

Microbiological quality of shredded Cheddar cheese packaged in modified atmospheres

DECLARATION

I declare that the dissertation herewith submitted for the degree MSc (Agric) Food Science and Technology at the University of Pretoria, has not previously been submitted for a degree at any other university or institution of higher education.

By

Evonne Laura Adhiambo Oyugi

Submitted in partial fulfilment of the requirements for the degree

MSc (Agric) Food Science and Technology

in the

Faculty of Natural and Agricultural Sciences

University of Pretoria

Pretoria

November 2004

17/11/04
11/11/2004
11/11/2004

DECLARATION

I declare that the dissertation herewith submitted for the degree MSc (Agric) Food Science and Technology at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education.

Secretly I would like to dedicate this dissertation to my mother who supported me for the duration of my studies.

DEDICATION

This dissertation is firstly dedicated to my Lord and God who kept me going through the tough times.

Dr. E. M. M. M. for her guidance, advice, patience and encouragement during the study.

Secondly I would like to dedicate this dissertation to my mother who supported me for the duration of my studies.

Marratle Tluter at the department of Microbiology and Plant pathology for her assistance and guidance with the identification of moulds.

The post-graduate students of the department of Food Sciences, for their cooperation, encouragement and help during the course of the study.

The International Foundation for Science for funding this project.

Franklin SA Pty. for providing the chives used during the project and Liquid N₂ CryoCool for donating the nitrogen scavenging and control film.

ACKNOWLEDGEMENTS

I would sincerely like to thank:

Dr. E. M. Buys for her guidance, advice, patience and encouragement during the course of the study.

Marriette Trutter at the department of Microbiology and Plant pathology for her assistance and guidance with the identification of moulds.

The postgraduate students of the department of Food Science, for their constant encouragement and help during the course of the study.

The International Foundation for Science for funding this project.

Parmalat SA Pty. for providing the cheese used during the project and Liquid Air Cryovac for donating the oxygen scavenging and control film.

ABSTRACT

MICROBIOLOGICAL QUALITY OF SHREDDED CHEDDAR CHEESE PACKAGED IN MODIFIED ATMOSPHERES

By Evonne Laura Adhiambo Oyugi

Supervisor: Dr. E. M. Buys

Department: Food Science

Degree: MSc (Agric) Food Science and Technology

Modified atmosphere packaging (MAP) is a technology commonly used to extend the shelf life of cheese. However, 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 a result, oxygen scavengers in a laminate packaging film were combined with MAP in this study to lower the residual oxygen conditions in shredded Cheddar cheese packages. The study investigated the microbiological quality, shelf life extension and mycoflora of shredded Cheddar cheese packaged in different modified atmospheres with and without oxygen scavengers included in the packaging material.

Shredded Cheddar cheese samples were packaged with each of 3 atmospheres (air, 80% CO₂ / 17% N₂ / 3% O₂, 73% CO₂ / 27% N₂) combined either with an oxygen scavenging or control film. The samples were stored for 16 weeks at 5 ± 1°C and analysed for lactic acid bacteria (LAB), yeast and moulds counts. In addition, the time taken for the first visible signs of mould growth on the cheese was noted. Mould isolates from the cheese were identified initially (0 weeks) and at 16 weeks.

The LAB counts in the cheese were unaffected by the gaseous atmosphere or packaging film. The cheese packaged in the 73% CO₂ / 27% N₂ atmosphere combined with the oxygen scavenging film had the lowest mould counts and the cheese in this packaging

combination took 12 weeks to develop visible mould growth along with the cheese packaged in the 73% CO₂ / 27% N₂ atmosphere in the control and 80% CO₂ / 17% N₂ / 3% O₂ atmosphere in the oxygen scavenging film. The cheese packaged in the air atmosphere combined with the control film had the highest yeast and mould counts and took 4 weeks to develop visible mould growth.

The genus *Penicillium* predominated initially (week 0) at 41% of all mould isolates on the shredded Cheddar cheese. At 16 weeks, the mycoflora differed according to the treatment in which the cheese was stored and the species isolated were fewer in the different treatments indicating that selection took place. In addition, the number of species isolated from the shredded Cheddar cheese packaged in the film with oxygen scavengers were fewer than the isolates from the cheese packaged in the control film which indicated that the lower oxygen conditions further restricted the mould growth.

The results of the study indicate that the 73% CO₂ / 27% N₂ atmosphere in combination with the oxygen scavenging film, resulted in the cheese with the best microbiological quality. In addition, it had the fewest mould species causing spoilage indicating that the atmosphere was restrictive to the range of species causing spoilage. While in general, the use of the oxygen scavenging film in combination with MAP was more effective than the control film in combination with MAP against mould growth.

TABLE OF CONTENTS

DECLARATION	i
DEDICATION	ii
ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
LIST OF TABLES	ix
LIST OF FIGURES	x
1 INTRODUCTION AND LITERATURE REVIEW	1
1.1 Introduction	1
1.2 Literature review	2
1.2.1 Cheddar cheese	2
1.2.2 Modified atmosphere packaging	3
1.2.2.1 Mechanism of action of carbon dioxide	4
1.2.2.2 Factors affecting the anti-microbial activity of carbon dioxide	5
1.2.2.2.1 Temperature	5
1.2.2.2.2 Micro organisms	5
1.2.2.2.3 Initial CO ₂ concentration in the gas state	6
1.2.2.2.4 CO ₂ headspace to volume ratio	6
1.2.2.2.5 pH of the food	7
1.2.2.2.6 Packaging film permeability	7
1.2.2.2.7 Water activity of the food	7
1.2.3 Active packaging	7
1.2.3.1 Oxygen scavengers	8
1.2.4 Packaging of cheese under modified atmosphere conditions	9
1.2.5 Moulds	13
1.2.5.1 Moulds in cheese spoilage	13
1.2.5.1.2 Mould species isolated from various cheeses	15
1.3 Objectives	20
1.4 Hypotheses	21

2 RESEARCH.....	22
2.1 MICROBIOLOGICAL QUALITY OF SHREDDED CHEDDAR CHEESE PACKAGED IN MODIFIED ATMOSPHERES WITH OXYGEN SCAVENGERS.	24
2.1.1 Introduction.....	25
2.1.2 Materials and methods	25
2.1.2.1 Packaging materials	25
2.1.2.2 Packaging treatments of shredded Cheddar cheese	25
2.1.2.3 Storage period of shredded Cheddar cheese	25
2.1.2.4 Microbiological analysis of shredded Cheddar cheese	26
2.1.2.5 Water activity of the shredded Cheddar cheese samples	26
2.1.2.6 Visual inspection of the shredded Cheddar cheese for mould growth	26
2.1.2.7 Statistical analysis.....	26
2.1.3 Results.....	27
2.1.3.1 Oxygen scavenging activity of the laminate film	27
2.1.3.2 Shredded Cheddar cheese packaged in air + control film (treatment 1) and air + oxygen scavenging film (treatment 4) for 16 weeks at 5 ± 1 °C ..	28
2.1.3.3 Shredded Cheddar cheese samples packaged in 80% CO ₂ / 17% N ₂ 3% O ₂ + control film (treatment 2) and 80% CO ₂ / 17% N ₂ / 3% O ₂ + oxygen scavenging film (treatment 5) stored for 16 weeks at 5 ± 1 °C.....	32
2.1.3.4 Shredded Cheddar cheese samples packaged in 73% CO ₂ / 27% N ₂ + control film (treatment 3) and 73% CO ₂ / 27% N ₂ + oxygen scavenging film (treatment 6) and stored at 5 ± 1 °C for 16 weeks	34
2.1.4 Discussion	36
2.1.5 Conclusion	40
2.2 CHARACTERISATION OF MOULDS ON SOUTH AFRICAN CHEDDAR CHEESE PACKAGED IN MODIFIED ATMOSPHERES WITH AND WITHOUT OXYGEN SCAVENGERS	41
2.2.1 Introduction.....	42

2.2.2 Materials and methods	44
2.2.2.1 Packaging materials	44
2.2.2.2 Packaging treatments of shredded Cheddar cheese	44
2.2.2.3 Storage period of shredded Cheddar cheese	44
2.2.2.4 Mould isolation	44
2.2.2.5 Mould identification.....	44
2.2.3 Results.....	45
2.2.3.1 Mould species isolated from shredded Cheddar cheese at 0 weeks and after 16 weeks of storage at 5 ± 1 °C packaged in treatments 1 – 6.	45
2.2.4 Discussion	48
2.2.4.1 Mould species isolated from shredded Cheddar cheese packaged in treatments 1 – 6 at week 0.....	48
2.2.4.2 Mould species isolated from shredded Cheddar cheese packaged in air + control film (treatment 1) and air + oxygen scavenging film (treatment 4) and stored at 5 ± 1 °C for 16 weeks	50
2.2.4.3 Mould species isolated from shredded Cheddar cheese packaged in 80 % CO ₂ / 17% N ₂ / 3% O ₂ + control film (treatment 2) and 80% CO ₂ / 17% N ₂ / 3% O ₂ + oxygen scavenging film (treatment 5) and stored at 5 ± 1 °C for 16 weeks.....	51
2.2.4.4 Mould species isolated from shredded Cheddar cheese packaged in 73% CO ₂ / 27% N ₂ + control film (treatment 3) and 73% CO ₂ / 27% N ₂ + oxygen scavenging film (treatment 6) stored at 5 ± 1 °C for 16 weeks.....	54
2.2.5 Conclusions.....	57
3 GENERAL DISCUSSION	58
4 CONCLUSIONS AND RECOMMENDATIONS	66
5 REFERENCES	67

LIST OF TABLES

Table 1.1	Incidence of mould genera isolated from various cheeses.....	16
Table 2.1.1	Water activity values of shredded Cheddar cheese packaged in treatments 1 - 6 and stored at 5 ± 1 °C (n = 36).....	27
Table 2.1.2	Statistical analysis of lactic acid bacteria counts in shredded Cheddar cheese packaged in treatments 1 – 6 and stored for 16 weeks at 5 ± 1 °C	27
Table 2.1.3	Statistical analysis of yeast and mould counts in shredded Cheddar cheese packaged in treatment 1 - 6 stored for 16 weeks at 5 ± 1 °C	28
Table 2.1.4	Mould growth (log cfu/g) in shredded Cheddar cheese packaged in air + control film (treatment 1) and air + oxygen scavenging film (treatment 4) and stored at 5 ± 1 °C for 16 weeks (n=36).....	30
Table 2.1.5	Mould populations (log cfu/g) in shredded Cheddar cheese packaged in 80% CO ₂ / 17% N ₂ / 3% O ₂ + control film (treatment 2) and 80% CO ₂ / 17% N ₂ / 3% O ₂ + oxygen scavenging film (treatment 5) stored for 16 weeks at 5 ± 1 °C (n=36).....	33
Table 2.1.6	Mould growth (log cfu/g) in shredded Cheddar cheese packaged in treatment 73% CO ₂ / 27% N ₂ + control film (treatment 3) and 73% CO ₂ / 27% N ₂ + oxygen scavenging film (treatment 6) and stored at 5 ± 1 °C for 16 weeks (n=36).....	36
Table 2.2.1	Mould species isolated from shredded Cheddar cheese at 0 weeks and after 16 weeks of storage at 5 ± 1 °C packaged in modified atmospheres combined with an oxygen scavenging and control film (treatments 1 - 6)	46

LIST OF FIGURES

Figure 2.1.1	Lactic acid bacteria and yeast populations in shredded Cheddar cheese packaged in air + control film (treatment 1) and air + oxygen scavenging film (treatment 4) for 16 weeks at 5 ± 1 °C (n=36)...	29
Figure 2.1.2	Shelf life of shredded Cheddar cheese packaged in an oxygen scavenging and control film in 3 atmospheres (air, 80% CO ₂ / 17% N ₂ / 3% O ₂ , 73% CO ₂ / 27% N ₂) and stored at 5 ± 1 °C (n=108) based on visible mould growth. Control film Oxygen scavenging film 	31
Figure 2.1.3	Lactic acid bacteria and yeast populations in shredded Cheddar cheese packaged in 80% CO ₂ / 17% N ₂ / 3% O ₂ + control film (treatment 2) and 80% CO ₂ / 17% N ₂ / 3% O ₂ + oxygen scavenging film (treatment 5) for 16 weeks at 5 ± 1 °C (n=36).....	32
Figure 2.1.4	Lactic acid bacteria and yeast populations in shredded Cheddar cheese packed in 73% CO ₂ / 27% N ₂ + control film (treatment 3) and 73% CO ₂ / 27% N ₂ + oxygen scavenging film (treatment 6) and stored at 5 ± 1 °C for 16 weeks (n=36).....	35
Figure 3	Proposed model for the microbiological quality, shelf life and mould species isolated from shredded Cheddar cheese packaged in modified atmospheres with and without oxygen scavengers and stored at 5 ± 1 °C for 16 weeks	62

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 $\geq 70\%$ of all isolates

 ■ Present as $\leq 30\%$ 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 (air, 40% CO₂ / 15% N₂ / 45% O₂, 33% CO₂ / 27% 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 33% CO₂ / 27% 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.

CHAPTER 2

2 RESEARCH

2.1 MICROBIOLOGICAL QUALITY OF SHREDDED CHEDDAR CHEESE PACKAGED IN MODIFIED ATMOSPHERES WITH OXYGEN SCAVENGERS

Submitted to the *International Journal of Food Science and Technology*

Abstract

Shredded Cheddar cheese samples were packaged with each of 3 atmospheres (air, 80% CO₂ / 17% N₂ / 3% O₂, 73% CO₂ / 27% N₂) combined either with an oxygen scavenging or control film. The samples were stored for 16 weeks at 5 ± 1 °C and analysed for lactic acid bacteria (LAB), yeast and moulds counts. In addition, the time taken for the first visible signs of mould growth on the cheese was noted. The LAB counts in the cheese were unaffected by the gaseous atmosphere or packaging film. The cheese packaged in the 73% CO₂ / 27% N₂ atmosphere combined with the oxygen scavenging 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 atmosphere packaging, moulds.

2.1.1 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 usually involves the use of gas mixtures of CO₂, N₂ and O₂ (Farber, 1991). Carbon dioxide is the gas responsible for the bacteriostatic and fungistatic effect on micro organism growth in a modified atmosphere environment (Smith, Ramaswamy and Simpson, 1990). MAP reduces the growth rate of spoilage micro organisms e.g. mould species like *P. verrucosum* and pathogenic micro organisms e.g. *L. monocytogenes* (Farber, 1991).

MAP on its own is not always successful in the prevention of mould growth on cheese because of residual levels of O₂ that may occur in the package as well as the tolerance of some spoilage moulds to low O₂ concentrations and high CO₂ 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. *P. 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 O₂. Secondly, each packaging film has a characteristic O₂ permeability that allows the transfer of O₂ from the environment into the package. Thirdly, O₂ could enter the package through improper sealing (Smith, Oraikul, Koersen, Jackson and Lawrence, 1986). As a result MAP is sometimes used 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).

An oxygen scavenger is a substance that reacts with and removes O₂ from the environment in which it is placed (Floros, Nielsen and Farkas, 1999). Examples are iron oxidase, ascorbic acid, unsaturated fatty acids, photosensitive dyes and enzymes (Floros *et al.*, 1999). The absence of O₂ from a packaging environment prevents deleterious effects due to oxidation and growth of micro organisms (Floros *et al.*, 1999; Vermeiren *et al.*, 1999). Oxygen scavengers are mainly used to prevent the growth of moulds e.g. *Aspergillus niger* (Floros *et al.*, 1999) since most moulds are obligate aerobes, and require O₂ for metabolism and growth functions (Hocking and Taniwaki, 1997).

Oxygen scavengers are commonly used in the form of a sachet attached to the interior of the packaging material (Smith, Hoshino and Abe, 1995) or they may be incorporated into the packaging structure of materials (Floros *et al.*, 1999). Low molecular weight oxygen scavengers may be dissolved or distributed in a packaging plastic or the plastic may be made from a polymeric scavenger (Rooney, 1995). This enables the oxygen scavengers to have greater contact with the gaseous environment (Rooney, 1995).

The objective of this study was to investigate the microbiological quality of shredded Cheddar cheese packaged in different modified atmospheres with and without oxygen scavengers included in the packaging film.

