InvisiShield™

Redefining food protection

Case Study:

Evaluation of InvisiShield™ technology to reduce pathogenic *Escherichia coli*, *Salmonella* and *Listeria monocytogenes* using the antimicrobial Chlorine Dioxide

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InvisiShield™

Objective

To determine the influence of Aptar's InvisiShield™ technology against multiple strains of foodborne pathogens *Listeria monocytogenes, Salmonella,* and pathogenic *Escherichia coli* on commercially packaged tomato slices.

Highlights

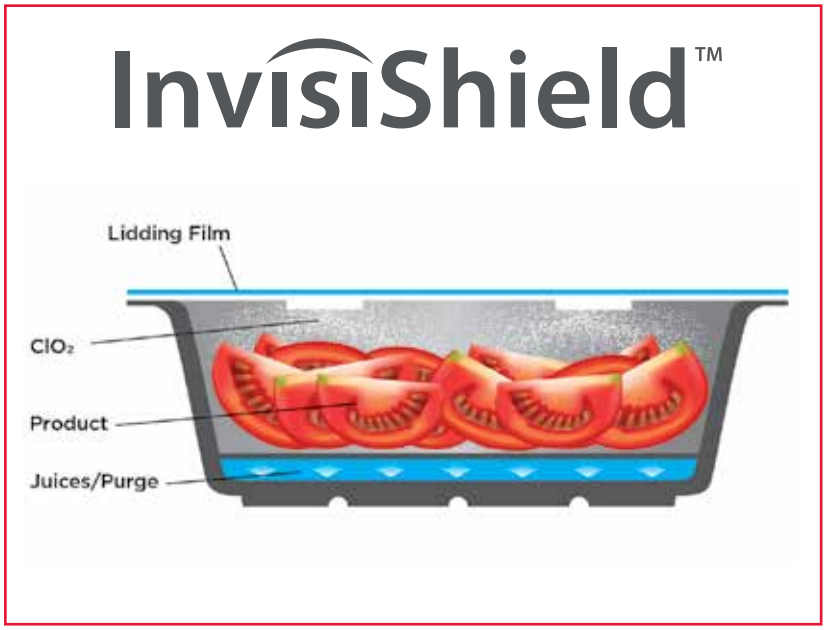
- Technology can reduce risk of foodborne illness
- Chlorine dioxide released from the InvisiShield™ system inhibits major pathogens up to 3 logs
- Sensory properties were not statistically different between treated and untreated tomatoes

Research Overview

Sliced tomatoes were inoculated with three foodborne pathogen cocktails and treated in trays utilizing Aptar's InvisiShield™ technology in temperature-controlled storage (7°C) for up to 14 days. Inoculated tomato slices experienced significant reductions of 3.6 logs, 4.5 logs and 4.2 logs for *Salmonella,* and pathenogenic *E. coli*, and *L. monocytogenes* after 7 days in the high inoculation test and 2.58, 2.67, and 1.82 log reductions for the low inoculation study respectively. Findings suggest that the InvisiShield™ technology demonstrated antimicrobial activity against *L. monocytogenes, Salmonella,* and pathenogenic *E. coli* as it was significantly different from the controls with p-values of 0.00 when compared per day. The levels of chlorine dioxide used were safe for food and did not negatively impact the tomatoes as demonstrated in the sensory panel results. InvisiShield™ technology offers a differentiated active packaging solution, protecting high-risk products such as fresh-cut produce.

Introduction

While chlorine and chlorine dioxide has been used for many years as an antimicrobial on food and water, there have been many challenges for both safety and quality that have prevented chlorine dioxide gas from being utilized commercially.[1] Chlorine dioxide gas is an antimicrobial of choice because it is very effective and is broad-spectrum, demonstrating efficacy against both gram-negative and gram-positive microorgamisms. Aptar's novel InvisiShield™ technology is able to fill a gap and offer this effective antimicrobial to the industry due to the specially-engineered delivery system, which can safely create ClO_2 and control the dosage in the package in order to reduce negative organoleptic properties. The InvisiShield™ material is extruded and remains stable throughout the supply chain distribution. It contains a base polymer, a channeling agent and the active ingredient (ClO_2). The release kinetics of the ClO_2 is triggered by relative humidity in the package. ClO_2 then migrates through the same channels or through the polymer blend itself into the surrounding environment in a controlled manner.



