Journal of Food Protection, Vol. 71, No. 3, 2008, Pages 653–656

Research Note

Sterility Testing of a Dispensing Valve for Aseptic Function in Food Service Applications

J. D. STOPFORTH, B. KOTTAPALLI, AND M. SAMADPOUR*

Institute for Environmental Health, Inc., 15300 Bothell Way N.E., Seattle, Washington 98155, USA

MS 07-344: Received 2 July 2007/Accepted 23 August 2007

ABSTRACT

Manual dispensing equipment for aseptically packaged beverages or foods requires refrigeration of the product package following breakage of the hermetic seal. The food service industry would benefit greatly by implementing dispensing equipment that maintains the sterility of products after continued use without the need for refrigeration. The purpose of this study was to evaluate the ability of a valve, designed to operate aseptically and dispense products with or without refrigeration, to maintain the sterility of products following breakage of the hermetic seal and continued use simulating that of food service. Plastic packages equipped with the ''aseptic'' dispensing valve in a bag-in-box (BIB) format were aseptically filled with enrichment media with and without the addition of 1% cornstarch to simulate high- and low-viscosity products, respectively. BIBs filled with media were left uninoculated or inoculated (10^4 CFU/ml) with *Staphylococcus aureus* or *Aspergillus niger* on the interior of the spout 1 cm from the opening to simulate consumer misuse. Uninoculated and inoculated BIBs were stored at 25°C, and media were dispensed once to twice per day, every day for up to 30 days to simulate food service use. Dispensates were observed for turbidity (compared with controls) indicating growth in BIBs and, thus, breach of sterility. Growth of samples taken aseptically through the package wall was checked microbiologically every 5 days by standard plating techniques. There was no breach in sterility until day 25. At day 25, uninoculated BIB (1 of 45 samples positive for growth) containing high-viscosity media and BIB inoculated with *S. aureus* (1 of 45 samples positive for growth) containing low-viscosity media became turbid. Viscosity and type of organism did not appear to influence the ability of the valve to maintain the sterility for ≥20 days. The results of this study provide evidence that the dispensing valve tested can maintain the sterility of aseptically filled products following initial dispensation and continued use under unrefrigerated conditions.

The food and beverage industries constantly strive to introduce new products and packages to grow market share in a highly competitive environment. One of the package platforms now gaining significant popularity is bag in box (BIB), which has been a successful package for many years for retail wine and food service dispensing because it can dispense ready-to-use liquids and has efficient flow as the inner bag collapses around the product during dispensing. The BIB package platform is beginning to find a niche in other retail and food service products, especially those aseptically prepared to maintain product integrity and quality.

The food service industry will have excellent success expanding their product ranges into BIB package platform and benefit greatly by implementing manual dispensing equipment that maintains the sterility of products after continued use without the need for refrigeration. The major food safety concern associated with manual dispensing equipment is the ability of the spout on the dispensing tap to become contaminated with pathogens. Many human pathogens associated with food processing and handling environments, including *Listeria monocytogenes* and *Staphylococcus aureus,* have the ability to attach to equipment surfaces *(1, 4, 5).* Colonization of dispensing equipment surfaces by pathogens that may form biofilms and eventually translocate from the exterior to the interior of the nutrient-rich package can result in a public health hazard. Traditionally, BIB packages equipped with valves have required refrigeration following the initial dispensation because the design of the valves did not ''reseal'' following actuation. According to National Sanitation Federation/American National Standards Institute Standards 18 (manual food and beverage dispensing equipment) and 20 (commercial bulk milk dispensing equipment), once a manual dispensing equipment has been actuated, the hermetic seal is broken, and the product is no longer considered aseptic and should thus be refrigerated to control the growth of microorganisms. Although refrigeration retards, if not prevents, most pathogenic organisms from growing, pathogens such as the ubiquitous *L. monocytogenes* are able to grow under refrigeration temperatures and can cause serious human illness and even result in death *(3).* For this reason, there is a need for a valve that can reseal following dispensation to form a hermetic seal that provides a barrier between the outside environment and the package contents, regardless of whether or not the package is refrigerated. Currently, there is only one valve on the market that has

* Author for correspondence. Tel: 206-522-5432; Fax: 206-306-8883; E-mail: mansour@u.washington.edu.

been designed to maintain the hermetic seal between the inside of the bag and the environment on the outside of the valve disc following continuous actuations *(2).*

The objective of this study was to evaluate the ability of a valve, designed to operate aseptically and dispense products with or without refrigeration, to maintain the sterility of products during continuous operation.