2.1.2 Materials and methods

2.1.2.1 Packaging materials

Two laminate packaging films were used during this study (Liquid Air Cryovac Pty (LTD), Johannesburg, South Africa). They consisted of:

1. A laminate film (control film), which consisted of Bx Nylon/ Linear low density polyethylene/ low density polyethylene/ Linear low density polyethylene. It had an oxygen transmission rate (OTR) $< 20 \text{ ml/ m}^2/ 24 \text{ hr/ atm. at } 22 \text{ }^\circ\text{C and } 75\% \text{ RH}$
2. A laminate film with an oxygen scavenger Ciba[®] SHELPLUS[™] O₂ (Ciba Specialty Chemicals, Sweden) incorporated into its multi layer structure at 3% of its total weight. It consisted of Bx Nylon/ Linear low density polyethylene/ low density polyethylene with master batch containing Ciba[®] SHELPLUS[™] O₂/ Linear low density polyethylene. It had an oxygen transmission rate (OTR) $< 20 \text{ ml/ m}^2/ 24 \text{ hr/ atm. at } 22 \text{ }^\circ\text{C and } 75\% \text{ RH}$.

2.1.2.2 Packaging treatments of shredded Cheddar cheese

Shredded Cheddar cheese samples were obtained from a cheese factory in the Western Cape region of South Africa. Ten kilogram blocks of vacuum matured Cheddar cheese all processed on the same day, were shredded and a total of 108 samples weighing 250g each were packaged (Multivac) with each of 3 atmospheres (air (20.8% O₂ / 0.3% CO₂ / 78.9%), 80% CO₂ / 17% N₂ / 3% O₂, 73% CO₂ / 27% N₂) combined either with an oxygen scavenging or control film as follows: treatment 1 = air + control film, treatment 2 = 80% CO₂ / 17% N₂ / 3% O₂ + control film, treatment 3 = 73% CO₂ / 27% N₂ + control film, treatment 4 = air + oxygen scavenging film, treatment 5 = 80% CO₂ / 17% N₂ / 3% O₂ + oxygen scavenging film, treatment 6 = 73% CO₂ / 27% N₂ + oxygen scavenging film. This resulted in 36 packages per treatment. The ratio of gas mixture to cheese was 2/3 to 1/3. After packaging, the shredded Cheddar cheese samples were transported by airfreight to the Department of Food Science, University of Pretoria.

2.1.2.3 Storage period of shredded Cheddar cheese

The samples were stored at a retail display temperature of $5 \pm 1^\circ\text{C}$ for 16 weeks in a Labcon low temperature incubator (Labex, Orange Grove, South Africa) at the Department of Food Science. Three shredded Cheddar cheese packages from

treatments 1 - 6, were selected at random and the samples analysed at 0, 4, 8, 12, 14 and 16 weeks for lactic acid bacteria (LAB), yeast and mould counts.

2.1.2.4 Microbiological analysis of shredded Cheddar cheese

Ten gram quantities of the cheese samples were weighed and macerated in 90 ml of sterile 2% (w/v) sodium citrate (Saarchem Ltd., Krugersdorp, South Africa) solution at a temperature of 45 °C with the aid of a Stomacher Lab Blender 400 (Seward Laboratory, London, UK) to achieve an initial 10^{-1} cheese emulsion. Further decimal dilutions were prepared in the same diluent and 0.1 ml portions were plated on the following solidified agar by use of the surface plate method.

LAB were enumerated on MRS agar (De Man, Rogosa and Sharp, 1960). The plates were incubated at 30 °C for 3 days.

Yeasts and moulds were enumerated on potato dextrose agar (PDA) (Biolab, Wadeville, South Africa) with the antibiotic Rifampicin (Lion Bridge, Pretoria, South Africa) added at a level of 50 mg/ litre of media (van Dyk, 2003). The plates were incubated at 25 °C for 5 days. The yeast and mould counts on week 0 and 4 were enumerated at levels of 10^5 to 10^8 while on week 8 to 16 they were enumerated at levels of 10^1 to 10^4 .

2.1.2.5 Water activity of the shredded Cheddar cheese samples

The water activity (a_w) was determined using a Pawkit portable water activity meter (Decagon devices, Inc. Wyoming, U.S.A.).

2.1.2.6 Visual inspection of the shredded Cheddar cheese samples for mould growth

All the shredded Cheddar cheese samples in the 6 treatments were visually inspected weekly for visible mould growth. This was done to determine the cut off point of shelf life based on visible mould growth.

2.1.2.7 Statistical Analysis

Analysis of variance (ANOVA) was used to determine whether the packaging film (control or oxygen scavenging film), atmosphere (air, 80% CO₂ / 17% N₂ / 3% O₂, 73% CO₂ / 27% N₂) and storage period (0, 4, 8, 12, 14 and 16 weeks) significantly affected ($p \leq 0.05$) the growth of LAB, yeasts and moulds. Duplicate samples were

evaluated during each analysis and the experiment was repeated three times to obtain a total of 6 observations for each analysis. ANOVA was performed using STATISTICA program for windows version 6.1 (Tulsa, Oklahoma, U.S.A, 2003).

2.1.3 Results

2.1.3.1 Oxygen scavenging activity of the laminate film

The oxygen scavenger used in this study, Ciba® SHELPLUS™ O₂, is moisture activated and thus requires foodstuffs with water activities of 0.7 or higher to initiate the absorption of oxygen (Ciba, 2002). The water activity of the shredded Cheddar cheese samples ranged between 0.926 - 0.940 (Table 2.1.1.) indicating that the water activity was high enough to activate the oxygen scavenger in all the packages.

Table 2.1.1 Water activity values of shredded Cheddar cheese packaged in treatment 1 - 6 and stored at 5 ± 1 °C (n = 36)

Treatment	Storage period	
	0 weeks (SD ±)	16 weeks
Air + control film (treatment 1)	0.926 (0.007)	0.940 (0)
80% CO ₂ / 17% N ₂ / 3% O ₂ + control film (treatment 2)	0.958 (0.003)	0.940 (0.007)
73% CO ₂ / 27% N ₂ + control film (treatment 3)	0.933 (0.007)	0.940 (0.006)
Air + oxygen scavenging film (treatment 4)	0.928 (0.003)	0.938 (0)
80% CO ₂ / 17% N ₂ / 3% O ₂ + oxygen scavenging film (treatment 5)	0.928 (0.006)	0.940 (0)
73% CO ₂ / 27% N ₂ + oxygen scavenging film (treatment 6)	0.933 (0.006)	0.943 (0.003)

Packaging film * storage period

Gas mixture * packaging film * storage period

2.1.3.2 Shredded Cheddar cheese packaged in air + control film (treatment 1) and air + oxygen scavenging film (treatment 4) for 16 weeks at 5 ± 1 °C

Table 2.1.2 Statistical analysis of lactic acid bacteria counts in shredded Cheddar cheese packaged in treatments 1 – 6 and stored for 16 weeks at 5 ± 1 °C

Variables	Degrees of freedom	P value
Atmosphere (air, 80% CO ₂ / 17% N ₂ / 3% O ₂ , 73%	2	0.239
Packaging film (oxygen scavenging and control)	1	0.754
Storage period (0, 4, 8, 12, 14, 16 w)	5	0.000
Gas mixture * packaging film	2	0.609
Gas mixture * storage period	10	0.088
Packaging film * storage period	5	0.797
Gas mixture * packaging film * storage period	10	0.450

Table 2.1.3 Statistical analysis of yeast and mould counts in shredded Cheddar cheese packaged in treatment 1 - 6 stored for 16 weeks at 5 ± 1 °C

Variables	Degrees of freedom	Yeasts	Moulds
		P value	P value
Atmosphere (air, 80% CO ₂ / 17% N ₂ / 3% O ₂ , 73% CO ₂ / 27% N ₂)	2	0.000	0.000
Packaging film (oxygen scavenging and control)	1	0.000	0.003
Storage period (0, 4, 8, 12, 14, 16 w)	3	0.055	0.000
Gas mixture * packaging film	2	0.102	0.001
Gas mixture * storage period	6	0.000	0.000
Packaging film * storage period	3	0.001	0.476
Gas mixture * packaging film * storage period	6	0.057	0.002

The yeast populations in the shredded Cheddar cheese in treatment 1 and 4 were highest in the 5th week at 5.1 and 5.0 log cfu/g in treatments 1 and 4 respectively. The counts then reduced gradually over the storage period to 3 and 2.7 log cfu/g in treatments 1 and 4 respectively during the 16th week (Figure 2.1.1).

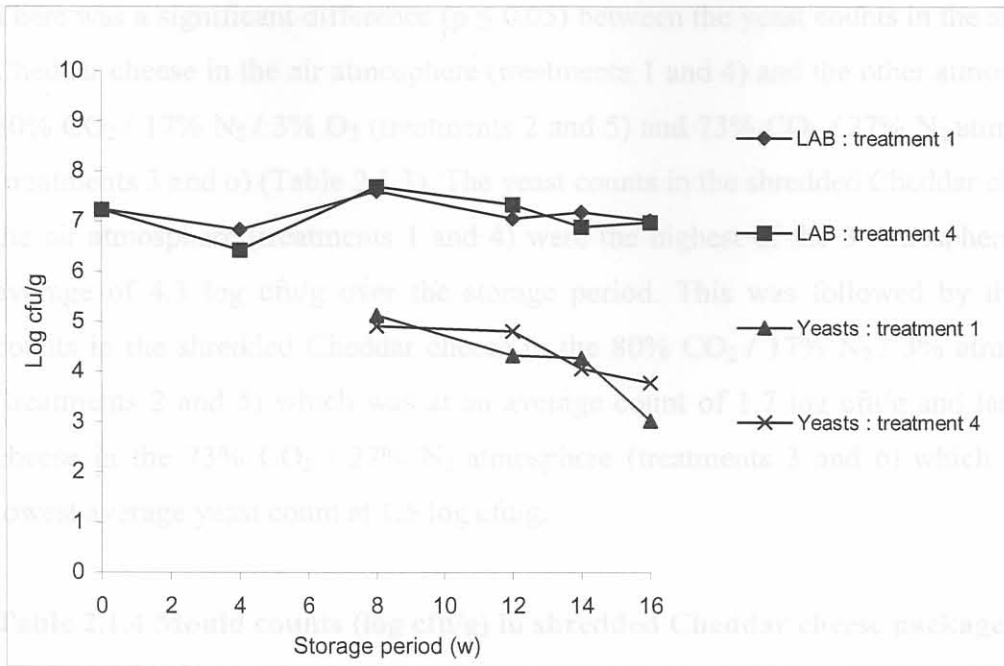


Figure 2.1.1 Lactic acid bacteria and yeast counts in shredded Cheddar cheese packaged in air + control film (treatment 1) and air + oxygen scavenging film (treatment 4) for 16 weeks at 5 ± 1 °C (n=36)

The LAB populations in treatment 1 and 4 were similar to each other during the 16 weeks storage period. The counts in the shredded Cheddar cheese in treatment 1 were detected initially (week 0) at 7.2 log cfu/g (Figure 2.1.1). The counts then reduced to 6.8 log cfu/g in the 4th week followed by an increase to 7.6 log cfu/g in the 8th week then a decrease to 7 log cfu/g in the 12th week. In the 14th and 16th week, the LAB counts in the cheese were detected at 7.2 log cfu/g and 7 log cfu/g respectively. In the shredded Cheddar cheese in treatment 4, from an initial 7.2 log cfu/g at week 0 the LAB counts were then detected at a level of 6.4 log cfu/g in week 4 followed by an increase in the 8th week to 7.7 log cfu/g. The counts then dropped to 7.3 log cfu/g in the 12th week and in the 14th and 16th weeks the counts were detected at 6.9 log cfu/g and 7.0 log cfu/g respectively (Figure 2.1.1).

The yeast populations in the shredded Cheddar cheese in treatment 1 and 4 were highest in the 8th week at 5.1 and 5.0 log cfu/g in treatments 1 and 4 respectively. The counts then reduced gradually over the storage period to 3 and 3.7 log cfu/g in treatments 1 and 4 respectively during the 16th week (Figure 2.1.1).

There was a significant difference ($p \leq 0.05$) between the yeast counts in the shredded Cheddar cheese in the air atmosphere (treatments 1 and 4) and the other atmospheres, 80% CO₂ / 17% N₂ / 3% O₂ (treatments 2 and 5) and 73% CO₂ / 27% N₂ atmosphere (treatments 3 and 6) (Table 2.1.3). The yeast counts in the shredded Cheddar cheese in the air atmosphere (treatments 1 and 4) were the highest of the 3 atmospheres at an average of 4.3 log cfu/g over the storage period. This was followed by the yeast counts in the shredded Cheddar cheese in the 80% CO₂ / 17% N₂ / 3% atmosphere (treatments 2 and 5) which was at an average count of 1.7 log cfu/g and lastly, the cheese in the 73% CO₂ / 27% N₂ atmosphere (treatments 3 and 6) which had the lowest average yeast count at 1.5 log cfu/g.

Table 2.1.4 Mould counts (log cfu/g) in shredded Cheddar cheese packaged in air + control film (treatment 1) and air + oxygen scavenging film (treatment 4) and stored at 5 ± 1 °C for 16 weeks (n=36)

Packaging treatment	Treatment 1		Treatment 4	
	log cfu/g	No. of samples with growth	log cfu/g	No. of samples with growth
0	ND ^a	0/6	ND ^a	0/6
4	ND ^a	0/6	ND ^a	0/6
8	3.6	5/6	3.4	5/6
12	2.7	4/6	ND	0/6
14	3.0	5/6	1.7	1/6
16	ND	0/6	ND	0/6

ND^a – Not detected below 10⁵ ND - Not detected below 10¹

There was a significant difference ($p \leq 0.05$) between the mould counts in the shredded Cheddar cheese packaged in treatments 1 and 4 (Table 2.1.3). The air packaged shredded Cheddar cheese samples in treatment 1 had higher mould counts over the storage period with a mean count of 2.2 log cfu/g while the samples in treatment 4 had a lower mean of 0.9 log cfu/g (Table 2.1.4). The shredded Cheddar cheese in treatment 1 also had a greater number of shredded Cheddar cheese packages positive for mould growth i.e. 14/36 vs. 6/36 (Table 2.1.4). Consequently the cheese packaged in treatment 1 developed visible mould faster i.e. within 4 weeks as

compared to the samples in treatment 4, which developed mould within 7 weeks (Figure 2.1.2). From the 8th week at 3.6 log cfu/g in treatment 1, the mould counts in the shredded Cheddar cheese, were detected at 2.7 log cfu/g in the 12th week and at 3.03 log cfu/g in the 14th week. However, the mould counts in the 16th week were below the detection level. In the shredded Cheddar cheese in treatment 4, the mould counts were only detected in the 8th and 14th week at 3.4 and 1.7 log cfu/g respectively, while in the 12th and 16th week, they were below the detection level (Table 2.1.4).

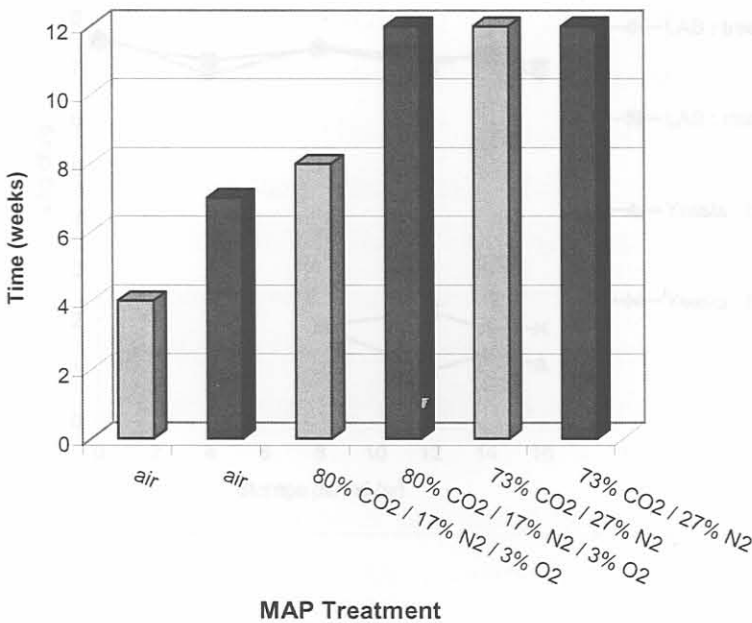


Figure 2.1.2 Shelf life of shredded Cheddar cheese packaged in an oxygen scavenging and control film in 3 atmospheres (air, 80% CO₂ / 17% N₂ / 3% O₂, 73% CO₂ / 27% N₂) and stored at 5 ± 1 °C based on visible mould growth (n=108). Control film Oxygen scavenging film

There was a significant difference ($p \leq 0.05$) between the mould counts in the cheese in the air atmosphere (treatments 1 and 4) and between the mould counts in the cheese in the other atmospheres of 80% CO₂ / 17% N₂ / 3% O₂ (treatments 2 and 5) and 73% CO₂ / 27% N₂ (treatments 3 and 6) (Table 2.1.3). The average mould counts in the shredded Cheddar cheese in the air atmosphere (treatments 1 and 4) was the highest over the storage period at 1.6 log cfu/g while in the 80% CO₂ / 17% N₂ / 3% O₂

atmosphere the average mould counts in the cheese was 0.3 log cfu/g and in the cheese in the 73% CO₂ / 27% N₂ atmosphere the average value was 0.2 log cfu/g.

