

CHAPTER 1

1 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Cheddar cheese is one of the most popular cheeses in South Africa. It is widely consumed in its natural state, while smaller quantities are further processed and consumed as pasteurised process cheese (Welthagen and Viljoen, 1999). The cheese is packaged in different forms as blocks, cuts, slices or shreds to suit the needs of the consumer. The shredded product is susceptible to post-contamination by air-borne micro organisms during shredding and it is therefore packaged in modified atmospheres comprising of CO₂ and N₂ (Elliot, Vuilleumard and Emond, 1998).

The shelf life of shredded packaged cheese can be compromised by the growth of moulds (Pitt and Hocking, 1997). Cheese is a good substrate for the growth of certain adaptive fungal species due to its low pH, elevated salt concentration and low water activity (Pitt and Hocking, 1997). Mould growth can occur on cheese during its ripening period or in the distribution chain under refrigerated storage and this can result in a safety and spoilage problem (Taniwaki and Dender, 1992). The moulds can produce mycotoxins, which have potential adverse health effects. In addition the moulds give the cheese an unsightly appearance, objectionable flavour and cause textural changes (Taniwaki and Dender, 1992; Kure, Wasteson, Brendehaug and Skaar, 2001).

Modified atmosphere packaging (MAP) is the enclosure of a food product in gas barrier materials in a gaseous atmosphere which has been changed with the purpose of extending the shelf life while maintaining product quality (Farber, 1991). MAP on its own is not always successful in the prevention of mould growth on cheese because of residual levels of oxygen that may occur in the package as well as the tolerance of some spoilage moulds to low oxygen concentrations and high carbon dioxide concentrations (Hocking, 1994;

Taniwaki, Hocking, Pitt and Fleet, 2001). Oxygen levels of 0.5% or lower are required to prevent the growth of many moulds e.g. *Penicillium commune* and *P. roqueforti* which are commonly found on Cheddar cheese (Hocking, 1994; Taniwaki *et al.*, 2001). The residual O₂ may occur firstly due to the ability of certain foods to trap air especially if the food is spongy or has interstice spaces (Alves, Isabel, Sarantopoulos, Fernandez and Faria, 1996). This will cause improper gas evacuation and the gas flushing will not totally remove all the oxygen. Secondly, each packaging film has a characteristic oxygen permeability that allows the transfer of oxygen from the environment into the package. Thirdly, air could enter the package through improper sealing (Smith, Ooraikul, Koersen, Jackson and Lawrence, 1986). As a result it is thus suggested that MAP should be in conjunction with oxygen scavengers (Alves *et al.*, 1996) albeit at lower levels than would be used in air packaging (Vermeiren, Devlieghere, Beest, Kruijf, and Debevere, 1999).

Knowledge on the fungi that cause spoilage of MAP South African Cheddar is lacking. With information on the principal fungal species causing spoilage in cheese, it would be possible to characterise the species in terms of growth requirements in order to control their growth on cheese (Haasum and Nielsen, 1996). In addition, hygienic measures during production can also be optimised for a longer shelf life of the product (Filtenborg, Frisvad and Thrane, 1996).

1.2 LITERATURE REVIEW

1.2.1 Cheddar cheese

Cheddar cheese is a popular cheese that is consumed the world over and it is widely traded internationally (Muir, Hunter, Banks and Horne, 1996). It originated in England in the town of Cheddar in the 16th century from where it spread to the whole world (Robinson, 1995). It has colour variations from pale to deep yellow while the flavour can be mild and creamy for the mild Cheddar to strong and biting for the mature Cheddar. It is also described as having a slightly nutty walnut flavour (Robinson, 1995; Kosikowski and Mistry, 1997). The differences in texture and flavour of Cheddar arise as a result of the length of the ripening period (Robinson, 1995). Mild Cheddar can be sold at around 3

- 4 months and it has a texture that is close and firm yet pliable and breaks down smoothly when small portions are kneaded between the fingers (Kosikowski and Mistry, 1997). Mature Cheddar is usually 12 - 24 months old, it has an intense flavour and the texture of the cheese at this stage is harder (Robinson, 1995). Cheddar cheese is a rennet coagulated cheese. The production of Cheddar cheese involves the mixture of milk, rennet, micro organisms and salt. These go through the process of coagulation, whey removal, acid production, salt addition and lastly ripening (Beresford, Fitzsimons, Brennan and Cogan, 2001; Kosikowski and Mistry, 1997).

1.2.2 Modified atmosphere packaging

MAP is the enclosure of a food product in gas barrier materials in a gaseous atmosphere, which has been changed with the purpose of extending the shelf life while maintaining product quality (Farber, 1991). MAP usually involves the use of gas mixtures of CO₂, N₂ and O₂ (Farber, 1991). Nitrogen is an inert tasteless gas. It is lipid and water insoluble and is thus used as a filler material to prevent pack collapse especially in products that absorb CO₂. It has no antimicrobial activity of its own and it is thought to mainly work by displacement of oxygen (Smith, Ramaswamy and Simpson, 1990; Farber, 1991). Oxygen is important in the packaging of meat, to maintain haemoglobin in the oxygenated state, it maintains respiration (in fruits and vegetables) and it inhibits the growth of anaerobic organisms in some types of fish and in vegetables (Farber, 1991).

