CHAPTER 2

2 RESEARCH

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

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Abstract

Shredded Cheddar cheese samples were packaged with each of 3 atmospheres (air, 80% CO_2 / 17% N_2 / 3% O_2, 73% CO_2 / 27% N_2) 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% CO_2 / 27% N_2 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 CO_2 and N_2 (Elliot, Vuillemard 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 CO_2, N_2 and O_2 (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 O_2 that may occur in the package as well as the tolerance of some spoilage moulds to low O_2 concentrations and high CO_2 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 O_2 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 O_2. Secondly, each packaging film has a characteristic O_2 permeability that allows the transfer of O_2 from the environment into the package. Thirdly, O_2 could enter the package through improper sealing (Smith, Ooraikul, 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 O_2 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 O_2 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 O_2 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 ml/ m^2/ 24 hr/ atm. at 22 °C and 75% RH
2. A laminate film with an oxygen scavenger Ciba® SHELPLUS™ O_2 (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™ O_2/ Linear low density polyethylene. It had an oxygen transmission rate (OTR) < 20 ml/ m^2/ 24 hr/ atm. at 22 °C and 75% 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% O_2 / 0.3% CO_2 / 78.9%), 80% CO_2 / 17% N_2 / 3% O_2, 73% CO_2 / 27% N_2) combined either with an oxygen scavenging or control film as follows: treatment 1 = air + control film, treatment 2 = 80% CO_2 / 17% N_2 / 3% O_2 + control film, treatment 3 = 73% CO_2 / 27% N_2 + control film, treatment 4 = air + oxygen scavenging film, treatment 5 = 80% CO_2 / 17% N_2 / 3% O_2 + oxygen scavenging film, treatment 6 = 73% CO_2 / 27% N_2 + 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 ± 1°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% CO_2 / 17% N_2 / 3% O_2, 73% CO_2 / 27% N_2) 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™ O_2, 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)

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 P value	Moulds 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

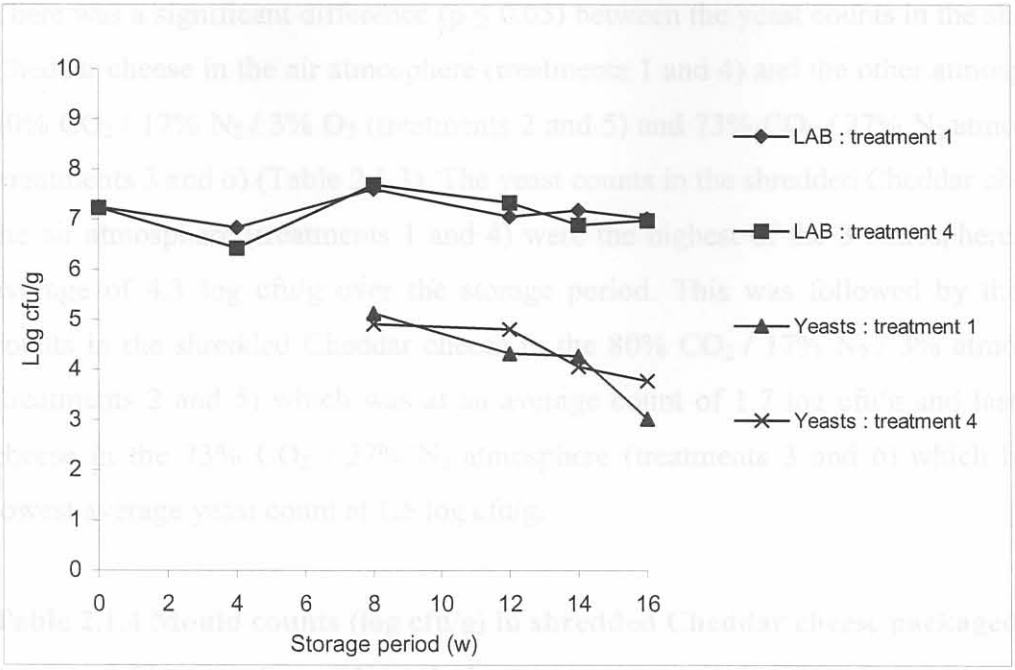

Figure 2.1.1 Lactic acid bacteria and yeast counts in shredded Cheddar cheese packaged in air + control film (treatment 1) and air + oxygen scavenging film (treatment 4) for 16 weeks at 5 ± 1 °C (n=36)