2.1.3.3 Shredded Cheddar cheese samples packaged in 80% CO₂ / 17% N₂ / 3% O₂ + control film (treatment 2) and 80% CO₂ / 17% N₂ / 3% O₂ + oxygen scavenging film (treatment 5) stored for 16 weeks at 5 ± 1 °C

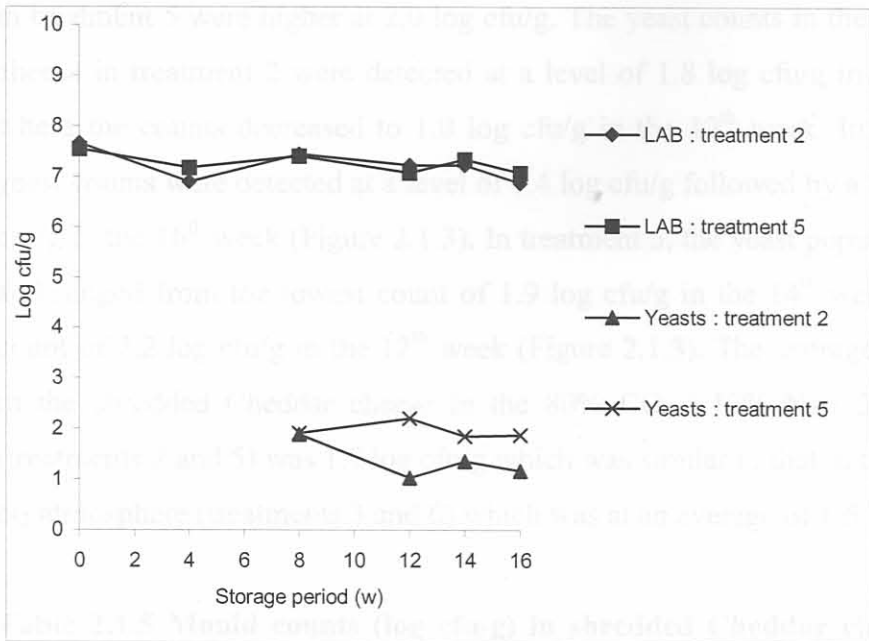


Figure 2.1.3 Lactic acid bacteria and yeast counts in shredded Cheddar cheese packaged in 80% CO₂ / 17% N₂ / 3% O₂ + control film (treatment 2) and 80% CO₂ / 17% N₂ / 3% O₂ + oxygen scavenging film (treatment 5) for 16 weeks at 5 ± 1 °C (n=36)

The LAB counts in the shredded Cheddar cheese in treatment 2 and 5 remained stable over the storage period (Figure 2.1.3) and were similar to each other as well as to the LAB counts of the other treatments (Table 2.1.1). The LAB counts in the shredded Cheddar cheese in treatment 2 ranged between the highest value of 7.6 log cfu/g in week 0 and the lowest value of 6.8 log cfu/g in the 16th week (Figure 2.1.3). In the shredded Cheddar cheese in treatment 5, the LAB counts in the shredded Cheddar cheese were initially at 7.5 log cfu/g in week 0. The counts then decreased to 7.1 log cfu/g in the 4th week, followed by an increase to 7.4 log cfu/g in the 8th week. In the

12th week, the counts decreased to 7.0 log cfu/g then increased slightly to 7.3 log cfu/g in the 14th week and in the 16th week, the counts dropped to 7.0 log cfu/g (Figure 2.1.3).

There was a significant difference ($p \leq 0.05$) between the yeast counts in the cheese in treatments 2 and 5 (Table 2.1.3), with the shredded Cheddar cheese in the treatment 2 having lower yeast counts at an average of 1.4 log cfu/g while the counts in the cheese in treatment 5 were higher at 2.0 log cfu/g. The yeast counts in the shredded Cheddar cheese in treatment 2 were detected at a level of 1.8 log cfu/g in the 8th week from where the counts decreased to 1.0 log cfu/g in the 12th week. In the 14th week the yeast counts were detected at a level of 1.4 log cfu/g followed by a decrease to 1.2 log cfu/g in the 16th week (Figure 2.1.3). In treatment 5, the yeast populations were stable and ranged from the lowest count of 1.9 log cfu/g in the 14th week and the highest count of 2.2 log cfu/g in the 12th week (Figure 2.1.3). The average yeast populations in the shredded Cheddar cheese in the 80% CO₂ / 17% N₂ / 3% O₂ atmosphere (treatments 2 and 5) was 1.7 log cfu/g which was similar to that in the 73% CO₂ / 27% N₂ atmosphere (treatments 3 and 6) which was at an average of 1.5 log cfu/g.

Table 2.1.5 Mould counts (log cfu/g) in shredded Cheddar cheese packaged in 80% CO₂ / 17% N₂ / 3% O₂ + control film (treatment 2) and 80% CO₂ / 17% N₂ / 3% O₂ + oxygen scavenging film (treatment 5) stored for 16 weeks at 5 ± 1 °C (n=36)

Packaging treatment	Treatment 2		Treatment 5	
Storage period (w)	log cfu/g	No. of samples with growth	log cfu/g	No. of samples with growth
0	ND ^a	0/6	ND ^a	0/6
4	ND ^a	0/6	ND ^a	0/6
8	2.0	2/6	ND	0/6
12	0.7	1/6	1.2	3/6
14	ND	0/6	ND	0/6
16	0.7	2/6	0.7	1/6

ND^a – Not detected below 10⁵ ND- Not detected below 10¹

There was a significant difference ($p \leq 0.05$) between the mould counts in the shredded Cheddar cheese packaged in treatments 2 and 5 (Table 2.1.3). The shredded Cheddar cheese in treatment 2 had a higher average mould population at 0.4 log cfu/g than the cheese in treatment 5 which had an average count of 0.2 log cfu/g over the storage period. Consequently the cheese samples in treatment 2 developed mould growth faster i.e. within 8 weeks while the cheese in treatment 5 developed mould within 12 weeks (Figure 2.1.2). The shredded Cheddar cheese in treatment 2 also had a greater number of shredded Cheddar cheese packages positive for mould growth i.e. 5/36 vs. 4/36 (Table 2.1.5). The mould counts in the shredded Cheddar cheese samples in the treatment 2 were highest in the 8th week at 2.0 log cfu/g from when they were detected at 0.7 log cfu/g in the 12th week. In the 14th week the mould counts were below the detection level while in the 16th week the mould count was detected at a level of 0.7 log cfu/g (Table 2.1.5). In the shredded Cheddar cheese samples in the treatment 5, the mould populations were only detected in the 12th and 16th week at 1.2 and 0.7 log cfu/g respectively while in the 8th and 16th week the counts were below detection level (Table 2.1.5). The average mould count in the shredded Cheddar cheese packaged in the 80% CO₂ / 17% N₂ / 3% O₂ atmosphere (treatments 2 and 5) was 0.3 log cfu/g which was similar to the average mould count in the shredded Cheddar cheese in the 73% CO₂ / 27% N₂ atmosphere (treatments 3 and 6) which was 0.2 log cfu/g.

2.1.3.4 Shredded Cheddar cheese samples packaged in 73% CO₂ / 27% N₂ + control film (treatment 3) and 73% CO₂ / 27% N₂ + oxygen scavenging film (treatment 6) and stored at 5 ± 1 °C for 16 weeks

The LAB counts in the shredded Cheddar cheese in treatment 3 were stable over the 16 weeks storage period (Figure 2.1.4). The LAB counts in treatment 3 were within a 0.3 log range of each other. The values ranged from the highest value of 7.3 log cfu/g initially (week 0) and the lowest value of 7.0 log cfu/g which was detected in the 8th, 14th and 16th week. In the shredded Cheddar cheese in treatment 6, the LAB counts in the cheese were detected at a level of 7.1 log cfu/g initially (week 0), then at 7.0 log cfu/g in the 4th week, followed by an increase to 7.5 log cfu/g in the 8th week. Thereafter the counts gradually decreased from 7.2 log cfu/g in the 12th week, to 7.0 log cfu/g in the 16th week.

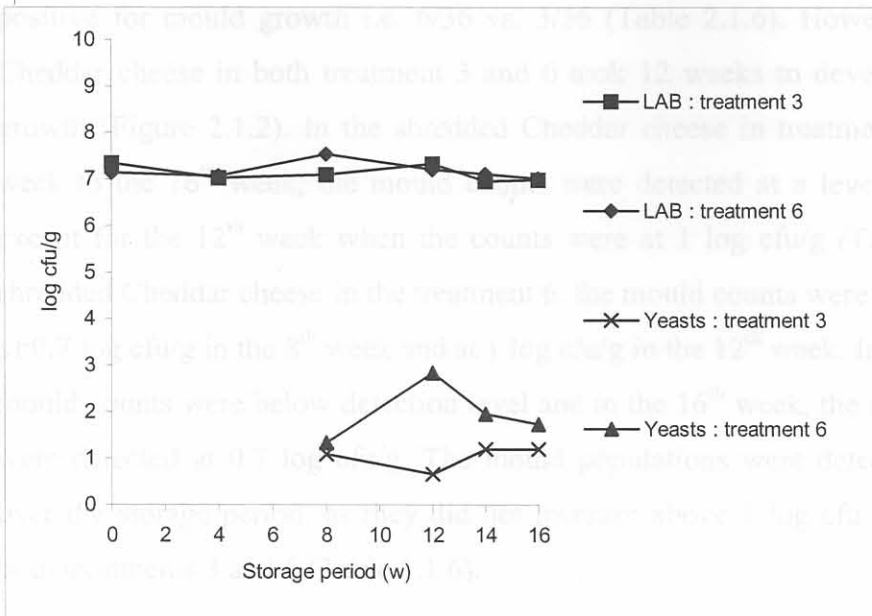


Figure 2.1.4 Lactic acid bacteria and yeast populations in shredded Cheddar cheese packed in 73% CO₂ / 27% N₂ + control film (treatment 3) and 73% CO₂ / 27% N₂ + oxygen scavenging film (treatment 6) and stored at 5 ± 1°C for 16 weeks (n=36).

There was a significant difference ($p \leq 0.05$) between the yeast counts in the shredded Cheddar cheese packaged in treatments 3 and 6 (Table 2.1.3). The yeast populations in the cheese in treatment 6, were higher at an average of 2 log cfu/g while in the cheese in treatment 3, they were at an average of 1 log cfu/g over the storage period. In the shredded Cheddar cheese in treatment 3, the yeast populations decreased from 1 log cfu/g in the 8th week to 0.6 log cfu/g in the 12th week. This was followed by an increase to 1.2 log cfu/g in week 14 where the counts remained constant in the 16th week (Figure 2.1.4). In the shredded Cheddar cheese packaged in treatment 6, the yeast populations increased from 1.3 log cfu/g at 8 weeks to 2.8 log cfu/g at 12 weeks. This was then followed by a decrease to 1.9 log cfu/g at week 14 and another decrease to 1.7 log cfu/g in the 16th week (Figure 2.1.4).

The LAB populations remained stable in the shredded Cheddar cheese in treatments 3 and 6. The shredded Cheddar cheese packaged in treatment 6 had a lower mean mould population over the storage period at a value of 0.2 log cfu/g compared to the cheese in treatment 3 which had an average value of 0.3 log cfu/g. The shredded Cheddar cheese in treatment 3 also had a greater number of shredded Cheddar cheese packages

positive for mould growth i.e. 6/36 vs. 3/36 (Table 2.1.6). However, the shredded Cheddar cheese in both treatment 3 and 6 took 12 weeks to develop visible mould growth (Figure 2.1.2). In the shredded Cheddar cheese in treatment 3, from the 8th week to the 16th week, the mould counts were detected at a level of 0.7 log cfu/g except for the 12th week when the counts were at 1 log cfu/g (Table 2.1.6). In the shredded Cheddar cheese in the treatment 6, the mould counts were detected at a level of 0.7 log cfu/g in the 8th week and at 1 log cfu/g in the 12th week. In the 14th week the mould counts were below detection level and in the 16th week, the mould populations were detected at 0.7 log cfu/g. The mould populations were detected at low levels over the storage period, as they did not increase above 1 log cfu/g in the cheese in both treatments 3 and 6 (Table 2.1.6).

Table 2.1.6 Mould growth (log cfu/g) in shredded Cheddar cheese packaged in treatment 73% CO₂ / 27% N₂ + control film (treatment 3) and 73% CO₂ / 27% N₂ + oxygen scavenging film (treatment 6) and stored at 5 ± 1°C for 16 weeks (n=36)

Packaging treatment	Treatment 3		Treatment 6	
	log cfu/g	No. of samples with growth	log cfu/g	No. of samples with growth
0	ND ^a	0/6	ND ^a	0/6
4	ND ^a	0/6	ND ^a	0/6
8	0.7	1/6	0.7	1/6
12	1.0	2/6	1.0	1/6
14	0.7	1/6	ND	0/6
16	0.7	2/6	0.7	1/6

ND^a – Not detected below 10⁵ ND - Not detected below 10¹

2.1.4 Discussion

The LAB populations remained stable in the shredded Cheddar cheese in treatments 1 – 6 during the storage period of 16 weeks and the counts were similar between the treatments (Figures 2.1.1, 2.1.3 and 2.1.4). Elliot *et al.*, (1998) similarly found that LAB counts in shredded MAP Mozzarella cheese stored at 10 ± 1 °C for 8 weeks, did

not vary except in the air atmosphere where they decreased initially and then increased to the same level as the other atmospheres. Maniar, Marcy, Bishop and Duncan (1994) also found that LAB populations in MAP cottage cheese over 28 days, were within a log range of 0.1 except the counts in the air samples that increased above the other atmospheres. LAB are gram positive and gram positive bacteria are generally less sensitive than gram negative bacteria to the inhibitory effects of CO₂ (Parry, 1993). Thus LAB are unaffected or slightly inhibited by CO₂ atmospheres (Parry, 1993; Farber, 1991) This study also indicates that packaging in the oxygen scavenging or control film in any of the three gaseous atmospheres would not affect the LAB populations in shredded Cheddar cheese.

The yeast populations in the shredded Cheddar cheese samples, packaged in air (treatment 1 and 4) were significantly different from the cheese packaged in 80% CO₂ / 17% N₂ / 3% O₂ atmosphere (treatments 2 and 5) and 73% CO₂ / 27% N₂ atmosphere (treatments 3 and 6) (Table 2.1.3). The cheese packaged in air (treatments 1 and 4) had the highest yeast counts of the 3 gaseous atmospheres. The lack of inhibition in the air atmosphere may have been due to the absence of CO₂ to inhibit the growth of yeasts (Day, 1992). Yeasts have an ability to grow in cheese during maturation and refrigerated storage in the retail chain (Fleet, 1990). This can be explained by their ability to grow at low temperatures, high salt concentrations, assimilation and fermentation of lactose and lactic acid, resistance to low pH and water activity values (Fleet, 1990).

The growth of yeasts in shredded Cheddar cheese was inhibited by the modified atmospheres containing CO₂ (80% CO₂ / 17% N₂ / 3% O₂, 73% CO₂ / 27% N₂). The yeast populations in the shredded Cheddar cheese in these two atmospheres were not significantly different from each other but were significantly different from the air atmosphere (treatments 1 and 4). The shredded Cheddar cheese in the 73% CO₂ / 27% N₂ atmosphere (treatments 3 and 6) had the lowest average yeast populations at 1.5 log cfu/g. The inhibition of yeasts in this atmosphere may have been due to the absence of oxygen which prevented the growth of yeasts and the high CO₂ concentrations which inhibited their growth (Day, 1992). The yeast populations in the shredded Cheddar cheese in the 73% CO₂ / 27% N₂ atmosphere were similar to the yeast populations in the shredded Cheddar cheese in the 80% CO₂ / 17% N₂ / 3% O₂

atmosphere (treatments 2 and 5) which had an average yeast count of 1.7 log cfu/g. This similarity may have been due to the higher level of CO₂ in the 80% CO₂ / 17% N₂ / 3% O₂ atmosphere inhibiting the growth of yeasts (Taniwaki *et al.*, 2001). The most effective gas atmosphere and film combination that inhibited the growth of yeasts was treatment 3 as it had the lowest average yeast populations although the yeast populations were also low in treatments 2, 5 and 6.

