Journal of Food Protection, Vol. 55, No. 5, Pages 367-369 (May 1992)

Inhibition of Surface Molds on Cheese by Polyethylene Film Containing the Antimycotic Imazalil

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(Received for publication August 26, 1991)

ABSTRACT

Imazalil, an antimycotic agent, was incorporated into low density polyethylene (LDPE) film and the resulting films tested for ability to inhibit *Penicillium* sp., and *Aspergillus toxicarius* growth by measuring the rate of carbon dioxide (CO_2) production in sealed jars containing either inoculated potato dextrose agar (PDA) or Cheddar cheese. Inhibition of surface mold growth on cheese was also determined in open systems. An imazalil concentration of 2000 mg/kg LDPE film delayed *A. toxicarius* growth on PDA while LDPE film containing 1000 mg/kg imazalil markedly delayed *Penicillium* sp. growth. Furthermore, LDPE film containing 1000 mg/kg imazalil inhibited both molds growing on Cheddar cheese. These data suggest that incorporation of an antimycotic agent such as imazalil into food contact packaging films would inhibit surface mold growth.

Surface contamination is a major factor in the microbial deterioration of several foods. Antimicrobial agents are either mixed directly with foods or applied to the surface of foods such as cheese to inhibit spoilage. If mixed directly, all added substances will be consumed even though they might not be needed in the interior of the food. However, if applied only to the surface, food loses the protection when it is partially consumed. Surface application also requires an additional step in the manufacturing process. Antimicrobial agents which are added into packaging materials and then migrate in small amounts to food may be a useful alternative.

It is a common practice to incorporate additives into synthetic polymers (plastics) for the purpose of modifying the polymer's chemical and/or physical properties and of facilitating manufacturing. Antioxidants, UV light absorbers, and other compounds have been added into polyethylene (*4*). Low density polyethylene is the most commonly used food contact polymeric material in food packaging. Halek and Garg (*3*) have chemically coupled an antimicrobial agent (methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate) to polymers and demonstrated inhibition of mold growth.

Preliminary tests in our laboratory indicated that low density polyethylene (LDPE) film incorporated with several common food-use antimicrobial agents including the free acids and salts of sorbic acid, propionic acid, and benzoic acid showed no antimicrobial activity. Imazalil (1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole) is thermally stable at the temperatures used in LDPE processing and has antimicrobial activity at low concentrations (*5*). Imazalil is a fungal sterol biosynthesis inhibitor (*6*) and is permitted (*1*) for use on fresh fruits to prevent mold infection. The objective of our work was to investigate the general principle that antimycotic-incorporated LDPE film could be used to inhibit surface mold growth in packaged foods.

MATERIALS AND METHODS

Imazalil-incorporated low density polyethylene (IMLDPE) film

LDPE (approximate mol wt 50,000, density 0.92) was purchased from Scientific Polymer Products, Inc. (Ontario, NY). Imazalil was a gift from Janssen Pharmaceutica (Piscataway, NJ). Two g of LDPE, 50 ml toluene, and an appropriate amount of imazalil were heated to reflux until LDPE dissolved. The solvent was evaporated at room temperature and the residue further dried in a vacuum oven (1 h, approximately -730 mm Hg pressure, 45°C). A small LDPE film (approximate thickness of 5.1 μm) was formed by pressing the residue between Mylar-covered heated (120°C) platens of a Loomis hydraulic press (Caldwell, NJ). Test chips were cut from the resulting films. The amount of imazalil incorporated into the films was confirmed by HPLC analysis.

Molds and spore suspension

Penicillium sp. isolated from potato by the Department of Plant Pathology, Cornell University and *Aspergillus toxicarius* ATCC 15517 were used. Cultures were maintained on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI) slants and subcultured every 10-14 d. The concentration of mold spores in the inoculum was approximately 10^1/μl. Preparation of mold spore suspensions has been described (*7*).

Antimycotic activity of IMLDPE film on PDA

The rate of increase in CO_2 in the headspace of sealed glass jars (ca. 120 ml [4 oz.], Ball Corporation, Muncie, IN; Model 14400-80400) was used, with modification, as a measure of the lag, log, and stationary phases of mold growth. These procedures have been described in detail (*7*). Seven ml of PDA was added to each jar (thickness ca. 3.5 mm), an approximate 2.5 x 2.5 cm piece of film placed on the solidified PDA, and 1 μl of mold

suspension placed on the film. After the mold suspension dried, the mold and film were covered with an additional 3 ml of PDA (thickness ca. 1.5 mm), the jar sealed, and incubated at 25°C. Proper controls, jars containing inoculated PDA without LDPE film and jars containing inoculated PDA/LDPE film without imazalil, were included. Headspace gas composition was measured by gas chromatography by sampling each jar through a rubber septa in the lid as described (7). Gas was sampled for 14 d.