Carbon dioxide is the gas responsible for the bacteriostatic and fungistatic effect on micro organism growth in a modified atmosphere environment. It is both lipid and water soluble (Smith *et al.*, 1990; Farber, 1991) thus when it is applied to a food product, a proportion of it dissolves in the aqueous phase and the fat phase of the food where it then exerts its antimicrobial activity (Devlieghere, Debevere and Impe, 1998). The concentration of CO₂ that dissolves thus influences the growth inhibition of micro organisms in the food (Devlieghere *et al.*, 1998).

1.2.2.1 Mechanism of action of carbon dioxide

According to Daniels, Krishnamurthi and Rizvi (1984) and Farber (1991), the mechanism of action of CO₂ on micro organisms to prevent their growth is thought to be exerted in four ways. Firstly it dissolves into the liquid phase of the system and it is converted to carbonic acid. This causes a drop in the pH thus affecting the metabolic activity of the micro organisms. Secondly CO₂ works by cellular penetration. It changes the structure of the cell membrane leading to an alteration in cell permeability. Thirdly it interferes with the cell metabolism. It has been found to have an inhibitory effect on certain enzymes. Fourthly it displaces some or all of the oxygen available for the growth of micro organisms though this is not a very limiting factor (Daniels *et al.*, 1984).

MAP has been found to extend the shelf life of cheese by reducing the growth rate of spoilage micro organisms e.g. *Pseudomonas* sp. and pathogenic micro organisms e.g. *Listeria monocytogenes* (Farber, Warburton, Gour and Milling, 1990). In addition it slows respiration and delays enzymatic and oxidative changes (Floros, Nielsen and Farkas, 1999). The effectiveness of MAP in cheese thus depends firstly on the removal of O₂ which prevents the growth of aerobic micro organisms and secondly on the inhibitory influence of CO₂ (Farber, 1991).

1.2.2.2 Micro organisms

When a food product is packaged in a modified atmosphere, the gases in the package, then change due to interactions between the food, the package environment and the external environment. The changes depend on the permeation of O₂, CO₂, and water vapour through the package material or through improper sealing and defective areas of the packaging material, the temperature of storage (which influences the permeability of packaging material to different gases), the surface area of the packaging material and the thickness of the packaging material (Skandamis and Nychas, 2002). The choice of film is thus an important part of an MAP system as its diffusion rates to different gases will influence its ability to maintain the desired ratio of gases in the package (Tsigarida and Nychas, 2001). Thus, the key parameters involved in maintaining the quality of MAP

products are the initial quality of the products, composition of the gas atmosphere, packaging material and machine properties and lastly temperature control (Alves *et al.*, 1996).

1.2.2.2 Factors affecting the anti-microbial effect of CO₂

When a food product is packaged in an atmosphere containing CO₂, the CO₂ partly dissolves in the water phase and the fat phase of the food where it exerts its anti-microbial activity (Devlieghere *et al.*, 1998). The amount of CO₂ that dissolves in a food system is dependent on the pH, a_w, temperature, initial CO₂ concentration in the gas state, packaging film permeability and headspace to volume ratio (Lowenadler and Ronner, 1994).

1.2.2.2.1 Temperature

Carbon dioxide is more soluble at lower temperatures thus its effectiveness will decrease at higher temperatures. The storage temperature of a MAP product should thus be kept as low as possible (Farber, 1991). However at a chilling temperature range of 4 - 12° C, the range in solubility is low (Devlieghere *et al.*, 1998).

1.2.2.2.2 Micro organisms

The type and growth phase of the micro organisms influences the action of CO₂ (Smith *et al.*, 1990). It has been found to be more inhibitory towards aerobic spoilage micro organisms and at the exponential growth phase of micro organisms its inhibitory effects are reduced (Brody, 1989). Gram-negative organisms are strongly inhibited by higher CO₂ concentrations while lactic acid bacteria (LAB) which are gram positive are minimally inhibited or unaffected (Farber, 1991; Day, 1992). Yeasts remain unaffected or mildly inhibited by atmospheres containing CO₂ except for non-fermentative yeasts (Farber, 1991; Day, 1992).

Most mould species are sensitive to the inhibitory effects of CO₂ and are generally

inhibited by CO₂ levels above 15% (Farber, 1991; Day, 1992). In order to inhibit mould growth in modified atmospheres, the important factors to consider are the concentration of CO₂ which influences its inhibitory effects and the minimum amount of O₂ needed for growth of the moulds (Hocking and Taniwaki, 1997). Fungi are obligate aerobes, and require oxygen for metabolism and growth function including spore formation and germination (Hocking and Taniwaki, 1997). However, some species e.g. *P. roqueforti* are able to grow at low oxygen concentrations e.g. below 0.5% (Gibb and Walsh, 1980; Magan and Lacey, 1984; Taniwaki, *et al.*, 2001) and high CO₂ concentration above 60% (Taniwaki *et al.*, 2001). At low oxygen concentrations of 0 - 2% fungi grow much slower due to a depression in their lag phase, in addition they have a different appearance (Floros *et al.*, 1999). *Penicillium* species with the exception of *P. roqueforti*, have been found to be greatly inhibited by low oxygen atmospheres of 1% or less O₂ (Yanai, Ishitani and Kojo, 1980 as cited by Hocking and Taniwaki, 1997; Magan and Lacey, 1984).