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% CO_2 / 17% N_2 / 3% O_2 (treatments 2 and 5) and 73% CO_2 / 27% N_2 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% CO_2 / 17% N_2 / 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% CO_2 / 27% N_2 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	
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	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 10^5 ND - Not detected below 10^1

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).

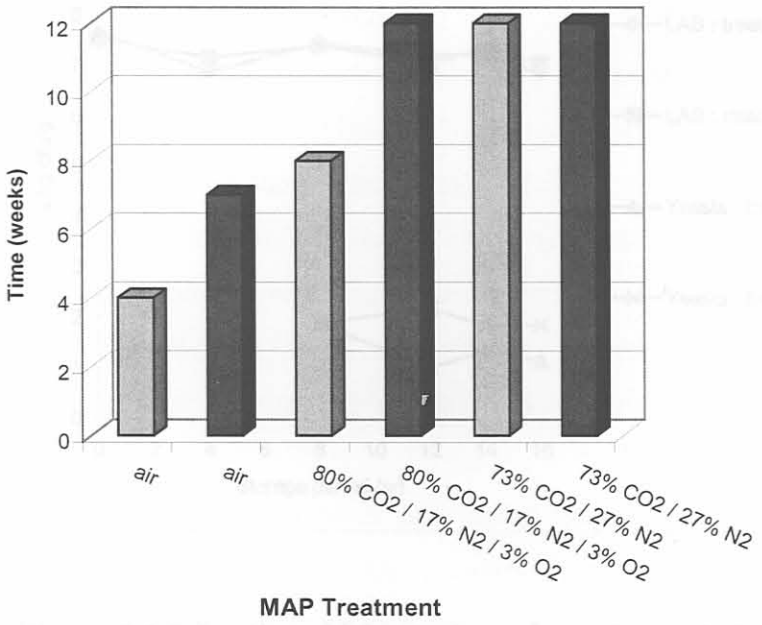

Figure 2.1.2 Shelf life of shredded Cheddar cheese packaged in an oxygen scavenging and control film in 3 atmospheres (air, 80% CO_2 / 17% N_2 / 3% O_2, 73% CO_2 / 27% N_2) and stored at 5 ± 1 °C based on visible mould growth (n=108). Control film ▭ Oxygen scavenging film ■

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% CO_2 / 17% N_2 / 3% O_2 (treatments 2 and 5) and 73% CO_2 / 27% N_2 (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% CO_2 / 17% N_2 / 3% O_2

atmosphere the average mould counts in the cheese was 0.3 log cfu/g and in the cheese in the 73% CO_2 / 27% N_2 atmosphere the average value was 0.2 log cfu/g.

