Active, Antimicrobial Packaging for Pet Food

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April 2013

Why Antimicrobial Packaging?

- Trend toward fresh pet foods
- Incidents of recalls
  - 12 Recalls involving suspected *Salmonella* spp.
  - Feb 19th to April 3, 2013 (FDA website)
- Antimicrobial Packaging – another hurdle
- Questions to ask for implementation
  - Regulatory Status and Liability
  - Economic – cost/benefit analysis
  - Technical Challenges

What is Active Packaging?

- Active Packaging
  - change condition/environment in the package to extend shelf life, enhance sensory properties or improve food safety.
  - senses change and changes package properties

- Intelligent Packaging
  - Senses and signals

Active Packaging

- Purge absorbers
- Moisture absorbers or emitters
- O_2 scavengers/emitters indicators
- CO_2 absorbers/emitters indicators
- Odor absorbers or emitters
- Ethylene absorbers
- Ethanol emitters
- Antimicrobials

Active Packaging

- **Moisture Absorbers**

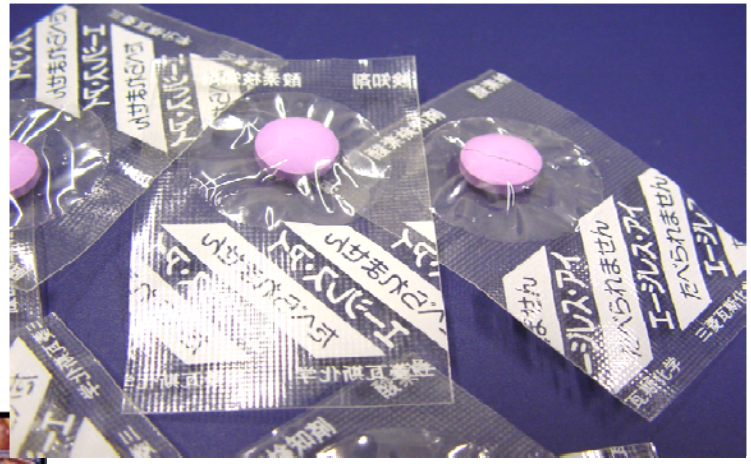
- **Packets**
- **Canisters**
- **Moldable products**

Oxygen Absorbers/Indicators

Sachets

Mitsubishi Ageless

Multisorb
FreshPax



Toppan - oxygen indicator

Oxygen Scavengers

- **Oxygen scavenging film**
  - **Cryovac OS – UV light activated**
- Bottles
  - Oxbar, Amcor, Darex, Aegis Ox, etc.
  - Caps and liners

Types of Antimicrobials Studied

Type	Specific examples/derivatives
Enzymes	Lyzoyme, Peroxidase
Chitosan	Derived from Shellfish
Bacteriocins	Nisin, Pediocin
Antibiotics	Imazalil
Organic acids	Benzoic, Sorbic, Ascorbic, Propionic
Spices	Rosemary, Garlic, Thymol
Citrus extracts	Grapefruit seed extract, limonene
Isothiocyanates	Allyl isothiocyanate
Metals	Silver ions
Fungicides	Benomyl, Ethanol
Oxidizers	Ozone, Chlorine dioxide

Screening Methods

- First step in formulation
- Performed in microbiological media
- Minimum effective level of antimicrobial agent
- Methods:
  - Spot on lawn
  - Agar diffusion
  - Kirby Bauer method

Options for Incorporation into Packaging

- Biopolymer films as carriers of antimicrobial agents.
- Biopolymer films as antimicrobial agents themselves.
- Incorporation of a antimicrobial delivery system for use in existing packaging systems.
- Incorporate into synthetic polymer
- Utilize a multi-system approach.

