Development of Antimicrobial Edible Coatings to Reduce Microbial Contamination of Broiler Carcasses

By

Ghadeer Fawzi Mehyar

A thesis

Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements
for the Degree of

Doctoral of Philosophy

Food and Nutritional Sciences
University of Manitoba
Winnipeg, Manitoba

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

OF

Doctor of Philosophy

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FORWARD

This thesis has been prepared in a paper format style. It is composed of a general introduction (Chapter 1), a literature review (Chapter 2), manuscripts published or to be published in refereed journal (Chapters 3, 4, 5 and 6) and discussion and conclusions.

Chapter 3 “Physical and mechanical properties of high-amylose rice and pea starch films as affected by relative humidity and plasticizer” was published in the Journal of Food Science, 2004, 69(9): E449-E454, with authorship by Mehyar, G. F. and Han, J. H.

Chapter 4 “Effectiveness of trisodium phosphate, lactic acid and commercial antimicrobials against pathogenic bacteria on chicken skin” was published in the journal Food Protection Trends, 2005, 25(5): 351-362, with the authorship by Mehyar, G. Blank, G. Han, J., Hydamaka, A and Holley, R. A.

Chapter 5 “Suitability of pea starch and calcium alginate as antimicrobial coatings on chicken skin” was submitted for publication to the Journal of Poultry Science in February 2006, with authorship by Mehyar G., Holley, R. A, Han, J., G. Blank, G. and Hydamaka, A.

Chapter 6 “Characterization of mechanical properties and release of antimicrobial agents from hydrogels into physiological saline” is in preparation for submission to Journal of Control Release in June 2006, with authorship by Mehyar, G, Liu, Z. and Han, J.
ABSTRACT

Broiler carcasses are a significant source of pathogenic microorganisms which can cause food-borne illnesses if not properly cooked. The extent of broiler flock contamination varies, but normally increases by contact of birds with equipment, and by scalding, defeathering and chilling. The currently used on-line approved chemical antimicrobials vary in their activity and do not effectively inactivate contaminating microorganisms, presumably as a result of microbial cell entrapment inside the carcass skin. In this work, antimicrobial coatings were designed to be sprayed or coated on broiler carcasses during processing. The resistance of physical and mechanical properties of high amylose pea starch (PS) and rice starch (RS) films to high relative humidity (RH) and plasticizer addition was investigated in the first part of the thesis. PS films which contained higher amylose showed more desirable properties than the RS films which contained low amylose at the same RH. The addition of glycerol to the films helped to form a more continuous structure. Increasing the RH from 51 to 90 % reduced tensile strength and increased the elongation of both films with PS films being more resistant to deformation. Both films were excellent oxygen barriers at low RH (≤ 34 %) but had relatively low water vapor permeability compared to other edible films in the literature.

The inhibitory activity of the most commonly used commercial antimicrobials was examined against *E coli* 0157:H7, *Salmonella* and *Campylobacter* spp. in the second part of the thesis. The commercial antimicrobials used were trisodium phosphate (TSP), Sanova (acidified sodium chlorite, ASC), Safe2O, Inspexx 100 and Cecure. Factors such as antimicrobial concentration, contact time, sequential use of two antimicrobials and the most appropriate stage of processing for use of the antimicrobials were studied. Results showed
that TSP (10 %) and acidified sodium chlorite (0.12 %) were the most effective for reducing viability of the pathogens and this was related to their effect on surface pH of the skin. Increasing the antimicrobial concentration and contact time up to 20 min enhanced the antimicrobial effectiveness. Sequential use of antimicrobials also increased activity especially for compounds used before cetylpyridinium chloride (CPC), with TSP being the most effective. Antimicrobials were also more effective on warm (before chilling) than on cold (after chilling) carcasses, indicating the use of antimicrobials in early stages of processing would be more efficient. When TSP (10 %) and ASC (0.12 %) were stabilized by incorporation into coatings made of pea starch (3 %) or calcium alginate (1.0 %), respectively, their antimicrobial activity significantly (p < 0.05) increased. Salmonella inoculated on the skin were reduced about 1.5 log cfu/g and < 1.0 log cfu/g by the stabilized and unstabilized antimicrobials, respectively, after storage at 4 °C for 24h.

Current Canadian standards require that carcasses be free of any additive before leaving the processing plant. This is why the third objective of the study was to formulate coatings containing antimicrobials that would interact with broiler skin but disappear during processing. PS coating containing TSP and alginate coating containing ASC were designed with polymer concentrations that were able to absorb into the chicken skin and gradually dissolve once their pHs equilibrated with skin pH. The interactions of the coatings with the skin were determined by measuring coating drop contact angle with the skin (surface energy difference between the coating and the skin) and coating absorption rate into the skin at different polymer concentrations. Compared to alginate, the PS coating had higher skin contact and faster absorptiveness.
The kinetics of coating dissolution from the skin were studied in the fourth part of thesis. Gels made of pea starch and alginate-containing antimicrobials were prepared and placed in a solution of 0.8 % saline that mimicked broiler skin osmotic strength. The rheological properties (dissolution rate) of these gels and antimicrobial concentrations in the solution were measured over 8h. The alginate coating had rapid initial (15 min) dissolution and antimicrobial release rates followed by even faster changes in solubility and release. The PS coating was more stable than the alginate coating in solution. Experimental data for mechanical changes and antimicrobial release fit with established models \( R^2 > 0.95 \). These results indicated that PS coating containing TSP had better interaction with skin and slowly dissolved after application. This coating would increase the contact time with the target microorganisms but disappear from the carcass before chilling was completed in actual processing, which means it is possible that it may have commercial value.
ACKNOWLEDGEMENTS

I would like to express my deepest thanks to...

My parents (to whom this thesis is dedicated) for their unique support and for their patience while being separated from them.

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TABLE OF CONTENTS

Forward iii
Abstract iv
Acknowledgments vii
Table of content ix
List of Tables xii
List of Figures xiv
List of Appendices xviii
1.0 Introduction 1
2.0 Review of Literature 6
  2.1. Contamination of broiler carcases with pathogenic and spoilage microorganisms 8
  2.2. Studies on antimicrobial activity of commercial antimicrobials on broiler carcases 8
    2.2.1. Trisodium phosphate (TSP) 8
      2.2.1.1. Factors affecting antimicrobial activity of TSP on poultry 12
        2.2.1.1.1. Application temperature 12
        2.2.1.1.2. TSP concentration and duration of application 13
        2.2.1.1.3. Method of application 14
        2.2.1.1.4. Skin topography/sample type 15
      2.2.2. Acidified sodium chlorite (ASC) 16
    2.2.3. Cetylpyridinium chloride (CPC) 19
  2.3. Stabilization of antimicrobial agents in films and coatings 20
    2.3.1. Effect of immobilization on antimicrobial activity 21
    2.3.2. Effect of food constituents on antimicrobial activity 22
    2.3.3. Structural compatibility of the carrier and the antimicrobial agent 23
    2.3.4. Other factors 24
  2.4. Controlled release systems 25
    2.4.1. Diffusion systems 26
      2.4.1.1. Reservoir 26
      2.4.1.2. Matrix 27
      2.4.1.3. Hydrogel delivery systems 27
    2.4.2. Degradable delivery systems 28
      2.4.2.1. Erosion 28
  2.5. Molecular characteristics of selected hydrocolloids 29
2.5.1. Alginate 29
2.5.2. Pea starch 30
2.6. Rheological considerations of the gel-sol phase transition 30

3.0 Physical and mechanical properties of high-amylose rice and pea starch films as affected by relative humidity and plasticizer 33

3.1. Abstract 34
3.2 Introduction 35
3.3. Materials and Methods 37
   3.3.1. Materials 37
   3.3.2. Film preparation 37
   3.3.3. Photomicrography 38
   3.3.4. Tensile test 38
   3.3.5. Water vapor permeability (WVP) 39
   3.3.6. Oxygen permeability (OP) 40
   3.3.7. Percentage of soluble matter (%SM) 40
   3.3.8. Statistical Analysis 41

3.4. Results and Discussion 41
   3.4.1. Film preparation 41
   3.4.2. Photomicrography 41
   3.4.3. Moisture content (MC) 43
   3.4.4. Tensile test 45
   3.4.5. Water vapour permeability 48
   3.4.6. Oxygen permeability 49
   3.4.7. Total soluble matter 51
3.5. Conclusions 53

4.0 Effectiveness of trisodium phosphate, lactic acid and commercial antimicrobials against pathogenic bacteria on chicken skin 55

4.1. Summary 56
4.2. Introduction 57
4.3. Materials and Methods 58
   4.3.1. Cultures and inoculation of chicken drumettes 58
   4.3.2. Treatment of drumettes with antimicrobials 59
   4.3.3. Coating of drumettes with biopolymer films 61
   4.3.4. Microbial analysis and surface pH determination 61
   4.3.5. Statistical analysis 62
4.4. Results 62
   4.4.1. Trisodium phosphate and lactic acid treatments 63
   4.4.2. Commercial antimicrobial treatments 67
4.4.3. Psychrotrophs and shelf-life 74
4.4.4. Biopolymer films 79
4.5. Discussion 79
4.6. Conclusion 84