MATERIALS AND METHODS

Strains examined and preparation of inocula. *S. aureus* (ATCC 6538) and *Aspergillus niger* (ATCC 16404) were used to generate inocula. Strains were available as frozen (−80°C) stock cultures in tryptic soy broth (Difco, Becton Dickinson, Sparks, Md.) with 20% glycerol and were activated by inoculating *S. aureus* in tryptic soy broth and *A. niger* in Sabouraud dextrose broth (Difco, Becton Dickinson) and incubating at 35 ± 2°C for 72 h. Cultures were then streaked on tryptic soy agar with 5% blood agar (TSA II 5% SB; Difco, Becton Dickinson) and incubated at 35 ± 2°C for 48 h. Colonies from each organism were suspended in phosphate-buffered saline (pH 7.4; 0.2 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 7H_2O$, 8.0 g of NaCl, and 0.2 g of KCl in 1 liter of distilled water) to yield a suspension concentration of approximately 10^8 cells per ml.

Preparation of enrichment media. Growth-promoting media were selected for use in this study to simulate nutrient-rich food products. Tryptic soy broth was prepared for inclusion in packages containing valves destined to be inoculated with *S. aureus.* Sabouraud dextrose broth was prepared for inclusion in packages containing valves destined to be inoculated with *A. niger.* Each medium was formulated with or without the addition of 1% cornstarch to generate high- or low-viscosity media, respectively.

Aseptic filling of packages. Plastic packages (50 by 36 cm; Dupont Liquid Packaging Systems, Liqui-Box Division, Sacramento, Calif.) were fitted with preassembled aseptic dispensing valves (The Answer, International Dispensing Corporation, New York, N.Y.) and sterilized via gamma radiation with a target dosage of 35 to 45 kGy (cobalt-based gamma radiation; Steris Isomedix, Libertyville, Ill.). Construction of the aseptic dispensing valve included a hermetic seal around the actuator button and spout opening. The valve disc inside the body of the dispensing tap separating the interior of the package and the exterior of the valve was designed by International Dispensing Corporation to form and maintain a hermetic seal. The sterilized packages were sent through an aseptic filling machine (Liqui-Box model 2000 C1T-0-A, Liqui-Box, Worthington, Ohio). Inside the sterile aseptic chamber of the machine, the aseptic BIB filler is designed to remove the valve from the spout of the bag so that the product can be filled through the spout. Because part of the outside of the bag was exposed in the sterile filling environment, the filler resterilized the part to be exposed with hydrogen peroxide (35%) as a wet mixture. Following sterilization, the valves were automatically removed in the sterile environment of the machine, the bags were aseptically filled with 5.7 liters of enrichment media, and the valves were reapplied into the ''fully seated'' position, ensuring that they could not be removed again without destroying the package. The filled and sealed packages were then ejected from the sterile chamber to be loaded into the outer container. Following filling, individual bags containing enrichment media were inserted into dispensing boxes (18 by 18 by 25 cm; Inland Container, Indianapolis, Ind.) to generate the BIB packages. Forty-five BIB packages were set up for each test variable (inoculum × viscosity).

