Inhibition of Nonproteolytic, Psychrotrophic Clostridia and Anaerobic Sporeformers by Sodium Diacetate and Sodium Lactate in Cook-in-Bag Turkey Breast

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ABSTRACT

A nonproteolytic, psychrotrophic Clostridium isolate, designated strain OMFRI1, was recovered from cook-in-bag turkey breasts (CIBTB) that displayed an intense pink discoloration and an off-odor following extended refrigerated storage. The viability of strain OMFRI1 in CIBTB containing sodium diacetate (at 0, 0.25, and 0.5%) and/or sodium lactate (at 0, 1.25, and 2.5%) was subsequently evaluated. Raw CIBTB batter was inoculated with 9 to 30 spores of strain OMFRI1 per g, vacuum packaged, cooked to an instantaneous internal temperature of 71.1°C, chilled, and incubated at 4°C for up to 22 weeks. In the absence of food-grade antimicrobial agents, spoilage (i.e., an off-odor) occurred within 6 weeks, and anaerobic plate counts reached 6.6 log_{10} CFU/g. The CIBTB containing sodium diacetate (0.25%) and that containing sodium lactate (1.25%) required 12 weeks for spoilage to occur and for anaerobic plate counts to reach 7.0 and 6.0 log_{10} CFU/g, respectively. When sodium diacetate (0.25%) and sodium lactate (1.25%) were used in combination, no off-odor was detected and anaerobic plate counts did not exceed 2.3 log_{10} CFU/g over 22 weeks of storage at 4°C. In related experiments, sodium diacetate (at 0, 0.25, and 0.5%), sodium lactate (at 0, 1.25, and 2.5%), and combinations of both ingredients were evaluated in uninoculated CIBTB incubated at 25°C for up to 22 days. In the absence of antimicrobial agents and in CIBTB containing sodium diacetate (0.5%), spoilage occurred within 8 days and anaerobic plate counts reached 6.8 and 6.6 log_{10} CFU/g, respectively. Samples of CIBTB containing sodium lactate (2.5%) showed signs of spoilage within 22 days, and anaerobic plate counts for these samples ranged from ≤1.0 to 6.3 log_{10} CFU/g. In CIBTB containing both sodium lactate (2.5%) and sodium diacetate (0.25%), spoilage was not evident and anaerobic plate counts were ≤1.0 log_{10} CFU/g within 22 days. These data validate the efficacy of sodium lactate and sodium diacetate in extending the shelf life of CIBTB.

Cook-in-bag refrigerated turkey breast (CIBTB) products are fully cooked in gas- and moisture-impermeable bags and sold in these packages to food service and retail establishments. A CIBTB is manufactured from whole or ground turkey breast muscles and receives an instantaneous heat treatment of 71.1°C to inactivate vegetative cells of spoilage and pathogenic bacteria. The cooking process is not designed to destroy bacterial spores or to bring about commercial sterility; thus, spores survive and persist in the final product. Since CIBTB are low in salt (~1.5%) and have a near-neutral pH, refrigeration is the sole barrier to the outgrowth of surviving spores of anaerobic bacteria. A CIBTB has a minimum expected shelf life of 90 days when it is properly stored at ≤4°C. Occasionally, however, this 90-day shelf life is not realized because of spoilage, as manifested by a strong off-odor and an intense pink discoloration in the interior when the product is cut or sliced. We isolated nonpathogenic, nonproteolytic, psychrotrophic clostridia (prototype designated strain OMFRI1) from “spoiled” CIBTB reportedly stored at the proper temperature. As summarized by Kalinowski and Tompkin (9), psychrotrophic Clostridium spp. have been isolated from vacuum-packaged beef, pork, and lamb, as well as from pasteurized crab meat (4, 5, 7, 9, 10).

The repeated isolation of psychrotrophic spoilage organisms like Clostridium strain OMFRI1 prompted our interest in identifying interventions to control spoilage anaerobes in CIBTB products. The germination and outgrowth of proteolytic Clostridium botulinum spores and the subsequent toxin production of these spores is an additional concern associated with temperature-abused CIBTB products. There have been several reports on the efficacy of sodium diacetate and sodium lactate in inhibiting facultative bacteria and anaerobic spore-forming bacteria (6, 11, 15, 17). Although sodium nitrite or sodium lactate could be added to CIBTB as a secondary barrier, these antimicrobial agents impact the product’s flavor and/or appearance. Thus,
TABLE 1. Analytical values for cook-in-bag turkey batters used in the inoculation studiesa

