Research Note

Screening of Commercial and Pecan Shell–Extracted Liquid Smoke Agents as Natural Antimicrobials against Foodborne Pathogens

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ABSTRACT

Liquid smoke extracts have traditionally been used as flavoring agents, are known to possess antioxidant properties, and serve as natural alternatives to conventional antimicrobials. The antimicrobial efficacies of commercial liquid smoke samples may vary depending on their source and composition and the methods used to extract and concentrate the smoke. We investigated the MICs of eight commercial liquid smoke samples against Salmonella Enteritidis, Staphylococcus aureus, and Escherichia coli. The commercial liquid smoke samples purchased were supplied by the manufacturer as water-based or concentrated extracts of smoke from different wood sources. The MICs of the commercial smoke samples to inhibit the growth of foodborne pathogens ranged from 0.5 to 6.0% for E. coli, 0.5 to 8.0% for Salmonella, and 0.38 to 6% for S. aureus. The MIC for each liquid smoke sample was similar in its effect on both E. coli and Salmonella. Solvent-extracted antimicrobials prepared using pecan shells displayed significant differences between their inhibitory concentrations depending on the type of solvent used for extraction. The results indicated that the liquid smoke samples tested in this study could serve as effective natural antimicrobials and that their inhibitory effects depended more on the solvents used for extraction than the wood source.

The organic food standards of the U.S. Department of Agriculture (USDA) state that USDA Certified Organic meat, poultry, eggs, and dairy products should come from animals not given antibiotics or growth hormones (28). Consumer perception of these foods as “healthier,” as well as the “social” aspects, such as being locally grown or produced in clean and sustainable environments, have considerably increased the demand for organic foods (6, 14, 24). In terms of market trends, the sales of organic food and beverages in the United States have risen from $1 billion in 1990 to $26.7 billion in 2010, with 7.7% growth in sales over the 2009 figures (23).

Chemical contamination of foods is a primary food safety concern for a majority of consumers rather than bacterial contamination (7). However, organic foods are not always free of microbial contamination and may harbor high levels of foodborne pathogens, such as Escherichia coli O157:H7, Listeria monocytogenes, Salmonella spp., and Campylobacter jejuni, raising concerns about the safety of minimally processed foods. Since the standards for organic meat processing do not permit the use of synthetic chemicals and chemical preservatives, the permitted natural preservatives must be able to provide an additional hurdle to help ensure the safety of minimally processed meat and poultry products. There is high demand for effective alternatives to the conventional antimicrobials to minimize foodborne illness outbreaks (6, 29). New natural antimicrobial food additives are needed to combat foodborne pathogens, including dangerous antibiotic-resistant pathogens, such as Staphylococcus aureus, that show resistance to common antibiotics including penicillin and methicillin (2, 3, 17, 25). Thus, finding alternatives to conventional antimicrobials is essential to address the demands of organic meat and poultry consumers while at the same time minimizing the risk from foodborne pathogens.

Different natural or organic alternative antimicrobials, such as bacteriocins, bacteriophages, and natural plant extracts such as essential oils and liquid smokes, have been studied for their antimicrobial activities against foodborne pathogens and food spoilage microorganisms (4, 10, 16, 22, 26). The commercial production of liquid smoke is typically done using a pyrolysis process involving thermal decomposition of wood (containing 10% moisture at 400 to 500°C) in retorts or rotary ovens under absence of oxygen, followed by a water spray to capture the resulting smoke and separation of the light and heavy fractions by gravity (1). The liquid smoke flavorings are usually obtained by fractionating the resulting condensate by techniques involving sequential extraction or liquid-liquid partitioning and/or solid-phase extraction based on the polarity and acidity of the constituents. The liquid smokes used in the food processing industry are used as

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flavoring agents, browning colorants, antioxidants, and food texture enhancers and, also, as alternative antimicrobial agents (1, 5, 18, 27). Foodborne pathogens, such as L. monocytogenes, have been shown to decrease on food system surfaces treated with commercially available liquid smoke extracts (12, 13, 19). The ready-to-eat meat industry has widely accepted the use of commercial liquid smokes as antimicrobial agents to address the high susceptibility of ready-to-eat foods for postprocessing contamination before packaging (30).

