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# Bacterial Survival on Cornstarch-Containing Polyethylene Film Held Under Food Storage Conditions

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**ABSTRACT**

Plastics in which cornstarch is incorporated into the polymer network have been developed. The effect of cornstarch in plastic film on the survival of spoilage and pathogenic bacteria was evaluated. Cornstarch-containing polyethylene film (CSPE) and control polyethylene film (PE) were inoculated with *Salmonella typhimurium*, *Aeromonas hydrophila*, *Bacillus cereus*, and *Pseudomonas fragi* and held under various combinations of temperature and relative humidity to mimic food storage conditions. Bacterial recovery from film samples indicated that, in general, survival was not enhanced by the presence of cornstarch. Enhanced growth of *S. typhimurium*, *A. hydrophila*, and *P. fragi* was observed under saturated relative humidity at some storage temperatures when a CSPE-supplemented minimal salts medium was used as compared to PE-supplemented medium. Enhanced growth was not apparent when a nutritionally complex growth medium supplemented with CSPE or PE was used. These results indicated that, from a microbiological viewpoint, cornstarch-containing polyethylene film could be successfully used to package foods.

Increased public concern over environmental issues has prompted the development of rapidly degradable plastics. Theoretically, one method of rendering plastic degradable is the incorporation of cornstarch (*2,4*). The action of microbial starch-degrading enzymes would create gaps in the plastic. This would decrease the physical strength of the plastic and increase the surface area available for attack by plastic-degrading microorganisms. A significant amount of plastic is used to package food products. If cornstarch-containing film has no adverse effects on food quality or on food safety, its use in food packaging may lead to decreased petrochemical consumption and increased corn utilization. Some microorganisms are capable of utilizing cornstarch as a growth substrate. Enhanced growth or survival of spoilage or pathogenic microorganisms due to the presence of cornstarch in packaging material would be unacceptable. The purpose of this study was to compare the survival of selected microorganisms on cornstarch-containing plastic film to survival on control polyethylene film held under food storage conditions.

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## MATERIALS AND METHODS

### *Plastic films*

Rexene 320 (Rexene Corp., Odessa, TX), a low-density, virgin polyethylene (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. Experimental film was prepared by combining the same polymer with a master-blend concentrate that contained 40% cornstarch and 60% polyethylene to result in a final cornstarch concentration of 6% in the film (CSPE). The cornstarch-containing concentrate did contain pro-oxidant. 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.

### *Culture preparation*

*Bacillus cereus*, *Salmonella typhimurium*, and *Aeromonas hydrophila* were propagated in tryptic soy broth (TSB) (Difco, Detroit, MI) for 24 h at 35°C. *Pseudomonas fragi* was propagated in a similar manner, except incubation was at 21°C. Growth was harvested by centrifugation at 12,000 x g for 10 min. Cells were washed and resuspended in nutrient salts broth (NSB), a modification of the medium specified for evaluating the susceptibility of plastics to microbial attack (*1*). Serial dilutions of the suspension were prepared in NSB so that an initial population of approximately $10^4$ CFU per sample or ml would be obtained.

### *Inoculation and storage*

Control PE and CSPE film squares were placed into the bottom of sterile petri plates and were inoculated with 0.01 ml of the diluted bacterial suspensions. To spread the inoculum over the film, the inoculum droplet was covered with another plastic square of the same type. Sufficient samples were inoculated so that 30 samples of both film types were stored under each combination of temperature and humidity. The petri plates were covered and placed in glass chambers held at 35, 21, 4, and -23°C. Three different relative humidities (RH) were maintained at each temperature. The use of saturated solutions of $K_2SO_4$ (potassium sulfate), $(NH_4)_2SO_4$ (ammonium sulfate), or $NaNO_2$ (sodium nitrite) in the chambers generated room temperature RH of approximately 97, 80, and 66%, respectively (*3*). Inoculation of plastic film squares was repeated up to three times for each organism used.

Flasks that contained 100 ml of plastic-supplemented NSB were used to generate saturated RH conditions. Plastic was added at a level of 8.5% (wt/vol). This resulted in a broth that would contain 0.5% (wt/vol) carbohydrate as cornstarch when CSPE was used as the supplement. NSB, CSPE-supplemented NSB, and PE-supplemented NSB were inoculated with bacterial suspensions and stored at 35, 21, and 4°C. Inoculations of plastic-supplemented NSB were performed in triplicate.