Antimycotic activity of IMLDPE film on Cheddar cheese

Five g of Cheddar cheese was placed in a 20-ml beaker which was placed inside of a jar (described above). The jar was covered and sterilized (121°C, 15 min); after cooling, the resulting solidified cheese was ca. 6 mm thick. The surface of the cheese was inoculated with 1 µl of spore suspension, ca. 1.5 x 1.5 cm of LDPE was placed over the inoculation area on the cheese, and the jar sealed and incubated at 25°C. Sterilized cheese alone, sterilized/inoculated cheese not covered with LDPE film, and sterilized/inoculated cheese covered with LDPE film without imazalil, were included as controls. The headspace gas composition was measured (7) for 14 d. All data are the average of at least three jars. In separate experiments, cheese was aseptically cut into ca. 2.5 x 1.5 x 0.5-cm pieces, placed in sterile petri dishes, inoculated with mold spore suspension (1 µl) in the center, and covered with LDPE film (with or without imazalil, ca. 2 x 1 cm). The cheese was incubated at 25°C and examined daily for 10 d.

RESULTS AND DISCUSSION

The rate of increase in headspace CO_2 in jars containing only PDA and mold (i.e., without film) followed typical curves (i.e., lag period, log growth, and stationary phases) previously observed (7) for mold growth (Fig. 1). With *Penicillium* sp., the headspace concentrations O_2 declined (data not shown) and CO_2 increased to 1.5 and 22.5% in 14 d, respectively. With *Aspergillus toxicarius*, the O_2 (data not shown) and CO_2 concentrations were 2 and 30% on day 14. When LDPE film that did not contain imazalil was used, the headspace gas composition curves showed a longer lag period and a lower rate of log growth than samples without film, but the CO_2 concentrations for both *Penicillium* sp. and *A. toxicarius* were nearly the same as in jars without film at the end of incubation (Fig. 1). Spores were deposited directly on the film; hence, it is likely that the film reduced the mold spores access to nutrients contained in the lower media layer. Both molds grew in these two types of control jars indicating that the media as used supported mold growth.

Film containing 500 mg/kg imazalil further reduced the rate of CO_2 production in PDA inoculated with *Penicillium* sp. (Fig. 1). The lag period was extended to 4 d and rate of growth slowed, but the CO_2 concentration was similar to the control on day 14. Film containing 1000 mg/kg imazalil extended the lag phase to 7 d and further slowed the growth phase. The amount of CO_2 produced in 14 d was approximately half that of the controls. The inhibitory effect of LDPE film containing imazalil was less pronounced with *A. toxicarius* (Fig. 1). LDPE film containing 1000 mg/kg imazalil did not significantly extend the lag period and slowed growth slightly compared to LDPE controls without imazalil. When imazalil content was increased to 2000 mg/kg, the lag period increased to 3 d. The maximum rate of growth was similar in all cases.

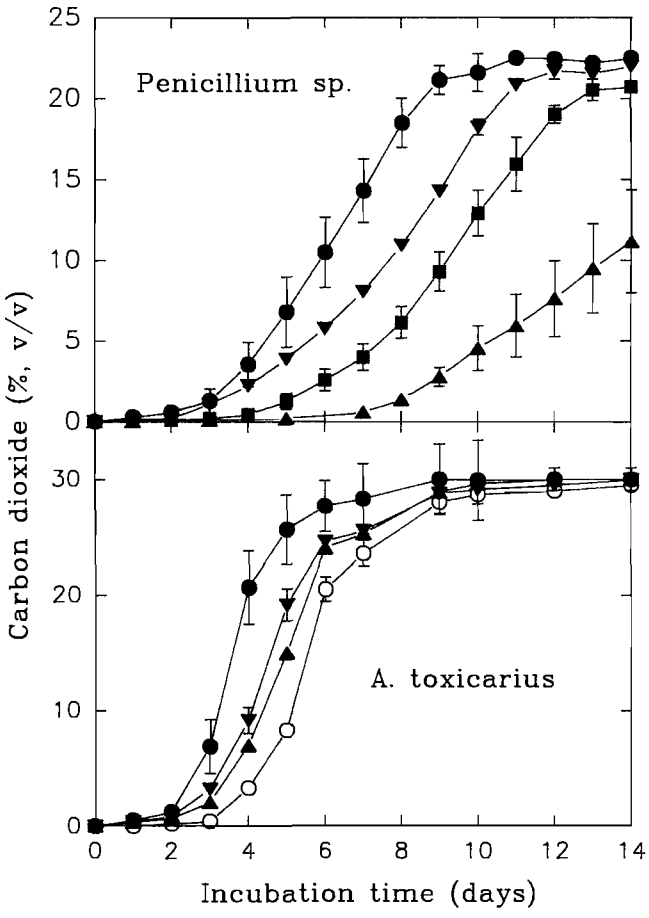

Figure 1. *Inhibition of* Penicillium *sp. and* A. toxicarius *growth on PDA by LDPE film in glass jars. Mean of three jars; error bar: standard error.*
● Control jars, without LDPE film.
▼ LDPE film without imazalil.
■ LDPE film containing 500 mg/kg imazalil.
▲ LDPE film containing 1000 mg/kg imazalil.
○ LDPE film containing 2000 mg/kg imazalil.

