Technical Brief:

Study Explores Efficacy of Novel InvisiShield™ Technology in Reducing Fungal Growth on Berries

Introduction & Objective

Fresh strawberries and blueberries, while a popular fruit choice, often experience mold growth after just a few days of sitting in a consumer's refrigerator. Aptar Food + Beverage – Food Protection recently optimized its proprietary InvisiShield™ antimicrobial technology to reduce pathogens/mold in berries. The technology delivers a controlled amount of ClO_2 into a sealed environment to reduce pathogen growth. InvisiShield™ technology is the latest innovation using our active material science platform technology, which can be engineered to adsorb moisture, oxygen, odors, VOCs or perform other actions to prevent product degradation inside a sealed package.

InvisiShield™ technology has been validated on a wide range of bacteria and viruses. However, until now, the company had not explored the antimicrobial technology's effect on fungal growth. A recent study conducted by Analytical Food Laboratories (AFL) tested the efficacy of Aptar's novel antimicrobial technology in inhibiting and reducing fungal growth in blueberries and sliced strawberries over a period of 14 days.

Protocol Overview

Samples of blueberries and sliced strawberries were purchased commercially, packaged in trays (with or without an antimicrobial), and surface inoculated with *Botrytis cinerea* and *Alternaria spp.* immediately upon packaging. Samples were held at refrigerated temperatures. At predetermined storage intervals, samples were evaluated for the amount of each challenge organism remaining. The results were used to determine the effect of the antimicrobial treatment in each matrix (blueberries and sliced strawberries).

Methods & Materials

Sample matrix and test materials

Samples of the product matrices (blueberries and sliced strawberries) were purchased commercially by Analytical Food Laboratories (AFL), while sealing equipment, lidding film, and product trays (1/6 steam tray including the InvisiShield™ treatment or an untreated control) were provided by Aptar Food + Beverage – Food Protection.

Challenge microorganisms and stock solution preparation

Botrytis cinerea (ATCC #11542) and *Alternaria sp.* (ATCC #20084) challenge organisms were prepared for this study. Each culture was prepared from a lyophilized preparation according to manufacturer's instructions or from stock plates. Cultures were transferred to Potato Dextrose Agar (PDA, Hardy Diagnostics, Santa Maria, CA) and incubated at 25 ± 2°C for 5 days. After incubation, cultures were harvested from the surface of the agar using sterile diluent. The resulting fungal suspensions were used as the inocula below.

Methods & Materials *(continued)*

Inoculation of samples

Samples of each matrix were surface inoculated by adding a volume of each challenge organism suspension to the sample surface. The combined inoculum was dispersed throughout a full tray of product (approximately 1.25 lbs. per tray). The target concentration for both matrices was approximately 10^5 cfu/sample.

Inoculated trays of samples were prepared, including InvisiShield™ test trays and control trays for both blueberries and strawberries. An additional uninoculated tray of each type of berry was also prepared.

Storage of samples and sampling intervals

Post-inoculation, one control tray, one InvisiShield™ test tray, and one uninoculated control tray for each berry type was sampled for Day 0 evaluation. The remaining trays were placed in refrigerated storage at 7°C, and one control tray and three InvisiShield™ test trays per berry type were sampled after 2, 4, 7, 9, 11, and 14 days of storage.

Sample enumeration and evaluation

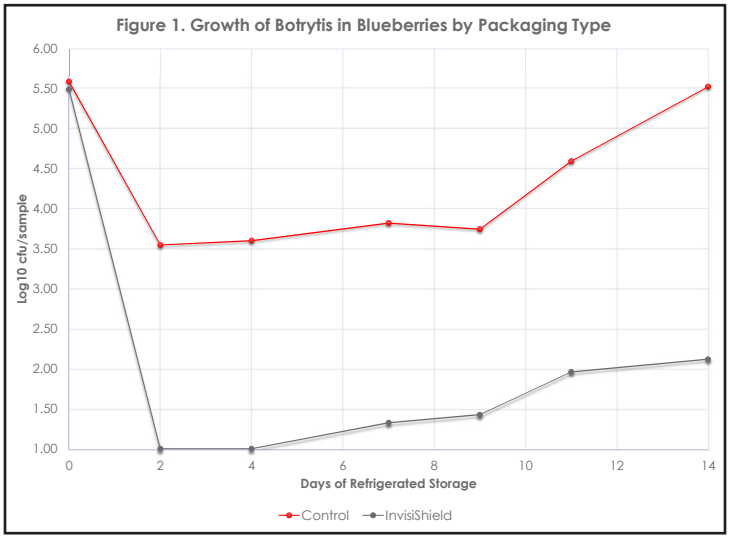
Three replicate samples from each tray (25g each) were combined with a volume of sterile diluent (Butterfield's Phosphate Buffer) equal to a 1:10 dilution of the sample. The sample was stomached for up to 30 seconds at high speed. Samples were spread plated at appropriate dilutions on PDA agar and incubated at 25 ± 2°C for 5 days. After incubation, plates were enumerated using a Quebec colony counter. The number of observed colonies for each challenge organism was multiplied by the dilution factor to determine the total count in cfu/sample.