<table>
<thead>
<tr>
<th>Treatment no.</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Sodium chloride (%)</th>
<th>Phosphate (%)</th>
<th>Sodium lactate (%)</th>
<th>Sodium diacetate (%)</th>
</tr>
</thead>
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<td>1 (control)</td>
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<td>17.0</td>
<td>1.2</td>
<td>1.56</td>
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<td>0.00</td>
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<td>0.23</td>
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<td>2.37</td>
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</tbody>
</table>

* Values presented are mean values for two trials.

sodium diacetate may be an acceptable alternative to sodium nitrite or sodium lactate if this compound proves to be more inhibitory to anaerobic spore-forming bacteria at abusive temperatures and does not have an impact on flavor and/or appearance.

Sodium diacetate is approved for use in certain foods (at levels of 0.05 to 0.4%) as an antimicrobial agent, a flavorant, and a pH control agent (2). At a level of 0.25%, sodium diacetate inhibited the growth of *Listeria monocytogenes* in turkey slurries during storage at 5°C (17). Schlyter et al. (15, 16) also established the efficacy of sodium diacetate with nitrite, lactate, and/or pediocin in controlling *L. monocytogenes* in turkey slurries during storage at 4 and 25°C. Likewise, sodium lactate is a generally-recognized-as-safe substance approved (with no limitation) for use in certain foods as an emulsifier, a flavorant, a humectant, and a pH control agent (3). The antibotulinal activity of sodium lactate has also been well established. For example, in temperature-abused CIBTB, sodium lactate (at 2.0 to 3.5%) delayed toxin formation by proteolytic strains of *C. botulinum* by 1 to 4 days (11). Also, sodium lactate (2.0%) and/or sodium diacetate (0.1%) showed potential for delaying the growth of psychrotrophic clostridia in a cooked turkey meat product (9). The U.S. Department of Agriculture’s Food Safety and Inspection Service has issued a final rule increasing the permissible levels of sodium lactate and potassium lactate to 4.8% and that of sodium diacetate to 0.25% for the inhibition of the growth of *L. monocytogenes* and *C. botulinum* in meat and poultry products (1). The purpose of this study was to determine whether sodium diacetate, sodium lactate, and/or a combination of these ingredients can delay CIBTB spoilage caused by strain OMFR1 during refrigerated storage. In addition, these compounds were also evaluated in uninoculated CIBTB subjected to temperature abuse conditions for the potential to delay spoilage caused by anaerobic sporeformers.

**MATERIALS AND METHODS**

**Bacterial strain and spores.** *Clostridium* strain OMFR1 was isolated from a spoiled CIBTB product. Subcultures of strain OMFR1 were sent to the Anaerobe Laboratory at Virginia Polytechnic Institute and State University (Blacksburg, Va.) for fatty acid analyses and biochemical characterization. Strain OMFR1 was identified as a nonproteolytic isolate that is closely related to *Clostridium subterminale* and is capable of growing at 4°C. The spore crop for the inoculation of CIBTB was prepared by the National Food Processors Association (Washington, D.C.) with the use of previously described methods (7, 8). The spores were washed several times with sterile deionized water, resuspended, and stored on glass beads at −65°C until they were used.

**Preparation of CIBTB batter.** A CIBTB product was developed from breast halves (73.5%), tenderloins (14.7%), and trimmings (11.8%) from turkeys to mimic a commercial food service product. The breast halves and tenderloins were ground to 2.5 cm and the trimmings were ground to 0.16 cm with a Hobart grinder (Model 4056, Hobart Manufacturing Co., Troy, Ohio). Sodium chloride (1.4%; HG Blending Salt, Morton International, Inc., Chicago, Ill.), sodium tripolyphosphate (0.5%; Rhone-Poulenc Inc., Cranbury, N.J.), kappa carrageenan (0.25%; FMC Corp., Rockland, Maine), 60% aqueous sodium lactate (0, 2.1, or 4.2%; Purac America, Wood Dale, Ill.), and/or sodium diacetate (0, 0.25, or 0.5%; American International Chemicals Inc., Natick, Mass.) were added to the ground turkey with a sufficient amount of water to achieve a target moisture level of about 76%. The sodium lactate levels were calculated on an anhydrous basis from 60% sodium lactate. The ground turkey and nonmeat ingredients were mixed under vacuum in a twin-paddle mixer. The CIBTB batter was held overnight at about 1°C to allow the ingredients to equilibrate before it was reground to 0.64 cm and samples were collected for chemical analyses. As shown in Table 1, the CIBTB batter was analyzed for moisture, protein, fat, sodium chloride, phosphate, sodium lactate, and sodium diacetate by the procedures of the Association of Official Analytical Chemists (12).