In order to compare the inhibitory activity of imazalil-incorporated LDPE film on media and cheese, the same mold species (*Penicillium* sp. and *A. toxicarius*) were used, although they are not the most common cheese spoilage molds. The headspace gas composition in jars containing only sterilized cheese did not change in 14 d (data not shown). The gas composition changes, when LDPE film was not present, for both *Penicillium* sp. and *A. toxicarius* on cheese (Fig. 2) were similar to those with PDA indicating that the cheese used in this study supported mold growth (no growth inhibition substances formed during sterilization). The growth was only slightly inhibited for both molds when covered by LDPE film without imazalil. This inhibition may have been a result of a reduction in the ability of the mold spores to obtain O_2. LDPE film containing 500 mg/kg imazalil extended the lag period to 6 d (*Penicillium* sp.) or to 4 d (*A. toxicarius*) and reduced the change rate in the log growth phase (Fig. 2). LDPE film containing 1000 mg/kg imazalil inhibited both molds on cheese suggesting that the imazalil-containing film was more effective on cheese than PDA. PDA may be a better

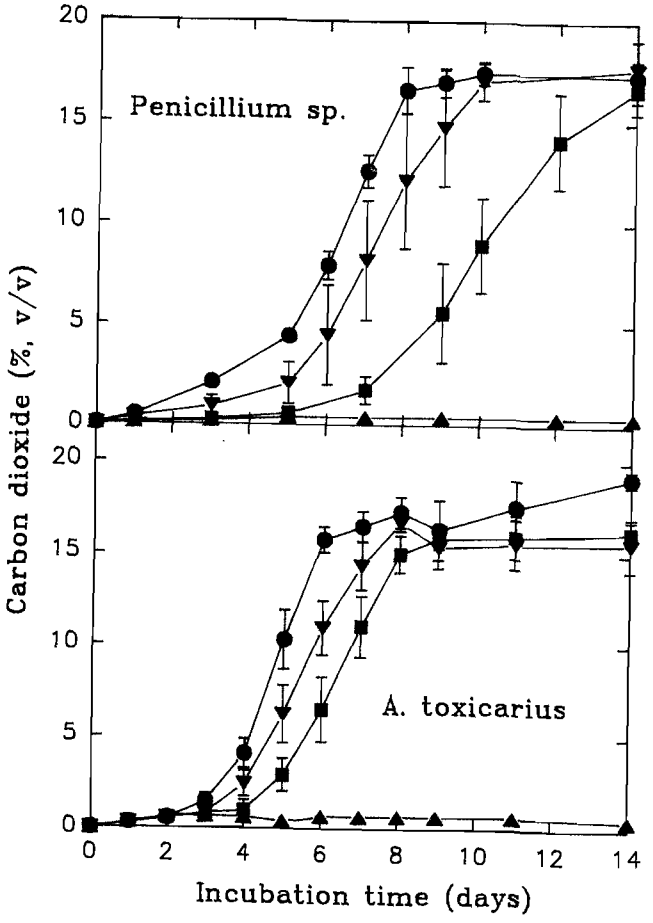

Figure 2. *Inhibition of* Penicillium *sp. and* A. toxicarius *growth on sterilized Cheddar cheese by LDPE film in glass jars. Mean of three jars; error bar: standard error.*

● Cheese inoculated with mold spores but not covered with LDPE film.

▼ Inoculated cheese covered with LDPE film without imazalil.

■ Inoculated cheese covered with LDPE film containing 500 mg/kg imazalil.

▲ Inoculated cheese covered with LDPE film containing 1000 mg/kg imazalil.

medium for molds (2) than cheese, or imazalil may migrate to cheese to a greater extent than PDA.

The inhibitory effect of LDPE film containing 1000 mg/kg imazalil was further tested on nonsterile Cheddar cheese inoculated with molds in unsealed petri dishes. Mold growth was visible after 2 d with both mold species in samples without LDPE film. When film without imazalil was used, mold growth was visible around the LDPE film on day 3 and on day 4 for *A. toxicarius* and *Penicillium* sp., respectively. The delay on cheese covered with LDPE film indicated that more time was needed for mold to access O_2 and to develop mycelium reaching out beyond the area covered by LDPE film. No growth was observed up to 10 d when LDPE film containing 1000 mg/kg imazalil was used.

These data demonstrate that an antimycotic agent such as imazalil can be successfully incorporated into LDPE and that this an effective way to control mold grow on the surface of cheese and media. While imazalil is approved only for mold inhibition on fruits and vegetables other inhibitors might behave similarly. The use of such films means that inhibition would be extended after the package was opened and a portion of the food was used as long as the film was used to rewrap the product. Additional studies are needed to determine the effect of films made with other antimycotics.

ACKNOWLEDGMENT

The authors wish to thank Dr. Y. D. Hang for his suggestions in preparation of this manuscript.

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