

# Antimicrobial Effects of Surface Treatments and Ingredients on Cured RTE Meat Products

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# Justification

- *Listeria monocytogenes* - Pathogen of major concern
- Administration's goal - Cutting Listeria caused illnesses in half by 2010
- RTE products (frankfurters and luncheon meats) identified as vehicles of food-borne *L. monocytogenes*

# Justification

- Federal Register, February 27, 2001  
USDA-FSIS - Proposed rule may require environmental and product testing for *L. monocytogenes*
- Rule will establish performance standards for all RTE products
- Sets levels of pathogen reduction and limits on *L. m.* growth to produce unadulterated products

# Justification

- An intervention step between cooking and packaging would be useful in meeting safety requirements for RTE products
- Residual bacteriostatic effect after packaging would be desirable



# Justification

Hypothesis -

Application of food grade GRAS preservatives to the surface of frankfurters and/or incorporation of these ingredients will retard the growth of *Listeria monocytogenes* during vacuum packaged storage at 4.5°C.

# Objectives

Specific objectives of the proposed research are to:

1. Determine the preservative properties of Safe<sub>2</sub>O™ + Lactic Acid (SWLA) or potassium lactate (KL) as ingredients for preserving quality and extending the shelf-life of vacuum packaged, cured, ready-to-eat (RTE) frankfurters.

# Objectives

2. Evaluate the effectiveness of a surface application of either lactic acid (LA), Safe<sub>2</sub>O™ + Propionic Acid (SWPA) or potassium lactate (KL) on frankfurters in combination with SWLA or KL as ingredients for suppressing the outgrowth of *Listeria monocytogenes*.

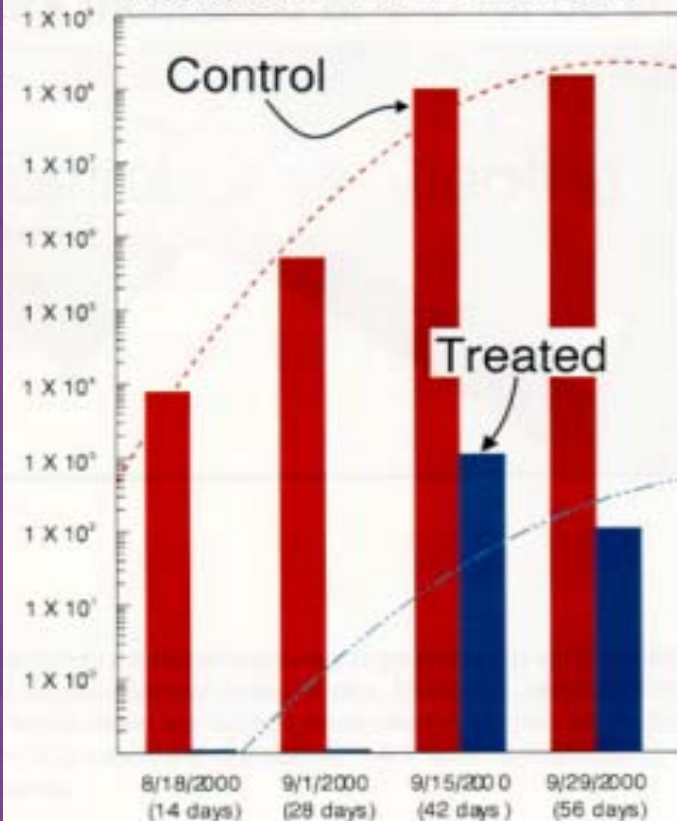
# Objectives

3. Evaluate the sensory, physical, chemical and microbiological properties of vacuum-packaged frankfurters containing SWLA or KL with a surface application of lactic acid, SWPA or potassium lactate.

# Literature Review

- **Safe<sub>2</sub>O™**, proprietary GRAS mixture of highly acidic propionic acid/lactic acid/calcium sulfate complex
- Surface application to franks - reduced Aerobic Plate Counts (APC's) to  $\sim 1 \times 10^2$  CFUs
- Untreated franks -  $> 1 \times 10^8$  CFUs after 8 weeks aerobic storage at 4°C (Proprietary Study, Mionix Corporation)

