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Aims: The following polymers were developed: polyethylene (PE), a PE and polyethylene oxide (70% PE and 30% PEO; PE + PEO) blend, PE and nisin (PE + nisin), PE, nisin, and EDTA (PE + nisin + EDTA), and PE + PEO with nisin (PE + PEO + nisin).

Methods and Results: Of the polymers tested, PE and PE + PEO did not exhibit any antimicrobial activity against *Brochothrix thermosphacta* (*BT*); however, PE + nisin, PE + nisin + EDTA, and PE + PEO + nisin did. Beef surfaces were experimentally inoculated with 3·50 $\log_{10}$ cfu/cm^2 of *BT*, vacuum packaged with each of the five polymers, and held at 4°C for 21 d. After 3 d at 4°C, *BT* was reduced > 1·70 $\log_{10}$ by PE + nisin and > 3·50 $\log_{10}$ with PE + nisin + EDTA or PE + PEO + nisin. By 21 d at 4°C, *BT* was reduced to 0·30 $\log_{10}$ cfu/cm^2 when treated with PE + PEO + nisin.

Conclusions: It appears that PE + PEO + nisin or PE + nisin + EDTA were more effective for reducing *BT*, as compared to polymers composed of PE + nisin.

Significance and Impact of the Study: Nisin-incorporated polymers may control the growth of undesirable bacteria, thereby extending the shelf life and possibly enhancing the microbial safety of meats.

INTRODUCTION

The use of films as antimicrobial delivery systems to reduce undesirable bacteria in foodstuffs is not a novel concept. Various approaches have been proposed and demonstrated for the use of edible and polymer films to deliver bacteriocins, such as nisin, to a variety of food surfaces, including muscle foods. Juhl *et al.* (1994) were issued a patent in which a thermoplastic polymer was blended with a 10% concentration of olefinic oxide polymer, and any of the following modifiers could be added: antioxidants (butylated hydroxy toluene, tocopherols, propyl gallate), fragrances (vanillin, clove, orange or citric extract), colourants or dyes, antimycotic agents (glycerine, propylene glycol, potassium sorbate, benzoic acid), or biocides (nisin, lysozyme, enzymes). Natrajan and Sheldon (2000) demonstrated a 4·3 log reduction of *Salmonella* Typhimurium on inoculated broiler skin exposed to nisin-coated polyvinyl chloride film. Wilhoit (1996) was issued a patent in which it was proposed using nisin or pediocin, with or without a chelating agent, in conjunction with a food packaging film to protect foodstuffs from harmful bacteria. Cutter and Siragusa (1996, 1998) reported that immobilization of nisin in an edible calcium alginate gel or a meat binding system may be a more effective delivery system of the bacteriocin to the beef carcass surface than direct application. Dawson *et al.* (1997)

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and Padgett *et al.* (1998) combined nisin with protein and/or corn zein films to inhibit micro-organisms on meat surfaces. We recently demonstrated that incorporation of nisin into polyethylene films not only retained nisin activity in plate overlay assays, but also effectively reduced the psychrotrophic, meat spoilage organism, *B. thermosphacta,* on vacuum packaged beef surfaces under long-term, refrigerated storage (Siragusa *et al.* 1999). The present study was conducted to determine if a change in the previously reported polymer formulation or addition of EDTA to a polyethylene-based polymer film could improve nisin activity against *B. thermosphacta* associated with beef.

MATERIALS AND METHODS

Polymer film preparation

In this study, five different polymer film compositions were developed. Of these, two control polymer films were made with polyethylene (PE) or a blend of polyethylene and polyethylene oxide (PE + PEO) and three polymer films were dry blended with nisin in dried milk solids (Nisaplin; Sigma Chemical Co., St. Louis, MO, USA) to a final concentration of 0·1%. Powdered low-density polyethylene (PE 3404B; Quantum Chemical Co., Houston, TX, USA) was mixed with nisin (PE + nisin), polyethylene oxide (Quantum Chemical Co., molecular weight = 100 000) and nisin (PE + PEO + nisin), or nisin and ethylenediaminetetraacetic acid (EDTA; Sigma Chemical Co.; PE + nisin + EDTA). Polymers were produced using a 19 mm diameter single screw extruder (Brabender) with a 0·5 inch blown film die at temperatures of 120°C for PE and PE + nisin and 125°C for PE + PEO, PE + nisin + EDTA, and PE + PEO + nisin. Screw speed was 20 r.p.m. and retention time in the extruder was approximately 7 min.