1.2.2.2.3 Initial CO₂ concentration in the gas state

The growth inhibition of micro organisms in modified atmospheres is also determined by the concentration of CO₂ which dissolves into the product (Devlieghere *et al.*, 1998). The effective CO₂ concentration required in the package headspace is 20 - 60%. As the level of CO₂ increases above 20%, the anti microbial effect against aerobic organisms increases. However, above 60% the anti microbial effect does not increase by much (Kotsianis, Giannou and Tzia, 2002; Farber, 1991).

1.2.2.2.4 CO₂ headspace to volume ratio

The final concentration of CO₂ dissolved in the foodstuff is influenced by the CO₂ headspace to volume ratio. The ratio between the volume of gas and the volume of the food product is usually as high as 2:1 or 3:1. This is necessary to prevent pack collapse because as CO₂ dissolves into the food, less of it will be present on the gas phase which will cause the package to get into closer contact with the product (Sivertsvik, Jeksrud, Vagane and Rosnes, 2003).

1.2.2.2.5 pH of the food

When CO₂ is dissolved in the water present in a food product, it will be hydrated to form carbonic acid (Devlieghere *et al.*, 1998). The carbonic acid then dissociates to form a bicarbonate ion and a hydrogen ion as shown in the equation below.



In a system with low pH/ high amounts of H⁺, the reaction will be pushed to the direction of CO₂ thus less CO₂ will dissolve in the water phase (Devlieghere *et al.*, 1998) while in a system with a high pH, CO₂ will be more soluble and thus exert a greater anti-microbial effect.

1.2.2.2.6 Packaging film permeability

The packaging film should have a low oxygen transmission rate (OTR). An OTR of about 28 cm³/ m²/ 24^h at 23 °C and 75% relative humidity should maintain the desired concentration of gases in the headspace (Tsigarida and Nychas, 2001).

1.2.2.2.7 Water activity (a_w)

The solubility of CO₂ in food increases with higher a_w, due to the presence of higher amounts of unbound water in the food. The CO₂ is thus able to dissolve in greater amounts and exert its antimicrobial effect (Lowenadler and Ronner, 1994).

1.2.3 Active packaging

Active packaging involves the interaction of a packaging material with the food enclosed within it and/or the gaseous atmosphere with the aim of extending shelf life and maintaining quality and safety characteristics (Floros *et al.*, 1999). Active packaging has many forms. It commonly includes substances that absorb oxygen, ethylene, moisture, carbon dioxide, flavour/odours and those which release carbon dioxide, anti-microbial agents, antioxidants and flavours. Other more sophisticated systems are enzyme inhibitors, stabilisers, light blockers/regulators, anti-fogging and anti-sticking agents and temperature sensing or controlling systems (Floros *et al.*, 1999; Vermeiren *et al.*, 1999).

1.2.3.1 Oxygen scavengers

An oxygen scavenger is a product that reacts with and removes oxygen from the environment in which it is placed (Floros *et al.*, 1999). Oxygen scavenging compounds that are used include powdered iron, ascorbic acid, enzymes (glucose oxidase and alcohol oxidase), unsaturated fatty acids (oleic and linolenic acid), photosensitive dyes that undergo oxidation, immobilized yeast on a solid material and ethylenically unsaturated hydrocarbons (Floros *et al.*, 1999; Vermeiren *et al.*, 1999).

The absence of oxygen from a packaging environment prevents deleterious effects due to oxidation and growth of moulds e.g. *P. commune* and aerobic spoilage bacteria e.g. pseudomonads, *Staphylococcus aureus*. It also prevents oxidation of food components thus preventing browning, rancidity of fats and oils and their related products (Floros *et al.*, 1999; Vermeiren *et al.*, 1999).

Oxygen absorbers have found a wide application in a wide variety of foods including cheese, dried fruit, fruit, coffee, fresh and pre cooked pastas, nuts bakery products e.g. bread, cakes, pizza crust, pastries, cookies, meat products e.g. ham, sausage, salami and dried beef jerky. In addition, oxygen scavengers are used in beverages e.g. beer, wine, soft drinks to prevent loss of flavour due to oxidation (Rooney, 1995; Floros *et al.*, 1999; Vermeiren *et al.*, 1999).

Oxygen scavengers can be used either in conjunction with MAP or vacuum packaging or on their own in air packaging. They absorb the residual oxygen that remains after packaging in addition to absorbing the oxygen that permeates through the packaging material (Floros *et al.*, 1999). Oxygen scavengers commonly used are in the form of a sachet that is attached to the interior of the packaging material. The sachets are sometimes not well accepted by consumers. Most consumers do not like the presence of a foreign material in their food package, as they prefer their foods to appear natural. In addition, there is the risk of ingestion of the sachet by children or pets, or contamination

of the food by the sachet tearing (Smith *et al.*, 1986; Smith, Hoshino and Abe, 1995). Another disadvantage of oxygen scavenging sachets is that in products that absorb CO₂, a vacuum can be created in the package, leading to the packaging film coming into closer contact with the product. This creates a stagnation of air in pockets around the product. In these pockets, the concentration of O₂ can rise due to permeation through the packaging film leading to proliferation of micro organisms (Smith *et al.*, 1986). Thus oxygen scavenging sachets need to be used to package products where there will be a free flow of air in the package with the package loosely wrapped around the product. Lastly, oxygen scavenging sachets cannot be used for liquid food.