Executive Summary

Foodborne illness is a major concern in the United States as it affects approximately 1 in 6 people, according to the Centers for Disease Control.[2] These estimated 48 million cases annually in the U.S. include 128,000 hospitalizations and 3,000 related deaths.[2] Of these, it is estimated that the 31 most pathogenic strains found in foods consumed in the United States each year caused nearly 9.5 million illnesses, 56,000 hospitalizations, and 1,351 deaths. Furthermore, the top seven strains accounted for 90% of all illnesses.[3] Nontyphoidal *Salmonella* bacteria are the leading cause of bacterial foodborne illnesses in the U.S.[4]

Active packaging can be designed to correct the deficiencies that exist in "passive" packaging. The importance of active packaging in food applications is that it can change the condition of the packaged food to extend shelf life and ensure microbiological safety. Typical food conditions that may be altered by active packaging include physiological processes (e.g., respiration of fresh fruits and vegetables), chemical processes (e.g., lipid oxidation), physical processes (e.g., dehydration), and microbiological aspects (e.g. spoilage by microorganisms).

Antimicrobial packaging systems can be used to increase the shelf life and improve the safety of food products by adding another hurdle that microorganisms must overcome. This packaging is not a replacement for good quality control. The main requirement of any antimicrobial agent are the ability to limit or eliminate the microorganism while maintaining the quality of the food.

Pathogens can occur in products such as minimally processed and refrigerated (MPR) fresh fruits and vegetables and ready to eat meats. MPR fruits and vegetables are defined as "those prepared by a single or any number of appropriate unit operations such as peeling, slicing, shredding, juicing, etc. given a partial but not end-point preservation treatment including the use of minimal heat, a preservative, or radiation."[5] There has been a public increase in consumption of MPR fruits and vegetables due to healthier eating habits and the need for convenience and longevity.[6]

Concerns about MPR fruits and vegetables are on the rise due to the large number of pathenogenic outbreaks associatied with these foods.[7] The main reasons for these outbreaks are insufficient sanitation or preservation treatment, temperature abuse during processing, distribution and marketing, and cross-contamination.

Chlorine dioxide (ClO_2) is an active food additive that inhibits activity against gram-positive microorganisms, gram-negative microorganisms, antiviral and antifungal, and is generally recognized as safe (GRAS) in the U.S. ClO_2 gas fumigation technology has shown promise as an effective and practical antimicrobial agent in the packaging of blueberries in a study by Sun et. al.[8] In another study by Park and Kang, the reduction of *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* were reduced to below the detectable limit (1 log) with 50 ppmv of ClO2 gas in 15 minutes under 90% relative humidity.[9]

Although it is very effective against pathogens and used in wash water, ClO_2 has safety and organoleptic concerns that have limited its commercial use in food packaging to date. The odor is detectable by humans at 17 mg/L and can be quite irritating to the respiratory system at 45 mg/L. In a study by Ellis et al., the color of chicken was adversely affected by the ClO_2 as areas close to the sachet were brown or green. The spoilage odor normally associated with 9-day old chicken was also masked based on sensory panelists' responses to samples treated with fast-release chlorine dioxide.[10]

Aptar has developed a novel InvisiShield™ system that can safely create ClO_2 and control the dosage in the package in order to reduce negative organoleptic properties. The InvisiShield™ material is extruded and remains stable throughout the supply chain distribution. It contains a base polymer, a channeling agent and the active ingredient (ClO_2). The release kinetics of ClO_2 are triggered by relative humidity in the package. ClO_2 then migrates out through the same channels or through the polymer blend itself into the surrounding environment in a controlled manner.

Materials and Methods

Sample Matrix and Containers

5 x 5 tomatoes were purchased commercially from a local wholesaler and used within 3 hours of purchase. A 200-ppm free chlorine solution using tap water (approximately the same temperature as the tomatoes) was prepared to clean the tomatoes. Tomatoes were:

- washed in chlorine solution for 2 minutes;
- rinsed with tap water; and
- aseptically sliced using a Tomato Saber® 943-D slicer (Prince Castle Carol Stream, IL) with the calyx facing down. *Note: This slicer was also washed and rinsed in the same 200-ppm bath and tap water.*

The blossom and calyx ends were discarded so that there were 42 slices packed into each tray, 6 tomatoes by 7 slices per tomato. Tomatoes were packed with slices vertically standing up in an Aptar 1/4 steam absorbent tray (Aptar Atlanta, GA). Treatment trays had Aptar's InvisiShield™ material included inside.

