ACTIVATED LACTOFERRIN - A NATURAL ANTIMICROBIAL INTERVENTION FOR BEEF SAFETY

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Background

The economic impact of food-borne illness and the less than desired shelf life of vacuum-packaged refrigerated products, call for the development of effective antimicrobial interventions. Currently used antimicrobial interventions by the food processors are cidal systems that kill microorganisms and leave behind an array of harmful toxic debris. Furthermore, post-processing protection, i.e. to prevent pathogen attachment and microbial proliferation is critical to ensure food safety at retailer and consumer level. Any new intervention to fulfill these unmet demands should therefore address bacterial adherence to food matrices and the mechanism(s) of microbial detachment. Such interventions aimed at detachment should also prevent microbial multiplication and eliminate any cellular appendages that anchor pathogen attachment to meat surface.

Microbial blocking agents are naturally occurring bio-active molecules that inhibit growth-multiplication and adhesion-colonization of microorganisms, as well as effectively neutralizing and detaching pathogens and their toxic debris from bio-surfaces (Naidu & Bidlack, 1998; Naidu, 2002). Activated lactoferrin (ALF) is a novel formulation of commercial lactoferrin for enhancing antimicrobial efficacy by using a specific molecular-milieu optimization process (Naidu & Nimmagudda, 2003). ALF is an effective inhibitor of microbial colonization factors such as fimbria on a *E.coli* cell surface, thereby blocking its attachment to a bio-surface such as meat tissue. Thus, ALF is the first all-natural antimicrobial intervention technology with 'microbial blocking activity' (Naidu et al., 2003).

Objectives

The main objective of this study is to evaluate the efficacy of ALF dry-blend formulation for food antimicrobial applications. Specific goals include (i) Applications of ALF in beef safety i.e. pathogen control including prevention of *E.coli* O157:H7 attachment to beef surface and inhibition of pathogen growth proliferation, (ii) Applications of ALF for inhibition of beef spoilage and extension of shelf life.

Methods

ALF dry blend (powder-form) prepared according to the patented ingredient specifications (Naidu, 2001) was evaluated for food antimicrobial applications. Dry blend was reconstituted in de-ionized water (with low content of iron and divalent cations) to make a 2% ALF formulation. The reconstituted ALF formulation was confirmed for enhanced antimicrobial activity against *E.coli* O157:H7 (Q/A criteria: 0.5% ALF should elicit >48-h total stasis of 4-log inoculum in tryptic soy broth). ALF formulation after passing the Q/A protocol was kept refrigerated until used.

Efficacy of ALF formulation to inhibit pathogen attachment to beef was performed by using ^{3}H-thymidine labeled *E.coli* O157:H7. Beef surface sprayed with 2% ALF formulation was challenged with *E. coli* O157:H7 in a contained tissue surface using a sterile bactainer (an open stainless-steel hollow with a sharp-edged square end of 1-in^2) and incubated for 2-h at room temperature. Beef without ALF treatment served as control. The inoculum was aspirated from the bactainer-contained meat surface and gently washed with 5-mL phosphate buffered saline. The bactainer-contained tissue area was excised into six pieces (about 0.5 g), placed into scintillation vials and each piece was digested with 4-mL of tissue homogenizer (Scintigest™, Fisher) overnight at 55°C in a shaking water bath. A 10-mL aliquot of scintillation cocktail (ScintiSafe™ Gel, Fisher) was added to the homogenate and the radioactivity (disintegrations/min, DPM) was measured using a liquid scintillation analyzer (Tri-Carb 2100 TR®, Packard Inc.). Efficiency of ^{3}H-thymidine uptake by *E.coli* O157:H7 was estimated at 1 dpm per 64 bacterial cells.

Antimicrobial protection of ALF-treated beefsteaks was tested by challenge studies with *E.coli* O157:H7 transformed with p-GLO plasmid to produce green fluorescent protein (gfp). The (gfp)-*E.coli* grown in Luria-Bertani broth containing arabinose (6 mg/mL) and ampicillin (50 µg/mL) were harvested and used for the challenge. A pre-marked 1-in^2 meat surface treated with 2% ALF was inoculated with ~4-log (gfp)-*E. coli* and the meat was either at room temperature for 18-h or was vacuum packaged and at room temperature for 48-h. Similarly challenged meat samples with no ALF treatment served as controls. Following the indicated times, pre-marked tissue portion was excised, stomached, appropriately diluted, plated on tryptic soy agar (containing arabinose/ ampicillin) and incubated at 37°C. After 24-h incubation, the (gfp)-*E.coli* were visualized against UV light as green fluorescent colonies and enumerated.

