Evaluation of the microbial inhibition performance of gas type antimicrobials (chlorine dioxide and allyl-isothiocyanate) with Modified Atmosphere Packaging (MAP), and the effect of chlorine dioxide exposure on the physical properties of plastic films

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Abstract

Listeria monocytogenes and S*almonella Typhimurium* are major pathogenic bacteria associated with poultry products. The consumption of pathogen contaminated food can cause illness or even death. In addition, spoilage microorganisms can lead to premature loss of product quality. The objectives of this study were to evaluate the effectiveness of a combination treatment of modified atmosphere packaging and antimicrobials (chlorine dioxide and allyl isothiocyanate) in inhibiting *Listeria monocytogenes* and S*almonella Typhimurium* on raw chicken breast at 4°C, and to determine the effect of chlorine dioxide (oxidizing gas) exposure on the mechanical, barrier, and thermal properties of plastic packaging films.

Chicken breast, obtained from a local source and handled to prevent gross contamination was inoculated with a mixture of *Listeria monocytogenes* and S*almonella Typhimurium* (10^6 log cfu/ml) and placed into a multilayer barrier tray. Sachets containing chlorine dioxide or allyl isothiocyanate were placed into the headspace of selected packages containing inoculated chicken breasts. The packages were gas flushed with 30%CO_2/70%N_2, and sealed In the same manner, control packages were prepared minus the antimicrobials. The sealed packages were then stored at 4°C. Microbial growth (pathogens and spoilage organism), color, and pH of chicken breast were evaluated every 3 days for 21 days. The effect of the antimicrobials on growth of pathogens and spoilage organisms and the effect of MAP and antimicrobials on the quality of fresh chicken breast were determined.

Package films were treated with chlorine dioxide gas at various concentrations, and their mechanical, barrier, and thermal properties evaluated. The treated films were evaluated using an Instron (universal testing machine), differential scanning calorimetry (DSC), and oxygen analyzers.

Introduction

Antimicrobial packaging is a significant technology which can improve food quality and enhance product safety against microbial agents. Chlorine dioxide (ClO_2) and allyl isothiocyanate (AIT) are attractive gas type antimicrobials because of their excellent microbial control performance at very low concentration. Gas type antimicrobials have the advantage in a packaging system that a minimum effective concentration can be maintained through constant release. An antimicrobial package system should also be more effective when it is combined with conventional modified atmosphere packaging.

Consumption of poultry meat in the US has an annual growth rate of 7% and is a $ 17.5 billon market. However, it is a highly perishable food and deteriorates within 10 days after slaughtering, even under chilled conditions. Microbial contamination of the product may occur in post processing and during distribution.

Salmonella *Typhimurium* and Listeria *monocytogenes* are two major pathogenic organisms associated with fresh chicken. Antimicrobial packaging has many potential advantages over conventional packaging in that it may be also reduce microbial risk and extend shelf life. Knowing the minimum inhibition concentration (MIC) is important to control these microorganisms in a package.

ClO_2 is a very strong oxidizing agent. An oxidizing gas can cause the formation of free radical functional groups and the degradation of polymer chains. Thus, exposure to ClO_2 may cause changes in the properties of polymeric materials. To avoid loss of polymer properties, knowledge of the relationship between ClO_2 gas concentration and physical degradation of polymers is important to understand

Objectives

1. To determine the minimum concentration of ClO_2 and AIT needed to control growth of Salmonella *Typhimurium* and Listeria *monocytogenes*
2. To evaluate the effects of ClO_2 and AIT in combination with 30%CO_2/70%N_2 modified atmosphere packaging on growth of Salmonella *Typhimurium* and Listeria *monocytogenes* at 4°C.
3. To investigate the effect of ClO_2 exposure at various concentrations and treatment times on the mechanical, barrier, and thermal properties of food packaging polymers.

Material & Method

Culture and growth conditions

L.monocytogenes (1002, 1176, 1304) and *S.Typhimurium* (G10127, G10601, G10931) cocktails were prepared, and subcultured twice in 10 ml tryptic soy broth (Difco Laboratories, Detroit, MI) containing 0.6% (w/v) yeast extract (Difco) at 35°C for 24 hr before being used. Cultures were serially diluted in 9 ml of 0.1 peptone water to yield 10^1-10^6 microorganisms.

