.75 (0.06)	5.20 (0.00)	4.45 (0.16)	57.67 (0.66)
52	39.77 (0.25)	2.10 (0.04)	30.88 (0.05)	1.90 (0.02)	5.00 (0.00)	5.29 (0.14)	51.27 (0.29)
54	41.69 (0.18)	1.92 (0.02)	30.58 (0.15)	1.89 (0.002)	5.10 (0.00)	4.62 (0.06)	52.43 (0.35)
55	41.48 (0.87)	1.86 (0.05)	27.63 (0.15)	1.85 (0.02)	5.00 (0.00)	4.48 (0.20)	47.22 (0.93)
57	39.95 (0.29)	2.12 (0.07)	28.82 (0.15)	2.18 (0.03)	5.00 (0.00)	5.31 (0.20)	48.00 (0.19)
59	39.91 (0.14)	2.12 (0.07)	29.35 (0.17)	2.17 (0.01)	5.00 (0.00)	5.32 (0.17)	48.84 (0.30)
60	39.55 (1.47)	2.02 (0.07)	27.78 (0.05)	2.17 (0.03)	4.95 (0.05)	5.12 (0.30)	45.97 (1.06)
62	42.62 (0.07)	2.16 (0.05)	28.02 (0.05)	1.88 (0.02)	5.10 (0.00)	5.07 (0.12)	48.84 (0.09)
63	40.22 (0.22)	1.47 (0.13)	28.32 (0.22)	2.17 (0.02)	5.00 (0.00)	3.66 (0.29)	47.39 (0.43)
66	41.33 (0.70)	1.98 (0.03)	31.30 (0.20)	2.10 (0.03)	5.00 (0.00)	4.80 (0.13)	53.36 (0.95)
67	39.67 (0.31)	1.85 (0.04)	30.70 (0.06)	2.14 (0.03)	4.99 (0.03)	4.68 (0.07)	50.97 (0.18)
68	40.24 (2.14)	1.53 (0.11)	30.32 (0.21)	2.13 (0.01)	5.00 (0.00)	3.81 (0.20)	50.80 (1.91)
69	39.54 (0.04)	2.15 (0.05)	31.37 (0.05)	2.18 (0.01)	5.00 (0.00)	5.44 (0.12)	51.90 (0.08)
70	38.05 (0.59)	2.08 (0.05)	28.82 (0.15)	2.17 (0.02)	5.00 (0.00)	5.48 (0.09)	46.50 (0.48)

*Figures in brackets are standard deviations

FFA = Free Fatty Acid

ADV = Acid Degree value

SM = Salt in Moisture

FIDM = Fat in Dry Matter

4.2.2 Proximate analyses of UP Cheddar and Gouda cheese

The mean moisture content for UP Cheddar and Gouda cheeses is outlined in Table 4.7. There was a significant ($p = 0.05$ and $p = 0.01$) decrease in moisture content for Cheddar and Gouda cheese respectively as ripening progressed but these decreases did not differ significantly ($p = 0.78$) between the two types of cheeses. Analyses were done in six-fold for the UP Cheddar and Gouda cheese samples.

Table 4.7 Means of moisture content for UP Cheddar and Gouda cheese during ripening (analysed in six-fold)

Days	Cheddar	Gouda
2	37.38 (0.78)*	40.06 (0.16)
14	37.14 (0.19)	38.27 (0.28)
28	37.05 (0.35)	37.86 (0.48)
42	34.02 (5.98)	37.57 (0.46)
56	35.84 (0.19)	36.80 (0.42)

