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Antibacterial Activity of LauriPure *In vitro* and on Skin of Processed Broilers

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Abstract: Studies were conducted to examine the ability of LauriPure to inhibit the growth of *Salmonella* Typhimurium, *Escherichia coli*, *Staphylococcus simulans* and *Listeria innocua* isolates recovered from processed broiler carcasses. *In vitro* studies were conducted using the Bioscreen C Microbiology Reader to measure the growth of isolates during incubation at 37°C for 48 h in Tryptic Soy Broth (TSB) supplemented with 0.0, 0.5, 1.0, or 2.0% (vol/vol) LauriPure. Also, the ability of LauriPure to reduce bacterial contamination of skin from processed broilers was examined by comparing the number of bacteria recovered from skin after 3 consecutive 1 min washes in distilled water or 1.0% LauriPure solutions. After each wash, bacteria recovered from the skin were enumerated by plating skin rinsates on Eosin Methylene Blue (EMB), *Staphylococci* (STA) and Plate Count (PC) agars and incubating plates at 37°C for 24-48 h. Results of *in vitro* experiments indicated that after 48 h of incubation the optical density (OD₆₀₀) of all isolates was significantly ($p < 0.05$) lower when cultured in media supplemented with either concentration of LauriPure than in media not supplemented with LauriPure. Results of skin washing experiments indicated that after each wash in LauriPure, significantly ($p < 0.05$) fewer bacteria were recovered on EMB, STA and PC agars from skin washed in 1.0% LauriPure than from skin washed in distilled water. Findings indicate that LauriPure possesses antibacterial activity towards several bacteria that may be found in the bacterial flora of processed poultry and that the sanitizer may be considered as a treatment for reducing bacterial contamination associated with some poultry processing operations.

Key words: LauriPure, poultry, sanitizer

INTRODUCTION

Processed poultry may be contaminated by a variety of pathogenic (Hinton *et al.*, 2007) and spoilage (Hinton *et al.*, 2002, 2004) microorganisms. Bacteria and other microorganisms associated with live poultry may remain attached to the skin of broiler carcasses after processing (Hinton *et al.*, 2007) and cause spoilage losses and human food borne diseases if poultry meat is not stored or handled properly before consumption (Mead *et al.*, 1999).

Several chemical sanitizers that reduce microbial contamination of broiler carcasses during processing are currently available to poultry processors; however, poultry is still considered to be a significant cause of human food borne illnesses (MMWR, 2010). LauriPure is a mixture of lauricidin (glycerol monolaurate), caprylic acid, capric acid, lactic acid and propylene glycol mono-fatty acid esters. Each of the components of LauriPure is classified as "Generally Recognized as Safe" (GRAS) by the Food and Drug Administration FDA (2010) and most of these components possess antimicrobial activity when utilized individually as sanitizers (Skrivanova *et al.*, 2006). LauriPure is primarily used as a moisturizing teat

dip to control mastitis in dairy cattle and the application of mixtures of lauricidin and lactic acid as a teat dip has been shown to reduce experimental intra-mammary infections of *Staphylococcus aureus* and *Streptococcus agalactiae* in dairy cattle (A and L. Laboratories, 2010; Boodie, 1992). Additionally, lauricidin and lactic acid have been proven to reduce the population of spoilage bacteria on fresh poultry meat (Anang *et al.*, 2010). The purpose of the present study was to examine the ability of LauriPure to inhibit the *in vitro* growth of several bacteria associated with processed broiler carcasses and to reduce bacterial contamination of processed broiler skin.

MATERIALS AND METHODS

In vitro studies. Sterile Tryptic Soy Broth (TSB, Becton Dickinson and Co., Sparks, MD) was prepared and supplemented with a final concentration of 0.0, 0.5, 1.0, or 2.0% (vol/vol) LauriPure (A and L. Laboratories, Minneapolis, MN). Suspensions (0.1 mL) of 18-24 h *Salmonella* Typhimurium, *Escherichia coli*, *Staphylococcus simulans*, or *Listeria innocua* cultures were added to 9.9 mL of TSB-LauriPure mixtures to

produce a final concentration of 10^6 cfu/mL of each isolate in separate test tubes. Volumes of 0.3 mL of the inoculated media were removed and placed in wells of a Honeycomb multi well plate (Labsystems, Inc, Franklin, MA). Filled multi well plates were then placed in the incubator tray of Bioscreen C Microbiology Reader (Thermo Electron Corp., West Palm Beach, FL) operated by a computer with Growth Curves Software, v.2.28 (Transgalactic Ltd., Helsinki, Finland). The microbiology reader recorded the optical density of cultures at 600 nm during incubation at 37°C for 48 h.