5.0 Suitability of pea starch and calcium alginate as antimicrobial coatings on chicken skin 85

5.1. Abstract 86
5.2. Introduction 87
5.3. Materials and Methods 89
  5.3.1. Antimicrobial coatings 89
  5.3.2. Chicken treatment 90
  5.3.3. Changes in drumette pH, weight and viable *Salmonella* after coating 91
  5.3.4. Coating absorptiveness 92
  5.3.5. Contact angle and skin wetting properties 93
  5.3.6. Statistical analysis 94
5.4. Results and Discussion 94
  5.4.1. Antimicrobial effectiveness, drumette weight and surface pH changes 94
  5.4.2. Coating absorptiveness 101
  5.4.3. Coating adhesion and skin wetting properties 103

6.0 Characterization of mechanical properties and release of antimicrobial agents from hydrogels into physiological saline 108

6.1. Abstract 109
6.2. Introduction 110
6.3. Materials and Methods 114
  6.3.1. Preparation of starch and alginate hydrogels 114
  6.3.2. Mechanical properties of hydrogels in air and in saline 115
  6.3.3. Determination of TSP and ASC concentrations in saline 117
  6.3.4. The hydrogel swelling ratio 118
  6.3.5. Modeling mechanical properties and antimicrobial release of hydrogel in saline 118
6.3. Results and Discussion 121
6.4. Conclusions 131

7.0 Discussion and Conclusions 132

8.0 Overall conclusion 141

9.0 Literature cited 143

10.0 Appendices 162
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1.</td>
<td>List of the most commonly used commercial antimicrobial agents.</td>
<td>3</td>
</tr>
<tr>
<td>Table 3.1.</td>
<td>Moisture content (MC), tensile strength (TS) and elongation-at-Break (E) of starch films at 51% and 90% relative humidity.</td>
<td>45</td>
</tr>
<tr>
<td>Table 3.2.</td>
<td>Water vapor permeability (WVP) and water solubility (soluble matters, SM) of starch films containing glycerol.</td>
<td>49</td>
</tr>
<tr>
<td>Table 3.3.</td>
<td>Comparison of the properties of edible films</td>
<td>53</td>
</tr>
<tr>
<td>Table 4.1.</td>
<td>Reductions in numbers of inoculated bacteria on unchilled drumettes treated 1 min with commercial antimicrobials and stored at 4 °C for 2 h.</td>
<td>69</td>
</tr>
<tr>
<td>Table 4.2.</td>
<td>Reductions in numbers of pseudomonads and psychrotrophic bacteria present on unchilled drumettes treated with commercial antimicrobials, then stored at 7 °C for 5 days.</td>
<td>75</td>
</tr>
<tr>
<td>Table 4.3.</td>
<td>Reductions of a Salmonellae cocktail inoculated on chicken drumettes then treated with TSP (10%) or sanova (0.12%) stabilized within polymers and stored at 4 °C.</td>
<td>76</td>
</tr>
<tr>
<td>Table 5.1.</td>
<td>Weight changes of chicken drumettes dipped in 10 % (w/v) trisodium phosphate (TSP) with or without 3 % (w/v) pea starch (PS), or in 1200 ppm acidified sodium chlorite (ASC) with or without 1 % (w/v) calcium alginate during storage at 4 °C.</td>
<td>99</td>
</tr>
<tr>
<td>Table 5.2.</td>
<td>Percentage absorptiveness (% A₀) of antimicrobial pea starch (PS+TSP) and calcium alginate (alginate+ASC) coatings containing different polymer concentrations applied to chicken skin and held at room temperature for ≤ 60 min.</td>
<td>102</td>
</tr>
</tbody>
</table>
Table 5.3. Linear regression analysis of changes in weight of chicken skin coated with antimicrobial pea starch (PS+TSP) and calcium alginate (alginate+ASC) coatings containing different polymer concentrations with time.

Table 6.1. Parameters for the storage modulus and antimicrobial release models of hydrogels.

Table 6.2. Swelling ratio of calcium alginate and pea starch gels with or without antimicrobials.

Table 6.3. Diffusion/erosion ratio\(^1\) of PS+TSP and alginate+ASC hydrogels during immersion in physiological saline (0.8 %; w/v) for 8h.
<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Figure 3.1.</strong> Rice and pea starch films after RH conditioning.</td>
<td>42</td>
</tr>
<tr>
<td><strong>Figure 3.2.</strong> Photomicrography (4X and 40X magnitude) of rice (R) starch and pea (P) starch films with (G+) and without (G-) glycerol at 51% RH. Scale bars in 4X and 40X are 100 µm and 10 µm, respectively.</td>
<td>44</td>
</tr>
<tr>
<td><strong>Figure 3.3.</strong> Oxygen permeability of rice and pea starch films containing glycerol at different relative humidity.</td>
<td>50</td>
</tr>
<tr>
<td><strong>Figure 4.1.</strong> Reduction of <em>E. coli</em> O157:H7 on chilled drumettes held at 4 °C for up to 120 h following 30 sec dipping in trisodium phosphate (TSP), lactic Acid (LA) or NaCl singly or sequentially. Drumettes were inoculated before antimicrobial treatment and were not rinsed between or after treatment. Means at the same storage time with different letters are significantly (p &lt; 0.05) Different. * Negative values were obtained at 120 h when NaCl was followed by LA treatment.</td>
<td>64</td>
</tr>
<tr>
<td><strong>Figure 4.2.</strong> Reduction of <em>E. coli</em> O157:H7 on chilled drumettes inoculated before antimicrobial treatment and held at 4°C for 2h (a) and 24 h (b) following dipping for 1, 20, 40 or 60 min with trisodium phosphate (TSP), lactic acid (LA) or NaCl. Means at the same storage time with different letters are significantly (p &lt; 0.05) different. * Negative values were obtained with NaCl.</td>
<td>65</td>
</tr>
<tr>
<td><strong>Figure 4.3.</strong> Reduction of <em>E. coli</em> O157:H7 on chilled drumettes inoculated before antimicrobial treatment held for up to 120 h at 4 °C following the various treatments. Contact times were 20 min for trisodium phosphate (TSP) and 10 min for lactic acid (LA) treatments. Means at the same storage time with different letters are significantly (p &lt; 0.05) different. * Negative values were obtained with NaCl.</td>
<td>66</td>
</tr>
<tr>
<td><strong>Figure 4.4.</strong> Reduction of <em>Salmonella</em> cocktail (<em>S. Heidelberg</em> plus two strains of <em>S. Typhimurium</em>) on unchilled or chilled drumettes treated 1 min and held at 4 °C for 2 h. Means within the same antimicrobial</td>
<td>66</td>
</tr>
</tbody>
</table>
treatment with different letters are significantly (p < 0.05) different.

**Figure 4.5.** Reduction of *Salmonella* cocktail (as in Fig. 4.1) on unchilled drumettes treated 10 min and held at 4 °C for 2 h. Means within the same antimicrobial treatment with different letters are significantly (p<0.05) different.

**Figure 4.6.** Reduction of *Campylobacter jejuni* on unchilled or chilled drumettes treated 1 min and held at 4 °C for 2 h. Means within the same antimicrobial treatment with different letters are significantly (p < 0.05) different.

**Figure 4.7.** Reduction of *E. coli* O157:H7 on chilled or unchilled drumettes treated 1 min and held at 4 °C for 2 h. Means within the same antimicrobial treatment with different letters are significantly (p < 0.05) different.

**Figure 4.8.** Reduction in viability of the *Salmonella* cocktail (as in Fig. 4.1) following dual 1 min treatments with cecure (0.5%) and other commercial antimicrobials on unchilled drumettes (38-40 °C) held at 4 °C for 2 h. Inoculation was done between the two treatments. Means within the same treatment with different letters are significantly (p<0.05) different.

**Figure 4.9.** Growth of naturally present pseudomonads and psychrotrophs on unchilled drumettes (38-40 °C) during storage at 7 °C for up to 120 h following dipping in 10 %TSP for 1 min. Means at the same incubation time with different letters are significantly (p < 0.05) different.

**Figure 5.1.** Application of coatings to chicken drumettes: A) 3.5 % (w/v) pea starch (PS) containing 10 % (w/v) trisodium phosphate (TSP); B) 1 % (w/v) alginate containing 1200 ppm acidified sodium chlorite (ASC). Solution (a) contained 1 (w/v) % CaCl₂ plus ASC; solution (b) contained 1 % (w/v) sodium alginate.

**Figure 5.2.** Effect of inclusion of commercial AMs in polymeric coatings on survival of inoculated *Salmonella* on chicken skin during storage at 4 °C for 5 d. TSP = Trisodium phosphate, ASC = Acidified sodium chlorite, PS = Pea starch. Columns with different letters at the
same sampling time are significantly ($P \leq 0.05$) different.

**Figure 5.3.** Surface pH of chicken drumettes dipped in 10 % (w/v) trisodium phosphate (TSP) and 1200 ppm acidified sodium chlorite (ASC) with and without inclusion in 3.5 % (w/v) pea starch (PS) or 1.0 % (w/v) calcium alginate (Algn), respectively during storage at 4 °C.

**Figure 5.4.** Effect of antimicrobial pea starch (PS+TSP) coating viscosity (prepared with different concentrations of PS) on the initial contact angle of coating drops applied to the chicken skin surface.

**Figure 5.5.** Effect of pea starch (PS) concentration change in the antimicrobial pea starch (PS+TSP) coatings on the initial contact angle of the coating drops on the chicken skin surface.

**Figure 6.1.** Schematic of (a) starch and (b) calcium alginate gel structures.

**Figure 6.2.** Schematic of assembly used for preparing calcium alginate gel.

**Figure 6.3.** Storage moduli ($G'$) plotted against shear stress ($\delta$) for pea starch gel with trisodium phosphate (PS+TSP) and calcium alginate gel with acidified sodium chlorite (alginate+ASC) in air.

