U.S. DEPARTMENT OF AGRICULTURE
FOOD SAFETY AND INSPECTION SERVICE

Petition for Rulemaking to Allow Sodium Benzoate and Sodium Propionate as Antimicrobial Agents in Meat and Poultry Products	)	Docket No. _____

Submitted by

Kraft Foods Global, Inc.

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January 19, 2007

FSIS Docket Clerk
U.S. Department of Agriculture
Food Safety and Inspection Service
Room 102, Cotton Annex Building
300 12th Street, S.W.
Washington, D.C. 20250-3700

Citizen Petition

I. INTRODUCTION

Kraft Foods Global, Inc. (Kraft) is proposing to expand the list of antimicrobial agents allowed in meat and poultry products to include sodium benzoate and sodium propionate, substances that permit reductions in sodium content and other important benefits without compromising food safety. This proposal stems from Kraft's research and development program, which aims to identify innovative ingredients and technologies that meet food safety, nutrition, consumer acceptance, and manufacturing needs. Our research has identified several combinations of antimicrobial ingredients that can be used to inhibit the growth of *Listeria monocytogenes* in hot dogs and deli meats in place of sodium and potassium lactate (lactate salts). These combinations provide the same antimicrobial effect as lactate salts at lower levels of use, making it possible to improve nutrition by reducing sodium, prevent off flavors that may be caused by potassium lactate, and improve manufacturing efficiencies by eliminating equipment and handling costs associated with lactate salts.

In support of this proposal, and as required by the Food Safety and Inspection Service (FSIS), Kraft conducted several studies and other research to confirm that the proposed antimicrobial ingredients are safe and suitable. Our research took into account the unique composition of diverse products such as hot dogs (including hot dogs made with chicken, turkey, beef, and pork), bologna, ham, and turkey breast. We developed an approach to predict the effect of antimicrobial ingredients on *L. monocytogenes* growth, and then confirmed our findings with tests of different formulations. We also assessed treated products for quality, analyzed the nutritional composition of planned formulations, and considered the status of sodium benzoate and sodium propionate as generally recognized as safe (GRAS) substances under Food and Drug Administration (FDA) requirements.

Our research revealed that differences in product composition, especially moisture, can influence antimicrobial activity and formulation needs. We identified three types of formulations as safe and effective for most processed meat and poultry products:

(1) A combination of sodium benzoate and sodium diacetate—for example, we found 0.1% sodium benzoate and 0.1% sodium diacetate to inhibit *L. monocytogenes* in most lower moisture products such as hot dogs.

(2) A combination of sodium benzoate, sodium diacetate, and sodium propionate—for example, a combination of 0.1% sodium benzoate,

0.15% sodium diacetate, and 0.2% sodium propionate inhibits *L. monocytogenes* in high moisture products such as ham.

(3) A combination of sodium benzoate, sodium diacetate, sodium propionate, and Lem-O-Fos® (a commonly used ingredient that contains sodium phosphate and lemon juice concentrate)—for example, a combination of 0.1% sodium benzoate, 0.15% sodium diacetate, 0.2% sodium propionate, and 0.56% Lem-O-Fos® inhibits *L. monocytogenes* in turkey.

These combinations are examples of formulations that can be used to control *L. monocytogenes* in ready-to-eat meat and poultry products. As with any antimicrobial use, the need for validation must be considered on a case-by-case basis to account for the many variables that can influence microbial growth in specific products.

To allow additional options for inhibiting *L. monocytogenes* in ready-to-eat meat and poultry products, this petition asks FSIS to amend 9 C.F.R. § 424.21(c) to list sodium benzoate and sodium propionate as acceptable antimicrobial agents that may be used in combination with other approved ingredients, such as sodium diacetate. This petition is based on sections 1(m) and 21 of the Federal Meat Inspection Act (FMIA) (21 U.S.C. §§ 601(m) and 621), sections 4(g) and 14 of the Poultry Products Inspection Act (PPIA) (21 U.S.C. §§ 453(g) and 463), and 7 CFR § 1.28. An amendment to FSIS regulations is necessary because current regulations state that sodium benzoate and sodium propionate may be used in meat or poultry products only if the use is specifically permitted in Title 9 of the Code of Federal Regulations.

II. REGULATORY ACTION REQUESTED

Kraft respectfully asks FSIS to amend 9 C.F.R. Part 424 to identify sodium benzoate and sodium propionate as safe and suitable antimicrobial agents. We ask FSIS to amend § 424.21(c), "Use of food ingredients and sources of radiation," to include the following specific uses:

§ 424.21 Use of food ingredients and sources of radiation

* * * *

(c) * * * *

Class of substance	Substance	Purpose	Products	Amount
* * * * Antimicrobial Agents				

Class of substance	Substance	Purpose	Products	Amount
* * * *				
	Sodium benzoate	To inhibit microbial growth	Various meat and poultry products	Up to 0.1% (by weight of total formulation) in combination with approved antimicrobial agents and adjuvants
	Sodium propionate	.....do.....	.....do.....	Up to 0.2% (by weight of total formulation) in combination with approved antimicrobial agents and adjuvants

Kraft requests expedited review of this petition and an interim or direct final rule in response to it. Expedited review is appropriate because the requested action will enable sodium reductions and control unavoidable *L. monocytogenes* in commonly consumed types of meat and poultry products.

III. STATEMENT OF GROUNDS

L. monocytogenes is a gram-positive, pathogenic bacterium that is ubiquitous in food and water. 1/ Plant materials, food-producing animals, and humans may come into contact with *L. monocytogenes* as a result of environmental sources, and may introduce the pathogen into food processing facilities. *L. monocytogenes* is widely regarded as unavoidable in the food processing environment and may be present in processing facilities despite strict adherence to current good manufacturing practices, sanitation standard operating procedures, and Hazard

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1/ *See, e.g.,* Fenlon, D.R., 1999. *Listeria monocytogenes* in the natural environment. In *Listeria*, Listeriosis, and Food Safety, 2d ed, pp. 21-37 (E.T. Ryser and E.H. Marth, editors).

Analysis and Critical Control Point programs. 2/ FSIS and FDA have identified control of *L. monocytogenes*, which has the potential to cause listeriosis, as an important public health objective.

Because *L. monocytogenes* may be unavoidably present in the food processing environment, it may be transmitted to some ready-to-eat foods like hot dogs and deli meats after cooking but before packaging. To help inhibit the growth of *L. monocytogenes* in meat and poultry products, FSIS has approved antimicrobial agents such as sodium and potassium lactate and sodium diacetate. These substances, however, can add to sodium content; in addition, potassium lactate can increase the potential for off-flavors in treated food products. The lactate salts also require substantial handling and equipment costs because they are used in a liquid form. Sodium benzoate and sodium propionate offer a better approach: sodium benzoate and sodium propionate are as effective as lactate salts and sodium diacetate in controlling *L. monocytogenes*, but achieve this result with a lower sodium content, less potential for off flavors, enhanced manufacturing efficiency, and good consumer acceptance.

A. Regulatory Status of Sodium Benzoate, Sodium Propionate, and Other Ingredients

Ingredients used in meat and poultry products must be safe and suitable. Safety is established by FDA and typically requires that an ingredient be an FDA-approved food additive or GRAS for the intended use. FSIS evaluates suitability for use in meat and poultry products, confirming that an ingredient is effective and does not cause a meat or poultry product to be adulterated or misbranded (e.g., the ingredient does not mask spoilage). Both safety and suitability require that the ingredient be used at an appropriate level—the level necessary to achieve a legitimate effect, such as inhibiting pathogen growth.

1. Safety of Sodium Benzoate

Sodium benzoate is affirmed as GRAS by FDA for use as an antimicrobial agent and a flavoring agent and adjuvant. 21 C.F.R. § 184.1733. FDA's GRAS affirmation allows use of sodium benzoate in food generally at levels not to exceed current good manufacturing practice. "Current good manufacturing practice" means that an ingredient is "food grade" (i.e., has appropriate specifications for purity and quality), is prepared and handled as a food ingredient, and is used at a level that does not exceed the amount reasonably required to accomplish the intended physical, nutritional, or technical effect. 21 C.F.R. § 184.1(b).

2/ *See, e.g.,* International Commission on Microbiological Specifications for Foods (ICMSF). 2002. Microorganisms in Foods 7 – Microbiological Testing in Food Safety Management, ch. 16, pp. 285-312.

FDA's regulation states that "current usage" of sodium benzoate results in a maximum level of 0.1% in food, and that FDA has not determined whether "significantly different conditions of use" would also be GRAS. Kraft is planning to use sodium benzoate in processed meat and poultry products at a level of 0.1%, which falls within the FDA regulation.

2. Safety of Sodium Propionate

Sodium propionate is affirmed as GRAS by FDA for use with no limitation other than current good manufacturing practice. 21 C.F.R. § 184.1784. FDA's GRAS decision was based on the use of sodium propionate as an antimicrobial agent and flavoring agent in a number of foods, including "meat products." "Meat products" include "all meats and meat containing dishes, salads, appetizers, frozen multicourse meat meals, and sandwich ingredients prepared by commercial processing or using commercially processed meats with home preparation." 21 C.F.R. § 170.3(n)(29). FDA's regulation identifies several additional food categories for which sodium propionate was determined to be GRAS, including baked goods, nonalcoholic beverages, cheeses, confections and frostings, gelatins, puddings, and fillings, jams and jellies, and soft candy. Kraft has carefully assessed the scope of this GRAS affirmation and finds it to support the safe use of sodium propionate at 0.2% in processed poultry products.

FDA affirmed sodium propionate as GRAS with no specific limitations, which means that the regulation is understood to cover both the listed uses and uses that are not significantly different. FDA explained the scope of general affirmations of GRAS status in the proposal to create this category of GRAS determinations:

> Where it is concluded after general evaluation of use of an ingredient that it is GRAS under conditions of use that presently exist or are reasonably foreseeable, it is sufficient that the regulation affirming GRAS status state that it may be used under good manufacturing practices. This type of regulation will contain the conditions and levels of use that have been reported by the 1972 NAS/NRC survey on food manufacturers pursuant to current good manufacturing practices. These reported conditions of use (the function for which it is used, the food categories in which it is used, and the maximum levels at which it is used) are not intended as rigid limitations. Variations in use of a GRAS ingredient subject to this type of regulation will be permitted as long as the new conditions of use are not significantly different from those on the basis of which the GRAS status of the substance was affirmed.

39 Fed. Reg. 34194, 34195 (Sept. 23, 1974). As FDA explained in the final regulation, "significantly different" uses would require an independent analysis of GRAS status or a food additive regulation:

> If a substance is affirmed as GRAS in part 184 . . . of this chapter with no limitation other than good manufacturing practice, it shall be regarded as GRAS if its conditions of use are not significantly different from those reported in the regulation as the basis on which the GRAS status of the substance was affirmed. If the conditions of use are significantly different, such use of the substance may not be GRAS. In such a case a manufacturer may not rely on the regulation as authorizing the use but must independently establish that the use is GRAS or must use the ingredient in accordance with a food additive regulation.

21 C.F.R. § 170.30(i); see also 21 C.F.R. § 184.1(b)(1). In other words, the use of sodium propionate in poultry products is reasonably viewed as GRAS if that category is not significantly different from the conditions identified in the GRAS regulation. From a GRAS perspective, the use of sodium propionate in processed poultry is not significantly different from Kraft's planned use in processed meat if the poultry use has a minimal effect on sodium propionate intake.

In Kraft's view, consumers are reasonably expected to treat processed meat and processed poultry as interchangeable options. Thus, a person might eat a turkey sandwich in place of a ham sandwich, but it would be unusual to consume two sandwiches, one turkey and one ham. Similarly, a person might eat a submarine sandwich that combines several different types of luncheon meats—such as turkey, ham, and bologna—but the total amount of meat consumed in such a sandwich is probably no greater than the amount consumed in a sandwich with just one type of meat.

To test these common sense conclusions, Kraft requested an analysis (provided in Appendix A) of the dietary intakes that may result from use of sodium propionate in processed meat and poultry. Based on food intake data from the National Health and Nutrition Examination Survey (NHANES), it makes little difference to dietary exposure if sodium propionate is used in processed meat only, processed poultry only, or both processed meat and poultry.

For the US population generally, consumers who eat large amounts of processed meats (i.e., consumers at the 90th percentile of intakes) and who consume meat with 0.2% sodium propionate would consume 244 mg sodium propionate per day; the corresponding intakes for processed poultry are an estimated 224 mg per day. When looking at consumers who reported eating both processed meat and processed poultry products in a single day, the combined intake of sodium propionate is estimated to be 269 mg per day—25 mg more than would be consumed from processed meat alone. Even for those populations who consume large amounts of processed meat and poultry, the use of sodium propionate in poultry has relatively little effect on dietary intakes. For example, teenage males at the 90th percentile of intakes were estimated to consume 263 mg of sodium propionate daily from processed meat, 257 mg daily from processed poultry, and 312 mg daily from processed meat and poultry combined. Thus, the addition of the processed poultry category is estimated to result in about a 49 mg increase in

sodium propionate intake as compared to processed meat alone. If consumers ate meat and poultry products in an additive way, instead of generally substituting one for the other, the increase would have been much larger.

Although a 25 to 49 mg increase might be significant to the exposure to some ingredients, for sodium propionate, this amount is not remarkable. Of particular importance, these amounts are very small as compared to intakes of sodium propionate from other food categories, such as bread and cheese. For example, for teenage males, the combined 90th percentile intakes for bread and cheese is estimated to be about 731 mg daily, and the mean for bread and cheese consumers is 389 mg daily. It is also noteworthy that both FDA and other authoritative bodies who have reviewed sodium propionate safety, such as the FAO/WHO Joint Expert Committee on Food Additives (JECFA), have found sodium propionate to be sufficiently safe that specific limitations on usage levels were unnecessary. Based on expert opinion, therefore, sodium propionate has a good safety profile.

In summary, the use of sodium propionate at 0.2% in processed meat is GRAS as provided in FDA's regulations, based on current good manufacturing practice. FDA and FSIS may view its use in processed poultry as mostly interchangeable with processed meat and equally acceptable in terms of GRAS status.

3. Safety of Antimicrobial Combinations

In addition to considering the GRAS status of individual antimicrobial ingredients, Kraft also considered the limit FDA has set on combining two or more ingredients that have the same technical effect and that are subject to specific limits when used individually. This limit is described in the GRAS regulations, which state that—

> The listing of more than one ingredient to produce the same technological effect does not authorize use of a combination of two or more ingredients to accomplish the same technological effect in any one food at a combined level greater than the highest level permitted for one of the ingredients.

21 C.F.R. § 184.1(d). Thus, if two ingredients are affirmed as GRAS for antimicrobial uses at specific, limited levels, those ingredients can be used in combination. If the GRAS regulation is relied upon as the legal basis for using the ingredients, however, the ingredients cannot be combined at the maximum levels. The combined level must be equal to or below the highest level allowed for either ingredient.

In contrast, if ingredients are affirmed as GRAS with no limits other than current good manufacturing practice, those ingredients may be used—individually or in combination—at the levels reasonably needed to provide the intended effect. For example, sodium lactate is affirmed as GRAS for use with no limitation other than current good manufacturing practice; sodium diacetate is also affirmed as GRAS consistent with current good manufacturing practice,

which the regulation states to result (at the time the regulation was adopted) in a maximum level in "meat products" of 0.1%. 21 C.F.R. §§ 184.1754, 184.1768. These ingredients are currently used in combination at levels exceeding 0.1%: in 2000, FSIS approved sodium lactate for use at levels up to 4.8% of the total formulation and sodium diacetate for use at levels up to 0.25% of the formulation. *See, e.g.*, 65 Fed. Reg. 3121 (Jan. 20, 2000).

Similarly, sodium benzoate is affirmed as GRAS at levels not to exceed current good manufacturing practice. The level on which the GRAS decision was based was 0.1%, but a higher level may lawfully be used if it is justified and not significantly different from the GRAS-affirmed level. This limit, therefore, does not restrict use of sodium benzoate alone, nor does it restrict the appropriate use of sodium benzoate in combination with other antimicrobial agents.

In developing alternative ingredients for *L. monocytogenes* control, Kraft was guided by current good manufacturing practice and did not consider the 0.1% level listed for sodium benzoate to restrict the use of other antimicrobial agents. Other antimicrobial ingredients may be used individually and in combination with sodium benzoate so long as the use is consistent with current good manufacturing practice. As described more fully in Section B below, Kraft's proposed use of sodium benzoate, sodium propionate, sodium diacetate, and other ingredients was carefully planned to meet good manufacturing practice conditions.

4. Historical Suitability of Sodium Benzoate and Propionate

Sodium benzoate has a long history of use in meat products. In the 1914 USDA Bureau of Animal Industry Regulations, "benzoate of soda" was allowed for use in meat products so long as it was declared on the label. Regulations Governing the Meat Inspection of the United States Department of Agriculture, Reg. 17, Sec. 9 and Reg. 18, Sec. 6 (1914). In the late 1940s, however, this allowance was removed without explanation. 13 Fed. Reg. 3071, 3073 (June 9, 1948).

In a 1970 final rule addressing ingredient approvals and other issues, FSIS expressed a concern that some antimicrobial agents may conceal damage or inferiority or make products appear to be better or of greater value than they are. Although the *Federal Register* preamble to the final rule did not specifically address sodium benzoate and sodium propionate, FSIS issued a regulation stating that sodium benzoate and sodium propionate may be used in or on meat and poultry products only if the use is expressly permitted in Title 9 of the Code of Federal Regulations. 9 C.F.R. § 424.23; *see also* 35 Fed. Reg. 15552 (Oct. 3, 1970). Kraft is proposing to list sodium benzoate and sodium benzoate in the FSIS regulations, and has carefully confirmed that these ingredients can be used in a way that is truthful, not misleading, and beneficial to consumers and industry.

5. Other Ingredients—Sodium Diacetate, Sodium Phosphate, and Lemon Juice Concentrate

In addition to sodium benzoate and sodium propionate, ingredients tested by Kraft included sodium diacetate and Lem-O-Fos®, a combination of 85% sodium phosphate and 15% lemon juice concentrate. Sodium diacetate is an approved antimicrobial that may be used in meat and poultry products, except infant formula and infant food, at a maximum level of 0.25% by weight of the total formulation. 9 C.F.R. § 424.21(c). Sodium phosphate is approved (as disodium phosphate) to decrease the amount of cooked-out juices in meat and poultry, and lemon juice concentrate is a common food ingredient. *Id.* Lem-O-Fos® is used in Kraft processed meat and poultry products as a combined source of sodium phosphate and lemon juice concentrate. Sodium phosphate would otherwise be added independently for the approved use, while lemon juice concentrate is used for its ability to enhance antimicrobial activity.

B. Suitability of Sodium Benzoate and Sodium Propionate for Use as Antimicrobial Agents in Processed Meats and Poultry

Through substantial product testing, Kraft has confirmed that sodium benzoate and sodium propionate are suitable for use in controlling *L. monocytogenes* in ready-to-eat meat and poultry products. Sodium benzoate and sodium propionate are useful antimicrobial agents, controlling *L. monocytogenes* as effectively as the lactate salts and sodium diacetate. These antimicrobial agents also do not conceal damage or inferiority (i.e., do not mask spoilage), do not negatively affect sensory attributes or consumer acceptance, and provide an improved nutritional profile by lowering sodium content.

1. Sodium Benzoate, Sodium Propionate, and Tested Combinations Are Effective

Current good manufacturing practice requires confirmation that an ingredient is effective for its intended use and is used at a level no greater than necessary. In searching for better ways to control *L. monocytogenes*, Kraft designed two studies to evaluate the ability of different antimicrobial agents to inhibit this pathogen while also contributing less sodium to finished products. These studies showed a need for three types of antimicrobial combinations—one for low moisture products such as hot dogs and bologna; one for higher moisture products such as ham; and one for turkey.

The first study (see Appendix B) used a statistical tool known as a response surface method design (RSM) to predict the effect of several variables, such as moisture and antimicrobial ingredients, on *L. monocytogenes* growth. As part of this study, thirty product treatments were formulated with varying levels of moisture, salt, sodium diacetate, and sodium benzoate, inoculated with *L. monocytogenes*, and evaluated for growth. All four variables are known to inhibit *L. monocytogenes* in ready-to-eat meat and poultry products, but the study was designed to help identify the optimal levels of sodium benzoate and sodium diacetate. Growth

(referred to as "time to growth" or TTG) was measured as the time it took for *L. monocytogenes* counts to grow to more than one log as compared to the inoculated level.

The resulting growth data were used to create a model to predict the effect of the chosen variables on *L. monocytogenes*. The model showed that a combination of sodium benzoate (0.1%) and diacetate (0.1%) can be used to inhibit growth in low moisture products such as hot dogs, and that both ingredients were important to achieve adequate control. At higher moisture levels, however, this combination was not effective, demonstrating that another antimicrobial would be needed.

To validate the predictive model created in the first study, a second study (see Appendix C) was designed to test specific formulations. The benzoate was locked in at 0.1% of each formula, but other ingredients varied according to anticipated need, based on the RSM model and Kraft experience. Other antimicrobial ingredients tested included sodium diacetate (tested at levels ranging from 0.05% to 0.15%), sodium propionate (added to higher moisture products at levels ranging from 0.1% to 0.2%), and Lem-O-Fos® (a combination of sodium phosphate and lemon juice concentrate tested in ham and turkey).

For most products, three types of antimicrobial combinations were found to be effective—in other words, to inhibit *L. monocytogenes* growth over the product shelf life:

(1) Sodium benzoate and sodium diacetate—a combination of 0.1% sodium benzoate and 0.1% sodium diacetate was effective in most lower moisture products such as full fat hot dogs.

(2) Sodium benzoate, sodium diacetate, and sodium propionate—a combination of 0.1% sodium benzoate, 0.15% sodium diacetate, and 0.2% sodium propionate was effective in higher moisture products such as ham. In products with greater moisture and a lower salt content, greater antimicrobial activity was generally needed to obtain the same level of inhibition as was shown for hot dog and bologna products.

(3) Sodium benzoate, sodium diacetate, sodium propionate, and Lem-O-Fos® (sodium phosphate and lemon juice concentrate)—a combination of 0.1% sodium benzoate, 0.15% sodium diacetate, 0.2% sodium propionate, and 0.56 percent Lem-O-Fos® was effective for products such as turkey. In contrast, Lem-O-Fos® did not seem to significantly affect the growth of *L. monocytogenes* in ham.

