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Bacterial Survival on Lean Beef and Bologna Wrapped With Cornstarch-Containing Polyethylene Film

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ABSTRACT

Cornstarch-containing plastic films could be used to package foods if the presence of cornstarch had no adverse effect on food safety. The survival of pathogenic bacteria on meat samples that had been wrapped with cornstarch-containing plastic film was evaluated. Cornstarch-containing polyethylene film and control polyethylene film were used to cover lean beef and bologna that had been inoculated with *Salmonella typhimurium, Staphylococcus aureus,* and *Bacillus cereus.* Additional samples were prepared in which inoculum was applied to the outer surface of plastic-covered meat. Samples were stored at 4 and 21°C. Bacterial recovery from meat samples indicated that survival was not enhanced by the presence of cornstarch. No migration through polyethylene film or cornstarch-containing polyethylene film into lean meat or bologna was observed. These results indicated that, from a microbiological viewpoint, cornstarch-containing polyethylene film could be successfully used to package foods.

In response to heightened public awareness of environmental issues, rapidly degradable plastics have been developed. Incorporation of cornstarch into plastic film is one method which would theoretically enhance biodegradation (*10,14*). Starch-degrading enzymes produced by microorganisms present in landfills would create gaps in the plastic and increase the surface area available for attack by plastic degrading microorganisms. Cornstarch-containing plastics have been used as shopping and garbage bags and as agricultural mulch. Before this type of plastic may be used to package food, the effects of cornstarch-containing plastic film on food quality and food safety must be examined (*3-6*). Because some organisms are capable of utilizing cornstarch as a growth substrate, enhanced bacterial growth or survival could occur on foods in contact with cornstarch-containing plastic. In addition, if cornstarch was degraded while foods are stored, contamination of the food product due to microbial migration from outside the package could occur (*3,16*).

Methods that have been described for evaluating the microbial permeability of food packages do not differentiate between migration that is due to penetration of the packaging film itself and migration through defects in package integrity. In the Bio-test method, filled packages are immersed into a tank of bacteria-inoculated water and are incubated for up to 3 weeks. If the package is permeable, microbial growth, gas production, or pH changes in the food would be evident (*11,13*). Chen et al. (*7*) inoculated aseptically filled juice packages with aerosolized *Lactobacillus cellobiosus* to evaluate microbial integrity of packages. Growth of the test organisms or pH changes in apple juice indicated compromised package integrity. Methods to determine the resistance of plastics to microbial growth have been described by the American Society for Testing and Materials (*1,2*). Plastic samples are placed onto the surface of agar plates and are inoculated with test cultures. Deterioration is measured on a semiquantitative basis, depending on the percentage of the plastic surface that is covered with growth. The American Society for Testing and Materials methods do not allow for determination of microbial migration through a plastic sample.

The objective of this study was to evaluate the survival of *Salmonella typhimurium, Staphylococcus aureus,* and *Bacillus cereus* on lean beef and bologna samples that were covered with cornstarch-containing polyethylene film (CSPE). The population of organisms inoculated onto the surface of meat samples which were subsequently covered with either CSPE or control polyethylene (PE) was compared. In addition, meat samples were covered with CSPE or PE, and the outer surface of the plastic was then inoculated. These samples were used to evaluate if microbial migration through the film had occurred.

MATERIALS AND METHODS

Plastic films

Low-density, virgin PE plastic film that contained food-grade slip and antiblock agents was extruded by a local plastic manufacturer (Polar Plastics, Oakdale, MN) and used as a control. CSPE film was prepared by combining the same polymer with a master blend that contained 40% cornstarch and 60% polyethylene to result in a final cornstarch concentration of 6% in the film. Both film types had nominal thickness of 0.05 mm (0.002 in) and were aseptically cut into 2.5 x 2.5-cm squares.

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Culture preparation

Salmonella typhimurium, *S. aureus*, and *B. cereus* were propagated for 24 h at 35°C in tryptic soy broth (Difco Laboratories, Detroit, MI). Cells were harvested by centrifugation at 12,000 g for 10 min. Cells were washed and resuspended in nutrient salts broth (NSB). NSB was prepared by omitting the solidifying agent from nutrient salts agar (*1*). Serial dilutions of the suspension were prepared in NSB so that an initial population of approximately 10^4 CFU per sample would be obtained.