*Enumeration*

Bacterial populations on films were monitored by periodically removing three replicate PE and CSPE film samples from each storage condition. Bacteria were removed from the films by agitating (Stomacher Lab Blender 400; Tekmar Co., Cincinnati, OH) each plastic sample for 2 min in 10 ml of 0.1% peptone water (Difco). Serial dilutions were prepared and pour plated in duplicate using tryptic soy agar (TSA, Difco). Plates were incubated at 35°C for 48 h. If *P. fragi* was the organism used, incubation was at 21°C. The bacterial population in NSB-containing flasks was monitored by diluting duplicate portions of the broth in peptone water and plating on tryptic soy agar as described above.

*Growth in TSB*

*S. typhimurium*, *A. hydrophila*, and *P. fragi* were used to inoculate flasks with 100 ml of TSB containing CSPE, PE, or no added plastic as described above. TSB flasks were incubated at the same temperatures at which enhanced growth at 100% RH was observed when these organisms were incubated in CSPE-supplemented NSB; i.e., *S. typhimurium* at 35 and 21°C, *A. hydrophila* at 21°C, and *P. fragi* at 21 and 4°C. Triplicate analyses were performed for each condition tested. Populations were monitored as described above.

## RESULTS AND DISCUSSION

Survival on plastic film was dependent on temperature, relative humidity, and organism in question, as well as film type. Because of variations in initial populations when each inoculation experiment was replicated, average values for the replicate trials could not be reported. Instead, data from those trials in which the longest period of survival of the greatest level of growth are reported. Populations of *S. typhimurium* decreased more rapidly on CSPE film samples than on PE film stored at 35°C (Fig. 1). It was possible that contact between the bacterial cell and the dry starch granules caused cell lysis. Populations decreased at either the same rate or more rapidly on CSPE than on PE when stored at 21 and 4°C. Populations declined more rapidly on PE than on CSPE when held at -23°C at 97% RH. Contrary to the effect seen at 35°C, the cornstarch granules may have exerted a cyroprotectant effect on the bacterial cells.

Under saturated RH conditions, growth of *S. typhimurium* was seen in NSB incubated at 35 and 21°C (Fig. 2). Because populations increased in NSB with no added plastic, it could be assumed that *S. typhimurium* was capable of utilizing some component in NSB as an energy source. Even greater increases in population were observed in CSPE-supplemented NSB at both 35 and 21°C. Trace amounts of oligosaccharides present on the surface of the plastic as a result of the manufacturing process may have accounted for this increase.

*A. hydrophila* and *S. typhimurium* are both gram-negative, enteric, pathogenic organisms. A significant difference between these two bacteria is the ability to produce amylase, an enzyme capable of starch degradation. *S. typhimurium* is amylase negative, while *A. hydrophila* is amylase positive. The ability of *Aeromonas* to degrade cornstarch could have serious food safety ramifications if food was packaged in cornstarch-containing plastics. Recovery of *A. hydrophila* was similar to recovery of *S. typhimurium* in that at 35, 21, and 4°C, recovery of *A. hydrophila* from CSPE was not possible for longer periods of time than from PE (Fig. 3). There were differences in the relative amounts of time for which these organisms could

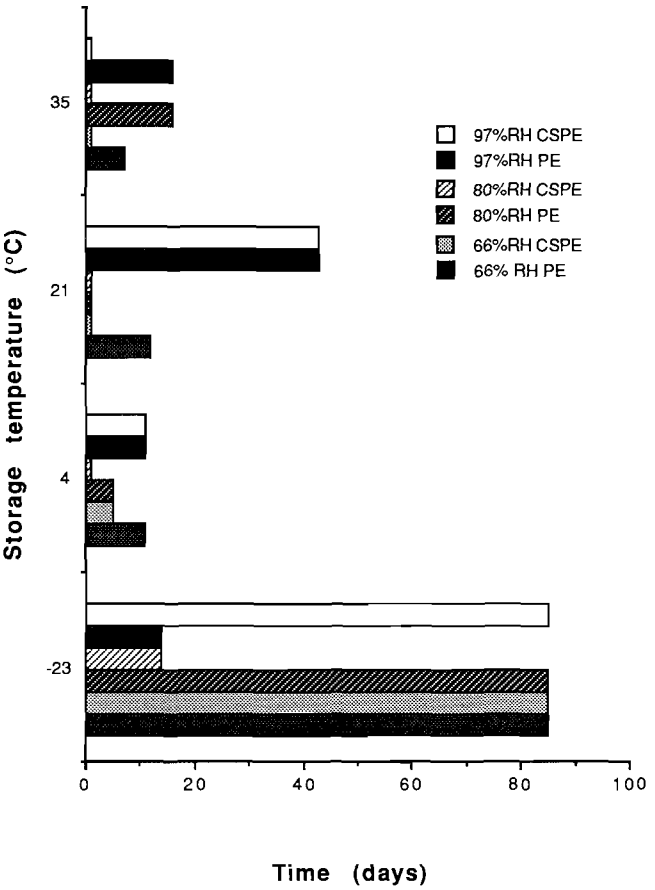


Figure 1. *Storage time required before no viable* S. typhimurium *cells were recovered from inoculated polyethylene (PE) and cornstarch-containing PE (CSPE) film samples held under food storage conditions. End point given is the day no colonies formed when the entire volume of diluent in which film samples were homogenized was plated.*