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

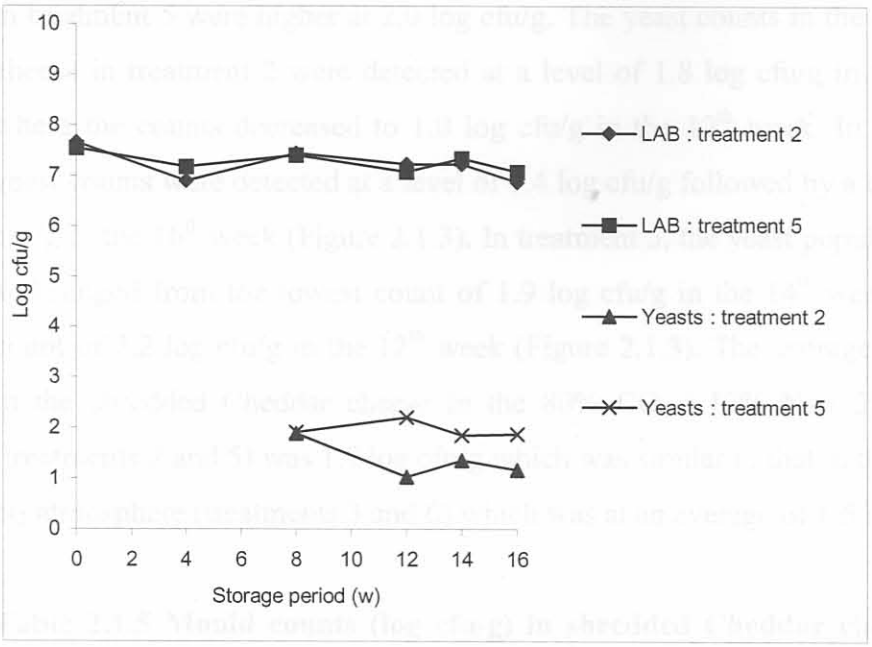

Figure 2.1.3 Lactic acid bacteria and yeast counts in shredded Cheddar cheese packaged in 80% CO_2 / 17% N_2 / 3% O_2 + control film (treatment 2) and 80% CO_2 / 17% N_2 / 3% O_2 + oxygen scavenging film (treatment 5) for 16 weeks at 5 ± 1 °C (n=36)

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% CO_2 / 17% N_2 / 3% O_2 atmosphere (treatments 2 and 5) was 1.7 log cfu/g which was similar to that in the 73% CO_2 / 27% N_2 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% 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)

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 10^5 ND- Not detected below 10^1

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% CO_2 / 17% N_2 / 3% O_2 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% CO_2 / 27% N_2 atmosphere (treatments 3 and 6) which was 0.2 log cfu/g.

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

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.

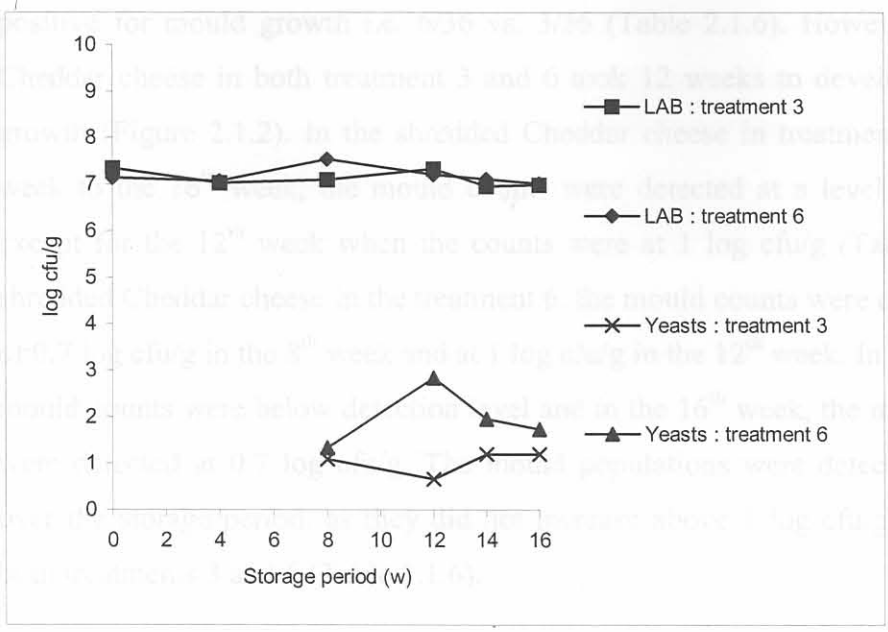

Figure 2.1.4 Lactic acid bacteria and yeast populations in shredded Cheddar cheese packed in 73% CO_2 / 27% N_2 + control film (treatment 3) and 73% CO_2 / 27% N_2 + oxygen scavenging film (treatment 6) and stored at 5 ± 1°C for 16 weeks (n=36).