Other authors have similarly packaged cheese in modified atmospheres to control yeast growth with a resulting inhibition of yeast growth. Elliot *et al.*, (1998) noted a complete inhibition of yeast growth in Mozzarella cheese packaged in different modified atmospheres of 10% CO₂ / 90% N₂, 25% CO₂ / 75% N₂, 50% CO₂ / 50% N₂, 75% CO₂ / 25% N₂ and 100% CO₂ stored at 10 °C for 8 weeks. Alves *et al.*, (1996) similarly found that in Mozzarella cheese packaged in 100% CO₂ and stored for 58 days at 7 ± 1 °C there was no growth of yeasts. However, in modified atmospheres of 100% N₂ and 50% CO₂ / 50% N₂, the growth of yeasts was detected in the cheese at levels between 6 to 7 log cfu/g.

The growth of moulds in the cheese packaged in the oxygen scavenging film was significantly different ($p \leq 0.05$) from that in the cheese packaged in the control film in all the gaseous atmospheres (Table 2.1.3). This was probably due to the absorption of oxygen by the oxygen scavenger leading to lower residual oxygen which resulted in an inhibition of mould growth because most moulds are obligate aerobes (Pitt and Hocking, 1997). The shredded Cheddar cheese in the air atmosphere (treatments 1 and 4) had the highest mould counts of all the atmospheres (Table 2.1.4). This may have been due to the lack of CO₂ to inhibit the growth of moulds as they are sensitive to the inhibitory effect of CO₂ (Day, 1992) and the presence of oxygen which allowed their growth. The cheese packaged in treatment 1 had the shortest shelf life of 4 weeks (Figure 2.1.2) probably due to the availability of more oxygen for mould growth in the control film, while in the cheese in treatment 4, the absorption of oxygen by the oxygen scavenger increased the shelf life to 7 weeks (Figure 2.1.2).

The shredded Cheddar cheese packaged in treatment 2 took 8 weeks to develop visible signs of mould growth as opposed to 12 weeks in the cheese in treatment 5 (Figure 2.1.2) which was packaged in the same gaseous atmosphere i.e. 80% CO₂ /

17% N₂ / 3% O₂. This was probably because the higher levels of oxygen in the control film allowed the growth of moulds faster leading to a higher average of 0.4 log cfu/g, while in treatment 5, oxygen was absorbed leading to a longer shelf life of the cheese and a lower mean mould count at 0.2 log cfu/g. The mould counts in the cheese in treatment 2 were at a level of 2.04 log cfu/g at 8 weeks when it developed visible mould growth which was higher than the level in the treatment 5 which wasn't detectable at the same time (Table 2.1.5). The cheese in the treatment 2 had signs of visible mould growth at 12 weeks (Table 2.1.5) when the mould count was at a level of 1.0 log cfu/g. No mould growth was detected in the shredded Cheddar cheese in treatment 2 and 5 in the 14th week although other samples had previously shown visible signs of mould growth (Table 2.1.5). This could have been as a result of variability within the shredded Cheddar cheese leading to some packages developing mould growth before the rest. The mould counts in the cheese at this time were also low i.e. below 1.0 log cfu/g (Table 2.1.5) so it was probable that there was no visible mould growth.

The shredded Cheddar cheese in treatments 5, 3 and 6 took the longest to show signs of visible mould growth i.e. 12 weeks (Figure 2.1.2) as compared to the other packaging treatments. In the cheese in treatments 3 and 6 (73% CO₂ / 27% N₂ atmosphere), this can be attributed to the inhibitory effect of high levels of CO₂ on mould growth (Day, 1992; Haasum and Nielsen, 1998a). The shredded Cheddar cheese packaged in treatment 5 may have taken 12 weeks to develop visible mould growth due to the oxygen scavengers in the film absorbing the 3% oxygen in the atmosphere, and the higher levels of CO₂ i.e. 80% inhibiting the growth of moulds. The cheese in the treatment 5 had a mean mould count of 0.2 log cfu/g which was similar to the means in the cheese in treatment 3 at 0.3 log cfu/g and treatment 6 at 0.2 log cfu/g. The film with oxygen scavenger would thus effectively absorb residual oxygen within a package environment as the shredded Cheddar cheese in 80% CO₂ / 17% N₂ / 3% O₂ atmosphere + oxygen scavenging film (treatment 5) obtained average mould counts similar to those in treatment 3 and 6 (73% CO₂ / 27% N₂ atmosphere) which did not have 3% oxygen within the atmosphere.

There was no difference between the time the cheese samples in treatment 3 and 6 took to develop visible mould growth i.e. 12 weeks (Figure 2.1.2), though they were

packed in the control and oxygen scavenging film respectively in the same atmosphere (73% CO₂ / 27% N₂). This may have been due to the low residual oxygen in this atmosphere leading to the atmosphere in the cheese in the treatment 6 (oxygen scavenging film) and treatment 3 (control film) not differing greatly as the oxygen scavenger may not have had much residual oxygen to absorb. However, the mean mould counts in the shredded Cheddar cheese in treatment 3 were slightly higher at 0.3 log cfu/g though similar to that in the treatment 6 at a mean of 0.2 log cfu/g. Both cheese samples in the treatment 3 and 6 developed visible mould growth, when the mould counts were at 1.0 log cfu/g (Table 2.1.6). Treatment 6 resulted in shredded Cheddar cheese with the lowest mould populations over the storage period.

Elliot *et al.*, (1998) similarly noted an inhibition of mould growth in modified atmospheres. They packaged Mozzarella cheese in modified atmospheres comprising 25% CO₂ / 75% N₂, 50% CO₂ / 50% N₂, 75% CO₂ / 25% N₂ and 100% CO₂ for 8 weeks at 10 °C and found that the mould counts remained below 1 log cfu/g while in the air atmosphere the mould counts increased from 0.4 log cfu/g at week 0 to 3.38 log cfu/g at week 8. Alves *et al.*, (1996) similarly noted a complete inhibition of mould growth in sliced Mozzarella cheese in modified atmospheres. Rosenthal, Rosen, Bernstein and Popel, (1991), found that CO₂, completely prevented the growth of yeasts and moulds in quarg and cottage cheese for 67 days of storage at 4 °C in an atmosphere of 67.1% CO₂ / 26.3% N₂ / 6.6% O₂. A parallel investigation with N₂ packaged cheese did not show any inhibitory effects on mould growth.

2.1.5 Conclusions

The packaging combination that best maintained the microbiological quality of the shredded Cheddar cheese was treatment 6. It resulted in shredded Cheddar cheese with the lowest mould counts. In addition, the shredded Cheddar cheese packaged in this treatment took 12 weeks to develop visible mould growth along with the cheese packaged in treatment 3 and 5 which also had low mould counts. In conclusion, the film with oxygen scavengers was more effective than the control film against mould growth while the 73% CO₂ / 27% N₂ atmosphere resulted in the cheese with the best microbiological qualities of the 3 atmospheres.

2. 2 CHARACTERISATION OF MOULDS ON SOUTH AFRICAN CHEDDAR CHEESE PACKAGED IN MODIFIED ATMOSPHERES WITH AND WITHOUT OXYGEN SCAVENGERS

Submitted to *Food Control*

Abstract

Shredded Cheddar cheese samples were packaged into an air atmosphere and two modified atmospheres combined either with an oxygen scavenging or control film. The samples were stored for 16 weeks at $5 \pm 1^\circ\text{C}$ and mould isolates from the cheese were identified initially (0 weeks) and at 16 weeks. The genus *Penicillium* predominated initially (week 0) at 41% of all isolates. At 16 weeks, the mycoflora differed according to the treatment in which the cheese was stored and the species isolated were fewer in the different treatments indicating that selection took place. In addition, the number of species isolated from the shredded Cheddar cheese packaged in the film with oxygen scavengers were fewer than the isolates from the cheese packaged in the control film which indicated that the lower oxygen conditions further restricted the mould growth.

Key words: Moulds, shredded Cheddar cheese, oxygen scavengers, modified atmosphere packaging.

The most commonly isolated fungal genus on cheese packaged in barrier packaging without modified atmosphere packaging (MAP) or vacuum packaging is *Penicillium* (Lund, Filtenberg and Frisvad, 1995; Hocking, 1997; Fitt and Hocking, 1994) and the most common species of *Penicillium* isolated from cheese of various types is *Penicillium commune* (Hocking, 1994; Lund *et al.*, 1995). Other commonly isolated genera are *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium* and *Mucor* (Northolt, Egrmond, Soetens and Deijff, 1980; Arin and Eke, 1987; Lund *et al.*, 1995; Kura *et al.*, 2001). On vacuum maturing cheese affected by thread mould spoilage, a different mycoflora has been found to exist due to the low oxygen levels in the package

2.2.1 Introduction

The shelf life of packaged semi-hard and hard cheese is commonly compromised by the growth of moulds (Pitt and Hocking, 1997). Cheese can become mouldy during its ripening period or in the distribution chain under refrigerated storage (Taniwaki and Dender, 1992). Mould growth on cheese can be a safety and spoilage problem as 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). The fungal growth has economic consequences for the cheese producers who have to absorb the financial loss due to fungal spoilage as well as the loss in sales due to negative brand image by consumers who are confronted by mould growth on the cheese within days of purchasing and opening the package (Bishop, Marcy and Moler, 1996).

According to Filtenborg, Frisvad and Thrane, (1996) a very limited group of fungi called mycobiota is responsible for the spoilage of individual types of food. Cheese parameters are restrictive in the range of species that can grow on them and produce spoilage (Hocking, 1994). 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). These characteristics along with extrinsic and processing conditions result in a specific habitat for the range of species that can grow on cheese (Filtenborg *et al.*, 1996; Pitt and Hocking, 1997).

The most commonly isolated fungal genus on cheese packaged in barrier packaging without modified atmosphere packaging (MAP) or vacuum packaging is *Penicillium* (Lund, Filtenborg and Frisvad, 1995; Hocking, 1997; Pitt and Hocking, 1997) and the most common species of *Penicillium* isolated from cheese of various types is *Penicillium commune* (Hocking, 1994; Lund *et al.*, 1995). Other commonly isolated genera are *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium* and *Mucor* (Northolt, Egmond, Soentoro and Deijji, 1980; Aran and Eke, 1987; Lund *et al.*, 1995; Kure *et al.*, 2001). On vacuum maturing cheese affected by thread mould spoilage, a different mycoflora has been found to exist due to the low oxygen levels in the package

(Hocking, 1994). 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). On vacuum maturing Cheddar cheese from Australia, the major species causing thread mould spoilage was found to be *C. cladosporioides* (Hocking and Faedo, 1992) and on a vacuum maturing Argentinean hard cheese similar to Cheddar, the major species causing thread mould spoilage was *Ph. glomerata* (Basilico, de Basilico, Chiericatti, and Vinderola, 2001). In vacuum packaged Norvegia and Jarlsberg cheese from Norway, the major mould species causing spoilage was found to be *P. roqueforti* (Kure and Skaar, 2000).

MAP has been found to reduce the growth rate of moulds, as most mould species are sensitive to increased levels of CO₂ (Smith, Oraikul, Koersen, Jackson, and Lawrence, 1986; Day, 1992). However, MAP on its own is not always successful in the prevention of mould growth on cheese (Floros, Nielsen and Farkas, 1999; Vermeiren, Devlieghere, Beest, Kruijf and Debevere, 1999) because of residual levels of O₂ that can occur in the package and the tolerance of some spoilage moulds e.g. *P. roqueforti* to low O₂ concentrations and increased CO₂ concentrations (Hocking, 1994; Haasum and Nielsen, 1998a; Taniwaki, Hocking, Pitt and Fleet, 2001). As a result MAP is sometimes used in conjunction with oxygen scavengers (Alves, Isabel, Sarantopoulos, Fernandez and Faria, 1996). Oxygen scavengers commonly used are in the form of a sachet that is attached to the interior of the packaging material (Smith, Hoshino and Abe, 1995) or they may be incorporated into the packaging structure of materials (Floros *et al.*, 1999). Low molecular weight ingredients may be dissolved or distributed in a packaging plastic or the plastic may be made from a polymeric scavenger (Rooney, 1995). This enables the oxygen scavenger to have greater contact with the gaseous environment (Rooney, 1995).

Knowledge on the mould species that cause spoilage of MAP South African Cheddar cheese is lacking. The objective of this study was thus to identify the mould species present on South African shredded Cheddar cheese before and after 16 weeks of storage in different modified atmospheres, with and without oxygen scavengers incorporated into the packaging film.

2.2.2 Materials and methods

2.2.2.1 Packaging materials

Refer to previous section (2.1.2.).

2.2.2.2 Packaging treatments of shredded Cheddar cheese

Refer to previous section (2.1.2.).

2.2.2.3 Storage period of shredded Cheddar cheese

Refer to previous section (2.1.2.).

2.2.2.4 Mould isolation

Ten gram quantities of the cheese samples were weighed and macerated in 90 ml of sterile 2% (w/v) sodium citrate (Saarchem Ltd., Krugersdorp, South Africa) solution at a temperature of 45 °C with the aid of a Stomacher Lab Blender 400 (Seward Laboratory, London, UK) to achieve an initial 10^{-1} cheese emulsion. Further decimal dilutions were prepared in the same diluent and 0.1 ml portions were surface plated on Malt extract agar (MEA) (Samson and Van Reenen-Hoekstra, 1988; Pitt, 1988) with the antibiotic Rifampicin added at a level of 50 mg/ litre of media (Van Dyk, 2003) and incubated at 25 °C for 14 days initially (week 0) and for 21 days at 16 weeks (Samson and Van Reenen-Hoekstra, 1988). The extended incubation time was used because it was assumed that the modified atmospheres would affect the fungal spores thus slowing down the rate of growth (Haasum and Nielsen, 1996; Haasum and Nielsen, 1998b). All of the resulting colonies from the dilution plates were then re-plated by taking a small amount of hyphae of each colony and plating them on MEA until pure colonies were achieved (Pitt and Hocking, 1997). Each colony represented one isolate.

2.2.2.5 Mould identification

The *Penicillium* species were identified to species level according to Pitt (1988) and Barnett and Hunter (1998). The *Fusarium* species were identified according to Nelson, Toussoun and Marasas (1983). The *Aspergillus* species were identified according to Kilch and Pitt (1988) and Ellis (1971). The *Cladosporium* and *Alternaria* species were identified according to Ellis (1971). *Phoma* and *Amerosporium* species were identified according to Sutton (1980) and Baxter, Rong, Roux, Schutte and Van

der Linde (1994). *Cylindrocarpon* species were identified according to Domsch, Gams and Anderson (1980) and Baxter *et al.*, (1994). *Sclerophoma* species were identified according to Sutton (1980).

2.2.3 Results

2.2.3.1 Mould species isolated from shredded Cheddar cheese at 0 weeks and after 16 weeks of storage at 5 ± 1 °C packaged in treatments 1 – 6.

The mould species isolated initially (week 0) from the 6 treatments were grouped together in Table 2.2.1 because it was assumed that the time between packaging and plating out the cheese i.e. 1 day, was not sufficient for the modified atmospheres to have an effect on the mycoflora of the cheese.

Initially (week 0), 17 isolates were obtained from the cheese and they belonged to 14 different species (Table 2.2.1). Of the 17 isolates, eight were from the genus *Penicillium*, making it the predominant genus at 41%. Three of the species isolated from the cheese at week 0 were also isolated at 16 weeks. *C. cladosporioides* was isolated at week 16 from the cheese in treatments 5 and 3. *P. expansum* was isolated from the shredded Cheddar cheese packaged in treatment 2 and *P. funiculosum* from the cheese packaged in treatment 5 at 16 weeks.

After a storage period of 16 weeks, the mycoflora differed according to the atmosphere in which the shredded Cheddar cheese was stored. In the shredded Cheddar cheese packaged in treatment 1, 17 isolates belonging to three species were isolated i.e. *P. crustosum*, *P. solitum* and *Am. polynematoides* (Table 2.2.1). The predominant mould species was *P. solitum* at 59% followed by *P. crustosum* at 29%. One isolate of *Am. polynematoides* was identified while one isolate was sterile i.e. did not produce spores and thus could not be identified (Table 2.2.1).