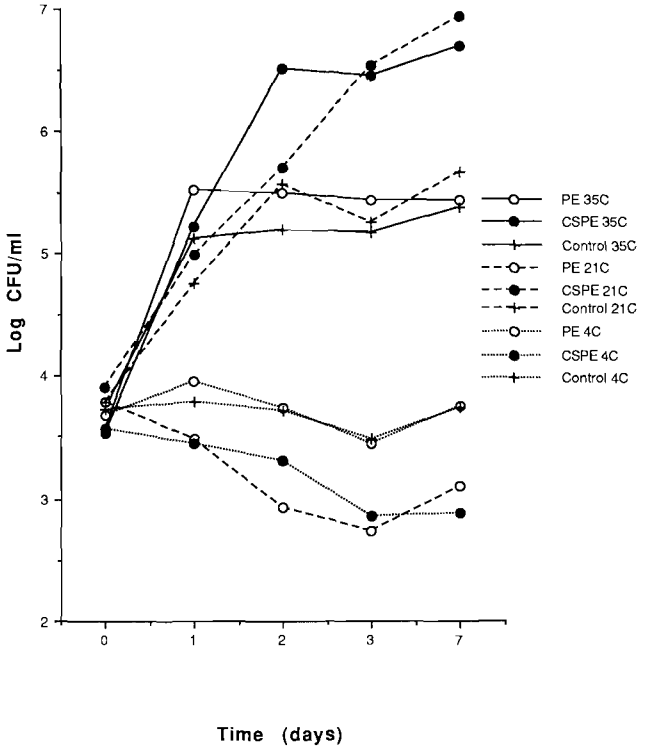


Figure 2. *Recovery of* S. typhimurium *from plastic-supplemented NSB.*

be recovered. In general, the time period in which recovery from frozen film samples was possible was shorter for *A. hydrophila* than for *S. typhimurium*. At -23°C, the *A. hydrophila* population declined more rapidly on PE than on CSPE under all humidities tested, while this pattern of recovery was observed only at 97% RH for *S. typhimurium*.

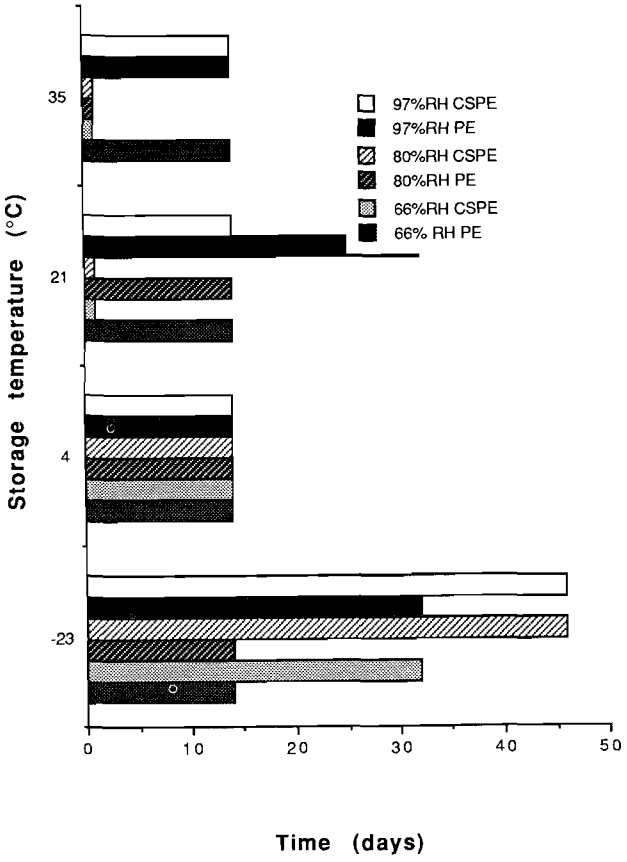


Figure 3. *Storage time required before no viable* A. hydrophila *cells were recovered from inoculated polyethylene (PE) and cornstarch-containing PE (CSPE) film samples held under food storage conditions. End point given is the day no colonies formed when the entire volume of diluent in which film samples were homogenized was plated.*

