Controlling *Listeria monocytogenes* on Ready-to-Eat Meat and Poultry Products Using Food-Approved Antimicrobials Benzoate, Propionate, and Sorbate

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Summary: The primary objective of this study was to identify levels of potassium sorbate, sodium benzoate, and sodium propionate that prevent growth of *Listeria monocytogenes* on sliced, cooked, uncured turkey breast (<1% fat) and cured ham (5-7% fat manufactured with 156 ppm sodium nitrite) products. Data revealed that ≥ 0.2% Propionate or combinations of ≥0.1% Proprionate+0.1% Sorbate will prevent listerial growth in uncured turkey stored at 4°C for 12 weeks. When used in conjunction with nitrite, lower concentrations of antimycotics are needed to control pathogen growth. Growth of *L. monocytogenes* was prevented in ham stored at 4°C for 12 weeks when formulated with 0.1% Benzoate, ≥0.2% Propionate, 0.3% Sorbate, or combinations of 0.1% Proprionate+0.1% Sorbate. Comparison with previous research in bologna suggests that relatively low moisture (55%) and pH (6.1) will also reduce the minimum concentration of antimycotic required to prevent listerial growth during 3 month storage at 4°C. Sensory analysis for products using the highest concentration of single, effective antimicrobials reported consumers preferred the flavor of ham with 0.3% Propionate or 0.1% Benzoate compared with the 1.6% Lactate + 0.1% Diacetate treatment, and no significant difference compared with the Control without antimicrobials added. The addition of 0.3% Sorbate rated lowest in consumer taste preference. For deli-style turkey, consumers rated 0.3% Sorbate treatments equivalent (P>0.05) to Controls without antimicrobials (rating and overall preference). In contrast, consumers preferred (P<0.05) the Control turkey over the turkey containing sodium propionate. Flavor appears to be a major limitation of the use sodium propionate in turkey. A literature review described the safety and sensitivities exhibited by individuals to the various antimicrobials. This research verified that propionate, benzoate, and sorbate will enhance the safety of high-moisture, RTE cured and uncured meat and poultry products and that addition of these antimicrobials should have minimal negative impact on consumer taste preference if used at the lowest effective levels. Data can be used to petition FSIS for approval of propionate, benzoate, and sorbate for use in product formulations to control *L. monocytogenes*.

Specific Objectives of Research Proposal:

- Identify levels of sorbate, benzoate, and propionate, individually and in combination, which prevent growth of *Listeria monocytogenes* on sliced, uncured cooked turkey breast and cured cooked ham products stored at 4, 7, and 10°C for up to 3 months.
- Determine the effect that select antimicrobial treatments have on sensory qualities.
- Conduct a literature search on the effect that sorbate, benzoate, and propionate have on human health in the presence and absence of nitrite in ready-to-eat meats.

INTRODUCTION

As a means to prevent outbreaks of listeriosis, the USDA-FSIS 2003 *Final Rule to Control of Listeria monocytogenes in Ready-to-Eat Meat and Poultry Products* permits the use of growth inhibitors for *Listeria* on RTE meat and poultry products so there is no more than 1.0 log CFU/g increase during its shelf life (Anonymous, 2003). Currently, many manufacturers have incorporated lactate and diacetate into formulations of RTE products to prevent growth of *L. monocytogenes* in high-moisture, high-pH products and prevent additional outbreaks and recalls (Anonymous, 2001). However, while studies have verified that the addition of lactate and diacetate combinations inhibit growth of *L. monocytogenes* in cured meat and poultry products (with sodium nitrite), these organic acid salts are less effective in uncured products (Glass et al., 2002; Legan et al., 2004; Mdandi and Shelef, 2001; Seman et al., 2002). High levels of lactate and diacetate which prevent listerial growth in uncured meat and poultry products may have a negative affect on sensory attributes. Therefore, alternate antimicrobial ingredients are needed to provide safe and acceptable options for manufacturing without adversely affecting product quality, especially in nitrite-free products.

Three GRAS additives, sorbate, benzoate, and propionate, approved for use to control mold growth in a variety of food products other than meats (US FDA 2004), have also been shown to inhibit growth of several Gram-positive bacterial pathogens, such as *Clostridium botulinum, Staphylococcus aureus,* and *Listeria monocytogenes* in media, as well as in and on meat systems (El-Shenawy and Marth, 1988; Islam et al., 2002a, 2002b; Samelis et al., 2001, Tompkin et al., 1974; Wederquist et al., 1994).

Preliminary research in our laboratory demonstrated that *L. monocytogenes* will not grow in wiener or turkey slurries supplemented with 0.25% potassium sorbate, propionic acid, or benzoic acid and stored at 4 or 10°C for up to 4 weeks (Glass et al., 2004). Additional experiments revealed that beef-pork bologna with nitrite that incorporated combinations of Benzoate+Propionate or Benzoate+Sorbate (total 0.1%; 0.05% of each compound, w/w) into the formulation will not support growth of *L. monocytogenes* on bologna stored 12 weeks at 4°C, compared with a >3.5-log increase in listerial populations in the control bologna without antimicrobials (Preston et al., 2005). Low levels of antimycotic agents were less effective in uncured turkey products, but they still slowed listerial growth. When uncured turkey was stored at 4°C for four weeks, populations of *L. monocytogenes* increased 2.5 and 4.5 log cfu/package in the Benzoate+Propionate and Benzoate+Sorbate treatments, respectively, compared with a 6.5 log cfu/pkg increase for the turkey control without antimicrobials.

While these antimycotic agents are not yet approved in the U.S. for use within formulations of processed meats, confirming their efficacy and safety in a variety of products will be useful in a successful petition for regulatory approval. This study was designed to identify the minimum levels of sorbate, benzoate, and propionate, that prevent growth of *Listeria monocytogenes* on sliced, uncured cooked turkey breast (<1% fat) and cured cooked ham (5-7% fat manufactured with 156 ppm sodium nitrite) products stored at 4, 7, and 10°C for up to 3 months, and to determine the effect that ingredients have on consumer taste preference.

MATERIALS AND METHODS

Production of ready-to-eat turkey and ham: Sixteen test formulations plus control formulations without antimicrobials were manufactured for each product type by commercial producers to provide the formulations listed on Table 1.

Ingredient statement for Control Turkey included: Turkey breast, water, 2% or less of modified food starch, salt, dextrose, carrageenan, sodium phosphate, turkey flavor (maltodextrin, salt, flavor). Ingredient statement for Control Ham included: Ham cured with water, salt, less than 2% dextrose, sodium phosphate, sodium erythorbate, sodium nitrite. Sodium lactate, sodium diacetate, sodium propionate, potassium sorbate, and sodium benzoate were added as defined in the experimental design (Table 1).

Cooked, uncured turkey breast and cured smoked composite ham were produced under Good Manufacturing Practices in USDA-inspected commercial facilities according to industry standard practices, stuffed into casings, and cooked to the desired endpoint temperature (>71.1°C, 160°F for ham and >73.9°C, 165°F for turkey). Cooked, chilled products were sliced on a commercial slicer, packaged (vacuum-package for turkey; nitrogen flush for ham) and stored at <4°C through transport to the Food Research Institute, UW-Madison, for inoculation and testing. The study was replicated twice. Replicate Control treatments were run for each inoculation day.

Sensory analysis: Consumer taste preference panels for ham and turkey were completed at the UW-Madison campus by the UW Department of Food Science Sensory Laboratory, Babcock Hall, and by the Meat Science and Muscle Biology Lab (ASTM, 1988; Berry et al., 1983), respectively. Freshly prepared products were sent directly from the manufacturer to the Meat Science and Muscle Biology Laboratory and used within 4 weeks after manufacture. For both product types, consumer preference was compared (pair-wise comparison) for Control without antimicrobials, with 0.3% Sorbate, and with 0.3% Propionate. In addition, ham treatments containing 0.1% Benzoate and 1.6% Lactate+0.1% Diacetate were also evaluated. The ballot used for consumer preference evaluation contained a structured 7-point hedonic scale (Amerine et al., 1965). The sensory anchors used were “dislike very much (1)” to “like very much (7). “Two hundred fifteen and 40 consumers were surveyed for the ham and turkey treatments, respectively. For the overall preference attribute, statistical analyses provided the mean score for each sample, the F-value for all samples, and the least significant difference (LSD) for making sample comparisons (significance level of $P < 0.05$).

Proximate and chemical analysis:
Moisture (5 h, 100°C, vacuum oven method, 950.46; AOAC, 2000), pH (1:10 dilution for 10 g homogenized portion, Accumet Basic pH meter and Orion 8104 combination electrode), NaCl (measured as % Cl^-, $AgNO_3$ potentiometric titration, Brinkman Metrohm autotitrator), nitrite (Colorimetric Method, 973.31, AOAC, 2000), and water activity (Decagon AquaLab CX-2 water activity meter, Pullman, WA) were assayed by the Food Research Institute for triplicate samples for each formulation; values for protein and fat were provided by the manufacturers. Chemical analysis for distribution of sorbate, benzoate, and propionate in the product matrix were determined in treatments with the maximum concentrations of each antimycotic agent and in the Lactate-Diacetate control. For each trial, two samples were taken from different regions of the ham or turkey and assayed by commercial laboratories (gas chromatography method, 983.13, AOAC, 2000; concentrations of benzoic acid, sorbic acid, and propionic acid used to determine percentage of potassium sorbate, sodium benzoate, and sodium propionate).

Table 1. Treatments for cured ham and uncured turkey manufactured with various levels of Sorbate, Benzoate, and Propionate. [1]

Ham[2]	Potassium sorbate	Sodium benzoate	Sodium propionate	Sodium lactate [3]	Sodium diacetate	Total antimycotic
1	0.05					0.05
2		0.05				0.05
3			0.05			0.05
4	0.1					0.1
5		0.1				0.1
6			0.1			0.1
7	0.05	0.05				0.1
8	0.05		0.05			0.1
9		0.05	0.05			0.1
10	0.075		0.075			0.15
11	0.1		0.1			0.2
12	0.2					0.2
13			0.2			0.2
14	0.3					0.3
15			0.3			0.3
16				1.6	0.1	0
Control	No additional antimicrobials; positive growth control					0
Turkey	**Potassium sorbate**	**Sodium benzoate**	**Sodium propionate**	**Sodium lactate**	**Sodium diacetate**	**Total antimycotic**
1	0.1					0.1
2		0.1				0.1
3			0.1			0.1
4	0.05	0.05				0.1
5	0.05		0.05			0.1
6		0.05	0.05			0.1
7	0.15					0.15
8			0.15			0.15
9	0.075		0.075			0.15
10	0.1		0.1			0.2
11	0.2					0.2
12			0.2			0.2
13	0.15		0.15			0.3
14	0.3					0.3
15			0.3			0.3
16				3.2	0.2	0
Control	No additional antimicrobials; positive growth control					0

[1] All percentages are given on a finished weight basis without excessive moisture loss expect for nitrite, which is meat-block basis to be in compliance with federal regulations
[2] Ham formulated with 156 ppm sodium nitrite and 550 ppm sodium erythorbate on meat-block basis in compliance with federal regulations
[3] Calculated on an anhydrous basis

Preparation of inocula: *Listeria monocytogenes* strains Scott A (clinical isolate, serotype 4b), LM 101 (hard salami isolate, 4b), LM 108 (hard salami isolate, 1/2a), LM 310 (goat milk cheese isolate, 4), and V7 (raw milk isolate, 4), were grown individually in 10 ml Trypticase® soy broth (BBL, Cockeysville, MD) at 37°C for 18 to 20 h. Cells were harvested by centrifugation (2,500 x *g*, 20 min) and suspended in 4.5 ml 0.1% buffered peptone water (pH 7.2). Equivalent populations of each isolate were combined to provide a five-strain mixture of *L. monocytogenes* to yield target level of 5-log CFU per 100-g package. Populations of each strain and the mixture were verified by plating on Trypticase® soy agar (TSA) and Modified Oxford agar (MOX; Listeria Selective Agar base, Difco).

Inoculation and testing:
Slices were surface-inoculated with *L. monocytogenes* to provide approximately 5-log CFU per 100 g package (equivalent to 3-log CFU per ml rinse material), vacuum-packaged (Multivac AGW, Sepp Haggemuller KG, Wolfertschewenden, Germany) in gas-impermeable pouches (3 mil high barrier EVOH pouches, Deli 1 material, oxygen transmission 2.3 cm^3 per cm^2, 24 h at 24°C; water transmission 7.8 g per cm^2, 24 h at 37.8°C and 90% relative humidity, WinPak, Winnepeg, Manitoba, Canada), and stored at 4, 7, and 10°C for up to 12 weeks.