*Figures in brackets are standard deviations

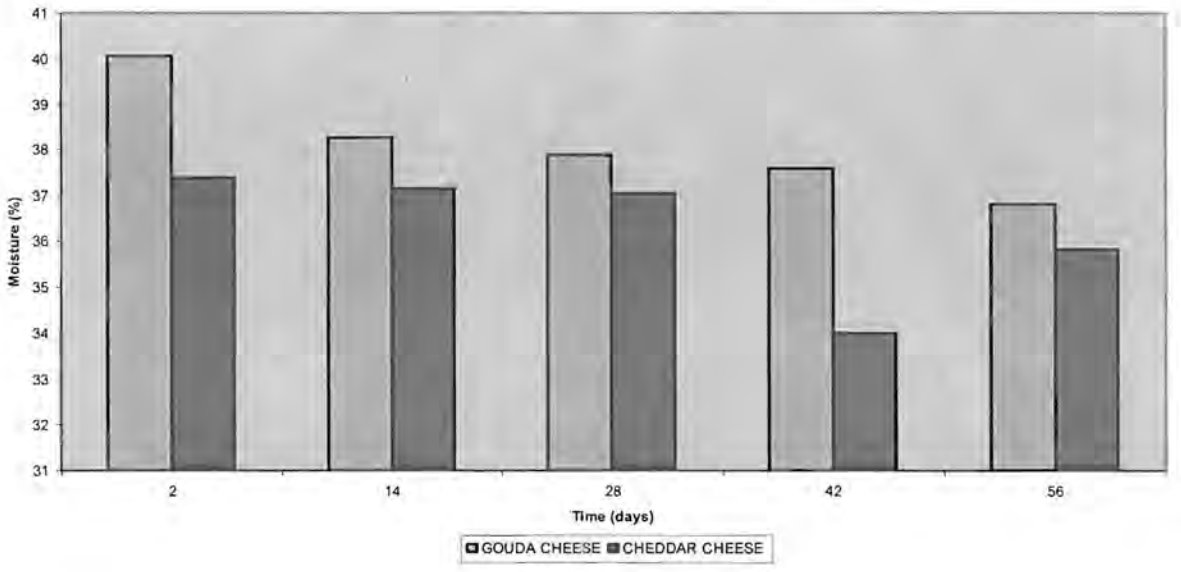

Figure 4.1 Changes in the moisture content of UP Cheddar and Gouda cheeses during ripening

The mean salt content for UP Cheddar and Gouda cheeses is outlined in Table 4.8. The increase in salt content was not significant ($p \geq 0.05$) during ripening, but there was a significant difference ($p = 0.04$) between the two types of cheeses. Analyses were done in six-fold on the samples of UP Cheddar and Gouda cheeses.

Table 4.8 Means of salt content of UP Cheddar and Gouda cheese during ripening (analysed in six-fold)

Days	Cheddar	Gouda
2	1.614 (0.05)*	0.275 (0.03)
14	1.826 (0.03)	1.396 (0.09)
28	1.831 (0.04)	1.712 (0.03)
42	1.856 (0.03)	1.707 (0.03)
56	1.874 (0.06)	1.707 (0.04)
p-value	0.9984	0.9982