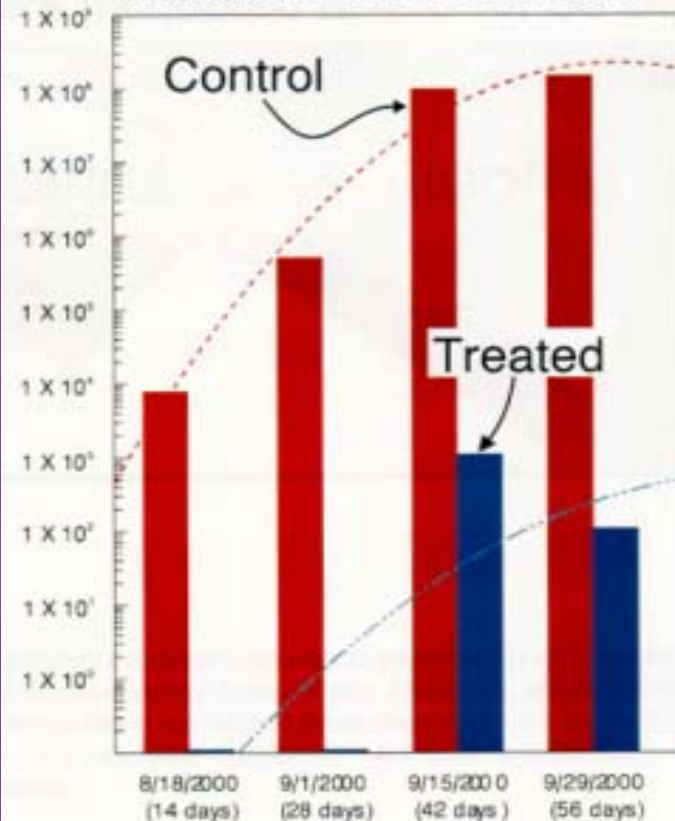
Effect of Safe<sub>2</sub>O™-PA Treatment and Time of Incubation at 4°C on Aerobic Bacteria Associated with Frankfurters



# Literature Review

- Incorporation or application to the surface of RTE products, may afford a degree of protection against pathogens

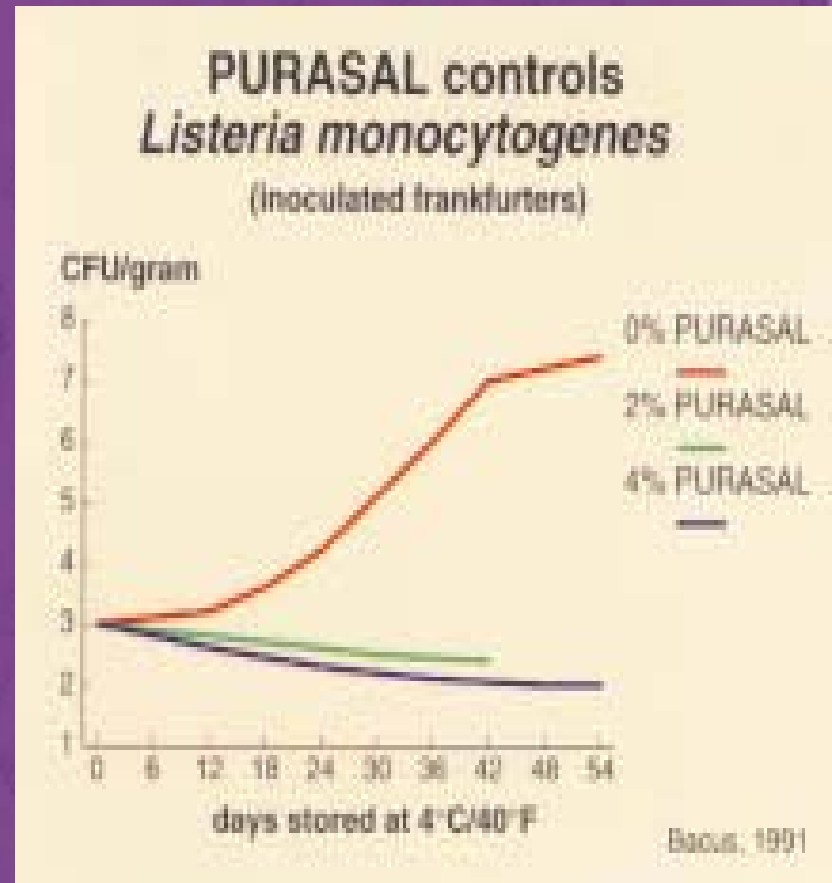
Effect of Safe<sub>2</sub>O™-PA Treatment and Time of Incubation at 4°C on Aerobic Bacteria Associated with Frankfurters



# Literature Review

- Sodium or potassium lactate incorporation into RTE meat products
- 2 to 4% serves as a bacteriostatic agent against *Listeria monocytogenes*, *Escherichia coli* O157:H7 and Salmonella

(Houtsma et al., 1996;  
Murano and Rust, 1995;  
Nerbrink et al., 1999;  
Shelef 1994)



# Experimental Procedures

- Ingredients

- 3.3% potassium lactate (KL, 60% solution) incorporated into 22.7 kg (50 lbs) batch of standardized frankfurter formulation
- Control processed without KL
- Each batch divided into inoculated (*L. monocytogenes*) and non-inoculated groups

