

Research Note

Use of Ozone To Reduce Molds in a Cheese Ripening Room

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ABSTRACT

Cheese ripening rooms have an unusual environment, an environment that encourages mold growth. Ozone has been applied in various ways in the food industry. One useful advantage of ozone is that it inactivates molds. In this study, a cheese ripening room was ozonated, and the effectiveness of this treatment was evaluated both in air and on surfaces through sampling on a weekly basis over a 3-month period. The results obtained indicate that ozone treatment reduced the viable airborne mold load but did not affect viable mold on surfaces. Only by wiping the surfaces with a commercial sanitizer was it possible to decrease the viable mold load on surfaces. To improve overall hygiene in the ripening room, a combination of cleaning regimes is recommended. The mold genera occurring most frequently in the air of the cheese ripening room were *Penicillium*, *Cladosporium*, and *Aspergillus*, which accounted for 89.9% of the mold isolates. *Penicillium* and *Aspergillus* were identified to the species level, and data showed that *P. brevicompactum* and *P. aurantiogriseum*, as well as *A. versicolor*, were the species most frequently isolated.

Many extraneous molds find cheese an excellent medium for growth. If the ripening process occurs in an environment rich in airborne mold spores or propagules, cheese can become moldy during the ripening process or later, on retail or domestic premises. Molds are usually considered undesirable contaminants that may lead to complete spoilage of food products; consequently, they are seen as responsible for industrial and commercial financial losses. Molds that develop on cheese during refrigerated storage are mainly *Penicillium* species, which, in contrast to *Aspergillus* species, can grow at low temperatures (1). *Penicillia* are also well-known producers of mycotoxins, including patulin, mycophenolic acid, ochratoxin A, citrinin, and penicillin, in cheeses (16, 17). Furthermore, the presence of *Penicillium* and *Aspergillus*, as well as *Cladosporium*, *Alternaria*, and *Fusarium*, in indoor air in the food industry indicates poor hygienic processes. Therefore, these molds are good indicators of food hygiene status (8). It is compulsory for Portuguese food producers to apply the hazard analysis critical control point system for food hygiene to guarantee a safe product. To implement this system, it is necessary to evaluate the ambient air in critical areas (such as cheese ripening rooms) in food-processing plants and to implement and document procedures for the attainment of good manufacturing practices.

The spraying of chemical disinfectants in the air is a routine practice for the control of mold spore and propagule loads in cheese ripening rooms (5). As an alternative, the use of ozone in the air has been widely used. In 1965,

Gammon and Kereluk (4) reported the work of Gibson et al. and stated that an atmosphere of 3 to 10 ppm of O₃ prevented mold growth on cheese. These authors also indicated that mold spore load was reduced in treated rooms without causing flavor defects in cheese. However, these ozone concentrations appear very high and, despite the absence of legislation on this matter, it is generally accepted that ozone levels in rooms where people are working should not exceed 0.1 ppm. Experiments with lower levels of ozone have been conducted successfully. For example, the inactivation of spores with the application of 0.02 ppm of ozone at 25°C and at a relative humidity of 80 to 85% increases the life of a stored product by several weeks and decreases odors otherwise present in storage rooms (12). Furthermore, the U.S. Food and Drug Administration has stated that ozone is generally recognized as safe for broad food applications, making ozone an important product for use as a disinfectant or sanitizer in food processing (7).

In this study, a sampling program was carried out to check the efficacy of ozonation and to evaluate the mold spore and propagule loads in the air and on surfaces at specific sites in a cheese ripening room.

MATERIALS AND METHODS

Cheese ripening room. The gross volume of the cheese ripening room was 3,500 m³. A constant airflow rate injection processed by an air conditioner system was used to keep the temperature at 5 ± 1°C and the relative humidity at >80%.

Ozonation conditions. After setting up the equipment (PL10, Ambiozone, Spain; maximum ozone generation capacity, 10 g h⁻¹), we decided to use 8 g h⁻¹ as the rate of ozone generation. Three levels of ozonation were tested (Table 1). During

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TABLE 1. Ozone production rates tested during the treatment period

Period	Ozone production rate (g h ⁻¹)	Period of ozonation
Weeks 1 through 5	8	From 6:00 p.m. to 6:00 a.m.
Weeks 6 and 7	4	From 6:00 p.m. to 6:00 a.m.
Weeks 8 through 20	8	40 min of each hour

the first 7 weeks, the treatment took place during non-work time only with an ozone generation rate of either 8 or 4 g h⁻¹. In the following 12 weeks, the treatment took place all day with ozone being generated at a rate of 8 g h⁻¹ for the first 40 min of each hour. The ozone gas flowed from the generator to the cheese ripening room via manifold tubes, the same tubes used to control the air temperature and humidity of the room.

