CONTROL OF *LISTERIA MONOCYTOGENES* POST-PROCESSING CONTAMINATION IN PORK PRODUCTS

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Background

The fatal 1998-99 listeriosis outbreak in the United States (2) and the numerous recalls associated with cured meat products repackaged after cooking and found to be contaminated indicate that *Listeria monocytogenes* cross-contamination of such products can not be prevented (5). Thus, there is a need for hurdles to control the pathogen during product storage (5, 7). Since irradiation is not approved for use on packaged ready-to-eat meats, and physical methods, such as high pressure technologies, are under development, expensive or of unknown effectiveness on these products, the application of chemical antimicrobials remains the most effective hurdle technology against this deadly pathogen in cured meat products. For this reason, the United States Department of Agriculture (USDA) announced (3) the increase of permissible levels of sodium lactate to 4.8% of a commercially available 60% potency product, and approved the use of 0.25% sodium acetate and sodium diacetate as antimicrobial agents in meat products, while it indicated that it could consider further increases of these levels, or use of additional chemical preservatives, if research data supported such approvals. Several studies have evaluated additives for control of *L. monocytogenes* in meat products (1, 4, 6, 7). However, the effects of antimicrobials applied post-processing but pre-packaging as well as post-packaging thermal pasteurization treatments against *L. monocytogenes* introduced on meat products after peeling or slicing need to be evaluated. Such thermal treatments may be effective alone, or may enhance inhibition by antimicrobials, as their activity is targeted onto the product surface where *L. monocytogenes* cells usually attach post-processing.

Objectives

Studies in our laboratory have evaluated the antilisterial activity of the currently permissible, and of increased, levels of chemicals, such as sodium lactate, sodium acetate, sodium diacetate, as single or combined antimicrobials, in frankfurter formulations; they have also been tested in combination with other chemicals and/or post-packaging thermal pasteurization. In addition, individual or combined antimicrobials have been evaluated for their antilisterial effects when applied as post-processing immersion solutions on inoculated sliced bologna.

Materials and Methods

Pork frankfurters (24 mm in diameter) and bologna (65 mm) were prepared using the same formulation and standard commercial practices. All bologna were prepared without antimicrobials, while frankfurters were formulated with or without antimicrobials. Both products were surface-inoculated (10^2-10^4 CFU/cm^2; ten-strain composite) with *L. monocytogenes* after peeling or slicing. After inoculation, frankfurters were vacuum-packaged (1 or 2 links per bag; 20 x 25 cm, 3 mil std barrier, Nylon/PE vacuum pouch; Koch, Kansas City, MO) and stored at 4°C, either directly or after packages received a 30, 60 or 90-sec immersion in a water bath (80°C). For sliced bologna, inoculation was done before or after immersion for 1 min in filter-sterilized solutions of antimicrobials. After treatment, bologna slices were vacuum packaged (2 slices per bag; 6 x 8.5 cm vacuum pouches) and stored at 4°C. Triplicate samples from each of 1-3 replicates were plated at 0, 10, 20, 35, 50, 70 and 90 d of storage on tryptic soy agar with 0.6% yeast extract (TSAYE) and PALCAM (Difco) agar plates, and incubated at 30°C for 48 h. Products were also analyzed for pH, fat and moisture contents, and water activity. Microbiological results are reported as time (days) of storage at 4°C needed for *L. monocytogenes* to exceed specified levels (log CFU/cm^2) of growth.