Incorporation into Packaging

- Coating
- Compound into polymer resin
- Blend into polymer through mix port in extruder
- Coextrude
- Encapsulation

Incorporation into Packaging

- Considerations
  - Miscibility
  - Viscosity
  - Heat sensitivity
  - Ability to release
  - Drying rates
  - Acidity
  - Converting on commercial equipment
  - Blocking and/or blooming in the roll form
  - Heat sealable
  - Even release of volatile active compounds
  - Effect of antimicrobial on food
  - Effect of food on antimicrobial effectiveness
  - Can material remain effective after storage, prior to use as a package

12

Screening methods for packaging film

- Methods differ depending upon method of antimicrobial delivery
  - Film on lawn
  - Agar overlay
  - Shake Flask
  - Extraction methods

- Measure over time to determine release
  - Quantitative – above methods
  - Kinetics - models

13

Antimicrobial Studies Vary

- Systematic approach
  - Level of antimicrobials used
  - Screening methods need to move to packaging applications
- Consistent Methods for Reporting Results
  - Positive/negative
  - % reduction comparison to control
  - Log reduction

14

Antimicrobials Studied

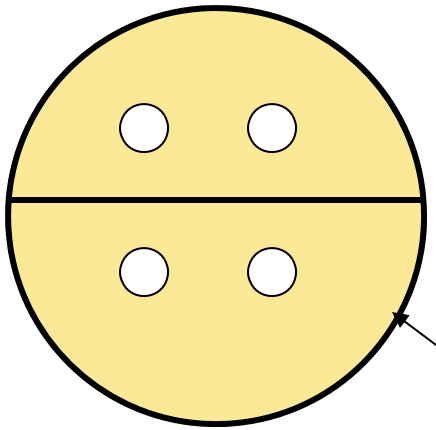
- Nisin
- Nisin Combinations
- Chitosan
- Chitosan Combinations
- Organic acids
- Chlorine dioxide
- Silver Ions

Nisin

- Methylcellulose and Hydroxypropyl methylcellulose coating as a carrier
- LDPE or barrier bags used as substrate
- Tested antimicrobial potential
  - Drop assay
  - Diffusion assay
  - Direct application of coated film to inoculated plate
  - Inoculated hot dogs, individually wrapped in coated film
- *Listeria monocytogenes*

