71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

ENHANCING SANITATION IN RED MEAT PROCESSING: A NOVEL ENZYMATIC APPROACH

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I. INTRODUCTION

The red meat processing sector contributes significantly to global food security and the economy yet presents opportunities for improving food safety and reducing environmental footprint. In particular, the meat processing sector uses sizable amounts of freshwater applied at high temperatures (55-60°C) to maintain operating standards [1]. However, current water usage is tied to cleaning and sanitation procedures vital for food safety. Water-saving methods are needed to alleviate some of the ecological burden. The present study explores enzymes as effective, eco-friendly agents to optimize water usage and food contact surface (FCS) hygiene. Four lipases and five proteases were tested for their activity under eco-friendly conditions, namely through reduced operating temperatures (40°C and 50°C) and neutral pH (7.2). The research aimed to select enzymes with high activity under the conditions described, as well as in the presence of surfactants to develop a novel enzymatic formulation. The formulation was applied to FCS experimentally contaminated with beef to assess its potential to enhance surface hygiene. Moreover, the solution was applied to single-species *Escherichia coli* biofilms to assess its ability to reduce biofilm mass. It was hypothesized that enzymatic treatments could reduce proteinaceous contamination and disrupt biofilms in the meat fabrication environment, while providing a biodegradable and less harsh alternative to strong caustic chemicals.

II. MATERIALS AND METHODS

Nine enzymes (lipase from *Aspergillus oryzae, Aspergillus niger,* Wheat germ, *Pseudomonas cepacia,* and protease from *Aspergillus oryzae, Streptomyces griseus, Bacillus amyloliquefaciens*, bromelain and papain) were included in a preliminary trial to assess enzymatic activity and select suitable candidates to develop a cleaning formulation. Lipolytic activity was assessed utilizing 4-nitrophenyl palmitate as a substrate [2], whereas proteolytic activity was assessed with azocasein substrate [3]. Enzymes were prepared in a neutral pH buffer and tested at 40 °C and 50 °C. Two lipases and two proteases were selected for further testing based on their superior activity. Enzymes were tested for retained activity when combined with each other, and with conventional cleaning reagents (ammonium lauryl sulfate, sodium lauryl sulfate, sodium borate, and poloxamer-407). Ultimately, a solution was selected which included *Aspergillus oryzae, Pseudomonas cepacia, Streptomyces griseus*, papain (0.5 mg/ mL) 0.5% (w/v) sodium borate, and 0.5% (w/v) sodium lauryl sulfate. Food contact surfaces (stainless steel (SS) and thermoplastic polyurethane (TPU)) were contaminated with beef for 16 hours at 10°C to recreate exposure conditions like those in meat processing facilities. Surfaces were then treated with one of the following treatments: enzymatic formulation (EF), enzyme control (EC), surfactant control (SC), hot water (HW), PowerFoam Plus (PF), or were not treated to serve as a control (POS). Negative controls were exposed to distilled water. After treatment for 5 minutes, surface hygiene was assessed with ProteinPlus and ATP swabbing, and microscopically observed using scanning electron microscopy. Cleaning potential of EF was additionally assessed on mature single-species biofilms produced by *Escherichia coli* O157:H7 in 96-well microplates. After treatment with described solutions, the crystal violet method was performed to quantify retained mean biofilm mass[4]. Statistical analyses were performed using ANOVA and pairwise comparison of least means square (Statistical Analysis System Software). Data from the ProteinPlus swab analysis were assessed with Chi-Square distribution analysis.

III. RESULTS AND DISCUSSION

The enzymatic formulation, when applied at a reduced operating temperature, was able to reduce the frequency of protein contamination detected by the ProteinPlus swabs and reduce the levels of ATP on both SS and TPU ($p<0.001$) (Table 1). Furthermore, EF was effective in reducing the mean biofilm mass ($p<0.001$), showing similar reduction to the conventional caustic treatment (PF). Overall, the enzymatic formulation was effective in reducing overall contamination on FCS and reducing biofilm matrix. Apart from the efficacy of EF, benefits of the enzymatic solution include reduced energy consumption to heat solutions, and an eco-friendlier profile compared to traditional sodium hypochlorite-based treatments like PF.

Table 1- Least means square analysis of ATP (measured in relative light units) detected with swabbing assays of contact surfaces contaminated with beef and treated with experimental sanitation solutions.

	Surface type	
	Stainless steel	Polyurethane Conveyor Belt
Enzymatic Formulation (40°C)	101.00$^{b/y}$	112.00$^{b/y}$
Enzyme Control (40°C)	566.00$^{b/y}$	337.22$^{b/y}$
Surfactant Control (40°C)	208.67$^{b/y}$	295.22$^{b/y}$
PowerFoam Plus (60°C)	267.44$^{b/y}$	509.00$^{b/y}$
Hot water (60°C)	561.89$^{b/y}$	623.78$^{b/y}$
POS	2032.89$^{a/y}$	1146.33$^{a/z}$
NEG	31.56$^{b/y}$	20.22$^{b/y}$

^{a,b,c} Least means square within columns lacking a common superscript letter differ within columns (p=0.05). ^{y,z} Least means square within columns lacking a common superscript letter differ within columns (p=0.05).

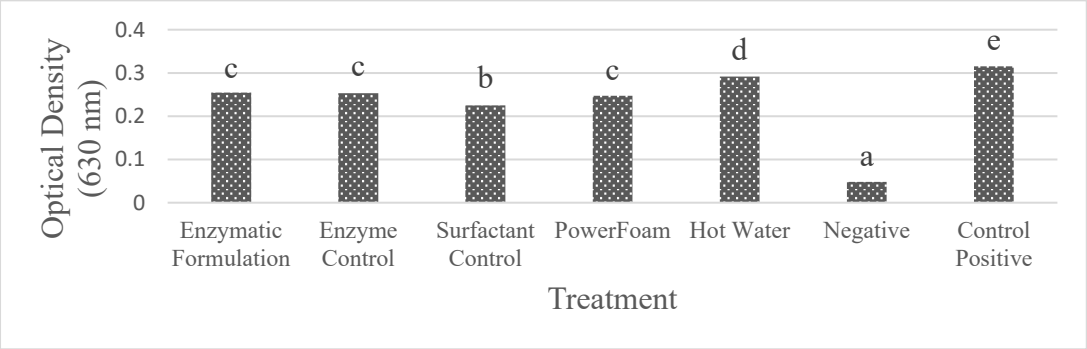


Figure 1. Mean optical density of biofilms following experimental sanitation treatments. *Escherichia coli* O157:H7 R508 biofilms were grown in microplates for 5 days at 25 °C before being washed thrice with Butterfield's phosphate buffer and either treated for 10 minutes with enzymatic formulation, enzyme control, or surfactant control (40 °C), or 5 minutes with PowerFoam plus or hot water (60°C), and stained with 0.1% crystal violet.

IV. CONCLUSION

A novel enzymatic formulation improved hygienic properties of SS and TPU surfaces experimentally contaminated with beef, and mitigated *E. coli* biofilm mass, while operating at a reduced temperature (40°C), neutral pH, and demonstrating a milder wastewater profile. Future research should assess the efficacy of the enzymatic formulation in a pilot-plant scale and explore ways to optimize the potential of enzymatic sanitation strategies for meat processing plants. Enzymatic sanitation solutions represent an eco-friendly and innovative method to enhance sanitation and sustainability in the meat processing industry.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Beef Cattle Research Council, the Agricultural Funding Consortium, and the University of Manitoba Graduate Fellowship for funding and supporting this project.

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