*Figures in brackets are standard deviations

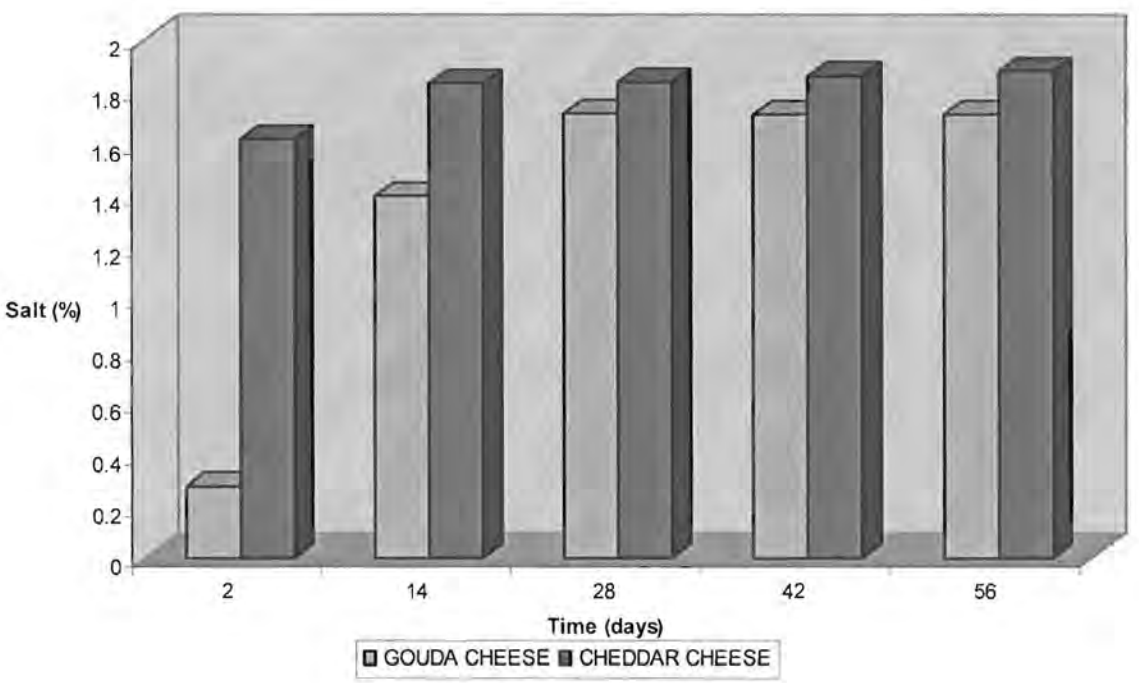

Figure 4.2 Changes in the salt content of UP Cheddar and Gouda cheeses during ripening

The fat content of UP Cheddar and Gouda cheeses is outlined in Table 4.9. There was a significant ($p \leq 0.05$) decrease in fat content of Gouda cheese during ripening but not for Cheddar cheese ($p \geq 0.05$). Changes in fat content differed significantly ($p = 0.006$) between the two types of cheeses. Analyses were done in six-fold on the samples of UP Cheddar and Gouda cheeses.

Table 4.9 Means of fat content of UP Cheddar and Gouda cheese during ripening (analysed in six-fold)

Days	Cheddar	Gouda
2	37.50 (0.50)*	33.47 (0.25)
14	37.47 (0.36)	33.18 (0.53)
28	36.98 (0.63)	32.38 (0.26)
42	36.95 (0.67)	32.68 (0.30)
56	37.08 (0.51)	32.58 (0.44)
p-Values	0.11	0.03

*Figures in brackets are standard deviations

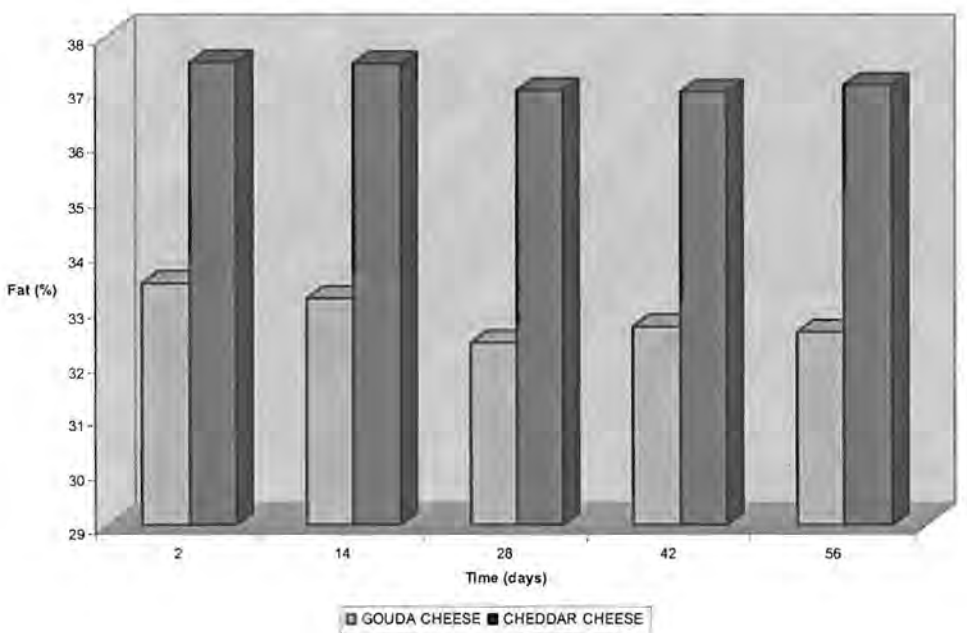

Figure 4.3 Changes in the fat content of UP Cheddar and Gouda cheeses during ripening