Data analysis

The raw count observed for each sample was converted to $\log_{10}$ cfu/sample. The amount of each challenge organism present at each testing interval was compared to the amount present in the initial samples to determine the ability of the antimicrobial delivered by InvisiShield™ technology to control the outgrowth of each organism in each matrix.

Results

In the blueberry samples, InvisiShield™ technology reduced both challenge organisms over the first four days of refrigerated storage, followed by a period of recovery. Figure 1 illustrates the reduction of *Botrytis* growth in blueberries stored in the control container versus an InvisiShield™ treatment tray. The blueberries in the InvisiShield™ tray showed a reduction of 4.5 logs when sampled at days two and four versus the control tray's reduction of 2 logs. The InvisiShield treatment tray kept growth of *Botrytis* low through day 14, ultimately resulting in a 3.5 log difference between the blueberries in the treatment tray versus the control tray.



Results *(continued)*

The immediate reduction and recovery behaviors related to *Alternaria* growth on blueberries in the InvisiShield™ and control trays paralleled the results with *Botrytis* (Figure 2).

In the strawberry samples, both inocula were heavily reduced (Figures 3 and 4), even in the control samples. However, both inocula were suppressed below the level of detection using the InvisiShield™ system. Minimal non-specific fungal organisms were detected in both types of berries, at an average value of 27 cfu/g for the blueberries, and 30 cfu/g for the strawberries.

Conclusions

The data in this study indicate that the InvisiShield™ antimicrobial delivery system is able to control the outgrowth of potential spoilage organisms, including *Botrytis cinerea* and *Alternaria spp.* In blueberries, InvisiShield™ technology reduced *B. cinerea* by an average of 1.38 logs and 2.67 logs greater than what was seen in the untreated control samples over the course of 14 days of refrigerated storage. Against *Alternaria spp.*, the increased reduction in this matrix was 1.39 and 2.48 logs. Increased reductions in the sliced strawberry matrix were lower (0.77 and 1.72 logs against *B. cinerea* and 0.72 and 1.72 logs against *Alternaria*).

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Figure 2. Growth of Alternaria in Blueberries by Packaging Type

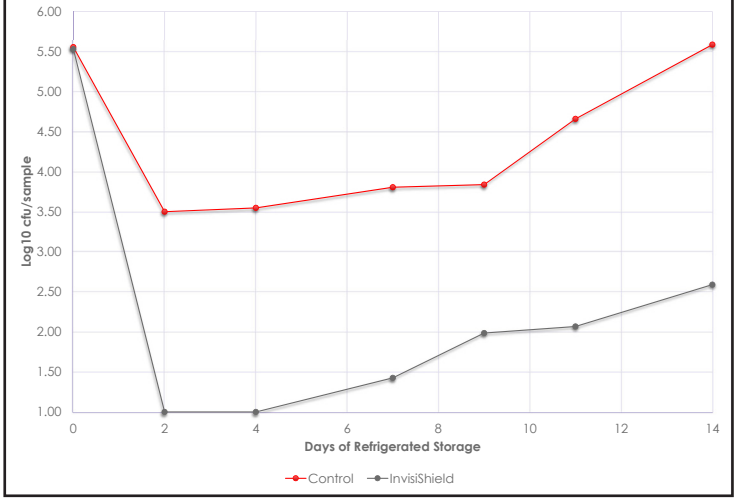

Figure 3. Growth of Botrytis in Strawberries by Packaging Type

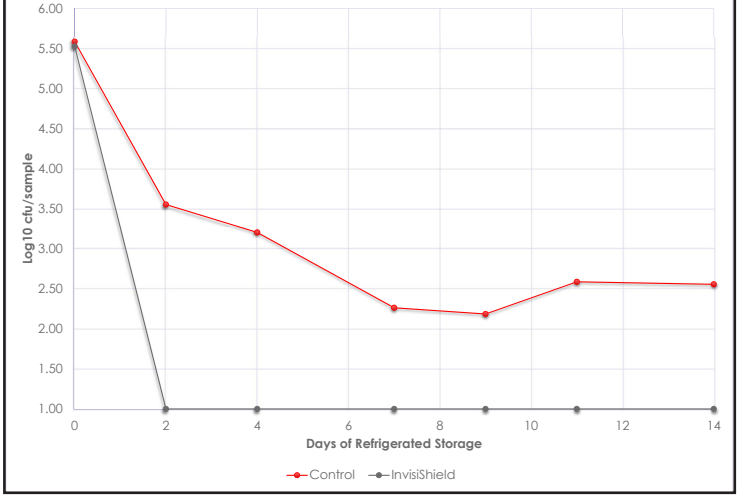

Figure 4. Growth of Alternaria in Strawberries by Packaging Type