Food systems

Beef top-round steak [72.7% moisture, 4.7% fat (12)] and beef bologna [56.2% moisture, 27.5% fat (12)] were purchased on the day of use and aseptically cut into 2.5 x 2.5-cm squares. Bologna samples were presliced, while beef samples were cut to a thickness of approximately 3 mm. Beef and bologna were used to represent moist and high-fat systems, respectively.

Inoculation and storage

Meat samples were placed into sterile plastic petri dishes and inoculated in two different orientations. On one set of samples, diluted bacterial suspensions were spread on the meat surface, and meat was covered with either CSPE or PE film. The remaining samples were covered with plastic, and inoculum was applied to the surface of the plastic. This second orientation was used to determine if microorganisms were able to migrate through the plastic film. Separate trials were performed with samples stored at 4 or 21°C. Storage at room temperature (21°C) represented abuse conditions. Bacterial populations were monitored until samples became aesthetically unacceptable due to development of off-odors, slime formation, or dessication. Triplicate trials were performed with each microorganism. Duplicate samples were evaluated for each condition at each sampling time.

Enumeration

The initial bacterial population on meat samples was determined by stomaching (Stomacher Lab Blender 400; Tekmar Co., Cincinnati, OH) samples for 2 min in 10 ml of 0.1% peptone water (PW) (Difco). If inoculum had been applied to the surface of the meat, the entire sample, meat and plastic, was stomached. If the inoculum had been applied to the plastic, the plastic was removed and the meat was analyzed. Additional dilutions were prepared in PW if necessary. Total aerobic bacterial populations were determined by plating with tryptic soy agar (Difco). Populations of the inoculated organisms were determined by plating aliquots from the same dilutions with selective media: Xylose lysine desoxycholate (Difco), Baird Parker (Difco), and mannitol yolk polymyxin (*17*) agars were used for *S. typhimurium*, *S. aureus*, and *B. cereus* samples, respectively. When very low levels of the organism in question were suspected to be present, the entire 10 ml of PW in which the sample was stomached was plated on the selective medium. The diluent was divided and used to inoculate multiple plates. Two samples were analyzed for each condition at each sampling time. All plates were incubated at 35°C for 48 h before colonies were counted.

RESULTS AND DISCUSSION

When *S. typhimurium* was inoculated so that bacterial cells were in contact with both lean beef and plastic, populations increased slightly on both PE and CSPE samples held at 21°C (Fig. 1a). A similar increase in total aerobic population was observed. At 4°C, the salmonellae population remained stable when in contact with beef, while total flora gradually increased (Fig. 1b). *S. typhimurium* was not recovered from samples in which the inoculum was applied to the plastic; therefore, migration through PE or CSPE into beef did not occur (Fig. 1a, b). The increase in *S. typhimurium* population observed on samples held at 21°C with inoculum in contact with bologna closely paralleled the increase in total flora (Fig. 2a), while a slight decline in recovery was observed on both PE- and CSPE-covered samples stored at 4°C (Fig. 2b). No migration through the PE or CSPE films onto bologna samples was apparent at either temperature (Fig. 2a, b).