**Preparation and sampling of inoculated CIBTB stored at 4°C.** The CIBTB batter (4.5 kg) was inoculated with spores (9 to 30 spores per g) of strain OMFR1 suspended in 10 ml of Butterfield’s phosphate buffer (Hardy Diagnostics, Santa Maria, Calif.) and mixed in a Hobart mixer (Model A-0200) for 5 min. Portions (100 g each) of inoculated raw CIBTB batter were vacuum packaged in Curwood pouches (layered nylon and linear low-density polyethylene, with an O₂ transmission rate of 0.08 cm³ of O₂ per 645 cm² per 24 h at 7.2°C; Curwood Inc., New London, Wis.) with a Multivac packaging machine (Model AG800, Multivac Inc., Sepp Haggenmüller, Germany). The packaged CIBTB batter was submerged in a 72.2°C steam-injected water cook tank until it achieved an internal temperature of 71.1°C to ensure the inactivation of any vegetative bacterial cells present in the CIBTB batter. It took 15 to 20 min of heating for the batter to reach...
TABLE 2. Analytical values for cook-in-bag turkey used in temperature abuse studies (n = 2)\textsuperscript{a}

<table>
<thead>
<tr>
<th>Treatment no.</th>
<th>Moisture (%)</th>
<th>Sodium chloride (%)</th>
<th>Sodium lactate (%)</th>
<th>Sodium diacetate (%)</th>
<th>pH</th>
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<tr>
<td>1 (control)</td>
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\textsuperscript{a}Values presented are mean values for duplicate samples.

71.1°C internally. The water cook tank was aerated to provide even heating of the packages. The heated packages were rapidly chilled in ice water and then moved to a 4°C cooler for storage.

Packages of CIBTB were sampled for strain OMFRI1 0 and 4 weeks after processing and every 2 weeks thereafter. When the packages were opened, they were evaluated subjectively, by sniffing, for the development of any obvious off-odors. A 30- to 40-g sample was aseptically removed and diluted (1:10) with Butterfield’s phosphate buffer in a stomacher bag (Seward Ltd., London, UK). The diluted sample was macerated for 2 min in a stomacher blender (Model 400; Seward). Additional dilutions in Butterfield’s phosphate buffer were prepared as necessary. For each sample, a total aerobic plate count was obtained with APT (all purpose Tween) Agar (BD Diagnostic Systems, Sparks, Md.) supplemented with 2% sucrose and 0.0032% bromoresol purple (BD Diagnostic Systems), and a total anaerobic plate count was obtained with Brewer Anaerobic Agar (BD Diagnostic Systems) in conjunction with anaerobe jars (BD Diagnostic Systems) and H\textsubscript{2}CO\textsubscript{2} gas-generating envelopes (BD Diagnostic Systems). The APT plates were incubated for 72 h at 25°C, and the Brewer plates were incubated for 72 h at 30°C prior to the enumeration of colonies. This study was replicated twice with three packages at each sampling interval for each treatment and duplicate platings from each dilution thereafter.

Preparation and sampling of uninoculated CIBTB stored at 25°C. The CIBTB batter (4.5 kg) was vacuum packaged in 0.7- to 0.8-kg portions with a Multivac packaging machine (Model R7000) and with forming and nonforming films (layered nylon and linear low-density polyethylene, with an O\textsubscript{2} transmission rate of 2.0 to 3.0 cm\textsuperscript{2} of O\textsubscript{2} per 645 cm\textsuperscript{2} per 24 h at 22.8°C; Curwood). The packaged CIBTB batter was heat processed in a stationary smokehouse (Model Rondair; Maurer and Sohne GmbH and Co., K.G., Reichenau, Germany) to an instantaneous internal temperature of 71.1°C, showered with cold water to an internal temperature of ≤32°C, and chilled in a 0°C cooler overnight. After chilling, two packages per treatment were analyzed for moisture, sodium chloride, sodium lactate, sodium diacetate, and pH (see Table 2) by the procedures of the Association of Official Analytical Chemists (12).