# Experimental Procedures

- Dip Solutions

- Distilled water (Control)
- Safe<sub>2</sub>O™ with propionic acid (SWPA); used 1:2, Safe<sub>2</sub>O™ :H<sub>2</sub>O dilution
- 3.3% potassium lactate (KL)
- 3.4% L-lactic acid (LA) (88% concentration)

- Storage

- Under refrigeration (4.5°C) or 12 weeks (vacuum packaged)

# Experimental Procedures

## Experimental design with formulation ingredients and dip preservatives

Ingredient	Control		3.3% Potassium Lactate (60% solution)	
	No Inoculation	Inoculation	No Inoculation	Inoculation
Dip				
Control	X	X	X	X
Safe <sub>2</sub> O™HOH Propionic Acid	X	X	X	X
3.3% KL*	X	X	X	X
3.4% (d,l) LA*	X	X	X	X

\*Treatments – PURAC HiPure, 60% solution of KL at 3.3% = 2.0% of 100% KL; 88% lactic acid solution at 3.4% = 3.0% of 100% solution.

# Analytical Procedures

- Process Yield - Finished Product Weight / Raw Product Weight x 100
- Vacuum Purge Release - Purge Weight/ Product Weight + Purge Weight x 100
- Composition - Percent moisture, fat, protein, ash (AOAC, 2000)
- Water Activity ( $a_w$ ) – Aqua Lab™, Model Series 3, 25g sample

# Analytical Procedures

- Residual Nitrite - Ion specific electrode
- Salt content - Ion specific electrode
- Total Lactate - Enzymatic, colorimetric method, Sigma Diagnostics Kit # 500
- Calcium - Ion specific electrode
- Phosphate - Inductively coupled plasma atomic emission spectrophotometry

# Analytical Procedures

- pH - Slurry, standardized at pH 4.00 and 7.00
- Objective Color – L\*, a\*, b\* color space values, Minolta Colorimeter, Model CR-200
- Sensory Evaluation – Descriptive attribute trained panel (aroma, flavor, texture, color)
- Microbiological Shelf-Life - Aerobic Plate Counts (APCs), *Listeria monocytogenes* ATCC 15313, 51414, 43256 49594 (surface inoculation at  $10^8$  CFU/ml prior to vacuum packaging)

# RESULTS

## Physical and Chemical Evaluation

Least squares means for percent process yield and sodium, calcium and phosphorus content of frankfurters containing KL

<u>Ingredient Treatment</u> <sup>1</sup>	Percent Process Yield	Percent Sodium	Calcium (mg/100g)	Phosphorus (mg/100g)
Control	90.20 <sup>a</sup>	0.77 <sup>a</sup>	4.15 <sup>a</sup>	209.63 <sup>a</sup>
KL	90.66 <sup>a</sup>	0.75 <sup>a</sup>	4.23 <sup>a</sup>	198.81 <sup>a</sup>
Std. Error <sup>2</sup>		0.005	0.15	1.28

<sup>abc</sup> Means within a column per main effect with different superscript letters are significantly different ( $P < 0.05$ ).

<sup>1</sup>Control = no KL, KL = 3.3% of a 60% solution.

<sup>2</sup>Std Error LS Mean for Process yield (%) - control = 0.33, KL = 0.35

# RESULTS

## Physical and Chemical Evaluation

Least squares means for percent vacuum-package purge, pH,  $a_w$ , lactate and residual nitrite content of frankfurters containing KL

	Percent Vacuum-package Purge	pH	$a_w$	Lactate (mg/g tissue)	Residual Nitrite (ppm)
<u>Ingredient Treatment</u> <sup>1</sup>					
Control	1.84 <sup>a</sup>	6.05 <sup>a</sup>	0.978 <sup>a</sup>	4.56 <sup>a</sup>	1.55 <sup>a</sup>
KL	1.98 <sup>a</sup>	6.09 <sup>a</sup>	0.973 <sup>a</sup>	11.60 <sup>a</sup>	1.36 <sup>a</sup>
Std. Error	0.03	0.008	0.0003	1.16	0.13

<sup>abc</sup> Means within a column per main effect with different superscript letters are significantly different ( $P < 0.05$ ).

<sup>1</sup>Control = no KL, KL = 3.3% of a 60% solution.