Triplicate inoculated and duplicate uninoculated samples for each treatment were assayed for changes in *L. monocytogenes* populations, and changes in lactic acid bacteria and pH, respectively, at 0-time, and at 2, 4, 6, 8, 10 and 12 weeks storage at 4°C, and at 4, 8, and 12 weeks only at 7 and 10°C. Bacterial populations were determined in rinse material obtained after adding 100 ml of sterile Butterfield phosphate buffer to each package and massaging the contents externally by hand for about 3 minutes (Glass and Doyle, 1989; Glass et al., 2002). *L. monocytogenes* was enumerated by surface plating serial (1:10) dilutions of rinse material on MOX. Select colonies were confirmed as *L. monocytogenes* by Gram-stain, tumbling motility, CAMP test, hemolysis on Trypticase soy agar with sheep blood, and biochemical analysis using MICRO-ID® *Listeria* (Remel, Lenexa, KS). The pH was measured by removing representative 10 g of the uninoculated samples and homogenizing with 90 ml hot deionized water using a Stomacher. Homogenized sample was allowed to cool to room temperature and the pH measured on the slurry. Changes in populations of lactic acid bacteria were assayed for the remaining uninoculated sample by plating rinse material on plate count agar with bromcresol purple (25°C, 48-72 h). Testing of a treatment was discontinued if listerial growth (2-log increase) was confirmed for packages tested for two consecutive sampling intervals.

RESULTS AND DISCUSSION

Proximate Analyses: Proximate analyses demonstrate variability that can be expected in normal commercial production (Table 2). Average moisture (75.04±1.08%) and pH (6.42±0.1) values for turkey were typically greater than that found in ham (73.65±0.27% moisture and pH 6.39±0.02), and salt values were typically lower in turkey (1.71±0.20%) compared with ham (2.59±0.02%). However, no consistent correlation was found between proximate analysis and microbial growth for the treatments tested (data not shown).

Table 2. Proximate analysis of turkey and ham.[4]

	Turkey	*Ham*
Moisture %	75.04±1.08 (Range 71.9-77.1)	73.65±0.27 (Range 71.4-79.9)
NaCl %	1.71±0.20 (Range 1.14-2.01)	2.59±0.02 (Range 2.24-2.90)
pH	6.42±.01	6.39±0.02
Water Activity	0.972±0.001	0.967±0.000
Nitrite (ppm)	Not added	34.6±13.8
Protein %[5]	17.9	17.9
Fat %	0.80	5.35

Levels of benzoate and sorbate were within expected target range for both the turkey and ham products, with little variation found between samples taken from different regions of the manufacturer's package or between samples from the two trials (Table 3). In contrast, propionate levels reported by a commercial laboratory using gas chromatography method yield significantly lower than expected results. Given the consistent inhibition of *L. monocytogenes* in the treatments with 0.3% sodium propionate, reported results are considered invalid and are either due to the commercial lab error or decomposition of propionic acid during processing or storage.

Table 3. Analysis of antimycotic agents for select formulations[6], [7]

Formulation	*Sodium Benzoate %*	*Potassium Sorbate %*	*Sodium Propionate %*
Ham 5[8]	0.11±0.01		
Ham 14[9]		0.31±0.03	
Ham 15[10]			<0.0060
Ham 16[11]	<0.0045	<0.0040	<0.0060
Turkey 2[8]	0.11±0.01		
Turkey 14[9]		0.29±0.07	
Turkey 15[10]			0.10±0.02
Turkey 16[11]	<0.0045	<0.0040	<0.0060

[4] Results are an average ± standard deviation for analysis of triplicate samples for each formulation
[5] Protein and fat levels reported by manufacturer

[6] Gas chromatography method, 983.13, AOAC, 2000; measured as acid form and converted to a percentage basis of salt form. Benzoate and Sorbate analyzed by R-Tech Laboratories, Arden Hills, MN; Propionate analyzed by Covance Laboratories, Madison, WI.
[7] Average for four samples ± standard deviation.
[8] Target 0.1% sodium benzoate
[9] Target 0.3% potassium sorbate
[10] Target 0.3% sodium propionate
[11] No antimycotic agent added, Lactate-Diacetate control

***Control of L. monocytogenes*:** Results from this study confirmed that antimycotic agents control growth of *L. monocytogenes* on the surface of high-moisture, high-pH processed meat and poultry products when used at levels which are deemed safe and acceptable for other food products.

When used in conjunction with nitrite in ham, low concentrations of antimycotics are needed to inhibit pathogen growth. Growth of *L. monocytogenes* was consistently prevented in cured ham stored at 4°C for 12 weeks when formulated with 0.1% Benzoate, ≥0.2% Propionate, 0.3% Sorbate, or combinations of 0.1% Propionate+0.1% Sorbate or 1.6% Lactate + 0.1% Diacetate (Figure 1). Other treatment, including combinations of ≥0.1% total antimycotic agents, 0.1% Propionate, and 0.1 and 0.2 % Sorbate, delayed growth until 6 to 10 weeks, but permitted >1 log growth for sporadic samples in at least one trial at or prior to 12 weeks, even though the overall average for both trials appeared to have <1 log increase. The Control treatment without antimicrobials and treatments with only 0.05% of any individual antimycotic agents supported 0.6 to 1.1-log growth at the 2-week sampling interval.

Antimicrobial treatments that prevented growth at 4°C were less effective when the ham was stored higher temperatures. Products supplemented with 0.1% Sorbate+0.1% Propionate, ≥0.2% Propionate, ≥0.2% Sorbate, and 1.6% Lactate + 0.1% Diacetate prevented listerial growth for 4 weeks at 7°C, but all formulations supported >1-log increase at 8-weeks (data not shown). For products stored at 10°C, only 0.3% Propionate delayed pathogen growth for 4 weeks (average 1 log increase), whereas all the other treatments supported a 2-4 log increase in the same period (data not shown).

For uncured turkey, products supplemented with ≥ 0.2% Propionate, combinations of ≥0.1% proprionate+0.1% Sorbate, and combination of 3.2% Lactate+0.2% Diacetate consistently inhibited growth of *L. monocytogenes* (<1-log increase) when stored at 4°C for 12 weeks (Figure 2). Combination of 0.075% Propionate+0.075% Sorbate prevented pathogen growth in samples from only one replicate for the duration of the study. Surprisingly, turkey with 0.3% Sorbate supported a 1-log increase starting at 8 weeks at 4°C, whereas 0.2% Sorbate delayed growth for 10 weeks. Chemical analysis of the Turkey with 0.3% Sorbate did not suggest insufficient addition or uneven distribution of sorbate within the product matrix. Additional study may be required before discounting the use of 0.3% Sorbate as an antilisterial agent in high-moisture, uncured products.

As observed for the ham treatments, inhibition was less pronounced when formulation was stored at abuse temperatures. Products formulated with 0.15% Sorbate+0.15% Propionate, 0.2% Propionate, 0.3% Propionate, and 3.2% Lactate+0.2% Diacetate delayed listerial growth 4 weeks (<1-log increase) when stored at 7°C, but supported significant growth (2-log increase) at 8 weeks. None of the treatments delayed listerial growth when stored at 10°C, with 1.5 to 5.5-log increase within 4 weeks.

Growth of spoilage lactic acid bacteria was inconsistent among samples within and between treatments. While most samples assayed contained populations fewer than detectable limit by direct plating (<1-log CFU/ml rinse), populations ranged to >8 log CFU/ml rinse (data not shown). The pH of the uninoculated samples tested did not decrease appreciably for any sample, with <0.15 pH unit decrease observed throughout the testing interval. No

correlation between growth of spoilage microflora and pH reduction was observed for the uninoculated samples assayed. Therefore, there is no clear evidence that the antimycotic treatments will also inhibit spoilage microflora and mask spoilage.

These results support previous studies indicating that antimycotic agents inhibit growth of *L. monocytogenes* on RTE meats, but demonstrate that they are more effective when used in combination with nitrite in cured meat products than in uncured products. Of the three antimycotic agents used, sorbate appears to have the least inhibitory effect on *L. monocytogenes* growth at any given concentration.

In addition to the presence of nitrite, product moisture and pH also appear to have a significant effect on efficacy of low concentrations of antimicrobials. Previous research (see Final Report to AMIF, Glass et al., Antimicrobial Combinations in RTE Meats, June 10, 2005) revealed that combinations of 0.05% Benzoate+0.05% Sorbate or 0.05% Benzoate+0.05% Propionate prevented growth of *L. monocytogenes* in cured beef-pork bologna (~57% moisture, pH 6.1), but the same concentrations have less inhibitory effect in the cured ham (~73-75% moisture, pH 6.3) or uncured turkey (~75-76% moisture, pH 6.3-6.4) products evaluated in this study.

Sensory Analysis: Sensory analysis for products using the highest concentration of effective antimicrobials reported consumers preferred the flavor of ham with 0.3% Propionate or 0.1% Benzoate compared with the Lactate-Diacetate treatment, and no significant difference compared with the Control ham without antimicrobials added (Table 4). The addition of 0.3% Sorbate rated lowest in consumer preference (see Appendix A for details on ham sensory analysis). Consumers rated deli-style turkey containing potassium sorbate equivalent ($P>0.05$) to Controls turkey without antimicrobials (rating and overall preference; Table 5). In contrast, consumers did not prefer turkey containing sodium propionate over Control turkey ($P<0.05$). However, when a direct comparison of potassium sorbate was made to sodium propionate, no preference was noted ($P>0.05$).

Table 4. Consumer evaluation of smoked ham containing sodium lactate+sodium diacetate blend (1.6+0.1%), sodium benzoate (0.1%) potassium sorbate (0.3%) or sodium propionate (0.3%).

	Sensory Response[1]	
Treatment comparison	**Preference Rating**	**Overall preference**
Control	5.8[A]	0.63
Lactate-Diacetate (1.6+0.1%)	5.5[B]	0.37
Control	6.0[A]	0.49
Sodium Benzoate (0.1%)	6.0[A]	0.51
Control	6.1[A]	0.56
Potassium Sorbate (0.3%)	5.9[B]	0.44
Control	6.0[A]	0.49
Sodium Propionate (0.3%)	6.0[A]	0.51
Lactate-Diacetate (1.6+0.1%)	5.9[A]	0.35
Sodium Benzoate (0.1%)	6.2[B]	0.65
Lactate-Diacetate (1.6+0.1%)	5.9[A]	0.56
Potassium Sorbate (0.3%)	5.8[A]	0.44
Lactate-Diacetate (1.6+0.1%)	5.9[A]	0.39
Sodium Propionate (0.3%)	6.2[B]	0.61

[1]Consumer response: Preference rating (1=dislike very much to 7= like very much; 4=neither like or dislike). For overall preference, consumers responses for preferred were entered as a 1 and not preferred as 0. More than 160 consumers per treatment comparison were used.
[A, B] Means within a comparison and sensory response with unlike superscript letters are different ($P<0.05$)

Table 5. Consumer evaluation of Deli-Style Turkey containing potassium sorbate (0.3%) or sodium propionate (0.3%).

	Sensory Response[1]	
Treatment comparison	**Preference Rating**	**Overall preference**
Control	5.6[A]	0.39[A]
Potassium Sorbate (0.3%)	6.0[A]	0.59[A]
Std. error	0.28	0.11
Control	6.1[A]	0.69[A]
Sodium Propionate (0.3%)	5.3[B]	0.31[B]
Std. error	0.26	0.11
Potassium Sorbate (0.3%)	5.5[A]	0.54[A]
Sodium Propionate (0.3%)	5.5[A]	0.46[A]
Std. error	0.28	0.11

[1]Consumer response: Preference rating (1=dislike very much to 7= like very much;4=neither like or dislike). For overall preference, consumers responses for preferred were entered as a 1 and not preferred as 0. Forty different consumers per treatment comparison were used.
[A, B] Means within a comparison and sensory response with unlike superscript letters are different ($P<0.05$)

Literature Review:
A literature review describing the safety and sensitivities exhibited by individuals to the various antimicrobials is detailed in Appendix B. Overall, data indicate that these compounds are of low toxicity with little or no genotoxic or carcinogenic potential.

CONCLUSIONS

This research verifies that propionate, benzoate, and sorbate will enhance the safety of high-moisture, RTE cured and uncured meat and poultry products and that addition of these antimicrobials will have little negative impact on consumer taste preference. Data can be used to petition FSIS for approval of propionate, benzoate, and sorbate for use in product formulations to control *L. monocytogenes*.

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Figure 1. Changes in populations of *L. monocytogenes* on cured ham prepared with various levels of sodium benzoate, sodium propionate, or potassium sorbate, and stored at 4°C for 12 weeks (averages for duplicate trials; standard deviations not shown).