A combination of interventions such as acid rinses, hot/cold water washes, and steam pasteurization are currently used as antimicrobial hurdles in beef processing. A meat processing simulation (MPS) system designed to mimic in-plant beef processing, was used to test the efficacy of current interventions with or without ALF spray to decontaminate ^{3}H-thymidine labeled-*E. coli* O157:H7 from beef. Samples were subjected to a sanitizing treatment typical of commercial beef processing consisting of five spray-wash steps, i.e. cold water (10-sec), 2% lactic acid (10-seconds), hot water (180°F for 30-seconds), cold water (10-seconds), and 2% lactic acid (10-seconds). Samples were also treated using an additional 1% ALF (10-seconds) wash step between cold water and lactic acid washes. After treatment, the loading frame was dismounted, the bactainer-contained tissue area was excised and measured for radioactivity (DPM) as described above.

Results and Discussion

Beef Safety Applications: Based on an average of three experimental runs, about 6.5 x10^5 *E.coli* cells/in^2 were found attached to the beef tissue surface. After rinsing with saline about 3.9 x 10^4 bacteria/in^2 remained on beef tissue causing a 1.2-log pathogen reduction compared to the control. Following an ALF rinse the beef surface showed no residual radioactivity (<64 bacterial cells based on the sensitivity of isotope label/detection) suggesting >2.8-log pathogen reduction compared to the saline-wash.

ALF spray reduced the growth of (gfp)-*E.coli* O157:H7 on beef by ~2 logs (99%) and ~3 logs (99.9%), when at room temperature directly for 18-h, and in the vacuum-pack for 48-h, respectively.

Finally, ALF demonstrated a potent antimicrobial spectrum against several major food pathogens, including, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp., *Vibrio* spp., *Aeromonas hydrophila*, and *Staphylococcus aureus*; and common food spoilers such as *Bacillus* spp., *Pseudomonas* spp., and *Klebsiella* spp. ALF also shown efficacy against multi-drug-resistant *Salmonella typhimurium* DT104, vancomycin-resistant *Enterococcus faecium*, and methicillin-resistant *Staphylococcus aureus*. Furthermore, ALF strongly inhibited radiation-resistant bacteria such as *Brochothrix thermospacta, Deinococcus radiopugnans, Deinococcus radiodurans, Acinetobacter*

radioresistens, and *Methylobacterium radiotolerans*. These antimicrobial attributes make ALF a potential intervention for beef, poultry, ready-to-eat meats and other food processing applications (Naidu, 2001).

Control of *E.coli* O157:H7 in multi-hurdle MPS system: Regular MPS system averaged 72.2% of *E. coli* detachment/in^2 of beef tissue. The MPS system combined with the 2% ALF spray demonstrated almost 100% efficacy - an average 99.9% *E. coli* detachment/g of beef tissue - in three experimental runs *(Figure-1A)*. Acid rinses and hot water washes seemed to reduce *E. coli* O157:H7 to a significant level, but scanning electron microscopy of the processed beef revealed that most of the bacterial debris remained firmly attached to the tissue *(Figure-1B)*. An additional 10-sec spray/rinse with 2% ALF effectively sanitized the contaminated beef surface by removing debris and residual bacteria.*(Figure-1C)*.

ALF was also evaluated directly on beef carcass as an antimicrobial intervention step on a kill floor process line, using an electrostatic spraying system (ESS). This spray system has been designed to deliver a thin uniform coating of ALF on a target surface. ALF solution was applied in 2-second bursts, using 16 ESS nozzles that delivered ~50-mL of ALF onto a beef carcass. After ALF spraying, the carcass passed through an organic acid rinse and then was placed in the "hot box" for a period of 18-h.

Beef tissue samples for application coverage were collected immediately after ESS and also after the organic acid rinse step to measure residual ALF on the beef carcass. Exogenous (ALF application) and endogenous (tissue-borne LF) levels were measured by solid-phase immuno-blotting and ELISA techniques, respectively, using a polyclonal anti-bovine LF antibody for capture and detection. Approximately 8.7 µg/in^2 of exogenous ALF on carcass and 0.2 µg/gm beef tissue of endogenous LF were detected. Seven random carcass sites excised and assayed for ALF, suggested a uniform distribution of this antimicrobial formulation by ESS. Beef sprayed with ALF, when subjected to a 4-log *E.coli* O157:H7 challenge for 24-h at room temperature, showed a potent stasis effect, thus ~2-log (~99%) reduction in pathogen proliferation compared to the untreated beef (control). The ALF-treated beef also effectively blocked the attachment of *E.coli* O157:H7 to its tissue surface, when tested at different time points of challenge, and the activity extended up to 6 weeks of post-ALF application, compared to the controls.