In NSB, PE-supplemented NSB, and CSPE-supplemented NSB, growth of *A. hydrophila* was observed at 21°C. The largest increase in population at 21°C was observed in CSPE-supplemented NSB (Fig. 4). Initially, the number of viable cells remained constant in all broths incubated at 4°C. However, the *A. hydrophila* population in CSPE-supplemented NSB declined until no recovery was possible after 7 d. When incubated at 35°C, growth was observed in NSB and PE-supplemented NSB, followed by a period in which the population slowly declined. No growth was observed in the CSPE-supplemented broth incubated at 35°C. Here the number of viable cells declined until at day 6, no recovery was possible. It is possible that diffusion of pro-oxidants contained in the cornstarch master blend into CSPE-supplemented NSB occurred. Inhibition of *A. hydrophila* by the pro-oxidant may have decreased viability at 35 and 4°C. *S. typhimurium* may not have been sensitive to this pro-oxidant. Another difference between the two enteric organisms was that CSPE-supplemented NSB supported enhanced growth of *S. typhimurium* at both 21 and 35°C. The ability to produce starch-degrading enzymes did not appear to enhance bacterial growth or survival.

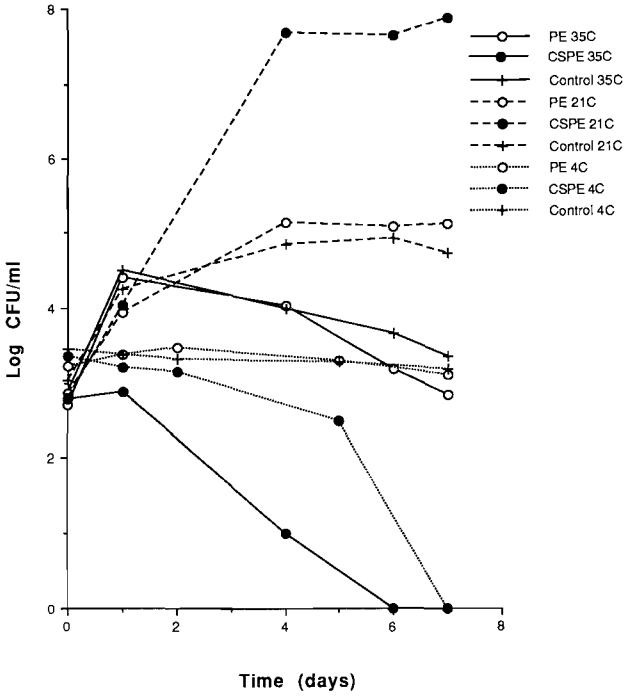


Figure 4. *Recovery of* A. hydrophila *from plastic-supplemented NSB.*

*S. aureus* was not recovered after 1 d from inoculated films held at 97, 80, or 66% RH regardless of type of film used. Under saturated RH conditions, no increases in *S. aureus* population were observed (Fig. 5). Populations remained stable in all combinations of NSB and plastic when incubated at 4°C and in NSB plus PE stored at room temperature. No enhanced survival was observed due to the presence of CSPE; in fact, populations declined more rapidly in CSPE-supplemented NSB incubated at 35 and 21°C than in PE-supplemented NSB.

Because of the psychrotrophic nature of pseudomonads, it was not surprising that *P. fragi* did not survive for extended periods at 35°C (Fig. 6). The longest period of survival at 35°C was observed on control film held at 97% RH, and then recovery was possible for only 5 d. No recovery was observed from samples stored for 2 weeks at 21°C. Extended survival of this spoilage organism was not observed on frozen and refrigerated samples.

As expected, a decline in *P. fragi* population was observed in NSB when incubated at 35°C (Fig. 7). In NSB with PE, growth of *P. fragi* at 21 and 4°C was similar to that in NSB with no added plastic. It could be assumed that *P. fragi*, like *S. typhimurium*, metabolized components of the NSB rather than the polyethylene polymer. A 3-log cycle increase in *P. fragi* population was observed in CSPE-containing NSB when incubated at 21 and 4°C.