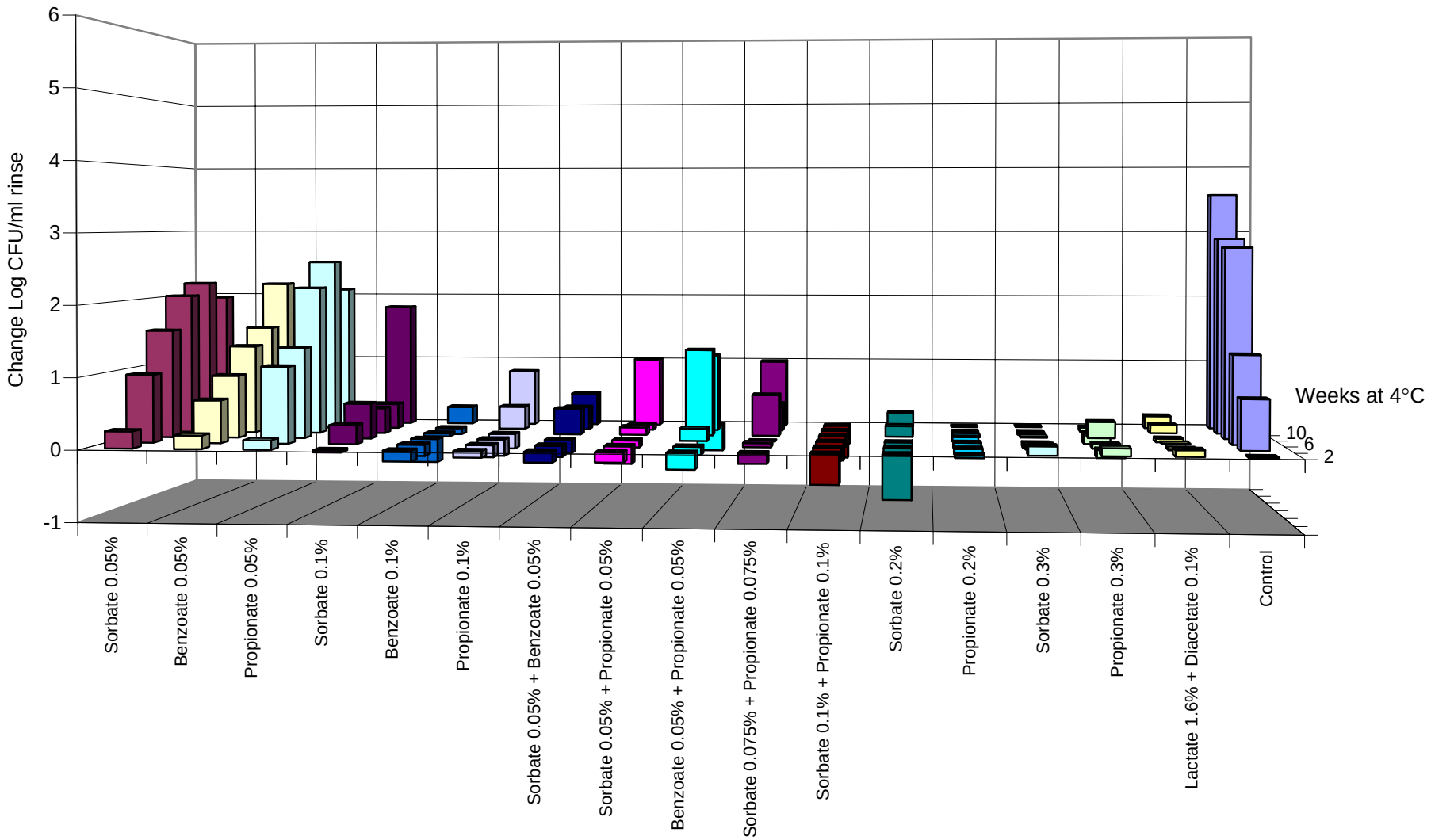
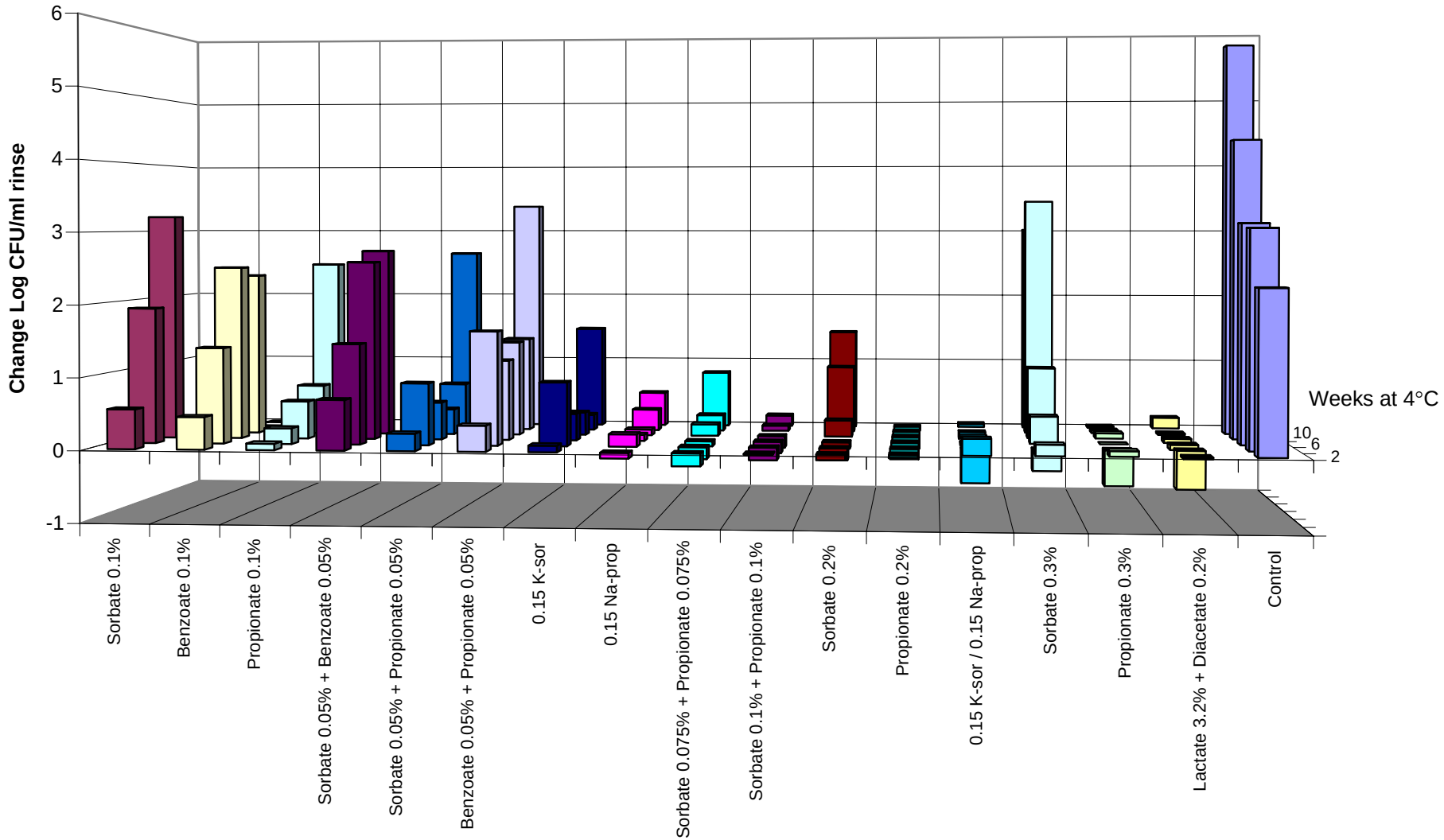


Figure 2. Changes in populations of *L. monocytogenes* on uncured turkey prepared with various levels of sodium benzoate, sodium propionate, or potassium sorbate, and stored at 4°C for 12 weeks (averages for duplicate trials; standard deviations not shown).



APPENDIX A

Report on the

Consumer Preference Evaluations of Sliced Hams

for

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by the

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Date: June 28, 2006 revised
Project No: #337
Sensory Laboratory Manager:
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Introduction

The Sensory Analysis Laboratory, Department of Food Science, University of Wisconsin-Madison, was contacted by Dr. James R. Claus, UW-Madison, Meat Science and Muscle Biology Laboratory, Madison, WI., regarding consumer preference evaluations of sliced hams. The objective of the evaluations was to determine consumer preferences for four or three pairs of sliced ham samples in each of two series of testing, respectively. Standard sensory evaluation conditions were slightly modified with agreement between Mr. Sungjoon Jang (UW-Madison, Sensory Laboratory) and Dr. James Claus (UW-Madison, Meat Science & Muscle Biology) to meet the specific requests by Dr. Claus for his experimental purposes. The agreements included that each session was to be composed of four pairs of sliced hams for series I or three pairs of sliced hams for series II, the total numbers of panelists at a session to be served each pair of samples would be 50 or 66 for each series (I and II) of evaluation, after the number of panelist evaluating the first pair of samples was met, the second and third, or fourth pair of sliced hams would be consecutively evaluated. In a session, each panelist would evaluate only one of the three or four pairs, and each session should be completed within the testing for a day. This report summarizes the consumer preference evaluations that were conducted on February 7, 9, 14, and 20, 2006 for the first series and on February 23, 27, and 28, 2006 for the second series. Tabulated panel results were provided to Dr. Claus earlier as they were accumulated (March 15, 2006).

Materials and Methods

Samples

Sample lots of sliced hams were delivered to the Sensory Analysis Laboratory by a graduate student of Dr. Claus on each morning of sensory evaluation sessions. Sliced hams for each sample lot were individually sealed, and were designated by Dr. Claus as "Control", "LD", "AM1", "AM2", and "AM3" for first series evaluated on February 7, 9, 14, 20, and as "LD", "AM1", "AM2", and "AM3" for second series evaluated on February 23, 27, and 28, 2006. The Control did not have a test antimicrobial added. The test antimicrobials were: 0.1% sodium benzoate (AM1), 0.3% potassium sorbate (AM2), and 0.3% sodium propionate (AM3). The samples among lots were paired appropriately for each evaluation. Samples were in an excellent cold condition upon receipt. Sample pairs for first series of consumer preference evaluation were "Control vs LD", "Control vs AM1", "Control vs AM2", and "Control vs AM3", and each pair was evaluated (maximum 50 judgments each) on February 7, 9, 14, and 20, 2006. Sample pairs for second series of consumer preference evaluation were "LD vs AM1", "LD vs AM2", and "LD vs AM3", and each pair was evaluated (maximum 66 judgment each) on February 23, 27, and 28, 2006.

Preparation of Samples for Serving

Samples of sliced ham were served after further preparation to cut each slice to four pieces as requested by Dr. James Claus (UW-Madison, Meat Science & Muscle Biology). Each refrigerated sliced ham (oval-shape) was center-cut both vertically and horizontally into quadrants to obtain four similar slice-pieces (ca 5.0 cm X 6.0 cm, one-quarter of original oval shape; ca 0.1 cm thickness). Sample preparation was conducted ca 20 min before each test. The slice samples were placed in the original plastic zip-closure bags, and stored in a household refrigerator (ca 4 °C) until served.

Taste Panel Testing Conditions

Consumer preference panels were held in the Consumer Testing Laboratory adjacent to the Dairy Products Salesroom located in Babcock Hall on the University of Wisconsin-Madison campus. For the first series of evaluations, unscreened participants (total of 215, 202, 217, and 164, respectively) evaluated each sample pair ("Control vs LD", "Control vs AM1", "Control vs AM2", and "Control vs AM3", respectively) during four consecutive sessions on February 7, 9, 14, and 20, 2006. For the second series of evaluations, unscreened participants (total of 197, 193, and 189, respectively) evaluated each sample pair ("LD vs AM1", "LD vs AM2", and "LD vs AM3", respectively) during three consecutive sessions on February 23, 27, and 28, 2006. At the time of each panel evaluation, samples were portioned by placing two pieces (ca 5.0 cm X 6.0 cm one-quarter of an oval shape; ca 0.1 cm thickness) of each sliced ham into separate 2 oz plastic portion cups which were each coded with a three-digit random number for identification of the sample. The sliced ham samples were placed on a serving tray as they were removed from the refrigerator immediately after each panelist was seated in a testing booth. Each panelist received a ballot (Appendixes I, II, III, and IV for first series and V, VI, and VII for second series) for recording responses, a tray containing two pieces of sliced ham samples, and two plastic forks. Cold drink cups and napkins were placed in a corner of each sensory booth for panelist free-choice use.

Ballot

The ballot for consumer preference evaluation of sliced ham samples was specially designed for this project, and it was finalized in collaboration with Dr. James Claus. The top part of the consumer preference ballot contained a structured 7-point hedonic scale (Amerine et al., 1965). After tasting both samples, panelists were also asked to answer two exit questions which were requested by Dr. Claus. Question 1 was “Which of the two samples you tasted do you prefer?” Question 2 was “Why do you prefer the sample checked in question 1 better than the other sample?” The ballot also contained an “Other comments” section to collect voluntary comments by panelists. To minimize the sample presentation sequence effects, the ballots for each pair samples were constructed with two permutations of two code numbers. Each group contained an equal numbers of ballots, and each ballot contained instructions for the tasting sequence of the samples. Copies of the ballots used in the test are presented in Appendixes I, II, III, IV, V, VI, and VII.