Sensory Analysis

The samples served for the triangle test of difference were:

- **Control trays** having no active ingredient *(Aptar 1/4 steam tray; Aptar Atlanta, GA)*
- **Treatment trays** that had Aptar's InvisiShield™ material included inside

The trays were stored at 4°C for 3 days as this was determined to be the possible first point that they would be opened commercially. The samples were single slices of tomato from either the control tray or the treatment tray. All samples were served at room temperature.

Triangle Test of Difference

The methods for this test are as given by Poste et al.[11] to evaluate the difference between samples within treatment trays and control trays. 42 panelists were given instructions prior to the evaluation as they were not trained panelists. Produce used for these studies was not inoculated with pathogens.

Challenge Microorganisms and Stock Solution Preparation

Salmonella cocktail prepared with five *Salmonella* isolates (ATCC Manassas, VA) including:

- *Salmonella* Heidelberg (ATCC 8326)
- *Salmonella* Enteritidis (ATCC 13076)
- *Salmonella* Typhimurium (ATCC 14028)
- *Salmonella* Typhimurium (ATCC 13311)
- *Salmonella* Senftenberg (ATCC 43845).

L. monocytogenes cocktail prepared with five *L. monocytogenes* isolates (ATCC Manassas, VA), including strains:

- ATCC 19112 – Serotype 2
- ATCC 19113 – Serotype 3
- ATCC 19111 – Serotype 1/2a
- ATCC 19115 – Serotype 4b
- ATCC 7644 – Serotype 1/2c

Pathogenic *E. coli* cocktail prepared with five pathogenic *E.coli* strains (ATCC Manassas, VA), including:

- *E.coli* O157:H7 ATCC 35150
- *E.coli* O111 ATCC BAA-2440
- *E.coli* O103:H11 ATCC BAA-2215
- *E.coli* O145 ATCC BAA-2192
- *E.coli* O121:H19 ATCC BAA-2219.

Each culture was prepared from a lyophilized preparation according to the manufacturer's instructions. Cultures were transferred into Tryptic Soy Broth (TSB, Neogen. Lansing, MI) and incubated at 35 ± 2°C for 24h. After incubation, the cultures were verified by streaking onto the following agars:

- *Salmonella* was streaked onto Xylose Lysine Desoxycholate Agar (XLD, Neogen Lansing, MI)
- *L. monocytogenes* was streaked onto PALCAM Agar (PALCAM, Neogen Lansing, MI)
- Pathogenic *E. coli* was streaked onto Sorbitol MacConkey Agar (SMAC, Neogen Lansing, MI).

The strains were individually cultured and subcultured in 10 ml TSB for 18-24h at 35°C, then combined into their respective cocktails and diluted in TSB to the concentration needed for the inoculums below.

Inoculation of Samples and Sealing

Each sample within a tray, consisting of 2 slices of tomato, were spot inoculated (6 slices per challenge cocktail per tray, 18 sample slices per tray total) by adding a 10µL volume of the challenge organism suspension to the sample surface. The target concentration on the surface of the product was approximately 2-3 log CFU/ml for the low inoculation study and approximately 9-10 log CFU/ml for the high inoculation study. Inoculated samples on each tray were identified by marking with a wax pencil (Newell Brands Inc., Atlanta, GA). A total of 56 trays (50 test, 6 control) of samples were prepared for the low inoculation study and 33 trays (30 test, 3 control) for the high inoculation study. After inoculation, trays were heat-sealed on the flange of the tray with an MTS tray sealer (Aptar Atlanta, GA) with polypropylene lidding film (Aptar Atlanta, GA) with an approx. 100 (cc/in²/day) oxygen transmission rate.

Storage of Samples & Sampling Intervals

Post-inoculation, 1 control and 1 test tray were sampled. The remaining trays were placed in refrigerated storage at 7°C and sampled (1 control tray, 10 test trays) after 2, 4, 7, 10 and 14 days of storage for the high inoculation study and 2, 4 and 7 days for the low inoculation study.

Sample Enumeration

Samples (2 tomato slices per sample ~40-50g; 9 samples per tray) were combined with a volume of BPB (3M, Maplewood, MN) supplemented with a direct chlorine neutralizer (sodium thiosulfate, at a concentration of 1%) equal to three times the weight of the sample. The sample was then stomached (Smasher, bioMerieux, Marcy-l'Etoile, France) for 1 minute at high speed. Samples were spread plated at appropriate dilutions on the following agars and incubated, depending on the challenge organism inoculated:

- *Salmonella*: XLD; 48 ± 2 hours, 35 ± 2°C
- *L. monocytogenes*: PALCAM; 48 ± 2 hours, 35 ± 2° C
- Pathogenic *E. coli*: SMAC; 48 ± 2 hours, 35 ± 2°C.