The mean free fatty acid content for UP Cheddar and Gouda cheeses is outlined in Table 4.10. The trend showed an increase in free fatty acid content during ripening, but these increases were not significant ($p \geq 0.05$). There was no significant difference ($p = 0.75$) between the two types of cheeses. Analyses were done in six-fold on the samples of UP Cheddar and Gouda cheeses.

Table 4.10 Means of free fatty acid (ADV) of UP Cheddar and Gouda cheese during ripening (analyses in six-fold)

Days	Cheddar	Gouda
2	1.147 (0.04)*	1.148 (0.01)
14	1.613 (0.04)	1.647 (0.01)
28	1.683 (0.04)	1.762 (0.05)
42	1.966 (0.05)	1.962 (0.05)
56	2.065 (0.05)	2.098 (0.06)
p-Value	0.9996	0.9994

*Figures in brackets are standard deviations

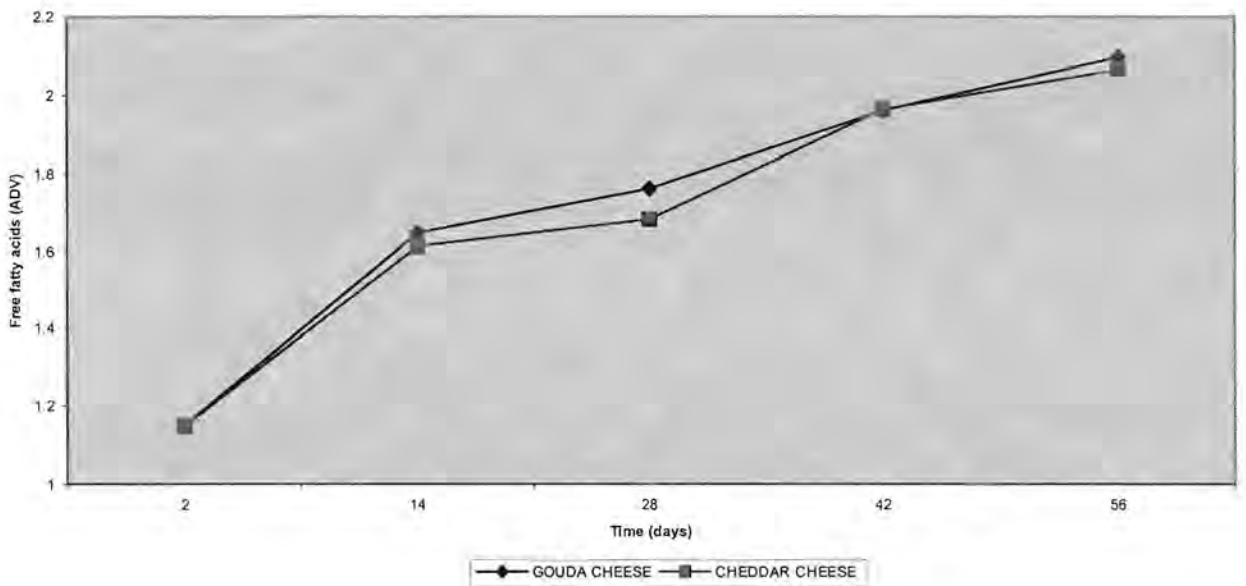

Figure 4.4 Changes in the free fatty acid content of UP Cheddar and Gouda cheeses during ripening

Figure 4.5 outlines the changes in pH during ripening of UP Cheddar and Gouda cheese samples. The pH for both samples remained constant throughout the ripening process. Analyses were done five times fore each sample during ripening.