Statistical Analysis

For calculation of overall preference scores, a code value of 1.0 was assigned to the category of “dislike very much” and a value of 7.0 to the category of “like very much’ with appropriate whole-number code values assigned to the intermediate categories.

The coded values from the panel sessions were subjected to analysis of variance appropriate for a randomized complete block design (Steel and Torrie, 1960). For the overall preference attribute, statistical analyses provided the mean score for each sample, the F-value for all samples, and the least significant difference (LSD) for making sample comparisons.

If the F-value is statistically significant, then the null hypothesis of no difference among population means is rejected. A significant F-value implies that the evaluation provided evidence of real differences among treatment means that would not be expected to occur by chance more than 5 % of the time. Because the F-value does not indicate which of theses differences can be considered significant, the LSD value computed for a 5 % level of significance is used for comparisons of paired means. If the difference between any pair of treatment mean scores within a sensory attribute exceeds the LSD, then that difference is considered statistically significant. If the F-value for treatments is not significant, then the evidence is not strong enough to indicate a real difference among treatment means at a 5 % level of significance, and specific treatment comparisons cannot be made.

Results and Discussion

Results and discussion of the consumer preference evaluations of sliced ham samples are organized into a sequence providing the combined results for each pair of samples evaluated on four or three consecutive days in series I and II, respectively. The order of presentation is Sample pair I: Control vs LD, Sample pair II: Control vs AM1, Sample pair III: Control vs AM2, and Sample pair IV: Control vs AM3 for first series of evaluations and Sample pair V: LD vs AM1, Sample pair VI: LD vs AM2, and Sample pair VII: LD vs AM3 for second series of evaluations.

Sample Pair I: Control vs LD Sliced Ham (Evaluated on February 7, 9, 14, and 20, 2006; maximum 50 observations for a pair on each day)

Results of the consumer preference evaluation of the Control and LD sliced ham samples are summarized in Table 1. The results show that the preference mean score of the Control sliced ham sample was statistically significantly higher at the 5% level than that for the LD sliced ham sample (mean preference scores were 5.83 and 5.47, respectively).

The panelist response distribution data (Table 1) revealed that the numbers of panelist response for the Control sliced ham sample (72) was much greater than for the LD sliced ham (52) in the top preference rating of "like very much" category. A relatively larger number of panelists also rated the LD sliced ham sample (85) in the "like moderately" category compared to the LD sliced ham sample (77). In contrast, larger numbers of panelists rated the LD sliced ham sample in the "like slightly" compared to responses for the Control sliced ham sample (47 vs 28; Table 1).

A summary of panelist responses to exit question #1 (“Which of the two samples you tasted do you prefer?”) is shown in Table 2. One hundred thirty two (132) out of 208 panelists expressed their preferences for the Control sliced ham sample than those for the LD sliced ham sample (76).

The responses to question #2 (“why do you prefer the sample checked in question #1...?”) were summarized into two groups (i.e., those who preferred the Control sliced ham sample, and those who preferred the LD sliced ham sample). The responses from the panelists who preferred the Control sample are listed in Table 3. The responses from the panelists who preferred the LD sample are listed in Table 4.

Of the comments from panelists who preferred the Control sliced ham sample the most (Table 3), and who commented, a large number of panelist responses indicated that the Control sample tasted “less salty” (52), "better” (24), had "better flavor” (20), "better texture" (8), and tasted "sweeter" (8). Some panelists commented that the Control sliced ham sample tasted "saltier" (4), has "more smoked flavor” (4), and tasted "richer" (2).

The major comments for panelists who preferred the LD sliced ham sample the most (Table 4) were that the LD sample had "better flavor" (24), “better texture” (11), tasted "better" (10), "saltier" (10), and "less salty" (8). Some comments from panelists included that the LD sliced ham

sample tasted "sweeter" (6), had "more smoked flavor" (6), tasted "richer" (5), "less tart" (1), and had " less smoked flavor" (1).

Panelist optional voluntary comments for this pair of samples are summarized in Table 5. Some comments indicated that the Control sliced ham sample tasted "too bland" (2), had "bad texture" (1), tasted "too sweet" (1), and had "off-flavor" (1). On the other hand, a notable number of panelists commented that the LD sliced ham sample had "bad aftertaste" (5), tasted "bland" (3), and "too salty" (2). Several other minor comments for the LD sample are listed in Table 5.

Sample Pair II: Control vs AM1 0.1% Sodium benzoate Sliced Ham (Evaluated on February 7, 9, 14, and 20, 2006; maximum 50 observations for a pair on each day)

Results of the consumer preference evaluation of the Control and AM1 sliced ham samples are summarized in Table 6. The results show that there was no statistically significant preference for either the Control sample or the AM1 sample at the 5% level, and the mean preference scores were 6.03 and 6.02, respectively. The panelist response distribution data (Table 6) show that similar response distribution patterns were obtained for most of the preference rating categories for both the Control and the AM1 sliced ham samples.

The summary of panelist responses to exit question #1 ("Which of the two samples you tasted do you prefer?") is shown in Table 7. Of the 197 panelists, ninety seven (97) panelists preferred the Control sliced ham sample, and one hundred (100) preferred the AM1 sample. The responses to question #2 ("why do you prefer the sample checked in question #1...?") are summarized in Tables 8 and 9 for the Control and the AM1 sliced ham samples, respectively. The responses from panelists who preferred the Control sliced ham sample (Table 8) and commented, showed that the Control sample had "better" flavor (26), tasted "less salty" (21), had "better texture" (15), and tasted "better" (8). Some minor responses from panelists who preferred the Control sample the most included that the Control sliced ham sample tasted "less fatty" (3), had "more smoked flavor" (3), "better aftertaste" (2), tasted "mild" (1), "sweeter" (1), had "less smoked flavor" (1), and "more moisture" (1) than those for the AM1 sliced ham sample (Table 8).

In comparison, a number of panelists preferring the AM1sample (Table 9) responded that the AM1 sliced ham sample tasted "less salty" (33), had "better flavor" (29), tasted "better" (15), and tasted "sweeter" (9) than did those for the Control sample. Several comments from panelists who preferred the AM1 sliced ham sample the most included that the AM1 sample tasted "saltier" (7), had "better texture" (7), "more smoked flavor" (7), tasted "richer" (3), "milder" (2), "spicier" (1), "less sweet" (1), and had more moisture" (1).

General voluntary comments for this pair of samples (Control vs AM1) are summarized in Table 10. A few panelists commented that the Control sample had "bad aftertaste" (3), "artificial taste" (1), "a tough casing" (1), had "too strong pork smell" (1), tasted "raw meat-like" (1), and "not fresh" (1). Only two panelists commented that the AM1 sliced ham sample had "off-flavor" (1), and tasted "too salty" (1).

Sample Pair III: Control vs AM2 0.3% Potassium Sorbate Sliced Ham (Evaluated on February 7, 9, 14, and 20, 2006; maximum 50 observations for a pair on each day)

Results of the consumer preference evaluation of the Control and the AM2 sliced ham samples are summarized in Table 11. The results indicated that the Control sliced ham sample was statistically significantly more preferred than the AM2 sliced ham sample at the 5% level, and the mean preference scores were 6.06 and 5.88, respectively.

The panelist response distribution data (Table 11) revealed that seventy four (74) out of 217 panelists scored the Control sliced ham sample in the category of "like very much" compared to sixty four (64) of 217 panelists for the AM2 sliced ham sample. The Control sliced ham sample received three (3) more responses from panelists for “like moderately” category than did the AM2 sample (95 and 92, respectively). In contrast, the Control sample received fewer panelist responses for the "like slightly" category than the AM2 sliced ham sample (38 and 46, respectively). Somewhat larger numbers of panelists rated the AM2 sliced ham sample in the lower preference categories compared to parallel ratings for the Control sliced ham sample (Table 11).

The summary of panelist responses to exit question #1 (“Which of the two samples you tasted do you prefer?”) is shown in Table 12. One hundred nineteen (119) out of 213 panelists expressed their preferences for the Control sliced ham sample than those for the AM2 sliced ham sample (94).

Of the comments from panelists who preferred the Control sliced ham sample the most (Table 13), and who commented, a large number of panelist responses indicated that the Control sample in Pair III had "better flavor” (25), tasted "saltier” (18), "better” (17), "less salty" (15), and had "more flavor" (13). Several additional comments for the Control sample also were given, and included that the Control sliced ham sample had "better texture" (7), tasted "sweeter" (5), had "less smoked flavor" (3), "better aftertaste" (2), tasted "juicer" (2), and "less sweet" (2).

The comments from panelists who preferred the AM2 sliced ham sample the most are summarized in Table 14, and relatively fewer responses were obtained from panelists for question #2, "why do you prefer the sample...?" than those for the Control sliced sample. The comments from panelists who preferred the AM2 sliced ham sample the most indicated that the AM2 sample (Table 14) tasted "less salty" (19), "sweeter" (14), had "more flavor" (11), "better texture" (10), tasted "better" (9), "saltier" (8), and had "more smoked flavor" (8). Several comments also indicated that the AM2 sliced ham sample had "less aftertaste" (4), tasted "juicier" (2), "less buttery" (1), and "milder" (1).

General voluntary comments for this session (Sample Pair III) are summarized in Table 15. Some panelists commented that the Control sliced ham tasted "too salty" (4), had "rubbery texture" (2), tasted "too sweet" (1), had "bad aftertaste" (1), and "too much smoked flavor" (1). The voluntary comments from panelists for the AM2 slice ham sample included that the AM2 sample had " off flavor" (7), tasted "too sweet" (4), "too salty" (3), had "slimy texture" (2), "slightly more smoked flavor" (1), was "tender" (1), and tasted "bland" (1).

Sample Pair IV: Control vs AM3 0.3% Sodium Propionate Sliced Ham (Evaluated on February 7, 9, 14, and 20, 2006; maximum 50 observations for a pair on each day)

Results of the consumer preference evaluation of the Control and AM3 sliced ham samples are summarized in Table 16. The results show that there was no statistically significant preference for either the Control or the AM3 sliced ham sample at the 5% level, and the mean preference scores were 5.99 and 6.02, respectively.

The Control sliced ham sample received four (4) more panelist responses for both “like moderately” and "like slightly" categories than did the AM3 sample (Table 16). However, the Control sliced ham sample received fewer panelist responses for "like moderately" than did the AM3 sample (65 and 75, respectively).

The summary of panelist responses to exit questions #1 (“Which of the two samples you tasted do you prefer?) is shown in Table 17. Panelists gave merely equal preference responses to the Control and AM3 sliced ham samples for exit question #1. Seventy eight (78) panelists stated they

preferred the Control sliced ham sample compared to eighty one (81) who expressed their preference for the AM3 sample.

The responses to question #2 (“why do you prefer the sample checked in question #1...?”) were summarized into two groups (i.e., those who preferred the Control sliced ham sample, and those who preferred the AM3 sample). The responses from the panelists who preferred the Control sliced ham sample are listed in Table 18. The responses from the panelists who preferred the AM3 sample are listed in Table 19.

Of the comments from panelists who preferred the Control sliced ham sample the most (Table 18), and who commented, a notable number of panelist responses indicated that the Control sample tasted “less salty” (24), tastes "better” (15), and had "better flavor” (15). Some comments for panelists who preferred the Control sliced ham sample the most included that the Control sample had "more smoked flavor" (6), tasted "saltier" (5), "sweeter" (4), had "more moisture" (4), and tasted "less fatty" (3).

The major comment categories for panelists who preferred the AM3 sliced ham sample the most (Table 19) included that the AM3 sample had "better flavor" (23), tasted “less salty" (18), "better” (12), "saltier” (9), and "sweeter" (8). Several comments also indicated that the AM3 sliced ham sample had "more smoked flavor" (6), "better texture" (6), tasted "milder" (3), had "better aftertaste" (1), "better appearance" (1), and "less smoked flavor" (1).

A few general voluntary comments for this pair of samples are summarized in Table 20. The comments for the Control sliced ham sample included that it tasted "greasy" (4), had "better aftertaste" (3), and tasted "bland" (2). Panelist comments for the AM3 sliced ham sample included that it tasted "bitter" (2), had "dry mouthfeel" (1), and "too strong aftertaste" (1).

Sample Pair V: LD vs AM1 0.1% Sodium benzoate Sliced Ham (Evaluated on February 23, 27, and 28, 2006; maximum 66 observations on each day)

Results of the consumer preference evaluation of the LD and AM1 sliced ham samples are summarized in Table 21. The results show that there was a statistically significant greater preference for the AM1 sliced ham sample than for the LD sample at the 5% level, and the mean preference scores for the AM1 and LD ham slice samples were 6.15 and 5.64, respectively.

