Microbiological quality of shredded Cheddar cheese packaged in modified atmospheres

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Abstract

The microbiological quality of shredded Cheddar cheese packaged in different modified atmospheres with and without oxygen scavengers included in the packaging film was studied with the aim of determining how modified air packaging would affect the mould species present on shredded Cheddar cheese. The film with oxygen scavengers was more effective than the control film against mould growth, whereas the 73% CO_2/27% N_2 atmosphere resulted in the cheese with the best microbiological qualities. The three modified atmospheres and the packaging film influenced the mycoflora of shredded Cheddar cheese as the mould species isolated initially differed, from those isolated at 16 weeks in the six treatments. This study demonstrated that O_2 scavengers were effective in controlling the growth of moulds on shredded Cheddar cheese.

Introduction

Cheddar cheese is one of the most popular cheeses in South Africa. The cheese is packaged in different forms as blocks, slices or shredded to suit the needs of the consumer. The shredded product is susceptible to post-production contamination by air-borne micro-organisms during shredding and it is therefore packaged in modified atmospheres comprising of CO_2 and N_2 (Elliot *et al*. 1998).

The shelf life of shredded packaged cheese can be compromised by the growth of moulds (Pitt and Hocking 1997). Modified atmosphere packaging (MAP) reduces the growth rate of mould species like *Penicillium verrucosum* (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 remain 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 *et al*. 2001). Oxygen levels of 0.5% or lower are required to prevent the growth of many moulds, such as *Penicillium commune* and *Penicillium roqueforti*, which are commonly found on Cheddar cheese (Hocking 1994; Taniwaki *et al*. 2001). 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 *et al*. 1999).

An oxygen scavenger is a substance that reacts with and removes O_2 from the environment in which it is placed (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 and to determine how MAP would affect the mould species present on shredded Cheddar cheese.

Materials and methods

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 h/atm at 22°C and 75% relative humidity (RH).
2. A laminate film with an oxygen scavenger Ciba® SHELPLUS™ O_2 (Ciba Specialty Chemicals, Sweden) incorporated into its multilayer 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 h/atm at 22°C and 75% RH.

Packaging treatments of shredded Cheddar cheese

Shredded Cheddar cheese samples were obtained from a cheese factory in the Western Cape region of South Africa. A total of 108 samples weighing 250 g each were packaged (Multivac) with each of three atmospheres (air (20.8% O_2/0.3% CO_2/78.9% N_2), 80% CO_2/17% N_2/3% O_2, 73% CO_2/27% N_2) combined with either an oxygen-scavenging or a 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; and treatment 6 = 73% CO_2/27% N_2 + oxygen-scavenging film. This resulted in 36 packages per treatment. After packaging, the shredded Cheddar cheese samples were transported by airfreight to the Department of Food Science, University of Pretoria, Pretoria, South Africa.

Storage period of shredded Cheddar cheese

The samples were stored at 5 ± 1°C for 16 weeks. Three shredded Cheddar cheese packages from treatments 1–6 were selected at random, and samples analysed at 0, 4, 8, 12, 14 and 16 weeks.

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 Laboratory Blender 400 (Seward Laboratory, London, UK).

Lactic acid bacteria (LAB) were enumerated (30°C) for 3 days on MRS agar (DeMan, Rogosa and Sharp 1960). Yeasts and moulds were enumerated (25°C) for 5 days on potato dextrose agar (PDA) (Biolab, Wadeville, South Africa) with 50 mg/L rifampicin (Lion Bridge, Pretoria, South Africa) (Van Dyk 2003).

Mould isolation

Mould isolates were identified at 0 and 16 weeks on malt extract agar (MEA) 25°C for 14 days (Samson and van Reenen-Hoekstra 1988; Pitt 1988) with 50 mg/L rifampicin

added (van Dyk 2003). All resulting colonies were then re-plated on MEA until pure colonies were achieved (Pitt and Hocking 1997).

Mould identification

The *Penicillium* species were identified according to Pitt (1988) and Barnett and Hunter (1998). The *Fusarium* species were identified according to Nelson *et al.* (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 *et al*. (1994). *Cylindrocarpon* species were identified according to Domsch *et al.* (1980) and Baxter *et al*. (1994). *Sclerophoma* species were identified according to Sutton (1980).

Visual inspection of the shredded Cheddar cheese samples for mould growth

All the shredded Cheddar cheese samples in the six treatments were weekly visually inspected for visible mould growth.

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 ($P = 0.05$) 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 six observations for each analysis. ANOVA was performed using the STATISTICA program version 6.1 (Tulsa, OK, USA, 2003) for Microsoft Windows (Microsoft Corp., Redmond, WA, USA).