Validated combinations like those described in Appendix C represent conditions of use consistent with current food manufacturing practice. As with any antimicrobial use, the combinations we tested are necessarily linked to the specific formulations. Differences in species, moisture, ingredient composition, and other factors may significantly affect

antimicrobial activity, emphasizing the importance of a case-by-case approach to product formulation.

2. Assessment of Normal Indicators of Spoilage

Kraft also assessed whether the proposed uses of sodium benzoate and sodium propionate would affect normal indicators of spoilage. To investigate the effect of proposed antimicrobial combinations on spoilage, two shelf life studies were undertaken at the Kraft Foods Research Pilot Plant in Madison, Wisconsin. These studies are described in Appendix D.

In the first study, packages of bologna, beef franks, ham, and turkey breast were prepared using three types of formulas: a formula with no antimicrobial ingredients; a formula with sodium lactate and diacetate; and a formula containing sodium benzoate and sodium diacetate with and without sodium propionate and Lem-O-Fos® (sodium phosphate and lemon juice concentrate). All treatments for each product type contained the same level of salt and other ingredients. Each treatment was inoculated with spoilage organisms and assessed for spoilage (measured by total plate count and sensory-related signs of spoilage such as appearance and odor). In the second study, spoilage was evaluated after packages were opened and inoculated with *L. monocytogenes*.

In both cases, there was little to no difference in spoilage characteristics among the treatments evaluated, supporting a conclusion that these treatments do not mask spoilage. Normal signals of spoilage, such as slime, fading/off color, and mold growth were similar among all groups. There was also little difference in the total plate count with sodium benzoate and propionate formulations as compared to sodium lactate.

3. Effects on Sensory Attributes

To confirm that meat and poultry products made with the proposed antimicrobial ingredients are acceptable in terms of taste, aroma, and similar attributes, Kraft conducted both a trained sensory panel and consumer tests.

A five-person trained panel evaluated proposed treatments for hot dogs (Appendix E), bologna (Appendix F), ham (Appendix G), and turkey breast (Appendix H). Treatments were evaluated for basic taste, aroma, flavor, texture and other characteristics using a 15 point descriptive analysis scale in two sessions. Data were statistically analyzed and refined to eliminate scores that were too far outside the norm and thus potentially unreliable. Results showed that the tested antimicrobial combinations had no negative impact on basic taste or flavor factors for hot dogs, bologna, ham, or turkey.

To confirm consumer acceptance, Kraft conducted central location consumer tests (CLT) for bologna (Appendix I), hot dogs (Appendix J), ham (Appendix K), and turkey breast (Appendix I). The bologna CLT included questions to assess consumer opinion of various ingredients used in processed meats as compared with sodium benzoate and sodium propionate.

The results showed that consumer liking of hot dogs, ham, and turkey was not adversely affected by the proposed antimicrobial combinations. Sodium benzoate had a very minimal effect on consumer liking of bologna, most likely because the removal of sodium lactate resulted in a less salty flavor. For ham, products treated with sodium benzoate and propionate were described as having more ham and smoky flavor, less salty flavor, and a less unpleasant aftertaste. The sodium benzoate and propionate treatment for turkey had closer to "just about right" ratings in uniformity, turkey breast flavor, and oven roasted flavor. Consumer opinion of sodium benzoate or sodium propionate, as ingredients in processed meats, was no different than other commonly used ingredients.

4. Effects on Nutritional Composition

Effects of the proposed uses on nutritional composition were also evaluated with analytical testing of the hot dogs and bologna made for the central location consumer test. Moisture, protein, fat, ash, and sodium content were measured.

Sodium was reduced by 10.6% for hot dogs and 15.1% for bologna and ham as compared to formulations containing sodium lactate. Other than a reduction in ash and an increase in moisture as lactate solids are replaced by water, no other differences in nutritional composition were found. The results are shown in Appendix M.

Kraft is aware of no evidence in the scientific literature of interactions between sodium benzoate or sodium propionate and vitamins or minerals.

5. Effects of Packaging Systems

Kraft intends to use sodium benzoate and sodium benzoate/sodium propionate in ready-to-eat meat and poultry products in vacuum packaging or modified atmosphere packaging (MAP) with nitrogen and carbon dioxide. To ensure that our studies reflected realistic conditions, we used these packaging systems in evaluating efficacy and spoilage characteristics. For example, in our study of the influence of sodium benzoate and sodium diacetate on *L. monocytogenes*, products were vacuum-sealed and stored at 4°C for 18 weeks (Appendix B). In our study of spoilage, products were packaged in both vacuum packaging and MAP packages with 75% nitrogen and 25% carbon dioxide (Appendix D). We did not see any differences in the technical effect of the tested combinations as compared to sodium lactate in vacuum, MAP, or aerobic (*i.e.*, opened) storage. The type of packaging system used, therefore, is not expected to present any concern.

IV. CONCLUSION

The proposed uses of sodium benzoate and sodium propionate in combination with other acceptable ingredients offer a unique opportunity to reduce sodium while protecting food safety. The antimicrobial combinations described in this petition are as effective as ingredients like lactate salts in inhibiting *L. monocytogenes* growth, but at lower levels of use,

making it possible to reduce sodium and improve nutrition. Moreover, the proposed uses achieve these desirable results while maintaining good product quality and improving manufacturing efficiency.

The requested action has the potential to enhance the public health by prompting sodium reductions and adding new options for inhibiting *L. monocytogenes* that may be unavoidably present in commonly consumed meat and poultry products. To achieve these important benefits, Kraft asks for expedited review of this petition.

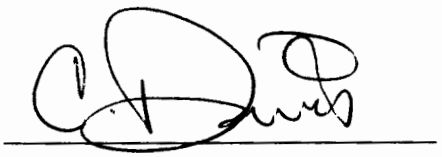
V. ENVIRONMENTAL IMPACT

The action requested by the Petition is not expected to have a significant effect on the quality of the human environment. The requested action addresses the presence of a substance in food regulated by FSIS and, therefore, is categorically excluded from any requirement to prepare an Environmental Assessment (EA) or Environmental Impact Statement (EIS) pursuant to 7 CFR § 1b.4.

VI. CERTIFICATION

To the best of our knowledge, this Petition includes all information and views on which the Petition relies, and it includes representative data and information known to Kraft that are unfavorable to the Petition.

Respectfully submitted,



Chuck Davis
Vice President
Global Convenient Meals, Technology
Kraft Foods Global Technology and Quality

APPENDIX A

Appendix A

Exponent®

Consumer Intakes from Sodium propionate uses as an antimicrobial in processed meats and poultry products: impact of adding the processed poultry category

Prepared for

Kraft Foods Global, Inc.

January 19, 2007

Consumer Intakes from Sodium propionate uses as an antimicrobial in processed meats and poultry products: impact of adding the processed poultry category

Prepared for

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January 19, 2007

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Acronyms and Abbreviations

CFR	Code of Federal Regulations
NHANES	National Health and Nutrition Examination Survey
FARE	Foods Analysis and Residue Evaluation program
GRAS	Generally Recognized As Safe
USDA	United States Department of Agriculture

Introduction and Background

Kraft Foods Global, Inc. (Kraft) asked Exponent, Inc. (Exponent) to estimate dietary intakes for the use of sodium propionate as an antimicrobial in processed meat and processed poultry products at a concentration of 0.2%. Kraft specifically asked Exponent to evaluate whether use of the antimicrobial in processed poultry products would result in intakes that are significantly different as compared to use in processed meat products alone. The question arises because the applicable Food and Drug Administration (FDA) regulation for sodium propionate (21 C.F.R. § 184.1784) identifies sodium propionate as generally recognized as safe (GRAS) for use in "meat products," but does not list poultry. Kraft approached Exponent to understand whether a proposed use in processed poultry products would be additive or competitive with the existing meat category—*i.e.*, do consumers eat foods from both categories in an additive way or do they typically choose one category or the other?

Intake estimates were derived for the entire US population and for two additional subgroups (teen age males and children from 1 to 6 years of age). The additional subgroups were selected to confirm that conclusions for the US population would be similar among subgroups of the population with high food intake levels (teenagers) and consumers who might have high intakes of a few foods (children 1-6 yrs).

FDA has affirmed sodium propionate as generally recognized as safe (GRAS) for use "in food with no limitation other than current good manufacturing practice" (GMP). 21 C.F.R. § 184.1784(c). The GRAS affirmation is based on a broad range of food categories considered to be consistent with GMP conditions of use, including baked goods, nonalcoholic beverages, cheeses, confections and frostings, gelatins, puddings, and fillings, jams and jellies, meat products, and soft candy. In addition, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the toxicity data for sodium propionate and concluded that it was essentially non-toxic and assigned it to the category of additives for which the "ADI is not specified." JECFA provided an additional explanation of the term "ADI not specified":

> "JECFA documents note that an "ADI 'not specified' is applied to a food substance of very little toxicity which, on the basis of the available chemical, biological, toxicological, and other data and the total dietary intake of the substance from its use at the levels necessary to achieve the desired effect and from its acceptable background level in food, does not, in the opinion of the Committee, represent a hazard to health. For this reason and for reasons stated in the individual evaluations, the establishment of an ADI in numerical form is deemed unnecessary." (WHO Food Additives Series, 2000)

Substances assigned an ADI not specified are assumed by JECFA to be allowable at GMP levels and no maximum limits are recommended by JECFA or established by the Codex Alimentarius.

Methods

Dietary consumption of sodium propionate was estimated using the following sources: Exponent's Foods Analysis and Residue Evaluation Program (FARE™) software version 7.98, data from the National Center for Health Statistics, National Health and Nutrition Examination Survey (NHANES) and a 0.2% use level for sodium propionate in all processed meat and poultry products. It was conservatively assumed that all foods within each of the categories would be treated at this level and that every time a consumer ate a food from one of these categories it would have been treated.

Food Consumption Data

The most recent national food consumption data - the NHANE Survey - that allows estimation of processed meat and poultry products was selected for this analysis. The NHANES 1999-2002 survey is a complex multistage probability sample of the civilian US population. It is designed to give annual samples that are nationally representative of the US population. The survey collects 1-day food intake data, in addition to nutrition, demographic, and health information. The NHANES survey over-samples minorities, low-income groups, and children, and statistical weights are provided by NCHS to adjust for the differential probabilities of selection. The NHANES surveys are administered in different locations in the US over the 2-year period and involve interviews, a physical exam, and laboratory tests done on location in mobile vehicles.

Participants included 9,965 subjects in the 1999-2000 survey and 11,039 subjects in the 2001-2002 survey. Three populations were selected for the current analyses: the entire US population, teen age males and children 1-6 years of age.

Consumer dietary practices vary from individual to individual and from day to day. The variation among consumers is captured by the large numbers of individuals who were surveyed in the NHANE survey. Variations in diet by the same consumer from day to day are more difficult to estimate. Long term (many days) of information about dietary practices provide more realistic (and reliable) estimates of intake. However, the available survey data for NHANES contain only a single day's dietary record for each respondent. Multiple studies have reported that single-day surveys overestimate the intake of foods. Therefore, the estimates contained in this report are conservative overestimates of potential consumer intake.

The National Center for Health Statistics at the Centers for Disease Control (NCHS, 1996) has published guidelines on statistical reporting standards for the NHANES survey. These guidelines identify the minimum sample size needed to estimate various statistics of distributions derived from simple random samples as well as surveys, such as the NHANES survey, which use a more complex sample design. Exponent follows those guidelines in reporting results.

Intake Methodology

To determine the intake of sodium propionate, Kraft's proposed use level (0.2% or 2000 ppm) was multiplied by the amount of the selected food consumed. Where a mixed dish was consumed and the sodium propionate was used on only some of the ingredients, Exponent applied "recipes" to estimate the proportion of the mixed dish that is reasonably expected to contain processed meat or poultry. These "recipes" are based on recipes released by the US Department of Agriculture (USDA) along with the survey. Where USDA did not identify all of the ingredients in a particular food, Exponent had developed additional "recipes" to further disaggregate the food. These "recipes" are incorporated into FARE and have been vetted extensively and the results used for regulatory submissions for more than 20 years.

Three measures of consumption were determined: Per capita, per user and the 90^{th} percentile user intake. Per user estimates look at intake of respondents who consumed the food(s) of interest only, whereas *per capita* estimates include intakes from all respondents irrespective of whether they consumed the food(s) of interest or not. A "user" is anyone who reported consuming at least one of the selected foods on the day that they were surveyed by NHANES. Per capita and per user estimates are presented in this report.

For each individual, the total daily intake of sodium propionate was determined by summing the intake of sodium propionate from each processed meat or poultry item they ate that could contain sodium propionate. To determine the impact on consumer intake of adding processed poultry products to the categories of food treated with sodium propionate, four analyses of sodium propionate intake were conducted: (1) processed meat products (which are included in the category of "meat products" identified in the GRAS regulation); (2) processed poultry products; (3) combined processed meats and poultry and (4) baked goods and cheeses. Sandwiches and meat-containing cheese dishes were excluded from category four. The categories of foods included in each of the analyses are presented in Table 1. The individual food codes within each of these categories are listed in Appendix 1.

Table 1. Food Categories Included in the Analyses

Food Category
Processed meat products (beef and pork) with sodium propionate at a level of 0.2%
Processed poultry products (chicken and turkey) with sodium propionate at a level of 0.2%
Combined processed meat and poultry products with sodium propionate at a level of 0.2%
Baked goods and cheese with sodium propionate at a level of 0.2% and .27% respectively (sandwiches and mixed cheese/meat dishes were excluded)

Results and Discussion

The mean per capita, mean and 90th percentile per user dietary intake of sodium propionate use in processed meat, processed poultry and baked goods and cheeses are presented in Table 2.

Table 2. Sodium propionate intake by the US population, teenagers and children (1-6 yrs of age) from processed meats, processed poultry, combined processed meats and poultry and baked goods/cheeses (mg/day)

Population		Processed meats alone	Processed poultry alone	Combined processed meats and poultry	Baked goods and cheeses alone[1]
US Population	Mean per capita	39	19	58	258
	Mean per user	119	109	136	296
	90th percentile user	244	224	269	590
Teen Age Males 13-19 years	Mean per capita	45	31	75	296
	Mean per user	132	157	165	351
	90th percentile user	263	257	312	678
Children 1-6 yrs	Mean per capita	29	19	48	172
	Mean per user	84	85	98	195
	90th percentile user	168	146	188	385

The results presented in Table 2 demonstrate that the addition of the processed poultry category will not substantially change the intake of sodium propionate even under the worst case assumptions used in this report. This finding is not unexpected. It would be anticipated that these food product categories are largely competitive—in other words,

[1] Sandwiches and mixed cheese/meat dishes were excluded in order to avoid double counting exposures

most consumers will eat foods from one category or the other, but not both. In those instances where a consumer might have more than one processed meat or poultry product, the products would be consumed at different meals or in divided portions (e.g., a sandwich with half the meat as processed beef and half as processed poultry).

In order to understand the likely importance of small increments in sodium propionate intake, consumer intakes from other food categories were evaluated. Two categories – baked goods and cheeses – were identified as having GMP levels similar to those in processed meats. The GMP level for ba[2]ked goods has been reported as 2000 ppm and for cheeses 2700 ppm (FASEB, 1979). Baked goods and cheeses are eaten frequently. The estimated intakes of sodium propionate are presented in Table 2. As can be seen, the baked goods/cheese categories contribute intakes that are substantially higher than the meat and poultry contributions.

For the entire US population, the mean per user dietary intake of sodium propionate from FDA-listed uses in processed meats is 119 mg/day. The corresponding mean per user for uses in processed poultry products would be 109 mg/day. The intake from uses in both processed meat and poultry products would be 136 mg/day. By comparison, the mean per user intakes from breads and cheeses is 318 mg/day.

Table 2 also provides the estimates of intake by consumers who eat the more of these categories than the typical consumer. The 90th percentile consumer of processed meats with 0.2% sodium propionate would consume 244 mg sodium propionate per day. The corresponding intakes for the poultry and combined intake estimates are 224 and 269 mg/day, respectively. The 90th percentile consumer of baked goods and cheeses with 0.2% sodium propionate would consume at least 567 mg/day[3]. The estimated combined intake for the high (90th percentile) consumer is only 10% higher than the intakes from the FDA-listed processed meat categories and less than half of the intake that could come from baked goods and cheese.

Males ages 13-19 have somewhat higher intakes than the US population (Table 2), but the relative differences between the categories are similar. The conservative "worst case" intake of 312 mg/day from processed meats and poultry is only 40% of the corresponding intakes from baked goods and cheeses (678 mg/day), further supporting a conclusion that a use in both categories does not represent a meaningful increase in sodium propionate intake.

Children 1-6 years of age would have lower daily intakes of sodium propionate from all of the uses that were evaluated. Like the US population as a whole and teen age males, there are only very small incremental increases due to the expansion of the processed meat category to include poultry. As with the US population and teen age males, breads and cheeses represent a much larger source of sodium propionate intake.

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[3] This estimate excluded breads eaten as part of a sandwich, cheeses consumed in meat-containing dishes and all fried snack foods

It should be noted that the absolute values presented in this report represent conservative overestimates of potential intakes. In particular, it was assumed that all foods within each of the categories would contain 0.2% sodium propionate and that every time a consumer ate a food from one of these categories it would have been treated.

CONCLUSIONS

The intake assessments presented in this report demonstrate that there is no meaningful additional increase in daily consumer intakes of sodium propionate when FDA-listed uses in processed meats (i.e., FDA's regulation identifying sodium propionate as GRAS in meat products with no limit other than GMP) are expanded to include processed poultry. These conditions of use—processed meats and processed poultry—tend to substitute for one another and are not significantly different from the perspective of dietary exposure and safety. Further, within each population group, the highest intake levels reported for the *90th percentile* consumer of the combined meat and poultry category are actually lower than the *mean* intake of sodium propionate from the consumption of the bread and cheese categories.

References

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Appendix 1: Foods included in the analyses: (intakes calculated for meat component of the food)

Food code or group	NFCSCODES "processed beef, pork, veal, and poultry products"	USED IN ANALYSIS of: MEAT Intakes INTAKES	POULTRY INTAKES	COMBINED MEAT and POULTRY
20	MEAT, NS AS TO TYPE	X		X
21002000	BEEF, PICKLED	X		X
216	OTHER BEEF ITEMS (BEEF BACON; DRIED BEEF; PASTRAMI)	X		X
223	HAM	X		X
22421000	PORK ROAST, SMOKED OR CURED, COOKED, NS AS TO FAT	X		X
22421010	PORK ROAST, SMOKED OR CURED, COOKED, LEAN & FAT	X		X
22421020	PORK ROAST, SMOKED OR CURED, COOKED, LEAN ONLY	X		X
225	CANADIAN BACON	X		X
226	BACON, SALT PORK	X		X
24198540	CHICKEN, CANNED, MEAT ONLY, NS AS TO LIGHT OR DARK		X	X
24198550	CHICKEN, CANNED, MEAT ONLY, LIGHT MEAT		X	X
24198570	CHICKEN, CANNED, MEAT ONLY, LIGHT & DARK MEAT		X	X
24198640	CHICKEN ROLL, ROASTED, NS AS TO LIGHT OR DARK MEAT		X	X
24198700	CHICKEN PATTY/FILLET/TENDERS, BREADED, COOKED		X	X
24198740	CHICKEN NUGGETS		X	X
24198840	CHICKEN CRACKLING, P.R. (CHICHARRON DE POLLO)		X	X
24201000	TURKEY, NFS		X	X
24201500	TURKEY, SMOKED, NS AS TO SKIN		X	X
24201510	TURKEY, SMOKED, SKIN EATEN		X	X
24201520	TURKEY, SMOKED, SKIN NOT EATEN		X	X
24206000	TURKEY, CANNED		X	X
24208000	TURKEY NUGGETS		X	X
24208500	TURKEY BACON, COOKED		X	X
2521 EXCEPT 25210210 25210220 25210230 25210310 25210410 25210610	FRANKFURTER OR HOT DOG	X		X
25220010	COLD CUT, NFS	X		X
25220100	BEEF SAUSAGE, NFS	X		X
25220110	BEEF SAUSAGE, BROWN & SERVE, LINKS, COOKED	X		X
25220120	BEEF SAUSAGE, SMOKED, STICK (INCLUDE BEEF JERKY)	X		X
25220130	BEEF SAUSAGE, SMOKED	X		X

25220140	BEEF SAUSAGE, FRESH, BULK, PATTY OR LINK, COOKED	X		X
25220210	BLOOD SAUSAGE	X		X
25220350	BRATWURST, COOKED	X		X
25220360	BRATWURST W/ CHEESE	X		X
25220390	BOLOGNA, BEEF, LOW FAT	X		X
25220400	BOLOGNA, PORK AND BEEF	X		X
25220410	BOLOGNA, NFS	X		X
25220420	BOLOGNA, LEBANON	X		X
25220430	BOLOGNA, BEEF	X		X
25220440	BOLOGNA, TURKEY		X	X
25220450	BOLOGNA RING, SMOKED	X		X
25220460	BOLOGNA, PORK	X		X
25220470	BOLOGNA, BEEF, LOWER SODIUM	X		X
25220480	BOLOGNA, CHICKEN, BEEF, & PORK	X	X	X
25220490	BOLOGNA, W/ CHEESE	X		X
25220500	BOLOGNA, BEEF & PORK, LOWFAT	X		X
25220510	CAPICOLA	X		X
25220650	CHICKEN & BEEF SAUSAGE, SMOKED	X		X
25220710	CHORIZOS	X		X
25220910	HEAD CHEESE	X		X
25221210	MORTADELLA	X		X
25221250	PEPPERONI	X		X
25221310	POLISH SAUSAGE	X		X
25221350	ITALIAN SAUSAGE	X		X
25221400	SAUSAGE (NOT COLD CUT), NFS	X		X
25221410	PORK SAUSAGE, FRESH, BULK, PATTY OR LINK, COOKED	X		X
25221420	PORK SAUSAGE, BROWN & SERVE, COOKED	X		X
25221450	PORK SAUSAGE RICE LINKS,BROWN & SERVE, COOKED	X		X
25221460	PORK & BEEF SAUSAGE	X		X
25221470	PORK & BEEF SAUSAGE, BROWN & SERVE, COOKED	X		X
25221500	SALAMI, NFS	X		X
25221510	SALAMI, SOFT, COOKED	X		X
25221520	SALAMI, DRY OR HARD	X		X
25221530	SALAMI, BEEF	X		X
25221610	SCRAPPLE, COOKED	X		X
25221650	SMOKED LINK SAUSAGE, PORK	X		X
25221660	SMOKED LINK SAUSAGE, PORK & BEEF	X		X
25221680	SMOKED SAUSAGE, PORK	X		X
25221810	THURINGER (INCLUDE SUMMER SAUSAGE)	X		X
25221840	TURKEY BREAKFAST SAUSAGE, BULK		X	X
25221850	TURKEY SAUSAGE, SMOKED		X	X
25221860	TURKEY SAUSAGE, REDUCED FAT, BROWN & SERVE, COOKED		X	X