A larger number of panelists expressed that they preferred the AM1 sliced ham sample more than the LD sample in "like very much" preference category (77 and 47, respectively). Correspondingly, the LD sliced ham sample received fifteen (15) more panelist responses for "like slightly" category than the AM1 sliced ham (41 and 26, respectively).

The summary of panelist responses to exit questions #1 (“Which of the two samples you tasted do you prefer?) is shown in Table 22. A larger number of panelists again expressed their preference for the AM1 sliced ham sample compared to the LD sliced ham sample (124 and 67 responses, respectively).

Responses to question #2 (“why do you prefer the sample checked in question #1...?”) are summarized into two groups, i.e., those who preferred the LD sliced ham sample, and those who preferred the AM1 sliced ham sample. The responses from the panelists who preferred the LD sample are listed in Table 23. The responses from the panelists who preferred the AM1 sample are listed in Table 24.

Of the comments from panelists who preferred the LD sliced ham sample the most, and who commented, a large number of panelist responses indicated that the LD sliced ham sample had “better flavor” (20), tasted "better” (10), "saltier” (10), "sweeter" (8), had "better texture" (6), tasted "milder" (5), and "less salty" (4).

The major comment categories for panelists who preferred the AM1 sliced ham sample the most (Table 24) included that the AM1 sample tasted “less salty" (49), had “better flavor” (34), tasted "better" (16), had "better texture" (13), and tasted "sweeter" (6). A few panelists also commented that the AM1 sliced ham tasted "saltier" (3), had "more smoked flavor" (3), and "better aftertaste" (3).

A summary of panelist voluntary comments for this pair of samples (LD vs AM1) is summarized in Table 25. The comments for the LD sliced ham sample included that the LD sample tasted "too salty" (11), "slightly bitter" (3), had "unclean off-flavor" (3), tasted "bland" (2), had "discolored edge" (1), and "soft/mushy texture" (1). Panelists also commented that the AM1 sliced ham sample tasted "too salty" (2), had "too chewy texture" (1), tasted "greasy" (1), and "slightly bland" (1).

Sample Pair VI: LD vs AM2 0.3% Potassium Sorbate Sliced Ham (Evaluated on February 23, 27, and 28, 2006; maximum 66 observations on each day)

Results of the consumer preference evaluation of the LD and AM2 sliced ham samples are summarized in Table 26. The results show that there was no statistically significant preference for either the LD or the AM2 sliced ham sample at the 5% level, and the mean preference scores were 5.90 and 5.82, respectively.

The panelist response distribution data (Table 26) revealed that the numbers of panelist responses showed parallel distributions of patterns in most of the preference rating categories between the LD and AM2 sliced ham samples. However, a slightly larger number of panelists rated the LD sample in both the "like very much" and "like slightly" categories compared to those responses for the AM2 sample (Table 26). In contrast, a slightly larger number of panelists rated the

AM2 sliced ham sample (75) in the "like moderately" category compared to responses for the LD sliced ham sample (67). In addition, a slightly larger number of panelists rated the AM2 sliced ham sample (10) in the "neither like nor dislike" category compared to responses for the LD sliced ham sample (5).

A summary of panelist responses to exit questions #1 ("Which of the two samples you tasted do you prefer?) is shown in Table 27. A relatively larger number of panelists expressed their preference for the LD sliced ham compared to those response for the AM2 sliced ham sample (Table 27).

The responses to question #2 ("why do you prefer the sample checked in question #1...?") were summarized into two groups (i.e., those who preferred the LD sliced ham sample, and those who preferred the AM2 sliced ham sample). The responses from the panelists who preferred the LD sliced ham sample are listed in Table 28. The responses from the panelists who preferred the AM2 sliced ham sample are listed in Table 29.

Of the comments from panelists who preferred the LD sliced ham sample (Table 28) the most, and who commented, a notable number of panelist responses indicated that the LD sample had "better flavor" (36), tasted "saltier" (18), "better" (14), had "better texture" (13), and tasted "less salty" (7). The major comment categories for panelists who preferred the AM2 sliced ham sample (Table 29) the most included that the AM2 sample tasted "less salty" (20), had "better flavor" (18), tasted "better" (12), "sweeter" (10), had "more smoked flavor" (6), and "better texture" (6).

Panelist voluntary comments for this pair (VI) of samples (LD vs AM2) are summarized in Table 30. The comments included that the LD sliced ham sample had "off-flavor" (5), "bad aftertaste" (3), tasted "too salty" (2), "too fatty" (1), and "bland" (1). Several panelists commented that the AM2 sliced ham sample had "bad aftertaste" (5), tasted "too salty" (4), "too fatty" (3), "too sweet" (1), "soapy" (1), and had "tough texture" (1).

Sample Pair VII: LD vs AM3 0.3% Sodium Propionate Sliced Ham (Evaluated on February 23, 27, and 28, 2006; maximum 66 observations on each day)

Results of the consumer preference evaluation of the LD and AM3 sliced ham samples are summarized in Table 31. The results show that the preference score of the AM3 sliced ham sample was statistically significantly higher at the 5% level than that for the LD sliced ham sample (mean preference scores were 6.17 and 5.86, respectively).

The panelist response distribution data (Table 31) revealed that the numbers of panelist responses for the AM3 sliced ham sample (87) was much greater than for the LD sliced ham (56) in the top preference rating of "like very much" category. In contrast, larger numbers of panelists rated the LD sliced ham sample in both the "like moderately" and "like slightly" preference categories compared to responses for the AM3 sliced ham sample (Table 31). A somewhat notable numbers of panelists rated the LD sliced ham sample in the "dislike slightly" category compared to parallel rating for the AM3 sliced ham sample (Table 31).

A summary of panelist responses to exit question #1 ("Which of the two samples you tasted do you prefer?") is shown in Table 32. One hundred fifteen (115) out of 188 panelists expressed their preferences for the AM3 sliced ham sample while 73 preferred the LD sliced ham sample.

The responses to question #2 ("why do you prefer the sample checked in question #1...?") were summarized into two groups (i.e., those who preferred the LD sliced ham sample, and those who preferred the AM3 sliced ham sample). The responses from the panelists who preferred the LD sample are listed in Table 33. The responses from the panelists who preferred the AM3 sample are listed in Table 34.

Of the comments from panelists who preferred the LD sliced ham sample the most (Table 33), and who commented, a notable number of panelist responses indicated that the LD sample had "better texture" (13), "better flavor" (12), tasted "better" (11), "less salty" (9), "saltier" (8), and had "more smoked flavor" (5). Several minor comments from panelists who preferred the LD sliced ham sample were also listed in Table 33.

Relatively larger numbers of panelists who preferred the AM3 sliced ham sample the most (Table 34) commented that the AM3 sample tasted "less salty" (36), had "better flavor" (28), tasted "better" (15), had "better texture" (14), tasted "saltier" (7), and had "more smoked flavor" (6). Some minor comments from panelists included that the AM3 sliced ham sample tasted "sweeter" (3), "juicier" (2), "less fatty" (2), "tangier" (2), had "less smoked flavor" (1), "better color" (1), and "less chewy" (1).

Panelist optional voluntary comments for this pair of samples (VII) are summarized in Table 35. Some comments indicated that the LD sliced ham sample tasted "too salty" (5), had "a little slimy texture" (3), "off-flavor" (2), and tasted "slightly bitter" (2). On the other hand, a few number of panelists commented that the AM3 sliced ham sample tasted "sour" (1), "too fatty" (1), and had "off-flavor" (1).

Summary

Results of consumer preference evaluations of sliced ham samples showed statistically significant differences between common Control sample and experimental sample (LD or AM2) in Pairs I and III, respectively, and also revealed that the common Control sample was statistically significantly more preferred than both the LD or AM2 sliced ham samples. Panelists perceived that the Control sample was notably or significantly less salty, better flavor, and better taste than the LD or AM2 sample in both Pairs I and II. For Sample Pair II: Control vs AM1 and Sample Pair IV: Control vs AM3 sliced ham samples, consumer preference evaluations did not show statistically significant preferences for either pair of samples. Greater numbers of panelists generally responded that saltiness and flavor were major influential factors for their preference choices of sliced ham products in the Sample Pair II and IV.

The preference evaluations of Sample Pairs V (LD vs AM1) and VII (LD vs AM3) revealed that the LD sliced ham sample was statistically significantly less preferred than both the AM1 and AM3 sliced ham samples. Results of both Pairs V and VII of sliced ham samples indicate that saltiness intensity play a key role in the preference for the experiment sliced ham sample (AM1 and AM3) versus the common LD sliced ham sample. Besides responses for the saltiness attribute, panelists gave notably large responses for flavor and texture descriptors for their preference choice of sliced ham product in both Sample Pair V and VII sensory evaluations. For Sample Pair VI, panelists showed no statistically significant preference between the LD and AM2 sliced ham samples. Panelists commonly cited flavor, saltiness, and taste descriptors as the main reasons for their preference choices expressed in Sample Pair VI.

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Appendix A
Sample Descriptive Sensory Analysis Ballot used for Evaluation of Sliced Hams

PERSONS WITH KNOWN FOOD ALLERGIES MUST REQUEST SPECIFIC INFORMATION ABOUT FOOD INGREDIENTS AND PREPARATION. PLEASE ASK THE PANEL ATTENDANT FOR THIS INFORMATION BEFORE PARTICIPATING IN TASTING

SENSORY ANALYSIS LABORATORY
Department of Food Science
University of Wisconsin-Madison

Ham
Date: February 7, 2005

Directions: 1. Taste Sample #824
2. Check the box below which best expresses your opinion of the sample
3. Repeat with Sample #563

Overall Preference	824	563
like very much	☐	☐
like moderately	☐	☐
like slightly	☐	☐
neither like nor dislike	☐	☐
dislike slightly	☐	☐
dislike moderately	☐	☐
dislike very much	☐	☐

1. Which of the two samples you tasted do you prefer? (check one box only)

824 ☐ 563 ☐

2. Why do you prefer the sample checked in Question 1 better than the other sample?

3. Other comments:

STATEMENT OF CONDITIONS FOR VOLUNTARY PARTICIPATION IN TASTE TESTING OF FOODS IN THE SENSORY EVALUATION LABORATORY, DEPARTMENT OF FOOD SCIENCE, UNIVERSITY OF WISCONSIN-MADISON. The sensory evaluation laboratory is engaged by various food research groups to evaluate the flavor, texture, and color of foods. In most instances the food samples are similar to commercially available products. In other instances food samples are the result of new product development or product modification efforts. However foods served to taste panelists are considered safe, wholesome, and prepared under good manufacturing practices. Participation in taste panels is voluntary, and panelists may withdraw participation at any time. Information concerning product composition and manufacturing history will be provided upon specific request.

Sample Pair I (Control vs. LD): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 1. Response frequency and mean scores for the consumer preference evaluation of ham.

	Assigned Numerical	Ham	
Preference Rating	Score	Control	LD
		(----------Number of Responses----------)	
Like very much	7	72	52
Like moderately	6	85	77
Like slightly	5	28	47
Neither like nor dislike	4	14	12
Dislike slightly	3	13	18
Dislike moderately	2	2	3
Dislike very much	1	1	6
Total number of responses		N = 215	
Mean Score		5.83^{A}	5.47^{B}
Statistical Analysis			
F-value		S	
LSD (at 5% level)		(0.18)	

S= significant at the 5% level; NS = not significant at the 5% level.
^{A,B} Mean scores in the same row with the same superscript are not significantly different at the 5% level.

Table 2. Summary of panelist responses to exit questions #1.

Exit questions	Number of response
#1: Which of the two samples you tasted do you prefer? (check one box only)	
Control	**132**
LD	**76**

Sample Pair I (Control vs. LD): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 3. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the Control sample the most.

The Control Ham sample:	Number of responses[1]
Tastes less salty	52
Tastes better	24
Has better flavor	20
Has better texture	9
Tastes sweeter	8
Tastes saltier	4
Has more smoked flavor	4
Tastes richer	2

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.

Table 4. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the LD sample the most.

The LD Ham sample:	Number of responses[1]
Has better flavor	24
Has better texture	11
Tastes better	10
Tastes saltier	10
Tastes less salty	8
Tastes sweeter	6
Has more smoked flavor	6
Tastes richer	5
Tastes less tart	1
Has less smoked flavor	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.

Sample Pair I (Control vs. LD): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 5. Summary of optional voluntary comments.

Control Ham sample:	**Number of responses[1]**
Tastes too bland	2
Has bad texture	1
Tastes too sweet	1
Has off-flavor	1
LD Ham sample:	
Has bad aftertaste	5
Tastes bland	3
Tastes too salty	2
Has bad texture	1
Has piggy off-flavor	1
Tastes fatty	1
Tastes sour	1
Tastes metallic	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.

Sample Pair II (Control vs. AM1 0.1% Sodium benzoate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 6. Response frequency and mean scores for the consumer preference evaluation of ham.