A gradual decline in *B. cereus* population was observed over a 6-month period when stored at 35°C (Fig. 8) and 21°C (Fig. 9). An initial sharp decline in *B. cereus* population was also seen on films held at 4°C (Fig. 10) and

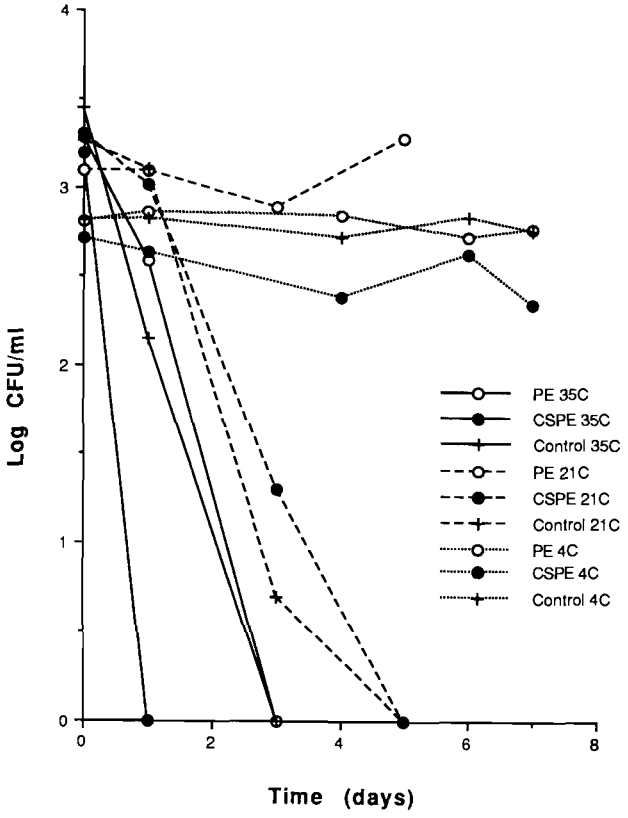


Figure 5. *Recovery of* S. aureus *from plastic-supplemented NSB.*

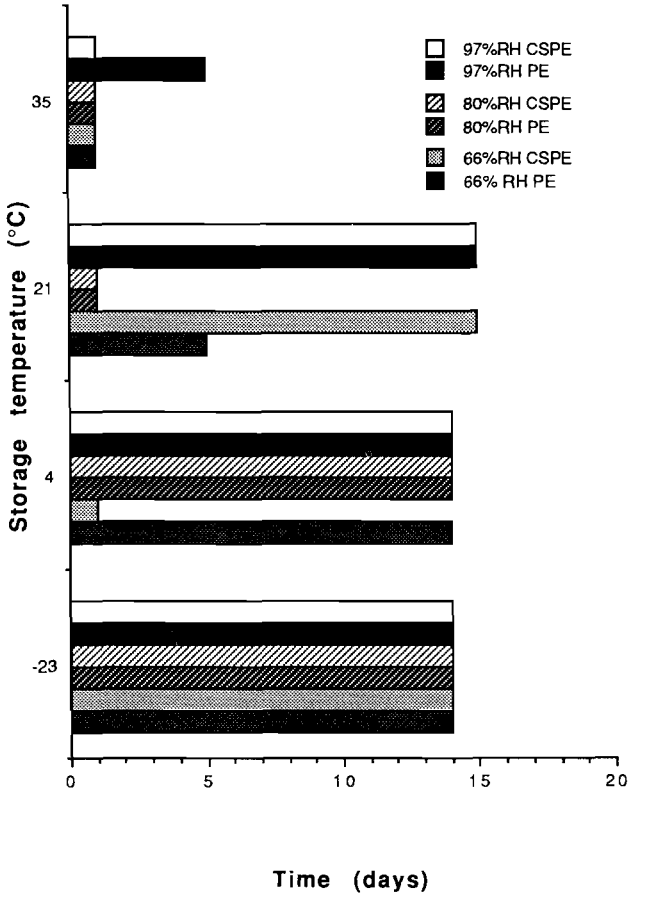


Figure 6. *Storage time required before no viable* P. fragi *cells were recovered from inoculated polyethylene (PE) and cornstarch-containing PE (CSPE) film samples held under food storage conditions. End point given is the day no colonies formed when the entire volume of diluent in which film samples were homogenized was plated.*