Antimicrobials

Chlorine dioxide was obtained from ICA Trinova. The release rate was designed (ICA Trinova) to be 3ug/hr.g (at 25°C) for 31 days. Allyl isothiocyanate was obtained from Sigma-Aldrich Chemical Co., Milwaukee, WI. For the AIT sachets, the AIT regent was mixed with corn oil, and added to a 20 ml glass vial. The vial was closed with an orificed screw cap on PE film. The corn oil was used to depress AIT vapor pressure in the vial. The AIT release rate was adjusted to 0.6-1.2ug/hr using the relationship between AIT permeability through a PE film and vapor pressure.

Minimum concentration of the chlorine dioxide (ClO_2) and allyl isothiocyanate (AIT)

950 ml glass canning jars were purchased and sterilized with 70% ethanol. The metallic lids of the jars were modified to accommodate headspace gas concentration analysis. The ClO_2 concentrations were measured using a detector tube and pump system (MSA Inc., Pittsburgh, PA). The lids were modified to contain two holes (0.25 in) and tygon tubing was attached to the holes using 3/8 in diameter brass fittings. The tubes were closed using laboratory pinch clamps. To determine AIT concentration, the metal lids were equipped with 3/8 in diameter septum adaptors. An orifice in the middle, sealed by silicon rubber, served as

a septum for sampling the headspace gas. 1ml of sample gas was injected into a GC (HP 6890) which was attached to a 30 m long, 0.32 mm o.d., 0.25 um crosslinked 5% PHME column (Supelco, Bellefonte, PA). The flow rates of the nitrogen carrier gas, hydrogen and air to the FID detector were 30, 30, and 240 ml/min, respectively. The column temperature was increased at 60°C /min from 45°C (4.5 min) to 230°C (5 min). The temperature of the injection port and detector were set at 250°C and 290°C respectively. For the standard curve,1ul of a known amount of AIT in hexane was used. 0.1 ml each of prepared cocktails (L.monocytogene and S.Typhimurium) were inoculated onto agar plates (60 mm x 15 mm). Glass jars were used to hold plates with different dilutions, ranging from 10^1 to 10^6 CFU. Modified Oxford agar (MOX) for *L.monocytogenes* and Xylose Lysine Deoxycholate (XLD) for *S.* Typhimurium were used as the growth media. After the lids were tightly closed, the glass jars were flushed with 100% nitrogen to simulate MAP, and stored in an incubator (37°C) for 48 hrs. Lack of growth in the inoculated plates (10^1-10^6) was considered to be due to inhibition of the inoculum by the respective antimicrobials.

Preparation of chicken and packaging

Fresh boneless, skinless chicken breast was obtained from a local retail store. The chicken was aseptically cut into a standard size, weighting approx. 200g. The two and three-strain cocktails were diluted to 10^6 CFU/ml, and 1 ml of each cocktail was inoculated evenly onto the chicken. The cocktails from *L. monocytogenes* and *S. Typhimurium* were inoculated onto separate chicken pieces in order to avoid any undesirable interaction. The inoculated chicken was placed into a multilayer barrier tray (5 in x 6 in x 1.5 in), obtained from Cryovac Sealed Air Corporation (Duncan, SC). The tray was sealed with a multilayer barrier lid (Cryovac lid 1050) after a 30% CO_2/70% N_2 gas mixture was flushed into the tray using a T-200 vacuum tray sealer (Multivac Inc, Kansas, MO). The chicken was stored at 4°C, and 3 trays from each treatment were removed on days 0, 3, 6, 9, 12, 15, 18, and 21 and analyzed for microbial counts, color, and pH change as a function of storage time.