Oxygen scavengers can also be incorporated into the packaging structure of materials. Low molecular weight ingredients may be dissolved or dispersed in a packaging plastic or the plastic may be made from a polymer scavenger (Rooney, 1995). This enables the oxygen scavenger to have greater contact with the gaseous environment thus overcoming the disadvantages of the oxygen scavenger sachets. However, Day (1998) according to Vermeiren *et al.*, (1999), stated that oxygen scavenging films work at a slower pace and have a lower capacity than iron based oxygen scavenger sachets. Another important consideration with oxygen scavenging films is that the film should have a low oxygen permeability otherwise the scavenger will become saturated and stop absorbing oxygen (Abe and Kondoh, 1989).

1.2.4 Packaging of cheese under modified atmosphere conditions

When cheese is packaged in a modified atmosphere the optimal gas composition used depends on the characteristics of the cheese (Alves *et al.*, 1996; Elliot *et al.*, 1998; Fandos, Sanz and Olarte, 2000). For semi-soft and hard cheeses either whole, sliced or shredded, the optimal gas conditions are minimal O₂ along with a controlled level of CO₂ (Floros *et al.*, 1999). It is important that the levels of CO₂ are controlled because for certain cheeses, high levels of CO₂ have been found to impart off flavours to the cheese (Chen and Hotchkiss, 1991; Manheim and Soffer, 1996; Fandos *et al.*, 2000). This is

because, when CO₂ applied to a food some of the gas dissolves and exists as carbonic acid, which can cause a sour flavour due to the rise in acidity (Chen and Hotchkiss, 1991). In addition, if the levels of CO₂ are too high without N₂ in the package, the CO₂ can be absorbed by the product leading to pack collapse. However, if the levels of CO₂ are too low, the bacteriostatic and fungistatic effect of CO₂ will not be experienced (Floros *et al.*, 1999). For certain soft cheeses e.g. cottage cheese, levels of up to 100% CO₂ are used and have been found to give the longest shelf-life and best sensory qualities as compared to those packaged in air, combinations of N₂ and CO₂ or N₂ alone (Maniar, Marcy, Bishop and Duncan, 1994). In studies of cheese packaged under MAP, on the whole, the shelf life has been found to increase compared to conventional packaging, with higher levels of CO₂ giving longer shelf lives (Alves *et al.*, 1996; Elliot *et al.*, 1998).

Cameros cheese (a fresh cheese from Spain) has been packaged in modified atmospheres with a resulting increase in shelf life (Fandos *et al.*, 2000). The cheese was packaged under conditions of 100% CO₂, 50% CO₂ / 50% N₂, 40% CO₂ / 60% N₂, 20% CO₂ / 80% N₂, in air (control) and under vacuum. Higher levels of CO₂ were found to be more inhibitory to the micro organisms (psychrotrophs, moulds and yeasts) involved in the spoilage of this cheese and the microbial populations took longer to reach spoilage levels due to an increase in their lag phase. They found that the growth of mesophiles and psychrotrophs were retarded in the cheese in all the MAP combinations and after 28 days of storage at 4 °C, the populations reached were 1.5 - 2 log units lower than the control. Moulds and yeasts were not detected in the cheese in any of the modified atmospheres or in the vacuum packaged cheese indicating that CO₂ had an inhibitory effect on the mould and yeast growth. However, the mould and yeast growth in the control cheese was also low indicating that the cheese had a low initial contamination of these micro organisms. Vacuum packaging was however found to be comparable to conventional air packaging as no significant retardation of the microbial population was noted. Although the 100% CO₂ atmosphere resulted in the cheese with the best microbial quality, the sensory scores were the lowest based on taste scores after seven days. The packaging atmospheres with lower levels of CO₂ resulted in better sensory scores which led to the 50% N₂ / 50% CO₂,

and 40% CO₂ / 60% N₂ atmospheres being found to be the most effective packaging atmospheres that would increase the shelf-life of the cheese while not affecting the sensory characteristics (Fandos *et al.*, 2000).

In a similar study on the stability of shredded Mozzarella cheese in modified atmospheres, Elliot *et al.*, (1998) found that CO₂ levels greater than 75% were most suitable for packaging the cheese. The cheese was packaged in 6 modified atmospheres of 100% CO₂, 100% N₂, 10% CO₂ / 90% N₂, 25% CO₂ / 75% N₂, 50% CO₂ / 50% N₂ and 75% CO₂ / 25% N₂. They found that atmospheres containing CO₂ and vacuum packaging, retarded the growth of *Staphylococci*, yeasts and moulds in the cheese. However, MAP was not as effective in inhibiting the growth of LAB (which are facultatively anaerobic), mesophiles and psychrotrophs. The investigators partly attributed the lack of inhibition to the high temperature of storage i.e. 10 °C ± 1 since the solubility of CO₂ decreases with increasing temperature. They also concluded that mesophilic bacteria comprise a wide group, thus the action of CO₂ on this group may not have been effective on certain species. Under conditions of 100% CO₂ the growth of moulds and yeast was totally inhibited in the cheese for 8 weeks, in addition they found that the high concentrations of CO₂ did not cause undesirable changes in the flavour as the cheeses packaged under 100% CO₂ had the best sensory scores.