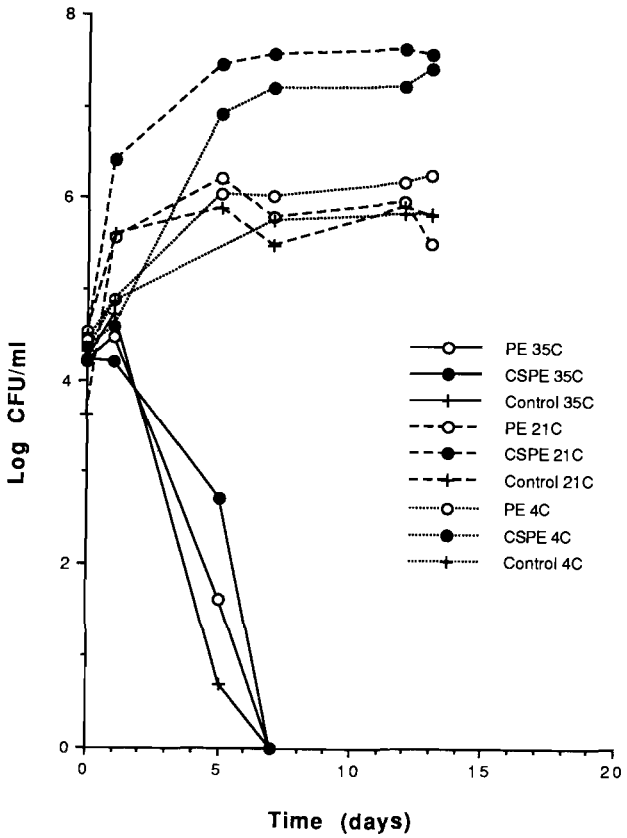


Figure 7. *Recovery of* P. fragi *from plastic-supplemented NSB.*

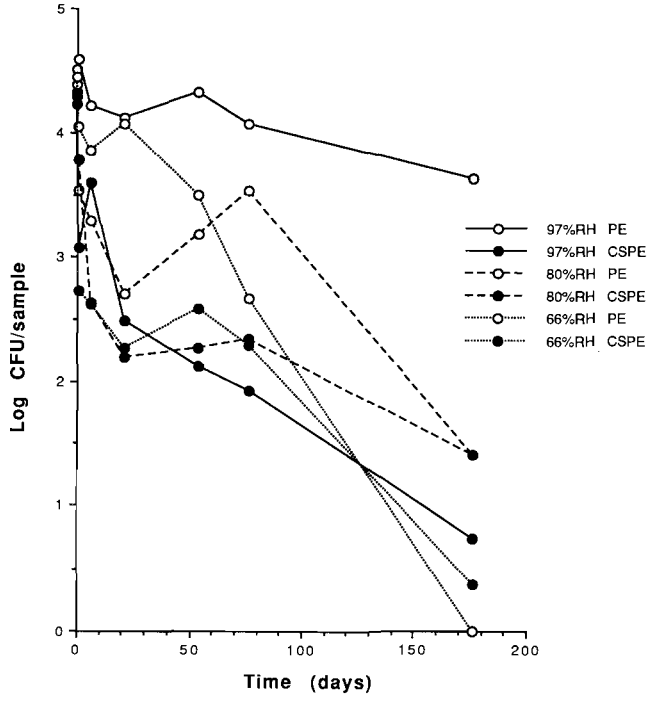


Figure 8. *Recovery of* B. cereus *from plastic films stored at 35°C.*

-23°C (Fig. 11). The decline was followed by an extended time interval in which the populations were stable. The *B. cereus* populations on CSPE were lower than on PE. After 6 months of storage, *B. cereus* was still viable on film samples held at each storage temperature. It was likely that sporulation of the *Bacillus* cells accounted for this persistence. At refrigeration and freezer temperatures, recovery from PE was slightly higher than from CSPE samples.