25221870	TURKEY&PORK SAUSAGE,FRESH,BULK,PATTY OR LINK,COOKED		X	X
25221880	TURKEY, PORK, & BEEF SAUSAGE, REDUCED FAT, SMOKED		X	X
25221890	TURKEY, PORK, & BEEF SAUSAGE, LOWFAT, SMOKED		X	X
25221910	VIENNA SAUSAGE, CANNED	X		X
25221920	VIENNA SAUSAGE, CHICKEN, CANNED		X	X
25221950	PICKLED SAUSAGE	X		X
2523	**LUNCHMEATS (entire category used in combined; subcodes as listed below in other analyses):**			X
25230110	LUNCHEON MEAT, NFS	X		X
25230310	CHICKEN/TURKEY LOAF, PREPACK/DELI, LUNCHEON MEAT		X	X
25230550	HAM, PORK & CHICKEN, LUNCHEON MEAT, CHOPPED, CAN, RED SODIUM	X		X
25230610	LUNCHEON LOAF (OLIVE, PICKLE OR PIMIENTO)	X		X
25230710	SANDWICH LOAF, LUNCHEON MEAT	X		X
25230790	TURKEY HAM, SLICED, XTRA LEAN, PKG'D, DELI		X	X
25230800	TURKEY HAM		X	X
25230820	TURKEY PASTRAMI		X	X
25230840	TURKEY SALAMI		X	X
25230900	TURKEY OR CHICKEN BREAST, PKG'D/DELI, LUNCHEON MEAT		X	X
2524 EXCEPT 25240210 25240220 25240310	MEAT SPREADS	X		X
27220010	MEAT LOAF MADE W/ HAM (NOT LUNCHEON MEAT)	X		X
27220080	HAM CROQUETTE	X		X
27246300	CHICKEN OR TURKEY CAKE, PATTY OR CROQUETTE		X	X
27246500	MEAT LOAF MADE W/ CHICKEN OR TURKEY		X	X
27500300	WRAP SANDWICH FILLED WITH MEAT, POULTRY, OR FISH, AND VEGETABLES	X	X	X
2754	SANDWICHES WITH POULTRY		X	X
2756	**SANDWICHES WITH MEAT & POULTRY (entire category used in combined: subcodes as listed below in other analyses: X**			X
27560300	CORN DOG (FRANKFURTER/HOT DOG W/ CORNBREAD COATING)	X	X	X
27560350	PIG IN A BLANKET (FRANKFURTER OR HOT DOG WRAPPED IN DOUGH)	X	X	X
27560360	FRANKFURTER/HOT DOG ,W/ CHILI, ON BUN (INCL CHILI DOG)	X	X	X
27560370	FRANKFURTER /DOG CHILI & CHEESE,BUN (CHILI CHEESE DOG)	X	X	X
27560710	SAUSAGE SANDWICH	X	X	X
27560910	SUB, COLD CUT SANDWICH, ON BUN, W/ LETTUCE	X	X	X

END

APPENDIX B

Appendix B: Influence of Sodium Benzoate in RTE Meat Products

Numerous factors inhibit the growth of *Listeria monocytogenes* in ready-to-eat meat products, including reduced moisture, salt, sodium lactate and sodium diacetate. At levels capable of suppressing *L. monocytogenes* growth, however, sodium lactate contributes more sodium than other substances that may be equally effective, such as sodium benzoate. To help identify levels at which sodium benzoate may be used in place of sodium lactate to inhibit the growth of *L. monocytogenes*, Kraft undertook a study at the Kraft Foods Research Pilot Plant in Madison, Wisconsin.

The study used a statistical tool known as a response surface method design to predict the effect of several variables on *L. monocytogenes* growth. Thirty product treatments were formulated with varying levels of moisture, salt, sodium diacetate, and sodium benzoate, inoculated with six strains of *L. monocytogenes*, and evaluated for growth. Results were used to develop a model to predict the effect of the identified variables, including sodium benzoate content, on *L. monocytogenes* growth.

MATERIALS AND METHODS

Sample Preparation

The statistical design required 30 treatments. The product for each treatment was formulated using a variety of raw material sources including 42 pork trimmings, trimmed turkey breast halves, and four muscle ham (including the *M. semimembranosus, M. semitendinosus, M. adductor, and M. gluteus medius*) purchased from commercial sources. All meats were ground 0.634 cm immediately prior to use. Each product also contained dry corn syrup solids, sodium erythrobate, modified starch (Firmtex®, National Starch, Bridgewater, NJ), sodium nitrite, carrageenan (Gelcarin® PS 4302, FMC Corporation, Princeton, NJ, 08543), sodium tripolyphosphate, and water (amounts varied depending upon the desired finished product moisture content). The products were made over three days.

The 30 separate product treatments were formulated using a linear program (*What's Best!*, Lindo Systems, Inc., Chicago, IL 60614) to determine the proper amount of water to include to obtain the formulation set points shown in Table 1. These products required mixtures of the various meats listed above. All meats, dry ingredients, and water were blended under vacuum for 30 minutes at 45 rpm using a 220 kg Keebler mixer (Keebler Engineering, Inc.). The meat batters were subsequently stuffed into non-permeable casings (3.3 cm diameter) and cooked in water tanks using the following schedule: one hr at 49°C, one hour at 60°C, and approximately 2 hr at 85°C until the products reached in internal temperature of 74°C. The products were chilled in 4°C water and subsequently chilled in a 0°C cooler so that the internal temperature of the products was less than 4°C within 8 hours of cooking.

After chilling, the products were stripped of their casings and sliced into 25 gram slices; four slices were placed into pouches (Curlon® Grade 863, nylon structure with polyethylene seal and PVDC barrier, OTR = <1.0 cc/645 cm^2/24 hr @ 23°C and 0% relative humidity, MVTR = <0.5 g/645 cm^2/24 hr @ 38°C and 90% relative humidity, Curwood, Oshkosh, WI) and inoculated with a six strain cocktail of *L. monocytogenes*. The pouches were immediately vacuum-sealed (Multivac C1400) and stored at 4°C for 18 weeks. Three randomly selected pouches per treatment were removed from storage every two weeks for 18 weeks and tested for *L. monocytogenes.*

Analytical Procedures

Chemical analysis

Samples of each treatment were submitted for analysis of moisture, protein, fat, ash, NaCl, lactate, diacetate, and pH.

Microbiological Methods

Bacterial strains and growth conditions. Four *L. monocytogenes* isolates from foodborne outbreaks (CDC861, F2379, an NFPA strain, and one from an outbreak associated with hot dogs) and two environmental isolates from a ready-to-eat meat manufacturing facility (MAD328, MAD225) were used as a six-strain cocktail throughout the studies. Strains were grown aerobically, without shaking, in 10-ml of Brain Heart Infusion broth (Becton Dickinson, Sparks, MD) for 24 h at 35°C, allowing the cultures to reach late stationary phase. Culture (0.1-ml) was transferred to 10-ml of fresh brain heart infusion broth and the incubation repeated.

Inoculum preparation and procedure. The inoculum was prepared by transferring 0.2-ml of each strain into 99-ml of Butterfield's phosphate buffer (Weber Scientific, Hamilton, NJ). Serial dilutions were made to achieve the desired inoculum level, approximately 10 - 100 cfu/g or 1,000 - 10,000 cfu/package. The inoculum (100-µl total) was applied to the surface of 100g of meat. One 50-µl aliquot was placed between slices and one was placed on the surface of the top slice. The pouches containing the meat were immediately vacuum-sealed (Multivac C1400) and stored at 4°C for up to 18 weeks.

Evaluation and enumeration of *L. monocytogenes.* Three samples of each treatment were analyzed every other week for *L. monocytogenes* by appropriately diluting the samples in Butterfield's phosphate buffer, direct plating onto Total Plate Count agar (TPC) and Modified Oxford agar (MOX; EMD Chemicals, Gibbstown, NJ) and incubating plates for 48 h at 35°C. Colonies producing a black precipitate on MOX, indicative of *Listeria* spp., were considered *L. monocytogenes* colonies.

Experimental Design

The statistical analysis method used is called a response surface method (RSM) design. An RSM study characterizes the relationship between several variables (in this case, moisture, salt, sodium diacetate, and sodium benzoate) and a response variable (time to growth of *L. monocytogenes*). The specific RSM used was a four-factor central composite design complete with star points and replicated center points. The factors and levels used are reported in Table 1, and the design matrix is shown in Table 3 at the end of this appendix.

This four-factor experiment was designed as a rotatable cube central composite RSM design. The design consisted of 16 factorial treatments augmented with eight star points (axial points) and six center points for a total of 30 treatments (Table 1). The radius for the star points was calculated by $2^{k/4}$ where k = the number of variables in the model (Cochran and Cox, 1957).

Establishing time-to-growth (TTG)

Growth curves of all modeling treatments were plotted using Microsoft Excel (Microsoft, Redmond, Wash.). The time-to-growth (TTG) was determined to be when the *L. monocytogenes* counts observed were greater than a one-log increase from the inoculated level. Given the inherent variability in the data, a one log increase was the smallest that could be reliably detected. An expert panel of food microbiologists has specified a one log increase as an acceptable criterion for *L. monocytogenes* (Institute of Food Technologists, 2001).

Four individuals reviewed the growth curves for each product and assigned a time to growth when, in their judgment, growth had a sustained one-log increase from the original inoculation level. The TTG values were averaged to determine the TTG for that particular treatment. In samples exhibiting no growth over one log at 18 weeks (the maximum code date that would be used at this time), TTG was recorded as 18 weeks and the observation was coded as "censored". Only observations where all four judges considered the TTG to be greater than 18 weeks were considered to be censored. Of the total 30 observations, 18 were censored (60%) and 12 were not (40%). The entire data set is shown in Table 4.

Statistical analysis

The experimental variables were normalized by assigning the codes shown in Table 1. These values were used to develop an equation to predict TTG at any combination of the four factors (salt, benzoate, diacetate, moisture). The equation was developed by inputting the variables and data in the MINITAB statistical analysis program (MINITAB® release 14, Minitab, Inc.).

Table 1. Experimental Design

Factor Code	**-2**	**-1**	**0**	**1**	**2**
Salt (%)	0.2	0.8	1.4	2.0	2.6
Benzoate (%)	0	0.08	0.165	0.25	0.335
Diacetate (%)	0	0.05	0.1	0.15	0.2
Moisture (of finished product) (%)	45	55	65	75	85

In Minitab, the coded values were input as factors in the "Regression with Life Data Analysis" option using the Weibull distribution. It uses what is called a maximum likelihood estimation function, which is a best fit model that takes the censored data into account. This option allowed development of a predictive model of the natural logarithm of TTG as a function of the four factors plus their two- and three-way interactions.

RESULTS

Analytical Data

The targeted and actual levels for each experimental variable are shown in Table 5. Most of the actual results were within experimental error, so the original targets were used in the data analysis. Treatment number 22, however, missed the targeted moisture of 45% with an actual moisture content of 64%. Changing the coding coefficient to match the actual moisture content did not change the results appreciably. The product pH was highly correlated with the sodium diacetate content.

Data Analysis

Table 2 shows the regression coefficients for the equation that can be used to predict TTG. The table includes the main factors, their two- and three-way interactions plus their regression coefficients, standard errors, Z values, and probability values. All main factors significantly influenced the TTG except product moisture. Moisture, however, was a significant factor when it interacted with the other factors in both the two-way and three-interactions. Consequently, it was left in the final model.

Due to the large number of censored data points, the statistical program could not analyze the squared terms and interaction terms at the same time. The model was calculated both ways (with the squared terms in place of some of the interaction values and with the interaction values without the squared terms). The squared terms (salt^2, benzoate^2, diacetate^2 and moisture^2) and the day of manufacture effect term were not statistically significant and therefore were not included in the final model.

Table 2. Regression coefficients for the Four-Factor RSM model[1]

Variable[1]	*Estimate*	*Standard error*	*Z*	*Probability*[2]
Intercept	2.91691	0.01835	158.94	0.000
Salt	0.56124	0.01498	37.46	0.000
Benzoate	1.00151	0.01409	71.10	0.000
Diacetate	0.54083	0.04198	36.10	0.000
Moisture	-0.002001	0.01393	-0.14	0.886
Salt x Benzoate	0.166310	0.01979	8.41	0.000
Salt x Diacetate	0.31550	0.02864	11.02	0.000
Salt x Moisture	-0.101357	0.02863	-3.54	0.000
Benzoate x Diacetate	-0.071808	0.01979	-3.63	0.000
Benzoate x Moisture	0.356149	0.02186	16.29	0.000
Diacetate x Moisture	-0.265609	0.02863	-9.28	0.000
Salt x Benzoate x Diacetate	-0.080852	0.03147	-2.37	0.018
Salt x Benzoate x Moisture	0.221129	0.02829	7.82	0.000
Salt x Diacetate x Moisture	-0.373129	0.01808	-20.64	0.000
Benzoate x Diacetate x Moisture	0.126852	0.02829	4.48	0.000
Shape	43.7990	12.2618		

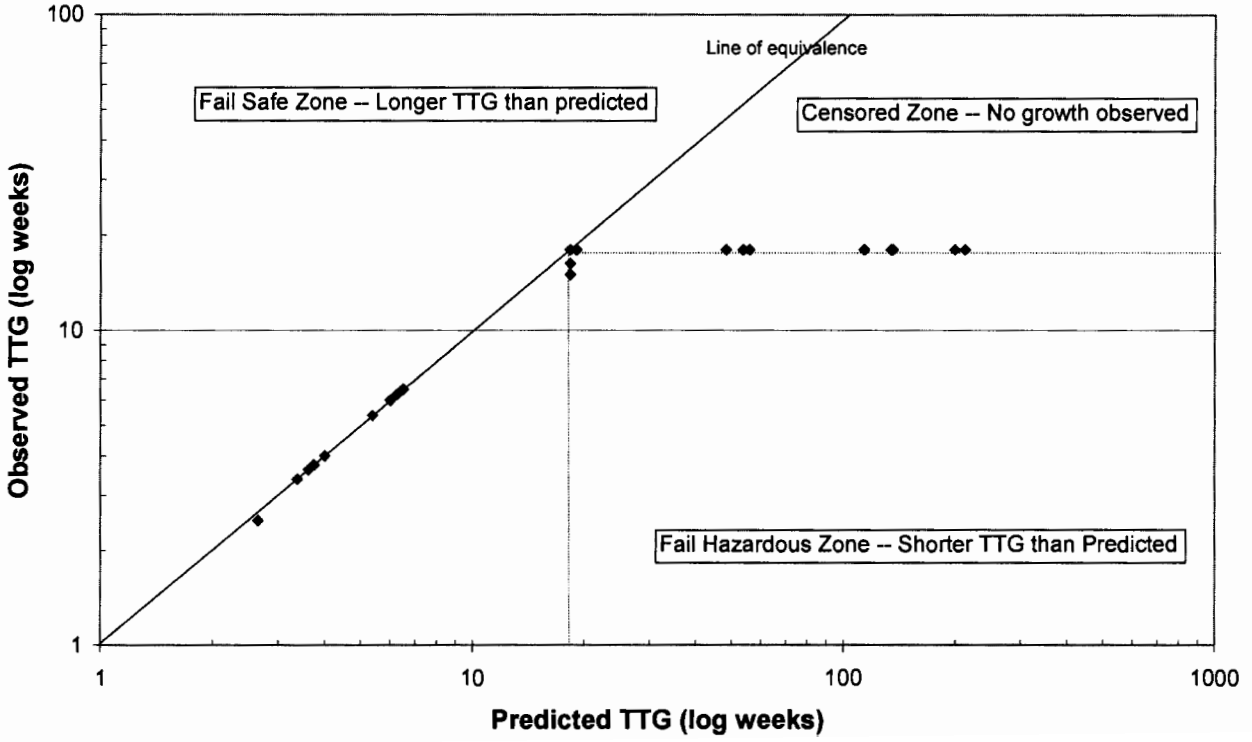
[1] Variable analyzed using the coded values (-2, -1, 0, 1, 2).

[2] Variables with probability values less than 0.05 were included in the model except for moisture. Moisture was included because all the interactions with moisture were significant.

Observed vs. Predicted Values

The usefulness of the model can be determined by comparing the observed vs. predicted values of the samples. These values are shown in Table 6 and plotted in Figure 1. Note in Figure 1 below that the points fall along the equivalence line (points where observed TTG values equal predicted values) until 18 weeks. Because the study stopped at 18 weeks, there are no observed values beyond 18 weeks, and the predicted values fall on a horizontal line. Significantly, there is good agreement between the observed and predicted values.

Fig. 1. Observed vs. Predicted TTG Values

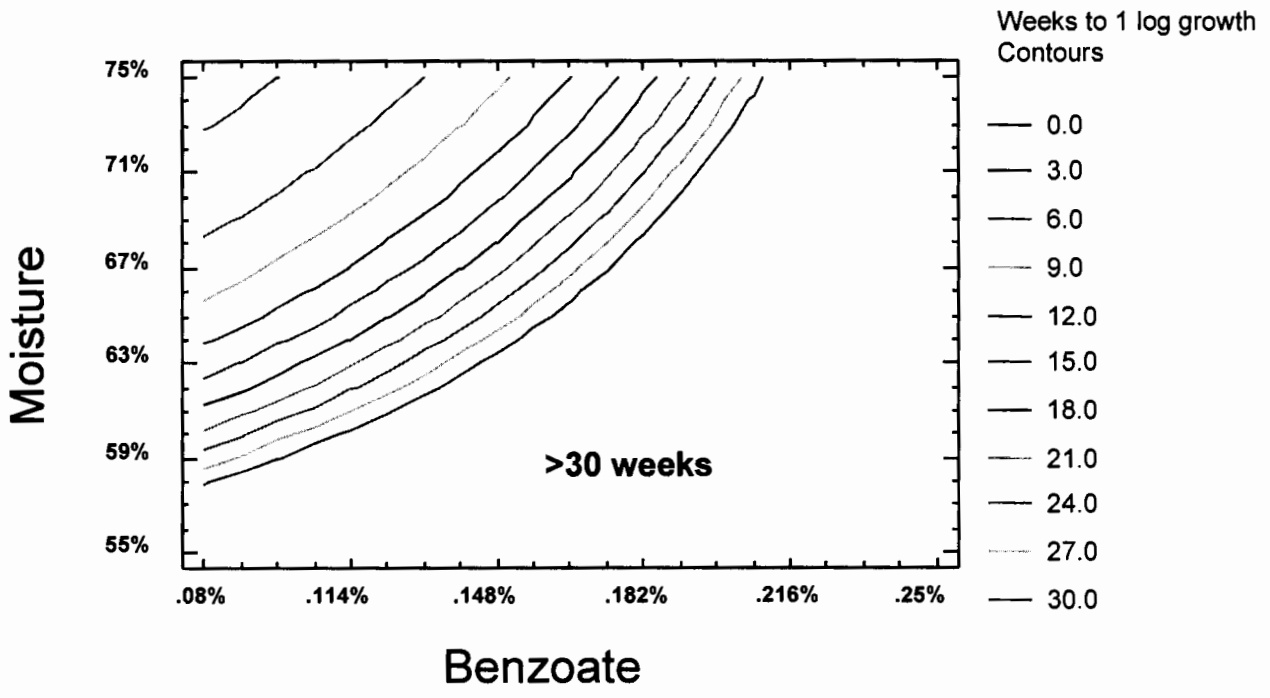


Response of TTG to benzoate and moisture

Fig. 2 shows predicted TTG (in the form of a contour plot) at varying concentrations of moisture and sodium benzoate with fixed concentrations of salt (2%) and sodium diacetate (0.1%). Note that there is a large area when TTG exceeds 30 weeks. This region could also be called a "no growth" region since it would exceed the shelf life of products stored at 4°C.

The no growth region could be maintained by various combinations of benzoate and moisture. If benzoate usage were restricted to 0.1%, values in the no growth region could only be obtained with lower moisture products such as hot dogs and bologna. In such products, lactate could be replaced with 0.1% sodium benzoate. However, products formulated to higher moisture contents such as ham and turkey breast, would have a much shorter predicted TTG when using 0.1% benzoate (about 3 weeks). For these products, either diacetate must be increased and/or one or more other antimicrobial ingredients are needed in addition to sodium benzoate to provide a sufficiently long TTG.

Fig. 2. Contour plot of TTG (time to growth) for a sample formulated to contain 2% salt and 0.1% sodium diacetate.

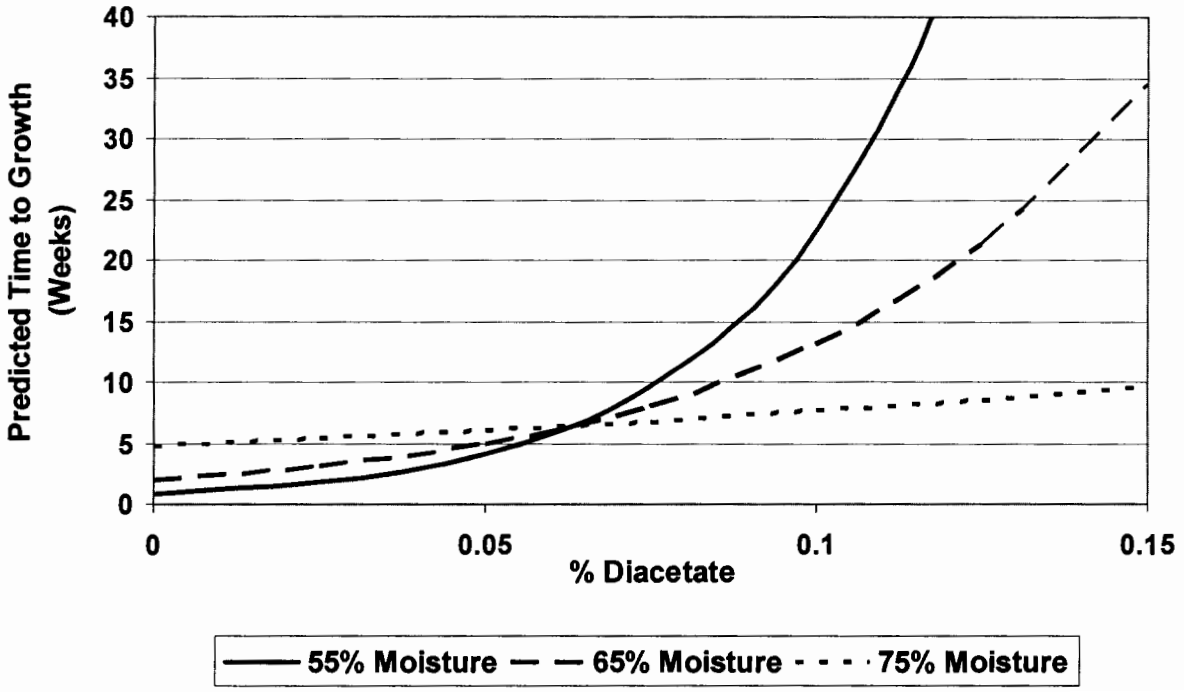


Response of TTG to diacetate and moisture

Figure 3 shows predicted time to growth of *L. monocytogenes* from the RSM model at varying levels of sodium diacetate. For the graph, salt was fixed at 2% and sodium benzoate was fixed at 0.1%. Three finished product moisture levels are plotted.