The internal temperature of one package of CIBTB was monitored for the first 24 h with the use of a data squirel (Model 6200 LTFN06, Data Trace, Littleton, Colo.) to determine the time required for the CIBTB to reach 25°C. Total aerobic and total anaerobic plate counts were determined for packages as described above after 0, 1, and 2 days of temperature abuse and at several additional times until the CIBTB was visually spoiled (i.e., gassy with an off-odor). Treatments that did not result in visual spoilage after 22 days of temperature abuse were discontinued. Tryptic soy agar (BD Diagnostic Systems) pour plates were used for the determination of total aerobic plate counts. The anaerobic plate count was determined as described above. All plates were incubated at 25°C for 72 h prior to the enumeration of colonies. This study was replicated twice with three packages at each sampling interval for each treatment and duplicate platings from each dilution thereafter.

RESULTS AND DISCUSSION

Viability of strain OMFRI1 inoculated into CIBTB containing sodium lactate and/or sodium diacetate during storage at 4°C. Biochemical characterization and fatty acid profiling identified strain OMFRI1 as nonproteolytic, psychrotrophic, and related to \textit{C. subterminale}. As detailed below, both sodium diacetate and sodium lactate effectively inhibited strain OMFRI1 and delayed the development of off-odors in CIBTB. In control CIBTB containing neither sodium diacetate nor sodium lactate, strain OMFRI1 grew and produced off-odors within 6 weeks at 4°C. The anaerobic plate count increased from 1.3 log\textsubscript{10} to 6.6 log\textsubscript{10} CFU/g within this period. Aerobic plate counts for the control and experimental treatments were negligible (<1.5 log\textsubscript{10} CFU/g) and are not reported herein.

The CIBTB containing 0.25% sodium diacetate did not develop off-odors until it had been stored for 12 weeks at 4°C; the anaerobic plate count increased from 1.3 to 7.0 log\textsubscript{10} CFU/g (Fig. 1A). The effect of sodium lactate was similar to that of sodium diacetate. The CIBTB containing 1.25% sodium lactate did not develop off-odors until it had been stored for 12 weeks at 4°C (Fig. 1B); the anaerobic plate count increased by 4.7 log\textsubscript{10} CFU/g. At levels of 2.5% sodium lactate, CIBTB did not develop off-odors for 22 weeks, and the anaerobic plate count increased by <0.5 log\textsubscript{10} CFU/g from the initial level of 1.3 log\textsubscript{10} CFU/g. The combination of sodium diacetate and sodium lactate had an additive effect. The inhibition of strain OMFRI1 was more extensive for CIBTB containing both 0.25% sodium diacetate and 1.25% sodium lactate than for CIBTB containing sodium diacetate or sodium lactate alone at these levels. Off-odors did not develop with this treatment, and the anaerobic plate count increased by <2 log\textsubscript{10} CFU/g over 22 weeks at 4°C (Fig. 1C).

The cook-in-bag manufacturing process results in products with a shelf life of ≥90 days owing to a combination of vacuum packaging, heating to 71.1°C to eliminate vegetative cells of spoilage and pathogenic bacteria, rapid chilling, and refrigerated storage. Postprocessing recontamination by non–spore-forming psychrotrophs such as lactic acid bacteria, \textit{Enterobacteriaceae}, and \textit{L. monocytogenes} is prevented, since the cook-in-bag package remains intact after processing. The outgrowth of most heat-resistant spores is controlled by the anaerobic environment and/or subsequent refrigerated storage. However, heat-resistant spores of psychrotrophic anaerobes have the potential to raise concerns about CIBTB.

The addition of sodium diacetate alone or in combi-
CONTROL OF CLOSTRIDIA IN TURKEY

FIGURE 1. Viability of Clostridium strain OMFRI1 in CIBTB formulated with and without sodium lactate and/or sodium diacetate during storage at 4°C (n = 2). (A) ■, control; ▲, 1.25% sodium lactate; ●, 2.5% sodium lactate. (B) ■, control; ▲, 0.25% sodium diacetate; ●, 0.5% sodium diacetate. (C) ■, control; ▲, 1.25% sodium lactate + 0.25% sodium diacetate; ●, 1.25% sodium lactate + 0.5% sodium diacetate; Δ, 2.5% sodium lactate + 0.25% sodium diacetate; ○, 2.5% sodium lactate + 0.5% sodium diacetate.