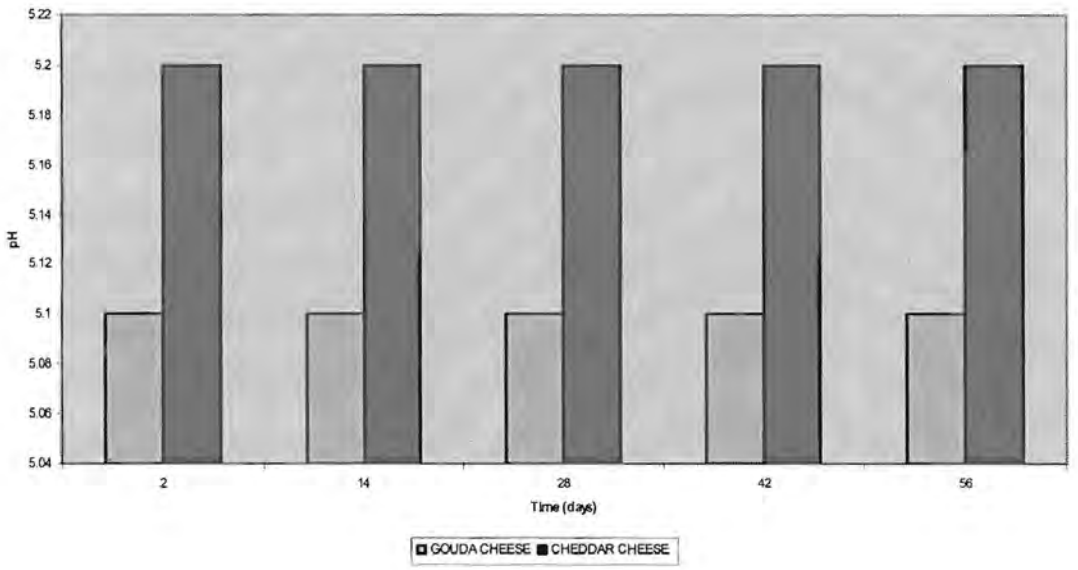

Figure 4.5 Changes in pH for UP Cheddar and Gouda cheeses during ripening

The SM and FIDM for UP Cheddar and Gouda cheeses are outlined in Table 4.11. The trend showed an increase in SM and a decrease in FIDM but there was no significant difference ($p \geq 0.05$) between the cheeses during ripening

Table 4.11 SM and FIDM of UP Cheddar and Gouda cheese during ripening.

Days	Cheddar		Gouda	
	SM	FIDM	SM	FIDM
2	4.31 (0.12)*	59.89 (1.09)	0.68 (0.07)	55.83 90.37)
14	4.92 (0.10)	59.60 (0.54)	3.65 (0.19)	53.75 (0.67)
28	4.94 (0.07)	58.75 (1.14)	4.52 (0.14)	52.12 (0.74)
42	5.66 (1.37)	56.38 (5.06)	4.54 (0.09)	52.36 (0.55)
56	5.22 (0.14)	57.80 (0.81)	4.64 (0.13)	51.56 (0.74)

*Figures in brackets are standard deviations

SM = Salt in moisture

FIDM = Fat in dry matter

CHAPTER 5

DISCUSSION

5.1 Microbiological Analyses

During the early stages of ripening, conditions for microbial growth are suitable, but later, conditions like high salt content, decreased lactose content and lower pH slows down the growth of microorganisms (Fox, 1987). The observed trend for UP Cheddar cheese agrees with what is reported in the literature, since there was a decrease in microbiological counts from day 2 to day 56 at all the pH levels of MRS. UP Gouda cheese showed a slight difference in the trend where there was growth in microbial load from day 14 to day 28 and after day 28 the numbers levelled off on MRS at pH 7.5 and pH 8.5. The increase might be due to the fact that strains other than starter strains were able to grow in the cheese. Beuvier, according to Bouton *et al.* (1998), reported a decrease in the starter bacteria population and then an increase after 3 weeks of ripening, attributed to the development of adventitious thermophilic lactobacilli.

