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Growth of fungi and mycotoxin production on cheese under modified atmospheres

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Abstract

The use of modified atmospheres to prevent fungal growth and mycotoxin production in cheese was evaluated. Eight fungal species: *Mucor plumbeus*, *Fusarium oxysporum*, *Byssochlamys fulva*, *B. nivea*, *Penicillium commune*, *P. roqueforti*, *Aspergillus flavus* and *Eurotium chevalieri* were inoculated onto cheese and incubated under conditions of decreasing concentrations of O_2 (5% to < 0.5%) and increasing concentrations of CO_2 (20–40%). Fungal growth was measured by colony diameter and ergosterol content. All fungi examined grew in atmospheres containing 20% and 40% CO_2 with 1% or 5% O_2, but growth was reduced by 20–80%, depending on species, compared with growth in air. The formation of aflatoxins B_1 and B_2, roquerfortine C and cyclopiazonic acid was greatly decreased but not totally inhibited in these atmospheres. At 20% or 40% CO_2 with < 0.5% O_2, only *B. nivea* exhibited growth, which was very slow. Growth of *F. oxysporum*, *B. fulva*, *P. commune* and *A. flavus* showed good correlations between colony diameter and ergosterol content. However, for the other species correlations were inconsistent. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cheese; Modified atmospheres; Spoilage; Fungi; Moulds

1. Introduction

The use of modified atmospheres to prevent fungal growth is considered a suitable method to extend the shelf life of some kinds of food. Modified atmosphere packaging (MAP) is a modern food process developed for extending the shelf life of relatively high moisture foods (Wolfe, 1980; Smith et al., 1990; Farber, 1991). The gases used for MAP include carbon dioxide, nitrogen, carbon monoxide and sulphur dioxide. The most commonly used and perhaps the most effective gas is CO_2, with or without other gases. MAP has been found to be effective in preventing or reducing fungal growth in bakery products (Smith et al., 1986; Powers and Berkowitz, 1990), fresh broccoli (Wang, 1979), cakes (Seiler, 1978) and strawberries (Chambroy et al., 1993).

Although many MAP studies have been conducted with bacteria (Hotchkiss, 1988; Farber, 1991; Farber et al., 1990), relatively few have been conducted on the behaviour of foodborne fungi (Smith et al., 1986; Powers and Berkowitz, 1990; Ellis et al., 1993).

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Many types of cheese are an excellent substrate for the growth of fungi. Commonly isolated fungi from cheese include *Penicillium*, *Aspergillus*, *Alternaria*, *Eurotium*, *Fusarium* and *Mucor* (Bullerman and Olivigni, 1974; Beranová et al., 1986; Aran and Eke, 1987; Hocking and Faedo, 1992; Taniwaki and van Dender, 1992; Lund et al., 1995).

Mycotoxigenic fungi have been reported in cheese. The most common mycotoxins that are stable in cheese are citrinin, cyclopiazonic acid, penitrem A, roquefortine C, sterigmatocystin and aflatoxin. Others, including patulin, penicillic acid and PR toxin do not persit in cheese (Scott and Kennedy, 1976; Stott and Bullerman, 1976; Lieu and Bullerman, 1977; Zerfiridis, 1985; Taniwaki and van Dender, 1992).

Vacuum packaging is sometimes used to inhibit fungal growth on cheese, but some fungal species are able to grow under these conditions. Hocking and Faedo (1992) isolated several fungal species from vacuum packaged cheeses, the most commonly occurring being species of *Cladosporium*, *Penicillium* and *Phoma*.

The use of modified atmospheres to protect high water activity foods such as cheese from fungal growth and mycotoxin production has not been extensively studied. This study investigated growth and mycotoxin production by eight fungal species in cheese under conditions of decreasing concentrations of O_2 and increasing concentrations of CO_2. The fungi were chosen for their reported ability to grow under MAP conditions, rather than because they commonly cause cheese spoilage.