In an investigation on the shelf life extension of sliced Mozzarella cheese in modified atmospheres, Alves *et al.*, (1996) packaged the cheese in MAP combinations of 100% CO₂, 100% N₂ and 50% CO₂ / 50% N₂, vacuum packaging and packaging in an air (control) and stored at 7 °C ± 1. It was found that greater levels of CO₂ inhibited the growth of aerobic psychrotrophs, moulds and yeasts. In the cheese packaged in the 100% CO₂ packaging atmosphere, it was found that the growth of moulds was totally inhibited for 58 days, however after 60 days the sensory characteristics of the cheese started to deteriorate due to sensory degradations inherent to the product. The cheese packaged in the 100% CO₂ atmosphere also had the best sensory attributes due to total inhibition of

yeast and mould development Under 100% N₂ and 50% CO₂ / 50% N₂ packaging conditions, the deterioration in the sensory attributes of the cheese was emphasised when the mould and yeast counts were highest. 100% CO₂ increased the product shelf life by 51 days, 50% CO₂ / 50% N₂ increased it by 31 days while N₂ was found to have little benefit as only a minor shelf increase was noted and it was found to be comparable to the conventional air packaging system. Each package registered an initial oxygen content of 0.8 - 1.4% after gas flushing and packaging which the investigators attributed to the air between the cheese slices and air that was trapped in the expanded polystyrene (EPS) packaging tray (Alves *et al.*, 1996).

Maniar *et al.*, (1994) found that cottage cheese packaged under 100% CO₂ had the best sensory and microbiological qualities as opposed to the cheese packaged in air or other combinations of 75% N₂ / 25% CO₂ and 100% N₂. All the modified atmospheres inhibited psychrophiles and mesophiles with the 100% N₂ atmosphere exhibiting the least inhibition of the 3 and the 100% CO₂ atmosphere having the greatest inhibition. In a similar investigation on the shelf life extension of cottage cheese by 100% CO₂, Mannheim and Soffer (1996) found that yeast and mould counts were reduced compared to the control cheese packaged in air. However, at a ratio of CO₂ to cheese (v/v) in the package of 1:2.5, the cheese developed a sour taste. At lower ratios of 1: 4.2 and 1: 3.1, the taste of the cheese was unaltered and the same as the control. Moir, Eyles and Davey (1993) similarly found that when creamed cottage cheese was packaged in an atmosphere of 100% CO₂ at a ratio of cheese to CO₂ of 1: 1 the cheese developed a fizzy taste and the texture was altered. In addition part of the CO₂ dissolved in the cheese which caused a partial vacuum to be formed resulting in the collapse of the polystyrene cups in which the cheese was packaged.

MAP has been found to inhibit pseudomonads, which are the micro organisms that commonly cause spoilage of cottage cheese (Moir *et al.*, 1993). These investigators inoculated pseudomonads in cottage cheese, incubated the cheese at 5 °C and 10 °C and

studied their growth patterns. They found that the lag phases of growth were increased and the growth rates were slower than the control. In addition, the inhibitory effect of CO₂ was more noticeable at 5 °C due to the greater solubility of CO₂ at lower temperatures (Moir *et al.*, 1993).

Rosenthal, Rosen, Bernstein and Popel, (1991), investigated the shelf life extension of quarg cheese in a modified atmosphere composed of 67.1% CO₂ / 26.3% N₂ / 6.6% O₂ and stored at 4 °C. It was found that CO₂ completely prevented the growth of yeasts and moulds as the levels remained below 1 log cfu/g for 67 days. After 67 days when the modified atmosphere was replaced by air until the 95th day, the mould counts then increased to 6.2 log cfu/g. A parallel investigation with N₂ packaged cheese did not show any inhibitory effects on mould growth. In addition the air packaged cheeses reached mould counts of ≥ 6.7 log cfu/g within 42 days.

1.2.5 Moulds

Moulds can be described as filamentous microscopic fungi (Hocking, 1997). They reproduce by the means of spores. These spores can easily be spread in the air and thus contaminate a whole factory environment (Hocking, 1997). When a fungal spore (which is about 2 - 5µm in diameter) germinates, a hyphae grows which branches continuously as it elongates to form a mass which is referred to as a mycelium. This mycelium is what is visible on the surface of foods as mould spoilage (Hocking, 1997).

1.2.5.1 Moulds in cheese spoilage

According to Filtenborg *et al.*, (1996) a very limited group of fungi called mycobiota is responsible for the spoilage of individual types of food. Individual mycobiota for different foods have also been found to differ greatly. Cheese parameters are restrictive in the range of species that can grow on them and produce spoilage. This is as a result of the combination of intrinsic, extrinsic and processing conditions of cheese, which gives a specific habitat for the range of species that can grow on cheese (Filtenborg *et al.*, 1996;

Pitt and Hocking, 1997). This impacts the food industry in that it has the potential to simplify actions to prevent and control contamination of cheese. In addition, hygienic measures during production can also be optimised for a longer shelf life of the product (Filtenborg *et al.*, 1996).