Microbial analysis

The whole chicken piece was placed into a stomacher filter bag after 10 g was removed for pH analysis. It was diluted with 200ml of 0.1% peptone water, and homogenized for 1 min in a stomacher. Serial dilutions were then prepared in peptone water and plated on Modified Oxford agar (MOX) with TSAYE overlaying for *L. monocytogenes,* and Xylose Lysine Deoxycholate (XLD) with TSAYE overlaying for *S*. Typhimurium. For total aerobic bacteria analysis TSAYE was also used. All colony counts were expressed as $\log_{10}$ colony forming unit (cfu/g).

pH

10g from each chicken piece was removed prior to microbial analysis, diluted with 90ml of distilled water (1:10 dilution), and homogenized in a blender for 2 min. Measurements were taken with a Corning model 430 pH meter and electrode. (Corning, USA).

Color

The surface color of the chicken breast, taken from three random locations on the chicken was measured with a colorimeter (Minolta Chroma Meter Measuring Head CR-300). An average value was obtained for each sample surface. They were recorded as "L","a", and "b" values, and total color change (ΔE).

ClO_2 treatment for physical properties analysis

Pre-conditioned (23°C/50% RH) films (PE, PP, PS, Nylon, and Cyovac 1050) were placed into a 4 liter glass container. 1 liter aqueous ClO_2 solution (ICA Trinova) was added and sealed tightly with a metal lid. Films were hung from the lid of the container above the solution, and exposed to ClO_2 gas. The equilibrium headspace concentrations of ClO_2 ranged from 10-1000 ppm using different concentrations in an aqueous solution. The headspace concentration was checked using an Idometric titration method. 200 ml headspace gas was withdrawn through a 1/4" silicon septum in the container lid, and titration analysis performed.

Mechanical properties

Tensile properties (TS) and elongation at break (EB) were determined according to ASTM D882. Tests were performed using an Instron 4201 (INSTRON Corporation, Canton, MA). Film samples were tested in both CD and MD.

Barrier properties

To investigate effect of ClO_2 on barrier properties, the oxygen transmission rate (OTR) was determined. OTR (ASTM D 3985) was determined using an Oxtran 8001 (Illinois instruments, inc. Johnsburg, IL)

Thermal properties

Differential scanning calorimetery (TA Instrument, New Castle, DE) was used to investigate thermal changes of the packaging films. The heating rate used 10 C/min, the weight of sample 3 mg, and a nitrogen gas flow rate of 50 cc/min. Tg and Tm were measured based on ASTM D 3418-97

Result & disscussion

Inhibition effectiveness of ClO_2 and AIT

L.monocytogenes and S.Typhimuirum were inoculated on differential selective agar plates, and their inhibition effectiveness observed. Results (figure 2) show that both antimicrobials were effective in controling these two organisms. Particularly, ClO_2 was more effective on L.monocytogenes, and AIT was more effective in inhibiting S.Typhimuirum.

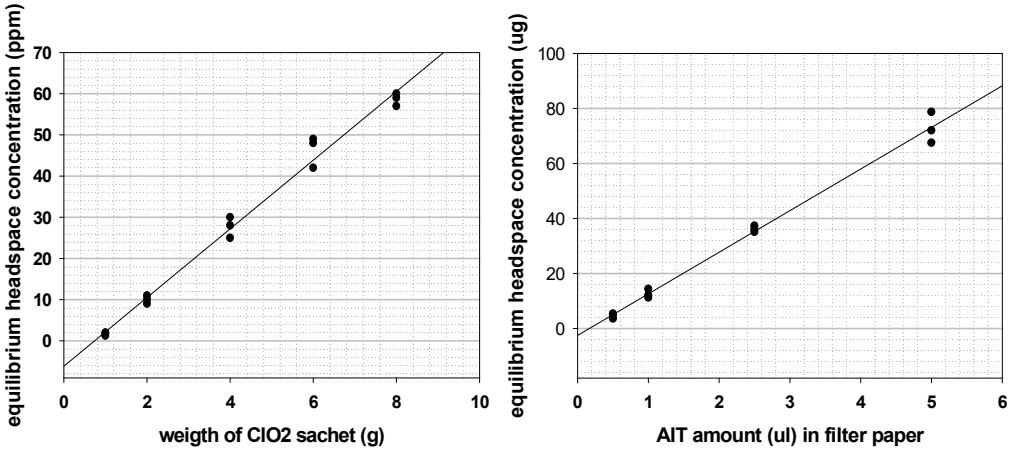

Figure 1. Equilibrium headspace concentration in empty jar (950ml) in incubator (37C)