**Figure 6.4.** Dimensionless storage moduli ($G'/G'_o$) plotted against soaking time ($t$) for (a) pea starch with and without trisodium phosphate, and (b) calcium alginate gel with and without acidified sodium chlorite. Solid lines represent estimates by models.

**Figure 6.5.** Changes in the storage ($G'$) and loss modulus ($G''$) of pea starch gel with trisodium phosphate (PS+TSP) (a) and calcium alginate gel with acidified sodium chlorite (alginate+ASC) (b) in the saline solution.

**Figure 6.6.** Concentration of antimicrobial agents in the saline
solution for pea starch gel with trisodium phosphate (PS+TSP) and calcium alginate gel with acidified sodium chlorite (alginate+ASC)
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix 1 Effect of antimicrobial treatments on surface pH of chilled drumettes after being inoculated with <em>E. coli</em> O157:H7 and stored at 4 °C.</td>
<td>162</td>
</tr>
<tr>
<td>Appendix 2 Surface pH of unchilled drumettes treated with commercial antimicrobials and stored at 7° C ≤ 5 days.</td>
<td>163</td>
</tr>
<tr>
<td>Appendix 3 Growth of naturally present pseudomonads and psychrotrophs on unchilled drumettes (38-40 °C) during storage at 7 °C for up to 120 h following dipping in 0.12 % ASC for 1 min. Means at the same incubation time with different letters are significantly (p &lt; 0.05) different.</td>
<td>164</td>
</tr>
<tr>
<td>Appendix 4 Growth of naturally present pseudomonads and psychrotrophs on unchilled drumettes (38-40 °C) during storage at 7 °C for up to 120 h following dipping in Safe2O for 1 min. Means at the same incubation time with different letters are significantly (p &lt; 0.05) different.</td>
<td>165</td>
</tr>
<tr>
<td>Appendix 5 Growth of naturally present pseudomonads and psychrotrophs on unchilled drumettes (38-40 °C) during storage at 7 °C for up to 120 h following dipping in Cecure for 1 min. Means at the same incubation time with different letters are significantly (p &lt; 0.05) different.</td>
<td>166</td>
</tr>
<tr>
<td>Appendix 6 Growth of naturally present pseudomonads and psychrotrophs on unchilled drumettes (38-40 °C) during storage at 7 °C for up to 120 h following dipping in Inspexx 100 for 1 min. Means at the same incubation time with different letters are significantly (p &lt; 0.05) different.</td>
<td>167</td>
</tr>
<tr>
<td>Appendix 7 Weight of drumettes (g) before and after coating with PS+TSP (3 %; w/v) and alginate+ASC (1%; w/v) during storage at 4 °C for 120h.</td>
<td>168</td>
</tr>
</tbody>
</table>
1. Introduction

The use of commercial antimicrobial agents in poultry processing plants has been recently implemented to replace traditional off-line reprocessing. Off-line reprocessing can involve the removal of physical contamination from the carcass usually by knife-trimming or with steam/hot water and vacuum. On-line processing includes carcass spraying with antimicrobial agents in the inside/ outside washer or in a separate cabinet before the final chilling (Sofos, 1998). Several investigations have shown that traditional off-line reprocessing is not more efficient than on-line reprocessing in reducing microbial loads (Kemp et al., 2000; Kemp et al., 2001; Bashor et al., 2004; Coppen et al., 1998). Off-line reprocessing, which included washing with chlorinated water, trimming and vacuuming, was ineffective in reducing Campylobacter and E. coli numbers more than 0.8 log cfu/ml and 0.5 log cfu/ml, respectively (Kemp et al., 2000; Kemp and Schneider, 2002). Furthermore, this method of reprocessing requires labor, time and processing tools, which increase processing costs.

The on-line method of reprocessing was shown to be more effective in reducing the microbial load on chicken by more than 2 logs without the need to remove the contaminated carcass from the processing line, which saves time and labor (Kemp et al., 2000; Kemp and Schneider, 2002). During on-line reprocessing, effective amounts of a commercial antimicrobial agent are sprayed or misted on chicken in a separate cabinet installed on-line following the inside/outside washer, which reduces the overall consumption of water in the carcass washer (Bashor et al., 2004). The advantages of on-line reprocessing reduce processing cost per carcass and yield carcasses with a lower microbial content. However, commercial antimicrobial agents should not be used as a
substitute for poor hygienic practices, but rather as an overall strategy to control unavoidable microbial contamination (Capita et al., 2002a).

The use of commercial antimicrobials is approved by the United States Food and Drug Administration (FDA) under the code of Federal Regulations (CFR) title 21, part 173 and part 182 as secondary direct food additives permitted in food for human consumption (FDA, 2003). The Food Safety and Inspection Service (FSIS) makes judgments regarding new substances to determine whether their application serves as a food processing aid or as a food ingredient based on the Food and Drug Administration’s definitions. If the substance is considered a processing aid, it does not need to be declared in the ingredient statement (Oyarzabal et al., 2004). Currently, the use of commercial antimicrobial agents used for on-line reprocessing are considered processing aids; thus, no labeling is required (Capita et al., 2002b). Table 1.1 contains a list of the most commonly used commercial antimicrobial agents, which can be used for on-line reprocessing to reduce bacterial numbers on raw carcasses (poultry, beef and pork). In the USA and Canada, commercial antimicrobials are commonly sprayed during poultry pre-chilling or post-chilling or are added directly to the chill tanks (Capita et al., 2002a; Oyarzabal 2005).
Table 1.1. List of the most commonly used commercial antimicrobial agents

<table>
<thead>
<tr>
<th>Number</th>
<th>Commercial name</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sanova</td>
<td>Acidified sodium chlorite</td>
</tr>
<tr>
<td>2</td>
<td>Cecure</td>
<td>Cetylpyridinium chloride and propylene glycol</td>
</tr>
<tr>
<td>3</td>
<td>Inspexx100</td>
<td>Peroxyacetic acid, hydrogen peroxide, octanoic acid and acetic acid</td>
</tr>
<tr>
<td>4</td>
<td>AvGard</td>
<td>Trisodium phosphate</td>
</tr>
<tr>
<td>5</td>
<td>Safe₂O</td>
<td>Lactic acid, propylene glycol, ethanol, sulfuric acid, calcium hydroxide and calcium sulfate dihydrate</td>
</tr>
</tbody>
</table>

Chicken skin consists of two layers: the upper layer (epidermis) and the lower layer (dermis) (Lucas and Stettenheim, 1972). The epidermis is divided into the *Stratum corneum* (cuticle) and *Stratum germinativum*. The cuticle of the epidermis consists of a waxy material that covers the skin surface, whereas the lower region is composed of a single layer of cells that can be differentiated and pushed toward the base of the *corneum* (the more superficial layer) (Lucas and Stettenheim, 1972). Scalding at high temperatures removes the cuticle layer from the skin, which affects the adhesive characteristics of the skin. Indeed, a thinner cuticle layer increases skin hydrophilicity and makes microbial contamination more likely because organisms may be deposited within the skin and its folds during the scalding process (Suderman and Cunningham, 1980). Thomas and McMeekin (1982, 1984) found that most (61%) *S. Typhimurium* cells get inside the skin when the chicken is immersed in contaminated water, which results in increasing the capillary-sized channels and water retention. Bacterial cells can penetrate the poultry skin by more than 150 μm. This penetration depends on the defeathering system used, which can change the skin topography and the amount of the residual *Stratum germinativum* layer (Kim et al., 1993). Furthermore, bacterial entrapment inside the skin increases with increasing exposure of the skin to the
contaminating microorganisms (Lillard 1986). As most bacterial cells reside pores the skin, traditional carcass washing with chlorinated water is ineffective in reducing bacterial contamination (Bashor et al., 2004). Removal of skin from the carcass before evisceration reduces the recovery of Campylobacter, E. coli, coliforms and total bacteria from the post-eviscerated broiler carcass outer surface (Berrang et al., 2002). This indicates the significant involvement of chicken skin in cell attachment and contamination.

Commercial antimicrobial agents have limited effectiveness when used to decontaminate poultry carcass (Coppen et al., 1998; Hwang and Beuchat, 1995; Schneider et al., 2002b), whereas they are able to cause noticeably higher reductions on pork, beef carcasses and biofilms and in broth systems (Lin and Chuang, 2001; Somers et al., 1994; Cutter et al., 2000; Kanellos and Burriel, 2005a). It can be concluded that factors specifically characteristic of chicken skin significantly contribute to greater bacterial resistance. These factors include bacterial attachment and physical entrapment inside the feather follicles. In addition, the presence of the waxy layer on the surface of the skin may also contribute by protecting the microorganisms inside the follicles and it may also interact with antimicrobial agents directly which may reduce their effectiveness (Capita et al., 2000a; Kim et al., 1994). Skin roughness may allow retention and absorption of large amounts of water during processing which could cause dilution of antimicrobial agents applied by spraying or dipping (Lucas and Stettenheim, 1972). An alternative approach to reduce surface contamination would be to stabilize active antimicrobial agents in edible coatings which could be sprayed on chicken carcasses during processing. These coatings could be designed to slowly release commercial antimicrobial agents
within desired time periods thus keeping the contaminating microorganisms in contact with the antimicrobial agent for a longer time than if it simply dripped from the carcass (Suppakul et al., 2003). One such approach involved synthesis of a coating from hydrophilic polymers which carried the antimicrobial agents and absorbed water on the carcass surface. This caused polymer swelling and was followed by release of the antimicrobial agents (Buonocore et al., 2004).