Generally, the mean microbial load for collected Gouda cheese samples was high compared to that of the collected Cheddar cheese samples. This might be due to the fact that the Gouda cheese had a lower salt in moisture content and the rate for salt uptake was slower compared to that for Cheddar cheese and this allowed for less inhibition of bacterial growth. Also, the cheeses were tested at different ripening times, and these may contribute to the difference in microbiological counts, since a decrease in microbiological counts is observed during cheese-ripening (Bouton *et al.*, 1998). UP Cheddar and Gouda cheese showed a different trend, whereby UP Cheddar cheese had a high microbial load and low pH but the salt content was high compared to UP Gouda cheese. The difference might mean that milk used to make cheeses with high microbial load was more contaminated (Litopoulou-Tzanetaki *et al.*, 1989). This goes to show that different factors like environmental factors, salt content, moisture content, etc. affects microbial growth.

Media with different pH values were used so as to ensure conditions suitable for the growth of most of *Pediococcus* spp (Garvie, 1986). No growth, or low growth, was observed for all cheese samples on MRS at pH 4.0 compared to that on MRS at the other two pH levels. This is due to the fact that pH 4.0 was less suitable for microbiological growth.

Bhowmik *et. al* (1990) and Tzanetakis & Litopoulou-Tzanetaki (1989) observed the occurrence of pediococci as part of the secondary microflora in cheese, with counts of 10^6 to 10^7/g. During a series of experiments with Cheddar and Gouda cheese samples, pediococci was frequently isolated in large numbers with counts of 1 x 10^6 /g from Gouda cheese.

Of the 1320 isolates, 24 (1.82%) isolates were spherical cells in pairs or tetrads, Gram-positive, non-motile and non-spore-forming, the colonies were smooth and round, and these isolates were classified as presumptive pediococci. Elliott & Mulligan according to Litopoulou-Tzanetaki *et al.* (1989), reported 20 pediococci among 2000 (1%) cultures examined from 20 different cheeses, whereas Litopoulou-Tzanetaki *et al.* (1989) found 4 pediococci isolates out of 390 (1%) cultures. The percentage of *Pediococcus* isolates found in our survey thus compares well with literature reports.

Most of the isolates described as presumptive pediococci were isolated from Gouda cheese (19) compared to Cheddar cheese (five). The difference in number of isolates between Cheddar and Gouda cheese is not easy to explain because literature reports are mostly on pediococci in Cheddar cheese and no reports were found on pedicocci in Gouda cheese. This might be explained by the fact that pediococci grow at different stages of ripening in different cheese samples. Bhowmik *et. al* (1990) reported that the maximum number of pediococci species appeared in a 1 m old cheese, whereafter the number remained nearly constant throughout the ripening period. On the other hand, Dacre (1958) observed that pediococci appeared within 18 d after manufacture of Cheddar cheese.

No *Pediococcus* spp. were isolated on MRS at pH 4.0. Most of the isolates were obtained on MRS at pH 8.5. This might be due to the fact that *P. pentosaceus* and *P. acidilactici* grow well at pH 8.5 (Garvie, 1986) and these are the two spp. which are usually isolated from dairy products (Garvie according to Tzanetakis & Litopoulou-Tzanetaki, 1989).

According to Litopoulou-Tzanetaki (1989), the Gram-positive streptococci were the dominant flora in the early stages of cheese ripening and Gram-positive rods became the dominant flora at about the fourth week of fermentation. Clark & Reinbold (according to Haque *et al.*, 1997) maintained that after the supply of lactose was depleted, numbers of streptococci started to decline and lactobacilli began to predominate. During microscopic examination, it was observed that the Gram-positive, chain-forming cocci (streptococci) predominated in collected cheese samples, and Gram-positive rods became dominant in UP Cheddar and Gouda cheese after day 14 of ripening (results not shown).

No statistically significant correlation was found between the number of presumptive pediococci species isolated and the proximate chemical composition of the cheese samples under investigation. This was due to the fact that a low number (1.82%) of *Pediococcus* spp. was isolated.

5.2 Chemical Analyses

Different trends were observed, most of which compared well with literature reports, for proximate chemical composition of Cheddar and Gouda cheese samples under investigation.