Different cheese varieties, are commonly made by concentrating the casein and fat contents of milk and thus they present a selective environment for the growth of micro organisms (Kosikowski and Mistry, 1997; Beresford, *et al.*, 2001). Most cheeses have a high content of protein, fat and volatile fatty acids combined with a low level of fermentable carbohydrates due to the fermentation of lactose to lactic acid resulting in a low pH and a low water activity due to the high salt content (Bullerman and Olivigni, 1974; Hocking, 1994). In vacuum packaged and MAP cheese, the reduced O₂ content and high CO₂ in the packaging environment and cheese also inhibit microbial growth (Hocking, 1994). Furthermore, most cheeses are refrigerated which reduces the range of micro organisms that can grow in them (Hocking, 1994). Thus spoilage will generally be caused by moulds that are psychrotolerant and can grow in low O₂ atmospheres (Pitt and Hocking, 1997).

Fungal contamination on cheese may originate from the air, equipment or the smear for smear ripened cheeses. Fungi are constantly present in the cheese making and storage environment and as such they will also commonly be found on the cheese (Zerfiridis, 1985; Basilico, de Basilico, Chiericatti, and Vinderola, 2001; Kure, Skaar and Brendehaug, 2004). Moulds are commonly found on cheese during the ripening process and cold storage. Only in mould ripened cheeses e.g. Roquefort cheese, Camembert, Brie and Gorgonzola is the presence of selected moulds desirable (Lopez-Diaz, Santos, Prieto, Garcia-Lopez, and Andres, 1996). Preservatives e.g. sorbate, can be added to the milk for cheese manufacture to inhibit mould growth but they impart undesirable flavour changes (Chen and Hotchkiss, 1991). In addition, a number of fungal species e.g. *P. roqueforti*

can grow in the presence of sorbate and cause a defect called kerosene flavour (Pitt and Hocking, 1997).

1.2.5.1.1 Mould species isolated from various cheeses

Production of cheese involves the mixture of milk, rennet, micro organisms and salt. These go through the process of coagulation, whey removal, acid production, salt addition and lastly ripening for rennet-coagulated cheeses (Beresford *et al.*, 2001). Manipulation in the blend of ingredients, variations in the processing parameters and the cheese microflora, play an important part singly or jointly or later in conjunction with ripening in determining the unique characteristics of each type of cheese (Beresford *et al.*, 2001; Kosikowski and Mistry, 1997). Due to the different characteristics of each kind of cheese, the mould species isolated from different types of cheese have been found to differ (Table 1.1).

Basilico *et al.*, (2001) studied thread mould spoilage of a vacuum maturing hard cheese similar to Cheddar cheese. The thread mould spoilage developed as dark stains on the surface of the cheese during ripening. It was found that the predominant mould was *Phoma glomerata* at 63.8% of all the isolates. *Penicillium* species made up 18.1% of the total isolates with *P. commune* as the predominant *Penicillium* species at 8.5% of *Penicillium* isolates. Other species included *Mucor hiemalis*, *Geotrichum candidum* and *Monilliella suaveolens*.

In a fungal examination to the genus level of mould species on Arzua (a soft cows milk cheese from Spain) the major genera causing spoilage were found to be *Penicillium* at 25%, *Rhizopus* at 24%, *Geotrichum* at 17%, *Cladosporium* at 16% and *Aspergillus* at 11% of all isolates (Fente-Sampayo, Vazquez-Belda, Franco-Abuin, Quinto-Fernandez, Rodriguez-Otero, and Cepeda-Saez, 1995).

Table 1.1 Incidence of mould genera isolated from various cheeses

Type of Cheese and its origin	INCIDENCE								Reference
	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Cladosporium</i>	<i>Fusarium</i>	<i>Mucor</i>	<i>Phoma</i>	<i>Geotrichum</i>	Other	
Cheddar substitute (Argentina)	■				■	○	■	■	Basilico <i>et al.</i> , (2001)
Arzua cheese (Spain)	■	■	■	■	■	■	■	○	Fente - Sampayo <i>et al.</i> , (1995)
Norvegia (shops Norway)	○		■		■	■		■	Kure and Skaar (2000)
Jarlsburg (shops Norway)	○				■		■	■	Kure and Skaar (2000)
Norvegia (factories, Norway)	○	■						■	Kure <i>et al.</i> , (2001)
Jarlsburg (factories, Norway)	○		■		■				Kure <i>et al.</i> , (2001)
Various cheeses (Europe)									Lund, Filtenborg, Frisvad and Thrane (1995)
packaged (shops)	○	■						■	
in factories	○	■	■	■	■			■	
Vacuum maturing Cheddar (Australia and New Zealand)	■	■	○	■	■		■	■	Hocking and Faedo (1992)
Van Herby (Turkey)	○	■					■	■	Kivanc (1990)
Kasar (Turkey) shops	○	■			■			■	Aran and Eke (1986)
Warehouses	○	■			■			■	
Moldy surplus cheeses (U.S.A.)	○								Tsai, Liewen and Bullerman (1988)
Teleme (Greece) Domestic	○	■	■	■	■			■	Zerfiridis (1985)
Imported	○	■	■	■	■			■	
Gouda and Edam (Netherlands)								■	Northolt, Egmond, Soentoro and Deiji (1980)
In shops and households	○	■	■		■				
Gouda and Edam (Netherlands)								■	Northolt <i>et al.</i> (1980)
In warehouses 1976-1977	○	■			■				
Gouda and Edam (Netherlands)									Northolt <i>et al.</i> (1980)
In warehouses 1977-1978	■	○							
Processed (Netherlands)	○	■						■	Northolt <i>et al.</i> (1980)