Sampling plan. Sampling of air and surfaces was carried out to monitor the treatment of the cheese ripening room. Five fixed sampling sites (three air sites [1.5 m above the floor] and two surface sites) were analyzed. The air sampling sites were the site opposite the room door (A1), the distal corner in relation to the door (A2), and the central site of the main corridor (A3). Samples taken before the start of ozonation treatment were collected for use as positive controls.

The sites for surface sampling were a shelf (S1) near the A1 site and a column (S2) near the A2 site. In addition, one mobile surface sampling site, i.e., one returnable cheese palette (S3) running randomly through different parts of the plant, was analyzed. The sampling was carried out during the morning on a weekly basis from February to July. Since it was determined that the ozonation treatment would not affect surface colony counts (see "Discussion"), after 2 months, surface disinfection (wiping with 4% chlorine solution) was carried out to reduce the fungal bioload. Samples taken before the wiping treatment were collected for use as positive controls.

Air sampling was carried out with the AirTEST-Omega de-

vice (L.C.A., France), which is based on the impaction method. The sampling apparatus allowed a selective airflow rate dependent on the mycological load in the room. The device was battery operated and portable. Two media were used to collect viable mold particles from the air: (i) dichloran rose bengal chloramphenicol (DRBC) (Oxoid, Basingstoke, Hampshire, UK) supplemented with 0.01% (wt/vol) chloramphenicol and (ii) malt extract agar (MEA) (Oxoid). The plates were incubated in the dark for 7 days at 25°C before they were examined. All samples were taken with the same sampler and were taken in duplicate.

The total number of viable mold spores and propagules on surfaces and in the air were determined in terms of CFU per surface unit or in terms of most probable number (MPN) per air volume unit according to the manufacturer's instructions.

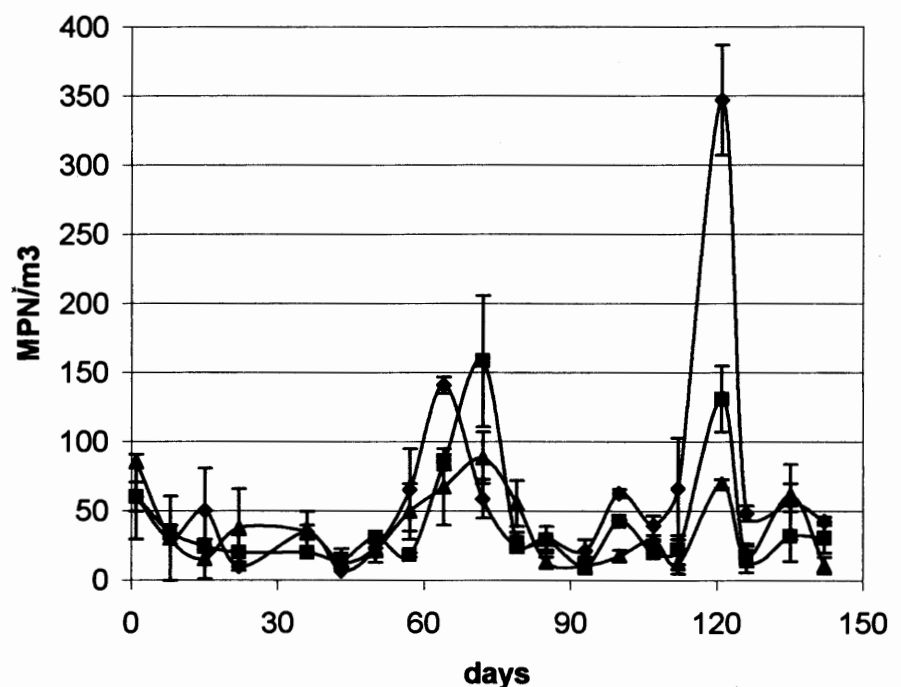
Isolation and identification of molds. Representative mold colonies were isolated, identified, and preserved in our culture collection (Micoteca da Universidade do Minho). Mold colonies were transferred to individual slants of MEA as they became visible by magnification (×4). The same medium was also used to maintain the isolates at 4°C. In this study, each strain isolated from the air plates was considered one isolate. Taxonomic identification of all isolates on the basis of macroscopic and microscopic distinctive features in accordance with the published literature (3, 13–15) was undertaken.

RESULTS

Figure 1 shows the fungal bioload results for the three air sampling sites in the cheese ripening room during the period studied. No differences between the MPNs for DBRC medium and those for MEA were observed; therefore, the values in Figure 1 represent the overall means for the data obtained. The mean control values for sites A1, A2, and A3 were 524 ± 195, 497 ± 235, and 176 ± 53 MPN/m³, respectively.