5.2.1. UP Cheddar and Gouda cheese

Results of the experiment showed that there was no significant difference ($p > 0.05$) in, salt content, fat content of the Cheddar cheese and FFA content, whilst the fat content of the Gouda cheese and moisture content showed significant differences ($p < 0.05$) within UP Cheddar and Gouda cheese. On the other hand, the fat content and salt content changed significantly ($p \leq 0.05$) between Cheddar and Gouda cheeses.

The mean moisture content of UP Cheddar and Gouda cheeses ranged between 37.38-35.84 and 40.06-36.80 respectively. Statistically there was no significant difference ($p > 0.05$) between the two types of cheeses but the moisture content decreased significantly during ripening. The decrease in moisture content of both cheeses can be ascribed to moisture losses during ripening, which is a normal process.

Generally, the moisture content of the Gouda cheese was higher compared to the Cheddar cheese samples. The Gouda cheese also had a lower salt in moisture (SM) than Cheddar. According to Fox (1987) an increase in moisture content of the cheese reduces SM. Also, the Gouda cheese had a lower fat in dry matter (FIDM) than the Cheddar which (according to Johnson *et al.*, 1998) allows for a higher moisture content without detrimental effects on the body.

The increase in the mean salt content was not significant ($p \geq 0.05$) during ripening, but there was a significant difference in salt content between the Cheddar and Gouda cheeses. The amount of salt uptake by Gouda cheese depends on the concentration of the brine, the moisture content of the cheese, the length and temperature of exposure, and ratio of surface area to volume of the cheese (Chapman & Sharpe, 1981). The fact that the rate of salt uptake decreased during ripening, may be due to the fact that there was a decrease in salt concentration on the surface of the curd (Fox, 1987).

Salt uptake was greater at the early stages of ripening (day 2 to day 14) and (day 2 to day 28) for Cheddar and Gouda respectively, and then it levelled off. During cheese-making, Cheddar curd is cut into smaller pieces before salting, hence the surface area is relatively larger than in bigger pieces (Gouda cheese-making), therefore salt

absorption occurs fast and the time required to attain the desired level is shorter (Fox, 1987). This explains the fact that the desired salt level was reached faster for Cheddar cheese as compared to Gouda cheese.

Generally, the UP Cheddar samples had higher salt content compared to the Gouda samples. According to Tzanetakis *et al.* (1991), the higher the moisture content the lower the salt content, which is the observed trend between Cheddar and Gouda cheeses whereby the Cheddar had lower moisture content compared to the Gouda cheeses.

During ripening there was a decrease in the mean fat content for UP Cheddar and Gouda cheese ranging between 37.50 – 36.95 and 33.47 – 32.58 respectively. Statistically there was a significant difference ($p \leq 0.05$) during ripening of the Gouda cheese and between the two types of cheeses. The decrease in fat content was not significant ($p \geq 0.05$) for the Cheddar cheese during ripening. The decrease in fat content may be explained by the fact that the salt content of the cheeses increased significantly, thus replasing some of the fat.

Even though Grandison.(1993) and Lolkema & Blaauw (1974), reported that the fat content for Cheddar and Gouda cheeses were in the ranges 33 – 34.4 % and 29.2 % respectively, it was observed that our experimental cheeses had significantly higher values than expected. The reason for these may be the presence of a high amount of fat originating from the cows milk. Also Mistry, Brouk, Kasperson & Martin (2002) recorded the Cheddar cheese fat content in the range 35.55 to 39.5, depending on the type of milk used (i.e. whether from Holstein or Brown Swiss cows).

The mean FFA content for UP Cheddar and Gouda cheeses increased during ripening but these increases, as well as the differences between the two types of cheeses, were not significant. The values ranged between 1.15 – 2.07 and 1.15 – 2.10 for UP Cheddar and Gouda cheeses respectively. The trend showed an increase in FFA content with a decrease in fat content, due to lipolysis.