According to the FDA standards for “food additives”, treatments should not leave any chemical residue or have any physical effect on the product as it leaves the processing plant (FDA, 2004). Thus treatments should have a minimum effect on product quality if they are to be used during processing. A desirable functional coating should adhere well, be absorbed into the skin, dissolve within its components, disintegrate, drip off and disappear from the skin after its antimicrobial action is completed. The physical and mechanical properties of food coatings can have an influence on product quality, such as change its color, reduce fat oxidation, and influence the survival and type of predominant microorganisms during product storage (Rindlav-Westling et al., 1998; Krochta and De Mulder-Johnston, 1997). If the coating is made of a hydrocolloid polymer, physical and mechanical properties of the coating material will be affected by the relative humidity, since these materials quickly pick up moisture from the surrounding atmosphere (Gontard et al., 1996). Addition of antimicrobial agents to edible coatings could also affect their rheological properties, if these agents interact with the coating components (Ayranci and Tunc 2001). During development of new coatings to achieve optimum antimicrobial effectiveness and minimal physical changes in the chicken skin, the effect of the conditions used for coating application on antimicrobial
activity and interactions with skin should also be investigated. This thesis investigated the hypothesis that inclusion of antimicrobials in surface coatings will reduce viability of spoilage and pathogenic bacteria on the surface of chicken skin.

The objectives of this thesis are to:

1. Study physical and mechanical properties of two edible coatings as affected by the relative humidity and plasticizer.
2. Determine which of the most commonly used commercial antimicrobial agents is most effective against spoilage organisms and pathogens on raw chicken, and investigate whether efficacy can be improved by combining agents, and how bacterial attachment to poultry skin influences their antimicrobial action.
3. Investigate the effect of stabilizing TSP and ASC (the most effective antimicrobials) in edible polymeric coatings on antimicrobial efficiency and determine how much of the residual coating remains on the skin surface post-treatment. Examine coatings for interactions with poultry skin surfaces. Interactions will be monitored through measurements of skin-coating adhesion and coating absorptiveness into the skin.
4. Monitor dissolution of antimicrobial coatings and the release of antimicrobial agents using a chicken skin model. Investigate the effect of antimicrobial agent addition on rheological properties of the developed coatings.

2. Literature Review

2.1. Contamination of broiler carcasses with pathogenic and spoilage microorganisms
The first step for implementing on-line reprocessing in the poultry industry is to gain information about the incidence and number of pathogenic and spoilage microorganisms which most influence product safety and shelf-life. *Campylobacter* and *Salmonella* are the major foodborne pathogens on raw poultry. The incidence of *Campylobacter* and *Salmonella* are 2-100% and 4.2-26%, respectively, on retail fresh poultry carcasses. The frequency of contamination is affected by sampling location, sample type (e.g., skin homogenate vs. carcass rinse) and sampling time (year and season) (Waldroup, 1996; Jorgensen *et al.*, 2002). *Campylobacter* numbers on poultry are much higher than *Salmonella* numbers (10^2-10^7 cfu/bird and 1-10^2 cfu/bird, respectively) (Zhao *et al.*, 2001; Jorgensen *et al.*, 2002). Pathogens that are less commonly isolated from raw poultry include *L. monocytogenes* (incidence: 2-50% of carcasses), *Clostridium perfringens* (incidence: 10-80%, number: 10^1 cfu/cm²), *Shigella* (5-10% of carcasses) and *Staphylococcus* (incidence: 35-90%, number: ≥ 10^3 cfu/g) (Waldroup, 1996). *E. coli* O157:H7 is rarely found on raw poultry and an incidence of 1.5% was reported only in one study (Waldroup, 1996). Non-pathogenic *E. coli* were isolated from 38.7% of raw chicken (Zhao *et al.*, 2001). In a survey of pathogenic and spoilage bacteria present on raw meat in Washington, DC, 91% of the stores visited (59) had *Campylobacter*-contaminated chicken which represented 70.7% of chicken samples collected (184 samples). About one half (53.6%) of these isolates were *C. jejuni*, whereas *E. coli* and *Salmonella* were isolated at lower rates (38.7% and 4.2%, respectively) from the samples (Zhao, *et al.*, 2001). In the UK, the prevalence of *Campylobacter* and *Salmonella* on raw chicken at retail outlets was 83% and 25%, respectively and 89% of *Campylobacter* isolates were *C. jejuni* (Jorgensen *et al.*, 2002).
In North America, there are about 76 million cases of foodborne illness every year. This results in 325,000 hospitalizations and 5000 deaths (Mead et al., 1999). Poultry was implicated in the transmission of 3.4%-7.1% of outbreaks and in 1.4-5.9% of individual cases of foodborne diseases in North America between the years 1993 and 1997 (Olsen, 2000). Accordingly, there is an opportunity to reduce foodborne disease by reducing the prevalence of zoonotic pathogens in poultry.

2.2. Studies on antimicrobial activity of commercial antimicrobials on broiler carcasses

The use of commercial antimicrobials is a new practice developed with the emergence of on-line reprocessing as a part of the HACCP system to reduce or eliminate microbial contamination (Capita et al., 2002a).

2.2.1. Trisodium phosphate (TSP)

TSP is an orthophosphate salt of phosphoric acid. It has pH of approximately 10.0 in a 1.5% solution (Sampathkumar et al., 2003). Trisodium phosphate is also available as the dodecahydrate form with the formula (Coppen et al., 1998):

\[ \text{Na}_3\text{PO}_4.12\text{H}_2\text{O} \]

TSP is the most commonly used antimicrobial agent in poultry processing plants. In October 1992, the United State Department of Agriculture (USDA) added TSP to its list of approved substances for use during the broiler slaughter process (Capita et al., 2002a). Since then, a large number of investigations have been conducted to determine the effectiveness of TSP in decontaminating raw poultry, including whole carcass, chicken parts (legs, wings, and drumsticks) and on skin samples (Yang et al., 1998; Wang et al.,
1997; Capita et al., 2002b; Slavik et al., 1994). Thus far, TSP is the most effective commercial antimicrobial agent tested. It works well against a wide spectrum of microorganisms including *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, *E. coli* O157:H7, and the natural microflora (including *Pseudomonas*, total aerobic bacteria and *Enterobacteriaceae*) (Kim et al., 1994; Capita et al., 2000a, Coppen et al., 1998; Lillard 1994; Rio et al., 2006). Besides being a very effective antimicrobial agent, TSP also has a minor effect on sensory properties of poultry meat. Treated raw chicken thighs with 8 and 10% w/v TSP for 15 min were selected favorably; they had better color, smell and overall acceptance than the controls directly after treatment and after 7 days storage at 2 °C (Capita et al., 2000b). However, to be effective, TSP in the processing plant must be added at high concentrations (8-12% w/v), which results in raising the pH of the processing water to ≥ 12.5 (Bashor et al., 2004). This may lead to corrosion of industrial equipment and environmental problems, in addition to the high costs resulting from using the high concentrations of TSP (Capita et al., 2002a).

The exact mechanism by which TSP kills bacteria on poultry skin is not fully understood. However, several factors have been proposed to explain its bactericidal action (Capita et al., 2002a, Sampathkumar et al., 2003).

I. The high pH (about 12) of the TSP solution may affect cell membrane components (e.g., enzymatic inactivation).

II. The high ionic strength of the TSP solution can cause cell autolysis.

III. It can enhance detachment of bacteria from food surfaces by sequestration of metal ions.
IV. It has the ability to remove a thin layer of lipids from the carcass surface, which may open feather follicles and eliminate bacterial protection inside the skin folds and crevices.

Investigations have demonstrated that high pH (alkalinity) significantly contributes to bacterial inactivation, and in addition ion chelation by TSP contributes to its antimicrobial action. Cells of S. Enteritidis developed similar physiological and morphological changes when exposed to the same pH values of solutions prepared with TSP or NaOH. The loss of cell viability and membrane integrity, cellular leakage and release of lipopolysaccharides increased proportionally when the pH was increased from 10.0 to 11.0. In contrast, cells treated with different concentrations of TSP or NaOH where pH was adjusted to 7.0 did not show any significant changes (Sampathkumar et al., 2003). Gram positive bacteria interact differently with TSP than NaOH. L. monocytogenes inoculated on chicken legs were inhibited more by TSP than by NaOH at the same pH (pH 12.9-12.75), although NaOH maintained significantly higher chicken surface pH (Capita et al., 2002b). In another study, L. monocytogenes was inhibited more by TSP than NaOH at the same pH and a lower recovery was recorded in both rinse water solution and on the skin while Salmonella inhibition was not affected by the type of treatment (Hwang and Beuchat, 1995). The authors concluded that besides the high pH effect, TSP could be involved in several modes of action that could be different between gram negative and gram positive bacteria. TSP seems to attach to the outer membrane of the cell wall by disruption of the lipopolysaccharide layer of gram negative bacteria. The addition of MgCl₂ to the growth medium makes TSP unavailable to act on the outer membrane, which suggests possible complex formation between TSP and
MgCl₂. Thus, TSP could act as a chelator of the metal ions of the cellular membrane (Sampathkumar et al., 2003). Pre-exposure of C. jejuni, E. coli, Pseudomonas fluorescens and S. Enteritidis to TSP increased cell susceptibility to lysozyme (10 μg ml⁻¹) and/or nisin (1 μM), whereas this increase in sensitivity was less pronounced in the gram positive St. aureus (Carneiro de Melo et al., 1998).