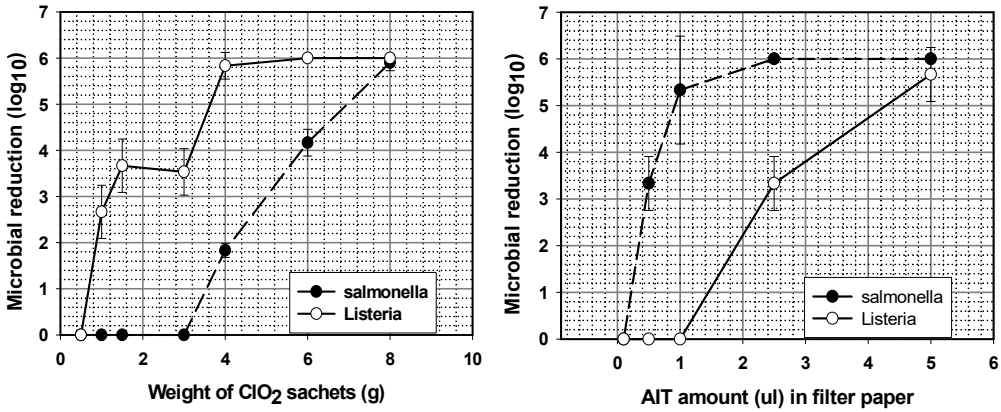

Figure 2. Reduction of salmonella & listeria on agar by antimicrobial treatment (n=3)

Microbial growth on inoculated chicken breast (at 4℃)

ClO_2 & AIT sachet release rates were designed to maintain a minimum inhibition concentration during storage. Almost all ClO_2/AIT treatments with MAP were significantly more effective during storage than conventional MAP. (4ug/hr was shown to be effective until the 12 th day against S.Typhimurium.

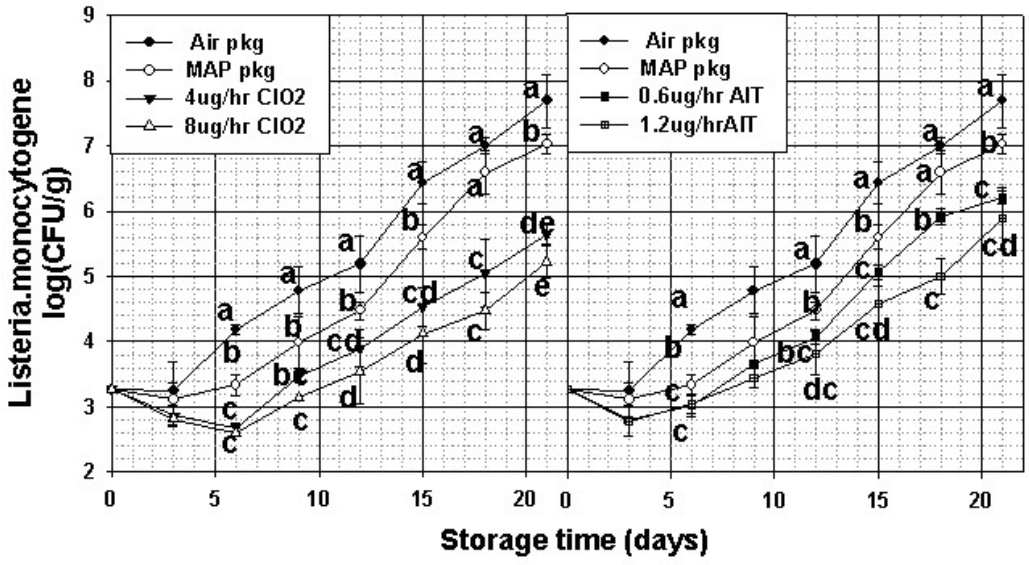

Figure 3. Growth of L.monocytognes on chicken breast during 21 days storage at 4±2˚C (n=3,P<0.05)