2. Material and methods

2.1. Fungal species

The fungal species used in this study are listed in Table 1. Most were isolated from processed foods of low O_2 content, including UHT fruit juice and vacuum packaged cheese.

2.2. Cultivation

Inocula for cheese samples were prepared from 5–7-day-old cultures grown on Czapek Yeast Extract Agar (CYA; Pitt, 1979), except for *B. nivea* and *E. chevalieri*, which were grown on Malt Extract Agar (MEA; Pitt, 1979) for 7–10 days and Czapek Yeast Extract 20% Sucrose Agar (CY20S; Pitt and Hocking, 1997) for 10–15 days, respectively.

Table 1
Origin of fungal species studied[a]

Species	Strain number	Origin	Mycotoxin production
M. plumbeus	FRR 2414	Apple juice	–
F. oxysporum	FRR 3414	Orange juice	–
Byssochlamys fulva	FRR 3792	Strawberry puree	–
B. nivea	FRR 4421	Strawberry	Patulin
P. commune	FRR 3932	Cheddar cheese	Cyclopiazonic acid
P. roqueforti	FRR 2162	Cheddar cheese	Roquefortine C
A. flavus	FRR 2757	Peanuts	Aflatoxins B_1, B_1
E. chevalieri	FRR 547	Animal feed	–

[a] FRR refers to the culture collection of Food Science Australia, North Ryde, NSW 2113, Australia.

2.3. Inoculation of fungi onto cheese

Commercial, sliced cheddar cheese was obtained from a local supermarket. Slices (1 mm thick) were cut to 75-mm diameter with a circular knife, placed in Petri dishes, then surface sterilised by exposure to UV light for 1 h in a laminar flow cabinet. Each slice was then inoculated in the centre with one fungal species. Sets of eight Petri dishes with inoculated cheese were placed in barrier plastic bags (polypropylene:ethylene vinyl alcohol:polypropylene) and incubated under modified atmosphere conditions. Control inoculated cheese slices in Petri dishes were placed in polyethylene plastic bags to permit air exchange while limiting a_w changes.

2.4. Incubation in various gaseous environments

The Petri dishes containing inoculated cheeses were incubated in various atmospheres: (i) air; (ii) 20% $CO_2/<0.5\%$ O_2; (iii) 20% $CO_2/1\%$ O_2; (iv)

20% CO_2/5% O_2; (v) 40% CO_2/< 0.5% O_2; (vi) 40% CO_2/1% O_2; or (vii) 40% CO_2/5% O_2; with the balance being N_2. To maintain atmospheres below 0.5% O_2, two O_2 scavenger sachets (Ageless, Mitsubishi, Japan) were placed in the packages, which were incubated at 25°C for 30 days. For atmospheres with higher levels of O_2, the dishes were incubated at 25°C for only 14 days as the cheese usually became visibly mouldy within that time.

The atmospheres inside the plastic bags were sampled using a gas tight pressure lock syringe. Samples for O_2:N_2 analysis (0.6 ml) and CO_2 analysis (0.4 ml) were taken through a silicone septum and were analysed using a Shimadzu Model 8AIT gas chromatograph (Shimadzu, Japan). The gas chromatograph was equipped with a 2 m stainless steel column, 3 mm OD, packed with molecular sieve 5A, 80–100 mesh, for O_2 and N_2 analysis, and a 2 m stainless steel column, 3 mm OD, packed with Porapak QS, 80–100 mesh, for the analysis of CO_2. Helium was used as the carrier gas with CO_2 pressure 1.9 kg/cm^2 and O_2 4.0 kg/cm^2. The column temperature was set at 70°C and the injector and detector at 90°C. The current was adjusted to 90 mA. Peaks were recorded and analysed with an NEC Multisync integrator (Model 2A).