Effect of LauriPure on the bacterial flora of the skin of broiler carcasses. Eviscerated broiler carcasses were obtained from a local commercial poultry processing facility, placed on ice and transferred to the laboratory. Breast skin from 3 broiler carcasses was cut into 1 g pieces, pooled and stored at 4°C until used. Skin samples that were not used within 5 days were discarded. Three pieces of 1 g broiler skin samples were placed in 50 mL sterile plastic Pro Cent Tubes with Screw Caps (Tyco Healthcare Group, Mansfield, MA, USA) and 20 mL of 1.0% LauriPure or distilled water (controls) were added to the samples. Skin was agitated in the liquids at medium speed for 1 min (Mistral Multimixer-Lab-line Instruments, Inc., Melrose Park, IL). After the first wash, samples in each tube were transferred to another tube containing 20 mL of Butter fields phosphate-buffered dilution water (FDA, 2010) and rinsed by agitation for 1 min. Rinsates were decanted for microbial analysis and skin samples were transferred to another tube containing a fresh volume of LauriPure or distilled water. The washing procedure and rinsate collection was repeated for a total of 3 washes in LauriPure or distilled water. Rinsates from each of the 3 consecutive washes were serially diluted and bacteria in the rinsates were enumerated on selective bacteriological media. Gram negative enteric bacteria were enumerated on Eosin Methylene Blue (EMB) Agar (Remel Inc., Lenexa, KS), Gram positive cocci were enumerated on *Staphylococci* (STA) Agar (Oxoid, Ltd., Basingstoke, Hampshire, England) and total aerobic plate counts were conducted on Plate Count (PC) Agar (Becton, Dickinson and Co., Sparks, MD). All plates were incubated aerobically at 37°C for 24-48 h and colony-forming-units (cfu) were counted. The experiment was repeated 3 times.

Statistical analysis: GraphPad InStat, Version 3.0 for Windows (GraphPad Software, San Diego, CA) was used to perform statistical analyses of data on culture OD from *in vitro* studies and log cfu/mL from skin washing studies. One-Way Analysis of Variance (ANOVA) of group means of culture absorbances and cfu/ml were performed to determine significant differences in data. When ANOVA detected significant variations in group means, the Tukey-Kramer Multiple

Comparison test was used to determine which means differed significantly. All significant differences were determined at $p < 0.05$.

RESULTS AND DISCUSSION

In vitro activity of LauriPure. Table 1 shows the growth of bacteria in TBS supplemented with different concentrations of LauriPure. Results indicated that LauriPure can significantly reduce *in vitro* growth of some bacteria associated with processed poultry. The OD of *E. coli*, *S. Typhimurium*, *S. simulans* and *L. innocua* cultures after 48 h incubation was significantly lower in media containing of 0.5% LauriPure than the OD of cultures grown in media containing no added LauriPure. Increasing the concentration of LauriPure in the media to 1.0 or 2.0% produced no further significant reductions in the OD of *S. simulans* or *L. innocua*; however, higher concentrations of LauriPure did produce additional significant reductions in OD of *E. coli* and *S. Typhimurium*. Furthermore, there was no significant increase in the OD of *S. simulans* or *L. innocua* during 48 h of incubation in media supplemented with 0.5, 1.0, or 2.0% LauriPure, while there were significant increases in the OD of *E. coli* and *S. Typhimurium* cultures grown in media containing these concentrations of LauriPure (data not shown). These findings support earlier studies that indicate that Gram positive bacteria are more susceptible than Gram negative bacteria to the antibacterial activity of fatty acids contained in LauriPure (Hinton, 2011; Kabara, 1977).