MIC of Nisin

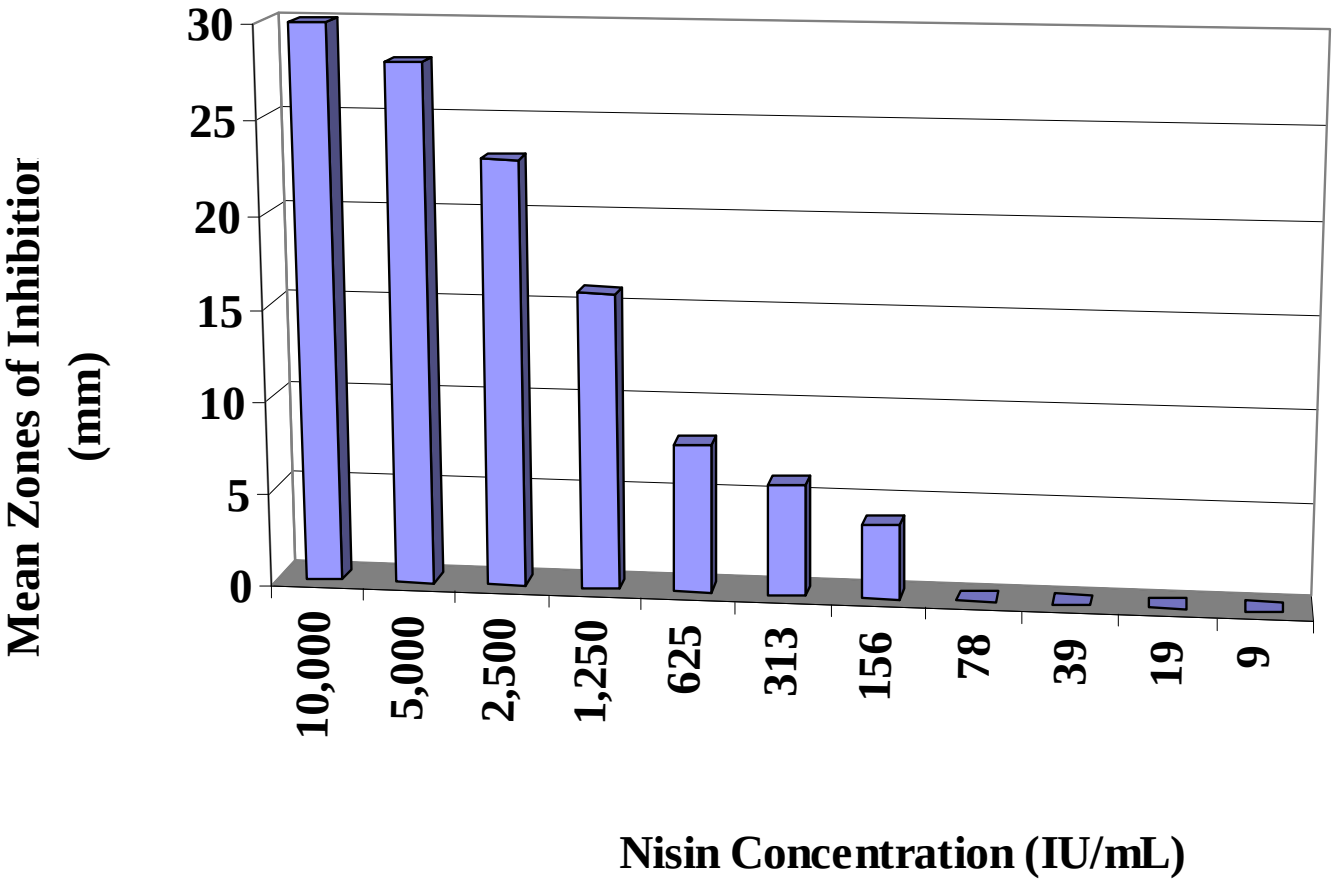
Spot on Lawn Assay



10μL drop taken from tubes containing film samples in buffer solution

buffer solution only

MIC of nisin in solution



Nisin

Film Coating Formulation

875mg Methyl cellulose

375mg Hydroxypropyl methylcellulose

0.75mL Polyethylene glycol (plasticizer)

1.25mL 0.02N Acetic acid

25mL Distilled water

25mL 95% Ethanol

Blend with homogenizer

Nisin

Film Production

- Thin layer chromatography plate coater.
- Gate set at 500μm thickness.
- Coat onto surface of 8” x 8” glass plates covered with low density polyethylene.
- Dry overnight at room temperature.
- Cut into 1 cm squares, place in plastic bag.
- UV light exposure for 7 mins. each side.

Nisin

LDPE film coated with cellulose-based coating

No nisin **10,000 IU/g nisin**

Inhibition of LM using a packaging film coating containing nisin

Film with
10,000 IU/mL nisin

Film with no nisin

Nisin

- Inhibition of LM in Hot Dogs using Nisin Coated Packaging Film
  - Nisin levels tested 10000, 7500, 2500 and 156
  - Coated onto barrier bags, hot dogs individually
    vacuum packaged
  - 5 strain LM cocktail, 5 log inoculum
  - Measured LM populations at days 0, 7, 15, 21 28 and 60

Inhibition of LM on the surface of hot dogs enumerated on TSA

Nisin (IU/mL)	Days of Storage					
	0	7	15	21	28	60
0	$5.29^{a,x}$	$5.51^{a,x}$	$6.13^{a,x}$	$6.33^{a,y}$	$8.01^{a,y}$	$9.11^{a,y}$
156.3	$4.84^{a,x}$	$4.9^{b,x}$	$4.90^{b,x}$	$5.37^{b,y}$	$7.50^{b,y}$	$9.52^{a,y}$
2500	ND^{b}	ND^{c}	ND^{c}	ND^{c}	ND^{c}	ND^{b}
7500	ND^{b}	ND^{c}	ND^{c}	ND^{c}	ND^{c}	ND^{b}
10,000	ND^{b}	ND^{c}	ND^{c}	ND^{c}	ND^{c}	ND^{b}

Nisin

- Overall summary
  - Effective at 2500 IU/mL or above
  - Diffuses from cellulose coating over time
  - Affects visual and heat sealing properties
  - 2,500 IU/ml ~$0.29/pouch
  - 7,500 IU/ml ~ $0.73/pouch

Nisin

- Forming stand alone film with nisin using cellulose formulation and compared to coating.
  - More effective when made as a film than as coating
  - Longer release

- Nisin/rosemary blend
  - Rosemary did not have synergistic effect in cellulose-based coating

Nisin

- Nisin or lauric acid with cellulose-chitosan film
  - Chitosan blended with cellulose alone, 2 log reduction
  - Chitosan blended with cellulose and lauric acid similar reduction
  - Chitosan blended with cellulose and nisin, 5 log reduction