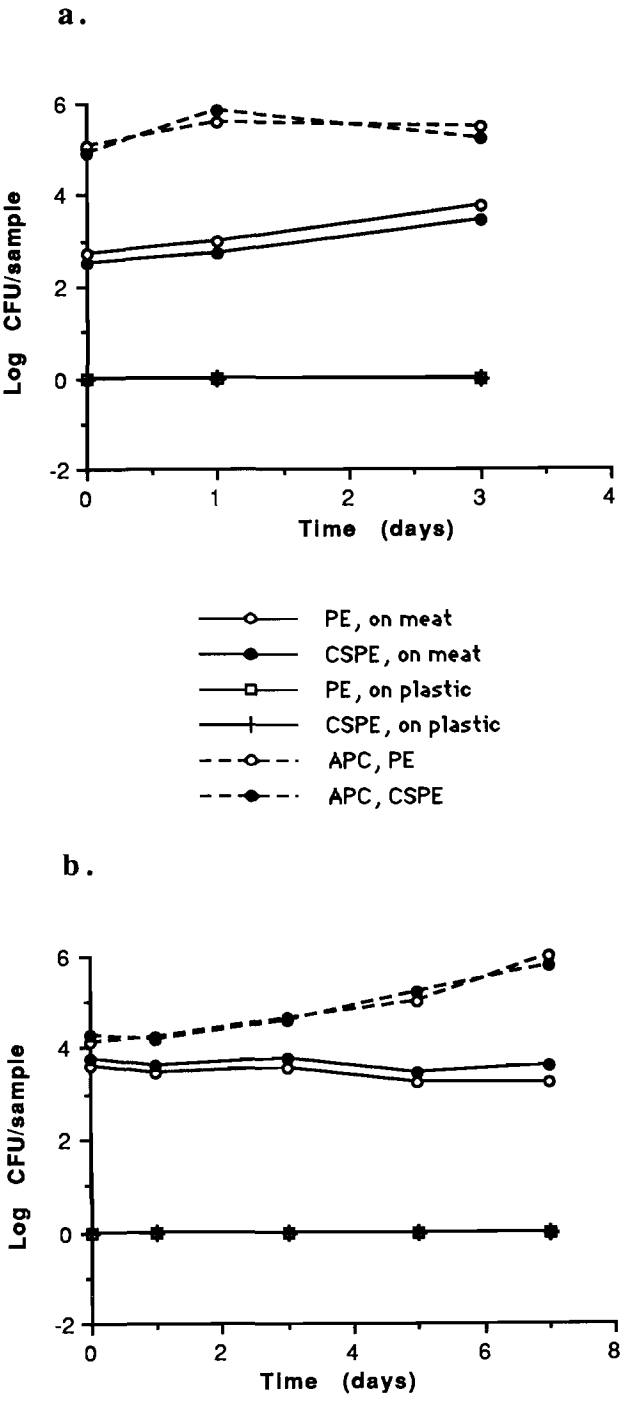

Figure 1. *Recovery of* S. typhimurium *and total aerobic population (APC) from plastic-covered beef stored at (a) 21°C or (b) 4°C. Inoculum was applied either directly to the meat surface or to plastic which covered the meat. Zero values indicate no recovery.*

Populations of *S. aureus* remained constant while total aerobic recovery increased on samples with inoculum ap