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 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% 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)

Packaging treatment	Treatment 3		Treatment 6	
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	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 10^5 ND - Not detected below 10^1

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 CO_2 (Parry, 1993). Thus LAB are unaffected or slightly inhibited by CO_2 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% CO_2 / 17% N_2 / 3% O_2 atmosphere (treatments 2 and 5) and 73% CO_2 / 27% N_2 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 CO_2 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 CO_2 (80% CO_2 / 17% N_2 / 3% O_2, 73% CO_2 / 27% N_2). 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% CO_2 / 27% N_2 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 CO_2 concentrations which inhibited their growth (Day, 1992). The yeast populations in the shredded Cheddar cheese in the 73% CO_2 / 27% N_2 atmosphere were similar to the yeast populations in the shredded Cheddar cheese in the 80% CO_2 / 17% N_2 / 3% O_2

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 CO_2 in the 80% CO_2 / 17% N_2 / 3% O_2 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% CO_2 / 90% N_2, 25% CO_2 / 75% N_2, 50% CO_2 / 50% N_2, 75% CO_2 / 25% N_2 and 100% CO_2 stored at 10 °C for 8 weeks. Alves *et al.*, (1996) similarly found that in Mozzarella cheese packaged in 100% CO_2 and stored for 58 days at 7 ± 1 °C there was no growth of yeasts. However, in modified atmospheres of 100% N_2 and 50% CO_2 / 50% N_2, 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 CO_2 to inhibit the growth of moulds as they are sensitive to the inhibitory effect of CO_2 (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% CO_2 /

17% N_2 / 3% O_2. 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% CO_2 / 27% N_2 atmosphere), this can be attributed to the inhibitory effect of high levels of CO_2 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 CO_2 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% CO_2 / 17% N_2 / 3% O_2 atmosphere + oxygen scavenging film (treatment 5) obtained average mould counts similar to those in treatment 3 and 6 (73% CO_2 / 27% N_2 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% CO_2 / 27% N_2). 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% CO_2 / 75% N_2, 50% CO_2 / 50% N_2, 75% CO_2 / 25% N_2 and 100% CO_2 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 CO_2, 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% CO_2 / 26.3% N_2 / 6.6% O_2. A parallel investigation with N_2 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% CO_2 / 27% N_2 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 ± 1°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.

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 mycobiotia 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 Deijii, 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 CO_2 (Smith, Ooraikul, 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 O_2 that can occur in the package and the tolerance of some spoilage moulds e.g. *P. roqueforti* to low O_2 concentrations and increased CO_2 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												
Atmosphere			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	Total
Penicillium thomii	1	5.9													1
Penicillium funiculosum	1	5.9							1	14.3					2
Penicillium expansum	2	11.8					1	16.7							3
Penicillium decumbens	1	5.9													1
Penicillium citreonigrum	1	5.9													1
Penicillium chrysogenum	1	5.9													1
Penicillium solitum			10	58.8	4	50					2	28.6			16
Penicillium crustosum			5	29.4	2	25	1	16.7							7
Penicillium verrucosum							1	16.7							1
Penicillium janthinellium											1	14.3			1
Penicillium roqueforti													1	33.3	1
Penicillium minioluteum							1	16.7							1
Sclerophoma sp.	1	5.9													1
Phoma epicoccina	1	5.9													1
Phoma eupyrena	1	5.9													1
Phoma sorghina											1	14.3			1
Cladosporium cladosporioides	1	5.9							3	28.6	1	14.3			5
Aspergillus ustus	1	5.9													1
Aspergillus puniceus	1	5.9													1
Fusarium oxysporum									1	14.3					1
Fusarium solani													1	33.3	1
Alternaria alternata	2	11.8													2
Cylindrocarpon sp.	2	11.8													2
Amerosporium polynematoides			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).