Nisin

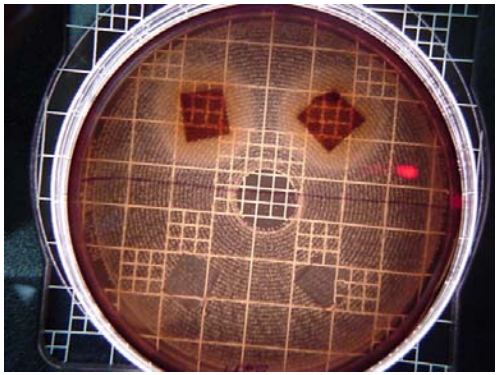
- Current work
  - Compounded with EVA, extruded film, inhibited spoilage bacteria on turkey bologna and cheese.
  - Coating onto paper, using pectin to control release, inhibiting L. monocytogenes.
  - Pattern coating cellulose based nisin blend for application on top web of form/fill/seal process along with spray on bottom forming web.

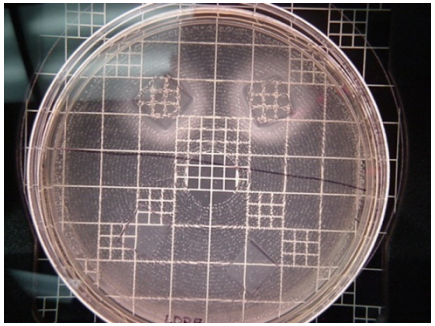
Chitosan

- Derived from shells of crustacean
- Inexpensive
- Requires corona treatment to coat onto film
- Reduces heat seal strength
- Shows effectiveness in liquid solution
- Minor reduction (2 log) in food
- Synergistic effect with nisin