Organism

Brochothrix thermosphacta ATCC 11509 was maintained in storage at −20°C in 75% glycerol until needed. *B. thermosphacta* was propagated three times in tryptic soy broth plus 0·5% w/v yeast extract (TSBYE) at 26°C for 24 h before the start of the experiments. Cells in late log or early stationary phase were used for all experiments.

Plate overlay studies

Nisin activity of the polymer films was determined by using the seeded lawn overlay spot assay (Siragusa and Cutter 1993). Briefly, TSBYE agar plates were overlaid with 8 ml of semisoft TSBYE agar (0·5% w/v agar) seeded with 8 μl of an overnight broth culture of *B. thermosphacta* ATCC 11509. The seed density was approximately 1×10^6 cfu ml^{-1} of overlay. Plates were evaluated for zones of inhibition after 24 h incubation at 26°C.

Extraction of antimicrobial activity from films

To determine the extractability of nisin, the following experiment was carried out using previously established protocols (Cutter 1999; Siragusa *et al.* 1999). Each of the different nisin-in-polymer-films (NIPF) was aseptically cut to 25 cm^2 pieces, placed into a sterile centrifuge tube and suspended in 25 μl of the following solutions and treated as follows: (a) distilled water for 6 h at 25°C, followed by boiling water bath, 10 min; (b) distilled water, followed by boiling water bath, 10 min; (c) 0·02 N HCl, followed by boiling water bath, 10 min; (d) physiological saline and 0·5% Tween 20, 6 h, 25°C, followed by boiling water bath, 10 min; and (e) physiological saline and 0·5% Tween 20, followed by boiling water bath, 10 min Tubes were cooled on ice for 30 min. Following treatments and cooling to 4°C, 1 cm^2 pieces were cut from the treated NIPF and assayed for activity using the plate overlay assay described above. Twenty microlitres of supernatant following the heat treatment also were spotted and evaluated in the plate overlay assay. Additional 1 cm^2 pieces of untreated NIPF were also placed onto the plate overlay assay and served as controls for the experiment. Experiments were replicated twice with duplicate plates and evaluated for zones of inhibition after 24 h incubation at 26°C.

Refrigerated storage challenge study

Sections (5 cm × 5 cm) of post-rigor beef carcass surface tissue, *cutaneous trunci* with intact superficial fascia, were ultraviolet light (UV) sterilized under a biosafety hood for 15 min (Cutter and Siragusa 1994). UV-sterilized tissue sections were inoculated by placing only the fascia side down into 10 ml of an overnight culture of approximately 3–4 log cfu ml^{-1} *B. thermosphacta* ATCC 11509 for 15 min at 25°C. Uninoculated UV-sterilized tissue sections also were monitored for sterility throughout the experiment. Inoculated sections were left untreated or prewrapped with a 5·5-cm × 11-cm piece of the polymer film with a particular composition in an envelope fashion (Siragusa *et al.* 1999). All treatments were then vacuum packaged (Hollymatic Model LV10G, Countryside, IL, USA) in a standard vacuum packaging bag (3·2 mil nylon/copolymer bag with oxygen transmission rate at 23°C of 52 cc/m^2; Hollymatic, Inc.) and held at 4°C until sampled at d 3, 7, 14, and 21. Only untreated control samples were sampled at d 0.

Microbiological analyses

Tissue sections were removed from the packs and placed in a filtered Stomacher bag along with 25 ml of buffered

peptone water (BBL, Cockeysville, MD, USA) with 0·1% v/v Tween 20 and pummeled for two minutes. *B. thermosphacta* numbers were determined by spiral plating (Model D Spiral Plater, Spiral Biotech, Bethesda, MD, USA) and/or spread plating (4×250 μl per plate) samples onto plates of STAA agar base with full selective supplement (Oxoid, Basingstoke, England, United Kingdom).

Calculations and statistical analyses

The refrigerated storage challenge study was repeated six times. After enumeration, bacterial populations from duplicate plates were averaged and converted to $\log_{10}$ cfu/cm^2. Least squared means (LSM) of bacterial populations ($\log_{10}$ cfu/cm^2) from each treatment were calculated from the six replications. Analysis of Variance and the General Linear Models procedure of SAS were used for analyses (SAS for Windows, release ver. 6·12, SAS Institute, Inc., Cary, NC). Inoculum counts were used as a covariant to normalize data between treatment replications. Statistical significance was defined as $P \leq 0{\cdot}05$, unless otherwise noted.