Figure 2 shows the results of surface sampling site S1, which exhibit patterns similar to those of sites S2 and S3. No differences were found in mold load values either be-

FIGURE 1. Airborne molds in the cheese ripening room during ozonation treatment. ♦, site A1; ▲, site A2; ■, site A3. Standard error bars are shown.



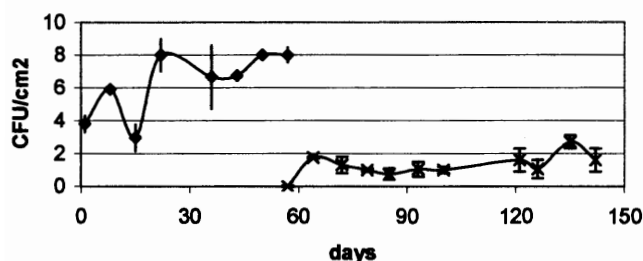


FIGURE 2. Surface mold counts for site S1 (as determined by replicate plating on DRBC medium) in the cheese ripening room during ozonation treatment without surface wiping (♦) and after surface wiping (×). Standard error bars are shown.

fore or during the first 8 weeks of ozonation treatment. In contrast, the mold load values decreased three- or fourfold after the surfaces were wiped with a commercial sanitizer. In addition, no differences were found between mold load values obtained with DRBC medium and those obtained with MEA.

A total of 69 molds were isolated from the air plates (Table 2). The mold most frequently isolated from the cheese ripening room was *Penicillium*, followed by *Cladosporium* and *Aspergillus*. These molds accounted for 42 of the 69 molds isolated.

For the 38 *Penicillium* strains isolated, 14 different species were identified. *Penicillium brevicompactum*, *P. aurantiogriseum*, *P. corylophilum*, *P. expansum*, and *P. neoehinulatum* were the species most frequently isolated from the cheese ripening room (Table 3).

Table 4 shows the distribution of aspergillus isolates comprising five species. *Aspergillus versicolor* was the species most frequently isolated.

DISCUSSION

The present study shows that ozonation of the cheese ripening room was very effective in reducing the viable airborne mold load. This treatment resulted in a 10-fold reduction compared with the level observed for the control. The values obtained were generally <50 MPN/m³ for the different intermittent scheduled treatments. However, two peaks can be observed in Figure 1. The first peak is due to an ozone generator breakdown, which led to a decrease in the ozone gas level in the room. The second peak is due to the room door system, which could not be kept closed during this time. Notwithstanding these problems, the values obtained for both incidents were still below the control values, and recovery profiles were achieved in a short period.

TABLE 2. Genera isolated from the cheese ripening room

Mold	No. of isolates
<i>Alternaria</i>	1
<i>Aspergillus</i>	10
<i>Cladosporium</i>	14
<i>Penicillium</i>	38
<i>Trichoderma</i>	2
<i>Mycelia sterilia</i>	4
Total (five genera)	69

TABLE 3. Identification of *Penicillium* strains isolated from the cheese ripening room

<i>Penicillium</i> sp.	No. of isolates
<i>P. aurantiogriseum</i>	6
<i>P. brevicompactum</i>	7
<i>P. corylophilum</i>	5
<i>P. digitatum</i>	1
<i>P. expansum</i>	5
<i>P. glabrum</i>	3
<i>P. griseofulvum</i>	1
<i>P. implicatum</i>	1
<i>P. minioluteum</i>	1
<i>P. neoehinulatum</i>	4
<i>P. pinophilum</i>	1
<i>P. rubrum</i>	1
<i>P. simplicissimum</i>	1
<i>P. spinulosum</i>	1
Total (14 species)	38

It is possible to conclude that ozone gas is very effective in decreasing viable airborne mold levels when it is present in the air of a closed or sealed room.

In contrast, over the surface swab sampling period, fungal counts for site S1 did not decrease (Fig. 2). Molds often adhere firmly to surfaces, and strong attachments are observed especially in the regions of cracks, holes, and irregularities (2). We surmise that ozone gas is not able to affect molds present on surfaces because their diffusion of these molds through the boundary layer near the surface is slow and because ozone will not penetrate the surfaces owing to its short half life. Ozone treatment may not prevent the development of molds that are already present on cheese surface before the cheese enters the ripening room, but it may reduce the sedimentation of viable airborne molds during ripening. This finding is in line with previous observations made by Schomer and McCulloch in the 1940s and quoted by Ingram and Barnes (6), who concluded that ozone was able to destroy most aerial *P. expansum* spores but failed to prevent their germination when they were lodged in apple surface wounds. Only after the surfaces were wiped was a decrease in mold load observed. In our opinion, no single method can solve the problem of mold load in a cheese ripening room, but a combination of different approaches is the key element in overcoming this hygiene-sanitary problem.