The antimicrobial effectiveness of TSP in reducing the viability of gram negative and gram positive bacteria is well documented. However, the comparison between results is less conclusive because of the complex interaction of experimental parameters. The extent of microbial inhibition by TSP may not represent the actual loss of cell viability. For example, Salmonella recovery from TSP-treated carcasses was affected by the buffering capacity of the recovery medium (Lillard, 1994). Campylobacter recovery changed with the recovery procedure, the presence or absence of pre-enrichment (Slavik et al., 1994), and injury of Salmonella and Campylobacter cells was recorded (Chantarapanont, et al., 2004). In general, TSP at 10% (applied at room temperature by a 5-10 sec dip) reduced bacteria naturally present on chicken such as Campylobacter and Salmonella by about 2 logs. Similar results were obtained after inoculation of chicken with E. coli or other Enterobacteriaceae. However, it was less effective against gram positive bacteria (Whyte et al., 2001; Coppen et al., 1998). In a comparative study of the resistance of different bacterial species to TSP in sterile distilled water, E. coli O157:H7 was the most sensitive; 30 s treatment with 1% TSP was enough for complete elimination (10³-10⁶ cfu/cm²). S. Typhimurium and C. jejuni showed intermediate resistance while L. monocytogenes was the most resistant; 8% TSP for at least 10 min was required to reduce biofilm cells 1 log (Somers et al., 1994). Different extents of
lethality of TSP against different Salmonella strains were recorded. Among five strains tested, S. Enteritidis pt4 was the most resistant with 50% of the cells remaining viable after exposure to 12% TSP for 10 min at 37 °C. Other strains were reduced by > 60% within the same period by similar exposure to TSP (Kanellos and Burriel, 2005b).

2.2.1.1. Factors affecting antimicrobial activity of TSP on poultry.

2.2.1.1.1. Application temperature

Increasing the temperature of TSP from 10 to 50 °C increased reduction of Campylobacter on chicken by 0.16 to 1.5 log/ml, respectively, after 1 d storage at 4 °C (Slavik et al., 1994). In another study (Rodriguez-de-Lebesma et al., 1996), Salmonella incidence on chicken wings was reduced by 6.4 and 99.67% by TSP was sprayed at 10 and 95 °C, respectively. The higher temperature of the antimicrobial solution could be important in solubilizing the outer skin layer, because of its waxy nature, bringing TSP into direct contact with the contaminating microorganisms. Therefore, it was proposed that TSP could be more effective in scalding (50 °C) than in chiller (4 °C) water (Slavik et al., 1994). L. monocytogenes, is however more resistant than Salmonella and E. coli to TSP than at low temperatures (<10 °C). Kim et al. (1994) found that Salmonella could be reduced by 1.2 and 1.6 log cfu/ml following treatment with TSP at 10 and 50 °C, respectively. Carneiro de Melo et al. (1998) found that L. monocytogenes in cell suspension was not significantly reduced by ≤ 50 mM TSP at 4 °C but was reduced by 96% when exposed to TSP at 37 °C. In contrast, E. coli was reduced more at the lower temperature (4 °C). Planktonic and attached cells of S. Typhimurium were also more sensitive to TSP at low temperatures (10 °C) than at room temperature. However, L. monocytogenes was more resistant to TSP at 10 °C (Somers et al., 1994). The
psychrotrophic nature of *L. monocytogenes* could account for its higher resistance to stresses at low temperature.

2.2.1.2. *TSP concentration and duration of application*

In general, increasing TSP concentration and application time increased its antimicrobial effectiveness. The effect of TSP concentration was more observable on *L. monocytogenes* than *S.* Typhimurium. Increasing TSP concentration from 5 to 10 % for 30 sec did not significantly increase the inhibition of *S.* Typhimurium (0.1 log), whereas increasing TSP concentration from 8 to 10% for 15 min resulted in significant inhibition of *L. monocytogenes* (reductions increased from 0.4 to 0.8 log, respectively) (Capita *et al*., 2001, 2002b; Xiong *et al*., 1998b). Furthermore, increasing the concentration of TSP to 12 % resulted in significantly greater inhibition of *L. monocytogenes* than when treated with 10% TSP (Capita *et al*., 2001). TSP at low concentration (1%) reduced *Salmonella, L. monocytogenes* and psychrophils by 1.3,1.0 and 1.1 log cfu/cm², respectively. However, longer (30 min) dipping time was required (Hwang and Beuchat, 1995). The effect of treatment duration was investigated by Mehyar *et al.* (2005). The latter found that dipping chicken drumettes in 10% TSP for 1 or 20 min reduced *E. coli* O157:H7 by 0.63 or 1.6 log cfu/g, respectively, directly after the treatment. Increasing the dipping time to more than 20 min or increasing TSP concentration showed no further reduction (Mehyar *et al*., 2005). The limited effectiveness of TSP found could be related to the fact that the majority of the microorganisms are protected inside the skin from the outside treatments (Thomas and McMeekin, 1984). In summary, the higher sensitivity of gram negative bacteria resulted in almost a complete inhibition at 5 % TSP for 15 min and no further inhibition occurred by increasing the concentration or treatment time.
Gram positive bacteria were more resistant and complete inhibition was not achieved under severe conditions of treatment time (15 min) and concentration (12 % TSP).

2.2.1.1.3. Method of application

TSP efficacy was tested on chicken by dipping the whole carcass (Lillard, 1994; Coppen et al., 1998) or spraying the whole carcass (Kemp et al., 2001; Kim et al., 1994) or the excised skin (Chantarapanont et al., 2004). Dipping (6 sec) with 10% TSP was more effective against Enterobacteriaceae and Pseudomonas than a 1L spray/carass for 10 sec or 2L spray/carass for 20 sec (Ellerbroek et al., 1998). The difference in effectiveness between dip and spray applications was also recorded with S. Typhimurium (Xiong et al., 1998b). The conclusion was that dipping induced better contact between the antimicrobial agent and the contaminating microorganisms, while spraying could generate a foam, which prevented direct contact with the target microorganisms. Increasing the spray pressure from 620.5 to 1034.2 KPa significantly increased the reduction from 1.6 to 2.3 log cfu/ml of S. Typhimurium inoculated on the skin of pre-chilled chicken carcasses (Wang et al., 1997). In another study Xiong et al. (1998a) found that the effectiveness of TSP did not increase against S. Typhimurium by increasing spraying pressure from 207 to 1034 KPa. In the former study, skin samples from unspecified sites were used; whereas, in the later study, breast skin samples were used. The high spray pressure may help in providing accessibility of the antimicrobial agents to bacterial cells inside the skin pores for samples with lower roughness, such as in the breast area, whereas it may push the cells inside the skin in the regions with higher roughness such as the leg and dorsal area (Capita et al., 2002c; Lucas and Stettenheim, 1972).
2.2.1.1.4. Skin topography/sample type

Reductions of *L. monocytogenes* inoculated on chicken were significantly greater on breast skin than on dorsal and leg areas when treated with 8-12%TSP and during storage at 2 °C for 5 days (Capita *et al.*, 2002c). No significant difference in surface pH of skin samples from the different areas was recorded. The authors concluded that *L. monocytogenes* might be attached more extensively and be more resistant on leg and dorsal skin than on breast skin as a result of differences in skin topography and specific physiochemical properties including exposed amino acid residues, surface proteins, lipids and surface energy (Kim *et al.*, 1996). Furthermore, cells may be prone to lower levels of entrapment in the breast area than on the leg and dorsal areas because of the larger featherless spaces in the former than in the later regions (Lucas and Stettenheim, 1972). In a related investigation, TSP was found to be more effective on chicken parts than on the whole carcass and was more effective on excised skin than on unexcised skin (Capita, *et al.*, 2000a, 2003). It was concluded that TSP could be physically more accessible to bacterial cells on chicken parts and excised skin than the whole carcass. An *in vitro* study showed that increasing substratum roughness of artificially established crevices on stainless steel plates increased the resistance of *S. enteritidis* in biofilms to TSP (Korber *et al.*, 1997).

TSP effectiveness against spoilage and natural flora of raw chicken was investigated in a number of studies. Psychrotrophs were reduced by only 1.1 log upon treatment of chicken with 1% TSP for 30 min. (Hwang and Beuchat, 1995) and 1.7 log by 15 sec dipping in 10% TSP (Whyte *et al.*, 2001). Total aerobic bacteria were reduced by 0.9 or
0.8 log/carcass by a 15 sec or 15 min. dip in 10% TSP, respectively (Yang, et al., 1998; Whyte et al., 2001; Coppen et al., 1998). E. coli and Enterobacteriaceae were reduced 1.9 and 2.17 log cfu/g, respectively by 10%TSP for 15 sec (Whyte et al., 2001). The significant suppression of natural psychotrophs and Pseudomonas by TSP treatment increased the shelf-life of chicken by 2.5 days (Mehyar et al., 2005). Some authors claimed that treatment with TSP, although reducing the number of gram negative bacteria, may increase the chance for the growth of gram positive psychrotrophic pathogens such as L. monocytogenes during refrigerated storage (Rio et al., 2006).