Atmospheres in the plastic bags were checked daily: the bags were flushed when variations from desired levels occurred that were greater than ±2% for CO_2 and ±0.5% O_2.

2.5. Growth measurement

Fungal growth was measured by colony diameters and ergosterol contents after 14 days of incubation. The diameters of colonies on the surface of the cheese slices were measured in millimetres with a ruler. Ergosterol was extracted directly from the colonies growing on the cheese. The area containing the colony was excised and transferred to a flask containing 100 ml of 95% ethanol:water (50:50 v/v) and potassium hydroxide (5 g), and extracted with refluxing for 30 min (Zill et al., 1988). Ergosterol was transferred to *n*-hexane by extracting three times in a separatory funnel. The combined hexane extracts were concentrated in a vacuum concentrator to near dryness, the residue redissolved in *n*-hexane (2 ml) then filtered through a polypropylene membrane (0.45 μm pore size, 13 mm diameter; Activon, Sydney, Australia). The filtrate was dried under N_2 and redissolved in *n*-hexane for quantification. Ergosterol concentrations were assayed by high-pressure liquid chromatography (HPLC) using a Shimadzu Model 10 A pump with a LiChrosorb SI 60 column (Activon Gold Pak, UK). The column was eluted with *n*-hexane:isopropanol (97:3, v/v) at 1 ml min^{-1} and ergosterol detected by absorption at 280 nm about 8–10 min after injection of the sample. Ergosterol was quantified by reference to an ergosterol standard (2 mg ml^{-1}; Sigma, St. Louis, MO, USA). Each analysis was carried out in duplicate.

2.6. Mycotoxin extraction

Cheeses were analysed for aflatoxins B_1 and B_2, roquefortine C and cyclopiazonic acid (CPA), with quantification by HPLC. All analyses were carried out in duplicate. Patulin was not analysed because it is not stable in cheese.

2.6.1. Aflatoxin

Cheese inoculated with *A. flavus* was analysed for aflatoxins B_1 and B_2. Each cheese slice (approximately 10 g) was extracted with chloroform (50 ml) by homogenization in a Colworth Stomacher for 5 min. The extract was filtered through a bed of anhydrous Na_2SO_4, concentrated under vacuum to approximately 3 ml and made up to a 5 ml in a volumetric flask. From 5 ml of extract, 50 μl were taken and the aflatoxins derivatised with trifluoroacetic acid before injection into the HPLC according to the method of Beebe (1978). The extract was dried and resuspended with chloroform (200 μl), filtered through a polypropylene membrane and analysed by HLPC. The column was eluted with water:acetonitrile:methanol (69:21:10 v/v/v) at 1.0 ml min^{-1} and the aflatoxin detected in a fluorescence detector (Perkin Elmer, San Francisco, CA) by excitation at 365 nm. Aflatoxins B_1 and B_2 were eluted at 5–7 and 11–13 min, respectively. Aflatoxin standards (10 ng ml^{-1}) were from Sigma. The lower limits for detection were 0.06 and 0.02 ng for aflatoxin B_1 and B_2, respectively. Recovery of aflatoxin standards from cheese was tested with 10 g of cheese spiked with 0.1 μg aflatoxin B_1 and 0.03 μg of B_2.

Recovery was 50–70% ($n = 3$) for aflatoxin B_1 and 66–94% ($n = 3$) for aflatoxin B_2.

2.6.2. Roquefortine C

Cheese inoculated with *P. roqueforti* was analysed for roquefortine C according to the method of Ware et al. (1980). Each slice was extracted with ethyl acetate (50 ml) by homogenization in a Stomacher (5 min). The extract was filtered through a bed of Na_2SO_4 and shaken (20 s) in a separatory funnel with 0.1% HCl (10 ml). The ethyl acetate layer was extracted with 0.1% HCl (10 ml) a second time. The acidic solutions were combined and extracted (20 s) twice with 3% aqueous $NaHCO_3$ (10 ml) and ethyl acetate (10 ml). The ethyl acetate extracts were combined, filtered through Na_2SO_4, then evaporated to near dryness on a steam bath with a few boiling chips, redissolved in ethyl acetate (3 ml) and filtered through a polypropylene membrane. The filtrate was dried under N_2 and redissolved in methanol for analysis by HPLC.