According to Farkye (1993) the concentration of FFA in bacteria-ripened cheeses like Cheddar and Gouda is low due to the fact that lipases contained in starter and non-starter bacteria has weak lipolytic activity toward milk fat. The results obtained for our experimental cheese was similar to those reported in the literature.

The mean pH differed significantly ($p < 0.05$) between the cheeses but there was no significant difference within the cheeses during ripening. Suprisingly, the pH for both Cheddar and Gouda cheeses remained constant during ripening. During cheese ripening, starter organisms ferment lactose to lactic acid resulting in a decrease in the initial pH of the cheese (Luyten & van Vleit, 1996). Then the metabolism of lactate and formation of alkaline nitrogen compounds by proteolysis result in an overall increase in the pH of most cheese varieties (Farkye, 1993). The pH of Cheddar cheese increases only slightly (~ 0.2 pH units) because the concentration of lactic acid remains high (1.2 – 1.9 %), even after 12 m of ripening (Farkye, 1993).

5.2.2. Cheddar and Gouda cheese collected from the retail outlets and AVI-Africa Expo.

Results of the experiment showed that there was no significant difference ($p > 0.05$) in fat content, moisture content, salt content pH and FFA content within most of the collected Cheddar and Gouda cheeses under investigation. Some exceptions were observed where there were significant differences, as in:

- The p-values for the mean moisture content for samples 4, 5, 7 and 8; and 2, 6, 69 and 70 were in the range $p < 0.004$ and $p > 0.013$; and $p < 0.003$ and $p > 0.044$ respectively.
- The p-values of the mean salt content for samples 8, 17, 21 and 37; and 1, 2 and 6 were in the range $p < 0.0009$ and $p > 0.026$; and $p < 0.002$ and $p > 0.027$ respectively.
- The p-values of the mean fat content for samples 7 and 15; and 6, 9, 10, 52 and 55 were in the range $p < 0.002$ and $p > 0.007$; and $p < 0.001$ and $p > 0.04$ respectively.

- The p-values of the mean free fatty acid content for samples 21, 23, 31, 37 and 39; and 1 and 2 were in the range $p < 0.0001$ and $p > 0.0009$; and $p < 0.003$ and $p > 0.034$ respectively.
- The p-value of the mean pH for sample 21 was in the range $p < 0.0025$ and $p > 0.034$.

One would expect to obtain some significant differences in the composition of the cheeses manufactured by different companies since milk used for cheese-making was obtained from different cows (breeds), which were given different treatments in terms of milking times and techniques, feeding practices and exposure to different geographical conditions. The absence of significant differences may be due to the fact that the milk composition was standardised according to specification in order to attain consistency, and that standard practices for manufacturing and ripening were used for production of these cheeses (Johnson *et al.*, 1998).

CHARPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Results of the present work confirm that pediococci do occur in Cheddar and Gouda cheese, although in minute numbers (1.82%). Most of the presumptive pediococci isolates were obtained from Gouda cheese as compared to Cheddar cheese, one possible reason being the fact that cheeses were sampled at different ripening times.

The average microbiological counts reached figures in the order of 10^6 cfu/g and for collected Gouda cheese samples the counts were higher as compared to that of the collected Cheddar cheese samples. This might be due to the fact that the Gouda cheese had a lower salt in moisture content and the rate for salt uptake was slower compared to that for Cheddar cheese and this allowed for less inhibition of bacterial growth.

Even if there were some differences in chemical composition, the composition of the experimental cheeses were in accordance with those recorded in the literature. No correlation was observed between the number of presumptive *Pediococcus* spp. isolated and the proximate chemical composition of the cheeses. This could be as a result of the small number of isolates obtained.

More definite studies are needed to research the occurrence of pediococci in Gouda cheese, since most of the literature concentrates on pediococci in Cheddar cheese. Also, the importance or role of pediococci in cheese-ripening and its effects on the quality of cheese needs to be researched. Bhowmik & Marth (according to Bouton *et al.*, 1998) stated that pediococci have proteolytic, lipolytic and esterolytic activities and therefore could play a beneficial role during the cheese-ripening process.

CHAPTER 7

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