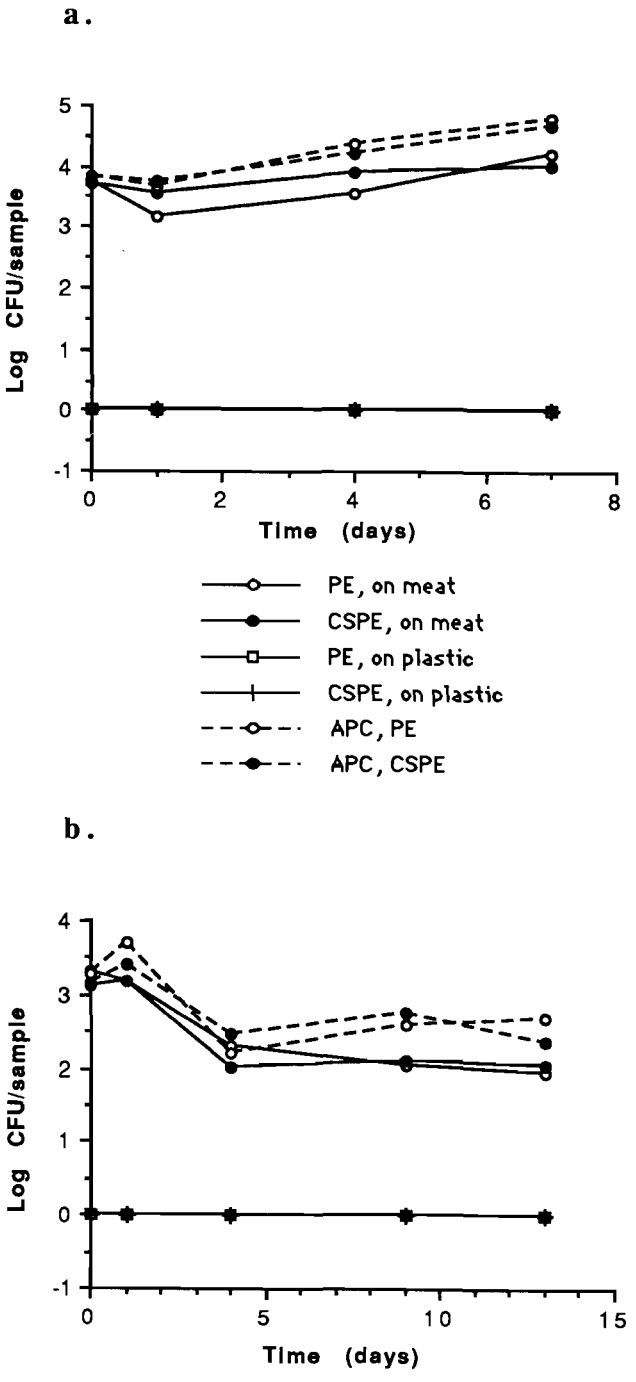

Figure 2. *Recovery of* S. typhimurium *and total aerobic population (APC) from plastic-covered bologna stored at (a) 21°C or (b) 4°C. Inoculum was applied either directly to the meat surface or to plastic which covered the meat. Zero values indicate no recovery.*

plied to lean beef when stored at 21°C (Fig. 3a). Recovery was not affected by film type. Recovery of *S. aureus* from lean beef samples in which the inoculum was applied to the plastic reflects the indigenous *S. aureus* present on this particular cut of beef. The indigenous *S. aureus* population declined when held at 21°C. No increase in *S. aureus* population was observed as a result of migration through the film. By the time beef samples held at 4°C were no longer acceptable, the total aerobic population had declined. Little change in the *S. aureus* population in contact with beef stored at 4°C was observed (Fig. 3b). Indigenous *S. aureus* were not recovered from this beef cut. No migration of *S. aureus* through either PE or CSPE into beef was apparent. The number of *S. aureus* cells in contact with bologna remained constant on samples covered with either film type at 21°C (Fig. 4a), while a decline in recovery on samples stored at 4°C was observed (Fig. 4b). No penetration through plastic film was observed at either storage temperature.