Others have claimed that growth of Brochothrix thermosphacta is greater than L. monocytogenes at refrigerated temperatures, and thus spoilage will probably occur prior to growth of pathogenic bacteria (Salvat et al., 1997).

2.2.2. Acidified Sodium chlorite (ASC)

The mode of ASC action is by generation of oxychlorous intermediates that act as broad-spectrum germicides by their oxidizing action at the microbial cell wall, attacking the sulfide and disulfide linkage of proteins and by non-specific attack on the amino acids of the cell membrane (Kemp et al., 2000). Commercial ASC consists of 1100 ppm sodium chlorite and 900 ppm citric acid. Citric acid acts as a proton donor for acidification of sodium chlorite and its transformation to hypochlorous acid (HOCl). This “active chlorine” is the antimicrobially active form (Kemp et al., 2000). The activity of ASC is pH dependent; at pH 4.0 to 6.0, sodium chlorite hydrolyses completely to HOCl, at which point ASC has the highest effectiveness. As pH increases above 6.0, a portion of the HOCl is converted to hypochlorite ions (OCl⁻), which have lower activity. At a pH greater than 9.0, all the HOCL is converted to OCl⁻ and no
antimicrobial activity occurs (Bashor et al., 2004). Sanova, the commercial preparation of ASC, has a pH of 2.5 and is applied in on-line reprocessing using a spray chamber (Mermelstein, 1998). At this pH, complete hydrolysis of ASC to hypochlorous acid is expected. Goncalves et al. (2005) found that L. monocytogenes on breast meat was inhibited to the same extent at the same level of active chlorine produced from two commercial preparations; calcium hypochlorite and a stabilized form, trichloroisocyanuric acid.

ASC has comparable antimicrobial activity to TSP; ASC at 1200 ppm reduced Campylobacter on broiler carcasses by 2.12 log cfu/cm² when used for on-line reprocessing (Kemp and Schneider, 2002). The only adverse effect of ASC is that it may cause a transient mild whitening of the skin surface of carcasses treated by dipping rather than by spraying (Schneider et al., 2002a). Subsequent tests in commercial settings have shown that this mild color change is lost during hydro-cooling and does not result in any organoleptic changes in raw or cooked poultry products (Schneider et al., 2002b).

The performance of ASC under conditions similar to those used in commercial poultry facilities is affected by a number of parameters. ASC was most effective against total aerobes, E. coli and total coliforms when a pre-washing treatment for 5 sec was used prior to ASC exposure. Dipping was more effective than spraying, and activation by phosphoric acid and citric acid were equally effective (Kemp et al., 2000). Concentrations of ASC between 500 and 850 ppm were equally effective against total coliforms and E. coli, but higher concentrations (1200 ppm) were required for
significant reductions of aerobes (Kemp et al., 2000). Post-chill application of ASC was more effective than when applied before chilling. The combined used of chlorinated (50 ppm) water and ASC on pre-chilled and post-chilled carcasses reduced *Campylobacter* 1.2 log cfu/ml and 1.75 log cfu/ml, respectively, during on-line spray (15 sec) application monitored by the whole carcass rinse procedure (Kemp et al., 2000; Oryazabal et al., 2004). *E. coli* was reduced to below the detection limits (0.01 cfu/ml) with the post chill procedure (Oryazabal et al., 2004). This increase in effectiveness was associated with dual stress imposed by the low temperature and ASC treatment on the microbial cells (Oyarzabal et al., 2004).

ASC was more effective in on-line reprocessing against *E. coli* and *Salmonella* than off-line reprocessing (Kemp et al., 2001). *E. coli* and *Campylobacter* numbers post off-line reprocessing were reduced to 0.59 and 1.14 log cfu/ml, respectively, compared to 2.37 and 2.89 log cfu/ml after on-line reprocessing. The on-line reprocessing resulted in the majority (94.1%) of the treated samples to be acceptable (< 100 cfu/ml) by the USDA performance standard. The zero fecal *E. coli* tolerance level that requires condemnation of carcasses that have visible signs of fecal contamination was met by all but two carcasses that underwent the continuous on-line reprocessing (Kemp et al., 2001). With regard to spoilage flora, ASC did not reduce the total aerobic plate count, total coliform count and *E. coli* numbers by more than 1 log cfu/ml during on-line reprocessing (Schneider et al., 2002b). In comparing ASC with other antimicrobials, ASC and peroxycetic acid (PAA) were equally effective against *C. jejuni* inoculated on breast skin (Chantarapanont et al., 2004). Each sanitizer resulted in about a 1 log reduction when applied at 100 ppm for 15 min and at 40 ppm for 2 min as a dip, and were
regarded as being slightly effective at those low concentrations. Application of 12% TSP or 1200 ppm ASC after the inside/outside carcass washer reduced *Campylobacter* equally (about 1.5 log cfu/ml) on carcasses in two different processing plants (Bashor *et al.*, 2004). In conclusion, ASC has antimicrobial activity which is comparable with other antimicrobial agents and its activity is affected by processing parameters such as pH, concentration, pre-washing and processing step at application. In contrast with other antimicrobials, the antimicrobial activity of ASC increases at low pH.

2.2.3. *Cetylpyridinium chloride (CPC)*

CPC is approved for use as a mouthwash and has a neutral pH (Cutter *et al.*, 2000). CPC is an amphiphilic molecule which can interact strongly with negatively (acidic groups) charged surfaces of bacteria to form weakly ionized compounds that subsequently inhibit bacterial metabolism (Kim and Slavik, 1996). CPC does not cause carcass bloating or skin discoloration, and it is non-corrosive (Xiong, *et al.*, 1998b). CPC (1%) was very effective on lean beef tissue, where *E. coli* O157:H7 and *S. Typhimurium* were reduced by 5 to 6 log cfu/cm². However, it had limited effectiveness on adipose tissue where 1.3-2.5 log cfu/cm² reductions were obtained (Cutter *et al.*, 2000). CPC was less effective on chicken skin. A 0.1% CPC spray reduced *S. Typhimurium* by 0.9 to 1.7 log units at 15 °C and 50 °C, respectively (Kim and Slavik, 1996). CPC was very effective in preventing cross-contamination. *S. Typhimurium* attachment to chicken skin was reduced by 4.9 log with 8 mg/ml CPC treatment for 10 min. (Breen *et al.*, 1997). CPC has lower activity at high temperature, especially when used with a high-pressure spray (Xiong, *et al.*, 1998b). Reduction in activity was believed due to foam formation under these conditions, which prevented direct contact with the contaminating microorganisms.
(Wang et al., 1997). Consistent antimicrobial activity of CPC on chicken carcasses has not been achieved. Xiong et al. (1998b) found that CPC spraying caused comparable reductions with TSP and lactic acid (LA) under the same processing conditions; spraying temperature, pressure, and exposure time. Similarly, 10 % TSP and 0.1 % CPC reduced S. Typhimurium on inoculated chicken skin by 1.5 to 2.3 log/38.5 cm² and 1.6 and 2.5 log/ 38.5 cm², respectively, after spraying for 30 sec (Wang, et al., 1997). Mehyar et al. (2005) found that CPC up to 0.5 % (w/v) did not reduce a Salmonella cocktail or C. jejuni on chicken drumettes. It was found that TSP and ASC were significantly more effective. Differences in strains of microorganisms and methods of application (spraying vs. dipping) may have contributed to the differences in the results from the different studies. Spraying at low pressure and application at an early step in processing would be best to achieve optimum the antimicrobial effectiveness of CPC on poultry carcasses.

2.3. Stabilization of antimicrobial agents in films and coatings

The increased demand for microbiologically safer food, greater convenience, smaller packages and longer product shelf-life has stimulated the food industry to develop new food preservation technology (Cagri et al., 2004). In foods, most contaminating microorganisms reside on the food surface, especially those that occur after processing. The theory behind effectiveness of antimicrobial packaging is based on stabilizing antimicrobial agents in compatible packaging materials. This allows a slow migration of the antimicrobial agent from the packaging material when it comes into contact with the food, thus maintaining adequate antimicrobial agent concentrations for a prolonged period at the food surface (Han, 2003; Han and Floros, 2000a). For effective action,
intimate contact between the food product and packaging material is required; therefore, vacuum-packaged products were proposed as good food applications for antimicrobial films (Vermeiren et al., 2002). Several films and coatings have shown promise as effective carriers for a wide range of antimicrobial agents and the former include starch, alginate, carrageenan, cellulose, WPI and collagen (Gennadios et al., 1997). Antimicrobial agents that have been successfully incorporated into edible and non-edible films and coatings include benzoates, propionates, sorbates, acetic acid, lactic acid, sodium chloride, sodium nitrite, bacteriocins, natural preservatives (such as essential oils), and liquid smoke (Gennadios et al., 1997; Guilbert et al., 1996; Cagri et al., 2004). Most commercial antimicrobials in solutions have limited effectiveness when used on the poultry processing line to reduce pathogens to below acceptable levels (Fries, 2002; Keener et al., 2004). Furthermore, these formulations lose their activity after prolonged storage as a result of interactions with skin components, the effects of the buffering capacity of the meat and metabolites produced by the contaminating bacteria (Capita et al., 2002a). Line speed and the dilution effect of water residues on the skin surface can contribute to the reduced effectiveness of antimicrobials applied in solutions (Oyarzabal et al., 2004).