The effectiveness of certain liquid smokes depends on the starting materials and methods of extraction, which affect the type and concentration of the chemical constituents. There will be differences in the effects of these individual components against the individual species of pathogens. Limited research has been done to explore the full potential of liquid smoke condensates originating from different sources and extraction methods to determine their antimicrobial efficacies against foodborne pathogens. In this study, we conducted experiments to determine the MICs of commercial liquid smoke condensates originating from hickory, apple, and mesquite woods and pecan nut shells in the inhibition of three foodborne pathogens. We also compared the effectiveness of extracts prepared in the laboratory using solvents that varied in polarity.

### MATERIALS AND METHODS

**Preparation of bacterial cultures.** The bacterial strains used for testing with commercial liquid smokes were Salmonella Enteritidis (PTA 13A), *E. coli* O157:H7 (ATCC 43888), *S. aureus* (ATCC 25923 and ATCC 6538), and two methicillin-resistant *S. aureus* (MRSA) strains (Table 1). For testing with solvent extractions prepared in the laboratory, the strains used were *L. monocytogenes* strain 174 (serotype 1/2a), *L. monocytogenes* 163 (serotype 4b), *Salmonella* Typhimurium 29, *Salmonella* Typhimurium LT2 (ATCC 19585), and *S. aureus* Col (MRSA). Strains with ATCC numbers were obtained from American Type Culture Collection (ATCC; Rockville, MD), and all other strains were obtained from the sources indicated in Table 1. Previously frozen stock cultures of these bacteria were grown in Difco tryptic soy broth (Difco Laboratories, Detroit, MI) at 37°C for 24 h before being subjected to treatments.

**Liquid smoke samples.** Eight commercial liquid smoke extracts commonly used in the food industry were supplied by three different commercial manufacturers. The liquid smoke types/treatments are referred to as A, B, C, D, E, F, G, and H in this study, with the first four being water-based (group I) and the rest being concentrated extracts (group II). As stated by the manufacturers, these commercial smokes were extracted from the woods of hickory, mesquite, apple, and pecan (group I); mesquite poly, code 100 (unknown), mesquite, and hickory comprised group II. No further data were available with regard to the pH or the chemical composition of the flavorings. The laboratory extraction of antimicrobial agents from pecan shells (roasted and unroasted) was done using solvents with a range of polarities (water, 30% acetic acid, methanol, and hexane). In the case of water and 30% acetic acid, high-temperature extraction was done at 85°C for 30 min, and for the methanol and hexane, the Soxhlet method of boiling extraction under vacuum was used (15). The antimicrobials extracted were held at 4°C until testing.

**Smoking treatments and microbiological analysis.** The effects of liquid smokes on the pathogenic bacteria mentioned in Table 1 were evaluated using a modification of the protocol of Friedly and others (9). Briefly, 200 μl of a 96% concentrated solution of each liquid smoke was prepared in 1× phosphate-buffered saline (PBS) (vol/vol) and placed in the first-row wells of a 96-well plate (NUNC, Rochester, NY). The remaining wells were filled with 100 μl of PBS, and two-fold dilutions of the liquid smokes were prepared by transferring a 100-μl aliquot of the 96% solution into the second row and mixing to obtain a 48% concentration. This solution was further serially diluted to obtain concentrations down to 0.375%. A control containing PBS solution was included, and all liquid smoke concentrations were tested in triplicates. All wells, including the control, were inoculated with 100 μl of bacterial culture and the plates were incubated at 37°C for 24 h. After the incubation period, a 3-μl suspension from each well was subsequently spotted on a tryptic soy agar plate and incubated further at 37°C for 24 h. Plates were subsequently read for visible growth to determine the MIC of the liquid smoke, which was defined as the concentration at which no