# RESULTS

## Physical and Chemical Evaluation

- Ingredient (KL)
  - No change in proximate composition with KL except slight decrease in moisture (~1%)
  - Process yield, vacuum purge, pH, lactate residual nitrite, sodium, calcium, phosphorus nor color were affected by addition of KL

# RESULTS

## Physical and Chemical Evaluation

Least squares means for percent process yield and sodium, calcium and phosphorus content of frankfurters dipped in antimicrobial solutions

<u>Dip Treatment</u> <sup>1</sup>	Percent Sodium	Calcium (mg/100g)	Phosphorus (mg/100g)
Control	0.78 <sup>a</sup>	3.48 <sup>ab</sup>	206.00 <sup>a</sup>
SWPA	0.75 <sup>a</sup>	<b>5.94<sup>c</sup></b>	206.25 <sup>a</sup>
KL	0.76 <sup>a</sup>	3.67 <sup>ab</sup>	203.00 <sup>a</sup>
LA	0.76 <sup>a</sup>	3.68 <sup>bc</sup>	201.63 <sup>a</sup>
Std.Error <sup>2</sup>	0.01	0.05	1.80

<sup>abc</sup> Means within a column per main effect with different superscript letters are significantly different ( $P < 0.05$ ).

<sup>1</sup>Control = Saline solution; SWPA = Safe<sub>2</sub>O™ with propionic acid; KL = Potassium lactate; LA = Lactic acid.

<sup>2</sup>Std Error LS Mean for Process yield (%) - control = 0.33, KL = 0.35

# RESULTS

## Physical and Chemical Evaluation

Least squares means for percent vacuum-package purge, pH,  $a_w$ , lactate and residual nitrite content of frankfurters dipped in antimicrobial solutions

	Percent Vacuum-package Purge	pH	$a_w$	Lactate (mg/g tissue)	Residual Nitrite (ppm)
<u>Dip Treatment</u> <sup>1</sup>					
Control	1.82 <sup>b</sup>	6.32 <sup>a</sup>	0.977 <sup>b</sup>	7.36 <sup>a</sup>	1.75 <sup>b</sup>
SWPA	<b>2.48<sup>a</sup></b>	<b>5.49<sup>c</sup></b>	<b>0.975<sup>a</sup></b>	9.23 <sup>a</sup>	<b>0.59<sup>a</sup></b>
KL	<b>1.52<sup>c</sup></b>	6.26 <sup>ab</sup>	0.977 <sup>b</sup>	7.69 <sup>a</sup>	1.83 <sup>b</sup>
LA	1.80 <sup>b</sup>	<b>6.20<sup>b</sup></b>	0.976 <sup>ab</sup>	8.03 <sup>a</sup>	1.66 <sup>b</sup>
Std. Error <sup>2</sup>	0.06	0.02	0.0004	0.71	0.08

<sup>abc</sup> Means within a column per main effect with different superscript letters are significantly different ( $P < 0.05$ ).

<sup>1</sup>Control = Saline solution; SWPA = Safe<sub>2</sub>O™ with propionic acid; KL = Potassium lactate; LA = Lactic acid.

<sup>2</sup>Std Error LS Mean for Process yield (%) - control = 0.33, KL = 0.35

# RESULTS

## Physical and Chemical Evaluation

- Dips (SWPA, KL, LA)
  - No effect on proximate composition
  - Vacuum purge and calcium increased slightly with SWPA
  - pH, water activity and residual nitrite decreased with SWPA; LA decreased pH slightly
  - Slight variations in L,a,b color space values, but not practically different

