# THE POTENTIAL USE OF HEN EGG WHITE LYSOZYME AS AN ANTIMICROBIAL AGENT IN FOODS.

A thesis
presented in partial fulfilment of the
requirements for the Degree of Master of Technology
in Food Technology at Massey University.

EDGAR RUSHIZHA

1995.

## ABSTRACT.

The potential use of lysozyme as an antimicrobial agent in foods was investigated in model food systems(brainheart infusion broth) using factorial designed experiments and in mussel and cottage cheese food systems. Optical density or absorbance was used as a tool to monitor the growth response of *Listeria monocytogenes* and *C. tyrobutyricum* in brain heart infusion broth under the combined influence of pH(5.5, 6.5), lysozyme (0.2mg/ml, 3mg/ml) and different chelating agents(ethylene diaminetetraacetic acid(EDTA), glycine, gluco delta lactone(GDL), citric acid, sodium phosphate dibasic(SPDB) and sodium hexametaphosphate(SHMP)(10mM, 25mM).

Using $2^3$ full factorial design experiments, the yield of the organisms (expressed as the area under the curve of a plot of change in optical density at 600nm vs time) was taken as the quantitative response variable for each treatment. These yield values were then used for (a) statistical analysis to determine which of the single or interactive factors tested significantly reduced the yield, (b) formulation of a mathematical regression equation which could be used to predict microbial growth within the limits of the factors studied. Diagnostic plots were constructed to evaluate further how well the statistical model fit the observed yield values. Plots of residuals versus predicted yield values appeared to suggest that a transformation of the response would improve the fit of the models. No other serious reservations were suggested by the diagnostic plots. Goodness of fit of the models was also evaluated by the R-squared values.

Significant two-way and three-way interactions between lysozyme, pH and EDTA, GDL, citric acid and glycine were exhibited. Response surface methodology(RSM) was used to (a) characterize the response of *L. monocytogenes* to variation in treatment combinations and (b) show non-linearity of models(or interaction of factors). Generally yield was minimal in treatment where pH was low, with high lysozyme and chelator.

Based on equal molar concentrations, the antimicrobial activity of the different chelating agents was in the order EDTA > GDL > citric acid > glycine > adipic acid > SHMP > SPDB. The same ranking was true for the degree to which each chelating agent

enhanced lysozyme activity. Based on broth culture studies, the chelating agents EDTA, GDL, glycine, citric acid and adipic acid were demonstrated to have potential for use as antimicrobial agents in combination with lysozyme in food systems.

Results of a $2^5$ factorial design indicated that the 5 factors, lysozyme, GDL, pH, inoculum level and temperature were important in the inhibition of *L. monocytogenes*. Results of the broth culture studies gave a good reflection of the survival of *L. monocytogenes* in the food system. The variable combinations interacted to decrease the growth of *L. monocytogenes* and extended the lag phase duration. However *C. tyrobutyricum* was more tolerant to the different treatment combinations other than EDTA.

A study of protein interference demonstrated that the antimicrobial activity of the lysozyme-GDL preservation system was not inhibited by the presence of proteins. The food system study demonstrated that the lysozyme-GDL treatment combination has potential for use as a preservative in refrigerated low pH ready-to-eat foods. The susceptibility of *L. monocytogenes* to lysozyme-GDL treatment in both broth culture and food systems increased as the temperature was reduced(25C-5C) and as the pH decreased(pH6.5-pH5.5).

Food system studies demonstrated that modified atmosphere packaging(96.58%$N_2$ , 2.09%O2 and 1.34% $CO_2$) has no influence of the growth of *L. monocytogenes*. The susceptibility of *L. monocytogenes* to lysozyme-GDL was a stable characteristic, remaining unchanged during the entire study. Attempts to select for greater lysozyme-GDL resistance by testing populations grown from lysozyme-GDL survivors isolated at the end of the food system study was unsuccessful. There was no evidence that *L. monocytogenes* was resistant to the lysozyme-GDL treatment.

## ACKNOWLEDGEMENTS.

- I wish to thank Dr. J. D. Brooks for the supervision of this work and for his constructive criticism.

- I wish to thank Professor Don Barnes for the valuable guidance he rendered in the statistical analysis of this work.

- I also wish to thank all members of staff in the Food Technology Department, Massey University, in particular all the technical staff for facilitating the successful completion of this work.

- I would like to thank my New Zealand host family, the Gartner family, for their encouragement, hospitality and friendship throughout my stay in New Zealand.

- I would like to thank the van Laar-Veth family for the wonderful friendship throughout my stay in New Zealand.

- Many thanks to my family and friends for encouraging me to take up further studies and for keeping in touch during the two years that we were apart.

- I would like to thank the Ministry of Foreign Affairs and Trade for funding this work.

# TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES.

## LIST OF APPENDICES.