Effect of LauriPure on bacterial flora of skin of broiler carcasses. LauriPure was also effective in reducing bacterial contamination of broiler skin (Table 2). After each of 3 consecutive washes, significantly fewer bacteria were recovered from skin washed in 1.0% LauriPure than from skin washed in distilled water. These results indicate that LauriPure was effective in reducing contamination by presumptive Gram negative Enterobacteriaceae that are the primary bacteria recovered on EMB agar, as well as presumptive Gram positive cocci that are the primary bacteria recovered on STA agar. Furthermore, LauriPure significantly reduced the total number of bacteria on the washed poultry skin as indicated by significant reductions in the number of bacteria recovered on PC agar after washing in LauriPure. These results also indicate that LauriPure possesses more antibacterial activity towards Gram positive bacteria than Gram negative bacteria, since no bacteria were recovered on STA agar after two washes in LauriPure although bacteria were recovered on EMB and PC agar.

Fatty acids are effective sanitizers because of their microbicidal activity due to the ability of these compounds to lyse the cellular membrane of bacteria and because of their cleansing abilities due to the surfactant activity of these compounds (Hinton *et al.*,

Table 1: Optical density (OD₆₀₀) of bacterial isolates after 48 h of growth in media supplemented with 0, 0.5, 1.0, or 2.0% LauriPure

Lauripure concentration(%)	Staphylococcus simulans	Listeria innocua	Salmonella typhimurium	Escherichia coli
0.0	0.65 ^b ±0.12	0.64 ^b ±0.34	1.37 ^d ±0.05	1.34 ^d ±0.10
0.5	0.10 ^a ±0.02	0.10 ^a ±0.03	0.49 ^b ±0.11	0.98 ^b ±0.06
1.0	0.08 ^a ±0.03	0.07 ^a ±0.02	0.57 ^b ±0.03	0.68 ^b ±0.05
2.0	0.06 ^a ±0.06	0.03 ^a ±0.02	0.64 ^c ±0.05	0.47 ^a ±0.09

¹Values are averages±SD deviation n = 15

^{a,d}Within columns, different superscripts indicate significant differences in culture OD (p<0.05)

Table 2: Log cfu/mL 1 of bacteria recovered from broiler skin after each of 3 consecutive washes in 0.0 or 1.0% (vol/vol) LauriPure

Recovery Medium ²	Wash#1		Wash#2		Wash#3	
	0.0%	1.0%	0.0%	1.0%	0.0%	1.0%
EMB	2.6 ^b	1.0 ^a	2.3 ^b	0.09 ^a	2.1 ^b	NR ^a
Staph	2.6 ^b	1.0 ^a	2.3 ^b	NR ^{a,3}	2.0 ^b	NR ^a
PC	3.1 ^b	1.5 ^a	2.7 ^b	0.09 ^a	2.5 ^b	NR ^a

¹Values are average number of colony-forming-units/ml bacteria recovered on agar media. n = 3

²Recovery media: Eosin Methylene Blue(EMB), Staphylococcus Agar (Staph) and Plate Count Agar (PC)

³NR-None recovered

^{a,b}For each wash, different subscripts indicate significant differences (p<0.05) in the number of cfu/ml recovered on agar media from skin after washing in distilled water (0.0%) or 1.0% LauriPure

2007). Therefore, fatty acids (glycerol monolaurate, caprylic acid and capric acid) contained in LauriPure may assist in physically removing bacteria found between the fat layers of broiler carcasses skin and in killing the bacteria attached to poultry skin. This antibacterial activity may contribute to the ability of LauriPure to inhibit bacterial growth and to decrease bacterial contamination of poultry skin. Further more, lactic acid, another component of LauriPure, may permeabilize bacteria cell membranes and enhance the activity of other antimicrobial substances (Anang, 2010; Rubin *et al.*, 1982; Salminen *et al.*, 1989). The low pH of LauriPure (2.38) that is partially due to lactic acid, may be effective in inhibiting the growth some important food borne bacteria associated with processed poultry. The final pH of LauriPure solutions used in these studies was not measured; however, the minimum pH value for growth of *Salmonella sp.* is 3.8 (Bell, 2002) and the minimum pH for growth *Listeria sp* is 4.2 (Farber *et al.*, 1989). Therefore, several individual components of LauriPure may utilize different mechanisms to inhibit bacterial growth or reduce bacterial contamination. Findings from this study indicate that this sanitizer is effective in inhibiting growth of several bacteria associated with processed poultry and might be used in some processing operations to reduce bacterial contamination.

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