This graph shows that unless diacetate is used, 0.1% benzoate will not provide practical inhibition of *L. monocytogenes* for product at every moisture level (TTG is less than 5 weeks). It also shows that diacetate has a much larger effect on growth in lower moisture items (55% moisture) versus higher moisture items (75% moisture).

Figure 3. Effect of Diacetate in the RSM Model
For Salt at 2% and Benzoate at 0.1%



SUMMARY of RSM DATA

This study used a response surface design methodology to gather data and develop a predictive model for estimating TTG at various concentrations of moisture, salt, sodium benzoate, and sodium diacetate. The model demonstrated that—

- All four factors included in the four-factor RSM, either singly or in the interaction terms, significantly affected TTG of *L. monocytogenes* in vacuum packaged samples stored at 4°C.

- A combination of benzoate and diacetate can be used to lengthen TTG; however, at higher moisture levels, the combination is not sufficient and the addition of another antimicrobial, such as sodium propionate, is needed to obtain acceptable TTG values.

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Institute of Food Technologists, 2001 Evaluation and definition of potentially hazardous foods: A report of the Institute of Food Technologists for the Food and Drug Administration of the U.S. Department of Health and Human Services. IFT/FDA contract no. 223-98-2333. Task order no. 4, chap. 6. Available at: http://www.foodprotect.org/pdf/hazard_foods/chapter6.pdf. Accessed 14 December, 2006.

Table 3. Design Matrix for the Four-Factor RSM

Block	Salt (%)	Benzoate (%)	Diacetate (%)	Moisture (%)
1	0.8	0.08	0.15	55
1	1.4	0.165	0.1	65
1	0.8	0.25	0.05	55
1	2	0.25	0.05	75
1	0.8	0.08	0.05	75
1	2	0.08	0.15	75
1	2	0.25	0.15	55
1	0.8	0.25	0.15	75
1	1.4	0.165	0.1	65
1	2	0.08	0.05	55
2	0.8	0.08	0.15	75
2	0.8	0.08	0.05	55
2	0.8	0.25	0.15	55
2	1.4	0.165	0.1	65
2	1.4	0.165	0.1	65
2	0.8	0.25	0.05	75
2	2	0.08	0.05	75
2	2	0.25	0.15	75
2	2	0.08	0.15	55
2	2	0.25	0.05	55
3	1.4	0	0.1	65
3	1.4	0.165	0.1	45
3	2.6	0.165	0.1	65
3	1.4	0.165	0.1	65
3	1.4	0.165	0.2	65
3	1.4	0.165	0.1	65
3	1.4	0.165	0	65
3	1.4	0.165	0.1	85
3	1.4	0.335	0.1	65
3	0.2	0.165	0.1	65

Table 4. RSM data set.[1]

Block	Salt	Benzoate	Diacetate	Moisture	Salt%	Benzoate %	Diacetate %	Moisture%	Treatment	Censor	Time to one log (eyeball)
1	-1	-1	1	-1	0.8	0.08	0.15	55	1	0	6
1	0	0	0	0	1.4	0.165	0.1	65	2	0	16.25
1	-1	1	-1	-1	0.8	0.25	0.05	55	3	1	18
1	1	1	-1	1	2	0.25	0.05	75	4	1	18
1	-1	-1	-1	1	0.8	0.08	0.05	75	5	0	3.625
1	1	-1	1	1	2	0.08	0.15	75	6	0	6.5
1	1	1	1	-1	2	0.25	0.15	55	7	1	18
1	-1	1	1	1	0.8	0.25	0.15	75	8	1	18
1	0	0	0	0	1.4	0.165	0.1	65	9	1	18
1	1	-1	-1	-1	2	0.08	0.05	55	10	0	3.375
2	-1	-1	1	1	0.8	0.08	0.15	75	11	0	5.375
2	-1	-1	-1	-1	0.8	0.08	0.05	55	12	0	3.75
2	-1	1	1	-1	0.8	0.25	0.15	55	13	1	18
2	0	0	0	0	1.4	0.165	0.1	65	14	1	18
2	0	0	0	0	1.4	0.165	0.1	65	15	0	15
2	-1	1	-1	1	0.8	0.25	0.05	75	16	1	18
2	1	-1	-1	1	2	0.08	0.05	75	17	0	4
2	1	1	1	1	2	0.25	0.15	75	18	1	18
2	1	-1	1	-1	2	0.08	0.15	55	19	1	18
3	1	1	-1	-1	2	0.25	0.05	55	20	1	18
3	0	-2	0	0	1.4	0	0.1	65	21	0	2.5
3	0	0	0	-2	1.4	0.165	0.1	45	22	1	18
3	2	0	0	0	2.6	0.165	0.1	65	23	1	18
3	0	0	0	0	1.4	0.165	0.1	65	24	1	18
3	0	0	2	0	1.4	0.165	0.2	65	25	1	18
3	0	0	0	0	1.4	0.165	0.1	65	26	1	18
3	0	0	-2	0	1.4	0.165	0	65	27	0	6.25
3	0	0	0	2	1.4	0.165	0.1	85	28	1	18
3	0	2	0	0	1.4	0.335	0.1	65	29	1	18
3	-2	0	0	0	0.2	0.165	0.1	65	30	0	6

[1] Treatment #22 was designed to contain 45% moisture, but the analytical moisture came back as 64.1%. Recoding the coefficient to correspond to 64.1% moisture did not change the analysis significantly. Consequently, the coefficient was left as –2. Proximate analysis data is shown in Appendix Table 3.

Table 5. Chemical Analyses of Treatments[1]

Treatment Number	Block	Factor Set Points (%)				Actual Values (%)						
		Salt	Benzoate	Diacetate	Moisture	Ash	Fat	Moisture	Protein	Salt	aw	pH
1	1	0.8	0.08	0.15	55	1.99	21.69	56.4	15.2	0.94	0.976	6.18
2	1	1.4	0.165	0.1	65	2.46	13.87	65.3	13.5	1.52	0.978	6.22
3	1	0.8	0.25	0.05	55	2.05	20.96	57.4	14.7	0.95	0.977	6.32
4	1	2	0.25	0.05	75	3.08	4.32	74	13.7	2.08	0.978	6.24
5	1	0.8	0.08	0.05	75	1.88	4.96	74.6	13.6	0.95	0.983	6.25
6	1	2	0.08	0.15	75	3.04	3.49	74.9	13.4	2.07	0.974	6.1
7	1	2	0.25	0.15	55	3.27	18.79	58.2	14.8	2.08	0.966	6.11
8	1	0.8	0.25	0.15	75	1.95	5.4	73.8	13.9	0.91	0.982	6.15
9	1	1.4	0.165	0.1	65	2.53	12.19	66.9	13.7	1.54	0.978	6.22
10	1	2	0.08	0.05	55	3.2	18.54	57.6	14.1	2.04	0.97	6.23
11	2	0.8	0.08	0.15	75	1.94	5.14	74.3	13.7	0.85	0.982	6.14
12	2	0.8	0.08	0.05	55	2.08	20.68	57	15.2	0.88	0.977	6.32
13	2	0.8	0.25	0.15	55	2.11	22.25	56.3	14.7	0.94	0.978	6.19
14	2	1.4	0.165	0.1	65	2.53	13.92	65.5	13.2	1.5	0.969	6.22
15	2	1.4	0.165	0.1	65	2.39	15.8	63.9	12.9	1.49	0.977	6.22
16	2	0.8	0.25	0.05	75	1.9	6	73.6	14	0.9	0.984	6.31
17	2	2	0.08	0.05	75	2.73	3.92	74.8	13.5	1.79	0.977	6.27
18	2	2	0.25	0.15	75	3.11	4.59	74	13.6	2.06	0.976	6.16
19	2	2	0.08	0.15	55	3.18	20.5	57.2	14.6	2.14	0.967	6.15
20	2	2	0.25	0.05	55	3.22	19.77	57.5	14.6	2.17	0.965	6.28
21	3	1.4	0	0.1	65	2.39	15.08	64.5	13.2	1.52	0.975	6.28
22	3	1.4	0.165	0.1	45	2.63	11.34	64.1	17.1	1.51	0.974	6.22
23	3	2.6	0.165	0.1	65	3.63	13.22	64.6	13.2	2.68	0.966	6.2
24	3	1.4	0.165	0.1	65	2.52	13.49	65.8	13.3	1.55	0.976	6.26
25	3	1.4	0.165	0.2	65	2.47	14.23	65.2	12.7	1.54	0.975	6.12
26	3	1.4	0.165	0.1	65	2.4	14.71	64.9	13.2	1.51	0.976	6.23
27	3	1.4	0.165	0	65	2.39	15.27	64.5	12.7	1.49	0.978	6.35
28	3	1.4	0.165	0.1	85	2.16	1.39	84.1	6.9	1.49	0.986	6.25
29	3	1.4	0.335	0.1	65	2.56	13.53	65.7	13.3	1.53	0.977	6.21
30	3	0.2	0.165	0.1	65	1.22	17.6	63.7	13	0.34	0.987	6.35

[1] See Appendix Table 2 for the appropriate coefficients for each treatment.

Appendix Table 6. Predicted and Observed TTG values for the RSM samples

No	Salt%	Benzoate %	Diacetate %	Moisture %	Salt	Benzoate	Diacetate	Moisture	Predicted time to one log (wk)	Actual TTG (wk)
1	0.8	0.08	0.150	55	-1.000	-1.000	1.000	-1.000	6.0	6
2	1.4	0.165	0.100	65	0.000	0.000	0.000	0.000	18.5	16.25
3	0.8	0.25	0.050	55	-1.000	1.000	-1.000	-1.000	19.2	18
4	2	0.25	0.050	75	1.000	1.000	-1.000	1.000	138.1	18
5	0.8	0.08	0.050	75	-1.000	-1.000	-1.000	1.000	3.6	3.625
6	2	0.08	0.150	75	1.000	-1.000	1.000	1.000	6.5	6.5
7	2	0.25	0.150	55	1.000	1.000	1.000	-1.000	218.7	18
8	0.8	0.25	0.150	75	-1.000	1.000	1.000	1.000	49.1	18
9	1.4	0.165	0.100	65	0.000	0.000	0.000	0.000	18.5	18
10	2	0.08	0.050	55	1.000	-1.000	-1.000	-1.000	3.4	3.375
11	0.8	0.08	0.150	75	-1.000	-1.000	1.000	1.000	5.4	5.375
12	0.8	0.08	0.050	55	-1.000	-1.000	-1.000	-1.000	3.7	3.75
13	0.8	0.25	0.150	55	-1.000	1.000	1.000	-1.000	19.2	18
14	1.4	0.165	0.100	65	0.000	0.000	0.000	0.000	18.5	18
15	1.4	0.165	0.100	65	0.000	0.000	0.000	0.000	18.5	15
16	0.8	0.25	0.050	75	-1.000	1.000	-1.000	1.000	19.2	18
17	2	0.08	0.050	75	1.000	-1.000	-1.000	1.000	4.0	4
18	2	0.25	0.150	75	1.000	1.000	1.000	1.000	202.7	18
19	2	0.08	0.150	55	1.000	-1.000	1.000	-1.000	117.4	18
20	2	0.25	0.050	55	1.000	1.000	-1.000	-1.000	19.2	18
21	1.4	0	0.100	65	0.000	-1.941	0.000	0.000	2.6	2.5
22	1.4	0.165	0.100	45	0.000	0.000	0.000	-2.000	18.6	18
23	2.6	0.165	0.100	65	2.000	0.000	0.000	0.000	56.8	18
24	1.4	0.165	0.100	65	0.000	0.000	0.000	0.000	18.5	18
25	1.4	0.165	0.200	65	0.000	0.000	2.000	0.000	54.5	18
26	1.4	0.165	0.100	65	0.000	0.000	0.000	0.000	18.5	18
27	1.4	0.165	0.000	65	0.000	0.000	-2.000	0.000	6.3	6.25
28	1.4	0.165	0.100	85	0.000	0.000	0.000	2.000	18.4	18
29	1.4	0.335	0.100	65	0.000	2.000	0.000	0.000	137.0	18
30	0.2	0.165	0.100	65	-2.000	0.000	0.000	0.000	6.0	6

APPENDIX C

Appendix C: Summary of Validation Samples for RTE Meat Products Containing Various Antimicrobial Agents and Sodium Diacetate

Appendix B describes a response surface model designed to predict whether *Listeria monocytogenes* will grow in ready-to-eat meat products containing various levels of sodium benzoate, sodium diacetate, salt, and moisture. A study to validate this model was undertaken at Kraft Foods Research Pilot Plant in Madison, Wisconsin.

This report covers comparisons of meat products ranging from those containing low moisture (i.e., hot dogs & bologna) to those containing high moisture (i.e., ham and cured turkey). The benzoate was locked in at 0.1% of the formula and the salt varied according to each formulation. The diacetate content varied between 0.05% and 0.15%. As shown in Appendix B, 0.1% sodium benzoate, alone, is not sufficient to inhibit *L. monocytogenes* in higher moisture items such as ham and turkey breast. Therefore, sodium propionate was added to the higher moisture products to provide additional inhibition, at levels ranging from 0.1% to 0.2%. A combination of sodium phosphate and lemon juice concentrate (Lem-O-Fos® 101, Innophos, Cranbury, NJ 08512) was added to some of the high moisture products as an additional inhibitor. For purposes of this study, time to growth (TTG) is defined as a sustained one log increase from the initial level of inoculation.

MATERIALS AND METHODS

Sample Preparation

Products were prepared in the Kraft Foods Madison, WI research pilot plant. The products were manufactured using procedures that were similar to typical production processes for the particular type of product. Bologna and hot dogs were mixed, chopped, stuffed into permeable casings and smoked/cooked in a batch smokehouse. Ham and turkey breast were mixed, cured overnight, stuffed into impermeable casings and cooked in a batch smokehouse. Packaging is described in the microbiological methods section below.

Analytical Procedures

Chemical Analysis

Samples of each treatment were submitted for analysis of moisture, protein, fat, ash, NaCl, lactate, and diacetate.

Microbiological Methods

Bacterial strains and growth conditions. Four *L. monocytogenes* isolates from food borne outbreaks (CDC861, F2379, an NFPA strain, and one from an outbreak associated with hot dogs) and two environmental isolates from a ready-to-eat meat manufacturing facility (MAD328, MAD225) were used as a six-strain cocktail throughout the studies. Strains were grown aerobically, without shaking, in 10-ml of Brain Heart Infusion broth (Becton Dickinson, Sparks, MD) for 24 h at 35°C, allowing the cultures to reach late stationary phase. Culture (0.1-ml) was transferred to 10-ml of fresh brain heart infusion broth and the incubation repeated.

Inoculum preparation and procedure. The inoculum was prepared by transferring 0.2-ml of each strain into 99-ml of Butterfield's phosphate buffer (Weber Scientific, Hamilton, NJ). Serial dilutions were made to achieve the desired inoculum level, approximately 10 - 100 cfu/g or 1,000 - 10,000 cfu/package. The inoculum (100-µl total) was applied to the surface of 100g of meat. One 50-µl aliquot was placed between slices and one was placed on the surface of the top slice. The pouches containing the meat were immediately vacuum-sealed (Multivac C1400) and stored at 4°C for up to 18 weeks.

Evaluation and enumeration of *L. monocytogenes.* Three samples of each treatment were analyzed every other week for *L. monocytogenes* by appropriately diluting the samples in Butterfield's phosphate buffer, direct plating onto Total Plate Count agar (TPC) and Modified Oxford agar (MOX; EMD Chemicals, Gibbstown, NJ), and incubating plates for 48 h at 35°C. Colonies producing a black precipitate on MOX, indicative of *Listeria* spp., were considered *L. monocytogenes* colonies.

RESULTS AND DISCUSSION

Results are arranged by specific products. Each section below describes the specific formulations used for each product (in terms of the addition of antimicrobial agents, salt content, and product moisture). Each section also contains a graph of $\log_{10}$ *L. monocytogenes* counts over 18 weeks of storage at 4°C.

Hot Dogs

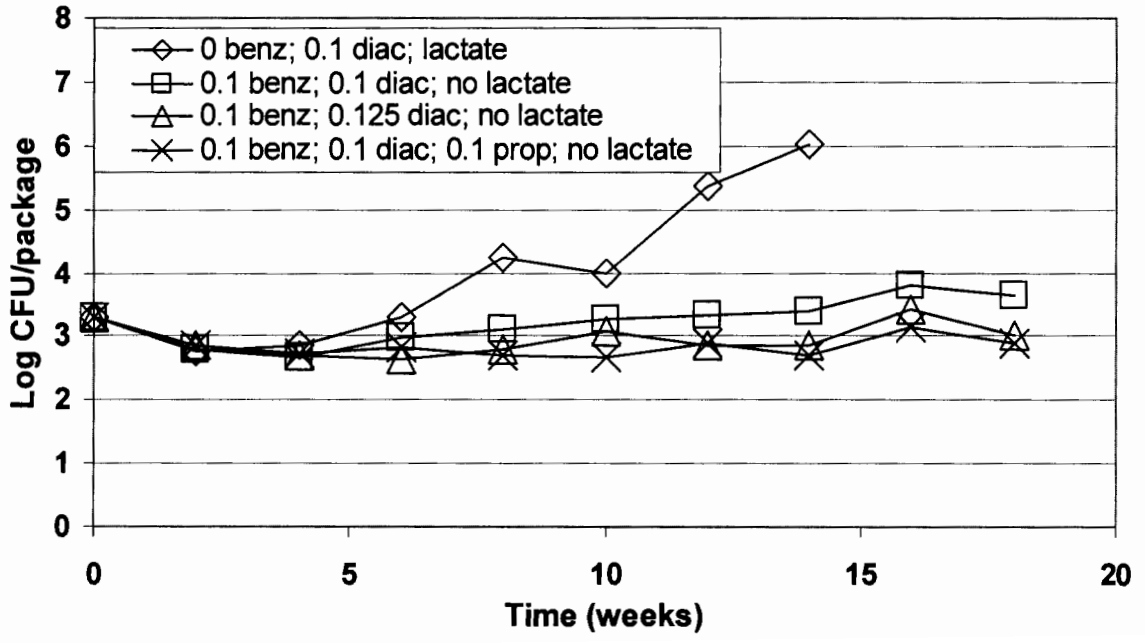
Table 1 shows the ingredients used for hot dogs in this test.

Table 1. Treatments for hot dogs (made with turkey, pork and chicken)

Ingredient	1	2	3	4
Salt (%)	1.799	1.799	1.799	1.799
Sodium lactate syrup (%)	1.4983	0	0	0
Sodium diacetate (%)	0.1	0.1	0.125	0.1
Sodium benzoate (%)	0	0.1	0.1	0.1
Sodium propionate (%)	0	0	0	0.1
Moisture (%)	57	57	57	57

Figure 1 indicates that adding 0.1% benzoate with 0.1% diacetate inhibited the growth of *L. monocytogenes* in inoculated samples of hot dogs over the entire 18 week test.

Figure 1. Plot of Listeria Counts in Hot Dogs



Bologna

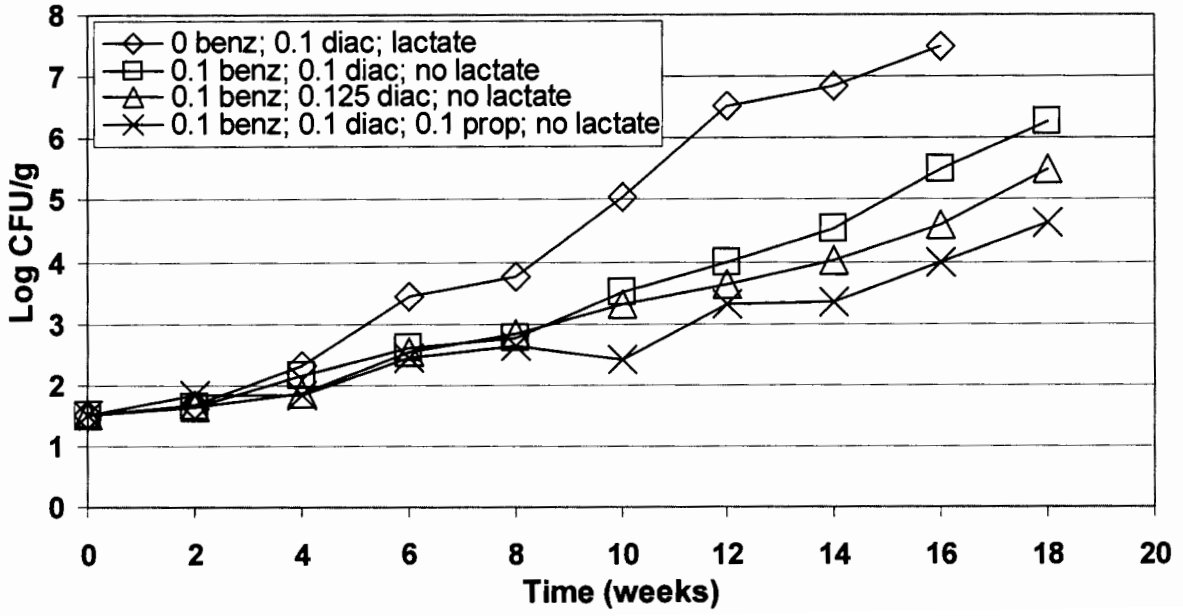
Table 2 shows the ingredients used for bologna in this test.