○ Present as ≥70% of all isolates

■ Present as ≤ 30% of all isolates

In a study on fungal growth occurring on 2 vacuum packaged semi-hard cheeses from Norway called Norvegia and Jarlsburg, Kure and Skaar, (2000) found that on both cheeses packed at the consumer level the most frequent contaminant was found to be *P. roqueforti* subspecies *roqueforti* at 25.8% and 39.5% of all isolates on Norvegia and Jarlsberg cheeses respectively. Other frequently isolated species were *P. commune* at 15.7% and 15.8%, *P. palitans* was at 15.1% and 6.68% and *P. solitum* at 13.2% and 19.1% all on Norvegia and Jarlsberg cheeses respectively. *Penicillium* was found to be the dominant genus making up 98.1% of isolates on Norvegia cheese and 89.2% of the isolates on Jarlsberg cheese (Kure and Skaar, 2000). Other genera identified included *Mucor*, *Phoma*, *Epicoccum*, *Alternaria*, *Cladosporium*, *Aureobasidium* and *Ulocladium*.

In a study on Norvegia and Jarlsberg cheese that were visibly mouldy at factory level, Kure *et al.*, (2001) found that *G. candidum* was the most common species on Jarlsberg cheese at 26.3% of all the isolates, while other dominant species included *P. palitans* and *P. roqueforti* ss. *roqueforti*. On Norvegia cheese the most common species was *P. commune* at 29.5% of all isolates and the other dominant species were *P. solitum*, *P. palitans*, and *P. roqueforti* ss. *roqueforti*. *Penicillium* species were the most common making up 84% of the total isolates on both cheeses. Kure *et al.*, (2001) noted that the fungi identified differed between the factory level and the consumer level. This indicated that contamination could have occurred during the cutting up of the cheese into smaller retail packages and that the refrigerated temperature of cold storage may have been more selective to the growth of *P. roqueforti* ss. *roqueforti* which was dominant on the cheese at the consumer level (Kure *et al.*, 2001).

Lund *et al.*, (1995) carried out a study on hard, semi hard and semi soft cheeses from all over Europe. The genus *Penicillium* was predominant comprising 91% of the isolates with *P. commune* as the most common species comprising 42% of the total isolates. The general associated mycoflora on the cheese was found to be *P. commune*, *P. nalgiovense*, *P. verrucosum*, *P. atramentosum*, *P. solitum*, *P. chrysogenum*, *P. roqueforti*, *P. crustosum*, *Aspergillus versicolor* and *P. echinulatum*. The other species were infrequent indicating that the origin of the cheese was greatly independent from the associated mycoflora (Lund *et al.*, 1995).

On Turkish Van herby and Van herby pickled white cheese, the *Penicillium* genus was the majority of the mycoflora at 65% of the total isolates (Kivanc, 1990). *P. roqueforti* was the highest of all the species identified on both types of cheese and it comprised 27.7% of total isolates on Van herby pickled white cheese and 38% of total isolates Van herby cheese. *P. verrucosum* var. *cyclopium* was the next dominant species at 20.1% of total isolates on Van herby pickled white cheese and at 10% of all isolates on Van herby cheese. The other species identified included *P. frequentans*, *As. flavus*, *As. niger*, *G. candidum*, *Trichoderma* sp. and *Mucor* sp. (Kivanc, 1990).

Hocking and Faedo, (1992) studied thread mould spoilage of vacuum maturing Cheddar cheese in Australia. Thread mould spoilage refers to fungal growth in the folds and creases of plastic bags used to wrap cheese and it is mainly associated with free whey drawn from the cheese blocks during vacuum packaging (Hocking and Faedo, 1992). It was found that *C. cladosporioides* was the most common species forming 35% of the total isolates while the *Penicillium* species formed 22% of the total isolates. The other commonly isolated species were *C. herbarum*, *P. commune*, *Ph. glaburum* and a *Phoma* species.

In a study on fungi causing spoilage of Brazilian cheese Taniwaki and Dender (1992), found that *Penicillium* was predominant in different brands of hard Parmesan cheese at 84 - 99% and in Prato cheese it was found to be 99% of total isolates.

Tsai *et al.*, (1988) found that 4 moulds of the genus *Penicillium* contaminated processed American and Cheddar cheese and a cream cheese substitute. The most dominant mould was *P. roqueforti* at 67% of total isolates followed by *P. cyclopium* at 18%, *P. viridicatum* at 12% and *P. crustosum* at 3%.

Aran and Eke (1987) investigated mouldy retail samples of a hard traditional Turkish cheese called Kasar in shops and households and found that *Penicillium* was the major genus at 87% of the total isolates from the cheese in the shops and at 96% of the total isolates from the cheese in the warehouses. Of the species isolated from the cheese in the shops, the most dominant species was *P. verrucosum* var. *cyclopium* at 45% of the total isolates. *P. roqueforti* was found to be the next dominant species at 15%. While in the cheese in the warehouses, the major species isolated was *P. verrucosum* var.

cyclopium at 48% of the total isolates followed by *P. verrucosum* var. *verrucosum* at 9% of the total isolates (Aran and Eke, 1987).