Results and Discussion

Inoculated *L. monocytogenes* exceeded 5 log CFU/cm^2 within 20 d of refrigerated (4°C) storage of sliced bologna or peeled frankfurters without antimicrobials. When used singly, the antimicrobials were effective either in the formulation (Table 1) or as post-processing immersion solutions (Table 2), depending on their nature and mode of action. Sodium diacetate was the only antimicrobial that, in single form, inhibited growth for up to 120 d by either method of application. In contrast, sodium acetate and GDL permitted growth within 20 (Table 2) to 35 d (Table 1), while sodium lactate was effective only when it was included in the formulation, especially by doubling its permissible level from 3% to 6% (Table 1). Conversely, sodium lactate in dipping solution (5-10%) had a limited effect on the pathogen (Table 2). Importantly, the antilisterial effect of 3% sodium lactate was increased by approximately two-fold when it was combined with 0.25% of sodium acetate, sodium diacetate or GDL in the formulation (Table 1). Post-packaging thermal pasteurization enhanced inhibition by 3% sodium lactate (Table 1), while it reduced surviving populations by approximately 0.5 to 1 log CFU/cm^2 throughout storage (120 d) when applied to products with the above combinations of additives (data not shown). In heat-treated frankfurters without antimicrobials, however, rapid growth of *L. monocytogenes* occurred (Table 1), especially when two links were placed in each vacuum bag, and the heating time was decreased from 90 to 60 to 30 sec (data not shown).

Organic acids and potassium benzoate or sorbate were tested as post-processing immersion solutions only (Table 2). At equal concentrations (2.5% in immersion solutions), acetic acid was more inhibitory than lactic acid; 5% lactic acid was needed to prevent growth (70 d). Likewise, at equal concentrations (5%), potassium benzoate controlled *L. monocytogenes* better than potassium sorbate during storage. Overall, the addition of nisin (0.5%) in dipping solutions of organic acids or salts enhanced their inhibitory effect, while nisin alone was ineffective after 20 d. However, nisin reduced counts of inoculated *L. monocytogenes* by 1-1.5 log CFU/cm^2 at day 0, irrespective of acid or salt presence. Notably, the immersion of uninoculated bologna in 0.5% nisin, followed by inoculation, increased inhibition by the bacteriocin itself, or by solutions of organic acid salts, but not lactic acid, applied after inoculation (Table 2). This result deserves further investigation in relation to the mode of action of nisin and the pH of chemical antimicrobials in solution.

Acetic acid, lactic acid and sodium diacetate were the only antimicrobials in dipping solutions that had a marked decreasing effect (0.5 to 1.5 pH units) on bologna pH (6.3 to 6.5) after immersion. Antimicrobials in the formulation did not affect the pH of frankfurters, except for 0.5% sodium diacetate, which slightly reduced (0.3 to 0.4 pH units) the initial pH (6.3) of the product.

Conclusions

Although shown to be effective, further increases of the currently permissible levels of sodium lactate and sodium acetate/diacetate in the formulation of processed meat products may not be necessary if additives are used in combination, or are combined with application of post-packaging thermal treatments. Immersion or spraying of meat products after cooking, but before repackaging, with combined solutions of antimicrobials may be an alternative method for controlling *L. monocytogenes* post-processing contamination in such products. These multi-hurdle approaches may also lessen any sensory defects on the product quality by using antimicrobials at lowered concentrations. Nevertheless, product development and sensory quality studies should be performed in plants, before commercial application of any of the effective treatments of this study. In addition, technological developments are needed for the application of antimicrobial treatments that are not part of the formulation.

Table 1. Time (days) of storage (4°C) needed for inoculated (3.1 to 4.0 log CFU/cm^2) *L. monocytogenes* to exceed specified levels of growth on peeled frankfurters with antimicrobials in the formulation and/or heat-treated (80°C, 60 sec) after packaging

Treatment	Initial	After heating	Days to exceed			No growth
	Log CFU/cm^2	Log CFU/cm^2	4 logs	5 logs	7 logs	
No antimicrobial	3.2		10	20	35	
No antimicrobial/in package heat-treatment	3.9	2.8	20	35	50	
Sodium acetate (0.25%)	3.3		35	35	70	
Sodium acetate (0.5%)	3.3		35	70	>120	
Sodium diacetate (0.25%)	3.2		50	70	>120	
Sodium diacetate (0.5%)	3.4					>120
Glucono-D-lactone (GDL) (0.25%)	4.0		10	20	35	
Sodium lactate (3%)	3.2		50	50	≥120	
Sodium lactate (6%)	3.2					>120
Sodium lactate (3%)+sodium acetate (0.25%)	3.1					>120
Sodium lactate (3%)+sodium diacetate (0.25%)	3.2					>120
Sodium lactate (3%)+ GDL (0.25%)	3.5					>120
Sodium lactate (3%)/in package heat-treatment	3.2	2.6	50	70	≥120	