The HPLC column was eluted with two solvents: (A) methanol:water (50:50 v/v) plus 0.05 M $(NH_4)_2HPO_4$ and (B) 100% methanol. The flow rate of solvent A was 0.65 ml min^{-1} and of solvent B 0.15 ml min^{-1}. Roquefortine C was detected by absorption at 326 nm about 12–15 min after injection and quantified by reference to a roquefortine C standard (3.6 μg ml^{-1}), prepared from a culture of *P. roqueforti*. The spectrum of roquefortine C was recorded in absolute methanol with a spectrophotometer (Gilford Instruments, Model 2600, USA) and quantified at 326 nm. The lower limit of detection was 2 ng.

The recovery of roquefortine C from cheese was tested with cheese (10 g) spiked with roquefortine C standard (9 μg), and the recovery was 63–70% ($n = 3$).

2.6.3. Cyclopiazonic acid

Cheese inoculated with *P. commune* was extracted for CPA according to Urano et al. (1992) with some modifications. Cheese (10 g) was extracted with methanol:2% $NaHCO_3$ solution (7:3; 50 ml) by homogenization in a Stomacher (5 min). The extract was filtered through Na_2SO_4 then shaken (1 min) with hexane (30 ml) in a separatory funnel. KCl (10%; 50 ml) was added and the lower phase drained into another separatory funnel. The extract was acidified with 6 N HCl (2 ml) and extracted twice with chloroform (30 ml). The chloroform extracts were combined, anhydrous Na_2SO_4 was added and filtered off, then concentrated under vacuum to near dryness. The residue was redissolved in chloroform (3 ml) then filtered through a polypropylene membrane. The filtrate was dried under N_2 and redissolved in methanol for quantification by HPLC. The column was eluted with two solvents: (A) methanol:water (85:15 v/v) and (B) methanol:water (85:15 v/v) plus 4 mM $ZnSO_4 \cdot 7H_2O$. The elution followed a linear gradient from 100% A to 100% B in 10 min, followed by 100% B for 10 min, at 1.0 ml min^{-1}. CPA was detected by absorption at 279 nm about 9–11 min after injection. CPA (1 μg ml^{-1}; Sigma) was used as standard.

The recovery of cyclopiazonic acid from cheese was tested with 10 g of cheese spiked with 6 μg of CPA: recovery was 54–64% ($n = 3$).

3. Results

3.1. Effect of increased CO_2 with reduced O_2 on the growth of fungi in cheese

After 30 days of incubation in atmospheres of 20% and 40% CO_2 with $< 0.5\%$ O_2, only *B. nivea* exhibited growth, which was very slow (Table 2).

All fungi showed growth in 20% or 40% CO_2 with 1% or 5% O_2, though much less than in air (Table 3). Only the growth of *B. nivea* was unaffected by 20% CO_2 with 5% O_2. Under these conditions, colony diameters of other species were re-

Table 2
Growth of *B. nivea* on cheese in 20% CO_2 or 40% CO_2 and $< 0.5\%$ O_2[a]

Gas concentration	Colony diameter (mm)	Ergosterol (μg)
20% CO_2, $< 0.5\%$ O_2	15.5	1.0
40% CO_2, $< 0.5\%$ O_2	7.0	0.07
Air	50.0	53.3

[a]Colony diameters were measured after 14 days incubation. Ergosterol figures result from assay of one Petri dish culture. Assays were carried out in duplicate.