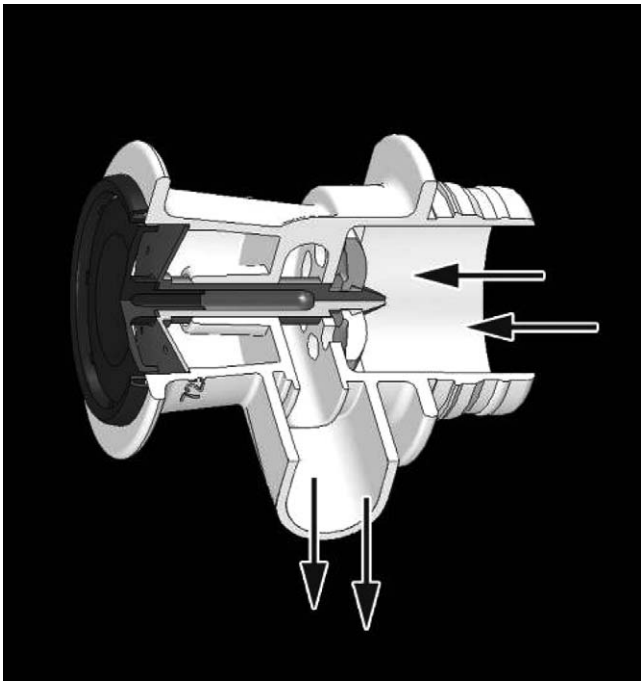

FIGURE 1. *Schematic of the tap with cross section showing the valve design and the direction of fluid movement.*

Inoculation of valves. The concentrated cell suspension for each organism was used to generate an inoculum for the study. A range of serial dilutions was prepared from the original cell suspensions by sequentially transferring 1 ml into sterile 0.1% buffered peptone water (Fisher Scientific, Houston, Tex.) to yield a cell suspension of approximately 10^4 CFU/ml for each organism. Final suspensions for *S. aureus* and *A. niger* were plated onto aerobic plate count Petrifilm (3M, St. Paul, Minn.) and yeast and mold count Petrifilm (3M), respectively, and incubated at 35 ± 2°C for 24 to 72 h for confirmation of culture concentration. Prior to inoculation, the hermetic seal covering the opening of the spout on each BIB package was removed to expose the spout opening. BIB packages containing tryptic soy broth were left uninoculated or inoculated by premoistening a sterile cotton swab with the diluted (10^4 CFU/ml) *S. aureus* cell suspension and swabbing an internal surface (25 mm^2) of the spout nozzle approximately 1 cm from the spout opening (Fig. 1). BIB packages containing Sabouraud dextrose broth were left uninoculated or inoculated by premoistening a sterile cotton swab with the diluted (10^4 CFU/ml) *A. niger* cell suspension and swabbing the internal surface of the spout nozzle approximately 1 cm from the spout opening (Fig. 1). BIB packages were incubated at 25 ± 2°C for 24 h to allow attachment of the inoculated organisms. The level of microbial attachment was determined by swabbing the inoculation spot (25 mm^2) with a premoistened sterile cotton swab after the 24-h incubation and after the first breach in sterility on additional BIB packages not used for dispensing. The premoistened sterile cotton swab used to swab the inoculation spot was suspended in buffered peptone water and plated onto aerobic plate count Petrifilm and yeast and mold count Petrifilm for *S. aureus* and *A. niger,* respectively, and incubated at 35 ± 2°C for 24 to 72 h.

Storage and dispensing from packages. Uninoculated and inoculated BIBs were stored at 25 ± 2°C for up to 30 days. Medium was dispensed (approximately 30 ml per dispensate) from

each BIB twice per day (with five actuations each time) for the first 10 days, after which time media were dispensed once per day for the remaining 20 days. Medium was dispensed to simulate typical food service use and to test the performance of the valve to maintain the sterility of the media inside the packages. Dispensates were observed for turbidity that would indicate growth in BIB and, thus, breach of sterility. Sterile enrichment media were used as negative controls, and inoculated enrichment media (*S. aureus* in tryptic soy broth and *A. niger* in Sabouraud dextrose broth) were used as positive controls for visual comparison of dispensate turbidity.

Microbiological analysis. Presence or absence of organisms in the enrichment media was checked microbiologically every 5 days by standard plating techniques. A volume (5 ml) of enrichment media was retrieved from the bags in each test case (inoculum × viscosity) by applying silicon gel (clear RTV silicone adhesive sealant; ITW Permatex, Inc., Solon, Ohio) to the bag, sanitizing the exterior surface of the bag (including the silicone gel) with 70% ethanol (Fisher), and inserting a sterile syringe (10-ml syringe, LuerLok, Difco, Becton Dickinson and 25-gauge 1½ PrecisionGlide needle, Difco, Becton Dickinson) through the gel to draw liquid from the interior. Media retrieved from BIBs containing tryptic soy broth were subsequently plated onto aerobic plate count Petrifilm (3M) and incubated at 35 ± 2°C for 24 h, while media from BIBs containing Sabouraud dextrose broth were plated onto yeast and mold count Petrifilm (3M) and incubated at 35 ± 2°C for 48 h. Following incubation, plates were observed, and the presence of colonies indicated internalization of organisms (a breach in sterility).