Table 2. Time (days) of storage (4°C) needed for inoculated (2.3 to 2.8 log CFU/cm^2) *L. monocytogenes* to exceed specified levels of growth on sliced, vacuum packaged bologna immersed in antimicrobial solutions post-processing

Solutions/sequence of inoculation/treatment	Initial	After treatment	Days to exceed			No growth
	Log CFU/cm^2	Log CFU/cm^2	3 logs	5 logs	7 logs	
Inoculated/No immersion (control)	2.3	2.3	10	20	20	
Inoculated/Water (control)	2.3	1.9	10	20	20	
Inoculated/Nisin (0.5%)	2.8	<1.9	20	20	35	
Nisin (0.5%)/inoculated	2.8	<1.9	20	50	70	
Inoculated/Acetic acid (2.5%)	2.3	1.9				>120
Inoculated/Lactic acid (2.5%)	2.3	1.9	10	20	70	
Inoculated/Lactic acid (5%)	2.3	1.5	90	>120	>120	
Inoculated/Lactic acid (5%)+nisin (0.5%)	2.5	<1.9	120	>120	>120	
Inoculated/Sodium acetate (5%)	2.3	1.9	20	35	70	
Inoculated/Sodium acetate (5%)+nisin (0.5%)	2.7	<0.9	35	70	>120	
Nisin (0.5%)/inoculated/sodium acetate (5%)	2.6	0.9	70	90	>120	
Inoculated/Sodium diacetate (5%)	2.3	1.9				>120
Inoculated/Sodium diacetate (3%)+nisin (0.5%)	2.7	0.9	120	>120	>120	
Nisin (0.5%)/inoculated/sodium diacetate (3%)	2.6	<0.9				>120
Inoculated/Sodium lactate (5%)	2.3	1.7	10	20	50	
Inoculated/Sodium lactate (10%)	2.3	2.0	10	35	50	
Inoculated/Potassium benzoate (5%)	2.3	2.1	120	>120	>120	
Inoculated/Potassium sorbate (5%)	2.3	1.9	50	90	>120	
Inoculated/Potassium sorbate (3%)+nisin (0.5%)	2.7	1.2	50	90	>120	
Nisin (0.5%)/inoculated/potassium sorbate (3%)	2.6	<0.9	90	120	>120	

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compositions and additives, protein vegetable preparations, including modified (extruded) ones made from peas, lentil and other leguminous appeared at domestic market.

At the end of 1998 there was a trend to the increase of Russian animal products production (due to high prices for foreign products), it was especially evident poultry production. As a result, local poultry processing enterprises provided their products to the domestic market, forcing out foreign poultry products from it. This situation gave impetus to Russian scientists to study the problems of growing and feeding of farm poultry, and to less extent – pigs and cattle.

It is interesting to note that up to the present time the work is being carried out in Russia on the development of feed additives from inedible slaughter products (blood, by-products). And this is in spite of the threat of bovine spongiofom encephalopathy . The explanation to this – Russian standards require high temperature treatment (118-122°C) for not less that 45 minutes.

There was a great deal of interest to problems of storage and development of new packaging materials.