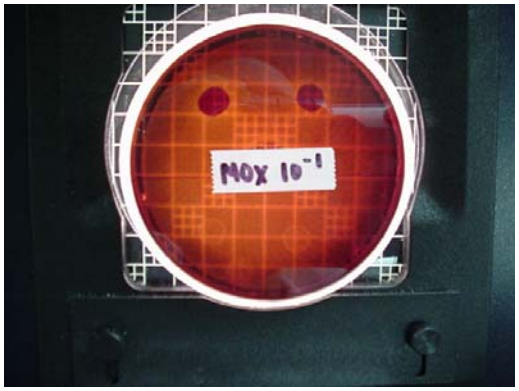
Chitosan

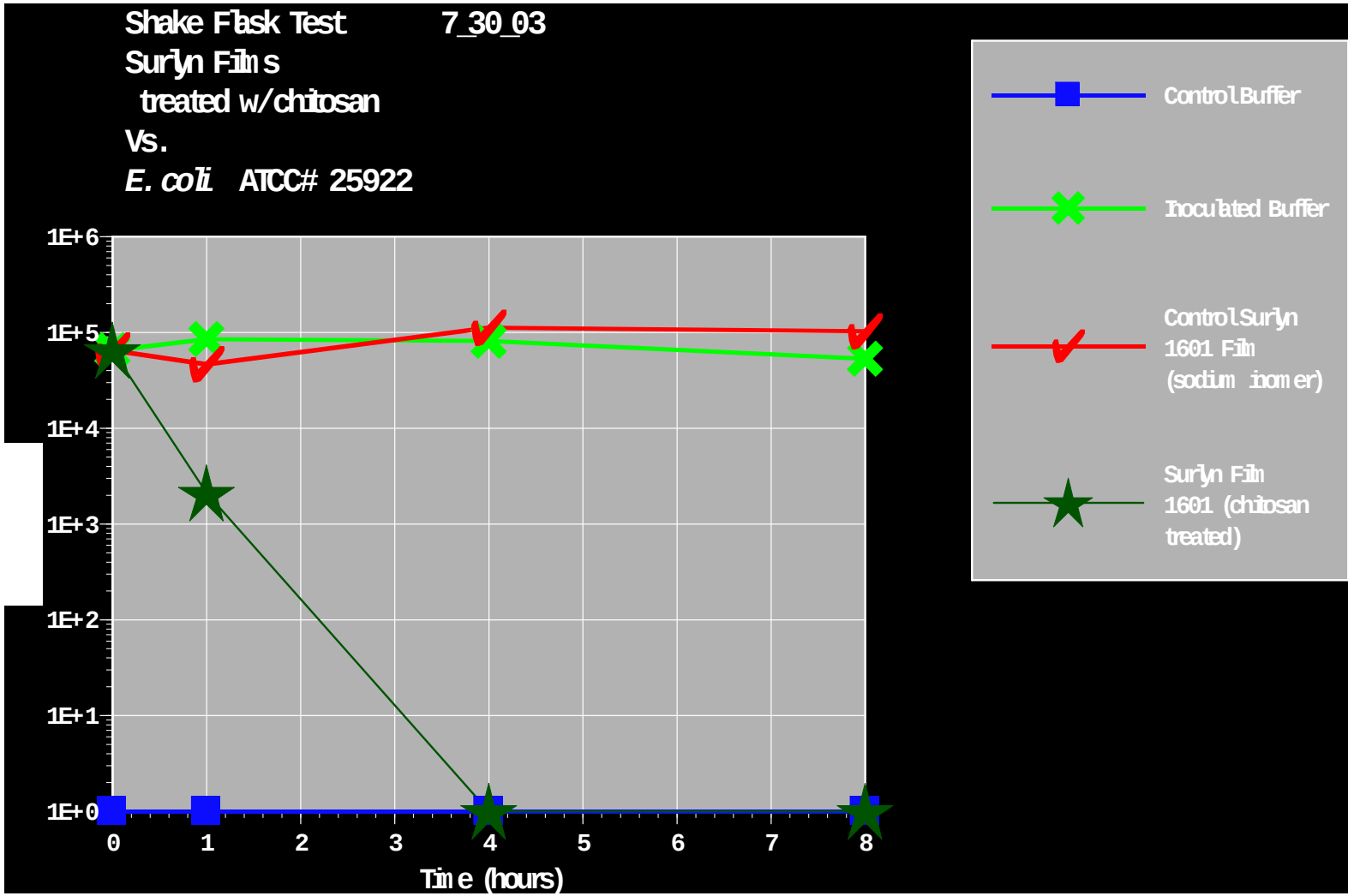
Film on Lawn





Spot on Lawn




Shake Flask Test 7_30_03
Surlyn Films
treated w/chitosan
Vs.
E. coli ATCC# 25922
Log CFU/mL
1E+6
1E+5
1E+4
1E+3
1E+2
1E+1
1E+0
0
1
2
3
4
5
6
7
8
Time (hours)
Control Buffer
Inoculated Buffer
Control Surlyn 1601 Film (sodium ionomer)
Surlyn Film 1601 (chitosan treated)

Chlorine dioxide

- Volatile compound, oxidizing agent
- Doesn’t require direct contact with product
- Extremely effective, biocide not biostatic
- Critical issues:
  - Release rate
  - Volume of package and headspace
  - Moisture content of product
  - Reduce effect of ClO_2 on product
  - Control to prevent oxidative reactions

