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% CO_2 / 27% N_2 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% CO_2 / 27% N_2 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 CO_2 (80% CO_2 / 17% N_2 / 3% O_2, 73% CO_2 / 27% N_2). 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% CO_2 / 17% N_2 / 3% O_2 and 73% CO_2 / 27% N_2 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% CO_2 / 17% N_2 / 3% O_2 and lastly in the cheese packaged in the 73% CO_2 / 27% N_2. 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.

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. crusotsum*, and *Am. polynematoides* were isolated from the cheese packaged in the control film while in the oxygen scavenging film, *P. solitum* and *P. crusotsum* 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% CO_2 / 27% N_2 atmosphere in the control and oxygen scavenging film (treatments 3 and 6) as well as in the 80% CO_2 / 17% N_2 / 3% O_2 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 CO_2 and the absence of O_2.

The cheese packaged in the 73% CO_2 / 27% N_2 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% CO_2 / 27% N_2, 80% CO_2 / 17% N_2 / 3% O_2) combined either with a control or oxygen scavenging film.

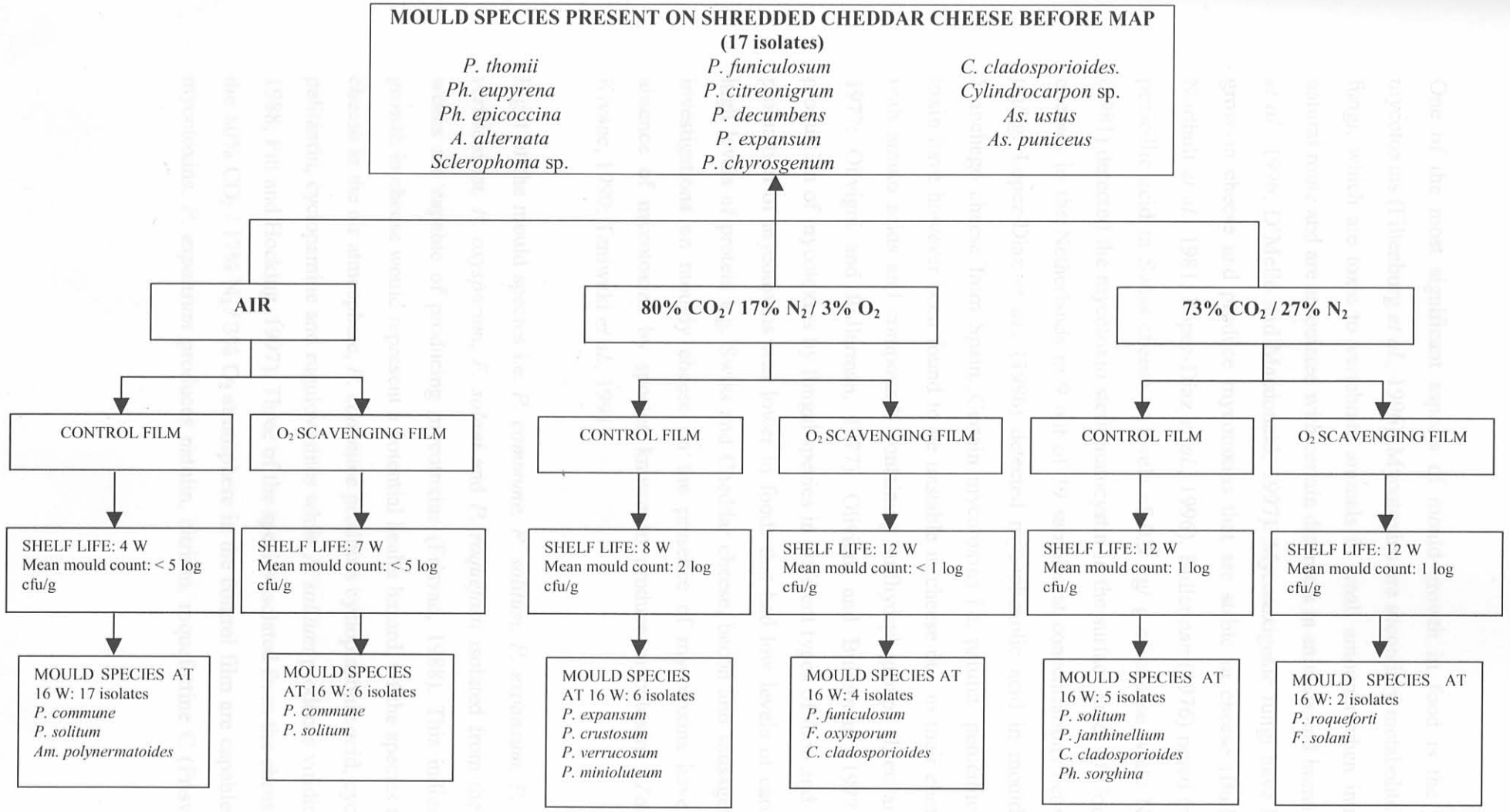

Figure 3 Proposed model for the mould counts, shelf life and mould species isolated from shredded Cheddar cheese packaged in modified atmospheres with and without oxygen scavengers and stored at 5 ± 1° C for 16 weeks.

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% CO_2 / 17% N_2 / 3% O_2 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% CO_2 / 27% N_2 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% CO_2 / 27% N_2 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, botryodiploidin (Frisvad, 1988).

In general, it has been found that elevated levels of CO_2 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 are 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.