Storage period	0 weeks		16 weeks												Total
			Air		Air		80% CO ₂ / 17% N ₂ / 3% O ₂		80% CO ₂ / 17% N ₂ / 3% O ₂		73% CO ₂ / 27% N ₂		73% CO ₂ / 27% N ₂		
Type of film	Isolates from all the treatments combined (n = 18)		Control film (n = 3)		Oxygen scavenging film (n = 3)		Control film (n = 3)		Oxygen scavenging film (n = 3)		Control film (n = 3)		Oxygen scavenging film (n = 3)		
Treatment	Treatments 1 - 6		Treatment 1		Treatment 4		Treatment 2		Treatment 5		Treatment 3		Treatment 6		
Mould species	Number of Isolates	% of Total	Number of isolates	% of total	Number of isolates	% of total	Number of isolates	% of total	Number of isolates	% of total	Number of isolates	% of total	Number of isolates	% of total	
<i>Penicillium thomii</i>	1	5.9													1
<i>Penicillium funiculosum</i>	1	5.9							1	14.3					2
<i>Penicillium expansum</i>	2	11.8					1	16.7							3
<i>Penicillium decumbens</i>	1	5.9													1
<i>Penicillium citreonigrum</i>	1	5.9													1
<i>Penicillium chrysogenum</i>	1	5.9													1
<i>Penicillium solitum</i>			10	58.8	4	50					2	28.6			16
<i>Penicillium crustosum</i>			5	29.4	2	25	1	16.7							7
<i>Penicillium verrucosum</i>							1	16.7							1
<i>Penicillium janthinellium</i>											1	14.3			1
<i>Penicillium roqueforti</i>													1	33.3	1
<i>Penicillium minioluteum</i>							1	16.7							1
<i>Sclerophoma sp.</i>	1	5.9													1
<i>Phoma epicoccina</i>	1	5.9													1
<i>Phoma eupyrena</i>	1	5.9													1
<i>Phoma sorghina</i>											1	14.3			1
<i>Cladosporium cladosporioides</i>	1	5.9							3	28.6	1	14.3			5
<i>Aspergillus ustus</i>	1	5.9													1
<i>Aspergillus puniceus</i>	1	5.9													1
<i>Fusarium oxysporum</i>									1	14.3					1
<i>Fusarium solani</i>													1	33.3	1
<i>Alternaria alternata</i>	2	11.8													2
<i>Cylindrocarpon sp.</i>	2	11.8													2
<i>Amerosporium polynematoides</i>			1	5.9											1
Sterile isolates			1	5.9	2	25	2	33.3	3	42.9	1	14.3	1	33.3	10
Unknown											1	14.3			1

Total	17	100	17	100	8	100	6	100	8	100	7	100	3	100
-------	----	-----	----	-----	---	-----	---	-----	---	-----	---	-----	---	-----

Table 2.2.1: Mould species isolated from shredded Cheddar cheese at 0 weeks and after 16 weeks of storage at 5 ± 1 °C packaged in modified atmospheres combined with an oxygen scavenging and control film (treatments 1-6).

shredded Cheddar cheese in treatment 4, six isolates were identified and they belonged to 2 species, which were *P. roqueforti* and *P. solitum* (Table 2.2.1). *P. roqueforti* was the major species making up 50% of the total isolates while *P. crumenosa* made up 25% of the total isolates. In treatment 5, 100% of the isolates were *P. roqueforti* and *P. solitum* were not identified. The species isolated in treatment 6 were *P. roqueforti* and *P. solitum* and they made up 100% of the total isolates. In treatment 1, 100% of the isolates were *P. roqueforti* and *P. solitum* and they made up 100% of the total isolates. In treatment 2, 100% of the isolates were *P. roqueforti* and *P. solitum* and they made up 100% of the total isolates. In treatment 3, 100% of the isolates were *P. roqueforti* and *P. solitum* and they made up 100% of the total isolates. In treatment 4, 100% of the isolates were *P. roqueforti* and *P. solitum* and they made up 100% of the total isolates. In treatment 5, 100% of the isolates were *P. roqueforti* and *P. solitum* and they made up 100% of the total isolates. In treatment 6, 100% of the isolates were *P. roqueforti* and *P. solitum* and they made up 100% of the total isolates.

In the shredded Cheddar cheese in treatment 4, six isolates were identified and they belonged to 2 species, which were *P. crustosum* and *P. solitum* (Table 2.2.1). *P. solitum* was the major species making up 50% of the total isolates while *P. crustosum* consisted of 25% of the total isolates.

Two isolates were sterile and could not be identified (Table 2.2.1). The species identified in treatments 1 and 4 were the same except that one isolate of *Am. polynematoides* was isolated from the cheese in treatment 1. In addition, the species isolated from the cheese in treatment 4 were fewer by 9 isolates. None of the species identified in treatments 1 and 4, was also isolated from the cheese initially (week 0). *P. solitum* was also isolated at week 16 from the cheese packaged in treatment 3 and *P. crustosum* was also isolated at week 16 from the cheese packaged in treatment 2.

In the shredded Cheddar cheese packaged in treatment 2, four isolates were identified, all belonging to the genus *Penicillium* (Table 2.2.1). They were *P. expansum*, *P. crustosum*, *P. verrucosum* and *P. minioluteum*. Two isolates were sterile and could not be identified (Table 2.2.1). In the shredded Cheddar cheese in treatment 5, there were a total of 8 isolates identified. Three were sterile and could not be identified while the other 5 were 3 isolates of *C. cladosporioides* and one isolate each of *P. funiculosum* and *Fusarium oxysporum* (Table 2.2.1). The species isolated in treatments 2 and 5 were not similar (Table 2.2.1). Apart from *P. expansum*, also isolated from the cheese initially and *P. crustosum* also isolated from the air packaged cheeses (treatments 1 and 4), none of the other species isolated from the cheese in treatment 2 was isolated from the shredded Cheddar cheese in initially (week 0) as well as from the other treatments. Out of the species isolated from the shredded Cheddar cheese in treatment 5, *C. cladosporioides* was also isolated initially (week 0) as well as from the cheese in treatment 3 at 16 weeks (Table 2.2.1).

Seven isolates were obtained from the shredded Cheddar cheese packaged in treatment 3. Three of the isolates (43%) were *Penicillium* species and they included, two isolates of *P. solitum* and one isolate of *P. janthinellium*. The other isolates were *Ph. sorghina* and *C. cladosporioides*. One isolate was sterile and could not be identified while the other one was unknown (Table 2.2.1). *P. roqueforti* and *F. solani* were the only moulds identified in the shredded Cheddar cheese packaged in

treatment 6 while one isolate was sterile and could not be identified (Table 2.2.1). Neither *P. roqueforti* nor *F. solani* were isolated from the shredded Cheddar cheese in the other treatments initially (week 0) or at 16 weeks. While of the species isolated from treatment 3, *P. solitum* was also isolated from the air packaged cheeses (treatment 1 and 4) and *C. cladosporioides* was also isolated from the cheese initially (week 0) and at week 16 in the cheese packaged in treatment 5.

2.2.4 Discussion

Three of the species isolated from the cheese initially (week 0) were also isolated at 16 weeks from the shredded Cheddar cheese packaged in the 6 treatments. *C. cladosporioides* was isolated at week 16 from the cheese in treatments 5 and 3. *P. expansum* was isolated from the shredded Cheddar cheese packaged in treatment 2 and *P. funiculosum* was isolated from the cheese packaged in treatment 5 at 16 weeks. Since only 3 species out of 17 isolated initially were isolated from the cheese packaged in treatments 1 - 6 at 16 weeks this indicates that the storage period, the gaseous atmospheres and the action of the oxygen scavenging film may have affected the mycoflora of the cheese since the species identified at 16 weeks differed from those isolated initially. The species isolated at 16 weeks also may have adapted to grow in cheese and survive in the atmosphere in which they were stored.

2.2.4.1 Mould species isolated from shredded Cheddar cheese packaged in treatments 1 – 6 at week 0.

Penicillium species were found to be predominant in the air atmosphere initially (week 0) at 41% of the 17 isolates (Table 2.2.1). Five of the species identified initially (week 0) have previously been isolated from different types of cheese. *P. funiculosum* has previously been isolated from Gouda and Edam cheese in the Netherlands (Northolt *et al.*, 1980). *P. expansum*, has previously been isolated from Norvegia and Jarlsberg cheese (Kure and Skaar, 2000), Kasar cheese (Aran and Eke, 1987) and as a contaminant in an indoor cheese-making environment (Kure, Skaar and Brendehaug, 2004). While *P. chrysogenum* has been isolated from Van herby cheese and Van herby pickled white cheese from Turkey (Kivanc, 1990) and hard, semi-hard and semi-soft cheeses of European origin (Lund *et al.*, 1995).

Alternaria alternata has previously been isolated from cheese as a minor contaminant. It has been isolated from vacuum maturing Cheddar cheese affected by thread mould spoilage (Hocking and Faedo, 1992), Norvegia and Jarlsberg cheese (Kure and Skaar, 2000) and as a contaminant from an indoor cheese-making environment (Basilico *et al.*, 2001). *C. cladosporioides* has been isolated from Gouda and Edam cheese in the Netherlands (Northolt *et al.*, 1980) and from indoor cheese making environments including the air, vats, processing, packaging and ripening rooms (Lund *et al.*, 1995; Basilico *et al.*, 2001; Kure *et al.*, 2004).

To the best of our knowledge, *P. thomii*, *P. citreonigrum*, *P. decumbens*, *Cylindrocarpon* sp., *Sclerophoma* sp., *Ph. sorghina*, *Ph. epicoccina*, *Ph. eupyrena*, *As. ustus* and *As. puniceus*, have not previously been isolated from cheese. *P. thomii* has been isolated from decaying wood, fruit (Pitt, 1988; Frisvad, 1988) as well as pistachios (Heperkan, Aran and Ayfer, 1994) and peanuts (Pitt, Hocking, Bhudhasamai, Miscamble, Wheeler and Tanboon-Ek, 1993), while *P. decumbens* has been isolated from soils, decaying vegetation and foods (Pitt, 1988) and *P. citreonigrum* has been isolated as a contaminant from an indoor cheese factory environment (Kure *et al.*, 2004). While *As. ustus* and *As. puniceus* have been isolated predominantly from soils (Kilch and Pitt, 1988). *Phoma* species are often isolated from soil and are associated with dead and living plant material (Sutton, 1980; Hocking and Faedo, 1992). However, *Phoma* species other than those isolated in this study have been previously isolated from other cheeses (Hocking and Faedo, 1992; Fente-Sampayo, Vazquez-Belda, Franco-Abuin, Quinto-Fernandez, Rodruguez-Otero and Cepeda-Saez, 1995; Kure and Skaar, 2000; Basilico *et al.*, 2001; Kure *et al.*, 2001).

The species isolated from the shredded Cheddar cheese initially indicate that the mycoflora of South African Cheddar cheese when initially packaged is similar to the mycoflora of other cheeses as reported in literature (Northolt *et al.*, 1980; Aran and Eke, 1987; Hocking and Faedo, 1992; Lund *et al.*, 1995; Kure and Skaar, 2000). However there were differences since 10 of the 17 species isolated initially have not previously been isolated from other cheeses or cheese making environments (Kilch and Pitt, 1988; Pitt and Hocking, 1997). This indicates that these species may be specific to the factory in the Western Cape of South Africa and could be contaminants

i.e. not a natural part of the cheese mycoflora and thus may not be adapted to growth conditions in cheese (Kure *et al.*, 2004)

2.2.4.2 Mould species isolated from shredded Cheddar cheese packaged in air + control film (treatment 1) and air + oxygen scavenging film (treatment 4) and stored at $5 \pm 1^\circ\text{C}$ for 16 weeks

Of the isolates obtained from the shredded Cheddar cheese packaged in treatment 1, 88% were *Penicillium* species while, 75% of isolates in the cheese in treatment 4 were *Penicillium* species. This is consistent with literature as most species isolated from cheese of various types are of the genus *Penicillium* (Lund *et al.*, 1995; Hocking, 1997; Pitt and Hocking, 1997). The predominance of *Penicillium* species in the air atmosphere (treatment 1 and 4) may be due to the fact that *Penicillium* species with the exception of *P. roqueforti*, have been found to be greatly inhibited by low oxygen atmospheres of 1% or less O_2 (Yanai, Ishitani and Kojo, 1980 as cited by Hocking and Taniwaki, 1997; Magan and Lacey, 1984) thus packaging in air may have selected for *Penicillium* species.

The isolates in the cheese in treatment 4 were fewer by 9 isolates than those in treatment 1 and the difference between the species isolated from the cheese in treatment 4 and from the cheese in treatment 1 was that one isolate of *Am. polynematoides* was isolated from the cheese in treatment 1. This may have been due to lower levels of oxygen, which inhibited mould growth, however the oxygen levels may not have been sufficiently reduced in the oxygen scavenging film to lead to a difference in the composition of the mycoflora.

P. solitum was isolated in week 16 on the shredded Cheddar cheese packaged in treatment 1 (59%) and in treatment 4 (29%) of all isolates (Table 2.2.1). It is a common spoilage mould on various types cheese (Frisvad, 1988). It has also been isolated from Norvegia and Jarlsburg cheeses at the retail level (11%) while at the factory level it was 13.2% on Norvegia cheese and 19.1% of all isolates on Jarlsburg cheese. Lund *et al.*, (1995) also identified *P. solitum* on various hard, semi-hard and semi-soft cheeses from different European countries. In a study on the predominant contaminant moulds present in an indoor cheese-making environment, Kure *et al.*, (2004) found it to be present as a contaminant in the air environment of a cheese

factory. Lund *et al.*, (1995) and Kure and Skaar (2000) noted that *P. solitum* species were mainly isolated from cheese stored at low temperatures of < 5 °C. The low storage temperature used during this study i.e. 5 ± 1 °C may have selected for the growth of *P. solitum* due to its ability to grow in cheese and cause spoilage at low temperatures.

P. crustosum was isolated from the shredded Cheddar cheese packaged in treatment 1 and treatment 4 (29% and 25% of all isolates respectively) (Table 2.2.1). It has been isolated from vacuum packaged Norvegia and Jarlsburg cheese both on retail packs and at the factory level (Kure and Skaar, 2000; Kure *et al.*, 2001). Aran and Eke (1987) isolated *P. crustosum* from mouldy Kasar cheese in Turkey, while Tsai, Liewen and Bullerman, (1988) isolated it from surplus commodity cheese and Lund *et al.*, (1995) isolated it from hard and semi-hard cheeses of European origin.

Three species were isolated in treatment 1 and 2 species in treatment 4 at 16 weeks as compared to 14 species isolated from the cheese initially (week 0). *P. solitum* and *P. crustosum* which were the predominant moulds in treatments 1 and 4 at 16 weeks were not among the species isolated initially (week 0). In the shredded Cheddar cheese packaged in treatments 1 and 4, oxygen was not a limiting factor nor was CO₂ present to inhibit mould growth. This indicates that the low temperature of storage and the ability to grow in cheese may have selected for *P. solitum* and *P. crustosum*.

2.2.4.3 Mould species isolated from shredded Cheddar cheese packaged in 80% CO₂ / 17% N₂ / 3% O₂ + control film (treatment 2) and 80% CO₂ / 17% N₂ / 3% O₂ + oxygen scavenging film (treatment 5) and stored at 5 ± 1 °C for 16 weeks.

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 moulds (Hocking and Taniwaki, 1997). However, when high levels of CO₂ are combined with O₂ the inhibitory effect of CO₂ is reduced (Taniwaki *et al.*, 2001). Thus, in the 80% CO₂ / 17% N₂ / 3% O₂ atmosphere, the high level of CO₂ i.e. 80% would inhibit the growth of moulds on cheese, however the 3% O₂ would allow the growth of certain moulds able to grow at this low O₂ oxygen and high CO₂ concentration.

In the shredded Cheddar cheese in treatment 2, all the 4 isolates identified were *Penicillium* species except for 2 unknown isolates (Table 2.2.1). The residual 3% oxygen in that atmosphere may have encouraged the growth of the *Penicillium* species as they are only sensitive to oxygen conditions below 1% (Yanai, *et al.*, 1980 as cited by Hocking and Taniwaki, 1997; Magan and Lacey, 1984). Three of the isolates i.e. *P. expansum*, *P. crustosum* and *P. verrucosum* have been found to be able to grow under low O₂ conditions (Golding, 1945; Hocking and Faedo, 1992; Basilico *et al.*, 2001).

One isolate of *P. verrucosum* was isolated from the shredded Cheddar cheese packaged in treatment 2 (Table 2.2.1). It has also been isolated from vacuum packaged Cheddar cheese affected by thread mould spoilage, which may indicate an ability to grow under low O₂ concentrations (Hocking and Faedo, 1992). According to Haasum and Nielsen (1998a), its growth is greatly inhibited by CO₂. An atmosphere of 25% CO₂ / 75% N₂ resulted in 68% inhibition in its colony diameter (Haasum and Nielsen, 1998a). Similarly Floros *et al.*, (1999) found that the colony diameter of *P. verrucosum* was increasingly reduced under levels of CO₂ ranging from 5 – 25%. Lund *et al.*, (1995) noted that *P. verrucosum* species were mainly isolated from hard, semi hard and semi soft cheeses from various European countries stored at low temperatures of < 5 °C which may indicate an adaptation to growth at low temperatures.