After incubation, plates were enumerated using a Quebec colony counter (Model #3325, Reichert Technologies, Depew, NY). The number of observed colonies was multiplied by the dilution factor to determine the total count in CFU/g. Sample bags were incubated at 35 ± 2°C until the plates were counted and viable counts determined. If no colonies grew on the plates, the corresponding sample bags were streaked onto the agar plates and incubated as instructed above. If the streaked plates came back with growth, the sample was marked as >3 CFU/sample as that was the detection limit on plating. If they came back with no growth again, the counts were considered <3 CFU/sample.

Statistical Analysis

Counts were averaged per sample type, per sample day, and standard deviation was calculated with Excel 2016 Version 16.0 (Microsoft Redmond, WA) from those samples per day and were recorded on the graphs as an average and error bars for standard deviation. Statistical differences ($P<0.05$) were analyzed using 1-way analysis of variance (ANOVA) and Tukey's test was done on Minitab 18.1 (Minitab, Inc. State College, PA) on samples versus the control for each sample day and pathogen type. The study was completed in triplicate.

Results

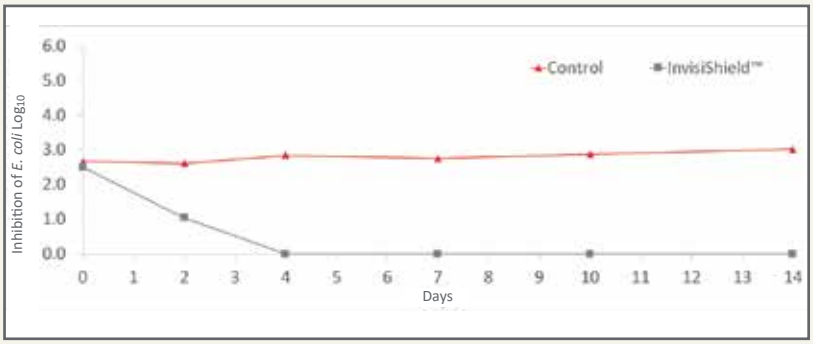
Sensory Analysis

Forty-two panelists were asked to rate the appearance, flavor and texture of sliced tomatoes stored at 4°C for 3 days. Treated tomatoes were rated as not significantly different in appearance, flavor, and texture attributes compared to the other sample. The data was analyzed by tabulating the number of correct responses and compared to values in tables for the minimum number of "correct" responses needed to conclude that a perceptible difference exists. For n=42 panelists, the number is 22 (a=0.01).[12]

Microbial Counts After 14 Days Refrigerated Storage of Product, Low Inoculation

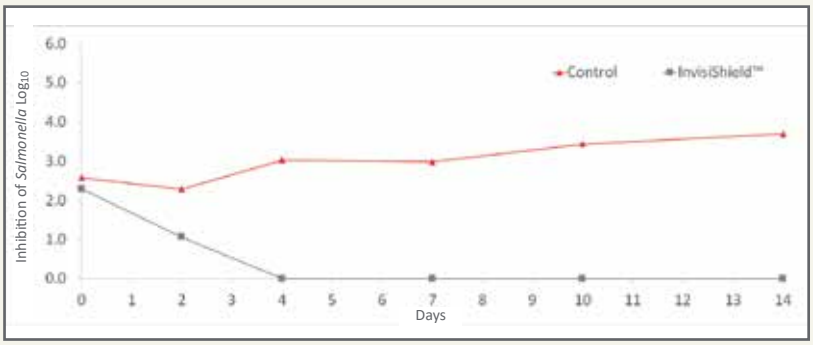
Salmonella, L. monocytogenes, and pathogenic *E. coli* saw similar levels of inoculation onto the sliced tomatoes in both the treatment and control trays on day 0 shown by no statistical difference in the Tukey test and a p-value of 0.156, 0.204, and 0.258 respectively. *Salmonella, L. monocytogenes,* and pathogenic *E. coli* levels on the surface of sliced tomatoes in the control trays were stable over the course of storage at 7°C, with an initial average reduction of 0.27 log CFU/g after 2 days, followed by an average increase of 0.87 log after 14 total days of storage (Figures 1, 2, and 3). *Salmonella* and *E. coli* levels were reduced on the surface of tomatoes in the treatment trays by 1.55 and 1.52 logs respectively after 2 days of storage and were not recoverable (after overnight enrichment and streak plating) from that point forward (Figures 1 and 2). The treatment trays were statistically different from controls after the initial day 0 testing point on all sampling days up to the end of the study on day 14. *L. monocytogenes* levels were also reduced in the treatment trays by 1.08 log after day 2, but at subsequent test points was recovered sporadically near the limit of detection, with an average reduction of 1.58 logs from that point forward (Figure 3). All treatment trays vs. control trays were statistically different after day 0 on each sampling day up to the end of the experiment on day 14.