### TABLE 1. Overview of the strains and sources

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>PT 13A, Poultry Science, University of Arkansas, Fayetteville</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em></td>
<td>ATCC 43888</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>ATCC 25923</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>ATCC 6538</td>
</tr>
<tr>
<td><em>S. aureus</em> Mu50, MRSA</td>
<td>Obtained from Dr. Brian Wilkinson’s laboratory, Illinois State University, Normal</td>
</tr>
<tr>
<td><em>S. aureus</em> Col, MRSA</td>
<td>Obtained from Dr. Brian Wilkinson’s laboratory, Illinois State University, Normal</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Strain 104035, wild type, obtained from Dr. Weidemann, Cornell University, Ithaca, NY</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Strain NADC (National Animal Disease Center) 2045, obtained from Dr. Aubrey Mendonca, Department of Food Science and Human Nutrition, Iowa State University, Ames</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium 29</em></td>
<td>Obtained from Dr. Michael Slavik, University of Arkansas, Fayetteville; source, CDC</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium LT2</em></td>
<td>ATCC 19585</td>
</tr>
</tbody>
</table>

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visible growth occurred in all three replicates. This value is reported as the MIC. The experiment was repeated three times for each of the bacterial strains.

**Statistical analysis.** All reported values of %MICs are the averages of three independent replications that were subjected to one-way analysis of variance (ANOVA) with Statistical Analysis Software (SAS 9.2, SAS Institute, Cary, NC) using PROC ANOVA, and mean comparisons were done using Tukey’s significance method where $P$ values of $<0.05$ were considered statistically significant. Data plotting was done using SigmaPlot 12.0.

**RESULTS AND DISCUSSION**

**MICs of commercial liquid smoke samples.** The effective concentrations of commercial liquid smoke extracts inhibiting the growth of *E. coli* O157:H7, *Salmonella* Enteritidis, and *S. aureus* strains are shown in Figure 1A, and the effectiveness of extracts prepared using acetic acid and methanol solvents against strains of *L. monocytogenes*, *Salmonella* Typhimurium, and *S. aureus* are shown in Figure 1B. The MICs of commercial liquid smoke samples ranged from 0.5 to 6.0 % for *E. coli* O157:H7, from 0.5 to 12.0 % for *Salmonella* Typhimurium, and from 0.38 to 6 % for *S. aureus*. These MICs are in agreement with previously reported results showing commercial liquid smoke extracts with MIC levels from 0.75 to 7 % against several pathogens (20). The inhibitory effects among group I (A, B, C, and D) or group II (E, F, G, and H) liquid smoke samples did not appear to depend on the bacterial type or the source of the extracts (Fig. 1A). However, the antimicrobial groups differed significantly ($P < 0.05$) from each other in that the bacterial MICs were less than 1 % for group II and approximately 6 % for group I liquid smoke samples. According to the manufacturer’s specifications, the group I smoke samples were water-based extractions, and the group II samples were concentrated extracts. Since the liquid smoke samples tested in this study were extracted differently, their different chemical compositions could have resulted in differences in their constituents of phenols, carbonyls, and organic acids, leading to different efficacies against the bacterial strains tested. Thus, the relative antimicrobial effectiveness of the liquid smokes may differ significantly depending on the composition of the smoke condensates in terms of the concentration of its constituents, such as phenols, carbonyls, isoeugenol, 4-methyguaiacol, guaiacol, organic acids, and other constituents (20, 21). More than the phenol constituents of the liquid smokes, the low pH ($<4.6$), high acidity ($>1.5\%$), and high carbonyl contents ($>110$ mg/ml) may have contributed to greater
bactericidal effects of the liquid smoke extracts against *Listeria innocua* M1 (20).