P. expansum was also isolated from the shredded Cheddar cheese packaged in treatment 2 (Table 2.2.1). According to Golding (1945), *P. expansum* has low requirements for O₂ oxygen and its growth is little affected by low levels of O₂. In a gaseous atmosphere of 2.1% oxygen with the balance as N₂, its growth was found to be 86% of its growth in air. This would indicate a possible ability to grow under the 3% oxygen in the 80% CO₂ / 17% N₂ / 3% O₂ atmosphere. Hocking (1994) also stated that *P. expansum* has been isolated from MAP Cheddar cheese in Australia as one of the less common species, which indicates an ability to grow under elevated levels of CO₂.

One isolate of *P. crustosum* was isolated from the shredded Cheddar cheese packaged in treatment 2 (Table 2.2.1). It has also been isolated from vacuum packaged Cheddar

cheese affected by thread mould spoilage (Hocking and Faedo, 1992) and a vacuum packaged hard cheese similar to Cheddar also affected by thread mould spoilage (Basilico *et al.*, 2001) at 2.1% of total isolates which would indicate an ability to grow under low oxygen concentrations.

Unlike the isolates from the shredded Cheddar cheese in treatment 2, only one isolate was isolated from the shredded Cheddar cheese packaged in the 80% CO₂/ 17% N₂/ 3% O₂ + oxygen scavenging film (treatment 5), the oxygen levels were expected to be lower than in the same atmosphere in the control film (treatment 2) so the species isolated from the cheese stored in this atmosphere would be more tolerant to low oxygen conditions than those isolated from the cheese treatment 2.

One isolate of *F. oxysporum* was isolated from the shredded Cheddar cheese packaged in treatment 5 (Table 2.2.1). *F. oxysporum* has an ability to grow in MAP conditions (Taniwaki *et al.*, 2001). It has been implicated in the spoilage of UHT processed fruit juice (Hocking, 1990) and it has been isolated from vacuum packaged maturing Cheddar cheese affected by thread mould spoilage (Hocking and Faedo, 1992) which would indicate an ability to grow under low oxygen conditions. Taniwaki *et al.*, (2001) found that *F. oxysporum* did not grow in an atmosphere of 20% or 40% CO₂ combined with 0.5% O₂ and the balance as N₂. However, it grew in 20% or 40% CO₂ combined with 1% O₂ or 5% O₂ indicating that it is able to grow at low oxygen concentration along with elevated levels of CO₂. In 20% CO₂ combined with 1% O₂ it had a 50% reduction in colony diameter compared to air and with 20% CO₂ combined with 5% O₂ the reduction in colony diameter was 40%. In the atmosphere containing 40% CO₂, the reduction in colony diameter was 55% when combined with 5% O₂ and a 48% reduction in colony diameter was experienced when 40% CO₂ was combined with 1% O₂ compared to the growth in air. The colony diameter of *F. oxysporum* was slightly affected by changes in O₂ from 5% to 1%, which indicates an adaptation to low oxygen concentrations (Taniwaki *et al.*, 2001).

Three isolates of *C. cladosporioides* were isolated at week 16 from the cheese packaged in treatment 5 (Table 2.2.1). According to Hocking (1994), *C. cladosporioides* has been found to be part of the spoilage mycoflora of MAP Cheddar cheese in Australia as one of the less frequent species. This indicates that it is able to grow under high CO₂ and low O₂ conditions. *C. cladosporioides* was found to be the

predominant species (30% of total isolates) causing thread mould spoilage of vacuum maturing Cheddar cheese (Hocking and Faedo, 1992) implying an ability to grow under low O₂ concentration.

Unlike the isolates from the shredded Cheddar cheese in treatment 2, only one isolate from the cheese in treatment 5 was a *Penicillium* species. The oxygen scavenger in treatment 5 may have lowered the oxygen levels to below 1%, which was unfavourable for the growth of *Penicillium* species (Yanai *et al.*, 1980 as cited by Hocking and Taniwaki, 1997; Magan and Lacey, 1984). The species isolated from the cheese in treatment 2 and treatment 5 were different indicating that the presence of oxygen scavengers changed the atmosphere which in turn influenced the mycoflora of the cheese. In addition, 3 species were isolated in the cheese in treatment 5 as opposed to 4 species identified in the shredded Cheddar cheese in treatment 2 (Table 2.2.1). This may be because the atmosphere in treatment 5 was more restrictive to the growth of moulds due to the lower oxygen levels (Pitt and Hocking, 1997).

2.2.4.4 Mould species isolated from shredded Cheddar cheese packaged in 73% CO₂ / 27% N₂ + control film (treatment 3) and 73% CO₂ / 27% N₂ + oxygen scavenging film (treatment 6) stored at 5 ± 1 °C for 16 weeks.

In the 73% CO₂ / 27% N₂ atmosphere the CO₂ levels were high and the oxygen levels were expected to be low i.e. below 0.05%. Thus the moulds that grew in this atmosphere would be those that are psychrotolerant, able to grow under low O₂ levels and resistant to the inhibitory effect of high CO₂ (Hocking and Taniwaki, 1997). In the cheese in treatment 3 and 6, no species were found to predominate as most species were only isolated once or twice.

Three isolates of the genus *Penicillium* were isolated from the shredded Cheddar cheese packaged in 73% CO₂ / 27% N₂ atmosphere in the control film (treatment 3) along with 1 isolate of *C. cladosporioides* and 1 isolate of *Ph. sorghina* (Table 2.2.1). Of the *Penicillium* isolates from the cheese in treatment 3 (73% CO₂ / 27% N₂ atmosphere in the control film), *P. janthinellium* has not previously been reported on cheese. It is described as a soil fungus (Pitt, 1988) and isolates have been found on various types of food including pistachios (Heperkan *et al.*, 1994) and peanuts (Pitt *et al.*, 1993). Two isolates of *P. solitum* were isolated from the cheese in treatment 3.

Hocking, (1994) noted that *P. solitum* along with *P. roqueforti* was the major species causing spoilage of MAP Cheddar cheese in Australia, which indicates an ability to grow under high CO₂ conditions. In addition, *P. solitum* has been isolated from vacuum maturing cheeses affected by thread mould spoilage (Hocking and Faedo, 1992; Basilico *et al.*, 2001), indicating an ability to grow under low oxygen concentrations. *Ph. sorghina* was isolated from the shredded Cheddar cheese in treatment 3 (Table 2.2.1). It hasn't previously been isolated from cheese however other *Phoma* species i.e. *Ph. glomerata* and *Phoma* sp. have been isolated from vacuum maturing cheeses affected by thread mould spoilage (Hocking and Faedo, 1992; Basilico *et al.*, 2001).

In the 73% CO₂ / 27% N₂ + oxygen scavenging film (treatment 6), any residual O₂ in the atmosphere was expected to be absorbed by the oxygen scavengers. So the species that would grow in this atmosphere were expected to be more tolerant to growth under low O₂ and high CO₂ atmospheres as compared to those isolated in the cheese in the 73% CO₂ / 27% N₂ atmosphere in the control film. One isolate each of *P. roqueforti* and *F. solani* were isolated from the cheese in treatment 6 (Table 2.2.1), while 7 isolates belonging to 4 species were isolated from the cheese in treatment 3. The atmosphere in treatment 6 may have been more restricting (unfavourable) to the growth of moulds due to the oxygen scavenging film absorbing residual O₂ in the atmosphere. The species isolated from the cheese in treatment 3 and 6 were not similar which indicates that the action of the oxygen scavengers may have resulted in an alteration of the mycoflora of the cheese between the two treatments. Of the species identified from the shredded Cheddar cheese in treatment 3, *P. solitum* was also isolated from the air packaged cheeses (treatments 1 and 4) while *C. cladosporioides* was also isolated initially (week 0) as well as from the cheese in treatment 3. None of the species isolated from the cheese in treatment 6, were isolated from the cheese at initially (week 0) or in the cheese packaged in the other treatments (1 – 5) at 16 weeks indicating that a selection may have taken place.

P. roqueforti, is the *Penicillium* species which has the lowest requirements for oxygen (Pitt and Hocking, 1997) which explains its ability to grow in the cheese in the modified atmosphere of 73% CO₂ / 27% N₂ in the oxygen scavenging film (treatment 6). Taniwaki *et al.*, (2001) found that *P. roqueforti* did not grow in an atmosphere of

20% or 40% CO₂ combined with 0.5% O₂, however, it grew in 20% or 40% CO₂ combined with 1% O₂ or 5% O₂. In 20% CO₂ combined with 1% O₂ it had a 40% reduction in colony diameter compared to air while in 20% CO₂ combined with 5% O₂, the reduction was 39% of its colony diameter in air. In the atmosphere containing 40% CO₂ and 5% O₂, the reduction was 27% of its colony diameter in air while with 1% O₂ the reduction was 11.5% of its colony diameter in air (Taniwaki *et al.*, 2001). Similarly Van den Tempel and Nielsen (2000) found that *P. roqueforti* could grow in an atmosphere of 25% CO₂ / 74.7% N₂ / 0.3% O₂ on cheese agar indicating that it is able to grow at low oxygen concentrations with elevated levels of CO₂.

Hocking (1994) stated that *P. roqueforti* was the most common mould species together with *P. commune* causing spoilage of MAP retail packs of Cheddar cheese in Australia which indicates a resistance to high levels of CO₂. *P. roqueforti* has also been isolated as the predominant mould species on vacuum packaged Norvegia and Jarlsburg cheeses at 25.8% and 39.5% of all isolates respectively (Kure *et al.*, 2001). Hocking and Faedo, (1992) isolated *P. roqueforti* from vacuum packaged maturing Cheddar cheese affected by thread mould spoilage which would indicate an ability to grow under low oxygen conditions. Taniwaki *et al.*, (2001) similarly noted that *P. roqueforti* is commonly found on cheese, as it is well adapted to growth inside cheese where high CO₂ and low O₂ concentration are experienced. In addition, *P. roqueforti* is a psychrophile and has been found to grow rapidly and vigorously at refrigeration temperatures (Pitt and Hocking, 1997). *P. roqueforti* along with *P. commune* is the most common spoilage mould on cheese of various types (Pitt and Hocking, 1997). This is due to its ability to grow at refrigeration temperatures, at low oxygen concentration, under lipolytic activity, at reduced a_w and it has a resistance to the preservative action of free fatty acids (Hocking, 1994; Pitt and Hocking, 1997).

F. solani was isolated from the shredded Cheddar cheese in the 73% CO₂ / 27% N₂ + oxygen scavenging film (treatment 6) (Table 2.2.1). It has previously been isolated from mouldy Kasar cheese (Aran and Eke, 1987) and from hard, semi hard and semi-soft cheeses from Europe (Lund *et al.*, 1995). Gibb and Walsh (1980) found that *F. solani* was able to grow at 1% O₂ / 99% N₂ to 4% of its colony diameter when grown in air. In 0.1% O₂ / 99.9% N₂ its growth was further inhibited. It formed micro

colonies and its growth was <1% of its diameter in air. This indicates an ability to grow under low O₂ levels.

3 GENERAL DISCUSSION

2.2.5 Conclusions

The 3 modified atmospheres and the packaging film, influenced the mycoflora of South African shredded Cheddar cheese as the mould species isolated initially differed, from those isolated at 16 weeks in the 6 treatments. Initially (week 0) the species isolated were diverse (15 species), however at 16 weeks, the species isolated were fewer in the different treatments indicating that selection took place. The number of species isolated from the shredded Cheddar cheese packaged in the film with oxygen scavengers were also fewer than in the cheese packaged in the control film in the 3 atmospheres at 16 weeks which indicated that the lower oxygen conditions further restricted the mould growth. This study demonstrated that only a small number of species are capable of causing spoilage of MAP and air packaged cheese after storage for 16 weeks and that MAP and O₂ scavengers were effective in controlling the growth of moulds on shredded Cheddar cheese.

cheese due to residual levels of oxygen that contribute to the spoilage and the tolerance of some spoilage moulds to low oxygen concentrations and high carbon dioxide concentrations. (Hocking, 1994; Tangwattana *et al.*, 2001; 2002) was combined with oxygen scavengers in this study to further reduce the residual oxygen levels in the packages and thus extend the shelf life of the cheese.

shredded Cheddar cheese was used in this study because it presents a greater problem than whole cuts of cheese in terms of shelf life as it is not suitable for vacuum packaging and it is exposed to post contamination by air borne micro-organisms after shredding (Alves *et al.*, 1998). Shredded cheese has an increased surface area which enhances its suitability for MAP as its contact with gases is higher. (Alves *et al.*, 1998). The shredded Cheddar cheese samples in this study were packaged in 3 atmospheres i.e. air, 80% CO₂ / 17% N₂ / 3% O₂ and 75% CO₂ / 23% N₂ combined either with an oxygen scavenging or control film. The air atmosphere was used as the control and to demonstrate the efficacy of the oxygen scavenger against large quantities of oxygen i.e. 21% O₂. The 80% CO₂ / 17% N₂ / 3% O₂ atmosphere was used to simulate a situation where the residual oxygen in a package would be 3% e.g.

CHAPTER 3

3 GENERAL DISCUSSION

The manufacture of cheese arose historically as a way to extend the shelf life and conserve the nutritional components of milk. This was done either by acid production and/ or whey removal (Beresford *et al.*, 2001). While cheese does have a longer shelf life than pasteurised milk, it still undergoes spoilage depending primarily on its moisture, salt and preservative content, a_w , pH, gaseous atmosphere surrounding the cheese and the temperature of storage (Day, 1992).

Cheddar, a hard cheese is susceptible to mould growth as the main mode of deterioration due to its low pH, elevated salt concentration and low a_w (Day, 1992; Pitt and Hocking, 1997). Most packages of Cheddar cheese are thus packaged in vacuum or in modified atmospheres in combinations of CO_2 / N_2 which inhibits mould growth due to the absence of O_2 and /or the inhibitory effects of CO_2 (Hocking, 1994). However, since MAP is not always successful in the prevention of mould growth on cheese due to residual levels of oxygen that can occur in the package and the tolerance of some spoilage moulds to low oxygen concentrations and high carbon dioxide concentrations, (Hocking, 1994; Taniwaki *et al.*, 2001) MAP was combined with oxygen scavengers in this study to further reduce the residual oxygen levels in the packages and thus extend the shelf life of the cheese.

Shredded Cheddar cheese was used in this study because it presents a greater problem than whole cuts of cheese in terms of shelf life as it is not suitable for vacuum packaging and it is exposed to post contamination by air borne micro organisms after shredding (Alves *et al.*, 1996). Shredded cheese has an increased surface area, which enhances its suitability for MAP as its contact with gases is higher, (Alves *et al.*, 1996). The shredded Cheddar cheese samples in this study were packaged in 3 atmospheres i.e. air, 80% $CO_2 / 17\% N_2 / 3\% O_2$ and 73% $CO_2 / 27\% N_2$ combined either with an oxygen scavenging or control film. The air atmosphere was used as the control and to demonstrate the efficacy of the oxygen scavenger against large quantities of oxygen i.e. 21% O_2 . The 80% $CO_2 / 17\% N_2 / 3\% O_2$ atmosphere was used to simulate a situation where the residual oxygen in a package would be 3% e.g.

due to machine inefficiency, ingress of oxygen through the package or oxygen trapped within the product. The gas mixture used to package shredded Cheddar cheese industrially is 73% CO₂ / 27% N₂ combined with the control film and this was compared to the other gas mixtures.

According to industrial reports (Fourie, Parmalat, 2003 - personal communication) the shelf life of shredded Cheddar cheese in a 73% CO₂ / 27% N₂ atmosphere in a control film is 12 weeks. Day (1992) also stated that the shelf life of hard cheeses packaged in air under refrigerated storage was 3 - 4 weeks while that of cheese packaged in modified atmospheres was 10 - 12 weeks. The extended time of storage i.e. 16 weeks was used to determine whether the oxygen scavenger would effectively increase the shelf life of the cheese.

The three microbiological groups studied were affected in various ways by the modified atmospheres and the type of film used for packaging. LAB were neither affected by the packaging film, the modified atmosphere nor by the storage period. The growth of yeasts in shredded Cheddar cheese was inhibited by the modified atmospheres containing CO₂ (80% CO₂ / 17% N₂ / 3% O₂, 73% CO₂ / 27% N₂). However, in the air atmosphere, no inhibition of yeasts was noted. The yeast counts in the air atmosphere were higher in the cheese in the control film than in the oxygen scavenging film. Conversely, in the 80% CO₂ / 17% N₂ / 3% O₂ and 73% CO₂ / 27% N₂ atmospheres, the yeast counts were higher in the cheese packaged in the oxygen scavenging film and lower in the control film. The mould counts were highest in the cheese packaged in the air atmosphere, followed by the cheese packaged in the 80% CO₂ / 17% N₂ / 3% O₂ and lastly in the cheese packaged in the 73% CO₂ / 27% N₂. In addition, the growth of moulds in the cheese packaged in the oxygen scavenging film was lower than that in the cheese packaged in the control film in all the gaseous atmospheres. This was probably due to the absorption of oxygen by the oxygen scavengers leading to lower residual oxygen, which resulted in an inhibition of mould growth (Pitt and Hocking, 1997).