Preference Rating	Assigned Numerical Score	Ham	
		Control	AM1 0.1% Sodium Benzoate
		(----------Number of Responses----------)	
Like very much	7	76	71
Like moderately	6	79	88
Like slightly	5	34	30
Neither like nor dislike	4	5	5
Dislike slightly	3	7	6
Dislike moderately	2	1	1
Dislike very much	1	0	1
Total number of responses		N = 202	
Mean Score		6.03^{A}	6.02^{A}
Statistical Analysis			
F-value		NS	
LSD (at 5% level)			

S= significant at the 5% level; NS = not significant at the 5% level.
^{A,B} Mean scores in the same row with the same superscript are not significantly different at the 5% level.
Date of evaluation: February 7, 9, 14, and 20, 2006.

Table 7. Summary of panelist responses to exit questions #1.

Exit questions	Number of response
#1: Which of the two samples you tasted do you prefer? (check one box only)	
Control	**97**
AM1 0.1% Sodium Benzoate	**100**

Date of evaluation: February 7, 9, 14, and 20, 2006.

Sample Pair II (Control vs. AM1 0.1% Sodium benzoate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 8. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the Control sample the most.

The Control Ham sample:	Number of responses[1]
Has better flavor	26
Tastes less salty	21
Has better texture	15
Tastes better	11
Tastes saltier	6
Tastes less fatty	3
Has more smoked flavor	3
Has better aftertaste	2
Tastes milder	1
Tastes sweeter	1
Has less smoked flavor	1
Has more moisture	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 7, 9, 14, and 20, 2006.

Table 9. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the AM1 0.1% Sodium Benzoate sample the most.

The AM1 0.1% Sodium Benzoate Ham sample:	Number of responses[1]
Tastes less salty	33
Has better flavor	29
Tastes better	15
Tastes sweeter	9
Tastes saltier	7
Has better texture	7
Has more smoked flavor	7
Tastes richer	4
Tastes fresher	3
Tastes milder	2
Tastes spicier	1
Tastes less sweet	1
Has more moisture	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 7, 9, 14, and 20, 2006.

Sample Pair II (Control vs. AM1 0.1% Sodium Benzoate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 10. Summary of optional voluntary comments.

Control Ham sample:	**Number of responses[1]**
Has bad aftertaste	3
Has artificial taste	1
Has a tough casing	1
Has too strong pork smell	1
Taste raw meat-like	1
Tastes not fresh	1
AM1 0.1% Sodium Benzoate Ham sample:	
Has off-flavor	1
Tastes too salty	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.

Date of evaluation: February 7, 9, 14, and 20, 2006.

Sample Pair III (Control vs. AM2 0.3% Potassium Sorbate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 11. Response frequency and mean scores for the consumer preference evaluation of ham.

Preference Rating	Assigned Numerical Score	Ham	
		Control	AM2 0.3% Potassium Sorbate
		(----------Number of Responses----------)	
Like very much	7	74	64
Like moderately	6	95	92
Like slightly	5	38	46
Neither like nor dislike	4	7	5
Dislike slightly	3	2	8
Dislike moderately	2	1	1
Dislike very much	1	0	1
Total number of responses		N = 217	
Mean Score		6.06^{A}	5.88^{B}
Statistical Analysis			
F-value		S	
LSD (at 5% level)		(0.17)	

S= significant at the 5% level; NS = not significant at the 5% level.
^{A,B} Mean scores in the same row with the same superscript are not significantly different at the 5% level.

Date of evaluation: February 7, 9, 14, and 20, 2006.

Table 12. Summary of panelist responses to exit questions #1.

Exit questions	Number of response
#1: Which of the two samples you tasted do you prefer? (check one box only)	
Control	**119**
AM2 0.3% Potassium Sorbate	**94**

Date of evaluation: February 7, 9, 14, and 20, 2006.

Sample Pair III (Control vs. AM2 0.3% Potassium Sorbate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 13. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the Control sample the most.

The Control Ham sample:	Number of responses[1]
Has better flavor	25
Tastes saltier	18
Tastes better	17
Taste less salty	15
Has more flavor	13
Has better texture	7
Tastes sweeter	5
Has less smoked flavor	3
Has better aftertaste	2
Tastes juicier	2
Tastes less sweet	2
Has more moisture	1
Has better color	1
Has more smoked flavor	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 7, 9, 14, and 20, 2006.

Table 14. Summary of the responses to exit question #3 (why do you prefer the sample....?) from panelists who preferred the AM2 0.3% Potassium Sorbate sample the most.

The AM2 0.3% Potassium Sorbate Ham sample:	Number of responses[1]
Tastes less salty	19
Tastes sweeter	14
Has more flavor	12
Has better flavor	11
Has better texture	10
Tastes better	9
Tastes saltier	8
Has more smoked flavor	8
Has less aftertaste	4
Tastes juicier	2
Tastes less buttery	1
Tastes milder	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 7, 9, 14, and 20, 2006.

Sample Pair III (Control vs. AM2 0.3% Potassium Sorbate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 15. Summary of optional voluntary comments.

Control Ham sample:	**Number of responses[1]**
Tastes too salty	4
Has rubbery texture	2
Tastes too sweet	1
Has bad aftertaste	1
Has too much smoked flavor	1
AM2 0.3% Potassium Sorbate Ham sample:	
Has off-flavor	7
Tastes too sweet	4
Tastes too salty	3
Has slimy texture	2
Has slightly more smoked flavor	1
Has tender	1
Tastes bland	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 7, 9, 14, and 20, 2006.

Sample Pair IV (Control vs. AM3 0.3% Sodium Propionate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 16. Response frequency and mean scores for the consumer preference evaluation of ham.

Preference Rating	Assigned Numerical Score	Ham	
		Control	AM3 0.3% Sodium Propionate
		(----------Number of Responses----------)	
Like very much	7	60	56
Like moderately	6	65	75
Like slightly	5	26	22
Neither like nor dislike	4	6	5
Dislike slightly	3	4	3
Dislike moderately	2	3	3
Dislike very much	1	0	0
Total number of responses		N = 164	
Mean Score		5.99^{A}	6.02^{A}
Statistical Analysis			
F-value		NS	
LSD (at 5% level)			

S= significant at the 5% level; NS = not significant at the 5% level.
^{A,B} Mean scores in the same row with the same superscript are not significantly different at the 5% level.

Date of evaluation: February 7, 9, 14, and 20, 2006.

Table 17. Summary of panelist responses to exit questions #1.

Exit questions	Number of response
#1: Which of the two samples you tasted do you prefer? (check one box only)	
Control	**78**
AM3 0.3% Sodium Propionate	**81**

Date of evaluation: February 7, 9, 14, and 20, 2006.

Sample Pair IV (Control vs. AM3 0.3% Sodium Propionate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 18. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the Control sample the most.

The Control Ham sample:	Number of responses[1]
Tastes less salty	23
Tastes better	15
Has better flavor	15
Has more smoked flavor	6
Tastes saltier	5
Tastes sweeter	4
Has more moisture	4
Tastes less fatty	3

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample. Date of evaluation: February 7, 9, 14, and 20, 2006.

Table 19. Summary of the responses to exit question #2 (why do you prefer the sample from panelists who preferred the AM3 0.3% Sodium Propionate sample the most.

The AM3 0.3% Sodium Propionate Ham sample:	Number of responses[1]
Has better flavor	23
Tastes less salty	18
Tastes better	12
Tastes saltier	9
Tastes sweeter	8
Has more smoked flavor	6
Has better texture	6
Tastes milder	3
Has better aftertaste	1
Has better appearance	1
Has less smoked flavor	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 7, 9, 14, and 20, 2006.

Sample Pair IV (Control vs. AM3 0.3% Sodium Propionate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 7, 9, 14, and 20, 2006.

Table 20. Summary of optional voluntary comments.

Control Ham sample:	**Number of responses[1]**
Tastes greasy	4
Has bad aftertaste	3
Tastes bland	2
Tastes watery	1
Tastes bitter	1
Has dry mouthfeel	1
AM3 0.3% Sodium Propionate Ham sample:	
Tastes bitter	3
Has dry mouthfeel	1
Has too strong aftertaste	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 7, 9, 14, and 20, 2006.

Sample Pair V (LD vs. AM1 0.1% Sodium Benzoate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 23, 27, and 28, 2006.

Table 21. Response frequency and mean scores for the consumer preference evaluation of ham.

Preference Rating	Assigned Numerical Score	Ham	
		LD	AM1 0.1% Sodium Benzoate
		(----------Number of Responses----------)	
Like very much	7	47	77
Like moderately	6	80	82
Like slightly	5	41	26
Neither like nor dislike	4	11	6
Dislike slightly	3	9	3
Dislike moderately	2	3	0
Dislike very much	1	3	0
Total number of responses		N = 194	
Mean Score		5.64^{A}	6.15^{B}
Statistical Analysis			
F-value		S	
LSD (at 5% level)		(0.19)	

S= significant at the 5% level; NS = not significant at the 5% level.
^{A,B} Mean scores in the same row with the same superscript are not significantly different at the 5% level.

Date of evaluation: February 23, 27, and 28, 2006.

Table 22. Summary of panelist responses to exit questions #1.

Exit questions	Number of response
#1: Which of the two samples you tasted do you prefer? (check one box only)	
LD	**67**
AM1 0.1% Sodium Benzoate	**124**

Date of evaluation: February 23, 27, and 28, 2006.

Sample Pair V (LD vs. AM1 0.1% Sodium Benzoate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 23, 27, and 28, 2006.

Table 23. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the LD sample the most.

The LD Ham sample:	Number of responses[1]
Has better flavor	20
Tastes better	10
Tastes saltier	10
Tastes sweeter	8
Has better texture	6
Tastes milder	5
Tastes less salty	4
Tastes juicier	2
Tastes less sweet	1
Has better aroma	1
Has more smoked flavor	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 23, 27, and 28, 2006.

Table 24. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the AM1 0.1% Sodium Benzoate sample the most.

The AM1 0.1% Sodium Benzoate Ham sample:	Number of responses[1]
Tastes less salty	49
Has better flavor	34
Tastes better	16
Has better texture	13
Tastes sweeter	6
Tastes saltier	3
Has more smoked flavor	3
Has better aftertaste	3

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 23, 27, and 28, 2006.

Sample Pair V (LD vs. AM1 0.1% Sodium Benzoate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 23, 27, and 28, 2006.

Table 25. Summary of optional voluntary comments.

LD Ham sample:	**Number of responses[1]**
Tastes too salty	11
Tastes slightly bitter	3
Has unclean off-flavor	3
Tastes bland	2
Has discolored edge	1
Has soft/mushy texture	1
AM1 0.1% Sodium Benzoate Ham sample:	
Tastes too salty	2
Has too chewy texture	1
Tastes greasy	1
Tastes slightly bland	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 23, 27, and 28, 2006.

Sample Pair VI (LD vs. AM2 0.3% Potassium Sorbate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 23, 27, and 28, 2006.

Table 26. Response frequency and mean scores for the consumer preference evaluation of ham.

Preference Rating	Assigned Numerical Score	Ham	
		LD	AM2 0.3% Potassium Sorbate
		(----------Number of Responses----------)	
Like very much	7	70	62
Like moderately	6	67	75
Like slightly	5	39	32
Neither like nor dislike	4	5	10
Dislike slightly	3	9	13
Dislike moderately	2	1	0
Dislike very much	1	2	1
Total number of responses		N = 193	
Mean Score		5.90^{A}	5.82^{A}
Statistical Analysis			
F-value		NS	
LSD (at 5% level)			

S= significant at the 5% level; NS = not significant at the 5% level.
^{A,B} Mean scores in the same row with the same superscript are not significantly different at the 5% level.

Date of evaluation: February 23, 27, and 28, 2006.

Table 27. Summary of panelist responses to exit questions #1.

Exit questions	Number of response
#1: Which of the two samples you tasted do you prefer? (check one box only)	
LD	**107**
AM2 0.3% Potassium Sorbate	**83**

Date of evaluation: February 23, 27, and 28, 2006.

Sample Pair VI (LD vs. AM2 0.3% Potassium Sorbate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 23, 27, and 28, 2006.

Table 28. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the LD sample the most.

The LD Ham sample:	Number of responses[1]
Has better flavor	36
Tastes saltier	18
Tastes better	14
Has better texture	13
Tastes less salty	7
Has more smoked flavor	4
Tastes sweeter	4
Has better aftertaste	2
Tastes fresher	1
Tastes less sweet	1
Tastes spicier	1
Has better appearance	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 23, 27, and 28, 2006.

Table 29. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the AM2 0.3% Potassium Sorbate sample the most.

The AM2 0.3% Potassium Sorbate Ham sample:	Number of responses[1]
Tastes less salty	20
Has better flavor	18
Tastes better	12
Tastes sweeter	10
Has more smoked flavor	6
Has better texture	6
Has better color	2
Has better aftertaste	2
Tastes less sweet	1
Tastes juicier	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 23, 27, and 28, 2006.