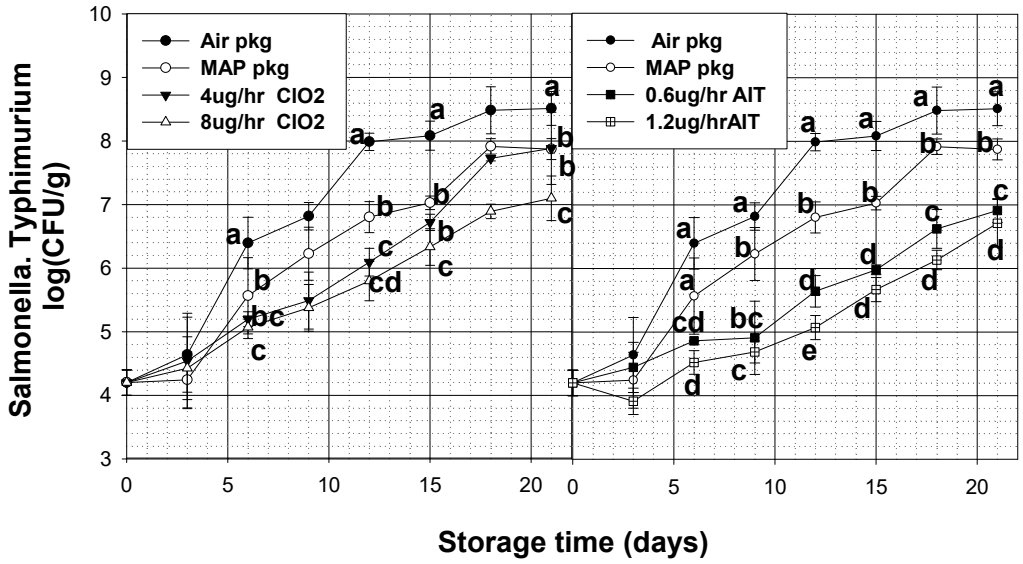

Figure 4.

Growth of S.Typhimuirum on chicken breast during 21 days storage at 4±2˚C (n=3, P<0.05)

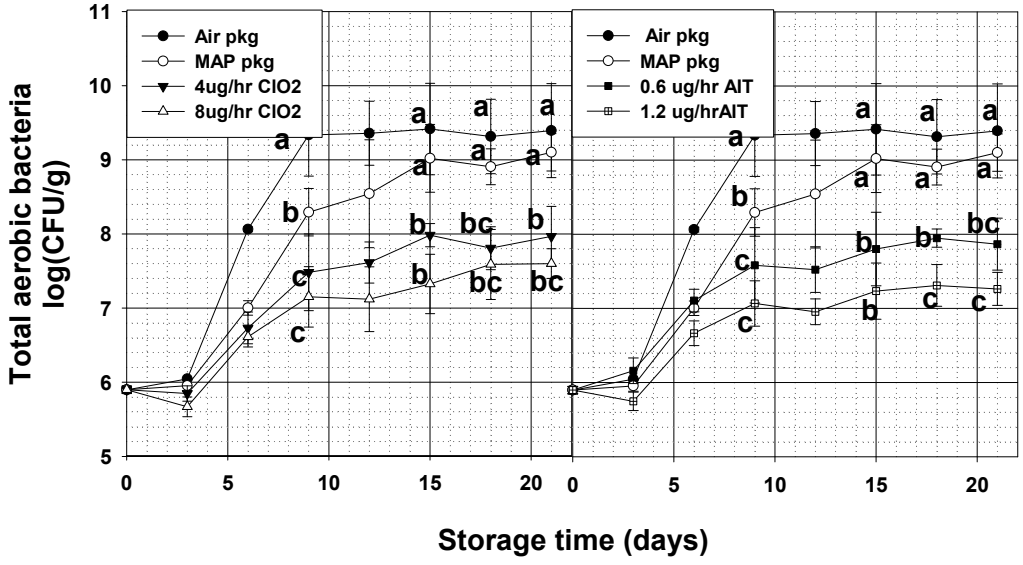

Figure 5.