An investigation into the moulds species causing spoilage on South African shredded Cheddar cheese in the 3 atmospheres combined either with the oxygen scavenging or control film was carried out. With information on these species causing spoilage in

conjunction with information on their growth requirements, it would be possible to exert greater control over the spoilage of the cheese as well as to optimise hygienic conditions during production. The moulds were isolated initially (0 weeks) to determine which mould species were present initially on the cheese and to compare these species to what grew on the cheese at 16 weeks in order to determine whether the air and modified atmospheres combined with the oxygen scavenging or control film would influence the mould species that grew and caused spoilage.

by using the oxygen scavenger.

The mycoflora in the air packaged cheeses both in the control and oxygen scavenging film were similar. However they took different amounts of time i.e. 4 and 7 weeks respectively to develop mould growth. Only *P. solitum*, *P. crustosum*, and *Am. polynematoides* were isolated from the cheese packaged in the control film while in the oxygen scavenging film, *P. solitum* and *P. crustosum* were isolated. The air packaged cheese in the oxygen scavenging film had lower mould counts than that packaged in the control film at 0.87 and 1.81 log cfu/g respectively. This may have been due to lower levels of oxygen, which inhibited mould growth, however the oxygen levels may not have been sufficiently reduced in the oxygen scavenging film to lead to a difference in the composition of the mycoflora.

The shredded Cheddar cheese packaged in the 73% CO₂ / 27% N₂ atmosphere in the control and oxygen scavenging film (treatments 3 and 6) as well as in the 80% CO₂ / 17% N₂ / 3% O₂ atmosphere in the oxygen scavenging film (treatment 5) took the same amount of time i.e. 12 weeks to develop mould growth however the species identified causing spoilage were different. The modified atmosphere and packaging film in each treatment may have affected the mycoflora of the cheese leading to the difference in the species identified. The average mould counts were however similar in the three treatments at 0.19, 0.25 and 0.16 log cfu/g in treatments 3, 5 and 6 respectively which could be the reason they took the same amount of time to develop visible mould growth. This indicates that both the mould counts and mould species causing spoilage as influenced by the atmosphere and packaging film influenced the time taken to develop visible mould growth in these treatments because the mould species had the ability to grow in that particular environment while the mould counts were influenced by the both the inhibition caused by the presence of CO₂ and the absence of O₂.

The cheese packaged in the 73% CO₂ / 27% N₂ atmosphere both in the oxygen scavenging and control film, had a shelf life of 12 weeks based on visible mould growth indicating that the shelf life with and without the oxygen scavenger was the same. However in the cheese packaged in the oxygen scavenging film the average mould count was slightly lower than that of the cheese packaged in the control film at 0.16 and 0.19 log cfu/g respectively. This implies that if packaging is done effectively resulting in low residual oxygen in this atmosphere, there is only a slight advantage to be gained by using the oxygen scavenger.

A model was thus proposed (Figure 3) to describe the shelf life and mould species causing spoilage of shredded Cheddar cheese packaged in air and the modified atmospheres (73% CO₂ / 27% N₂, 80% CO₂ / 17% N₂ / 3% O₂) combined either with a control or oxygen scavenging film.



Figure 3 Proposed model for the mould species, shelf life of 2-3 weeks and mould species causing spoilage of shredded Cheddar cheese packaged in air and the modified atmospheres (73% CO₂ / 27% N₂, 80% CO₂ / 17% N₂ / 3% O₂) combined either with a control or oxygen scavenging film.

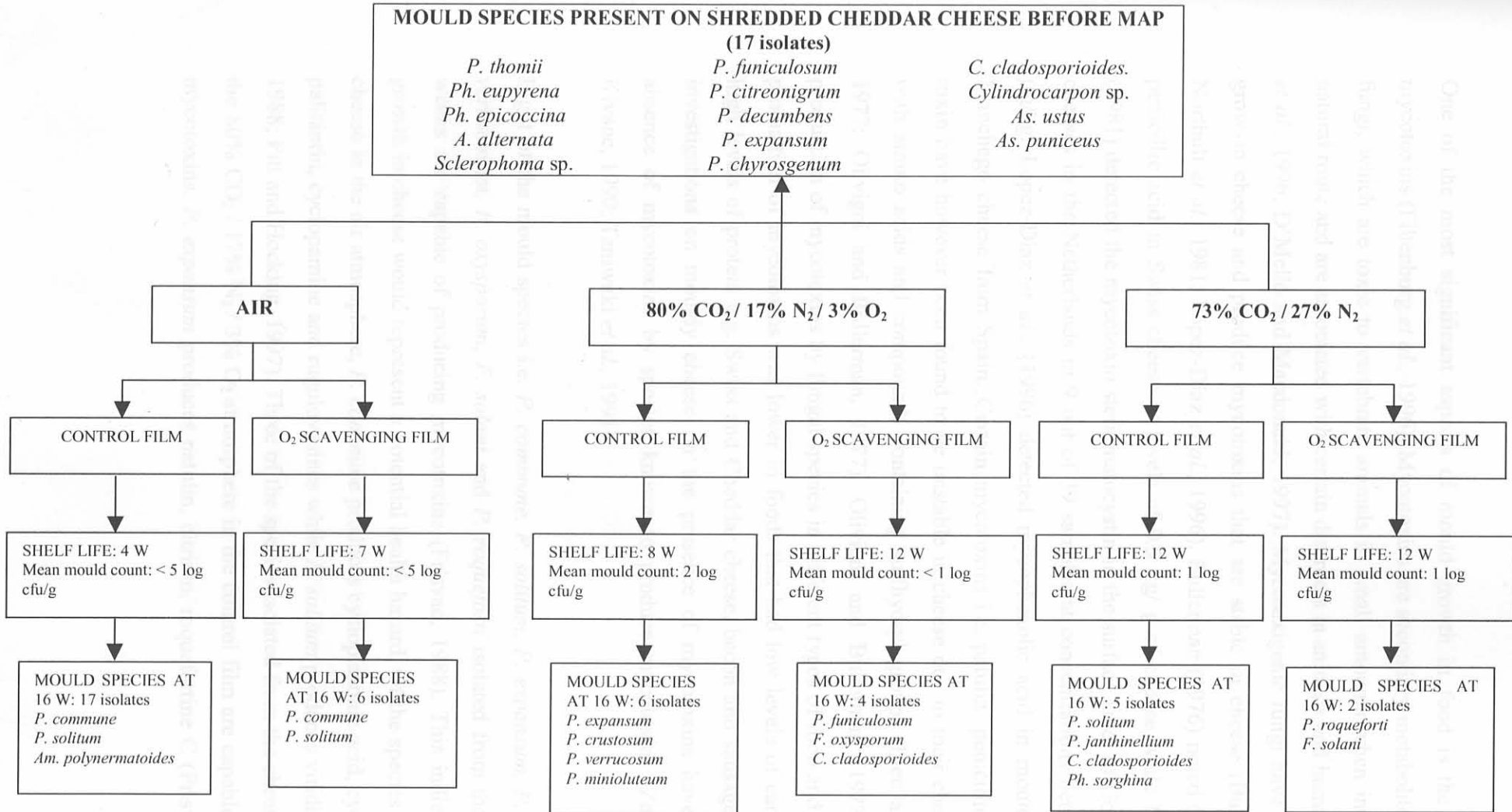


Figure 3 Proposed model for the mould counts, shelf life and mould species isolated from shredded Cheddar cheese packaged in modified atmospheres with and without oxygen scavengers and stored at $5 \pm 1^\circ \text{C}$ for 16 weeks.

One of the most significant aspects of mould growth in food is the production of mycotoxins (Filtenborg *et al.*, 1996). Mycotoxins are secondary metabolites produced by fungi, which are toxic to vertebrate animals in small amounts when introduced via a natural route and are associated with certain disorders in animals and humans (Filtenborg *et al.*, 1996; D'Mello and Macdonald, 1997). Mycotoxigenic fungi have been found to grow in cheese and produce mycotoxins that are stable in cheese (Bullerman, 1976; Northolt *et al.*, 1981; Lopez-Diaz *et al.*, 1996). Bullerman (1976) noted the presence of penicillic acid in Swiss cheese at levels of 0.5 µg/ g of cheese while Northolt *et al.*, (1981) detected the mycotoxin sterigmatocystin in the surface layer of Gouda and Edam cheeses in the Netherlands in 9 out of 39 samples at concentrations between 5 to 600 µg/kg. Lopez-Diaz *et al.*, (1996) detected mycophenolic acid in mouldy samples of Manchego cheese from Spain. Certain mycotoxins i.e. patulin, penicillic acid and PR toxin have however been found to be unstable in cheese due to their chemical reaction with amino acids and compounds containing sulfhydryl groups (Lieu and Bullerman, 1977; Olivigni and Bullerman, 1977). Olivigni and Bullerman (1977) studied the production of mycotoxins by fungal species in different types of food and found that the production of mycotoxins was lower in foods that had low levels of carbohydrate and high levels of protein e.g. Swiss and Cheddar cheese, bacon and sausages. In addition, investigations on mouldy cheese on the presence of mycotoxins have revealed the absence of mycotoxins by species known to produce mycotoxins (Zerfiridis, 1985; Kivanc, 1990; Taniwaki *et al.*, 1991)

Eight of the mould species i.e. *P. commune*, *P. solitum*, *P. expansum*, *P. crustosum*, *P. verrucosum*, *F. oxysporum*, *F. solani* and *P. roqueforti* isolated from the cheese at 16 weeks are capable of producing mycotoxins (Frisvad, 1988). This indicates that their growth in cheese would represent a potential health hazard. Of the species isolated in the cheese in the air atmosphere, *P. commune* produces cyclopiazonic acid, cyclopaldic acid, palitantin, cyclopamine and rugulovasines while *P. solitum* produces viridicatin (Frisvad, 1988; Pitt and Hocking, 1997). Three of the species isolated from the cheese packaged in the 80% CO₂ / 17% N₂ / 3% O₂ atmosphere in the control film are capable of producing mycotoxins. *P. expansum* produces patulin, citrinin, roquefortine C (Frisvad, 1988). *P.*

crustosum produces penitrem A, roquefortine C, isofumigaclavines, terrestric acid, viridicatin and *P. verrucosum* produces ochratoxin A and citrinin (Frisvad, 1988). *F. oxysporum* produces moniliformin, fusaric acid, enniatins and naphthoquinones (Frisvad, 1988). *Ph. sorghina* isolated from the cheese packaged in the 73% CO₂ / 27% N₂ atmosphere in the control film has been found to produce teuazonic acid (Shepard, Thiel, Sydenham, Vleggaar and Marasas, 1991). Both *F. solani* and *P. roqueforti* which were isolated from the shredded Cheddar cheese packaged in the 73% CO₂ / 27% N₂ atmosphere in the oxygen scavenging film are capable of producing mycotoxins. *F. solani* produces naphthoquinones and fusaric acid (Frisvad, 1988). *P. roqueforti* chemotype 1 produces PR-toxin, roquefortine c, mycophenolic acid while chemotype 2 produces patulin, penicillic acid, roquefortine c, mycophenolic acid, botryodiplodin (Frisvad, 1988).

In general, it has been found that elevated levels of CO₂ will inhibit the production of mycotoxins by fungi (Hocking, 1990). Taniwaki *et al.*, (2001) found that *P. commune* and *P. roqueforti* both commonly isolated from cheese, were capable of producing mycotoxins under modified atmospheres. However the amounts of mycotoxins produced were low i.e. were between 0.1% to 17% of the amounts produced in air (Taniwaki *et al.*, 2001). Hocking and Taniwaki (1997) similarly found the production of mycotoxins by several fungal species was greatly reduced and in some cases totally inhibited by packaging in modified atmospheres. Thus, given the instability of mycotoxins in cheese and the inhibited production of mycotoxins in modified atmospheres, the production of toxins could be expected to be a small hazard in the cheese in this study. However, further work should be done to investigate mycotoxin production on cheese in modified atmospheres by the species isolated that are capable of producing mycotoxins.

In order to control the growth of fungal contaminants, knowledge about the effects of environmental factors on fungal development and colonization is needed. A better understanding of the responses of these fungi to combinations of pH, temperature, water activity, salt content and gas mixtures would be of benefit to manufacturers to better control the mould spoilage of cheese as these are the main environmental factors

influencing fungal growth in and on cheese (Stadhouders, 1975; Haasum and Nielsen, 1996). These parameters greatly influence the sporulation and growth of fungi and determine whether or not mould growth will occur on cheese. Information on the growth and sporulation of the fungi under different conditions can be used. The data may be used to develop a mathematical model for the prediction of the shelf life of cheese.

This study has identified the mould species that cause mould contamination of South African Cheddar cheese. The ubiquity of fungi in the environment in which cheeses are manufactured and stored results in the presence of these micro organisms in and on cheese (Kure *et al.*, 2002; Kure *et al.*, 2004). In the interest of reducing the mould spoilage of these cheeses, environmental studies would be needed in the production plants to identify the critical control points for mould contamination in the production of cheese. Fungi should be identified from the cheese factory environment including cheese making equipment, factory air and compressed air, whey and curd, which will enable the source of fungal contamination to be pinpointed.

In addition, for future research, it is recommended that sensory evaluation of the cheese samples during the storage period should be carried out in order to determine the cut off point of shelf life based on sensory evaluation. This is because off flavours could be formed due to enzymatic activity caused by growth of moulds before the cheese is visibly mouldy. Thus, these results could be combined with the microbiological counts and time taken for visible mould growth to be noticed which would give a more accurate indication of the shelf life.

4 CONCLUSIONS

The film with oxygen scavengers was more effective than the control film against mould growth while the 73% CO₂ / 27% N₂ atmosphere resulted in the cheese with the best microbiological qualities of the 3 atmospheres. Thus the packaging combination that best maintained the microbiological quality of the shredded Cheddar cheese was the 73% CO₂ / 27% N₂ atmosphere in the oxygen scavenging film, because it resulted in shredded Cheddar cheese with the lowest mould counts. In addition, the shredded Cheddar cheese packaged in this treatment took 12 weeks to develop visible mould growth along with the cheese packaged in the 73% CO₂ / 27% N₂ atmosphere in the control and oxygen scavenging film and 80% CO₂ / 17% N₂ / 3% O₂ atmosphere in the oxygen scavenging film. Lastly, it had the fewest mould species causing spoilage indicating that the atmosphere was restrictive to the range of species causing spoilage.

The 3 modified atmospheres and the packaging film, influenced the mycoflora of South African shredded Cheddar cheese as the mould species isolated initially differed, from those isolated at 16 weeks in the 6 treatments. The species isolated at 16 weeks may have been adapted to growth in cheese and survival in the atmosphere in which they were stored while those isolated initially could have been contaminants or species not adapted to growth under the storage conditions. The number of species isolated from the shredded Cheddar cheese packaged in the film with oxygen scavengers were also fewer than in the cheese packaged in the control film in the 3 atmospheres at 16 weeks which indicated that the lower oxygen conditions further restricted the mould growth. This study demonstrated that only a small number of species are capable of causing spoilage of MAP and air packaged cheese after storage for 16 weeks at 5 ± 1 °C.

BISHOP, R., MARCY, J.E. AND MOLLER, T., 1996. Improved quality of shredded cheddar cheese, antimicrobials, oxygen scavengers and modified atmosphere packaging. *COA Annual report 92-93*.

5 REFERENCES

ABE, Y. AND KONDOH, Y., 1989. Oxygen absorbers. *In: Brody A.L. (Ed.) Controlled/Modified atmosphere / Vacuum packaging of foods.* Trumbull: Food and Nutrition Press Inc. pp. 149-158.

ALVES, R.M.V., ISABEL, C., SARANTOPOULOS, G., FERNANDES, A.G.F. AND FARIA J., 1996. Stability of sliced Mozzarella cheese in modified atmosphere packaging. *Journal of Food Protection* 59, 838-834.

ARAN, N. AND EKE, D., 1987. Mould mycoflora of Kasar cheese at the stage of consumption. *Food Microbiology* 4, 101-104.

BARNETT, H.L. AND HUNTER, B.B., 1998. *Illustrated Genera of the Imperfect Fungi.* 4th Edition. St. Paul: APS Press.

BASILICO, J.C., DE BASILICO, M.Z., CHERICATTI, C., AND VINDEROLA, C.G. 2001. Characterisation and control of thread mould in cheese. *Letters in Applied Microbiology* 32, 419-423.

BAXTER, A.P., RONG, I.H., ROUX, C., SCHUTTE, L. AND VAN DER LINDE, E., 1994. *Elementary keys to common fungi in South Africa.* Published by, National collection of fungi, Biosystematics Division, Plant Protection Research Institute, Pretoria.