Table 3
Effect of carbon dioxide (20% or 40%) with oxygen (1% or 5%) on colony diameters of fungi on cheese, compared with growth in air[a]

Species	Colonies in air (mm)	Colony diameter in 20% CO_2 + 5% O_2 (mm)	Colony diameter in 20% CO_2 + 1% O_2 (mm)	Colony diameter in 40% CO_2 + 5% O_2 (mm)	Colony diameter in 40% CO_2 + 1% O_2 (mm)
M. plumbeus	75.0	35.0 (53)	27.5 (63)	36.5 (51)	21.0 (72)
F. oxysporum	67.0	40.0 (40)	33.5 (50)	30.0 (55)	35.0 (48)
B. fulva	47.0	25.0 (47)	23.5 (50)	15.0 (68)	12.5 (73)
B. nivea	50.0	50.0 (0)	42.0 (16)	45.0 (10)	40.0 (20)
P. commune	50.0	25.0 (50)	20.0 (60)	25.0 (50)	12.0 (76)
P. roqueforti	65.0	40.0 (39)	45.0 (31)	47.5 (27)	57.5 (11.5)
A. flavus	75.0	38.0 (49)	40.0 (47)	32.5 (57)	26.0 (65)
E. chevalieri	75.0	40.0 (47)	51.0 (32)	28.0 (63)	16.0 (79)

[a]Figures in parentheses are percentage reductions in colony diameters in gas mixtures compared to those in air.

duced by 38–53% compared with growth in air. Growth of *P. roqueforti*, *A. flavus* and *E. chevalieri* was little affected by reduction of O_2 from 5% to 1% in the presence of 20% CO_2. Other species grew more slowly under these conditions (Table 3).

The growth of *P. commune*, *M. plumbeus* and *P. roqueforti* was unchanged by an increase of CO_2 from 20% to 40% in the presence of 5% O_2. Colony diameters of the other species were smaller after incubation in 40% CO_2 (Table 3).

Decreasing O_2 from 5% to 1% in an atmosphere of 40% CO_2 caused little or no change in colony diameters of *F. oxysporum* and *P. roqueforti*. Other species showed reductions of up to 50% (Table 3).

Ergosterol formation by these species when grown in cheese under the conditions described above is shown in Table 4. Results were rather variable, probably due to the small size of many of the colonies analysed, but in general ergosterol content was reduced under the influence of high CO_2 and low O_2. Ergosterol production by *B. nivea* and *P. roqueforti* was higher when grown in 20% CO_2 with 5% O_2 in comparison with growth in air. In other species, the ergosterol content was 54–88% lower when grown in this atmosphere compared with air.

Reduction in O_2 from 5% to 1% with 20% CO_2 had little effect on ergosterol production by *A. flavus*, *F. oxysporum*, *M. plumbeus* or *E. chevalieri*, but ergosterol content was lower for *B. fulva*, *B. nivea*, *P. commune* and *P. roqueforti* (Table 4).

Increasing CO_2 from 20% to 40% with 5% O_2 caused increases in ergosterol production by *M.*

Table 4
Effect of carbon dioxide (20% or 40%) with oxygen (1% or 5%) on ergosterol content (μg per Petri dish culture) on cheese, compared with ergosterol content in air[a]

Species	Ergosterol content in air (μg)	Ergosterol content in 20% CO_2 + 5% O_2 (μg)	Ergosterol content in 20% CO_2 + 1% O_2 (μg)	Ergosterol content in 40% CO_2 + 5% O_2 (μg)	Ergosterol content in 40% CO_2 + 1% O_2 (μg)
M. plumbeus	35.7	4.7 (87)	2.6 (93)	22.2 (38)	3.5 (90)
F. ocysporum	109.1	13.2 (88)	11.3 (90)	6.4 (94)	21.2 (81)
B. fulva	13.3	6.1 (54)	2.0 (85)	1.9 (86)	0.4 (97)
B. nivea	53.3	73.3 (138)	5.1 (90)	26.7 (50)	25.2 (53)
P. commune	81.1	9.0 (89)	2.0 (98)	5.4 (93)	2.0 (98)
P. roqueforti	13.3	17.3 (130)	8.8 (34)	9.4 (29)	16.4 (123)
A. flavus	123.5	18.8 (85)	22.2 (82)	13.3 (89)	4.8 (96)
E. chevalieri	72.1	11.3 (84)	17.3 (76)	22.1 (69)	7.6 (90)

[a]Figures in parentheses are percentage reductions ergosterol content in gas mixtures compared to those in air.