nation with sodium lactate can delay CIBTB spoilage caused by strain OMFRI1. A combination of both agents is also effective and may have less of an impact on the organoleptic properties of CIBTB because the required level of each compound is decreased with this approach. Sodium diacetate at 0.25% was detectable in CIBTB, and at 0.5% it was judged to be unacceptable in informal taste tests (data not shown). Some individuals also detected an off-flavor in CIBTB containing 2.5% sodium lactate. Presumably, although the possibility was not tested in this study, the addition of sodium lactate to CIBTB would have the added benefit of delaying the production of proteolytic C. botulinum toxin under temperature abuse conditions as reported previously (6, 11, 13, 14). The results of the present study are in agreement with results of a similar study conducted by Kalinowski and Tompkin (9). These investigators isolated several psychrotrophic clostridia strains from CIBTB, and three of these strains multiplied and spoiled the product by causing off-odors and a pink discoloration in an inoculated cooked turkey product within 70 days during storage at 3.3°C. In a second inoculated-product study, these same three strains displayed various behaviors in the presence of sodium lactate and/or sodium diacetate at levels of 2.0 and 0.1%, respectively. More specifically, one of the isolates was inhibited, another showed delayed growth, and the third grew rapidly. Kalinowski and Tompkin (9) also reported that different products typically harbor different species of clostridia. Beef products typically contain Clostridium laramie, whereas poultry products typically contain Clostridium ctm, a heretofore uncharacterized species. C. laramie tends to cause a strong odor and package swelling, and C. ctm tends to cause odor and pink discoloration without swelling. Further studies should be conducted to determine whether there is a species-specific correlation and/or whether discoloration is associated only with poultry.

Viability of anaerobic sporeformers in CIBTB containing sodium lactate and/or sodium diacetate during storage at 25°C. In related studies, we evaluated the efficacy of sodium lactate and sodium diacetate in delaying CIBTB spoilage caused by anaerobic spore-forming bacteria during storage at 25°C for up to 22 days. The internal temperature of CIBTB in both test groups increased from 0 to 25°C within 12 h after the samples were placed under abuse conditions (data not shown). Aerobic plate counts were negligible for the nine treatments tested and are not reported. The intention of the determination of aerobic plate counts was to differentiate between the growth of facultative organisms and that of obligate anaerobes. There was no difference between the control treatment (involving no sodium lactate or sodium diacetate) and the experimental treatments involving 0.25 and 0.5% sodium diacetate with respect to the rate of spoilage. Packages of CIBTB for these three treatments showed signs of spoilage (i.e., gas and off-odor) and contained approximately $10^7$ anaerobic organisms per g after 8 days at 25°C (Fig. 2A). The CIBTB containing 1.25% sodium lactate, with or without sodium diacetate, showed signs of spoilage after 10 days at 25°C and contained approximately $10^5$ anaerobic organisms per g at that time (Fig. 2B and 2C). The treatment involving a combination of 2.5% sodium lactate and 0.5% sodium diacetate was the most inhibitory treatment. Packages of CIBTB treated with this combination contained <10 anaerobic organisms per g after 22 days at 25°C. Anaerobic organisms grew in CIBTB containing 2.5% sodium lactate alone or 2.5% sodium lactate in combination with 0.25% sodium diacetate within 7 days at 25°C. By day 22, one of three packages of CIBTB containing 2.5% sodium lactate alone showed signs of spoilage, whereas all packages of CIBTB containing 2.5% sodium lactate in combination with 0.25% sodium diacetate remained visually acceptable for up to 22 days.

The antibotulinic activity of sodium lactate has already been documented. The objective of this study was to determine whether sodium diacetate could be used alone or
in combination with sodium lactate to inhibit the germination and outgrowth of spores of anaerobic bacteria that occur naturally in temperature-abused CIBTB. Although 2.5% sodium lactate in combination with 0.5% sodium diacetate was more inhibitory to such germination and outgrowth than was 2.5% sodium lactate alone; CIBTB formulated with this combination would probably be organoleptically unacceptable to many consumers. Other combinations of these two ingredients were not more inhibitory than sodium lactate alone. On the basis of these data, sodium diacetate would presumably be ineffective as an antibacterial agent. Regardless, the results of this study demonstrate the effectiveness of sodium diacetate and sodium lactate as food-grade antimicrobial agents for the prevention of CIBTB spoilage caused by anaerobic sporeformers, including nonproteolytic, psychrotrophic isolates such as strain OMFR11. Further studies are warranted to address

strain-to-strain variations in the effectiveness of sodium lactate and sodium diacetate, particularly for strains isolated from sources other than poultry. Further studies are also needed to optimize formulations to achieve the desired taste and the optimal antclostridial activity level.

ACKNOWLEDGMENTS

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REFERENCES