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. crusotsum* 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 ± 1°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 CO_2 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% 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.

In order to inhibit mould growth in modified atmospheres, the important factors to consider are the concentration of CO_2 which influences its inhibitory effects and the minimum amount of O_2 needed for growth of moulds (Hocking and Taniwaki, 1997). However, when high levels of CO_2 are combined with O_2 the inhibitory effect of CO_2 is reduced (Taniwaki *et al.,* 2001). Thus, in the 80% CO_2 / 17% N_2 / 3% O_2 atmosphere, the high level of CO_2 i.e. 80% would inhibit the growth of moulds on cheese, however the 3% O_2 would allow the growth of certain moulds able to grow at this low O_2 oxygen and high CO_2 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 O_2 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 O_2 concentrations (Hocking and Faedo, 1992). According to Haasum and Nielsen (1998a), its growth is greatly inhibited by CO_2. An atmosphere of 25% CO_2 / 75% N_2 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 CO_2 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 O_2 oxygen and its growth is little affected by low levels of O_2. In a gaseous atmosphere of 2.1% oxygen with the balance as N_2, 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% CO_2 / 17% N_2 / 3% O_2 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 CO_2.

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.

In the shredded Cheddar cheese packaged in the 80% CO_2 / 17% N_2 / 3% O_2 + 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% CO_2 combined with 0.5% O_2 and the balance as N_2. However, it grew in 20% or 40% CO_2 combined with 1% O_2 or 5% O_2 indicating that it is able to grow at low oxygen concentration along with elevated levels of CO_2. In 20% CO_2 combined with 1% O_2 it had a 50% reduction in colony diameter compared to air and with 20% CO_2 combined with 5% O_2 the reduction in colony diameter was 40%. In the atmosphere containing 40% CO_2, the reduction in colony diameter was 55% when combined with 5% O_2 and a 48% reduction in colony diameter was experienced when 40% CO_2 was combined with 1% O_2 compared to the growth in air. The colony diameter of *F. oxysporum* was slightly affected by changes in O_2 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 CO_2 and low O_2 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 O_2 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 *Penicillum* 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% 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.

In the 73% CO_2 / 27% N_2 atmosphere the CO_2 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 O_2 levels and resistant to the inhibitory effect of high CO_2 (Hocking and Taniwaki, 1997). In the cheese in treatment 3 and 6, no species where 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% CO_2 / 27% N_2 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% CO_2 / 27% N_2 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 CO_2 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% CO_2 / 27% N_2 + oxygen scavenging film (treatment 6), any residual O_2 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 O_2 and high CO_2 atmospheres as compared to those isolated in the cheese in the 73% CO_2 / 27% N_2 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 O_2 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% CO_2 / 27% N_2 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% CO_2 combined with 0.5% O_2, however, it grew in 20% or 40% CO_2 combined with 1% O_2 or 5% O_2. In 20% CO_2 combined with 1% O_2 it had a 40% reduction in colony diameter compared to air while in 20% CO_2 combined with 5% O_2, the reduction was 39% of its colony diameter in air. In the atmosphere containing 40% CO_2 and 5% O_2, the reduction was 27% of its colony diameter in air while with 1% O_2 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% CO_2 / 74.7% N_2 / 0.3% O_2 on cheese agar indicating that it is able to grow at low oxygen concentrations with elevated levels of CO_2.

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 CO_2. *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 CO_2 and low O_2 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% CO_2 / 27% N_2 + 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% O_2 / 99% N_2 to 4% of its colony diameter when grown in air. In 0.1% O_2 / 99.9% N_2 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 O_2 levels.

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 O_2 scavengers were effective in controlling the growth of moulds on shredded Cheddar cheese.