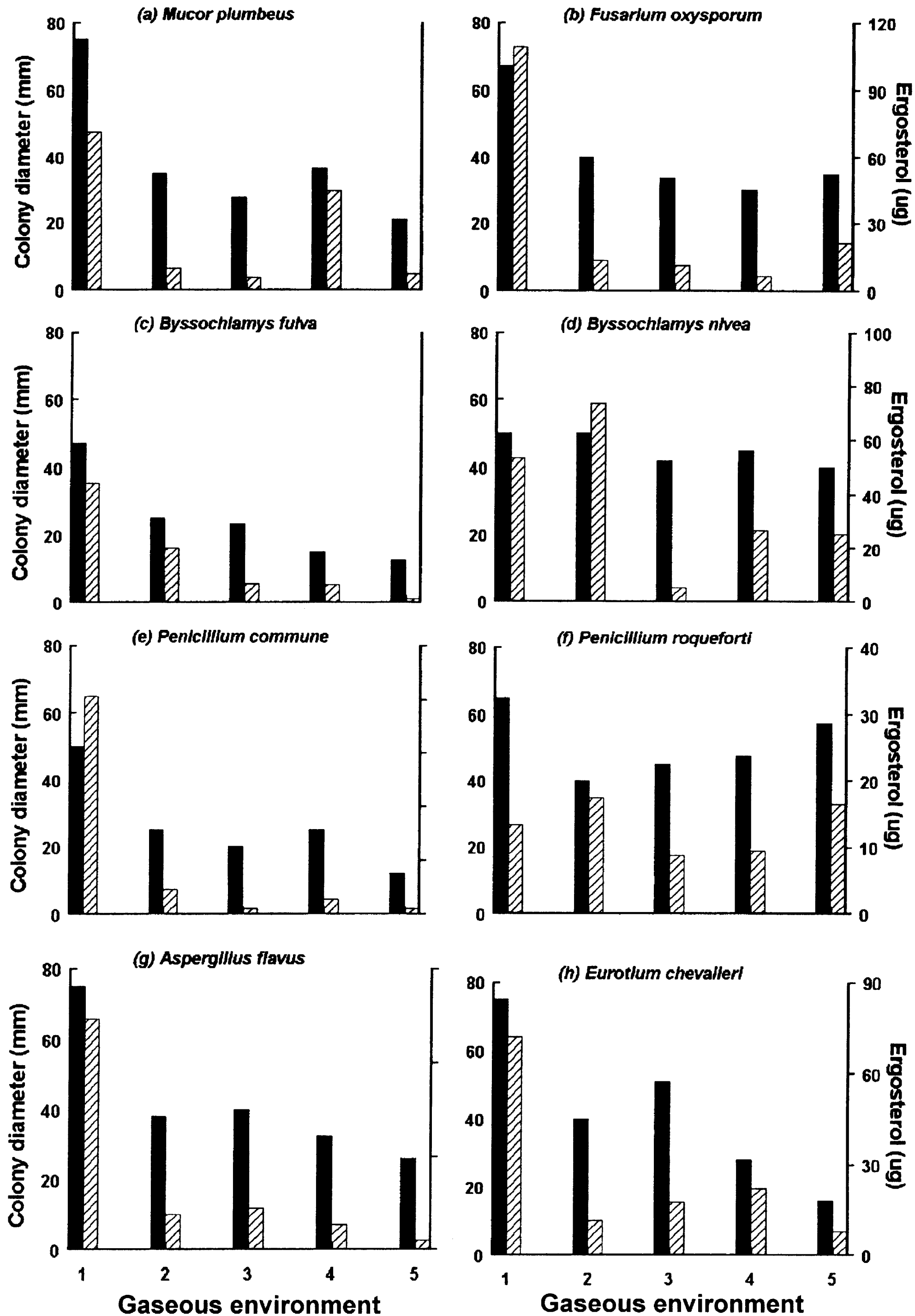

Fig. 1. Effect of modified atmospheres on the growth of fungi on cheese after 14 days at 25°C. (1) air; (2) 20% CO_2 + 5% O_2; (3) 20% CO_2 + 1% O_2; (4) 40% CO_2 + 5% O_2; (5) 40% CO_2/1% O_2; ■ Colony diameter (mm); □ ergosterol content (μg).

Table 5
Effect of carbon dioxide (20% or 40%) with oxygen (1% or 5%) on mycotoxin production (μg per 10 g) on cheese, compared with mycotoxin production in air[a]

Atmosphere	Aflatoxin production by *A. flavus*		Roquefortine C production by *P. roqueforti*	Cyclopiazonic acid production by *P. commune*
	B_1	B_2		
Air	5850	175	0.39	34.0
20% CO_2 + 5% O_2	4.0	2.0	0.02	2.8
20% CO_2 + 1% O_2	0.5	0.04	0.07	0.1
40% CO_2 + 5% O_2	0.07	0.01	0.06	0.4
40% CO_2 + 1% O_2	0.03	ND[a]	0.05	0.04

[a]ND, not detected; < 0.005 μg/kg per 10 g cheese.

plumbeus and *E. chevalieri* (Table 4). Ergosterol levels in the other species were lower by 40% or more when grown in the higher CO_2 concentration.

Decreasing O_2 from 5% to 1% in the presence of 40% CO_2 caused apparent increases in ergosterol production by *F. oxysporum* and *P. roqueforti*, but decreased ergosterol formation in the other species to varying extents.

3.2. Colony diameters vs. ergosterol content

The relationships between colony diameters and ergosterol production when grown on cheese are shown as histograms in Fig. 1. Reasonably good correlations between these two parameters were observed for *F. oxysporum*, *B. fulva*, *P. commune* and *A. flavus*. The other species showed less consistent responses.

3.3. Effect of increased CO_2 with reduced O_2 on mycotoxin production on cheese