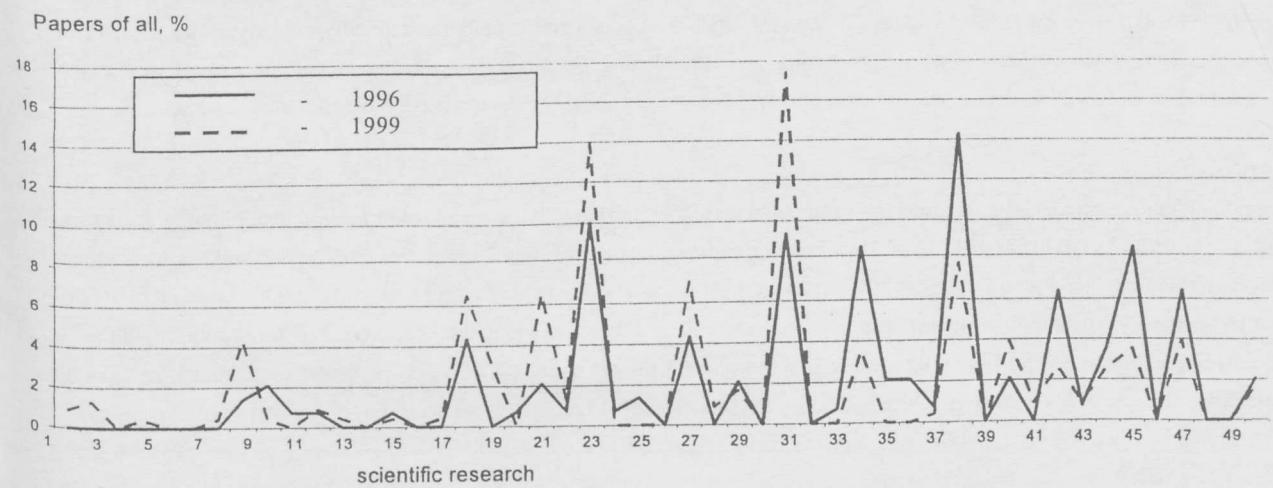
In general, it should be noted that socio-economic situation has had an impact on the field of research of Russian scientists, which primarily were directed to the development of meat products with different functional additives with the aim of cost reduction of final products, to the organization of marketing system and development of scientific bases of prediction of consumer demands - earlier, with planned economics, there had been no requirements in such investigations. At the same time one should note a high scientific potential in the field of development of functional products and processing of slaughterhouses wastes for food and feed materials.

Analysis of state of food engineering has shown backwardness of Russian developments, that were mainly aimed at automation and mechanization of individual stages of technological processes, while the leading foreign designers were creating fully automatic lines with the use of elements of robotic equipment. Unfortunately, backwardness of technical level of the domestic counterparts continues, which has a serious negative effect on competitiveness of Russian equipment and production cost of meat products manufactured in this country.

We compared the fields of scientific interests of Russia and countries of the world. There were not found a large amount of works devoted to the study of pre-slaughter handling of animals, slaughter, primary processing of the animals and the influence of these factors on quality of final products. It is explained by the absence of economical conditions for development of Russian animal husbandry during the indicated period. The Russian scientists more actively studied problems of storage and quality of meat raw materials and aspects of its safety, technologies of processing and obtaining of final products of guaranteed quality and safety , development of special food products, automation and mechanization of technological processes. While the efforts of foreign scientists were directed to revealing of life-time factors influencing the quality attributes of meat raw materials, their studying and predicting to provide high-quality raw materials to processors, development of objective methods of evaluation and a system of safety control of foods over the whole technological chain, evaluation of the meat composition and its influence on the health of humans, evaluation of safety of food additives and ingredients used in the meat industry.

However, the comparative analysis has shown that in the scientific development of such directions as creation of functional products (or curative-preventive foods according to Russian terms) Russian scientists have an obvious priority. This activity was originated in 1950s when creating the foods for cosmonauts. Based on it a methodology of curative-preventive nutrition has been developed, which is going on at the present time . Technogenic catastrophes that became more frequent in recent times, unfavorable ecological situation in a number of Russian regions, increase of the share of population with chronic diseases, obesity, cardiovascular metabolic diseases, etc. led the necessity of development of products containing ingredients capable to accumulate and remove harmful compounds from the organism, such as radionuclides, heavy metals and their salts, nitrosamines, toxins, etc. A wide range of meat-based curative-preventive products enriched with specific biocorrectors was developed for a wide circle of persons, primarily oncological patients, subjected to intensive radiation and chemical therapy, as well as for people who had suffered from radiation accidents or living at contaminated with radiation areas or in regions contaminated with genotoxic substances, inhibiting blood-forming and immune system. The investigations in this field are on the increase.

Trend of scientific interests in Russia for the period 1996 – 1999



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