BERESFORD, T.P., FITZSIMONS, N.A., BRENNAN, N.L. AND COGAN, T.M., 2001. Recent advances in cheese microbiology. *International Dairy Journal* 11, 259-274.

BISHOP, R., MARCY, J.E. AND MOLER, T., 1996. Improved quality of shredded cheese, antimycotics, oxygen scavengers and modified atmosphere packaging. *CDR Annual report* 92-93.

BRODY A.L., 1989. Modified atmosphere packaging of meat. In: Brody A.L. (Ed.) *Controlled/ Modified atmosphere/ Vacuum packaging of foods*. Trumbull: Food and Nutrition Press, Inc. pp. 149-158.

BULLERMAN L.B., 1976. Examination of Swiss cheese for incidence of mycotoxin producing moulds. *Journal of Food Science* 41, 26-28.

BULLERMAN, L.B., 1980. Incidence of mycotoxic moulds in domestic and imported cheeses. *Journal of Food Safety* 2, 47-58.

BULLERMAN L.B. AND OLIVIGNI, F.J., 1974. Mycotoxin producing potential of moulds isolated from Cheddar cheese. *Journal of Food Science* 39, 1166-1168.

CHEN, J.H. AND HOTCHKISS, J.H., 1991. Effect of dissolved carbon dioxide on the growth of psychrotrophic organisms in cottage cheese. *Journal of Dairy Science* 74, 2941-2945.

CIBA, 2002. Oxygen absorbers keep freshness in the package. Ciba® SHELPLUS™ O₂. *Ciba Specialty Chemicals*.

DANIELS, J.A., KRISHNAMURTHI, R. AND RIZVI, S.S.H., 1984. A review of effects of carbon dioxide on microbial growth and food quality. *Journal of Food Protection* 48, 532-537.

DAY, B.P.F., 1992. *Guidelines for the good manufacturing and handling of modified atmosphere packed food products*. Gloucestershire: Campden Food and Drink Research Association. pp. 6-9.

DAY, B.P.F., 1998. Active packaging of foods. *CCFRA New Technologies Bulletin* 17, 23-24.

- DEMAN, J.C., ROGOSA, M. AND SHARPE, M.E., 1960. A medium for the cultivation of lactobacilli. *Journal of Bacteriology* 23, 130-135.
- DEVLIEGHERE, F., DEBEVERE, J. AND IMPE, J.V., 1998a. Concentration of carbon dioxide in the water phase as a parameter to model the effect of a modified atmosphere on micro organisms. *International Journal of Food Microbiology* 43, 105-113.
- D'MELLO, J.P.F. AND MACDONALD, A.M.C., 1997. Mycotoxins. *Animal Feed Science and Technology* 69, 155-166.
- DOMSCH, K.H., GAMS, W. AND ANDERSON, T.H., (1980). *Compendium of soil fungi*. Vol 1, London: Academic Press.
- ELLIOT, S.C., VUILLEMARD, J.C. AND EMOND, J.P., 1998. Stability of Mozzarella cheese under modified atmospheres. *Journal of Food Science* 63, 1075-1080.
- ELLIS, M.B., (1971). *Dematiaceus Hyphomycetes*. Commonwealth Mycological Institute. Kew, Surrey: England.
- FANDOS, E.G., SANZ, S. AND OLARTE, C., 2000. Microbiological, physiochemical and sensory characteristics of Cameros cheese packaged under modified atmospheres. *Food Microbiology* 17, 407-414.
- FARBER, J.M., 1991. Microbiological aspects of modified atmosphere packaging technology. – A review. *Journal of Food Protection* 54, 58-70.
- FARBER, J.M., WARBURTON, D.W., GOUR, L. AND MILLING, M., 1990. Microbiological quality of foods packaged under MAP conditions. *Food Microbiology* 7, 327-334.

FENTE-SAMPAYO, C.A., VAZQUEZ-BELDA, B., FRANCO-ABUIN, C. QUINTO-FERNANDEZ, E., RODRIGUEZ-OTERO, J.L. AND CEPEDA-SAEZ, A., 1995. Distribution of fungal genera in cheese and dairies. *Archiv für Lebensmittelhygiene* 46, 49-72.

FILTENBORG, O., FRISVAD, J.C. AND THRANE, U., 1996. Moulds in food spoilage. *International Journal of Food Microbiology* 33, 85-102.

FLEET, G.H., 1990. Yeasts in dairy products. *Journal of Applied Bacteriology* 68, 199-211.

FLOROS, J.D., NIELSEN, P.V. AND FARKAS, J.K., 1999. Advances in modified atmosphere and active packaging with applications in the dairy industry. *Bulletin of the International Dairy Federation* 346, 22-28.

FRISVAD, J.C. 1988. Fungal species and their specific production of mycotoxins. In Samson R. A. and Van Reenen-Hoekstra E. S. (Eds.) *Introduction to Food-Borne Fungi*. Baarn: Centraalbureau voor Schimmelcultures. pp 242 – 243.

GIBB, E. AND WALSH, J.H., 1980. Effect of nutritional factors and carbon dioxide on growth of *Fusarium moniliforme* and other fungi in reduced oxygen concentrations. *Transactions of the British Mycological Society* 74, 111-118.

GOLDING, N.S., 1945. The gas requirements of moulds. IV. A preliminary interpretation of the growth rates of four common mould cultures on the basis of absorbed gases. *Journal of Dairy Science* 28, 737-750.

HAASUM, I., AND NIELSEN, P.V., 1996. Pre-incubation of *Penicillium commune* conidia under modified atmosphere conditions: Influence on growth potential as determined by an impedimetric method. *Journal of Stored Product Research* 32, 329-337.

HAASUM, I., AND NIELSEN, P.V., 1998a. Physiological characterisation of common fungi associated with cheese. *Journal of Food Science* 63, 157-161.

HAASUM, I., AND NIELSEN, P.V., 1998b. Ecophysiological characterization of common food-borne fungi in relation to pH and water activity under various atmospheric compositions. *Journal of Applied Microbiology* 84, 451-460.

HEPERKAN, D., ARAN, N., AND AYFER, M., 1994. Mycoflora and aflatoxin contamination in shelled pistachio nuts. *Journal of the Science of Food and Agriculture* 66, 273-278.

HOCKING A.D., 1990. Responses of fungi to modified atmospheres. *Australian centre for International Research* 25, 70-82.

HOCKING, A.D., 1994. Fungal spoilage of high fat food. *Food Australia* 46, 30-33.

HOCKING, A.D., 1997. Understanding and controlling mould spoilage in cheese. *The Australian Journal of Dairy Technology* 52, 123-124.

HOCKING, A.D. AND FAEDO, M., 1992. Fungi causing thread mould spoilage of vacuum packaged Cheddar cheese during maturation. *International Journal of Food Microbiology* 16, 123-131.

HOCKING, A.D. AND TANIWAKI, M.H., 1997. Growth of fungi in high carbon dioxide and low oxygen atmospheres. In: *Proceedings of the international congress on controlled atmosphere and fumigation in storage of products*. Printco Nicosia Cyprus pp 103-112.

KILCH, M.A. AND PITT, J.I., 1988. *A laboratory guide to common Aspergillus species and their teleomorphs*. North Ryde: Commonwealth Scientific and Industrial Research organization.

KIVANC, M., 1990. Mould growth and presence of aflatoxin in some Turkish cheeses. *Journal of Food Safety* 10, 287-294.

KOSIKOWSKI, F.V. AND MISTRY, V.V., 1997. *Cheese and fermented milk foods: Volume 1: Origins and principles*. Great Falls: Virginia. pp. 109-120.

KOTSIANIS, I.S., GIANNOU, V. AND TZIA, C., 2002. Production and packaging of bakery products using MAP technology. *Trends in Food Science and Technology* 13, 319-324.

KURE, C.F. AND SKAAR, I., 2000. Mould growth on the Norwegian semi-hard cheeses Norvegia and Jarlsberg. *International Journal of Food Microbiology* 62, 133-137.

KURE, C.F., WASTESON, Y., BRENDEHAUG, J. AND SKAAR, I., 2001. Mould contaminants from Jarlsberg and Norvegia cheese blocks from four factories. *International Journal of Food Microbiology* 70, 21-27.

KURE, C.F., SKAAR, I. AND BRENDEHAUG, J., 2004. Mould contamination in production of semi-hard cheese. *International Journal of Food Microbiology* 93, 41-49.

LIEU, F.Y. AND BULLERMAN, L.B., 1977. Production and stability of aflatoxins, penicillic acid and patulin in several substrates. *Journal of Food Science* 42, 1222-1224.

LOPEZ-DIAZ, T.M., SANTOS, J.A., PRIETO, M., GARCIA-LOPEZ, M.L., AND ANDRES, O., 1996. Mycoflora of a traditional Spanish blue cheese. *Netherlands Milk and Dairy Journal* 49, 191-199.

LOWENADLER, J. AND RONNER, U., 1994. Determination of dissolved carbon dioxide by coulometric titration in modified atmosphere systems. *Letters in Applied Microbiology* 18, 285-288.

LUND, F., FILTENBORG, O. AND FRISVAD, J.C., 1995. Associated mycoflora of cheese. *Food Microbiology* 12, 173-180.

MAGAN, N. AND LACEY, J., 1984. Effects of gas composition and water activity on growth of field and storage fungi and their interactions. *Transactions of the British Mycological Society* 82, 305-314.

MANIAR, A.B., MARCY, J.E., BISHOP, R. AND DUNCAN, E., 1994. Modified atmosphere packaging to maintain direct set cottage cheese quality. *Journal of Food Science* 59, 1305-1327.

MANNHEIM, C.H. AND SOFFER, T., 1996. Shelf life extension of cottage cheese by modified atmosphere packaging. *Lebensmittel-Wissenschaft und Technologie* 29, 767-771.

MOIR, C.J., EYLES, J. AND DAVEY, J.A., 1993. Inhibition of pseudomonads in cottage cheese by packaging in atmospheres containing carbon dioxide. *Food Microbiology* 10, 345-351.

MUIR D.D., HUNTER, E.A., BANKS, J.M. AND HORNE, D.S., 1996. Sensory properties of Cheddar cheese: changes during maturation. *Food Research International* 28, 561-568.

NELSON, P.E., TOUSSOUN, T.A. AND MARASAS, W.F.O., 1983. *Fusarium species. An Illustrated Manual for Identification*. University Park: Pennsylvania State University Press.

NORTHOLT, M.D., EGMOND, H.P.V., SOENTORO, P. AND DEIJII, E., 1980. Fungal growth and the presence of sterigmatocystin in hard cheese. *Journal of The Association of Official Analytical Chemists* 62, 115-119.

OLIVIGNI, F.J. AND BULLERMAN, L.B., 1977. Simultaneous production of penicillic acid and patulin by a *Penicillium* species isolated from Cheddar cheese. *Journal of Food Science* 42, 1654-1657.

PARRY, R.T., 1993. Introduction In: Parry, R.T. (Ed.). *Principles and Applications of Modified Atmosphere Packaging of Food*. Glasgow: Blackie Academic and Professional. pp. 14-15.

PITT, J.I., 1988. *A laboratory guide to common Penicillium species*. 2nd Edition. North Ryde: Commonwealth Scientific and Industrial Research organization, Division of Food Processing.

PITT, J.I., HOCKING, A.D., BHUDHASAMAI, K., MISCAMBLE, B.F., WHEELER, K.A., AND TANBOON-EK, P., 1993. The normal mycoflora of commodities from Thailand. 1. Nuts and oilseeds. *International Journal of Food Microbiology* 20, 211-226.

PITT, J.I. AND HOCKING, A.D., 1997. 2nd Edition. *Fungi and Food spoilage*. Blackie Academic and Professional. London. pp. 505-507.

ROBINSON, R.K. (Ed.) 1995. *A colour guide to cheese and fermented milks*. Chapman and Hall: London. pp. 47-48.

ROONEY, M.L., 1995. Active packaging in polymer films. In: Rooney, M. L. (Ed.) *Active Food Packaging*. Glasgow: Blackie Academic and Professional. pp. 74-109.

ROSENTHAL, I., ROSEN, B., BERNSTEIN, S. AND POPEL, G., 1991. Preservation of fresh cheeses in a CO₂-enriched environment. *Milchwissenschaft* 4, 706-708.

SUTTON, B.C., 1989. *The Coniomyxeta: Fungi imperfecti with Pyrenopeziza, Dothidea and Stromatium*. Kew: Commonwealth Mycological Institute.

SAMSON R.A. AND VAN REENEN-HOEKSTRA E.S., 1988. Identification of the common fungi. In Samson R. A and Van Reenen-Hoekstra, E. S. *Introduction to Food Borne Fungi* Baarn: Centraalbureau voor Schimmelcultures. pp. 3-4.

TANIWAKI, M.H. AND DENDER, A.G.F., 1992. Occurrence of toxicogenic moulds in SHEPARD, G.S., THIEL, P.G., SYDENHAM, E.W., VLEGGAR, R. AND MARASAS, W.F.O., 1991. Reversed-phase high-performance liquid chromatography of tenuazonic acid and related tetramic acids. *Journal of Chromatography* 556, 195-205.

SIVERTSVIK, J., JEKSRUD, W.K., VAGANE, A. AND ROSNES, J.T., 2003. Solubility and absorption rate of carbon dioxide onto non-respiring foods. Part 1: Development and validation of experimental apparatus using a monomeric method. *Journal of Food Engineering* 61, 449- 458.

SKANDAMIS, P. N. AND NYCHAS, G. E., 2002. Preservation of fresh meat with active and modified atmosphere conditions. *International Journal of Food Microbiology* 79, 35-45.

SMITH, J.P., OORAikul, B., KOERSEN, W.J., JACKSON, E.D. AND LAWRENCE, R.A. 1986. Novel approach to oxygen control in modified atmosphere packaging of bakery products. *Food Microbiology* 3, 315-320.

SMITH, J.P., RAMASWAMY H.S. AND SIMPSON, B.K., 1990. Developments in food packaging technology. Part 2: Storage aspects. *Trends in Food Science and Technology* 11, 11-18.

SMITH, J.P., HOSHINO, J. AND ABE, Y., 1995. Interactive packaging involving sachet technology. In: Rooney, M. L. (Ed.) *Active Food Packaging*. Glasgow: Blackie Academic and Professional. pp.143-173

SUTTON, B.C., 1980. *The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli and Stromata*. Kew: Commonwealth Mycological Institute.

STADHOUDERS, J., 1975. Microbes in milk and dairy products. An ecological approach. *Netherlands Milk and Dairy Journal* 29, 104-126.

TANIWAKI, M.H. AND DENDER, A.G.F., 1992. Occurrence of toxigenic moulds in Brazilian cheese. *Journal of Food Protection* 55, 187-191.

TANIWAKI, M.H., HOCKING A.D., PITT, J.I. AND FLEET, G.H., 2001. Growth of fungi and mycotoxin production on cheese under modified atmospheres. *International Journal of Food Microbiology* 68, 125-133.

TSAI, W.J., LIEWEN, M.B. AND BULLERMAN, L.B., 1988. Toxicity and sorbate sensitivity of moulds isolated from surplus commodity cheeses. *Journal of Food Protection* 5, 457-462.

TSIGARIDA, E. AND NYCHAS, G.J.E., 2001. Ecophysiological attributes of *Lactobacillus* sp. and *Pseudomonads* sp. on sterile beef fillets in relation to storage temperature and film permeability. *Journal of Applied Microbiology* 90, 696-705.

VAN DEN TEMPEL, T., AND NIELSEN, M.S., 2000. Effects of atmospheric conditions, NaCl and pH on growth and interaction between moulds and yeasts related to blue cheese production. *International Journal of Food Microbiology* 57, 193-199.

VAN DYK, K., 2003. Fungi associated with root and crown rot of wheat and barley in Tanzania. MSc dissertation, University of Pretoria.

VERMEIREN, L., DEVLIEGHERE, F., BEEST, M., KRUIJF, N. AND DEBEVERE, J., 1999. Developments in the active packaging of foods. *Trends in Food Science and Technology* 10, 77-86.

WELTHAGEN, J.J. AND VILJOEN, B.C., 1999. The isolation and identification of yeasts obtained during the manufacture and ripening of Cheddar cheese. *Food Microbiology* 16, 63-73.

YANAI, S., ISHITANI, T., AND KOJO, T. 1980. The effects of low-oxygen atmospheres on the growth of fungi. *Nippon Shokukin Kogyo Gakkai-Shi* 27, 20-24.

ZERFIRIDIS, G.K., 1985. Potential aflatoxin hazards to human health from direct mould growth on Teleme cheese. *Journal of Dairy Science* 68, 2184-2188.