Chlorine dioxide

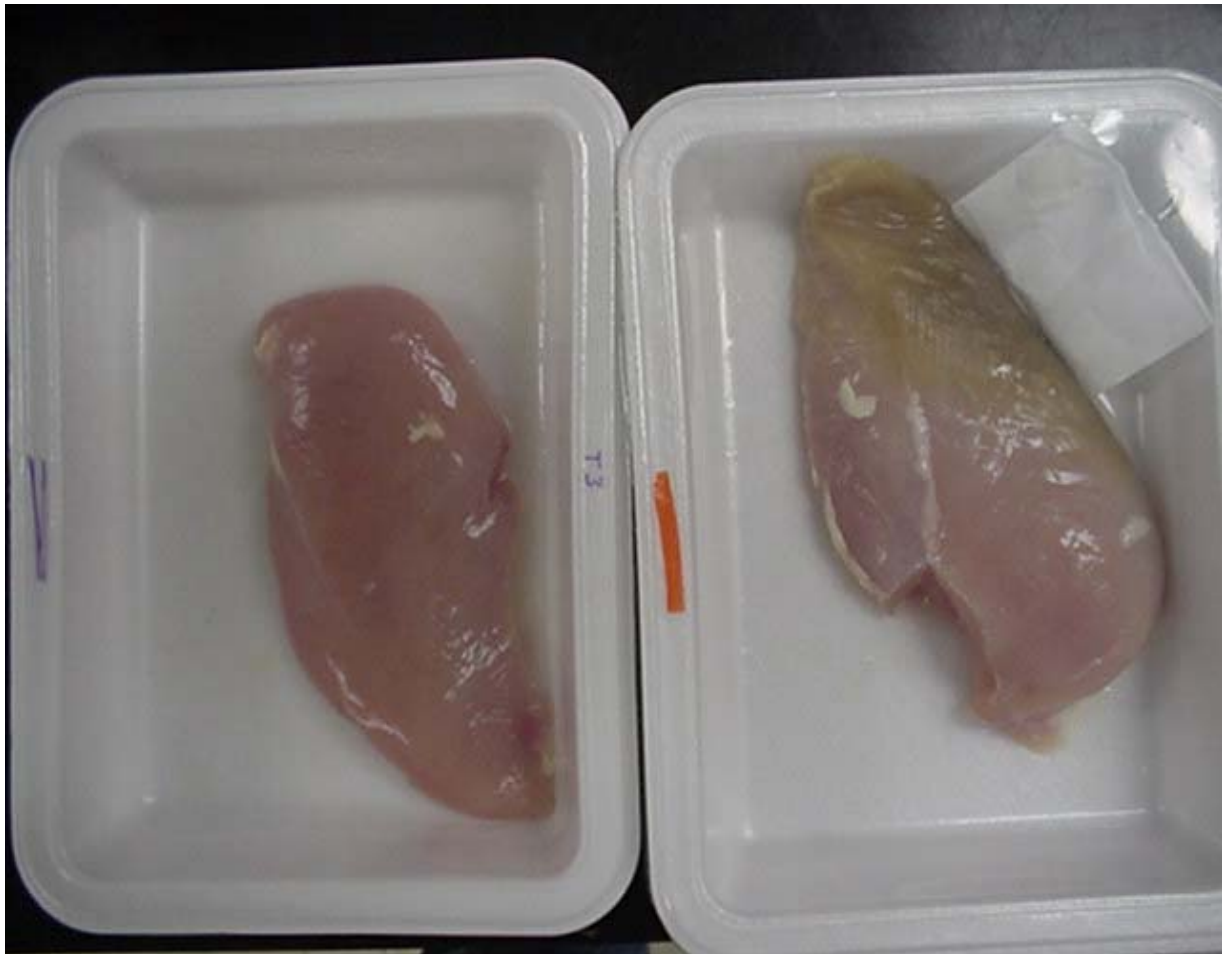
- Fresh chicken breasts inoculated with 4 log population *S. typhimurium* NAR
- Sachets:
  Fast release (6.6mg, 26 hours)
  Slow release (2.25mg, 22 days)
- Package atmosphere:
  100% N_2 or 75% N_2/25% CO_2
- TSA, TSA w/NA, L.a.b. color and sensory (odor and color) on days 0, 3, 6, 9, 12 and 15 of refrigerated storage.

Quality of Chicken using ClO_2 and MAP Packaging

Conclusions

- Total plate counts increased with storage time but those with ClO_2 were 1-1.5 log lower regardless of package atmosphere.
- After 15 days, *S. typhimurium* counts were significantly lower on samples treated with ClO_2 (fast and slow release sachets).

Quality of Chicken using ClO_2 and MAP Packaging



Chicken breasts after 15 days at 2.8°C

Quality of Chicken using ClO_2 and MAP Packaging

Conclusions

- Color was adversely affected but not reflected by instrumental or sensory evaluation due to statistical variability and sample location evaluated.
- Odor was significantly reduced by ClO_2 treated samples, a concern with regard to indication of spoilage.

Chlorine dioxide

- Current work
  - New sachets proven to work with bulk shipment of raspberries, blackberries and blueberries
  - Tomatoes - bulk shipment for foodservice
    - Reduce spoilage during shipment (temperature abuse)
    - Prevent outgrowth of pathogenic bacteria (*E. coli*)

Overall Summary

- Antimicrobial packaging can be effective.
- Method of testing effectiveness affects results
- What level of inhibition is “significant”?
- Combination systems show promise.
- Effect on food properties needs to be further studied.
- Focus on testing antimicrobials incorporated into package with food is critical to make progress
- Development of commercial production of antimicrobials packaging materials is our goal at Clemson

Questions?









Thank You!

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