# RESULTS

## Physical and Chemical Evaluation

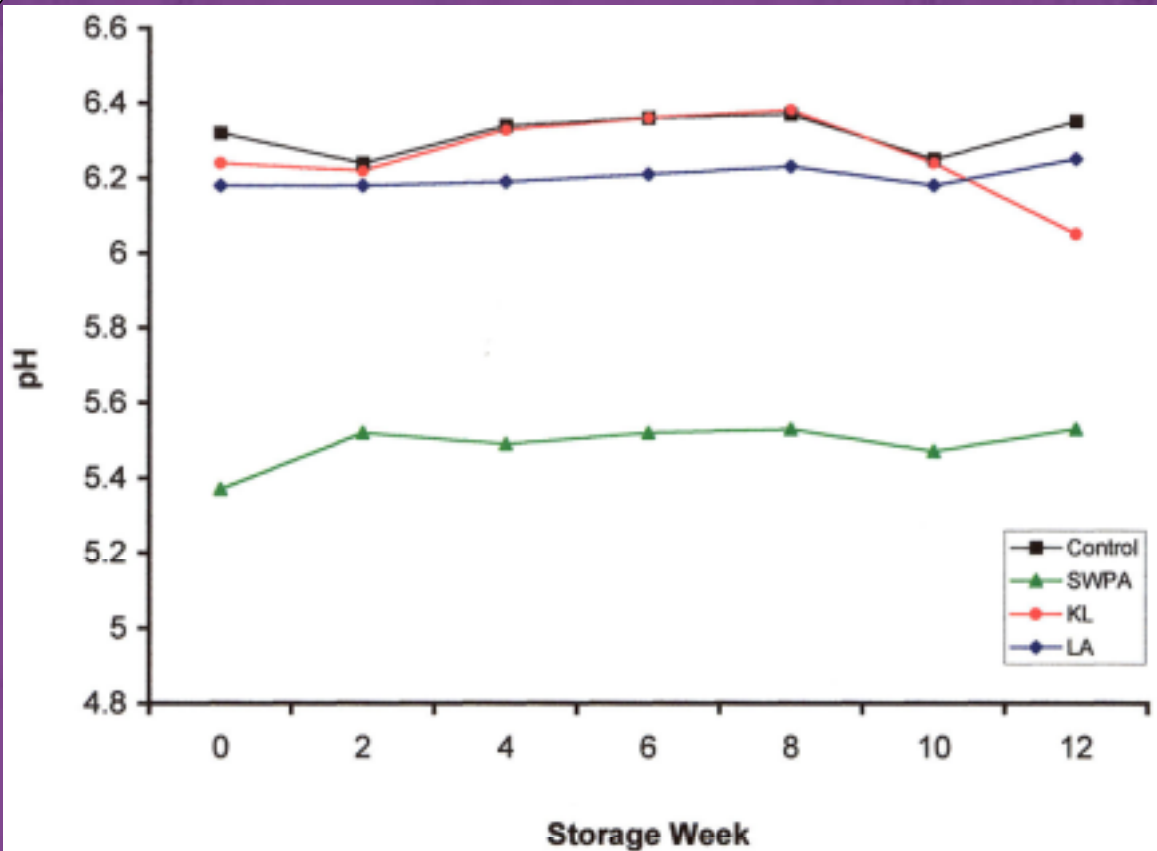


Fig 1. Least squares means for dip by storage week interaction for pH values

# RESULTS

## Physical and Chemical Evaluation

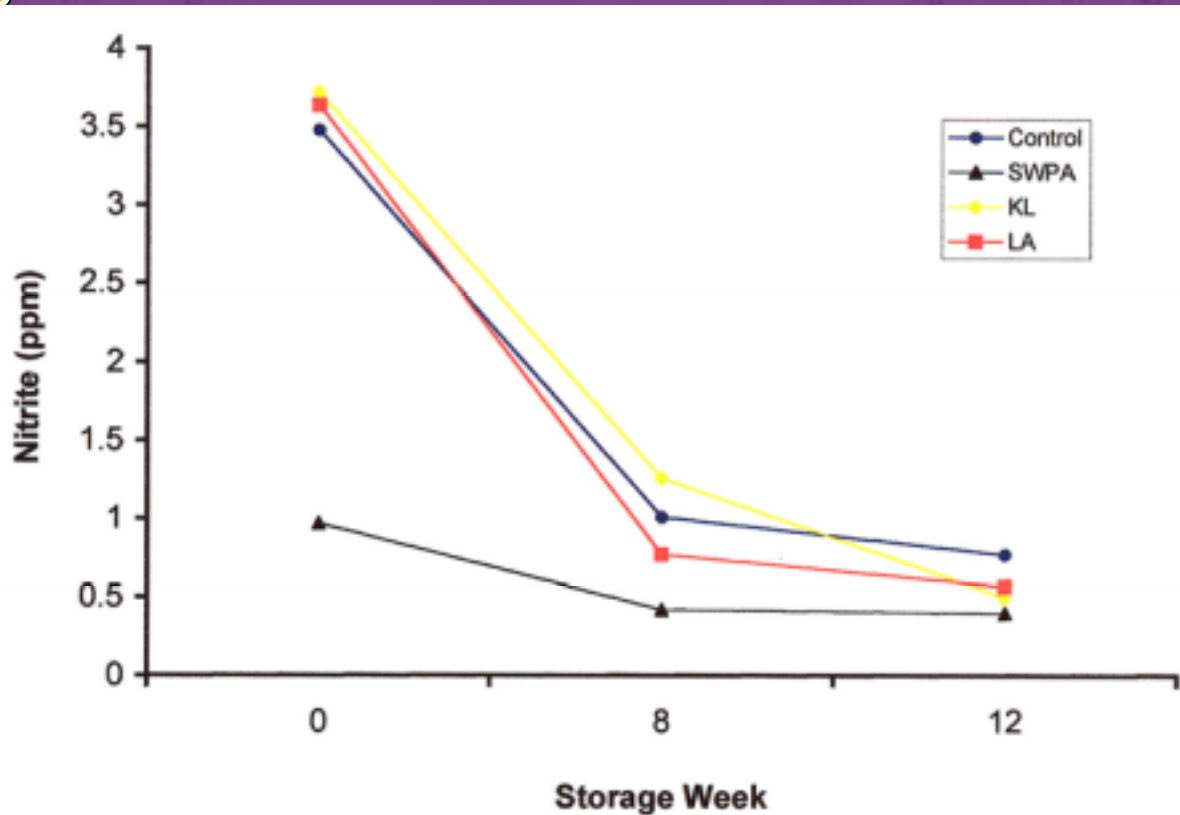


Fig 2. Least square means for dip by storage week interaction for nitrite

# RESULTS

## Sensory Evaluation

Least squares means of descriptive attribute sensory panel scores for aromatics<sup>1</sup>, feeling factors<sup>1</sup>, basic tastes<sup>1</sup>, aftertastes and textures<sup>1</sup> of frankfurters containing KL