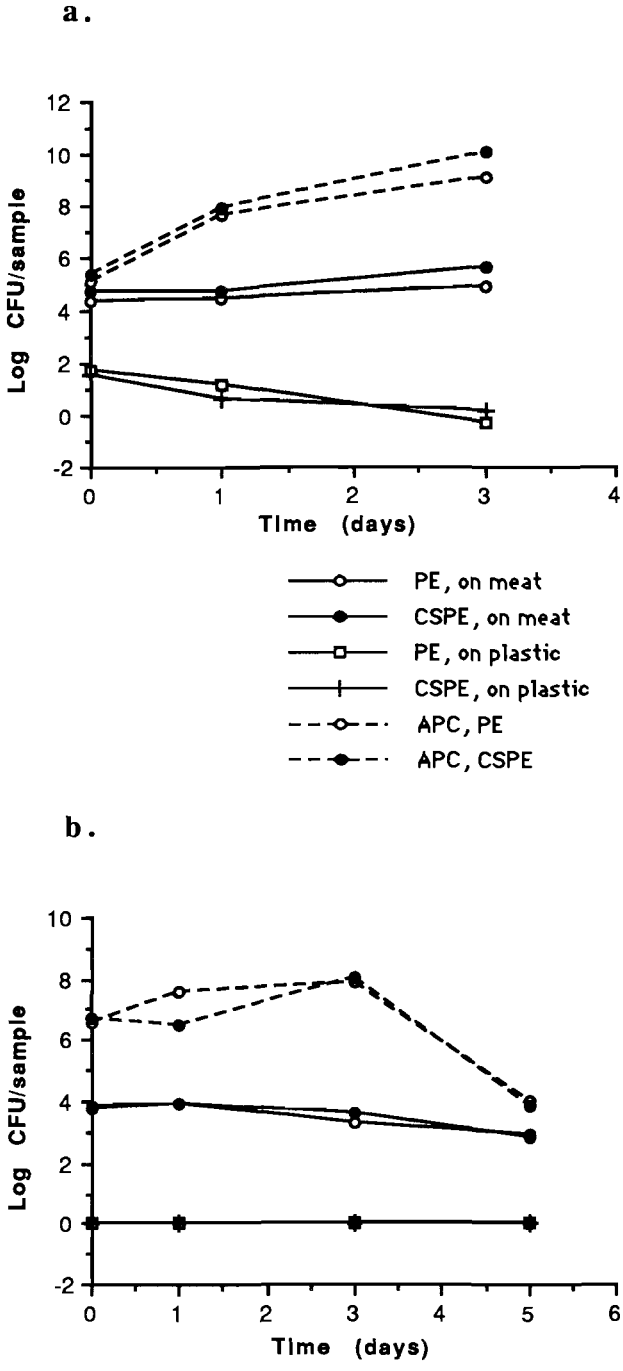

Figure 3. *Recovery of* S. aureus *and total aerobic population (APC) from plastic-covered beef stored at (a) 21°C or (b) 4°C. Inoculum was applied either directly to the meat surface or to plastic which covered the meat. Zero values indicate no recovery.*

A rapid increase in total flora corresponded to a smaller increase in *B. cereus* population on samples with inoculum

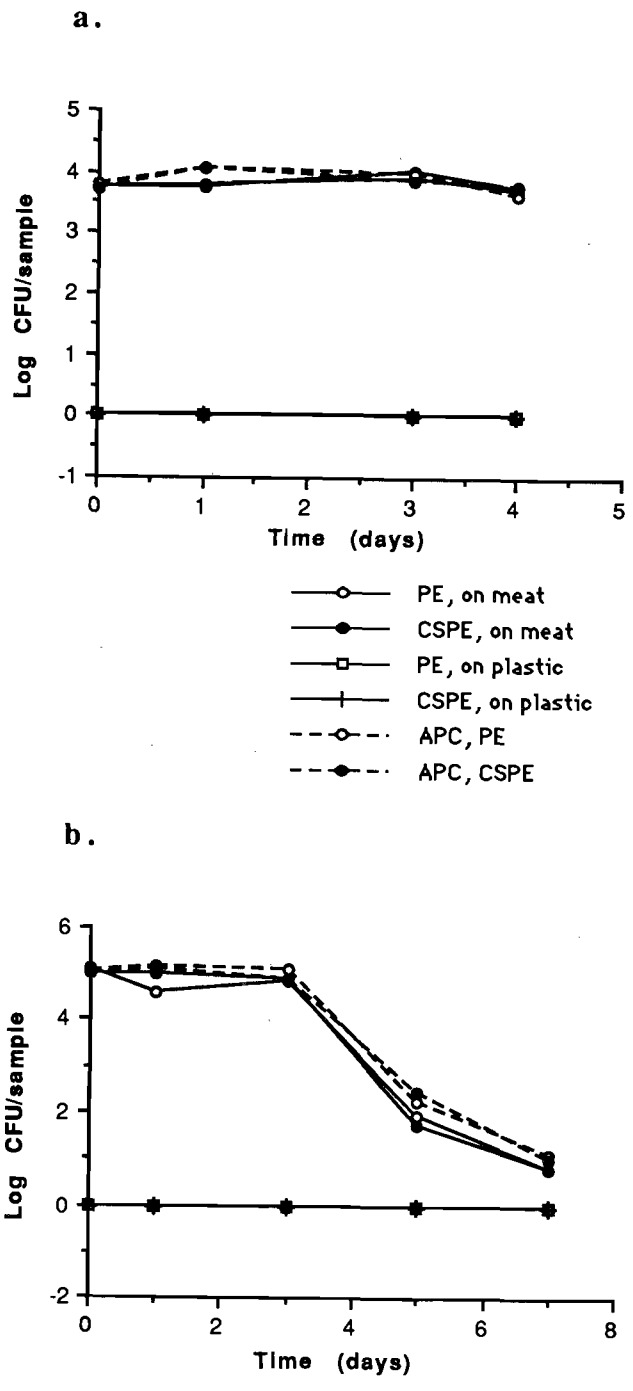

Figure 4. *Recovery of* S. aureus *and total aerobic population (APC) from plastic-covered bologna stored at (a) 21°C or (b) 4°C. Inoculum was applied either directly to the meat surface or to plastic which covered the meat. Zero values indicate no recovery.*