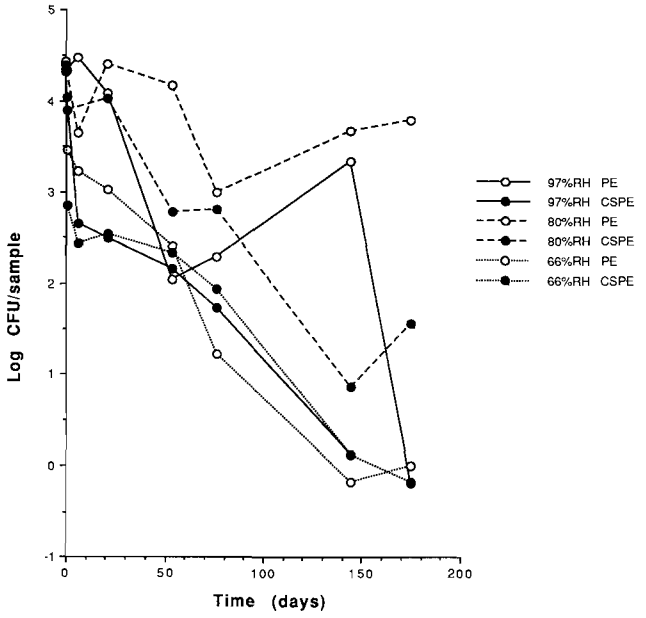


Figure 9. *Recovery of* B. cereus *from plastic films stored at 21°C.*

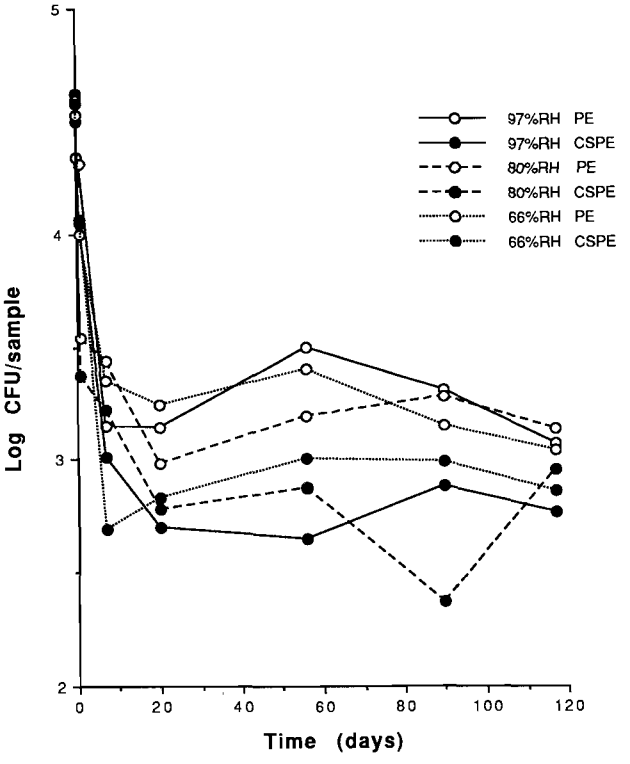


Figure 10. *Recovery of* B. cereus *from plastic films stored at 4°C*

*B. cereus* populations remained high in NSB that contained PE and were stored at 35 or 21°C (Fig. 12). The *B. cereus* population declined in NSB that was stored at 4°C. At each storage temperature, the population decreased more quickly when CSPE was present than when PE or no added plastic was present in NSB. This phenomenon may have been due to diffused pro-oxidants.

Survival of bacteria when inoculated directly onto cornstarch-containing plastic film varied with the organism present, storage temperature, and RH. The presence of cornstarch did not greatly enhance microbial survival. It was likely that the ability to metabolize cornstarch was not