	Control <sup>2</sup>	KL <sup>2</sup>	Std. Error
<u>Aromatics</u>			
Overall Meat Flavor	6.25 <sup>a</sup>	6.44 <sup>a</sup>	0.030
Fatty	2.14 <sup>a</sup>	<b>2.28<sup>b</sup></b>	0.010
Smoke	2.07 <sup>a</sup>	2.13 <sup>a</sup>	0.010
Spice	2.96 <sup>a</sup>	3.02 <sup>a</sup>	0.040
Cardboard	0.03 <sup>a</sup>	0.02 <sup>a</sup>	0.020
Painty	0.00 <sup>a</sup>	0.01 <sup>a</sup>	0.004
Fishy	0.01 <sup>a</sup>	0.00 <sup>a</sup>	0.010
Caramelized	0.06 <sup>a</sup>	0.08 <sup>a</sup>	0.002
Vinegar	0.58 <sup>a</sup>	0.46 <sup>a</sup>	0.020
<u>Feeling Factors</u>			
Astringent	2.52 <sup>a</sup>	<b>2.64<sup>b</sup></b>	0.004
Metallic	2.14 <sup>a</sup>	2.21 <sup>a</sup>	0.005

<sup>ab</sup> Means in the same row with different superscript letters are different ( $P < 0.05$ ).

<sup>1</sup> Based on a 16-point intensity scale (0 = absence of an attribute; 15 = extremely intense).

<sup>2</sup> Control = no KL, KL = 3.3% of a 60% solution.

# RESULTS

## Sensory Evaluation

Least squares means of descriptive attribute sensory panel scores for aromatics<sup>1</sup>, feeling factors<sup>1</sup>, basic tastes<sup>1</sup>, aftertastes and textures<sup>1</sup> of frankfurters containing KL (contd.)

	Control <sup>2</sup>	KL <sup>2</sup>	Std. Error
<u>Basic Tastes</u>			
Salt	3.83 <sup>a</sup>	4.43 <sup>a</sup>	0.090
Sour	2.52 <sup>a</sup>	2.56 <sup>a</sup>	0.030
Bitter	2.39 <sup>a</sup>	<b>2.43<sup>b</sup></b>	0.002
Sweet	0.72 <sup>a</sup>	0.69 <sup>a</sup>	0.060
<u>Aftertastes</u>			
Fat Mouthfeel	0.97 <sup>a</sup>	1.05 <sup>a</sup>	0.030
Sour	0.91 <sup>a</sup>	0.86 <sup>a</sup>	0.005
Bitter	0.73 <sup>a</sup>	<b>0.81<sup>b</sup></b>	0.003
Salty	1.20 <sup>a</sup>	1.52 <sup>a</sup>	0.030
<u>Textures</u>			
Springiness	6.81 <sup>a</sup>	6.74 <sup>a</sup>	0.044
Juiciness	3.88 <sup>a</sup>	4.01 <sup>a</sup>	0.050
Hardness	4.85 <sup>a</sup>	4.72 <sup>a</sup>	0.090
Cohesiveness of mass	4.29 <sup>a</sup>	4.21 <sup>a</sup>	0.030

<sup>ab</sup> Means in the same row with different superscript letters are different ( $P < 0.05$ ).

<sup>1</sup> Based on a 16-point intensity scale (0 = absence of an attribute; 15 = extremely intense).

<sup>2</sup> Control = no KL, KL = 3.3% of a 60% solution.

# RESULTS

## Sensory Evaluation

- Ingredient (KL)
  - KL increased the fatty aromatic, astringency, bitter taste, and bitter aftertaste but only at a minutely detectable level
  - Other sensory factors (meat flavor aromatics, basic tastes, aftertastes and texture) were not affected by KL

# RESULTS

## Sensory Evaluation

Least squares means of descriptive attribute sensory panel scores for aromatics<sup>1</sup>, feeling factors<sup>1</sup>, basic tastes<sup>1</sup>, aftertastes<sup>1</sup> and textures<sup>1</sup> of frankfurters dipped in antimicrobial solutions