Figure 1: Pathenogenic *Escherichia coli* Low Inoculation Study



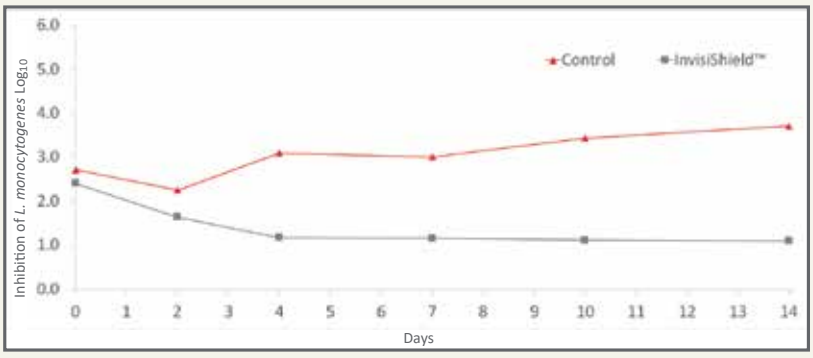
Changes in mean E. coli log CFU/g counts in control trays (▲) and treatment trays (■) over time in Low Inoculation Study.

Figure 2: *Salmonella* Low Inoculation Study



Changes in mean Salmonella log CFU/g counts in control trays (▲) and treatment trays (■) over time in Low Inoculation Study.

Figure 3: *L. monocytogenes* Low Inoculation Study



Changes in mean L. monocytogenes log CFU/g counts in control trays (▲) and treatment trays (■) over time in Low Inoculation Study.

Microbial Counts After 7 Days Refrigerated Storage of Product, High Inoculation

Salmonella, *L. monocytogenes,* and pathogenic *E. coli* saw similar levels of inoculation onto the sliced tomatoes in both the treatment and control trays on day 0 shown by no statistical difference in the Tukey test and a p-value of 0.149, 0.183, and 0.283 respectively. *Salmonella*, *L. monocytogenes*, and pathogenic *E.coli* levels on the surface of sliced tomatoes in the control trays were relatively stable with an initial average inoculation of 9 logs and reduction of 0.5 log over the 7 day span for *E.coli* and *L. monocytogenes*, and a 1.5 logs reduction for *Salmonella* over the same 7 day span with a final count of 7.5 logs respectively on day 7 (Figures 4, 5, and 6). The treatment trays showed a reduction to 3.8 logs for *Salmonella* and *E. coli*, with an average log reduction of 3.6 logs and 4.5 logs respectively (Figures 4 and 5). *L. monocytogenes* showed a similar reduction in the treatment trays with a count of 4.3 logs on day 7 and a reduction compared to the control trays of 4.2 logs (Figure 6).

Antimicrobial packaging systems can be used to increase the shelf life and improve the safety of food products by adding another hurdle that microorganisms must overcome. InvisiShield™ technology utilizes specialized engineering to strike the perfect balance between safety and quality.