Mycotoxin production was greatly decreased but not totally inhibited in 20% or 40% CO_2 and 1% or 5% O_2 in comparison with production in air (Table 5). Aflatoxin B_1 production by *A. flavus* was reduced by a factor of greater than 1000 compared to production in air, even in the most favourable atmosphere studied (20% CO_2 + 5% O_2). In 20% CO_2 with 1% O_2 and in 40% CO_2 with 5% O_2, production was reduced by a further factor of 10. The level produced in 40% CO_2 with 1% O_2 was insignificant. Growth was decreased by only 45–65% under these conditions (Table 3).

Roquefortine C production by *P. roqueforti* was decreased by factor of 7- to 20-fold by the various modified atmospheres studied. However, differences between the amounts produced under 20% or 40% CO_2 and in 1% or 5% O_2 were small.

CPA formation by *P. commune* decreased with increasing stringency of growth conditions (Table 5). In 20% CO_2 + 5% O_2, the level was 8% of that in air, while in 40% CO_2 + 1% O_2 it was 0.1% of that in air.

4. Discussion

It has previously been demonstrated that increased levels of CO_2 are not totally effective for preventing fungal growth in bakery products packaged under MAP (Smith et al., 1986). Total inhibition was only possible if head space O_2 was reduced and maintained at levels below 0.4%. Their conclusion has been confirmed here. Atmospheres with 20% or 40% CO_2 and less than 0.5% O_2 inhibited growth of most fungi in cheese for 30 days at 25°C. *B. nivea* was the only species able to grow (although this species has very rarely been found in cheese and is not of concern in this commodity).