Results

Shredded Cheddar cheese packaged in MAP for 16 weeks at 5 ± 1°C

The LAB levels for all the treatments (1–6) remained stable over the storage period (Figure 1) and were similar. The counts in the shredded Cheddar cheese in all treatments were log 7.2–7.6 cfu/g initially (week 0) and during week 16, the LAB counts were log 6.8–7.0 cfu/g (Figure 1).

There was a significant difference ($P = 0.05$) between the yeast counts in the shredded Cheddar cheese in the air atmosphere (treatments 1 and 4) and the other atmospheres, treatments 2–6 (Figure 1). The yeast counts in the shredded Cheddar cheese in treatments 1 and 4 were the highest, at an average of 4.3 log cfu/g, over the storage period. This was

followed by treatments 2 and 5, 1.7 log cfu/g, and lastly, treatments 3 and 6, 1.5 log cfu/g.

There was a significant difference ($P = 0.05$) between the mould counts in the shredded Cheddar cheese packaged in the different treatments. The air-packaged shredded Cheddar cheese samples in treatment 1 had higher mould counts over the storage period, 2.2 log cfu/g, whereas the samples in treatment 4 had a lower mean, 0.9 log cfu/g (Table 1). The shredded Cheddar cheese in treatment 1 also had a greater number of shredded Cheddar cheese packages positive for mould growth, that is, 14/36 vs 6/36 (Table 1). Consequently, the cheese packaged in treatment 1 developed visible mould faster, that is, within 4 weeks as compared with the samples in treatment 4, which developed visible mould within 7 weeks (Figure 2).

The average mould population in the shredded Cheddar cheese in treatments 1 and 4 was the highest over the storage period, 1.6 log cfu/g, whereas in the 80% CO_2/17% N_2/3% O_2 atmosphere the average mould population 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.

The shredded Cheddar cheese in treatment 2 developed visible mould growth faster, that is, within 8 weeks, whereas the cheese in treatment 5 developed visible mould within 12 weeks (Table 1). The shredded Cheddar cheese in treatment 2 also had a greater number of shredded Cheddar cheese packages positive for mould growth, that is, 5/36 vs 4/36. In the shredded Cheddar cheese samples in treatment 5, the mould populations were only detected after 12 and 16 weeks at 1.2 and 0.7 log cfu/g, respectively (Table 1).

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 with 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, that is, 6/36 vs 3/36 (Table 6). However, the shredded Cheddar cheese in both treatments 3 and 6 took 12 weeks to develop visible mould growth (Figure 2).

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 six treatments were grouped together because it was assumed that the time between packaging and plating out the cheese, that is, 1 day, was not sufficient for the modified atmospheres to have an effect on the mycoflora of the cheese (Table 2).

Initially (week 0), 17 isolates were obtained from the cheese that belonged to 14 different species (Table 2). 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. *Cladosporium cladosporioides* was isolated at week 16 from the cheese in treatments 5 and 3. *Penicillium expansum* was isolated from the shredded Cheddar cheese packaged in treatment 2 and *Penicillium 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, that is, *Penicillium crustosum*, *Penicililum solitum* and *Amerosporium polynematoides* (Table 2). The predominant mould species was *P. solitum* at 59%, followed by *P. crustosum* at 29%.

In the shredded Cheddar cheese in treatment 4, six isolates were identified and they belonged to two species, which were *P. crustosum* (50%) and *P. solitum* (25%) (Table 2). The species identified in treatments 1 and 4 were similar except for *A. polynematoides* isolated from the cheese in treatment 1. In addition, fewer species were isolated from the treatment 4 samples than from treatment 1. None of the species identified in treatments 1 and 4 was also isolated from the cheese initially (week 0).

In the shredded Cheddar cheese packaged in treatment 2, four isolates were identified, all belonging to the genus *Penicillium* (Table 2). In the shredded Cheddar cheese in treatment 5, there was a total of eight isolates identified. Three were sterile and could not be identified, whereas the other five isolates were three isolates of *C. cladosporioides* and one isolate each of *P. funiculosum* and *Fusarium oxysporum* (Table 2). 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 initially (week 0) as well as from the other treatments. 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).

Seven isolates were obtained from the shredded Cheddar cheese packaged in treatment 3. Three of the isolates (43%) were *Penicillium* species and the other isolates were *Phoma sorghina* and *C. cladosporioides. P. roqueforti* and *Fusarium solani* were the only moulds identified in the shredded Cheddar cheese packaged in treatment 6. Neither *P. roqueforti* nor *F. solani* were isolated from the shredded Cheddar cheese in the other treatments initially (week 0) or at 16 weeks.

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. 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).

Yeast counts in the shredded Cheddar cheese samples packaged in air (treatments 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). 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).