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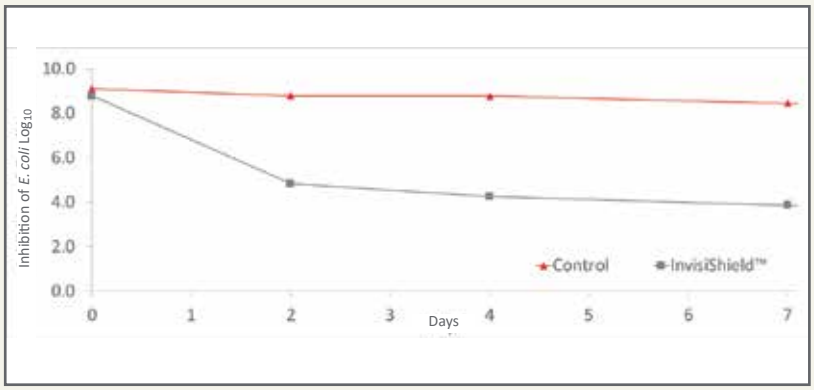
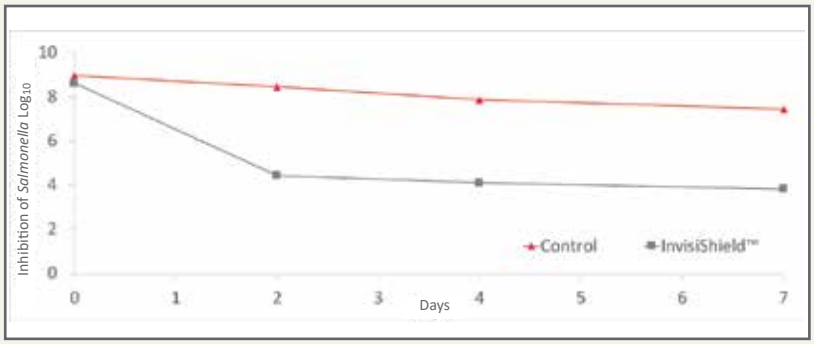
Figure 4: Pathogenic *Escherichia coli* High Inoculation Study



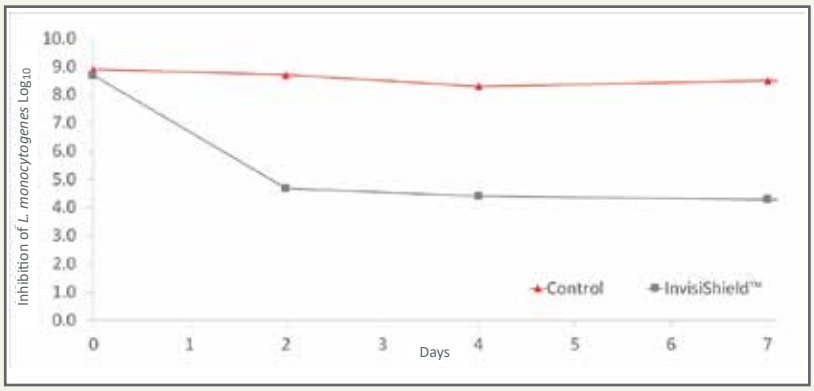
Changes in mean E. coli log CFU/g counts in control trays (▲) and treatment trays (■) over time in High Inoculation Study.

Figure 5: *Salmonella* High Inoculation Study



Changes in mean Salmonella log CFU/g counts in control trays (▲) and treatment trays (■) over time in High Inoculation Study.

Figure 6: *L. monocytogenes* High Inoculation Study



Changes in mean L. monocytogenes log CFU/g counts in control trays (▲) and treatment trays (■) over time in High Inoculation Study.

Conclusion

The lack of an adverse effect on sensory quality make this treatment promising for sliced tomato commercial application. This is similar to what Sy et al. saw in an earlier study but only had 20-30 min. exposure in a manual exposure tank with whole tomatoes compared to a sealed commercial product with sliced tomatoes in this study.[13] In a similar study, also by Sy, there was a whitening of the strawberries that decreased the sensory quality that was not seen with this controlled release technology on sliced tomatoes.[14] This further shows the advantage of this system over other methods of exposure to chlorine dioxide.

The data in this study indicated that the InvisiShield™ system can reduce the level of *L. monocytogenes, Salmonella* and pathenogenic *E. coli* on sliced tomatoes during elevated refrigerated storage. In the low inoculation study, treatment trays with sliced tomatoes surface inoculated with each pathogen and held at 7°C for up to 14 days showed complete inhibition of the *Salmonella* and *E. coli* during storage after 2 days and suppression to nearly the limit of detection for the *L. monocytogenes* inoculum after 4 days of storage. In the high inoculation study, treatment trays showed at least a 4 log reduction over the study with the most being a 4.6 log reduction, on pathenogenic *E. coli* on Day 7, and the least being a 3.6 log reduction on *Salmonella* on Day 7, compared to control trays.

The ability to control the kinetics and customize the release rate of the chlorine dioxide was vital to maintaining the tomato's color, flavor, aroma and texture while achieving efficacy. The technology allows for this specialized engineering in order to strike the perfect balance between safety and quality.

InvisiShield™

Aptar is actively seeking pilot partners to introduce InvisiShield™ technology in your market!
If you are interested, please contact:

Angela Morgan | angela.morgan@aptar.com | 770-845-6077

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