Zerfiridis (1985) examined domestic and imported samples of Teleme cheese in Greece and revealed that *Penicillium* was the predominant genus at 78% of total isolates on imported cheeses in Greece and 85% of total isolates on domestic cheeses. *Aspergillus* was the next dominant genus at 3.8% of all isolates on domestic cheeses and 3.9% of all isolates on imported cheeses. The other genera isolated included *Mucor*, *Cladosporium*, *Fusarium* and *Alternaria*, which were encountered in less than 10% of the total isolates. A study was also done on the occurrence of contaminating moulds at various sections of the cheese making plants. It was discovered that *Penicillium* species were dominant in all in the sections. Other genera identified were *Mucor*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Alternaria*. However in the packaging section, only *Penicillium* species were identified. This indicated that low temperatures in that section may have favoured the growth of *Penicillium* species over other species.

Northolt *et al.*, (1980) studied fungal growth on Edam and Gouda cheeses found in households, shops, warehouses and in factories. They found that *P. verrucosum* var. *cyclopium* was the major species at 79.3% of all isolates in cheese moulded in shops and at 57.9% of isolates in cheese moulded in households. On the cheese in shops, other major species included *As. repens* at 12%, *P. roqueforti* at 10%, *P. verrucosum* var. *verrucosum* at 9% of all isolates. While on the cheese moulded in the households, other major species included *P. roqueforti* at 15% and *Rhizopus nigricans* at 10% of all isolates. *Penicillium* species were the majority both on the cheeses in the shops and households. The same researchers also noted that during the ripening period of the cheese in the warehouses, the mycoflora changed during the early and late ripening stage. In the early ripening stage of the cheese *Penicillium* isolates were predominant with *P. verrucosum* var. *cyclopium* being the predominant species at 54.4%. However, in the latter stage of ripening *As. versicolor* became the major species at 53.5% of all isolates.

Bullerman (1980) investigated moulds found on hard, semi hard, and soft domestic US cheeses and imported cheeses. The majority of the species identified were

Penicillium species at 86% of all isolates in domestic cheeses and 80% of all isolates in imported cheeses. Other genera identified included *Cladosporium* at 2.7% and 4.6%, *Fusarium* at 1.2% and 0.6%, *Aspergillus* at 2.3% and 5.4% all on domestic and imported cheeses respectively.

1.4.2 Modified atmospheres will affect the composition of the mycoflora of cheese

The predominant moulds causing spoilage of Swiss cheese were found to be from the genus *Penicillium* at 87% of the isolates (Bullerman, 1976). The other genera were un-named and formed 13% of the total isolates. One isolate of *As. flavus* was also identified. The isolates of the *Penicillium* genus were found to be able to grow at 5 °C, which indicated that they were the major moulds of concern in refrigerated cheeses.

Bullerman and Olivigni (1974) found that *Penicillium* isolates were the highest of the mould species isolated from Cheddar cheese. The other genera identified were found to be *Alternaria*, *Fusarium*, *Hormodendrum*, *Oidium*, *Monilia*, *Stachybotrys* and *Chaetopsis*. The numbers isolated were not stated.

1.3 OBJECTIVES

The overall objective of the study was to determine the effect of MAP with different gas mixtures with and without oxygen scavengers on the growth and survival of moulds on shredded Cheddar cheese.

- 1.3.1 To investigate the microbiological quality and shelf life extension of shredded Cheddar cheese packaged in different modified atmospheres with and without oxygen scavengers included in the packaging material.
- 1.3.2 To identify the mould species present on South African shredded Cheddar cheese packaged in modified atmospheres with and without oxygen scavengers included in the packaging material before and after 16 weeks of storage.

1.4 HYPOTHESES

- 1.4.1 Since the majority of moulds are obligate aerobes, if oxygen is totally excluded from the packaging environment, their growth will be inhibited and the shelf life of the cheese extended.
- 1.4.2 Modified atmospheres will affect the composition of the mycoflora of cheese during storage by selecting for mould species able to grow and survive in a particular modified atmosphere because selected mould species are able to survive at reduced oxygen and high carbon dioxide levels and others are not.

Submitted to the International Journal of Food Science and Technology

Abstract

Shredded Cheddar cheese samples were packaged with each of 3 atmospheres (an air, 75% CO₂ / 25% N₂, 75% O₂ / 25% CO₂ / 25% N₂) combined either with an oxygen scavenger or control film. The samples were stored for 16 weeks at 4 °C and analysed for lactic acid bacteria (LAB), yeast and mould counts. In addition, the time taken for the first visible signs of mould growth on the surface was recorded. Mould counts in the cheese were influenced by the type of atmosphere or packaging film. The cheese packaged in the 75% CO₂ / 25% N₂ atmosphere combined with the oxygen scavenger film had the lowest mould counts while the cheese packaged in the air atmosphere combined with the control film had the highest yeast and mould counts along with the shortest shelf life i.e. 4 weeks based on visible mould growth.

Key words: Shredded Cheddar cheese, oxygen scavengers, modified atmospheres, packaging, moulds.