2.3.1. Effect of immobilization on antimicrobial activity

Immobilization of antimicrobial agents in films and coatings enhances antimicrobial activity. Immobilized lactic and acetic acids in calcium alginate had a greater inhibitory effect against L. monocytogenes, S. Typhimurium and E. coli O157:H7 inoculated on lean beef tissue during storage at 5 °C for 7 days than when not immobilized (Siragusa and Dickson, 1992; 1993). Studies on immobilized nisin showed that it retained more
activity against *L. monocytogenes* when added to corn zein film applied to the surface of ready-to-eat chicken (Janes *et al.*, 2002). Natrajan and Sheldon (2000b) found that nisin at 300 and 500 µg/ml in calcium alginate was significantly ($P \leq 0.05$) more inhibitory against *S. Typhimurium* on raw chicken as compared to treatments with nisin alone. The degree of cross-linking between the polymer molecules, which depend on polymer concentration, was shown to affect surface migration of antimicrobial agents. *Salmonella* was inhibited more (4.6 log cycles) by a 0.7% agar gel that contained 500 µg/ml nisin than by a 1.25% agar gel (1.8 log cycle) on raw chicken. Results suggest that the higher effectiveness at lower agar concentration could be the result of a more open network allowing for greater migration of nisin.

2.3.2. Effect of food constituents on antimicrobial activity

Antimicrobial packaging systems have been shown to be effective *in vitro* but often exhibit reduced effectiveness when used on edible foods. This could be due to interactions between food constituents, in particular adipose tissue and other active substances like proteins which exhibit buffering capacity, and microbial metabolites. Low density polyethylene (LDPE) films containing 1.0% w/w trichlosan exhibited strong antimicrobial effects *in vitro* in simulated vacuum-packaging against *L. monocytogenes*. However, trichlosan did not effectively reduce spoilage bacteria or *L. monocytogenes* when inoculated on vacuum packaged chicken breasts stored at 7 °C (Vermeiren *et al.*, 2002). In other work, acetic acid diffused from chitosan films more rapidly than propionic acid in an aqueous medium, but diffused to a lesser extent than propionic acid and had a lower activity when these films were in contact with vacuum packaged meat (Ouattara *et al.*, 2000a,b). Incorporating lauric acid in the formulation
significantly enhanced the retention of acetic acid within the films, but it did not improve the film antimicrobial activity (Ouattara et al., 2000a). The authors concluded that release that leads to antimicrobial activity is a complex phenomenon and is the net result of osmosis and interactions between acid and polymer molecules, which may include structural changes in the polymer induced by the acids present. Accordingly, the use of an antimicrobial mixture should be optimally formulated to reduce these interactions and as well, take into account the type of target microorganisms.

2.3.3. Structural compatibility of the carrier and the antimicrobial agent

The structural compatibility between the antimicrobial agent and the carrier material is one of the features that are most investigated and used to explain the usefulness or unsuitability of the treatment. Non-polar polymers are usually compatible with those of high molecular weight with low polarity. Furthermore, molecular weight, ionic charge and solubility of different additives in the polymer as well as food structure can affect the rate of additive diffusion (Han and Floros 2000a; Suppakul et al., 2003). Compounds with good chemical compatibility should be able to maintain threshold antimicrobial concentrations during contact with targeted microorganisms to allow for adequate antimicrobial action. Structural incompatibility between the polar compound sorbic acid and the non-polar ethylene vinyl alcohol(EVA)/linear low density polyethylene (LLDPE) led to insufficient release (< 7%, after 21 days) of sorbic acid from films when placed in distilled water, buffered water (pH 3.8-5.8) or on cheese cubes. These films were ineffective in suppressing growth of spoilage microorganisms on cheese (Devlieghere et al., 2000). However, the low concentration of sorbate present in an undissociated form at the pH of the film (5.8) may have contributed to the low
antimicrobial activity (Devlieghere et al., 2000). In a related study, sorbic acid retained in a whey protein isolate showed more activity in acidic (fermented sausage) meat against *L. monocytogenes* than meats with higher pH, such as bologna and hot dogs. Antimicrobial films with sorbic acid on high pH meat (pH ≤ 6.2) may have reduced activity because sorbic acid becomes dissociated at those pH values (Cagri et al., 2002, 2003). A more hydrophobic LLDPE film containing nisin, which is hydrophilic, had greater activity against *S. Typhimurium* on inoculated chicken drumsticks than the slightly hydrophobic polyvinyl chloride (PVC) and nylon films containing nisin. This was believed due to the repulsion of aqueous nisin by the LLDPE film to a greater degree, resulting in more localized inactivation of *Salmonella* on the surface of chicken drumsticks (Natrajan and Sheldon, 2000a). Film development based on the chemical compatibility of the added agents and the films themselves is a technology used to increase antimicrobial activity of modified films. When polyethylene-co-methacrylic acid (PEMA) film containing sorbic acid was pretreated with NaOH the release of sorbic acid was greater than when the film was treated with hydrochloric acid. The film was found primarily to have antifungal activity, presumably as a result of an increase in film polarity, which facilitated the release of sorbic acid (Weng et al., 1999). In the design of films as carriers for antimicrobials, a desirable carrier should maintain release of the active ingredient at an effective concentration for period of time that matches the growth profile of target microorganisms.

2.3.4. Other factors
Other factors that affect migration of active agents from the carrier to food are storage temperature and sample/film moisture content (Han et al., 2000b; Suppakul et al., 2003). According to the Arrhenius equation (Han and Floros 1998) that correlates diffusion (LnD) with the temperature (1/T), increasing the storage temperature increases diffusion of the antimicrobial agent. It was shown that the migration of β-lactoglobulin and bovine serum albumin from a hydrogel increased with increasing temperature up to 37 °C and no further increase was recorded beyond 37 °C, perhaps because of shrinking of the hydrogel at the higher temperature (Han and Floros, 1998). Diffusion of benzoic acid and sorbic acid from methyl cellulose films to an aqueous solution decreased from 60 % to 40 % by reducing the temperature from 25 °C to 4 °C, respectively. This was attributed to the lower molecular interactions and higher molecular mobility at the high temperature (Chen et al., 1996). The moisture content between the carrier and the food surface should not lead to excessive leaching of the active agents, particularly those which are water soluble (Saltzman, 2001). The drier surface of bologna compared to ham and pastrami was correlated with reduced release of sorbic acid and higher antimicrobial efficacy of sorbic acid on bologna was reported (Ouattara et al., 2000a). This may have been related to lower water activity (a_w) on the bologna surface. On the other hand, wet synthetic films (PVC, Nylon and LLDPE) were more effective in suppression of S. Typhimurium survival on chicken and extended its shelf-life by 0.6 to 2.2 days over the dry films at 4 °C as a result of increased antimicrobial migration from the wet films (Natrajan and Sheldon, 2000a).

2.4. Controlled release systems
Controlled release systems are designed to prolong or sustain antimicrobial concentration above the lethal dose for the target microorganisms. This can be achieved using slow release technology (Brannon-Peppas, 1993). Effective antimicrobial packaging should be designed in a way that the release matches the microbial growth rate. If the migration rate is too slow, the system will lose its antimicrobial activity because the concentration of the antimicrobial agent will be maintained below the minimum inhibitory concentration (MIC). Alternatively, if the release rate is very fast and does not coincide with rapid microbial growth, the antimicrobial agent may be depleted before active microbial growth begins, leaving the packaged material unprotected from microorganisms (Han, 2003).

Controlled release systems can be divided into two groups, diffusion systems and degradable delivery (erosion) systems.

2.4.1. Diffusion systems

2.4.1.1. Reservoir

In the reservoir system, a liquid reservoir of the active ingredient is enclosed in a polymer membrane which can provide controlled release of antimicrobial molecules (Staniforth and Baichwal, 1993; Brannon-Peppas, 1993). The release of the active agents from the reservoir into the external environment occurs in three steps (Saltzman, 2001).

1. Dissolution of the agent in the polymer.

2. Diffusion of the agent across the polymer membrane.

3. Dissolution of the agent into the external phase.
Therefore, the rate of release is controlled by the concentration difference across the reservoir wall, the wall thickness and its permeability (Saltzman, 2001).

2.4.1.2. Matrix

In the matrix system, the active ingredients are dissolved or dispersed throughout a solid polymer phase. The active ingredient is released by diffusion through small pores created by dissolution of the surface ingredients (Staniforth and Baichwal, 1993). The polymeric materials are usually the same as those used for the rate-limiting membrane in reservoir devices (Saltzman, 2001). Hydrophobic polymers are usually used with an internalized hydrophilic material for release in this system. When the matrix is placed in an aqueous environment, the water-soluble molecules diffuse at the rate determined by the polymer concentration and the degree of binding between the carrier and the active ingredient (Han and Floros, 2000a). The release of the active agent creates small pores in the polymer, which results in its slow migration and dissolution into the outer solution. Therefore, the system is called a solvent-activated system (Pitt, 1980).

2.4.1.3. Hydrogel delivery systems

A hydrogel is created by placing dehydrated polymers in water. Polymers absorb the water quickly, swell and undergo dimensional changes that usually reduce intermolecular spaces (Staniforth and Baichwal, 1993). In hydrogels the rate of active ingredient diffusion though the bulk material depends on the extent of cross-linking between polymer chains that determine intermolecular spaces (Han et al., 2000c). Besides polymer cross-linking, multilayer structures have been successfully used to control the delivery of agents from hydrogels (Buonocore et al., 2004). The active
ingredient could be either water soluble or insoluble. Nonetheless, in both systems water acts as a transporter for the active ingredients, leaching them from the polymer matrix (Han et al., 2000b). The release of the active ingredient from the swollen hydrogel depends on the intermolecular separation between polymers and the size of the diffusing agent (Han et al., 2000b). For efficient release, the molecular size of the agent should be smaller than the interchain space (Han et al., 2000b; Buonocore et al., 2004). Logical compromises between the degree of cross-linking and molecular size of the active ingredient should be established based on the type and number of target microorganisms and time needed for their elimination (Zastre, 1997).