Sample Pair VI (LD vs. AM2 0.3% Potassium Sorbate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 23, 27, and 28, 2006.

Table 30. Summary of optional voluntary comments.

LD Ham sample:	**Number of responses[1]**
Has off-flavor	5
Has bad aftertaste	3
Has too salty	2
Tastes too fatty	1
Tastes bland	1
AM2 0.3% Potassium Sorbate Ham sample:	
Has bad aftertaste	5
Tastes too salty	4
Tastes too fatty	3
Tastes too sweet	1
Tastes soapy	1
Has tough texture	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 23, 27, and 28, 2006.

Sample Pair VII (LD vs. AM3 0.3% Sodium Propionate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 23, 27, and 28, 2006.

Table 31. Response frequency and mean scores for the consumer preference evaluation of ham.

Preference Rating	Assigned Numerical Score	Ham	
		LD	AM3 0.3% Sodium Propionate
		(----------Number of Responses----------)	
Like very much	7	56	87
Like moderately	6	83	65
Like slightly	5	31	25
Neither like nor dislike	4	7	8
Dislike slightly	3	11	2
Dislike moderately	2	0	2
Dislike very much	1	1	0
Total number of responses		N = 189	
Mean Score		5.86^{A}	6.17^{B}
Statistical Analysis			
F-value		S	
LSD (at 5% level)		(0.19)	

S= significant at the 5% level; NS = not significant at the 5% level.
^{A,B} Mean scores in the same row with the same superscript are not significantly different at the 5% level.

Date of evaluation: February 23, 27, and 28, 2006.

Table 32. Summary of panelist responses to exit questions #1.

Exit questions	Number of response
#1: Which of the two samples you tasted do you prefer? (check one box only)	
LD	**73**
AM3 0.3% Sodium Propionate	**115**

Date of evaluation: February 23, 27, and 28, 2006.

Sample Pair VII (LD vs. AM3 0.3% Sodium Propionate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 23, 27, and 28, 2006.

Table 33. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the LD sample the most.

The LD Ham sample:	Number of responses[1]
Has better texture	13
Has better flavor	12
Tastes better	11
Tastes less salty	9
Tastes saltier	7
Has more smoked flavor	5
Has more moisture	3
Tastes less sweet	2
Tastes juicier	2
Tastes less fatty	1
Tastes less piggy	1
Tastes sweeter	1
Tastes less processed	1
Has better color	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 23, 27, and 28, 2006.

Table 34. Summary of the responses to exit question #2 (why do you prefer the sample....?) from panelists who preferred the AM3 0.3% Sodium Propionate sample the most.

The AM3 0.3% Sodium Propionate Ham sample:	Number of responses[1]
Tastes less salty	36
Has better flavor	28
Tastes better	15
Has better texture	14
Tastes saltier	7
Has more smoked flavor	6
Tastes sweeter	3
Tastes juicier	2
Tastes less fatty	2
Tastes tangier	2
Has less smoked flavor	1
Has better color	1
Has less chewy	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 23, 27, and 28, 2006.

Sample Pair VII (LD vs. AM3 0.3% Sodium Propionate): Consumer Preference Sensory Evaluation for Ham Evaluated on February 23, 27, and 28, 2006.

Table 35. Summary of optional voluntary comments.

LD Ham sample:	**Number of responses[1]**
Tastes too salty	5
Has a little slimy texture	3
Has off-flavor	2
Tastes slightly bitter	2
Has too strong aftertaste	1
Has metallic aftertaste	1
AM3 0.3% Sodium Propionate Ham sample:	
Tastes sour	1
Tastes too fatty	1
Has off-flavor	1

[1] Data reflect total number of comments offered for each descriptor category; some panelists commented about more than one descriptor category for a given sample.
Date of evaluation: February 23, 27, and 28, 2006.

APPENDIX B

Controlling *Listeria monocytogenes* on Ready-to-Eat Meat and Poultry Products Using Food-Approved Antimicrobials Benzoate, Propionate, and Sorbate

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Objectives for Literature Search:

- Conduct a thorough search on the effects that sorbate, benzoate, and propionate in ready-to-eat meats, in the presence and absence of nitrite, might have on human health
- Summarize recent (last 3 years) articles on effects of sorbate, benzoate, and propionate on *Listeria monocytogenes* and on spoilage organisms

Effects of Organic Acids on *L. monocytogenes* in RTE Meats: Recent Scientific Literature

Numerous recent research papers (2003–2006) have presented more data on the inhibitory effects of organic acids on growth and survival of *L. monocytogenes* in RTE meats. Important factors to consider in challenge testing were also investigated and discussed (*87*).

Frankfurters

Sodium or potassium lactate with or without sodium diacetate were used in frankfurter formulations to control growth of *L. monocytogenes* in frankfurters stored at 4–7ºC. When used alone, lactate concentrations were 3–3.3% (*14, 70*). When used in combination with diacetate, lactate levels were in the range of 1.4–1.8% and diacetate levels ranged from 0.05–0.25% (*6, 25, 48*).

In addition, organic acids have been used as dipping solutions to suppress growth of *L. monocytogenes* that may have contaminated the surface of frankfurters. The following dipping solutions were reported to aid in pathogen control on frankfurters that contained sodium or potassium lactate (with or without sodium diacetate) in their formulation: *i*) lactic acid (2.5%) and acetic acid (2.5%) (*6, 25*); *ii*) potassium benzoate (5%) (*25*); *iii*) mixture containing 2% acetic, 1% lactic, 0.1% benzoic, and 0.1% propionic acids (*60*); *iv*) mixture containing 3% sodium diacetate and 6% sodium lactate (*86*); *v*) 3% or 6% sodium diacetate, potassium benzoate, sodium lactate, alone or in combination (*46, 47*); *vi*) acidic calcium sulfate with propionic and lactic acids (*14*). If organic acids were not included in the formulation, the dipping solutions delayed growth of *L. monocytogenes* somewhat but did not effectively retard growth during the expected shelf life of the product. Dipping solutions containing 0.125–0.5% sodium diacetate reduced the irradiation dose required to suppress listerial growth on frankfurters (*80*).

Lactate and diacetate, incorporated into casings or coatings for frankfurters, also help to retard growth of *L. monocytogenes* (*48, 50*). However, sorbate in a coating did not significantly inhibit listerial growth (*51*).

Bologna

Organic acids have also been used to control listerial growth on bologna. Incorporation of 1.8% sodium lactate and 0.25% sodium diacetate into pork bologna was reported to be the best combination for inhibiting bacterial growth during 20 days at 4 or 10ºC (*7*). Dipping solutions containing 2.5% acetic acid, 2.5% lactic acid or 5% potassium benzoate all reduced populations of listeria on bologna slices during storage at 10ºC for 48 days. These slices did have lower sensory scores, however (*24*). A plastic (polyvinylidene chloride) film containing 1.5% or 3% sorbic acid, placed between slices of inoculated beef bologna, prevented growth of *L. monocytogenes* for 28 days at 4ºC (*44*). Sodium lactate (1–2%) and sodium diacetate (0.07–0.15%) decreased the dose of irradiation required to effectively suppress listerial growth on beef bologna during refrigerated storage. Use of these compounds allowed a lower dose of irradiation which decreased oxidation of lipids and minimized adverse effects on flavor (*81*).

Ham

Dipping solutions containing 2.5% acetic acid, 2.5% lactic acid or 5% potassium benzoate all reduced populations of listeria on ham slices during storage at 10ºC for 48 days. These slices did have lower sensory scores, however (*24*). Lactates were found to act synergistically with high hydrostatic pressure and low storage temperature to inhibit growth of *L. monocytogenes* (*3*). Sodium lactate (2%) and sodium diacetate (0.1%) in combination with 1 kGy of irradiation effectively suppressed listerial growth on turkey ham for 6 weeks at 4ºC. Use of these compounds allowed a lower dose of irradiation which minimized adverse effects of irradiation on flavor of the ham. Although potassium benzoate also had inhibitory effects, some benzene was detected in the irradiated ham containing benzoates, indicating that this is not an appropriate preservative to use in combination with irradiation (*94*).

Sausage

Addition of 0.125–0.5% sodium diacetate to fine-emulsion sausages reduced the irradiation dose required to suppress listerial growth (*80*). Sodium lactate (3.3%) was found to have anitlisterial effects similar to 0.05% or 0.1% potassium sorbate and sodium benzoate in sausage and delayed lag phase growth of *L. monocytogenes* by at least two weeks at 4ºC (*13*).

Poultry

Some organic acids decrease listerial populations on raw poultry. *L monocytogenes* levels on chicken breast were reduced by 3.88 logs following a 15 min dip in a solution of 2.5% sodium lactate (*26*). A solution of 1.54% potassium lactate and 0.11% sodium diacetate only somewhat inhibited listerial growth on cook in bag turkey breasts (*49*). Addition of 4.8% sodium lactate to ground chicken actually increased the heat resistance of *L. monocytogenes* added to the meat (*61*).

Predictive Models

Some recent research has also described broth-based predictive models with data on the listeriocidal or listeriostatic effects of lactate and diacetate (*36, 93*). Evaluations of predictive models for effective control of *L. monocytogenes* in meat emphasize that such models need to be validated in meat or predictive errors will result (*45, 53*). Effects of sodium lactate (0–4.8%) and sodium diacetate (0–0.25%) on heat resistance of *L. monocytogene*s in ground beef were tested at 60–73.9ºC (*37*). Sodium lactate alone was found to increase heat resistance of the bacteria (similar to the effect in ground chicken; *61*) while combinations of the two organic acids reduced heat resistance. Data were used to construct a predictive model with D values indicating the safety of different combinations of heat and organic acids for controlling *L. monocytogenes*. Another predictive model was constructed with data on the effects of sodium chloride (0.8–3.6%), potassium lactate (0.2–9.25%), and sodium diacetate (0–0.2%) on growth of *L. monocytogenes* at 4ºC in cured and uncured RTE meats (*42*).

Effects of Organic Acids on Spoilage Organisms

Spoilage organisms may affect growth of *L. monocytogenes* on RTE meats. Recently published research indicated that the population of listeriae inoculated on to frankfurters was highest at the lowest concentration of spoilage bacteria. However, the growth rate of listeriae at 10ºC was similar at all concentrations of spoilage bacteria (*71*).

Experiments in laboratory media have demonstrated that sodium lactate inhibits the growth of spoilage bacteria (*33, 34, 66*). This has also been demonstrated in several meat models. Sodium lactate (3%) and a combination of sodium lactate (2, 3, or 4%), 0.5% sodium acetate, and 0.15% potassium sorbate were most effective in retarding growth of a spoilage bacterium (*Lactobacillus curvatus*) on frankfurters and a "pariza" type meat product (*17*). Sodium lactate also effectively inhibited growth of *Lactobacillus sake* and *Lactobacillus curvatus* in frankfurters and ham at 4ºC (*82, 83*) and of *Brochothrix thermosphacta*, a spoilage bacterium, in poultry sausage (*43*). Growth of aerobic psychrotrophic bacteria and lactic acid bacteria on poultry sausage were inhibited by 1–2% sodium lactate (*11*). Shelf life of cooked meat products has been extended by sodium lactate and this has been modeled (*15*). Combinations of 1.8–3.0% sodium lactate and 0.2–0.30% sodium diacetate were reported to be effective at retarding growth of spoilage bacteria on pork bologna (*7*), beef bologna (*54, 69*), and cook-in-bag turkey (*55*). Sodium lactate has also been reported to inhibit germination and growth of *Clostridium perfringens* (*38, 39*) and some, but not all, psychrotrophic clostridia (*41*).

When used as dipping solutions, a solution of 2.5% sodium lactate and 0.2% sodium diacetate inhibited growth of spoilage organisms on beef (*76*) while with bologna and ham, sequential dipping in 2.5% acetic acid, 2.5% lactic acid, and 5% sodium benzoate was reported to inhibit growth of spoilage bacteria (*24*).

In laboratory media, acetic and propionic acids (0.1–1.0%) inhibited growth of six common meat spoilage bacteria. Because of their low solubility in broth sorbic and benzoic acids could be used only at low concentrations (<0.15%) and were not effective against these spoilage organisms (*66*). Sorbic acid containing plastic films inhibited growth of common spoilage organisms on beef bologna (*44*). Sodium diacetate appears to be ineffective in controlling spoilage bacteria on cook-in-bag turkey (*55*), frankfurters (*82*) and ham (*83*). However, sodium diacetate (0.3%) did inhibit some Gram negative spoilage bacteria (*77*).