Table 2. Treatments for bologna (made with chicken and pork)

Ingredient	1	2	3	4
Salt (%)	1.69	1.69	1.69	1.69
Lactate syrup (%)	1.75	0	0	0
Diacetate (%)	0.1	0.1	0.125	0.1
Benzoate (%)	0	0.1	0.1	0.1
Propionate (%)	0	0	0	0.1
Moisture (%)	57	57	57	57

Figure 2 indicates that the addition of 0.1 % benzoate and 0.1% to 0.125% diacetate inhibited the growth of *L. monocytogenes* in inoculated samples of bologna for 8 weeks. Addition of 0.1% benzoate, 0.1% diacetate and 0.1% propionate inhibited the growth of *L. monocytogenes* in inoculated samples for 10 weeks.

Figure 2. Plot of Listeria Counts in Bologna



Beef Hot Dogs

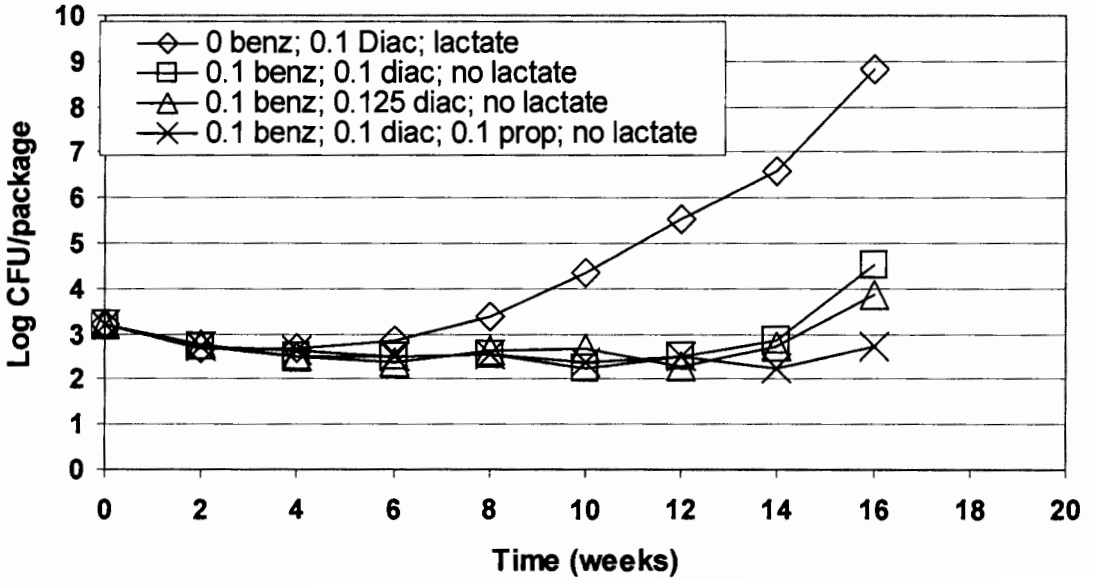
Table 3 shows the ingredients used for beef hot dogs in this test.

Table 3. Treatments for beef hot dogs

Ingredient	1	2	3	4
Salt (%)	1.90	1.90	1.90	1.90
Sodium lactate syrup (%)	1.3897	0	0	0
Sodium diacetate (%)	0.1	0.1	0.125	0.1
Sodium benzoate (%)	0	0.1	0.1	0.1
Sodium propionate (%)	0	0	0	0.1
Moisture (%)	56	56	56	56

Figure 3 indicates that adding 0.1% benzoate and 0.1% diacetate inhibited the growth of *L. monocytogenes* in inoculated samples of beef hot dogs for 14 weeks. Addition of 0.1% benzoate, 0.1% diacetate and 0.1% propionate inhibited the growth of *L. monocytogenes* in inoculated samples for 16 weeks.

Figure 3. Plot of Listeria Counts in Beef Hot Dogs



Beef Bologna

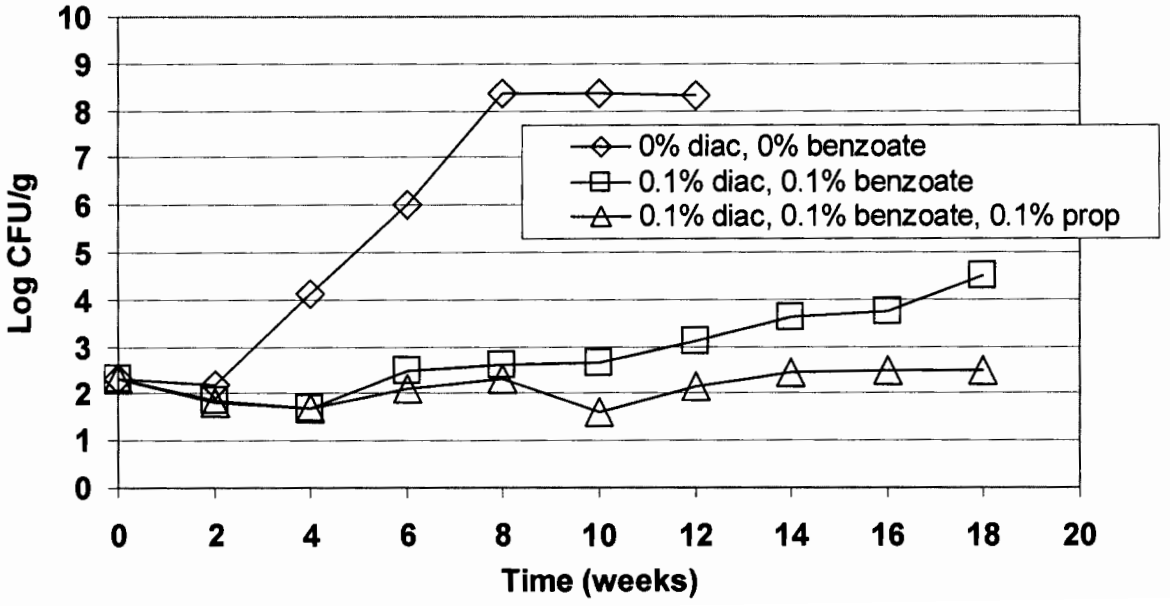
Table 4 shows the ingredients used for beef bologna in this test.

Table 4. Formulations for beef bologna

Ingredient	1	2	3
Salt (%)	2	2	2
Benzoate (%)	0	0.1	0.1
Diacetate (%)	0	0.1	0.1
Propionate (%)	0	0	0.1

Figure 4 indicates that adding 0.1% benzoate and 0.1% diacetate inhibited the growth of *L. monocytogenes* in inoculated samples of beef bologna for 14 weeks. Addition of 0.1% benzoate, 0.1% diacetate and 0.1% propionate inhibited the growth of *L. monocytogenes* in inoculated samples over the entire 18 week test.

Figure 4. Plot of Listeria Counts in Beef Bologna



Turkey breast

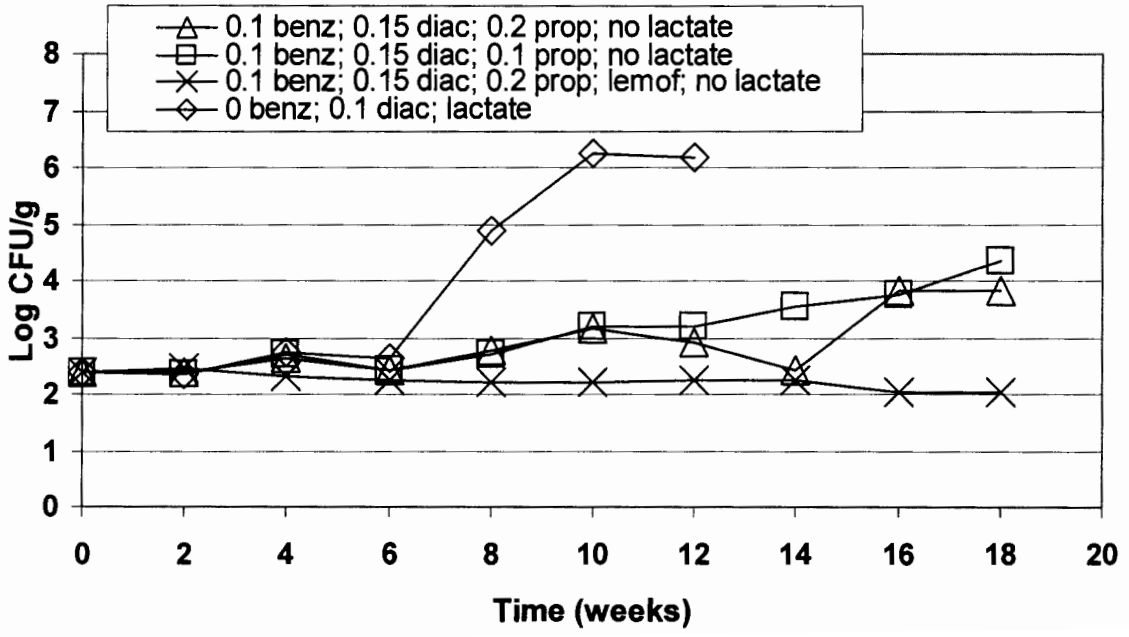
Table 5 shows the ingredients used for turkey breast in this test. All treatments of turkey breast were cured. Treatment 3 contained Lem-O-Fos® (sodium phosphate and lemon juice concentrate) to see whether the lemon juice concentrate it contained might assist the antimicrobial activity of the benzoate and propionate.

Table 5. Treatments for turkey breast

Ingredient	1	2	3
Salt (%)	1.1	1.1	1.1
Sodium diacetate (%)	0.15	0.15	0.15
Sodium benzoate (%)	0.1	0.1	0.1
Sodium propionate (%)	0.2	0.1	0.2
Lem-O-Fos® (%)	0	0	0.56
Moisture (%)	77	77	77

Figure 5 shows that treatments 1 and 2, containing 0.2% and 0.1% propionate, had similar *L. monocytogenes* growth patterns – a time to one log growth of about 16 weeks. The addition of the Lem-O-Fos® appeared to make the antimicrobial agents more effective, since treatment 3 inhibited the growth of *L. monocytogenes* in inoculated samples over the entire 18 week test.

Figure 5. Plot of Listeria Counts in Turkey Breast



Ham

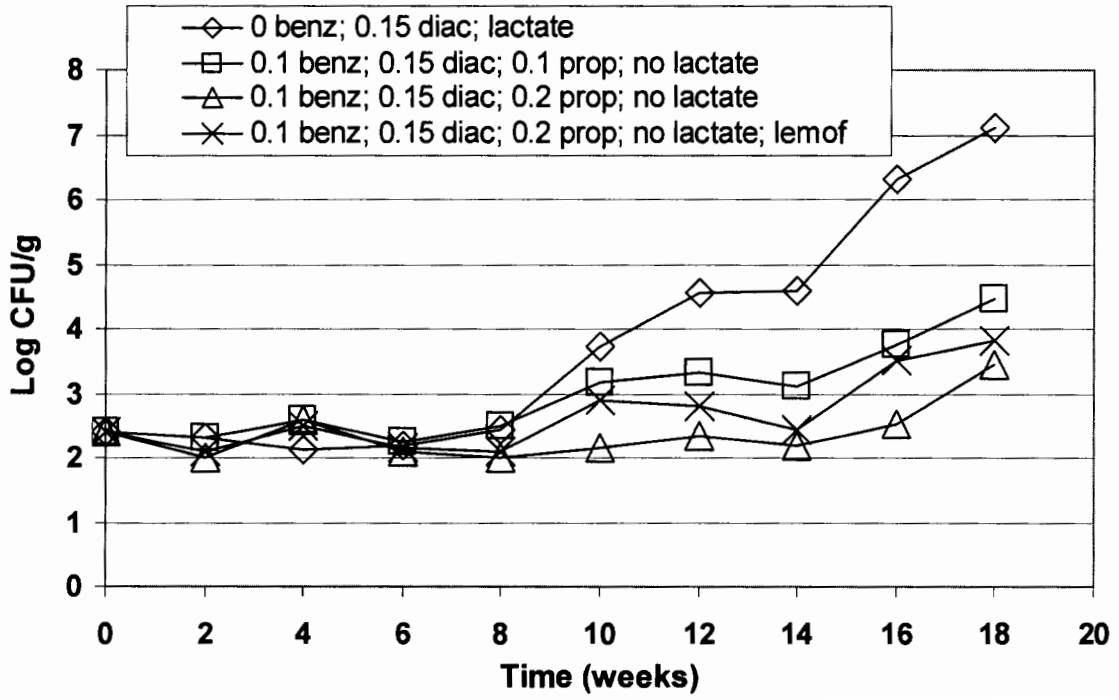
Table 6 shows the ingredients used for ham in this test. Treatment 4 also contained Lem-O-Fos® (sodium phosphate and lemon juice concentrate) to see whether the lemon juice concentrate it contained might assist the antimicrobial activity of the benzoate and propionate.

Table 6. Treatments for ham

Ingredient	1	2	3	4
Salt (%)	1.57	1.57	1.57	1.57
Sodium lactate syrup (%)	2.76	0	0	0
Sodium diacetate (%)	0.15	0.15	0.15	0.15
Sodium benzoate (%)	0	0.1	0.1	0.1
Sodium propionate (%)	0	0.1	0.2	0.2
Lem-o-fos® (%)	0	0	0	0.447
Moisture (%)	77	77	77	77

Figure 6 indicates that the adding 0.1% benzoate, 0.15% diacetate and 0.2% propionate inhibited the growth of *L. monocytogenes* in inoculated samples of ham for 16 weeks. The addition of Lem-O-Fos® did not significantly increase the time to growth for ham.

Figure 6. Plot of Listeria Counts in Ham



Other research in the literature

The Food Research Institute has conducted work for the American Meat Institute Foundation over the last few years into combinations of antimicrobial ingredients and their effect on the growth of *Listeria monocytogenes* (Glass 2005, 2006, 2007). They found that sodium benzoate and sodium propionate would inhibit the growth of *L. monocytogenes* in ready-to-eat meat products both singly and in combination with other antimicrobials. Differences in the effect of antimicrobials at specific levels in their studies versus the effects shown in this report are likely due to differences in formulation of the products tested.

CONCLUSIONS

- In each case described above, the combination of sodium benzoate and diacetate or sodium benzoate, propionate, diacetate and/or Lem-O-Fos® delayed the growth of *L. monocytogenes* in deliberately challenged packages of ready-to-eat meat and poultry products.

- In lower moisture meats such as full fat bologna and hot dogs, a combination of 0.1% benzoate and 0.1% diacetate will inhibit growth of *L. monocytogenes*. Bologna may require increased diacetate and/or propionate to achieve growth inhibition of *L. monocytogenes* over the desired shelf life.

- In ham, a combination of 0.1% benzoate, 0.1% diacetate and 0.2% propionate will inhibit growth of *L. monocytogenes*. Turkey breast required Lem-O-Fos® in addition to the above ingredients to have the same growth inhibition as for ham.

REFERENCES

Glass, K., Veesenmeyer, J., Eimerman, P., Preston, D., McDonnell, L. 2005. Control of *Listeria monocytogenes* in Processed Meat and Poultry by Combinations of Antimicrobials. American Meat Institute Foundation Final Report, June 2005. Available at: http://www.amif.org/AMIFResearch/2005Reports/GlassAMIFinalreport6805.pdf. Accessed 17 January, 2007.

Glass, K., Claus, J., McDonnell, L., Rassel, R., Zierke, K. 2006. Controlling *Listeria* monocytogenes on Ready-to-eat Meat and Poultry Products Using Food-Approved Antimicrobials, Benzoate, Propionate, and Sorbate. American Meat Institute Foundation Final Report, June 2006. Available at: http://www.amif.org/amifresearch/2006finalreports/05-226controllinglmusingfoodapprovedantimicrobials.pdf. Accessed 17 January, 2007.

Glass, K., Preston, D., Veesenmeyer, J, 2007. Inhibition of Listeria monocytogenes in Turkey and Pork-Beef Bologna by Combinations of Sorbate, Benzoate, and Propionate. Journal of Food Protection, Vol. 70, No. 1, 2007, Pages 214–217.

APPENDIX D

Appendix D: Effects on Spoilage

To investigate whether meat and poultry products formulated with antimicrobial combinations display normal signs of spoilage, two shelf life studies were undertaken at the Kraft Foods Research Pilot Plant in Madison, Wisconsin. In the first study, packages of bologna, beef franks, ham, and turkey breast were inoculated with spoilage organisms and assessed for spoilage (measured by total plate count and sensory-related signs of spoilage such as appearance and odor). In the second study, spoilage was evaluated after packages were opened and inoculated with *L. monocytogenes*. Both studies showed little to no difference in spoilage characteristics among the treatments evaluated, supporting a conclusion that these treatments do not mask spoilage.

I. INOCULATED SHELF LIFE

MATERIALS AND METHODS

Sample Preparation

Samples of bologna (made with chicken and pork), hot dogs (made with turkey, pork and chicken), ham, and turkey breast were made in the Madison, Wisconsin Research Pilot Plant using three different formulas: a formula with no antimicrobial ingredients, a formula with sodium lactate and diacetate, and a formula containing sodium benzoate and sodium diacetate with and without sodium propionate and Lem-O-Fos® (sodium phosphate and lemon juice concentrate). All three treatments for each product type contained the same level of salt and other ingredients.

For the no antimicrobial formula, water was used in place of sodium lactate and diacetate. The sodium lactate treatments each contained sodium lactate and sodium diacetate formulated according to the Purac OptiForm® Listeria growth suppression model. The sodium benzoate and propionate treatments were formulated as follows: bologna contained 0.1% sodium benzoate and 0.11% sodium diacetate; beef franks contained 0.1% sodium benzoate and 0.11% sodium diacetate; ham contained 0.1% sodium benzoate, 0.15% sodium diacetate, and 0.2% sodium propionate; turkey breast contained 0.1% sodium benzoate, 0.15% sodium diacetate, 0.2% sodium propionate, and Lem-O-Fos® (sodium phosphate and lemon juice concentrate). The sodium phosphate component of Lem-O-Fos® replaced phosphate otherwise added to the formulation.

Microbiological Analysis

Packages of the bologna, beef franks, ham, and turkey breast were inoculated with spoilage bacteria (*Leuconostoc mesenteroides* and *Weisella viridescens*) and sealed in vacuum packages. Ham and turkey breast were also inoculated and sealed in MAP packages (75% nitrogen, 25% carbon dioxide). The packages were then evaluated for total plate count, visual spoilage, and off odors until noticeably spoiled. Sensory spoilage was measured by appearance (including the presence of slime, fading/off

color, milky/cloudy purge and mold) and odor (sour, sulfur, or putrid odor). The following are general guidelines used in evaluating spoilage of product samples:

- Slime: Strands of slime are noticeable when slices are separated or product is removed from package.
- Fading/off color: Product has changed from its original cured meat color toward a grey-green color (can be in spots or the entire product).
- Milky/cloudy purge: Product purge has changed from transparent to containing areas of turbidity and/or opacity.
- Mold: Visible colonies of mold growth appear on the surface of the product.
- Odor: Headspace in the package exhibits a persistent atypical odor with sour, sulfur, or putrid notes.

RESULTS

Figure 1 shows a graph of the total plate count by week for hot dogs. There was no difference in counts between the sodium lactate and benzoate treatments, and only slightly more growth in the treatment with no antimicrobial ingredients. All three treatments were noticeably spoiled at three weeks.

Figure 2 shows a graph of the total plate count by week for bologna. There was almost no difference in counts between the lactate and benzoate treatments, and only slightly more growth in the treatment with no antimicrobial ingredients. All three treatments had a sour odor at three weeks.

Figure 3 shows a graph of the total plate count by week for ham. There was no difference between the sodium lactate and sodium benzoate/propionate treatments. They had similar growth curves and were noticeably spoiled at the same time (ten weeks). The vacuum packaged product also had similar growth compared to the MAP packaged product. The treatment with no antimicrobials had a faster rate of growth between weeks one and two, but was similar after that, and it was visibly slimy after three weeks. For ham only, the use of lactate or benzoate/propionate appeared to inhibit growth of spoilage organisms more than the no antimicrobial treatment. This resulted in the increased time until noticeable spoilage occurred.

Figure 4 shows a graph of the total plate count by week for turkey breast. There was very little difference between any of the treatments for turkey breast. All of the treatments were noticeably spoiled at three weeks.

The data reflect little to no difference in spoilage as measured by total plate count or sensory (appearance and odor) spoilage of ready-to-eat processed meat products when using no added antimicrobial ingredients, a combination of sodium lactate and diacetate, or a combination of sodium benzoate, propionate, and diacetate.