TABLE 4. Identification of *Aspergillus* strains isolated from the cheese ripening room

<i>Aspergillus</i> sp.	No. of isolates ^a
<i>A. candidus</i>	1
<i>A. flavus</i>	1
<i>A. ustus</i>	1
<i>A. versicolor</i>	4
<i>Eurotium chevalieri</i>	1
Total (five species)	8

^a Two isolates lost their viability during the isolation process.

In the present study, the molds most frequently isolated were identified in order to assess the molds responsible for the poor hygiene status of the cheese ripening room studied. The genera listed in Table 2 clearly show that *Penicillium*, *Cladosporium*, and *Aspergillus* dominate in this ripening room. These genera are regarded as indicators of poor factory hygiene, and they should be monitored to assess procedures to improve in hygiene (8). When penicillia were studied in more detail, *Penicillium commune*, *P. palitans*, *P. roqueforti*, and *P. solitum* were not found as airborne molds, although they have been described as important contaminants of cheese (9, 10, 17). However, *A. versicolor* was the prevailing *Aspergillus* species isolated, a finding that concurs with the results obtained by Lund et al. (11), who indicated that this mold often dominates the airborne flora of ripening rooms but rarely grows on cheeses. The fact that most of the penicillia and aspergilli identified in this study are potential mycotoxin producers represents an additional problem for the cheese industry.

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REFERENCES

1. Bullerman, L. B. 1981. Public health significance of molds and mycotoxins in fermented dairy products. *J. Dairy Sci.* 64:2439-2452.
2. Dhawan, S., and K. L. Grag. 1993. Fungal deterioration of paper materials, p. 119-128. In B. Rai, D. K. Arora, N. K. Dubey, and P. D. Sharma (ed.), *Fungal ecology and biotechnology*. Rastogi Publications, Meerut, India.
3. Ellis, M. B. 1971. *Dematiaceous Hypomycetes*. CAB International, Wallingford.
4. Gammon, R., and K. Kereluk. 1965. Gaseous sterilization of foods. *AIChE Symp. Ser.* 132:91-99.
5. Holah, J. T., S. J. Rogers, J. Holder, K. E. Hall, J. Taylor, and K. L. Brown. 1995. The evaluation of air disinfection systems. *R&D Rep. Campden Chorleywood Food Res. Assoc.* 13:1-40.
6. Ingram, M., and E. M. Barnes. 1954. Sterilization by means of ozone. *J. Appl. Bacteriol.* 17:246-271.
7. Kim, J.-G., A. E. Yousef, and S. Dave. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. *J. Food Prot.* 62:1071-1087.
8. Kurata, H. 1994. Mycological monitoring for sanitary evaluation in the Japanese food industry, p. 31-37. In R. A. Samson, B. Flannigan, M. E. Flannigan, A. P. Verhoeff, O. C. G. Adan, and E. S. Hoekstra (ed.), *Health implications of fungi in indoor environments. Air quality monographs, vol. 2*. Elsevier Applied Science, Amsterdam.
9. Kure, C. F., and I. Skaar. 2000. Mould growth on the Norwegian semi-hard cheeses Norvegia and Jarlsberg. *Int. J. Food Microbiol.* 63:133-137.
10. Kure, C. F., Y. Wasteson, J. Brendehaug, and I. Skaar. 2001. Mould contaminants on Jarlsberg and Norvegia cheese blocks from four factories. *Int. J. Food Microbiol.* 70:21-27.
11. Lund, F., O. Filtenborg, and J. C. Frisvad. 1995. Associated mycoflora of cheese. *Food Microbiol.* 12:173-180.
12. OZONET. Natural purification systems. Available at: http://www.ozonet.com/Ozone_applications.html#anchor91913. Accessed 12 May 2003.
13. Pitt, J. I. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, London.
14. Pitt, J. I., and A. D. Hocking. 1997. *Fungi and food spoilage*. Academic Press, London.
15. Raper, K. B., and D. I. Fennell. 1965. *The genus Aspergillus*. Williams and Wilkins, Baltimore.
16. Scott, P. M. 1989. Mycotoxigenic fungal contaminants of cheese and other dairy products, p. 193-259. In H. P. van Egmond (ed.), *Mycotoxins in dairy products*. Elsevier Applied Science, London.
17. Taniwaki, M. H., A. D. Hocking, J. I. Pitt, and G. H. Fleet. 2001. Growth of fungi and mycotoxin production on cheese under modified atmospheres. *Int. J. Food Microbiol.* 68:125-133.