Growth of total aerobic bacteria on L.monocytogene inoculated chicken breast during 21 day storage at 4±2˚C (n=3, P<0.05)

Color and pH

There was no significant color change observed due to microbial growth, however, high release treatments (8ug/hr ClO_2 and 1.2 ug/hr AIT) caused undesirable color changes on the chicken breast.

Table 1. color and pH values of chicken breast during storage time

pkg type		3 day	6 day	12 day	18 day
Air	L	$53.00\pm0.81_{a}$	$54.90\pm0.92_{a}$	$55.40\pm1.12_{a}$	$51.13\pm1.37_{b}$
	a	$1.54\pm0.02_{a}$	$2.53\pm0.54_{a}$	$3.00\pm0.67_{a}$	$2.85\pm0.57_{a}$
	b	$1.85\pm0.15_{a}$	$1.83\pm0.31_{a}$	$1.90\pm0.73_{a}$	$2.08\pm0.46_{a}$
	pH	$5.79\pm0.01_{a}$	$6.01\pm0.02_{b}$	$6.08\pm0.05_{c}$	$6.25\pm0.04_{d}$
MAP	L	$49.42\pm1.84_{a}$	$49.83\pm1.32_{a}$	$50.14\pm1.61_{a}$	$47.95\pm0.48_{a}$
	a	$1.54\pm0.02_{a}$	$1.40\pm0.82_{a}$	$1.70\pm0.73_{a}$	$1.88\pm0.74_{a}$
	b	$1.85\pm0.15_{a}$	$2.33\pm0.46_{a}$	$2.33\pm0.45_{a}$	$2.38\pm0.43_{a}$
	pH	$5.79\pm0.01_{a}$	$5.81\pm0.01_{a}$	$5.96\pm0.10_{b}$	$5.95\pm0.06_{b}$
ClO_2 (8ug/hr)	L	$49.37\pm2.15_{a}$	$48.60\pm3.07_{ab}$	$44.74\pm2.89_{c}$	$43.94\pm3.30_{c}$
	a	$1.54\pm0.02_{b}$	$1.09\pm0.67_{b}$	$1.40\pm0.46_{b}$	$2.25\pm0.25_{a}$
	b	$1.85\pm0.15_{a}$	$2.08\pm0.15_{a}$	$1.76\pm0.23_{a}$	$2.03\pm0.55_{a}$
	pH	$5.79\pm0.01_{a}$	$5.57\pm0.03_{b}$	$5.42\pm0.01_{c}$	$5.55\pm0.07_{c}$
AIT (1.2ug/hr)	L	$50.46\pm1.79_{b}$	$50.24\pm1.79_{b}$	$53.21\pm3.75_{ab}$	$55.74\pm1.15_{a}$
	a	$1.54\pm0.02_{a}$	$1.33\pm0.75_{a}$	$1.25\pm0.17_{a}$	$0.70\pm0.36_{a}$
	b	$1.85\pm0.15_{c}$	$2.45\pm0.33_{bc}$	$2.48\pm0.71_{bc}$	$3.38\pm0.48_{a}$
	pH	$5.79\pm0.01_{a}$	$5.80\pm0.08_{a}$	$5.82\pm0.03_{a}$	$5.75\pm0.01_{a}$

Mean±standard deviation, Mean in the same row value with different letter are significantly different, $P<0.05$

Mechanical properties

In the 10 ppm treatment, there were no significant physical propertiy changes after 1 month storage. However, in high concentrations (more than 500ppm), the mechanical properties of PS and Nylon were significantly decreased by ClO_2 treatment. High ClO_2 concentration consistently affected barrier properties of PS. Further study is needed to define effect of ClO_2 on polymer. The results shown that high ClO_2 treatment causes polymer property changes at high concentration. The high concentration (500ppm) is not a proper application for food packaging. However, It is important to know the relationship between ClO_2 concentration and modification of physical properties for sanitization of food and pharmaceutical package applications.