Toxicology Studies with Organic Acids

Since organic acids have been widely used as preservatives in some foods for many years, numerous tests have been conducted to determine their possible toxic effects in rodents and cell cultures. In addition, there have been clinical studies to assess possible allergenicity and there are some reports of adverse reactions to these compounds in humans. Overall, data indicate that these compounds are of low toxicity with little or no genotoxic or carcinogenic potential. Reports of significant adverse reactions are rare. However, four potentially significant issues should be mentioned:

i) Benzoate, more than any of the other organic acids, appears to provoke hypersensitivity reactions in sensitive individuals. These are not common but one should be aware of this possibility.

ii) Several reports indicate that under acidic conditions and during irradiation small amounts of the known carcinogen benzene can form from benzoates. Therefore, benzoates should probably not be used in some acidic foods or in foods that will also be irradiated.

iii) Under some conditions, sorbates have been reported to degrade during long storage times to form genotoxic compounds and sorbates were reported to form mutagens with nitrites. This is considered unlikely using current procedures of meat processing but should be reassessed if processes change.

iv) At very high dietary levels (4% of diet) over extended feeding periods, propionates have caused forestomach cancers in rodents. Again this is very unlikely to occur in humans,

but one should be aware that this has been reported in the literature. Currently available information is summarized below.

Sodium Diacetate

Sodium diacetate is an approved GRAS substance for use as an antimicrobial in several foods. No adverse reactions have been reported for humans or animals and no recent acceptable daily intake (ADI) has been established. In 1973, FAO stated that up to 15 mg/kg body weight/ day was acceptable (*16*). Sodium diacetate was approved by FSIS in 2000 for use as an antimicrobial in meat and poultry products up to a concentration of 0.25% by weight of total formulation (Federal Register 65: 3121–3123 and 65:17128–17129) Higher concentrations (up to 0.4%) are used in some bread and bakery products (*16*).

Lactate

Lactic acid is an approved GRAS substance for use in various foods. No acceptable daily intake has been established. Oral LD_{50} doses of lactic acid in rodents exceed 1.8 g/kg body weight (*16*). Sodium and potassium lactates were approved by FSIS in 2000 for use as antimicrobials in meat and poultry products, singly or in combination, up to a concentration of 4.8% by weight of total formulation (Federal Register 65: 3121–3123 and 65:17128–17129) A 2-yr. study demonstrated no toxic or carcinogenic effects in rats given water containing 2.5 or 5% calcium lactate in drinking water (*52*).

Propionic Acid

Propionic acid and its salts are approved GRAS substances for use in various foods, including cheese and bakery products. Sodium propionate is made from the reaction of sodium hydroxide with propionic acid. Propionic acid can be prepared by a variety of methods, but occurs naturally as the result of metabolic processes and can be obtained from fermentation by *Propionibacterium*. Use of sodium propionate has no limitations in a variety of food products (including cheeses, soft candies, baked goods, jams, jellies, nonalcoholic beverages) other than current good manufacturing practices (21CFR 184.1784). Acceptable daily intakes have not been established. Oral LD_{50} doses of propionates in rodents exceed 3 g/kg body weight (*16*).

Genotoxicity. Tests with propionic acid in the following genotoxicity assays were negative: SOS chromotest, *Salmonella*/microsome mutagenicity assay, sister chromatid exchange *in vitro*, and micronucleus test *in vivo*. Some positive results were obtained with the *E. coli* DNA repair assay. These data reinforce other evidence that propionic acid is not mutagenic (*8*).

Carcinogenicity. Rats fed diets containing 4% propionic acid develop hyperplastic lesions and tumors in the forestomach; tumors were not formed when propionic acid constituted 0.4% of the diet. Damage and cellular proliferation were detectable in the forestomach of rats, mice, and hamsters after 7 days of consuming diets containing 4% (but not 0.4%) propionic acid (*28, 29, 68*). In some other experiments, rats developed hyperplasia and severe inflammatory lesions only when fed propionic acid in a powdered diet but not when fed pellets containing propionic acid. Propionic acid accumulated in inflammatory lesions (*10*). Humans do not have forestomachs but they do have similar epithelial tissue in the esophagus and pharynx. Because food contact time in the forestomach is much longer than that in the pharynx and esophagus, propionic is not considered a carcinogenic risk for humans (*28*).

Benzoic Acid and Sodium Benzoate

Benzoic acid occurs naturally in some fruits and fermented products and has been used as a preservative since the early 20th century. Sodium benzoate, approximately 200 times more water soluble than benzoic acid, is produced by neutralization of benzoic acid with sodium bicarbonate, sodium carbonate, or sodium hydroxide. Both benzoic acid and sodium benzoate are GRAS in the U.S. and are permitted in certain foods as antimicrobial or flavoring agents, with current maximum usage level of 0.1% (21CFR 184.1733). CODEX specifies higher permitted levels in some foods such as liquid eggs (0.5%) and semi-preserved fish (0.2%) (http://www.codexalimentarius.net).

Benzoates are readily absorbed from the intestine but do not accumulate in the body since they are rapidly metabolized in the liver and excreted in the urine as hippuric acid. An oral dose of 250 mg benzoic acid was quantitatively excreted within four hours by a healthy male. An acceptable daily intake (ADI) of up to 5 mg/kg body weight has been established. Benzoates and benzoic acid scavenge hydroxyl free radicals (*12, 63*).

Under some conditions benzoic acid or benzoates may form small amounts of benzene, a volatile compound with known toxic and carcinogenic effects. This has been reported in some fruit drinks containing sodium benzoate and ascorbic acid (*12*) and during irradiation of a turkey breast roll (*95*) and ham (*94*) containing potassium benzoate.

Hypersensitivity reactions. Reported human reactions to benzoic acid and benzoates can be categorized as hypersensitivity reactions. These appear to be non-immunological reactions and may involve production of histamine and prostaglandins. In most cases, reactions appear to be mild-moderate but a few cases of anaphylaxis have been reported.

Dermatitis/urticaria. A number of challenge tests indicated that oral doses of 20–250 mg sodium benzoate can cause urticaria in some individuals including children (*23, 35, 56, 65, 84*). Sodium benzoate caused a significant increase in production of leukocyte inhibitory factor (a compound involved in cell-mediated immune responses) by mononuclear cells from persons who reported developing urticaria in response to benzoates (*89*). Sodium benzoate was reported to stimulate release of histamine and prostaglandin from human gastric mucosa in some patients (*12*). Oral reactions to toothpastes containing sodium benzoate have also been reported (*1, 59*).

Another study which involved careful testing of 47 patients who reported allergic reactions after consuming foods containing sodium benzoate, revealed that only one reacted to a challenge with 75 mg sodium benzoate but did not react to a placebo (*64*).

Asthma. Some reports indicate that benzoate induces asthmatic symptoms in some susceptible people (*23, 32, 91*). Four of 14 patients tested, reacted with asthmatic symptoms to sodium benzoate (20–120 mg) added to an orange drink (*21*). Two asthmatic children, whose symptoms worsened when treated with some antiasthmatic drugs and antibiotics, were found to be sensitive to the benzoates contained in these drugs. Treatment with benzoate-free versions of these drugs did not elicit respiratory symptoms (*4*).

Rhinitis. Sodium benzoate (50 mg oral dose) was implicated in a rigorously investigated case of rhinitis (*2*). A double-blind placebo-controlled trial investigating the effects of food additives demonstrated that sodium benzoate (100 and 200 mg oral doses) induced or aggravated symptoms of rhinitis in 8.8% of 226 subjects tested (*67*). Still another challenge study demonstrated that some people developed rhinitis after ingesting 250 mg benzoate (*91*).

Anaphylaxis. A few cases of severe anaphylactic reactions have been attributed to benzoate. One involved a patient undergoing general anesthesia. A challenge test later demonstrated that an oral dose of 100 mg sodium benzoate decreased peak expiratory flow rate (*57*). Another case patient apparently reacted to sodium benzoate used as a preservative in mustard and cheese with a severe systemic reaction. A later oral challenge with 20 mg sodium benzoate induced urticaria, but after the patient was treated for a sinus infection, she tolerated up to 160 mg benzoate before developing symptoms (*56*).

Genotoxicity. Benzoic acid and sodium benzoate tested negative in bacterial and most *in vivo* mammalian genotoxicity assays. They have also tested negative in most, but not all, *in vitro* mammalian assays (*63*). DNA adducts with benzoate were detected in liver and kidney after oral doses of 100, 500, and 1000 µg/kg body weight. Adduct levels declined by 75% within a day as the benzoate was metabolized to hippurate and excreted in the urine (*92*).

Carcinogenicity. No evidence for the carcinogenicity of sodium benzoate was detected in studies with rats (2% in pellets) (*79*) or mice (2% in drinking water) (*85*).

Toxicity. Some early studies (1907, 1908, 1924) reported adverse effects in some (but not all) human volunteers consuming very high levels of sodium benzoate (33 g/kg dose or a liter of juice containing 0.2% or 0.3%) or benzoic acid (2.5 g/day for 5 days) (*63*).

Consumption of a diet containing sodium benzoate (2.4% for rats, 3% for mice) for ten days caused some significant changes in hepatocytes and liver and kidney weights in male rodents. Some effects were

observed in females but these were less pronounced and often not significant (*22*). Benzoic acid appeared to depress weight gain in mice (3 months at 80 mg/kg/day or 17 months at 40 mg/kg/day) and in rats (18 months at 40 mg/kg/day) (*78*).

In subchronic toxicity tests with rats, decreased feed intake and growth were only observed in animals fed diets containing >1% benzoic acid. Other studies with mice showed that they did respond to benzoic acid concentrations <1% (*63*).

Neurotoxicity tests with rodents were negative. In teratogenicity tests, benzoic acid was negative in rats (up to 500 mg/kg/day) but positive in hamsters (600 mg/kg). Sodium benzoate caused no teratogenic effects in rats and mice at 175 mg/kg/day (*63*).

Oral LD_{50} values for sodium benzoate and benzoic acid in rodents, rabbits, cats, and dogs were all >1.5 g/kg body weight (*63*).

Sorbic Acid, Sodium Sorbate, Potassium Sorbate

Sorbic acid and potassium sorbate, are generally recognized as safe in the U.S. when used in accordance to good manufacturing practices (21CFR 182.3640). Allowable usage levels this antimycotic agent vary depending on the target food and range between 0.1% for fruit preserves (21CFR Part 150) and 0.3% for certain cheeses (21CFR Part 133).

Allergy. Some challenge tests with humans indicate that sorbic acid or sorbates can cause non-immunological contact urticaria in susceptible people (*27, 88*). A few other reports indicate that a small number of people may be sensitive to oral doses of sorbic acid (*74*).

Genotoxicity. Results from a number of *in vitro* and *in vivo* tests indicate that sorbic acid (alone) and potassium sorbate do not produce genotoxic effects (*5, 40, 62, 73*). Some other genotoxicity tests using Chinese hamster cells *in vitro* indicated that sorbic acid and sorbates exert weak genotoxic effects (*31*). Freshly prepared sodium sorbate also tested negative in a number of experiments (*90*). However, solutions of sodium sorbate stored for several weeks did exhibit weak genotoxic effects (*62, 73, 90*). This may be due to the formation of the degradation product, 4,5-oxohexenoate, which is a mutagen (*31, 40*).

Some other experiments have demonstrated genotoxic effects (increased levels of sister chromatid exchanges and micronuclei in mice) of sorbic acid at high concentrations (75–150 mg/kg body weight) in mice (*58*).

Carcinogenicity. Neither sorbic acid nor sorbates appear to be carcinogenic in rodent studies with animals fed diets containing as much as 10% sorbic acid in the diet (*88*).

In the presence of sodium nitrite, potassium sorbate was reported to have cytotoxic but not mutagenic effects (*9*). However, other experiments demonstrated that nitrite and potassium sorbate formed direct acting mutagens (*30*). Japanese researchers reported that interactions between sorbate and nitrite may induce production of mutagens when concentrations of 2200 ppm sorbic acid and 1300 ppm nitrites are present in high acid conditions (*72*). However, mutagenicity was blocked when pH values were higher than 3.5, or in the presence of ascorbate. Cured meat products produced in the United States typically contain erythorbate or ascorbate to reduce nitrosoamine formation, and this would prevent mutagen formation at pH values that mimic gastric conditions should otherwise optimal conditions exist (*30, 75*). Sorbic acid and nitrite exerted additive or synergistic effects in several in vivo genotoxicity assays in mice (*5, 58*). Sorbic acid can react with amines (that may be present in foods) but the products do not appear to be mutagenic or genotoxic (*18–20*).

Toxicity. Sorbic acid and sorbates are reported to exert a very low level of mammalian toxicity. Median lethal doses of sorbic acid and its potassium and sodium salts in rodents range from 4 to 10 g/kg body weight. This corresponds to about 500 g (over 1 pound) for an adult human. Short term toxicity assays with rodents and dogs demonstrated that dietary levels of 2–10% sorbic acid/sorbate had no adverse effects. No adverse reproductive or developmental effects have been observed in multigenerational rodent studies (*88*).

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