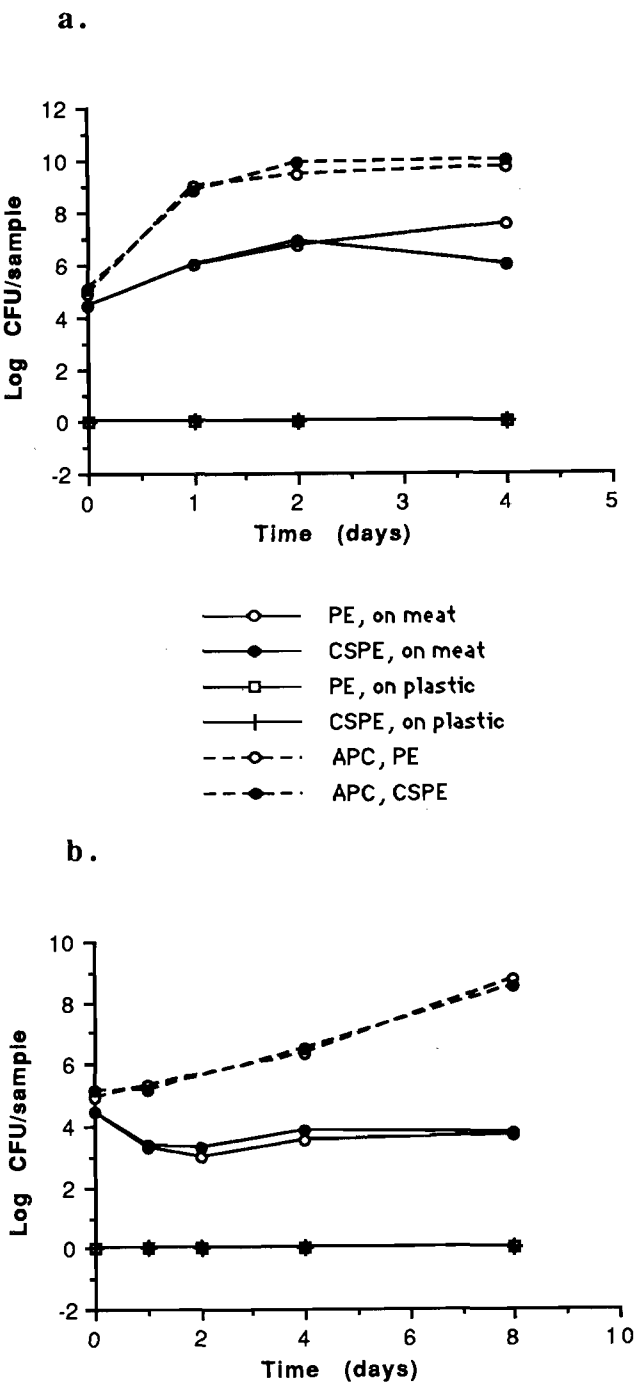

Figure 5. *Recovery of* B. cereus *and total aerobic population (APC) from plastic-covered beef stored at (a) 21°C or (b) 4°C. Inoculum was applied either directly to the meat surface or to plastic which covered the meat. Zero values indicate no recovery.*

applied to lean beef and covered with plastic when held at 21°C (Fig 5a). This increase was observed on samples in which the bacterial cells were in contact with both meat and PE or CSPE. No recovery of *B. cereus* was possible from samples in which inoculum was applied to the surface of PE or CSPE plastic film. At 4°C, a slight initial decline in *B. cereus* recovery was apparent, followed by period in which recovery remained constant (Fig. 5b). Populations remained constant on samples with either PE or CSPE. Total aerobic population gradually increased on samples covered with both film types. No migration through either PE or CSPE into lean meat was observed, regardless of the storage temperature. *B. cereus* levels gradually increased when in contact with bologna and stored at 21°C (Fig. 6a). The type of plastic used did not affect this increase. At 4°C storage, a gradual decline in population was observed on samples with *B. cereus* in contact with bologna (Fig. 6b). No migration through PE or CSPE into bologna occurred at either storage temperature (Fig. 6a, b).