RESULTS AND DISCUSSION

Of the polymer films tested in this study, the control polymer films, PE and PEO did not exhibit any antimicrobial activity against *B. thermosphacta* (data not shown); however, PE + nisin, PE + nisin + EDTA, and PE + PEO + nisin did exhibit activity as indicated by presence or absence of zones of inhibition in plate overlay assays (Figs 1, 2, and 3).

The antimicrobial activity was not extractable from the nisin-incorporated polymer film by prolonged exposure at 25°C followed by boiling in distilled water (data not shown). However, mild acid (PE + nisin; PE + PEO + nisin, and PE + nisin + EDTA) or surfactant extractions (PE + PEO + nisin, and PE + nisin + EDTA) under boiling conditions resulted in leaching of antimicrobial activity from the NIPF (data not shown). Thus, only boil treatments demonstrated extractability of nisin from the polymer films whether under acidic or surfactant conditions. This observation was observed previously with polymer films composed of PE + nisin (Siragusa *et al.* 1999).

A subsequent experiment with inoculated meat surfaces treated both without (Untreated) or with each of the five polymer films, and held at 4°C for up to 21 d indicated that *B. thermosphacta* could be inhibited on meat surfaces by the nisin-incorporated polymer films (Fig. 4). Specifically, after 3 d of refrigerated storage, levels of *B. thermosphacta* were

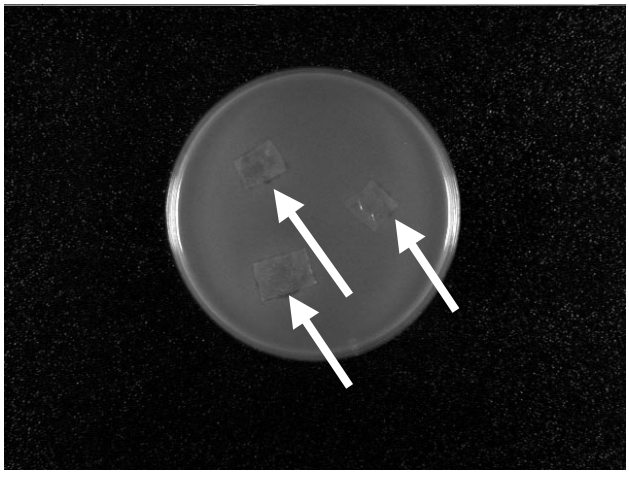

Fig. 1 Agar overlay of *Brochothrix thermosphacta* grown on STAA, 26°C, 48 h with 1 cm × 1 cm pieces of PE + nisin. Arrows indicate areas of detectable nisin activity

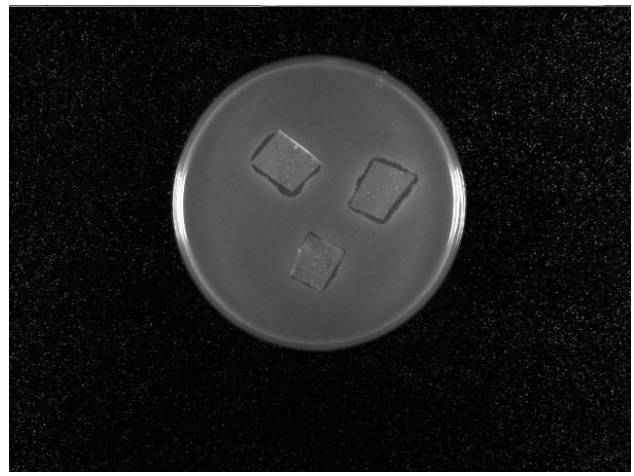

Fig. 2 Agar overlay of *Brochothrix thermosphacta* grown on STAA, 26°C, 48 h with 1 cm × 1 cm pieces of PE + EDTA + nisin