Figure 1. Graph of total plate count by week for hot dogs inoculated with spoilage organisms

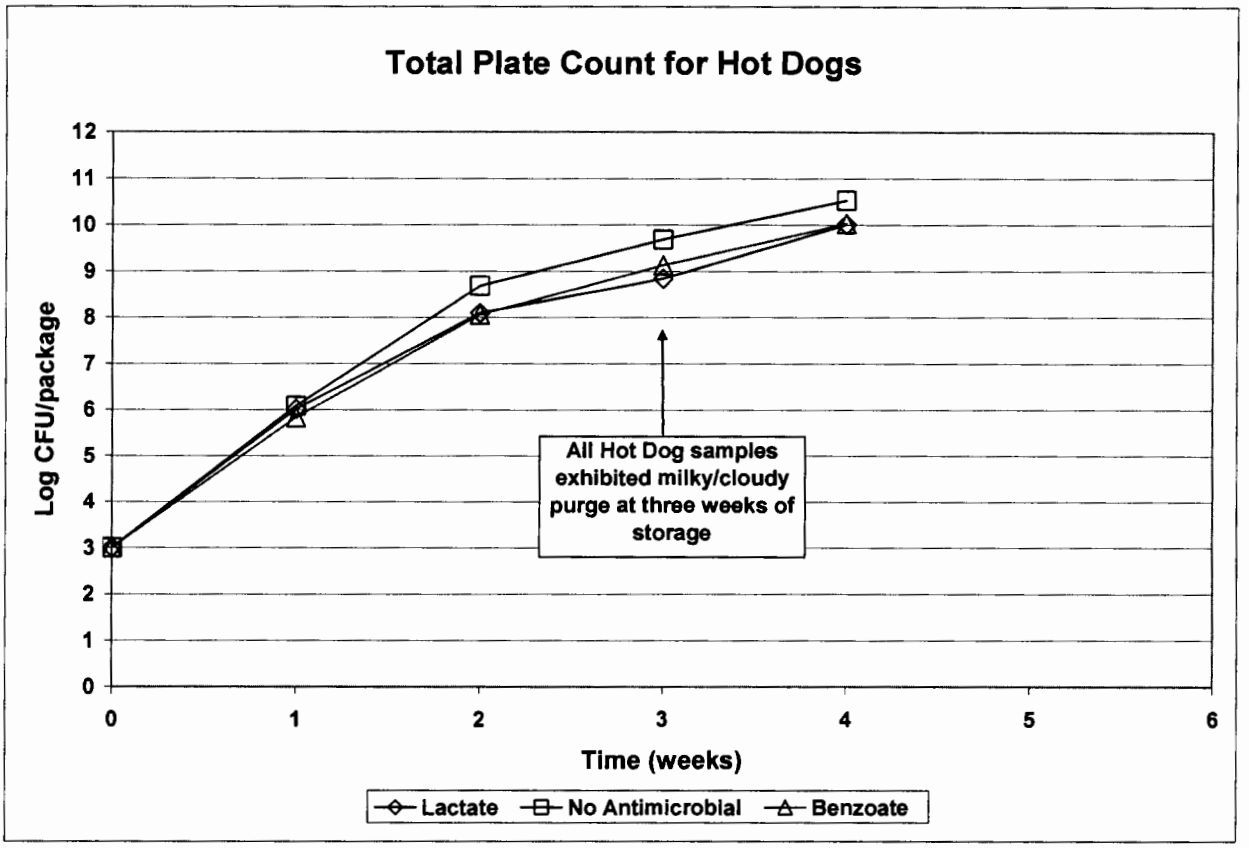


Figure 2. Graph of total plate count by week for bologna inoculated with spoilage organisms

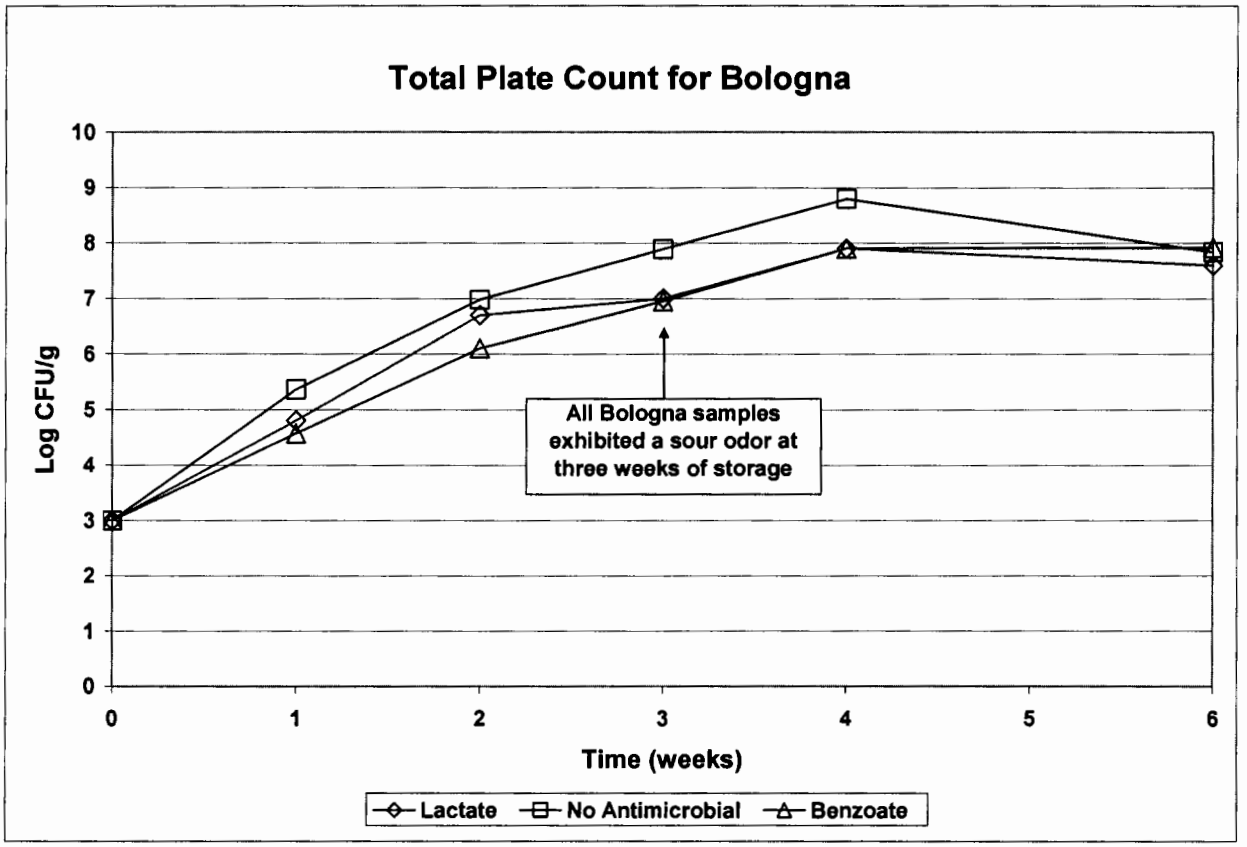


Figure 3. Graph of total plate count by week for ham inoculated with spoilage organisms

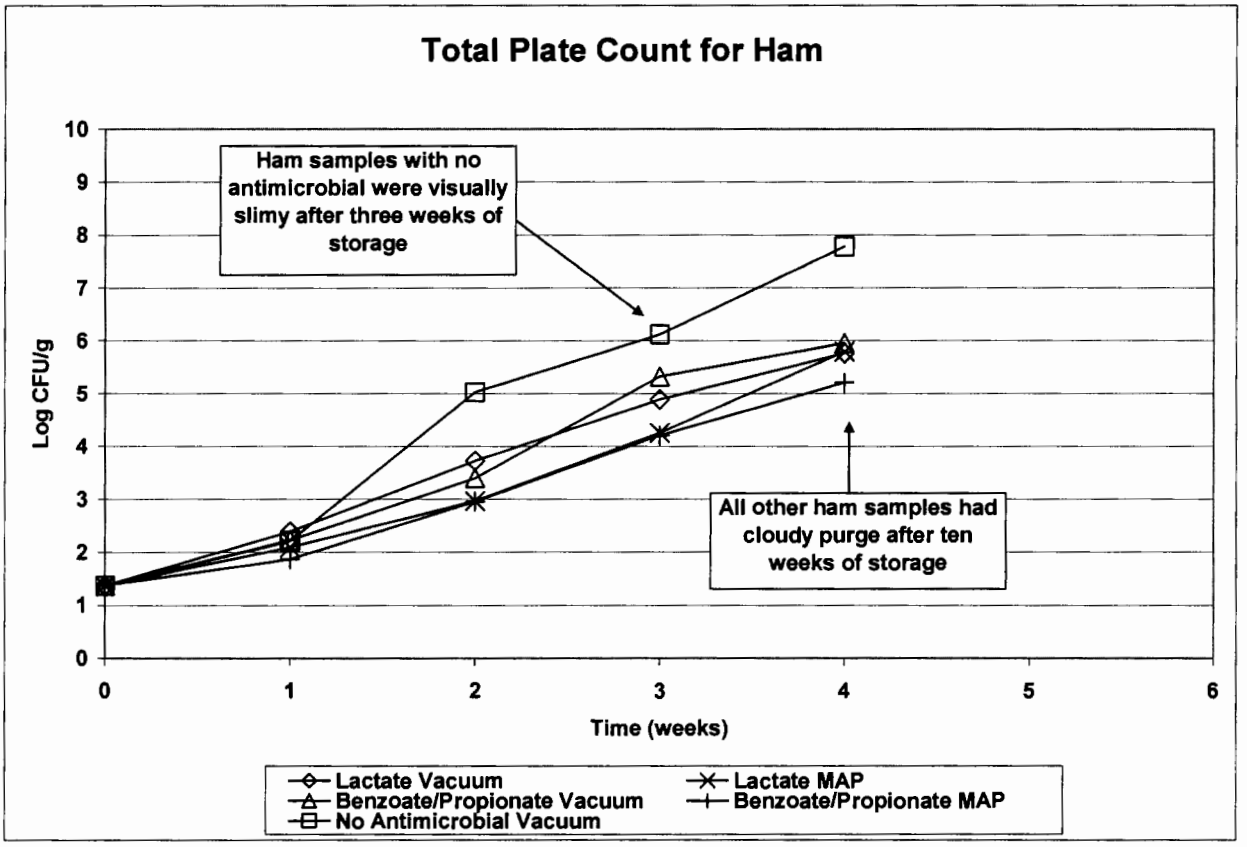
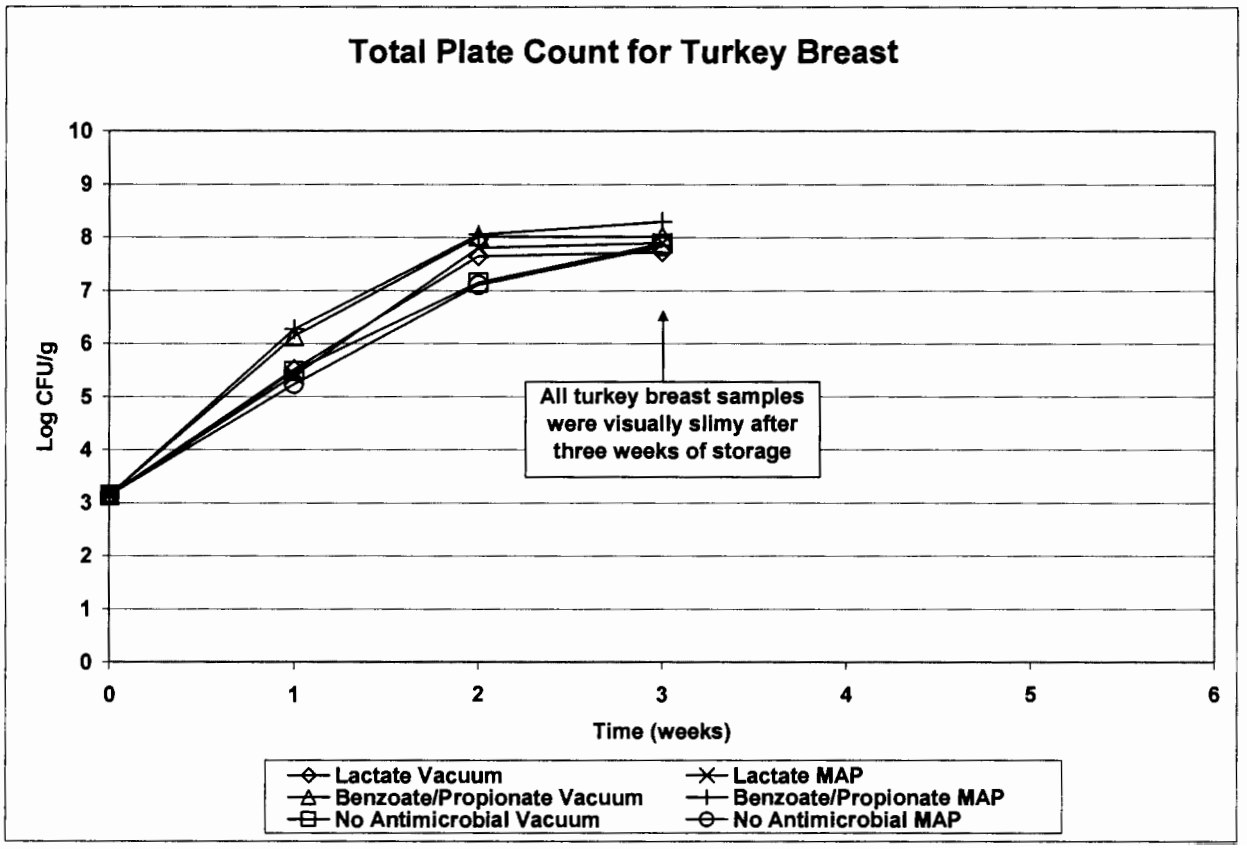


Figure 4. Graph of total plate count by week for turkey breast inoculated with spoilage organisms



II. OPENED PACKAGE SPOILAGE

MATERIALS AND METHODS

Sample Preparation

Samples of bologna, beef franks, ham, and turkey breast were made in the Madison, WI Research Pilot Plant using either the current sodium lactate and diacetate formula or a formula containing sodium benzoate and sodium diacetate with or without sodium propionate. The sodium lactate treatments each contained sodium lactate and sodium diacetate formulated according to the Purac OptiForm® Listeria growth suppression model. For the test treatments, bologna contained 0.1% sodium benzoate and 0.125% sodium diacetate; beef franks contained 0.1% sodium benzoate and 0.1% sodium diacetate; ham contained 0.1% sodium benzoate, 0.15% sodium diacetate, and 0.2% sodium propionate; and turkey breast contained 0.1% sodium benzoate, 0.1% sodium diacetate, and 0.1% sodium propionate.

Microbiological Analysis

Packages of the bologna, beef franks, ham and turkey breast were held for 60 days, then opened and inoculated with *L. monocytogenes*. The packages were then evaluated visually and for total plate count weekly for four weeks.

RESULTS

Figure 1 is a graph of average total plate count for the first three weeks of the test, and shows there is little difference between the sodium lactate and sodium benzoate/ propionate treatments. Turkey breast counts were not included in the graph as the counts of all treatments were over log 5 at the first week.

After four weeks, both the sodium lactate and the sodium benzoate/propionate treatments were visibly spoiled for bologna, ham, and turkey breast (see Table 1). Neither treatment of beef franks was visibly spoiled at four weeks.

The data support a conclusion of no difference in spoilage of ready-to-eat processed meat products when using sodium lactate as compared to sodium benzoate and/or sodium propionate. This remains true when product is inoculated with *L. monocytogenes*.

Figure 1. Graph of total plate count

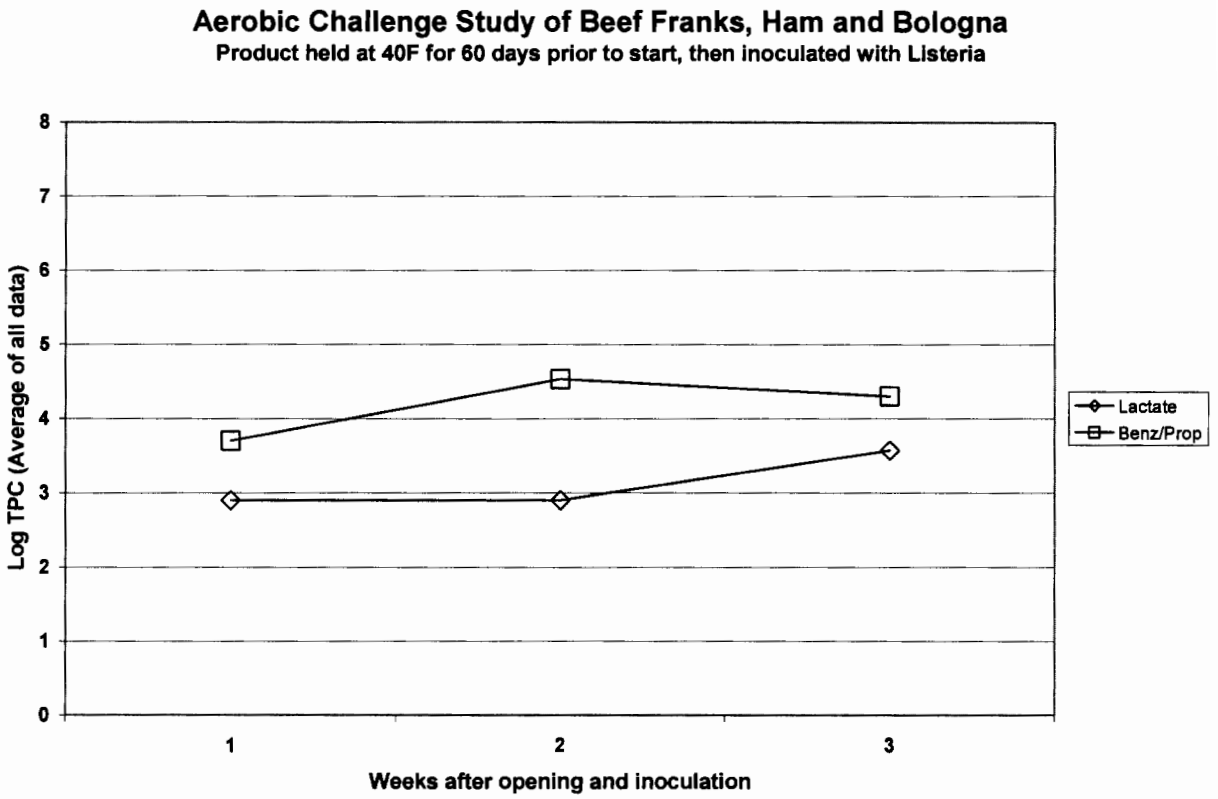


Table 1. Visual spoilage assessment at four weeks

	Product appearance at 4 weeks	
	Lactate	Benzoate and/or Propionate
Bologna	Visible spoilage Fading / off color Some mold	Visible spoilage Fading / off color Some mold
Beef Franks	No visible spoilage	No visible spoilage
Ham	Visible spoilage Mold	Visible spoilage Mold
Turkey Breast	Visible spoilage Fading / off color	Visible spoilage Fading / off color

APPENDIX E

Appendix E: Descriptive Analysis of Hot Dogs

To investigate possible effects of antimicrobial ingredients on product quality, Kraft asked trained sensory experts to evaluate the aroma, flavor, and texture profile of hot dogs formulated with different antimicrobial combinations.

MATERIALS AND METHODS

Sample Preparation

Hot dogs (made with turkey, pork and chicken) were made in the Kraft manufacturing facility in Columbia, MO on February 17, 2006. Two treatments were produced: a sodium lactate and diacetate treatment formulated according to the Purac OptiForm® Listeria growth suppression model, and a sodium benzoate treatment with 0.1% sodium benzoate and 0.11% sodium diacetate.

Descriptive Analysis

The two treatments were evaluated using a 15 point descriptive analysis scale in duplicate sessions by a trained descriptive panel (n=5) at the Madison Headquarters on March 9, 2006. Data were statistically analyzed using the SAS General Linear Model and mean separation was performed for both products and panelists with significant differences reported at $p \leq 0.05$.

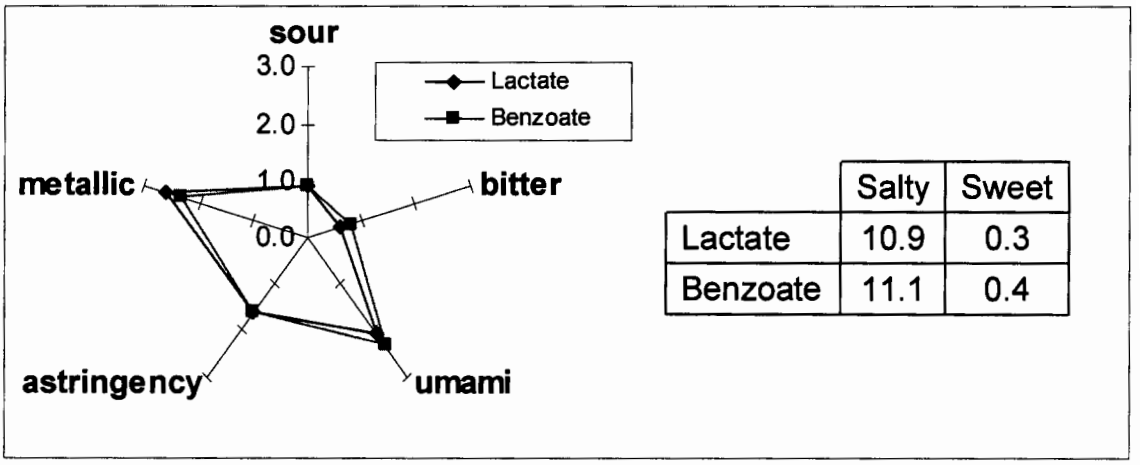
RESULTS

Figure 1 shows a spider plot of the basic taste and feeling factor results; Figure 2 shows a plot of a selection of the flavor factors. Table 1 provides the complete results from sensory descriptive profiling of the aroma, flavor, and texture attributes by the trained panel.

The spider plots show that hot dogs with lactate or benzoate have very similar flavor profiles. Table 1 shows that only two factors were statistically different: savory/brothy and pork impression.

The data support a conclusion that sodium benzoate used at 0.1% does not negatively impact the sensory attributes of hot dogs.

