MARCIA ENGLISH
BSc (Biology - Honors), University of New Brunswick, 2007

DEPARTMENT OF PROCESS ENGINEERING AND APPLIED SCIENCE

TITLE OF THESIS:	Interactions of Native and Modified Clupeine with *Escherichia coli* K-12 and *Salmonella enterica* serovar Typhimurium 14028 Cells and Model Biomembranes
TIME/DATE:	1:00 pm, Thursday, November 16, 2017
PLACE:	Room 3107, The Mona Campbell Building, 1459 LeMarchant Street

EXAMINING COMMITTEE:

Dr. Darren Korber, Department of Food and Bioproduct Sciences, University of Saskatchewan (External Examiner)

Dr. Paul Gratzer, Department of Biomedical Engineering, Dalhousie University (Reader)

Dr. Tom Gill, Department of Process Engineering and Applied Science, Dalhousie University (Reader)

Dr. Allan Paulson, Department of Process Engineering and Applied Science, Dalhousie University (Supervisor)

DEPARTMENTAL REPRESENTATIVE:	Dr. Alex Speers, Department of Process Engineering and Applied Science, Dalhousie University
CHAIR:	Dr. Roger McLeod, PhD Defence Panel, Faculty of Graduate Studies

ABSTRACT

Clupeine, a cationic antimicrobial peptide found in the sperm cells of fish, is of interest as a food additive because of its antimicrobial activity against several foodborne pathogens. But, non-specific binding of clupeine to anionic molecules reduces its antimicrobial activity. It has been shown that the overall positive charge of the native peptide can be reduced by blocking 10% of its arginine residues with 1,2-cyclohexanedione (CHD) to form CHD treated clupeine. CHD treated clupeine retains antimicrobial activity but it is not known if the modes of interaction against Gram-negative bacteria remain the same as the native peptide. The focus of this study was to investigate the effect of charge reduction on antimicrobial activity and peptide membrane interactions by comparing the effect of native and CHD treated clupeine on *Escherichia coli* K-12 and *Salmonella enterica* susp. *enterica* serovar Typhimurium 14028 cells and in model biomembranes.

E. coli K-12 cells were more susceptible to native (minimum inhibitory concentrations MIC, 500 µg/mL) and CHD treated (MIC, 400 µg/mL) clupeine than *S. enterica* serovar Typhimurium 14028 cells (MIC, 1250 µg/mL for both peptides). The relative expression of the outer membrane porin gene *ompF* was down-regulated in *E. coli* K-12 cells exposed to native or CHD treated clupeine, which was in strong contrast to the up-regulation ($P < 0.05$) of this gene observed when *S. enterica* serovar Typhimurium 14028 cells were exposed to minimum bactericidal concentrations (2500 µg/mL) of both peptides. Increased expression of the outer membrane porin protein OmpA, was identified by mass spectroscopy and the oxidative stress-related glyceraldehyde-3-phosphate dehydrogenase (GapA) protein was only observed when test strains were exposed to the CHD treated peptide.

Model biomembranes composed of lipids used to mimic the inner membrane of *E. coli* (PE, phosphatidylethanolamine: PG, phosphatidylglycerol: and CL, cardiolipin), in the following ratios: PE:PG:CL (79:17:4 mole %) were studied using Neutron Reflectometry (NR) and X-ray reflectometry (XRR). Symmetric bilayers were deposited on silicon blocks applying the Langmuir-Blodgett and Schaefer technique. Some lipid mixing was observed in the inner tail region (69 ± 0.24% DPPC (1,2-dipalmitoyl (d62)-sn-glycero-3-phosphocholine) and 24 ± 0.02% PE:PG:CL); and in the outer tail region (24 ± 0.02% DPPC and 56 ± 0.01% PE:PG:CL). Native and CHD treated clupeine were not able to cross the model PE:PG:CL:DPPC bilayer biomembrane, however, CHD treated clupeine showed increased interactions with the lipid head group.

Inspite of the different interactions observed in the test strains and model systems, a more comprehensive understanding of the safety and toxicology of both peptides is required before they can be used for food applications in Canada.