All fungi grew in 20% or 40% CO_2 in the presence of 1% or 5% O_2. This confirms the work of Ellis et al. (1993) who reported that some fungi can grow in the presence of elevated CO_2 if O_2 is present.

Previous studies in our laboratory have shown that *F. oxysporum*, *M. plumbeus* and *B. fulva* are able to grow on culture media under atmospheres of

40% and 60% CO_2 with less than 0.5% O_2 (Taniwaki, 1995; Hocking and Taniwaki, 1997). However, these species did not grow on cheese under the conditions used here. Apparently cheese is not a suitable substrate for these fungi under low O_2 concentrations, perhaps because of its reduced a_w (0.93) and high content of lipid.

P. roqueforti and *B. nivea* were especially resistant to atmospheres with 20% or 40% CO_2 and 1% or 5% O_2. *P. roqueforti* is apparently well adapted to growth inside cheese, where high CO_2 and low O_2 concentrations might be expected. *B. nivea* is well known for its ability to cause spoilage in pasteurised foods under conditions of low O_2 tension (Pitt and Hocking, 1997).

Atmospheres inside plastic bags are difficult to control. The variability in results obtained may be partly explained by this factor. The species studied were chosen for their ability to grow in low O_2 tensions or high CO_2, and small variations in gas concentration in the plastic bags would undoubtedly contribute to some of the observed inconsistencies in the data presented in Tables 3 and 4 and Fig. 1.

When cheese was used as a substrate, *A. flavus*, *P. roqueforti* and *P. commune* were capable of producing aflatoxin, roquefortine C and cyclopiazonic acid, respectively, when growth occurred under the modified atmospheres studied here (Table 5). However, production of these toxins was always greatly reduced below production in air, indicating that MAP has a strong inhibitory effect on production of these mycotoxin. The high minimum temperature for growth of *A. flavus* (12°C; Pitt and Hocking, 1997) precludes aflatoxin production in cheese stored under refrigeration. Aflatoxin formation in cheese produced under modern processing and distribution systems is not a hazard.

The fungi of most concern in cheese are *Penicillium* species: *P. roqueforti* and *P. commune* were the species more frequently found in cheese by Hocking and Faedo (1992). *P. roqueforti* is used in the production of Roquefort and other types of blue cheeses containing internal mould. Roquefortine C is a neurotoxin that has been detected in several samples of blue cheese (Scott and Kennedy, 1976). However, in the current study, roquefortine C was produced at only very low levels (5–6 $\mu g/kg$) in any of the modified atmospheres studied.

P. commune is particularly well adapted to growth on cheese (Hocking and Faedo, 1992). In this study, cyclopiazonic acid was produced in cheese at 25°C after 14 days (Table 5). This toxin was not synthesized in cheese by *P. commune* at 8–10°C after 1 month (Still et al., 1979), suggesting its formation is unlikely under refrigerated storage. Le Bars (1979) reported that CPA was formed at 25°C, 13°C and 4°C: at 4°C the toxin (0.04 to 0.05 $\mu g/ml$) was detected only after 1 month, but at 25°C between 7 and 22 days (0.04 to 1.5 $\mu g\ ml^{-1}$). Le Bars (1979) found CPA in 11 out of 20 Camembert cheese crusts in concentration from 0.05 to 1.5 $\mu g\ g^{-1}$, but this toxin was not found in the inner parts of the cheese. The production of cyclopiazonic acid in cheese by *P. commune* can be prevented if adequate modified atmosphere packaging is used. Atmospheres with $O_2 < 0.5\%$ will prevent growth, while 20–40% CO_2 and 1% O_2 will reduce CPA production to very low levels.

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