	Control <sup>2</sup>	SWPA <sup>2</sup>	KL <sup>2</sup>	LA <sup>2</sup>	Std. Error
<u>Aromatics</u>					
Overall Meat Flavor	6.44 <sup>b</sup>	<b>6.09<sup>a</sup></b>	6.40 <sup>b</sup>	6.45 <sup>b</sup>	0.064
Caramelized	0.07 <sup>a</sup>	<b>0.13<sup>c</sup></b>	<b>0.04<sup>b</sup></b>	<b>0.04<sup>b</sup></b>	0.070
Vinegar	0.16 <sup>a</sup>	<b>1.41<sup>b</sup></b>	0.26 <sup>a</sup>	0.23 <sup>a</sup>	0.080
<u>Feeling Factors</u>					
Astringent	2.53 <sup>ab</sup>	<b>2.74<sup>c</sup></b>	2.47 <sup>a</sup>	2.57 <sup>b</sup>	0.030
Metallic	2.15 <sup>a</sup>	<b>2.27<sup>b</sup></b>	2.14 <sup>a</sup>	2.16 <sup>a</sup>	0.023
<u>Basic Tastes</u>					
Salt	4.13 <sup>a</sup>	4.10 <sup>a</sup>	4.09 <sup>a</sup>	4.18 <sup>a</sup>	0.104
Sour	2.37 <sup>a</sup>	<b>2.84<sup>b</sup></b>	2.49 <sup>a</sup>	2.46 <sup>a</sup>	0.059
Bitter	2.39 <sup>ab</sup>	<b>2.45<sup>c</sup></b>	2.38 <sup>a</sup>	2.43 <sup>bc</sup>	0.011
Sweet	0.81 <sup>b</sup>	<b>0.57<sup>a</sup></b>	0.80 <sup>b</sup>	0.65 <sup>ab</sup>	0.045
<u>Aftertastes</u>					
Sour	0.62 <sup>a</sup>	<b>1.27<sup>b</sup></b>	0.83 <sup>a</sup>	0.82 <sup>a</sup>	0.074
<u>Textures</u>					
Hardness	4.72 <sup>ab</sup>	<b>4.94<sup>c</sup></b>	4.68 <sup>a</sup>	4.79 <sup>b</sup>	0.031

<sup>abc</sup> Means in the same row with different superscript letters are different ( $P < 0.05$ ).

<sup>1</sup> Based on a 16-point intensity scale (0 = absence of an attribute; 15 = extremely intense).

<sup>2</sup> Control = Saline solution; SWPA = Safe<sub>2</sub>O™ with propionic acid; KL = Potassium lactate; LA = Lactic acid.

# RESULTS

## Sensory Evaluation

- Dips (SWPA, KL, LA)
  - SWPA changes were noted for meat flavor, astringency, metallic, salt, bitter, sweet, sour and texture hardness but only at minutely detectable levels
  - Other sensory factors (meat flavor aromatics, basic tastes, aftertastes and texture) were not affected by dips

# RESULTS

## Microbiological Evaluation

Least squares means for APC, *Listeria monocytogenes* counts and log reduction on frankfurters containing KL and dipped in antimicrobial solutions

	APC (log <sub>10</sub> CFU/Frank)	<i>Listeria monocytogenes</i> (log <sub>10</sub> CFU/Frank)	<i>Listeria monocytogenes</i> log reduction
<u>Ingredient Treatment<sup>1</sup></u>			
Control	3.1 <sup>a</sup>	5.2 <sup>a</sup>	3.0 <sup>a</sup>
KL	2.9 <sup>a</sup>	4.7 <sup>a</sup>	2.9 <sup>a</sup>
Std. Error	0.13	0.32	0.16
<u>Dip Treatment<sup>2</sup></u>			
Control	3.6 <sup>ab</sup>	6.6 <sup>b</sup>	1.4 <sup>a</sup>
SWPA	1.8 <sup>a</sup>	1.7 <sup>c</sup>	5.8 <sup>c</sup>
KL	4.7 <sup>b</sup>	7.0 <sup>b</sup>	1.3 <sup>a</sup>
LA	2.0 <sup>a</sup>	4.5 <sup>a</sup>	3.2 <sup>b</sup>
Std. Error	0.61	0.44	0.40