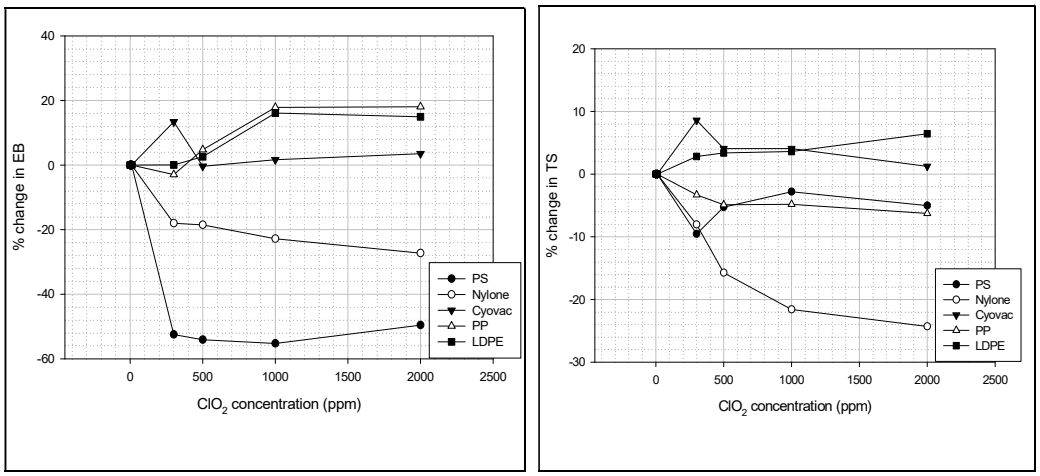

Figure 6. Percentage change in TS and EB of ClO_2 treated films (n=3)

Table 2. O2 permeability of PS film after 1 hour ClO_2 treatment

ClO_2 concentration (ppm)	OTR ($cc/100in^2.day$)
0	322.3
500	292.5
1000	287.5
2000	271.0

Thermal properties

No significant difference was observed between non-treated samples and ClO_2 treated samples

Conclusion

1. ClO_2 and AIT were effective against S.*Typhimurium*, L.*monocytogenes* on agar plates.

2. ClO_2 and AIT in combination with 30%CO_2/70%N_2 modified atmosphere packaging was more effective than conventional MAP to prevent growth of S.*Typhimurium*, L.*monocytogenes,* and total aerobic bacteria on chicken at 4°C.

3. ClO_2 treatment caused significant changes in the mechanical properties of PS, PP, Nylon, LDPE , and barrier properties of PS at high ClO_2 concentration.

Referece

1. Ahvenainen, 2003, *Novel food packaging techniques*, Woodhead pubishing ltd, p 50~65.

2. Han, Y., R.,H., etc., 2001, *Reduction of Listeria monocytogenes on green peppers by gaseous and aqueous chlorine dioxide and water washing and its growth at 7C*. Journal of food protection, 64, p1730~1738.

3. Han.J. H., 2000, A*ntimicrobial food packaging*. Food technology, 54(3), p56~65.

4. Isshiki, K., K. Tokuoka, R. Rori, and S. Chiba, 1992 *Preliminary examination of allyl isothiocyanate vapor for food preservation*. Biosci. Biotech. Biochem. 56,1476~1477.

5. Jimenez. S. M., 1997, *spoilage microflora in fresh chicken breast stored at 4C: influence of packaging methods*, Journal of Applied Microbiology, 83, p613~618.

6. Kraft , A.A., Reddy, etc., 1982, *Microbiological quality of vacuum packaged poultry with or without chlorine treatment*, journal of Food science, 47, p390~385.

7. Kim Y.S., etc, 2002, *Extension of shelf life by treatment with Allyl isothiocyanate in combination with acetic acid on cooked rice*, Journal of food science, 67(1), p274~279.

8. Lim, Loong-Tak, Marvin A. Tung, 1997, *Vapor pressure of Allyl isothiocyanate and its transport in PVDC/PVC copolymer packaging film*, Journal of food science, 62(5), p1061-1066

9. Banu F.O., etc., 2002, *Effects of ozone exposure on the structural, mechanical and barrier properties of select plastic packaging film*, Packaging technology and science, 15, p301-311

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