RESULTS AND DISCUSSION

The study was conducted to simulate realistic food service use (uninoculated taps) and operator misuse (spout nozzles inoculated with 10^4 CFU/ml) of an aseptic dispensing valve on BIB packages containing growth-promoting media (to represent nutrient-rich product) to test the ability of the valve design to prevent microorganisms from entering the sterile contents of the bag. *S. aureus* was selected for inoculation to represent a bacterium likely to contact the tap, because this organism is intimately associated with human skin, whereas *A. niger* was selected to represent a common environmental mold. The level of *S. aureus* (inoculated at 6.8×10^3 CFU/ml) cells attached to the plastic spout nozzle was 8.0×10^1 CFU/25 mm^2. The level of *A. niger* (inoculated at 6.2×10^3 CFU/ml) attached to the plastic spout nozzle was 1.8×10^1 CFU/25 mm^2. The results indicated that both organisms attached effectively to the interior surface of the plastic spout nozzle. By day 25 when the first breach in sterility was detected, the level of *S. aureus* attached to the plastic was 4.0×10^1 CFU/25 mm^2, whereas that of *A. niger* was 1.1×10^4 CFU/25 mm^2. The results indicated that both organisms readily survived and successfully colonized the interior surface of the plastic spout nozzle, most likely because of the nourishment from the residual growth-promoting media on the interior surface of the spout nozzle. It should be noted that the BIB packages inoculated with *A. niger* formed a visual cluster of black spores (observed by day 5) that appeared to spread within the interior of the spout nozzle, yet packages inoculated with this organism did not experience a breach in sterility up to day 30.

For purposes of this study, media (simulating nutrient-rich products) were formulated with or without 1% cornstarch to simulate high- and low-viscosity liquid products, respectively. The consistency of the high-viscosity media in this study represented products such as creamers, thick juices (i.e., orange), iced coffee and milk-based drinks, and coffee or tea with creamer, whereas the consistency of the low-viscosity media represented coffee or tea without creamer, clear juices (i.e., apple), water, liquors (i.e., wine), and thin broths or soups. These are the product categories that would most likely be used in the BIB format with the aseptic valve, and as such, the media were formulated to mimic these products. Overall, the viscosity and type of organism did not appear to affect the ability of the valve to maintain the sterility of the product; that is, there was no breach in sterility of any of the BIB packages (0 of 360 [45 bags × 2 viscosities × 4 inoculum schemes]) by day 20. There was no breach in sterility until day 25. By day 25, uninoculated BIB (1 of 45) containing high-viscosity media and BIB (1 of 45) containing low-viscosity media and inoculated with *S. aureus* experienced a breach in sterility. The rationale for studying differing viscosities was that BIBs containing thicker products would pose a greater risk of microorganisms entering the bag, because the product might interfere with the ability of the valve to close properly and form a hermetic seal as designed. Furthermore, thicker products would more likely form a film of product residue on the interior plastic surface and allow microorganisms to spread. There was no trend suggesting that the high-viscosity product used in this study resulted in a higher likelihood of microorganisms entering the packages. Indeed, the results seemed sporadic and unexplained in that an uninoculated package became contaminated by day 25. The result suggests that there is a ''use-life'' of the aseptic dispensing valve, after which the risk of the product inside the BIB package becoming contaminated with microorganisms increases. The use-life is proposed as the time that the aseptic dispensing valve is validated to maintain the sterility of high-risk products (nutrient-rich products), which, in this case, appears to be 20 days. The test is considered a conservative, worst-case scenario, because the liquid used for the study was growth-promoting media that would have nourished organisms on the interior surface of the plastic, whereas actual products intended for use with the aseptic dispensing valve, such as coffee, tea, water, and juices, would be less effective in supporting survival and growth of microorganisms on the plastic surface.