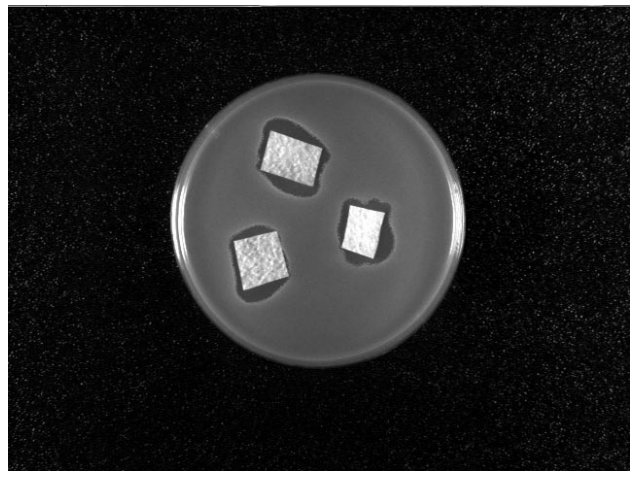

Fig. 3 Agar overlay of *Brochothrix thermosphacta* grown on STAA, 26°C, 48 h with 1 cm × 1 cm pieces of PE + PEO + nisin

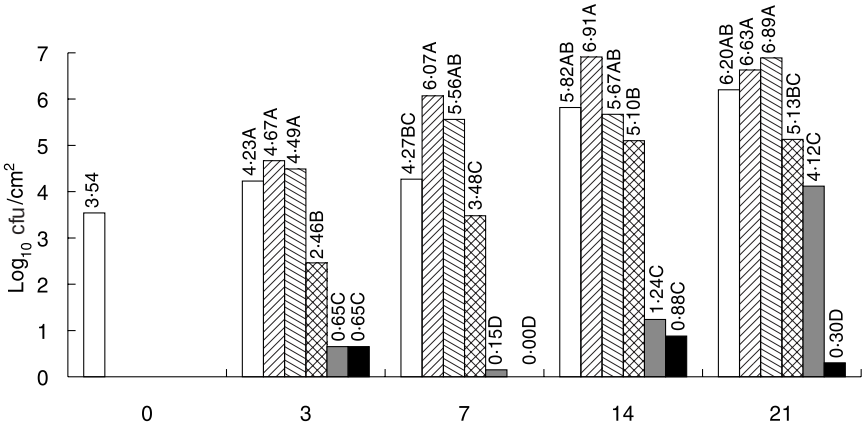

Fig. 4 Populations of *Brochothrix thermosphacta* on beef surfaces with the following treatments: untreated (□), PE (▨), PEO (▧), PE + nisin (▩), PE + nisin + EDTA (▩), and PE + PEO + nisin (■) and long-term, refrigerated (4°C), vacuum packaged storage

reduced > 1·70 $\log_{10}$ by PE + nisin and reduced > 3·50 $\log_{10}$ with PE + nisin + EDTA and PE + PEO + nisin, as compared with PE, PE + PEO and untreated controls. By d 21 of refrigerated storage, bacterial populations had increased to > 6 $\log_{10}$ cfu/cm^2 for untreated, PE-, and PE + PEO-treated surfaces. Conversely, PE + nisin and PE + nisin + EDTA reduced *B. thermosphacta* 1·07 and 2·08 $\log_{10}$ cfu/cm^2, respectively. More importantly, populations of *B. thermosphacta* were reduced to approximately 0·30 $\log_{10}$ cfu/cm^2 when surfaces were treated with PE + PEO + nisin. These results indicate that PE + PEO + nisin polymer films were more effective for reducing *B. thermosphacta,* as compared to polymer films composed of PE + nisin, PE + nisin + EDTA.

Polyethylene oxide is a water soluble, hygroscopic polymer (Juhl *et al.* 1994). As such, the rate of nisin release could be greater in films made from this polymer. This observation may explain why nisin activity was greater in PE + PEO + nisin polymer films as compared with PE + nisin + EDTA polymer films.

Combining the bacteriocin directly into a polymer material may provide several advantages as a bacteriocin delivery mechanism. First, only the necessary amount of bacteriocin would be used. Secondly, the agent would not be a direct additive to the food product, but may be classified as an indirect food additive. Thirdly, if a polymer material containing the bacteriocin was made from an edible and/or biodegradable polymer, environmental advantages associated with disposal, landfill waste, breakdown, etc. would be realized (Siragusa *et al.* 1999).

In this study, we have demonstrated that retention of nisin activity occurred when nisin was incorporated into a polymer film composed of a blend of polyethylene and polyethylene oxide (PE + PEO + nisin) or when EDTA was added to polyethylene and nisin (PE + nisin + EDTA). Additional variations of the PE and/or PEO compositions may further improve the inhibition observed in this study.

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