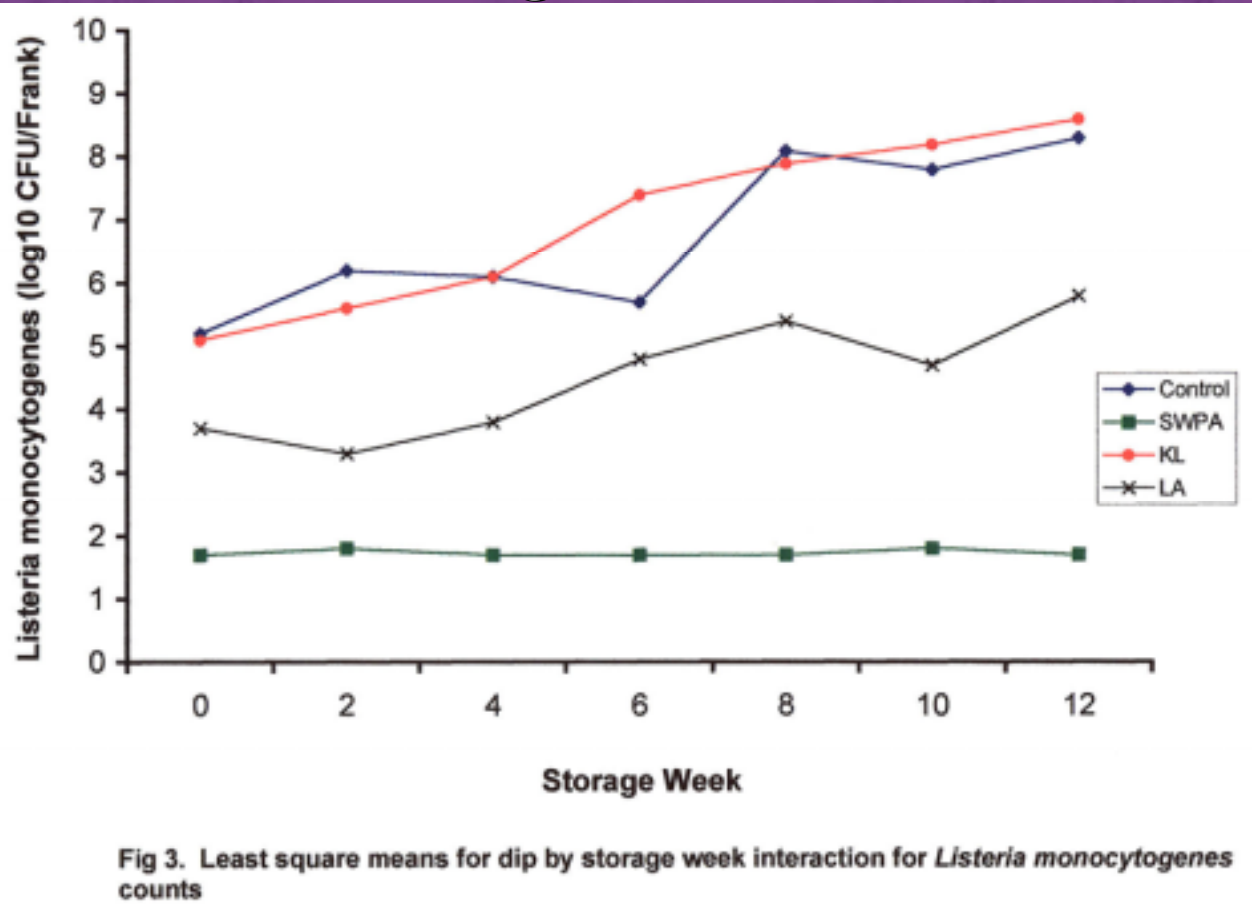
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<sup>1</sup>Control = no KL, KL = 3.3% of a 60% solution.

<sup>2</sup>Control = Saline solution; SWPA = Safe<sub>2</sub>O™ with propionic acid; KL = Potassium lactate; LA = Lactic acid.

# RESULTS

## Microbiological Evaluation



# RESULTS

## Microbiological Evaluation

- Ingredient (KL)
  - Surface APC and *L. m.* counts were not reduced by incorporation of KL
- Dips (SWPA, KL, LA)
  - SWPA and LA reduced surface *L. m.* counts by 5.8 and 3.2 logs, respectively
  - APC counts of SWPA and LA were lower than the KL dip

# RESULTS

## Microbiological Evaluation

- Storage (12 weeks)
  - SWPA reduced *L. m.* counts to 1.7 logs immediately after surface treatment
  - *L. m.* exhibited no growth on SWPA franks; counts remained at 1.7 logs for 12 weeks
  - 3% LA reduced *L. m.* initially to 3.7 logs, but incremental growth allowed *L. m.* to reach 5.8 logs by the end of storage

# CONCLUSIONS

- SWPA and 3% LA dips reduced *L. m.* on the surface of frankfurters by 5.8 and 3.2 logs, respectively
- SWPA appears to have residual bacteriostatic properties to suppress the growth of *L. m.* during refrigerated storage
- SWPA reduced pH ~0.82 unit likely causing increased vacuum-package purge (0.66%) and reduced residual nitrite

# CONCLUSIONS

- Antimicrobial dips had little or no affect on other physical, chemical and sensory parameters measured

# Benefits to the Industry

- Provides an inexpensive intervention that retards or eliminates pathogens on RTE pre-cooked sausages
- Utilizes antimicrobial ingredients that have GRAS status by the FDA
- Reduces the risk of food-borne disease and provides safe products to consumers

# Acknowledgement

This research was funded through an American Meat Institute Foundation grant in cooperation with the Texas Agricultural Experiment Station.