Figure 1. Basic Taste and Feeling Factors



	Salty	Sweet
Lactate	10.9	0.3
Benzoate	11.1	0.4

Figure 2. Selected Flavor Factors

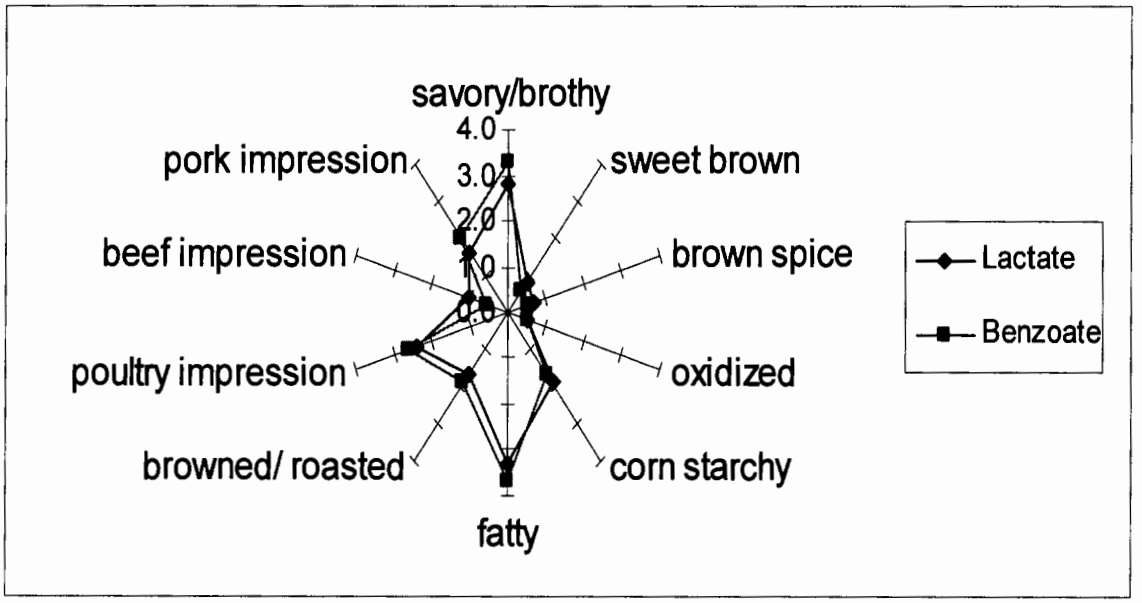


Table 1. Mean[1] aroma, flavor and texture attribute intensities[2] of hot dogs with sodium lactate versus benzoate

Aroma/Flavor	**Lactate**	**Benzoate**
smoke aroma	3.0	3.6
sulfur aroma	2.1	2.2
sweet brown aroma	0.8	0.6
liver/organ aroma	1.5	1.3
soured aroma	1.3	1.4
smoke	3.6	3.3
ash	1.6	1.6
creosote	1.3	1.7
sulfur	2.1	1.8
soured/lactic acid	2.0	1.8
savory/brothy	2.8 b	3.3 a
cured/tangy	3.5	3.2
sweet brown	0.5	0.9
brown spice	0.7	0.5
oxidized	0.5	0.5
corn/starchy	1.9	1.7
fatty	3.3	3.7
roasted/browned	1.7	1.9
poultry impression	2.4	2.6
beef impression	1.0	0.6
pork impression	1.6 b	2.0 a
liver organ	1.7	1.8
garlic/onion	2.4	2.4
pepper	2.0	1.9
mustard	2.6	2.6
piggy/boar taint	0.3	0.3
phenolic medicinal	1.4	1.3
barnyard/zoo	0.3	0.5
herb impression/other spices	0.6	0.5
Basic Tastes/Feeling factors		
salt	10.9	11.1
sweet	0.3	0.4
sour	0.9	0.9
bitter	0.6	0.8
umami	2.1	2.3
astringency	1.6	1.6
metallic	2.6	2.3
Texture		
moistness (surface)	3.9	4.4
oiliness/greasiness (surface)	6.2	6.2
roughness (surface)	4.6	4.9
firmness	4.1	3.8
deformability	4.5	3.9
juiciness	4.0	4.0
springiness/rubberiness	4.5	4.3
cohesiveness of mass	5.3	5.0
roughness/coarseness of mass	5.6	5.4
loose particles	3.7	3.7
oily/greasy film	5.0	4.6
chewiness 1	13	13
chewiness 2	13	13

Different letters within each row indicate significant differences ($p \leq 0.05$). [2]Intensities are based on a 15-point universal intensity scale, with the exception of 'chewiness' which is reported as the actual # of chews required to prepare the sample for swallowing.

APPENDIX F

Appendix F: Descriptive Analysis of Bologna

To investigate possible effects of antimicrobial ingredients on product quality, Kraft asked trained sensory experts to evaluate the aroma, flavor, and texture profile of bologna formulated with different antimicrobial combinations.

MATERIALS AND METHODS

Sample Preparation

Bologna (made with chicken and pork) was made in the Kraft manufacturing facility in Davenport, Iowa on February 1, 2006. Two treatments were produced: a sodium lactate and diacetate treatment formulated according to the Purac OptiForm® Listeria growth suppression model, and a sodium benzoate treatment with 0.1% sodium benzoate and 0.11% sodium diacetate.

Descriptive Analysis

The two treatments were evaluated using a 15 point descriptive analysis scale in duplicate sessions by a trained descriptive panel (n=5) at the Madison Headquarters on March 9, 2006. Data were statistically analyzed by two-way ANOVA and mean separation for both products and panelists with significant differences reported at $p \leq 0.05$.

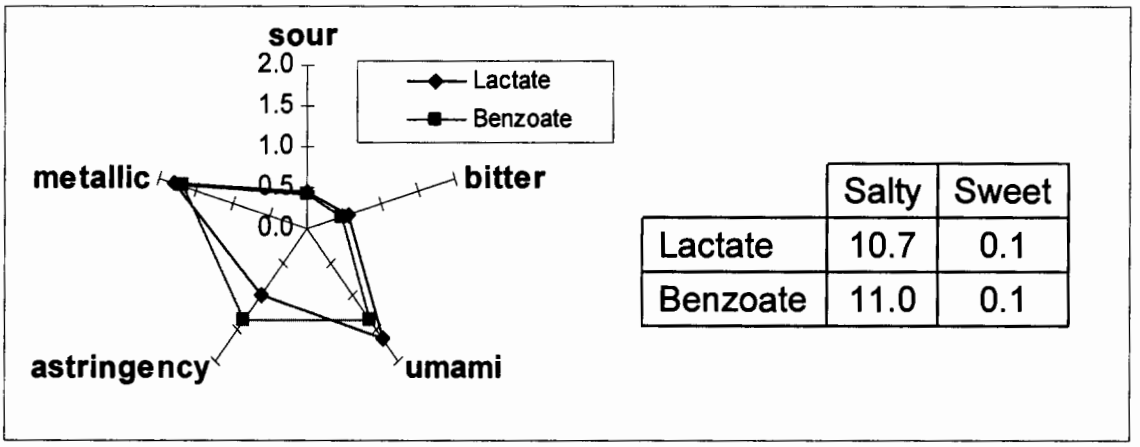
RESULTS

Figure 1 shows a spider plot of the basic taste and feeling factor results; Figure 2 shows a plot of a selection of the flavor factors. Table 1 provides the complete results from sensory descriptive profiling of the aroma, flavor and texture attributes by the trained panel.

The spider plots show that bologna with sodium lactate or benzoate have very similar flavor profiles. None of the factors was statistically different as shown in Table 1.

The data support a conclusion that sodium benzoate used at 0.1% does not negatively impact the sensory attributes of bologna.

Figure 1. Basic Taste and Feeling Factors



	Salty	Sweet
Lactate	10.7	0.1
Benzoate	11.0	0.1

Figure 2. Selected Flavor Factors

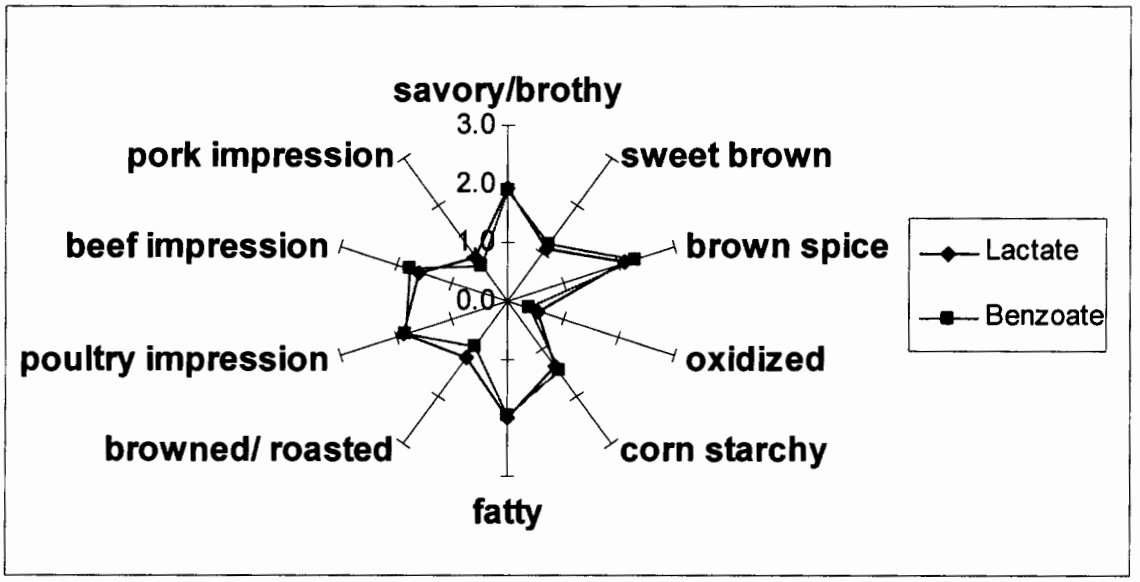


Table 1. Mean aroma, flavor and texture attribute intensities[1] of Bologna with lactate versus benzoate

Aroma/Flavor	**Lactate**	**Benzoate**
sulfur aroma	2.4	2.3
sweet brown aroma	0.8	1.0
pickle relish aroma	1.3	1.2
garlic/ onion aroma	2.2	2.2
smoke	0.1	0.0
sulfur	2.3	2.3
liver/organ aroma	1.9	2.2
savory/brothy	1.9	1.9
sweet brown	1.1	1.2
brown spice	2.1	2.3
oxidized	0.6	0.4
corn/starchy	1.4	1.5
fatty	2.0	2.0
browned/ roasted	1.2	1.0
poultry impression	1.9	1.8
beef impression	1.6	1.8
pork impression	0.9	0.8
liver/organ	1.7	1.9
garlic/onion	3.3	3.1
pepper	2.0	2.1
mustard	0.9	0.8
pickle relish	1.0	1.0
piggy/boar taint	0.4	0.4
barnyard/zoo	0.5	0.3
herb impression/other spices	0.8	1.1
Basic Tastes/Feeling factors		
salty	10.7	11.0
sweet	0.1	0.1
sour	0.4	0.4
bitter	0.6	0.5
umami	1.7	1.4
astringency	1.0	1.4
metallic	1.8	1.7
Texture		
moistness (surface)	3.3	3.4
oiliness/greasiness (surface)	4.9	4.9
roughness (surface)	2.1	2.4
firmness	3.9	3.8
deformability	4.3	4.1
juiciness	2.8	2.8
springiness/rubberiness	3.2	3.0
cohesiveness of mass	4.6	4.6
roughness/coarseness of mass	6.1	6.2
loose particles	4.0	3.9
oily/greasy film	4.0	4.1
chewiness 1	15	14
chewiness 2	15	15

[1]Intensities are based on a 15-point universal intensity scale, with the exception of 'chewiness' which is reported as the actual # of chews required to prepare the sample for swallowing.

APPENDIX G

Appendix G: Descriptive Analysis of Ham

To investigate possible effects of antimicrobial ingredients on product quality, Kraft asked trained sensory experts to evaluate the aroma, flavor, and texture profile of ham formulated with different antimicrobial combinations.

MATERIALS AND METHODS

Sample Preparation

Ham (Water Added) was made in the Madison, WI Research Pilot Plant on October 5, 2005. Two treatments were produced: a sodium lactate and diacetate treatment formulated according to the Purac OptiForm® Listeria growth suppression model, and a sodium benzoate treatment with 0.1% sodium benzoate and 0.15% sodium diacetate, and 0.2% sodium propionate.

Descriptive Analysis

The two treatments were evaluated using a 15 point descriptive analysis scale in duplicate sessions by a trained descriptive panel (n=6) at the Madison Headquarters on October 20, 2005. Data were statistically analyzed by two-way ANOVA and mean separation for both products and panelists with significant differences reported at $p \leq 0.05$.

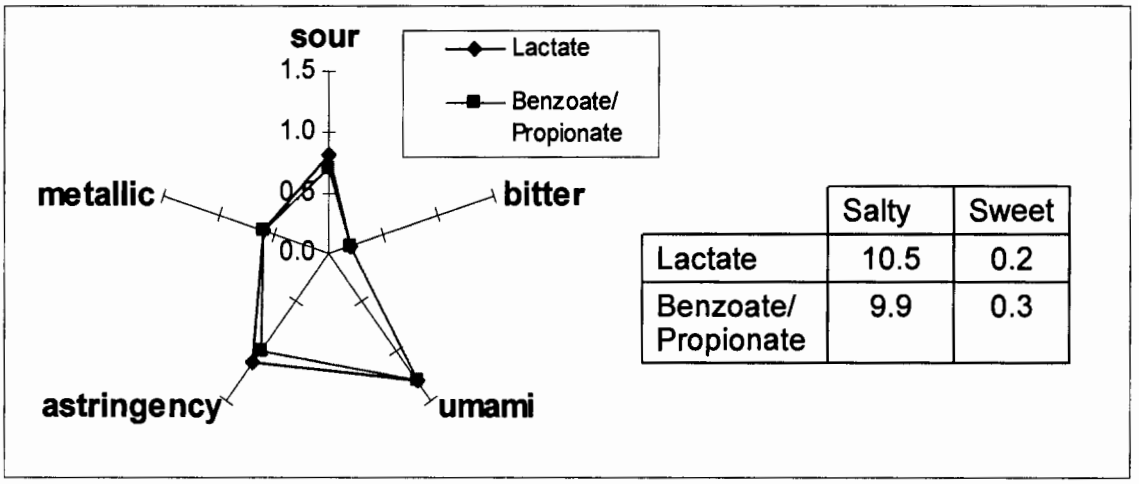
RESULTS

Figure 1 shows a spider plot of the basic taste and feeling factor results; Figure 2 shows a plot of a selection of the flavor factors. Table 1 provides the complete results from sensory descriptive profiling of the aroma, flavor and texture attributes by the trained panel.

The spider plots show that ham with lactate or benzoate/propionate have very similar flavor profiles. Only the herb impression/other spices attribute had a statistical difference as shown in Table 1.

The data support a conclusion that sodium benzoate and sodium propionate at the proposed levels of use do not negatively impact the sensory attributes of ham.

Figure 1. Basic Taste and Feeling Factors



	Salty	Sweet
Lactate	10.5	0.2
Benzoate/ Propionate	9.9	0.3

Figure 2. Selected Flavor Factors

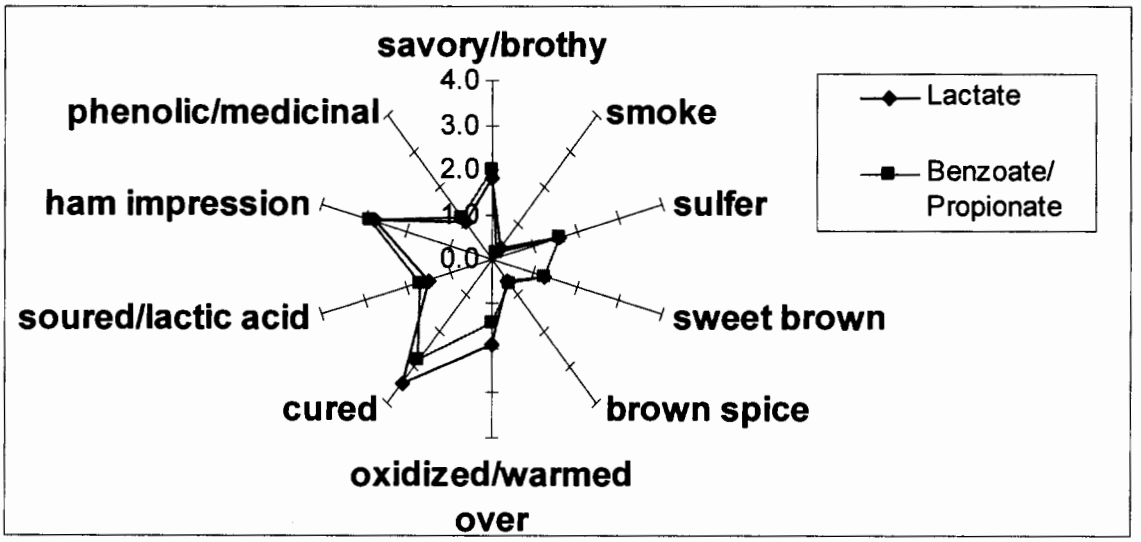


Table 1. Mean[1] aroma, flavor and texture attribute intensities[2] of Ham with lactate versus benzoate/propionate.

Aroma/Flavor	**Lactate**	**Benzoate/Propionate**
smoke aroma	0.4	0.4
sulfur aroma	1.9	2.2
sweet brown aroma	0.6	0.6
smoke	0.3	0.2
ash	0.1	0.1
creosote	0.1	0.0
sulfur	1.6	1.6
cured aroma	1.4	1.5
soured/lactic acid aroma	1.5	1.9
savory/brothy	1.8	2.0
sweet brown	1.2	1.2
brown spice	0.6	0.7
oxidized/warm over/refrigerated	1.9	1.4
cured	3.4	2.8
garlic/onion	0.2	0.1
pepper	0.1	0.0
soured/lactic acid	1.5	1.7
ham impression	2.8	2.9
phenolic/medicinal	1.0	1.1
piggy/boar taint	2.0	1.9
mothball	1.4	1.2
barnyard/zoo	0.8	0.9
herb impression/other spices	0.1 b	0.2 a
Basic Tastes/Feeling factors		
salty	10.5	9.9
sweet	0.2	0.3
sour	0.8	0.7
bitter	0.2	0.2
umami	1.3	1.3
astringency	1.1	1.0
metallic	0.6	0.6
Texture		
moistness (surface)	5.3	5.9
oiliness/greasiness (surface)	2.1	2.0
roughness (surface)	3.5	3.6
firmness	5.7	5.1
deformability	5.0	5.0
juiciness	5.3	5.8
springiness/rubberiness	4.4	4.3
cohesiveness of mass	4.8	4.8
roughness/coarseness of mass	6.1	5.9
loose particles	4.6	4.0
oily/greasy film	3.0	3.2
chewiness 1	33	32
chewiness 2	33	29

[1]Different letters within each row indicate significant differences ($p \leq 0.05$). [2]Intensities are based on a 15-point universal intensity scale, with the exception of 'chewiness' which is reported as the actual # of chews required to prepare the sample for swallowing.

APPENDIX H

Appendix H: Descriptive Analysis of Turkey Breast

To investigate possible effects of antimicrobial ingredients on product quality, Kraft asked trained sensory experts to evaluate the aroma, flavor, and texture profile of turkey breast formulated with different antimicrobial combinations.

MATERIALS AND METHODS

Sample Preparation

Turkey breast was made in the Madison, Wisconsin Research Pilot Plant on October 5, 2005. Two treatments were produced: a sodium lactate and diacetate treatment formulated according to the Purac OptiForm® Listeria growth suppression model, and a sodium benzoate treatment with 0.1% sodium benzoate and 0.15% sodium diacetate, and 0.2% sodium propionate. Both treatments were cured with sodium nitrite.

Descriptive Analysis

The two treatments were evaluated using a 15 point descriptive analysis scale in triplicate sessions by a trained descriptive panel (n=5) at the Madison Headquarters on October 26, 2005. Data were statistically analyzed by two-way ANOVA and mean separation for both products and panelists with significant differences reported at $p \leq 0.05$.

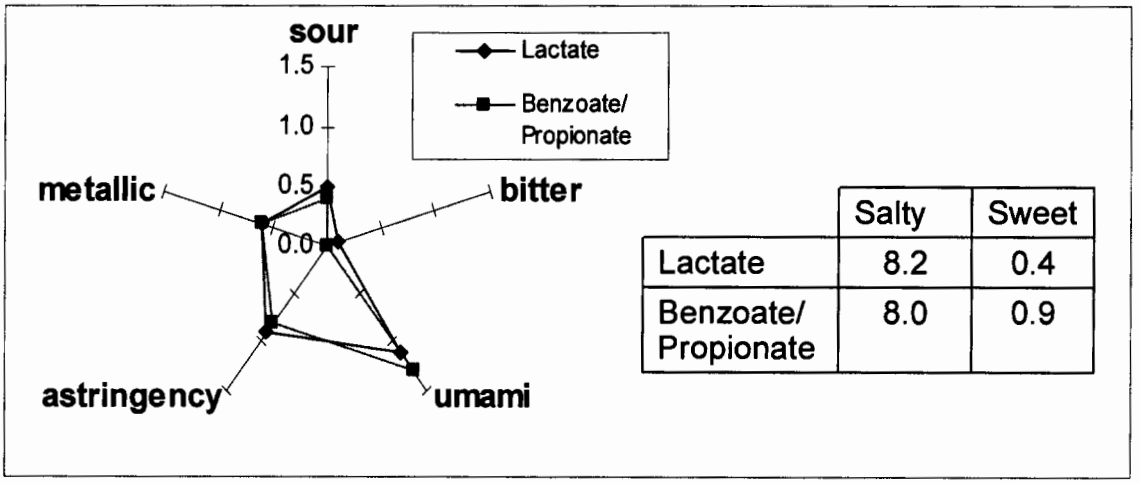
RESULTS

Figure 1 shows a spider plot of the basic taste and feeling factor results; Figure 2 shows a plot of a selection of the flavor factors. Table 1 provides the complete results from sensory descriptive profiling of the aroma, flavor and texture attributes by the trained panel.

The spider plots show that turkey breast with lactate or benzoate/propionate have very similar flavor profiles. Table 1 shows that only three of the factors were statistically different: cured flavor, cohesiveness of mass, and roughness/coarseness of mass.

The data support a conclusion that sodium benzoate and sodium propionate at the proposed levels of use do not negatively impact the sensory attributes of turkey breast.

Figure 1. Basic Taste and Feeling Factors



	Salty	Sweet
Lactate	8.2	0.4
Benzoate/ Propionate	8.0	0.9

Figure 2. Selected Flavor Factors

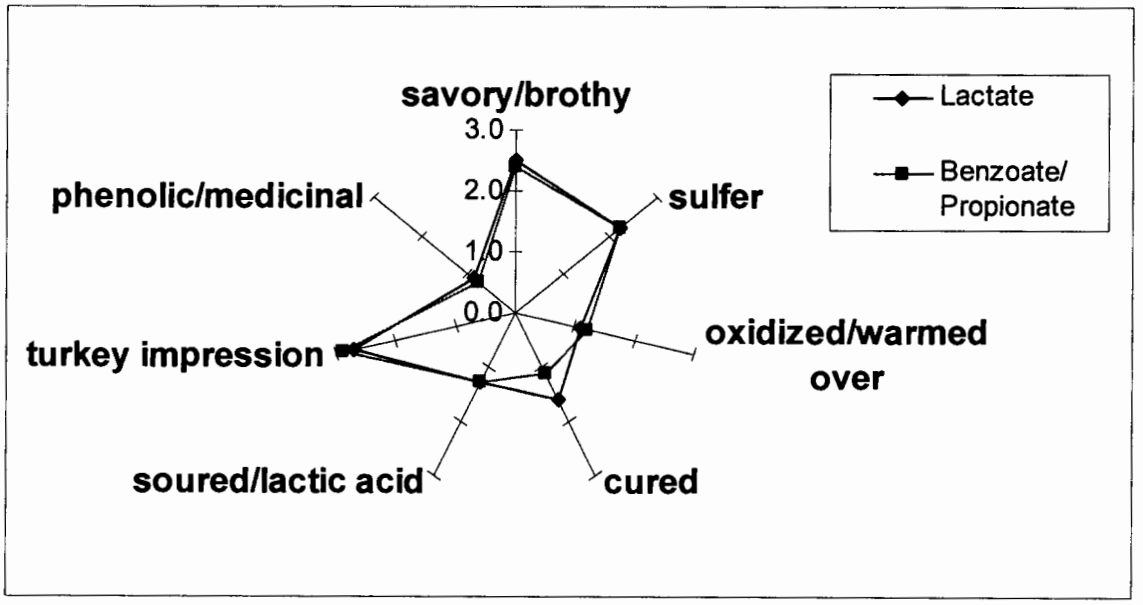


Table 1. Mean[1] aroma, flavor and texture attribute intensities[2] of Turkey Breast with lactate versus benzoate/propionate.