**MICs of solvent extracts of pecan shells.** The chemical composition of the smoke flavorings may generally depend on the type of solvents used for extraction, extraction time, temperature, and other factors. The effects of water, acetic acid, methanol, and hexane as solvents for the extraction of antimicrobials from unroasted and roasted pecan shells showed differences in antimicrobial effectiveness against various bacterial strains (Fig. 1B). There was no growth inhibition for any of the bacterial strains with either the water or hexane solvents (data not shown), indicating an inability of these solvents to extract antimicrobial agents from pecan shells. Acetic acid extracts of unroasted and roasted pecan shells yielded smokes that showed lower %MICs than the methanol-extracted smokes. The MICs against *L. monocytogenes* strains ranged from 1.5 to 12% for acetic acid and the acetic acid extracts of unroasted and roasted pecan shells, and the lowest MIC, 1.5%, was seen for *L. monocytogenes* 163 when roasted pecan shell extract was used (*P* < 0.05). The inhibition of *Salmonella* Typhimurium strains showed a similar trend between the acetic acid and methanol extracts. However, when roasted pecan shells were used, significantly lower %MICs were seen, with MICs of 1.5 and 24% against *S. Typhimurium* LT2 for acetic acid and methanol extracts, respectively (*P* < 0.05). The use of acetic acid alone inhibited the growth of all pathogens tested, meaning that the observed %MICs for acetic acid extracts may not be totally due to the antimicrobial compounds extracted from the pecan shells. However, when unroasted and roasted pecan shell extracts in acetic acid were compared, the roasted extracts performed better in inhibiting bacterial growth. When methanol and methanol extracts of pecan shells were compared, methanol alone did not show the same level of inhibition as acetic acid alone and required much higher concentrations to inhibit bacterial growth, while methanol alone showed no inhibition of both *L. monocytogenes* strains and one *S. aureus* strain. When the methanol extracts of roasted pecans shells were compared with methanol extracts of unroasted pecan shells, the inhibition of *Salmonella* Typhimurium LT2 and *S. aureus* Col (MRSA) strains was significantly lower, at 24%, than that of the unroasted pecan shell extracts, at 48% (*P* < 0.05). Overall, the extracts prepared using roasted pecan shells showed better growth inhibition of most of the bacterial strains, with lower %MICs than the extracts prepared with unroasted pecan shells. This could be due to a lower moisture content of roasted shells than of the unroasted ones that contributed to higher concentrations of chemicals in the respective extracts. Clearly, the significant variation in the MICs of antimicrobials used in this study depended mainly on the solvent used and roasting of the material subjected to extraction. For example, the roasting process of plant products such as coffee beans is known to influence the formation of *α*-dicarbonyl compounds and result in antibacterial activities higher than those of green coffee extracts against bacterial strains of *S. aureus* and *Streptococcus mutans* (8). Similarly, enhanced antioxidant activity of roasted coffee bean extract in comparison with the antioxidant activity of green coffee beans was attributed to the formation of Maillard compounds during thermal decomposition (11). In agreement with these findings, similar effects of roasted pecan shell extracts may have caused antimicrobial activities higher than those of the unroasted pecan shell extracts against the test organisms. It can be stated that the solvent chemistry, the moisture content, and the composition of the natural products may show unpredictable differences in every plant material-solvent system used to prepare the natural antimicrobials. However, future work in this area could involve efforts to identify the chemical constituents of the liquid smokes and test for their potential inhibitory effect on food pathogens. Future studies may also involve testing for the efficacy of these liquid smoke samples in a meat matrix to inhibit typical pathogens and normal contaminating bacteria. The findings of this study confirm the antilisterial activity of liquid smoke and indicate that the inhibitory effects of smoke flavorings against the tested organisms may differ with the extraction methods, which alter the chemical composition of the liquid smokes.

**ACKNOWLEDGMENTS**

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