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 most effective gas atmosphere and film combination, which inhibited the growth of yeasts, was treatment 3, as it had the lowest average yeast counts, although the yeast counts were also low in treatments 2, 5 and 6. Other authors have packaged cheese similarly 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 and 7 log cfu/g.

The growth of moulds in the cheese packaged in the oxygen-scavenging film was significantly different ($P = 0.05$) from 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 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. 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 that allowed their growth.

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, which was

packaged in the same gaseous atmosphere, that is, 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, whereas 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 shredded Cheddar cheese in treatments 5, 3 and 6 took the longest to show signs of visible mould growth, that is, 12 weeks 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 1998). 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 treatments 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 treatments 3 and 6 took to develop visible mould growth, that is, 12 weeks (Figure 2), although 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 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 although similar to that in the treatment 6 at a mean of 0.2 log cfu/g.

Penicillium species (41%) predominating in the air atmosphere initially (week 0) indicates that the mycoflora of South African Cheddar cheese when initially packaged is similar to the mycoflora of other cheeses, as reported in the 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 as, to the best of our knowledge, *Penicillium thomii*, *Penicillium citreonigrum*, *Penicillium decumbens*, *Cylindrocarpon* sp., *Sclerophoma* sp., *Ph. sorghina*, *Phoma epiccocina*, *Phoma eupyrena*, *Amerosporium ustus* and *Amerosporium puniceus*, 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, that is, not a natural part of the cheese mycoflora and thus may not be adapted to grow in cheese (Kure *et al*. 2004).

The isolates in the cheese in treatment 4 were fewer by nine isolates than those in treatment 1. This may have been due to lower levels of oxygen; 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. Unlike the isolates from the shredded Cheddar cheese in treatment 2, only one isolate from the cheese in treatment 5 was a *Penicillium* species, due to the oxygen scavenger in treatment 5 (Yanai *et al*. 1980 as cited by Hocking and Taniwaki 1997; Magan and Lacey 1984).

Of the *Penicillium* isolates from the cheese in treatment 3, *Penicillium janthinellium* has not previously been reported on cheese. 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*, isolated from the shredded Cheddar cheese in treatment 3, has not previously been isolated from cheese.

In treatment 6, any residual O_2 in the atmosphere was expected to be absorbed by the oxygen scavengers. One isolate each of *P. roqueforti* and *F. solani* was isolated from the cheese in treatment 6 (Table 2), whereas seven isolates belonging to four species were isolated from the cheese in treatment 3. None of the species isolated from the cheese in treatment 6 was isolated from the cheese 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.

Conclusions

The film with oxygen scavengers was more effective than the control film against mould growth, whereas the 73% CO_2/27% N_2 atmosphere resulted in the cheese with the best microbiological qualities. The three modified atmospheres and the packaging film influenced the mycoflora of shredded Cheddar cheese as the mould species isolated initially differed, from those isolated at 16 weeks in the six treatments. This study demonstrated that O_2 scavengers were effective in controlling the growth of moulds on shredded Cheddar cheese.

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Figures and tables

Figure 1 Lactic acid bacteria (LAB) and yeast populations in shredded Cheddar cheese packed in modified atmospheres, stored at 5 ± 1°C for 16 weeks (*n* = 36). (T1 = air + control film, T2 = 80% CO_2/17% N_2/3% O_2 + control film, T3 = 73% CO_2/27% N_2 + control film, T4 = air + oxygen-scavenging film, T5 = 80% CO_2/17% N_2/3% O_2 + oxygen-scavenging film, T6 = 73% CO_2/27% N_2 + oxygen-scavenging film).

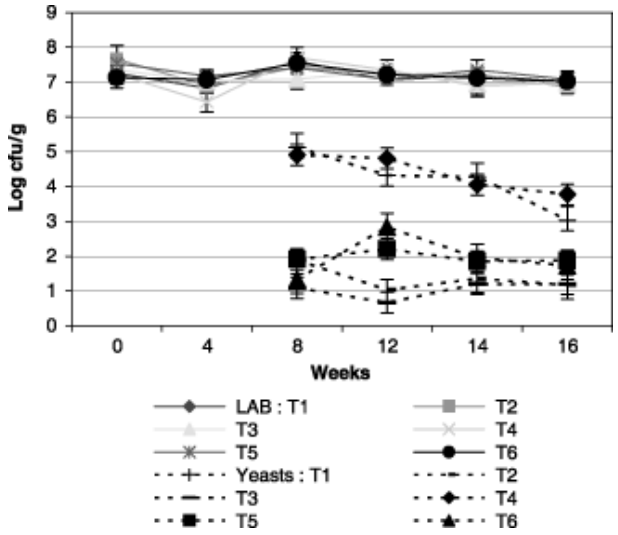

Figure 2 Shelf life of shredded Cheddar cheese packaged in an oxygen-scavenging and control film in air, 80% CO_2/17% N_2/3% O_2, 73% CO_2/27% N_2 and stored at 5 ± 1°C (*n* = 108) based on visible mould growth. Oxygen-scavenging film; ▒ Control film.