Conclusions

ALF is a functionally enhanced form of isolated lactoferrin that acts as a powerful microbial blocking agent against pathogenic bacteria that may be present on a food surface. ALF is an effective intervention to control adherent *Escherichia coli* O157:H7 on a beef tissue, tightly bound *Salmonella* Typhimurium or *Campylobacter jejuni* on a poultry broiler skin or proliferation of *Listeria monocytogenes* in ready-to-eat foods. Considered generally recognized as GRAS by the US-FDA and approved by the USDA for use on fresh beef, ALF can be sprayed onto carcasses to prevent bacterial contamination during processing or can be applied to a subprimal or finished beef surface prior to final packaging to inhibit bacterial growth and extend shelf life.

Most antimicrobial interventions currently in use are cidal systems that kill microorganisms and leave debris as well as potent toxins on processed foods. In contrast, ALF is an extremely powerful microbial blocking system that prevents proliferation and attachment of pathogens to bio-surfaces such as beef or poultry tissue. The ability to detach the leftover microbial debris and to inactivate the surface-splattered endotoxins makes ALF a highly effective intervention all by itself or as an additional step in current multi-hurdle sanitizing systems in food processing. In the ever-competitive food market, where consumer demand is steadily increasing for minimally processed foods that sustain functionality of naturally occurring bioactive ingredients, ALF clearly stands out as a potent natural antimicrobial system as an impressive microbial blocking agent that fulfills the unmet need for food safety applications.

References

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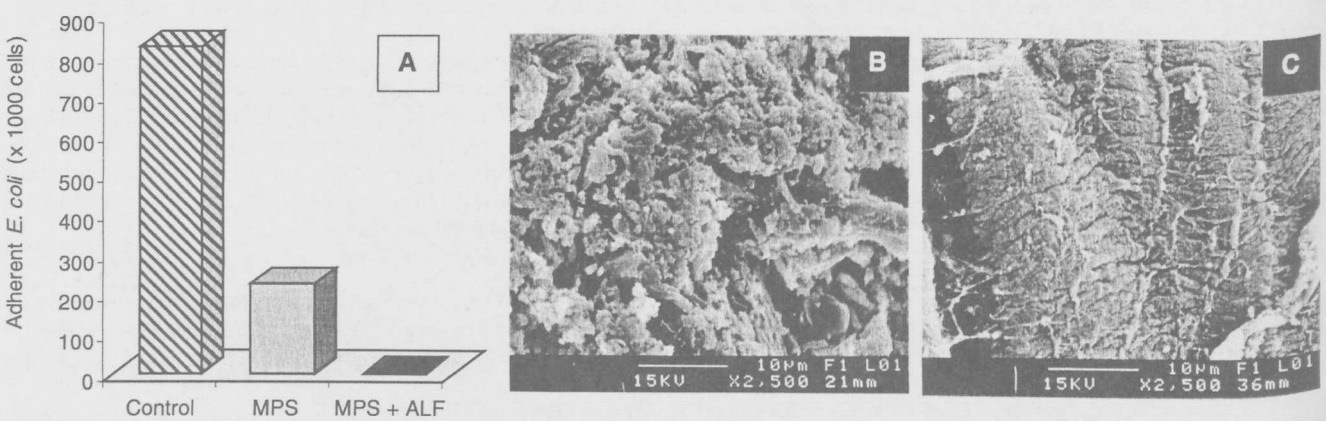

Figure-1. A meat processing simulation (MPS) system designed to mimic in-plant beef processing, was used to test the efficacy of current interventions with or without ALF spray to decontaminate *E. coli* O157:H7 from beef. **(A)** Regular MPS system averaged 72.2% of *E. coli* detachment per inch^2 of beef tissue. The MPS system combined with the 2% ALF spray demonstrated almost 100% efficacy - an average 99.9% *E. coli* detachment/g of beef tissue. Scanning electron microscopy of the processed beef revealed that **(B)** most of the bacterial debris remained firmly attached to the tissue after MPS and **(C)** an additional 10-sec spray/rinse with 2% ALF effectively sanitized the contaminated beef surface by removing debris and residual bacteria.