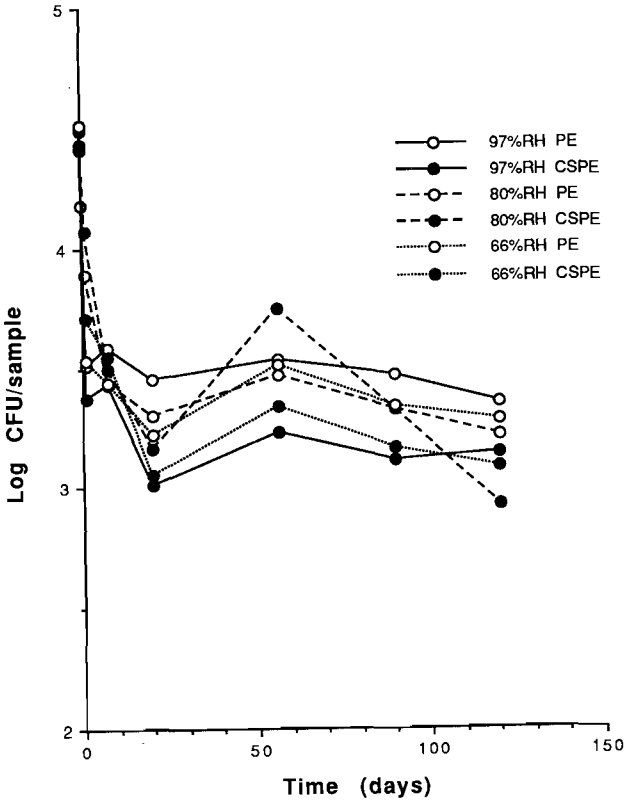


Figure 11. *Recovery of* B. cereus *from plastic films stored at -23°C.*

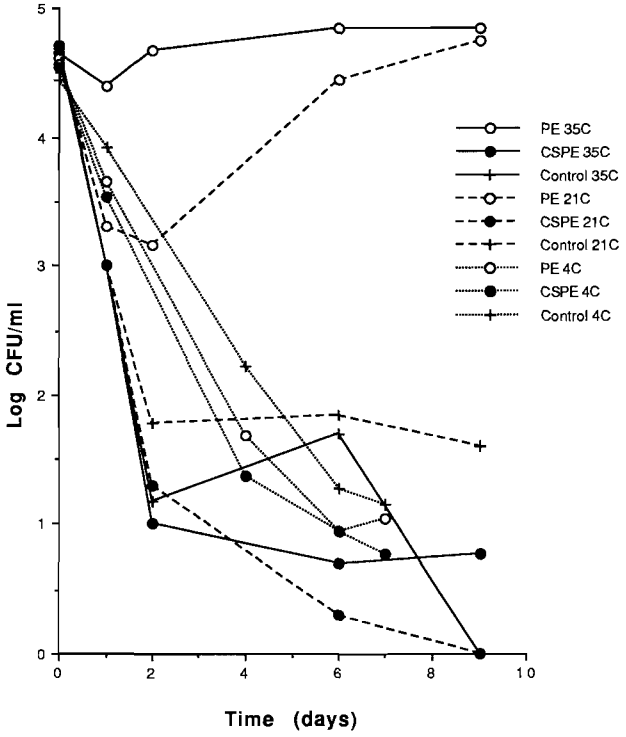


Figure 12. *Recovery of* B. cereus *from plastic-supplemented NSB.*

as important in affecting bacterial survival as the innate ability of the organism to survive conditions encountered in food storage. *B. cereus*, a sporeforming, amylase-positive pathogen, could be recovered from CSPE and PE films stored for 6 months. Limited survival of amylase-positive *A. hydrophila* and amylase-negative *S. typhimurium* was

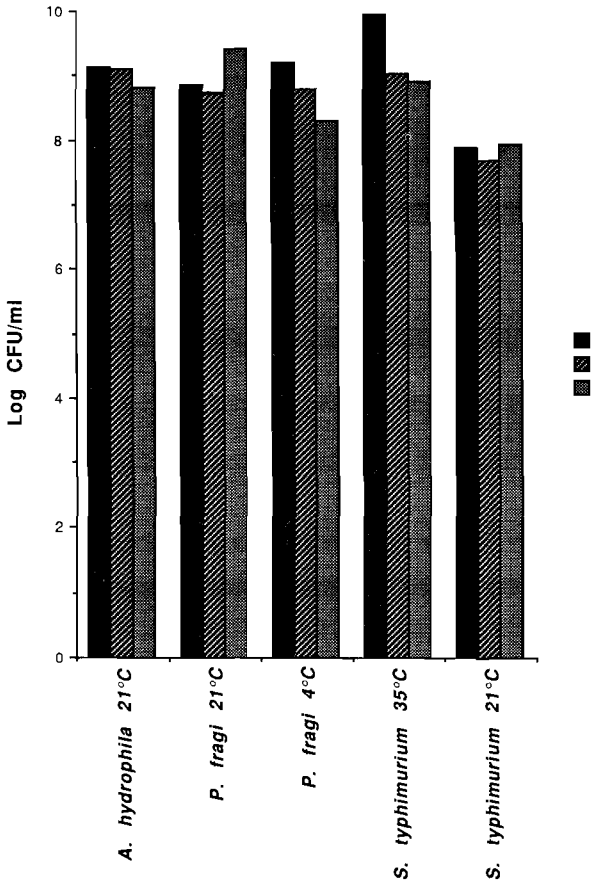


Figure 13. *Maximum bacterial population reached after 48 h incubation in plastic-supplemented TSB.*

seen. At freezer temperatures, there may have been a synergistic effect between low moisture and low temperature that enhanced survival of *Salmonella* and *Aeromonas*, but in both cases survival was for finite periods of time.

Higher populations of *S. typhimurium*, *A. hydrophila*, and *P. fragi* were observed in CSPE-containing NSB than in PE-containing NSB when incubated at temperatures that permitted growth of these organisms. NSB represents saturated relative humidity, a condition encountered in food storage conditions only when a liquid food is packaged. NSB is not representative of liquid food systems, except perhaps bottled water. Most fluid food systems, such as milk, juices, soups, and gravies, are nutritionally complex. TSB more closely simulates food systems. Growth in TSB was not enhanced by the presence of CSPE (Fig. 13); therefore, the significance of enhanced microbial growth in CSPE-supplemented NSB is minimal. Enhanced growth did not occur in CSPE-TSB. Therefore, it is unlikely that the presence of cornstarch in PE used to package foods would have adverse effects on food safety.

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