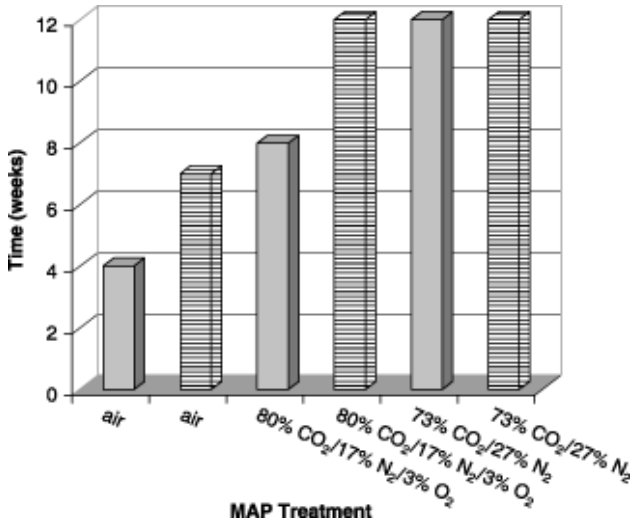

Table 1 Mould growth (log cfu/g) in shredded Cheddar cheese packaged in modified atmospheres with and without oxygen scavengers, stored at 5 ± 1°C for 16 weeks

Atmosphere Type of film Storage period (w)	***Air control***	***Air oxygen scavenging***	***80% CO_2/17% N_2/3% O_2 control***	***80% CO_2/17% N_2/3% O_2 oxygen scavenging log cfu/g***	***73% CO_2/27% N_2 control***	***73% CO_2/27% N_2 oxygen scavenging***
0	ND	ND	NDa	NDa	ND	ND
4	ND	ND	NDa	NDa	ND	ND
8	3.6	2.0	0.7	0.7	ND	0.7
12	2.7	0.7	1.0	1.0	1.2	1.0
14	3.0	ND	0.7	ND	ND	ND
16	ND	0.7	0.7	0.7	0.7	0.7
No. of samples with growth (n = 36)	14	5	6	3	4	6
ND, not detected.						

Table 2 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)

Storage period	***0 weeks***		***16 weeks***												***Total***
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 (n = 3)	***Isolates from all the treatments combined (n = 18)***		***control***		***oxygen scavenging***		***control***		***oxygen scavenging***		***control***		***oxygen scavenging***		
TreatmentMould species	***Treatments 1–6***		***Treatment 1***		***Treatment 4***		***Treatment 2***		***Treatment 5***		***Treatment 3***		***Treatment 6***		
	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***	
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	1	5.9													1

Storage period	***0 weeks***		***16 weeks***												***Total***
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 (n = 3)	***Isolates from all the treatments combined (n = 18)***		***control***		***oxygen scavenging***		***control***		***oxygen scavenging***		***control***		***oxygen scavenging***		
TreatmentMould species	***Treatments 1–6***		***Treatment 1***		***Treatment 4***		***Treatment 2***		***Treatment 5***		***Treatment 3***		***Treatment 6***		
	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***	
chrysogenum															
Penicillium solitum			10	58.8	4	50					2	28.6			16
Penicillium crustosum			5	29.4	2	25	1	16.7							8
Penicillium verrucosum							1	16.7							1
Penicillium janthinellium											1	14.3			1
Penicillium roqueforti													1	33.3	1
Penicillium minioluteum							1	16.7							1

Storage period	***0 weeks***		***16 weeks***												***Total***
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 (n = 3)	***Isolates from all the treatments combined (n = 18)***		***control***		***oxygen scavenging***		***control***		***oxygen scavenging***		***control***		***oxygen scavenging***		
TreatmentMould species	***Treatments 1–6***		***Treatment 1***		***Treatment 4***		***Treatment 2***		***Treatment 5***		***Treatment 3***		***Treatment 6***		
	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***	
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

Storage period	***0 weeks***		***16 weeks***												***Total***
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 (n = 3)	***Isolates from all the treatments combined (n = 18)***		***control***		***oxygen scavenging***		***control***		***oxygen scavenging***		***control***		***oxygen scavenging***		
TreatmentMould species	***Treatments 1–6***		***Treatment 1***		***Treatment 4***		***Treatment 2***		***Treatment 5***		***Treatment 3***		***Treatment 6***		
	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***	
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			
Total	17	100	17	100	8	100	6	100	8	100	7	100	3	100	66