Aroma/Flavor	**Lactate**	**Benzoate/Propionate**
sulfur aroma	4.2	4.1
sulfur	2.2	2.2
savory/brothy	2.5	2.4
oxidized/warm-over	1.1	1.2
cured	1.6 a	1.1 b
soured/lactic acid	1.3	1.3
turkey impression	2.7	2.9
phenolic/medicinal	0.9	0.8
Basic Tastes/Feeling factors		
salty	8.2	8.0
sweet	0.4	0.9
sour	0.5	0.4
bitter	0.1	0.0
umami	1.1	1.3
astringency	0.9	0.8
metallic	0.6	0.6
Texture		
moistness (surface)	5.2	4.4
roughness (surface)	3.8	4.3
firmness	3.0	3.5
deformability	3.4	3.6
juiciness	4.8	4.5
springiness/rubberiness	2.8	2.7
cohesiveness of mass	6.2 a	5.4 b
roughness/coarseness of mass	5.5 b	6.2 a
loose particles	3.3	3.5
chewiness 1	18	19
chewiness 2	18	19

[1]Different letters within each row indicate significant differences ($p \leq 0.05$). Intensities are based on a 15-point universal intensity scale, with the exception of 'chewiness' which is reported as the actual # of chews required to prepare the sample for swallowing.

APPENDIX I

Appendix I: Consumer Test of Hot Dogs

To investigate possible effects of antimicrobial ingredients on product quality, Kraft asked consumers to evaluate the aroma, flavor, and texture profile of hot dogs formulated with different antimicrobial combinations.

MATERIALS AND METHODS

Sample Preparation

Hot dogs (made with turkey, pork and chicken) were made in the Kraft manufacturing facility in Columbia, MO on February 17, 2006. Two treatments were produced: a sodium lactate and diacetate treatment formulated according to the Purac OptiForm® Listeria growth suppression model, and a sodium benzoate treatment with 0.1% sodium benzoate and 0.11% sodium diacetate.

Consumer Testing

A mall-recruited central location test was conducted in March 2006. One hundred and fifty six respondents (hot dogs users) were recruited across four cities: Chicago IL, Springfield MO, Charlotte NC, and Tampa FL. Respondents evaluated the hot dogs, one per sample, in a sequential monadic order on key attributes. A balanced presentation was used to avoid bias.

RESULTS

Table 1 shows overall liking scores, Table 2 shows an assessment of attributes using non "just about right" ratings (non-JAR), and Table 3 shows aftertaste ratings.

Table 1 shows that there is no significant difference in overall consumer liking or other key liking factors between hot dogs made with sodium lactate versus sodium benzoate. Table 2 shows that there is very little difference in the hot dog attributes with sodium lactate or sodium benzoate – only tenderness of the skin and firmness were statistically different. Table 3 shows that the aftertaste of both are also very similar.

The data support a conclusion that sodium benzoate does not affect consumer liking of hot dogs.

Table 1. Consumer liking ratings (10 point scale)

	Lactate	Benzoate
Overall Liking	6.8	6.8
Appearance Liking	6.6	6.6
Overall Color Liking	6.6	6.6
Aroma Liking	6.5	6.5
Flavor Liking	6.5	6.5
Smoke Flavor Liking	6.4	6.3
Texture Liking	6.5	6.3

Uppercase letters indicate column of significant difference at a 95% confidence level.
Lowercase letters indicate column of significant different at an 80% confidence level.

Table 2. Non - Just about right ratings (% agreeing with statement)

	Lactate	Benzoate
Color Amount (too light)	6	8
Color Amount (too dark)	13	13
Aroma (not strong enough)	15	12
Aroma (too strong)	10	11
Flavor (not strong enough)	15	17
Flavor (too strong)	11	13
Smokiness (not enough)	22	22
Smokiness (too much)	10	10
Meaty Flavor (not strong enough)	16	21
Meaty Flavor (too strong)	6	4
Spiciness (not enough)	30	31
Spiciness (too much)	4	4
Saltiness (not enough)	13	17
Saltiness (too much)	15	12
Sweetness (not enough)	15	21
Sweetness (too much)	3	3
Juiciness (not enough)	13	13
Juiciness (too much)	5	3
Tenderness of skin (not enough)	13	18
Tenderness of skin (too much)	3	10 A
Firmness (too soft)	8	6
Firmness (too firm)	4	11 A

Table 3. Aftertaste rating (% agreeing with statement)

	Lactate	Benzoate
A very noticeable aftertaste	19	17
A somewhat noticeable aftertaste	47	47
No aftertaste at all	33	36
Pleasantness (Mean)	3.3 b	3.1

APPENDIX J

Appendix J: Consumer Test of Bologna

To investigate possible effects of antimicrobial ingredients on product quality, Kraft asked consumers to evaluate the aroma, flavor, and texture profile of bologna formulated with different antimicrobial combinations.

MATERIALS AND METHODS

Sample Preparation

Bologna (made with chicken and pork) was made in the Kraft manufacturing facility in Davenport, Iowa on February 1, 2006. Two treatments were produced: a sodium lactate and diacetate treatment formulated according to the Purac OptiForm® Listeria growth suppression model, and a sodium benzoate treatment with 0.1% sodium benzoate and 0.11% sodium diacetate.

Consumer Testing

A mall-recruited central location test was conducted in February 2006. One hundred respondents (bologna users) were recruited across four cities: Chicago IL, Springfield MO, Charlotte NC, and Los Angeles, CA. Respondents were given one package of bologna to evaluate in a sequential monadic order. A balanced presentation was used to avoid bias.

RESULTS

Table 1 shows overall liking scores and Table 2 shows an assessment of attributes using "just about right" ratings (JAR). Figure 1 shows consumer rating of potential concern about various ingredients commonly used in bologna as well as sodium benzoate and sodium propionate.

Table 1 shows that there is a small difference in overall consumer liking and in a few other key liking factors between bologna made with sodium lactate versus sodium benzoate. Table 2 shows that the bologna with sodium benzoate was considered to be lower in bologna flavor, spiciness, and saltiness. This is felt to be caused by the lower salt intensity of the benzoate treatment due to removal of the lactate. The salt level of our bologna was reduced when lactate was added a number of years ago, and for this test, the salt in the sodium benzoate treatment was not increased. Pilot plant testing suggests that this slight flavor decrease can be made up by adding back a small amount of potassium chloride.

Figure 1 shows that consumer concern about sodium benzoate or sodium propionate, as ingredients in bologna, was no different than for other commonly used ingredients.

The data support a conclusion that sodium benzoate (as compared to sodium lactate) has only a minimal effect on consumer liking of bologna, most likely due to decreased saltiness.

Table 1. Consumer liking ratings (10 point scale)

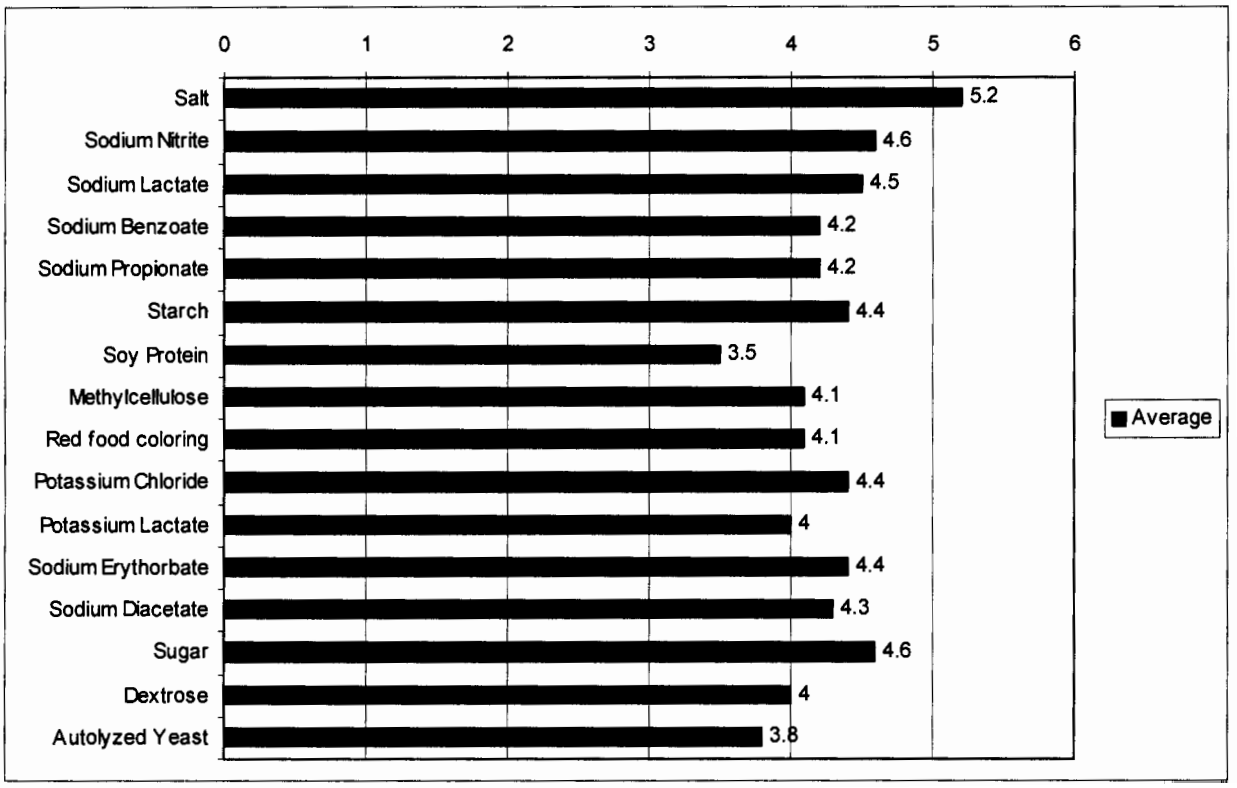
	Lactate	Benzoate
Overall Liking	7.26 a	6.95
Appearance Liking	7.3 a	6.9
Surface Wetness Liking	6.9 A	6.4
Uniformity Liking	7.3 A	6.8
Color Liking	7.2 a	6.8
Aroma Liking	6.9	6.7
Flavor Liking	7.1 a	6.7
Spice Liking	6.4	6.3
Smoked Flavor Liking	6.4 a	6.1
Saltiness Liking	6.6 a	6.3
Texture Liking	7.0	6.8
Moistness Liking	6.6	6.5
Firmness Liking	7.1 a	6.8

Uppercase letters indicate column of significant difference at a 95% confidence level.
Lowercase letters indicate column of significant different at an 80% confidence level

Table 2. Non - Just about right ratings (% agreeing with statement)

	Lactate	Benzoate
Surface wetness (not enough)	3	7 b
JAR	79	75
Surface wetness (too wet)	18	18
Uniformity (not enough)	4	11 b
JAR	93	84
Uniformity (too much)	3	5
Color (too light)	9	13
JAR	88	86
Color (too dark)	3	1
Aroma (not strong enough)	8	12
JAR	75	81
Aroma (too strong)	17 A	7
Bologna Flavor (not strong enough)	8	17 b
JAR	80	75
Bologna Flavor (too strong)	12	8
Spiciness (not enough)	22	34 b
JAR	72	64
Spiciness (too much)	6 a	2
Smokiness (not enough)	24	34 b
JAR	71	62
Smokiness (too much)	5	4
Saltiness (not enough)	12	23 B
JAR	78	71
Saltiness (too much)	10	6
Moistness (not enough)	4	9 b
JAR	77	80
Moistness (too much)	19 a	11
Firmness (too soft)	6	9
JAR	88	88
Firmness (too firm)	6	3

Figure 1. Concern about ingredients (10 point scale, 10 = extremely concerned)



APPENDIX K

Appendix K: Consumer Test of Ham

To investigate possible effects of antimicrobial ingredients on product quality, Kraft asked consumers to evaluate the aroma, flavor, and texture profile of ham formulated with different antimicrobial combinations.

MATERIALS AND METHODS

Sample Preparation

Ham (Water Added) was made in the Kraft manufacturing facility in Kirksville, MO the week of October 21 2006. Two treatments were produced: a sodium lactate and diacetate treatment formulated according to the Purac OptiForm® Listeria growth suppression model, and a sodium benzoate treatment with 0.1% sodium benzoate and 0.15% sodium diacetate, and 0.2% sodium propionate. Both treatments were stuffed into impermeable cook-in- bag casings and cooked in a hot water deluge process.

Consumer Testing

A mall-recruited central location test was conducted in November 2006. One hundred and fifty two respondents (ham users) were recruited across four cities: Charlotte NC, Springfield MO, Dallas TX, and Baltimore MD. One serving of six slices (51gm) of each treatment was presented in a balanced sequential monadic design. Liking and attribute responses were obtained for each sample.

RESULTS

Table 1 shows overall liking scores and Table 2 shows an assessment of attributes using just about right ratings.

Table 1 shows that ham made with sodium benzoate and propionate is liked directionally more than ham made with sodium lactate. Saltiness liking was significantly higher for the sodium benzoate and propionate treatment, and it had a less unpleasant aftertaste. Table 2 shows that the only significant difference in ham attributes is slightly more ham and smoky flavor, and less salty flavor, in the sodium benzoate/propionate treatment.

The data support a conclusion that a combination of sodium benzoate and sodium propionate does not adversely affect consumer liking of ham.

Table 1. Consumer liking ratings (10 point scale)

	Lactate	Benzoate & Propionate
Overall Liking	7.03	7.28
Appearance Liking	7.1	7.1
Surface Wetness Liking	6.6	6.8
Color Liking	6.9	7.0
Aroma Liking	6.8	6.9
Smoky Aroma Liking	6.5	6.7
Flavor Liking	6.9	7.2
Smoked Flavor Liking	6.8	7.0
Saltiness Liking	6.2	6.7 A
Texture Liking	6.8	7.0
Moistness Liking	6.6	6.8
Firmness Liking	6.6	6.9
Aftertaste Liking (% agreeing with statement)		
Pleasant aftertaste	66%	70%
Neither pleasant nor unpleasant	19%	22%
Unpleasant aftertaste	14% b	8%

Uppercase letters indicate column of significant difference at a 95% confidence level.
Lowercase letters indicate column of significant different at an 80% confidence level.

Table 2. Just about right ratings (% agreeing with statement)

	Lactate	Benzoate & Propionate
Fatty Appearance (too lean)	7	9
Just about Right	84	83
Fatty Appearance (too fatty)	10	8
Surface Wetness (not wet enough)	5	4
Just about Right	83	82
Surface Wetness (too wet)	13	14
Uniformity (not uniform enough)	7	7
Just about Right	88	90
Uniformity (too uniform)	5	3
Pink Color (not enough pink)	10	10
Just about Right	86	83
Pink Color (too pink)	4	7
Aroma (not strong enough)	9	9
Just about Right	84	86
Aroma (too strong)	7	6
Ham Flavor (not strong enough)	10 b	5
Just about Right	83	88 a
Ham Flavor (too strong)	7	7
Smokiness (not smoky enough)	13	12
Just about Right	84	81
Smokiness (too smoky)	3	7 c
Saltiness (not salty enough)	8 b	4
Just about Right	70	79 a
Saltiness (too salty)	22	17
Moistness (not moist enough)	6	5
Just about Right	84	80
Moistness (too moist)	11	14
Firmness (too soft)	5	2
Just about Right	86	87
Firmness (too firm)	9	11

Uppercase letters indicate column of significant difference at a 95% confidence level.
Lowercase letters indicate column of significant different at an 80% confidence level.

APPENDIX L

Appendix L: Consumer Test of Turkey Breast

To investigate possible effects of antimicrobial ingredients on product quality, Kraft asked consumers to evaluate the aroma, flavor, and texture profile of turkey breast formulated with different antimicrobial combinations.

MATERIALS AND METHODS

Sample Preparation

Turkey breast was made in the Kraft manufacturing facility in Newberry, South Carolina the week of October 21, 2006. Two treatments were produced: a sodium lactate and diacetate treatment formulated according to the Purac OptiForm® Listeria growth suppression model, and a sodium benzoate treatment with 0.1% sodium benzoate, 0.15% sodium diacetate, 0.2% sodium propionate, and Lem-O-Fos® (sodium phosphate and lemon juice concentrate). Both treatments were stuffed into impermeable cook-in-bag casings and cooked in a stationary smokehouse oven.

Consumer Testing

A mall-recruited central location test was conducted in November 2006. Two hundred and five respondents (turkey breast users) were recruited across four cities: Charlotte NC, Springfield MO, Dallas TX, and Baltimore MD. One serving of six slices (51gm) of each treatment was presented in a balanced sequential monadic design. Liking and attribute responses were obtained for each sample.

RESULTS

Table 1 shows overall liking scores and Table 2 shows an assessment of attributes using just about right ratings.

Table 1 shows that turkey breast made with sodium benzoate and propionate is equally liked overall compared to turkey breast made with sodium lactate. Oven roasted flavor, texture and aftertaste were all liked significantly more for the benzoate and propionate treatment. Table 2 shows that the benzoate/propionate treatment was closer to "just about right" in uniformity, turkey breast flavor, and oven roasted flavor.

The data support a conclusion that a combination of sodium benzoate, sodium propionate and Lem-O-Fos® does not adversely affect consumer liking of turkey breast.

Table 1. Consumer liking ratings (10 point scale)

	Lactate	Benzoate & Propionate
Overall Liking	6.88	6.86
Appearance Liking	6.9	7.0
Surface Wetness Liking	6.5	6.8
Color Liking	6.6	6.8
Appearance Outer Edge Liking	6.3	6.5
Aroma Liking	6.5	6.7
Oven Roasted Aroma Liking	6.2	6.4
Flavor Liking	6.7	6.8
Oven Roasted Flavor Liking	6.5	6.8 a
Saltiness Liking	6.4	6.6
Texture Liking	6.5	6.8 a
Moistness Liking	6.3	6.6
Firmness Liking	6.6	6.8
Aftertaste Liking (% agreeing with statement)		
Pleasant aftertaste	56%	63% a
Neither pleasant nor unpleasant aftertaste	31% b	23%
Unpleasant aftertaste	13%	14%

Uppercase letters indicate column of significant difference at a 95% confidence level.
Lowercase letters indicate column of significant different at an 80% confidence level.

Table 2. Just about right ratings (% agreeing with statement)

	Lactate	Benzoate & Propionate
Fatty Appearance (too lean)	7	5
Just about Right	89	89
Fatty Appearance (too fatty)	4	6
Surface Wetness (not wet enough)	8	7
Just about Right	83	82
Surface Wetness (too wet)	8	10
Uniformity (not uniform enough)	11	7
Just about Right	85	90 a
Uniformity (too uniform)	4	3
Color (not enough pink)	9	10
Just about Right	86	86
Color (too pink)	5	4
Aroma (not strong enough)	12	11
Just about Right	80	81
Aroma (too strong)	8	8
Turkey Breast Flavor (not strong enough)	20 b	13
Just about Right	78	80
Turkey Breast Flavor (too strong)	3	7
Oven Roasted Flavor (not oven roasted enough)	20 b	14
Just about Right	78	81
Oven Roasted Flavor (too oven roasted)	3	5
Saltiness (not salty enough)		
Just about Right		
Saltiness (too salty)		
Moistness (not moist enough)	11	8
Just about Right	83	84
Moistness (too moist)	6	7
Firmness (too soft)	11	8
Just about Right	84	86
Firmness (too firm)	5	5

Uppercase letters indicate column of significant difference at a 95% confidence level.
Lowercase letters indicate column of significant different at an 80% confidence level.

APPENDIX M

Appendix M: Effect on Nutritional Composition

Kraft evaluated the nutritional composition of hot dog, bologna, and ham products formulated with sodium benzoate, sodium diacetate, and sodium propionate as compared to similar products formulated with lactate salts.

MATERIALS AND METHODS

Sample Preparation

The hot dogs and bologna manufactured for consumer testing as described in previous appendices were analyzed for moisture, protein, fat, ash and sodium using standard AOAC methods for meats (see Appendices I and J). The ham was manufactured in the Kraft Foods Research Pilot Plant in Madison, Wisconsin in October 2006 with the same formulas used in the ham consumer tests (see Appendix K).

RESULTS

Table 1 shows the analysis of products made with sodium lactate versus sodium benzoate (or sodium benzoate and propionate). The only significant differences are a decrease in sodium and a decrease in ash and an increase in moisture as lactate solids are replaced by water. Sodium was decreased by about 10% in hot dogs, 15% in bologna, and 15% in ham—amounting to a decrease of about 70 to 120 mg per 55 g reference amount. This 10 to 15% reduction of sodium in the product corresponds to 3 to 5% of the 2400 mg daily value for sodium. When viewed on a population-wide basis, this incremental reduction in commonly consumed foods can advance important public health goals.

Table 1. Composition of Products made with Lactate versus Benzoate/Propionate

		Moisture	Protein	Fat	Ash	Sodium
		%	%	%	%	mg/100g
Hot Dogs	Lactate	59.2	11.9	23.8	3.9	1230
	Benzoate	59.7	11.9	23.8	3.6	1100
Bologna	Lactate	53.9	11.5	26.5	3.7	1190
	Benzoate	54.5	11.5	26.4	3.5	1010
Ham	Lactate	74.9	17.5	2.4	Not determined	1460
	Benzoate / Propionate	77.0	17.8	2.2	Not determined	1240
	AOAC Method	950.46Bb	992.15	960.39	920.153	984.27

REFERENCES

Omitted from electronic copy
(See hard copies)