To summarize, when inoculum was applied to beef and covered with either PE or CSPE, pathogen populations remained stable at 21 and 4°C. Beef samples had high levels of normal flora present. It was likely that the growth

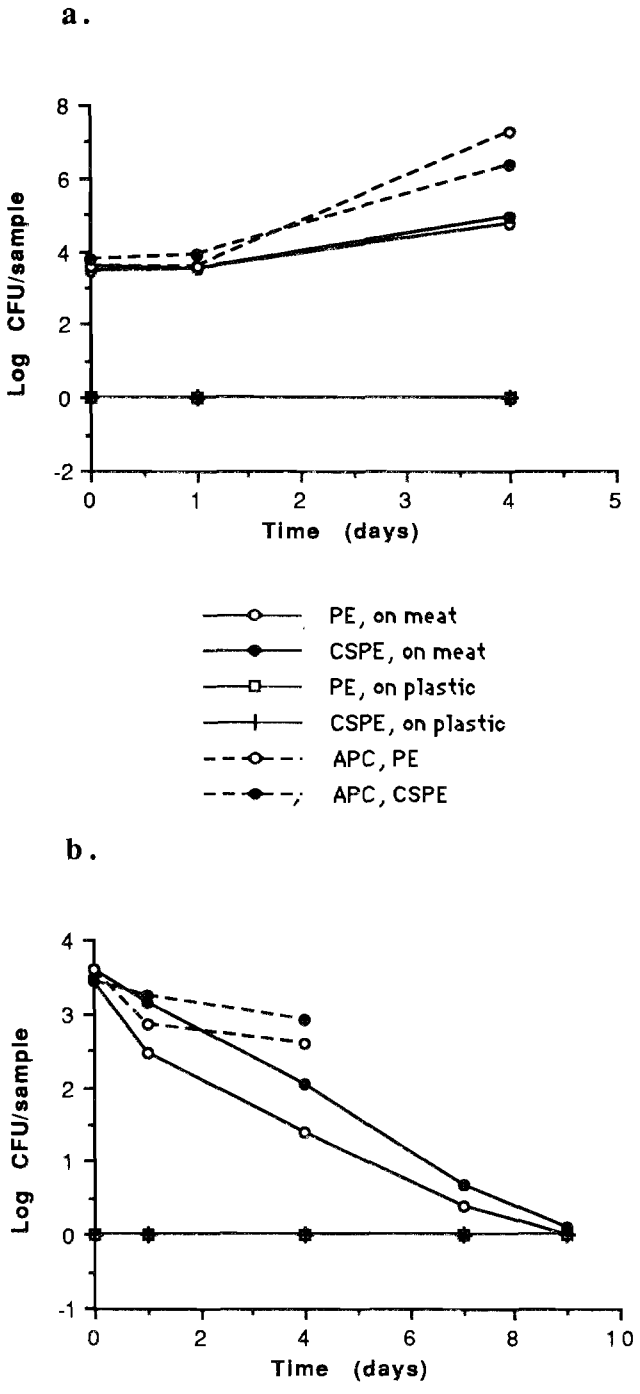

Figure 6. *Recovery of* B. cereus *and total aerobic population (APC) from plastic-covered bologna stored at (a) 21°C or (b) 4°C. Inoculum was applied either directly to the meat surface or to plastic which covered the meat. Zero values indicate no recovery.*

of these microorganisms prevented rapid growth of inoculated pathogens. Very low levels of initial contamination were apparent on bologna samples, as evidenced by the similarity in recovery on the selective and nonselective media. When inoculated onto bologna and stored at 4°C, bacterial recovery declined. Prolonged exposure to high fat content of bologna may have affected bacterial cell membranes resulting in decreased recovery. The additional carbohydrate provided by any cornstarch granules that may have been present on the surface of CPSE did not serve as a nutritive supplement for inoculated bacteria. Enhanced growth or survival of bacteria inoculated, so that cells were in contact with both meat and CSPE, was not observed.

The methods used in this study permitted evaluation of the ability of the bacteria to penetrate the film. Meat samples were covered and inoculum was localized on the outer surface of the plastic cover. Because packages were not formed, defects in package integrity was not an experimental factor. The presence of microorganisms in the food could only be the result of migration through the film, not the result of package failure. Bacterial activity is reduced at low temperatures. Therefore, abusive storage at room temperature, which would be more condusive to growth and enzyme synthesis, was used. The rate of migration of plasticizers, antioxidants, and other compounds from plastic packaging films into foods has been shown to be directly related to the fat content of the food (*8,15,18*). Direct contact between the packaging material and a fatty food also increases the rate of migration (*9*). In this study, bologna was used as a high fat system to promote bacterial migration through the films used. None of the organisms tested, including amylase-positive *B. cereus*, were able to migrate through PE or CSPE into the meat samples, regardless of the temperature of storage. These results indicated that the microbiological quality of foods was not affected by the presence of cornstarch in PE film.

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