The concept of aseptic dispensing without refrigeration is relatively new, and there are inherent concerns regarding public health, because any microorganisms entering the sterile product in the bag would not be temperature controlled and would be able to proliferate, provided the intrinsic properties (water activity, pH, etc.) and nutrient content of the product supported microbial growth. There is particular concern with potential hazards associated with spore-forming pathogens, specifically toxins of nonproteolytic and proteolytic *Clostridium botulinum.* These spore-forming bacteria are able to grow in low-acid (pH > 4.6) foods, and control of these organisms is usually afforded

by refrigeration of the product; however, in nonproteolytic strains, refrigeration is insufficient to prevent germination of the spores. For this reason, it is essential that facilities that process and pack such products incorporate validated control measures that will ensure that spore-forming bacteria do not grow and produce toxins should the product, as offered for sale by the processor, be kept unrefrigerated in distribution or by consumers. There is a need to highlight the fact that the industry currently uses dispensing valves (not designed to function aseptically) on BIB packages that require refrigeration following initial actuation. The requirement for refrigeration of BIB packages with nonaseptic dispensing valves (National Sanitation Federation Standards 18 and 20) accepts that microorganisms may be internalized and rationalizes that refrigeration inhibits growth of microorganisms; however, this is not a control strategy for psychrotrophic organisms, especially pathogens such as *L. monocytogenes* or the nonproteolytic strains of *C. botulinum.* There is thus a need for technology that prevents microorganisms from entering BIB packages rather than attempting to minimize or inhibit the growth of organisms that may enter such packages. The design of an aseptic dispensing valve that reseals by forming a hermetic seal following multiple actuations provides a solution for manual dispensing equipment that may be used with or without refrigeration. Results of this study provide evidence that an aseptic dispensing valve is able to maintain the sterility of the product in BIB packages subjected to realistic food service and operator misuse scenarios without refrigeration for 20 days.

There are two primary mechanisms preventing microorganisms from entering BIB packages fitted with aseptic dispensing valves: (i) the design of the valve disc is such that release of the actuator following dispensing allows the silicone disc to form a hermetic seal with the plastic valve body and (ii) the positive pressure of liquid caused by the bag collapsing around the product as it dispenses prevents liquid from flowing back into the bag. The design of International Dispensing Corporation's The Answer tap (Fig. 1) incorporates a check valve that differentiates it from other taps, which are a spring-loaded cork valve design (a plastic plug forced into a tapered plastic cylinder), a modified ball valve (a threaded sleeve with a dispensing outlet), or a plug valve (usually a one-piece plastic molded part that relies on the ''memory'' of the plastic to shut off the product flow). The design of the check valve allows the product to be sealed against liquids but, most importantly, to clear away the liquid seal surface with every actuation, ensuring against possible wicking of the liquids. The maintenance of product sterility due to the function of the aseptic dispensing valve assumes the following: (i) the entire BIB package, including bag, spout, and dispensing tap, is constructed of plastic sufficient to withstand high doses of radiation; (ii) irradiation protocols ensure the minimum dosage penetration for the entire lot of packaging material treated; (iii) aseptic processing and filling follow strict sterilization protocols to ensure packages are filled aseptically; and (iv) hermetic seals are installed on the spout opening, around the actuator for the valve, and around the valve seal that separates the interior of the bag from the environment.

Under the conditions of this study, the viscosity of growth-promoting media mimicking products, such as coffee, tea, coffee or tea with creamer, creamers, juices, water, and broths, did not appear to influence the ability of the aseptic dispensing valve to maintain the sterility of the product within BIB packages. The ability of the aseptic dispensing valve to maintain the sterility of the product within BIB packages was not affected by inoculation of the spout nozzle with a bacterium (*S. aureus*) or a mold (*A. niger*) when compared with uninoculated controls following multiple actuations and storage at room temperature. In summary, the results of this study validate the ability of the aseptic dispensing valve to maintain the sterility of growth-promoting products in BIB packages following multiple actuations for 20 days without refrigeration under conditions simulating realistic use and operator misuse. Use of the aseptic dispensing valve provides the food service industry options to expand its product ranges while alleviating the need for refrigeration and also provides facilities that require the refrigeration of packages for organoleptic purposes the option of a safer alternative to dispensing products.

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