2.4.2. Degradable delivery systems

2.4.2.1. Erosion

In this system, the supporting matrix is made of biodegradable polymers that will dissolve with or after the active ingredient is released such that no residual material remains in the treated tissue (Saltzman, 2001). Erosion systems consisting of pregelatinized starch digested by enzymatic hydrolysis have been reported (Witt and Sauter, 1996). During degradation, the solid material slowly loses mass by dissolution, decreasing in weight and surface area, and eventually disappears (Xin et al., 2002; Dimantov et al., 2004). Water-soluble polymer material could be designed with a degree of hydrophobicity and desirable morphology to allow only a certain level of water penetration with a pre-determined concentration of active ingredient released (Xin et al., 2002; Saltzman, 2001). The disappearance of the polymer could be either by surface erosion, where the degradation begins from the matrix surface, or by bulk erosion, where the degradation is uniform throughout the matrix. The material eventually becomes
spongy. The erosion creates holes inside the matrix, which become larger until the matrix is no longer mechanically stable (Saltzman, 2001). Most materials are designed to be degraded by a combination of the two processes (surface and bulk erosion).

Polymer erosion occurs in an oscillatory fashion when placed in distilled water (Makino et al., 1996). This process starts with swelling of the polymer layer that is in contact with the erosion solution and leads to its erosion. Sequentially the next layer begins to swell and is eroded during the swelling of the third layer. This process continues until all the polymer molecules are liberated from the hydrogel matrix (Makino et al., 1996). Before and while the polymer matrix is degraded, the release of incorporated active agent follows accepted diffusion law. Most erosion systems, therefore, have two release mechanisms, erosion and diffusion, but because erosion occurs in steps, these systems yield more rapid release kinetics than reported from simple diffusion systems.

2.5. Molecular characteristics of selected hydrocolloids

2.5.1. Alginate

Alginites are salts of alginic acid, which is an unbranched binary copolymer of (1-4)-linked β-D-manuronic acid (M) units, poly-α-L-glucopyranosyluronic acid (G) units and their combinations (Nisperos-Carriedo, 1994). Calcium ions react with the dissolved alginate salts to form gels of calcium alginate, which are insoluble (Rhim, 2004). At a pH less than 5, the residual calcium becomes more charged and increases its intermolecular interactions. Increased protonation of the carboxyl groups of alginate at low pH also increases solution viscosity and gelation may occur as a result of reduced
electrostatic repulsion between the molecules (King, 1982). At relatively higher pH (>5.5), a greater concentration of calcium is needed for gelation (King, 1982).

2.5.2. Pea starch

Starch granules consist of two distinct polymers, amylose and amylopectin. Amylose is a linear polymer composed of (1-4)-α-D-glucopyranosyl monomers. Amylopectin contains an amylose backbone with linked D-glucopyranosyl side units (Nisperos-Carriedo, 1994). Amylopectin has a larger molecule size than amylose and it is highly branched. Raw starch contains 18-30% amylose and 70-82% amylopectin (Parker and Ring, 2001, Elliasson, 2004). For stronger gel applications, high amylose starches are usually used. This starch is difficult to disperse in water and requires a high temperature (ca 133 °C) for gelation (Ratnayake et al., 2002). Pea starch granules are insoluble in water due to the presence of H-bonding between the polymer chains. Upon heating at 95 °C, the granules gradually swell and absorb water, which increases the polymer water solubility (Ratnayake et al., 2002). At a very high pH (>10), alkaline degradation of pea starch polymers occurs, reducing its viscosity and increasing its solubility (BeMiller, 1965).

2.6. Rheological considerations of the gel-sol phase transition

The transition from the solid to liquid phase in food materials occurs as a result of an increase in molecular mobility. At elevated temperature solubilization occurs following phase transition (Roos, 1995). Water acts as a plasticizer with compatible polymers that makes the system softer presumably by increasing the molecular mobility (Gontard et al.,
During the sol-gel transition or during gel formation, more and more chemical bonds are added to the system which reduce molecular motion and the material is eventually transformed to a gel phase. At the gelation point, the relaxation time (the time for the polymer to relax in response to an imposed stress) becomes infinitely large in response to formation of large molecular clusters (Winter and Mours, 1997). The gelation point can be defined as the instant at which molecular cross-linking occurs as the average molecular weight of the polymer increases to infinity and the number of potential cross-linking points becomes saturated. This is expressed by the formula \(1/(f-1)\), where \(f\) is the potential number of cross-linking points per chain (Ross-Murphy, 1995; Winter and Mours, 1997). Physical gels are reversible and have hydrophobic, hydrogen and ionic interactions involved in the network structure. In these gels, the interactions have infinite lifetime but easily open and close in response to environmental changes (temperature, pressure and pH) (Ross-Murphy, 1995; Winter and Mours, 1997).

Gel strength is measured by the shear modulus, \(G'\), which is proportional to the number of the elastic network chains (Ross-Murphy, 1995). Storage modulus \(G'\) represents the amount of energy stored in the network structure (Rector et al., 1989) while the loss modulus, \(G''\), reflects the energy dissipated in the system and is believed to be associated with the relaxation of the dangling chain ends (Ross-Murphy, 1995). Therefore, when the \(G'\) of a system is lower than \(G''\), this indicates that a fluid state exists. Rector et al. (1989) investigated the conditions for gel reversibility of whey protein isolate at different pH and protein concentrations. They found that network formation by molecular aggregation and bond breaking was a reflection of the net molecular charge.
(pH effect) and protein concentration. The network properties at different conditions were monitored by measuring rheological parameters. Changes in $G'$ during gelation of low methoxyl (LM) pectin as a function of the sucrose level added and protein concentration were used to estimate the average molecular weight of the chain between cross-links, $M_c$ (Fu and Rao 2001). Increasing the sucrose concentration reduced $M_c$. The latter workers concluded that the OH group of the sugar may be involved in stabilizing the structure of junction zones by promoting H-bonding. The effect of sorbitol addition to LM pectin on the structure loss rate ($-d G'/dt$) during the melting process of LM pectin was studied by Fu and Rao (1999). Results showed that sorbitol addition increased the rate of structure loss and this could have been the result of sorbitol competition with LM pectin for calcium in the formation of complexes (Fu and Rao 1999). Similarly, melting of margarine and cheese were monitored by $G'$ changes at different product soluble solids (Borwankar et al., 1992; Mounsey O’Riordan 1999).
CHAPTER 3

Physical and mechanical properties of high-amylose rice and pea starch films as affected by relative humidity and plasticizer
3.1. Abstract

The tensile properties, water vapor and oxygen permeabilities at different relative humidity (RH) as well as the water solubility of edible films made from high-amylose rice starch (RS) or pea starch (PS) were measured and compared to the most commonly used edible films. Photomicrography of starch films showed amylopectin-rich gels containing amylose-rich granules. The addition of glycerol to starch films caused amylose-rich granules to become swollen and these were continuously dispersed among the amylopectin-rich gels. Tensile strength of RS and PS films decreased when the RH increased from 51% to 90%, while elongation-at-break (E) of both films increased when the RH increased. Water vapor permeabilities of both films were similar with observations of 130 – 150 g mm m⁻² d⁻¹ kPa⁻¹. Oxygen permeability of RS and PS films were very low (< 0.5 cm³ μm m⁻² d⁻¹ kPa⁻¹) at RH values of < 40% RH, and were 1.2 – 1.4 cm³ μm m⁻² d⁻¹ kPa⁻¹ at 45% RH. The water solubility of PS films at 32.0% was lower than the 44.4% of RS films. Overall, high-amylose rice and pea starch films possessed excellent oxygen barrier properties with extremely high stretchability.

Key Words: Starch film, Edible film, Oxygen permeability, Water vapor permeability, Tensile property
3.2. Introduction

The interest in biodegradable and edible packaging films and coatings is increasing because they consist of natural materials, which do not contribute to environmental pollution (Webb-Jenkins, 2002; Ahvenainen et al., 1997). These films and coatings may be applied on a food or between heterogeneous food components to prevent mass transfer phenomena that could cause deterioration of food quality (Guilbert 2000). Since these materials are both packaging and food components, they should have properties that fulfill the requirements of both packages and foods. Edible packaging should be tasteless, free of toxic substances, serve as a barrier to intrusion of water, gasses and aroma, and have mechanical properties that allow efficient handling of the food products (Krochta and De Mulder-Johnson, 1997). These packaging materials can be used as a carrier or encapsulating material for functional ingredients such as antioxidants, antimicrobial agents, pigments or nutritional substances contributing, respectively, to the microbial and chemical stability as well as the nutritional quality of packaged foods (Krochta and De Mulder-Johnson, 1997; Natrajan and Sheldon, 2000a).

Protein-based films and coatings such as those based on pea, soy and whey protein have stimulated increased interest in recent years (Krochta 2002; Choi and Han 2001; Choi and Han 2002). These films generally have good oxygen barrier properties, are strong and preserve nutritional quality which allows them to either be substituted for synthetic packaging materials or be used to simplify total packaging requirements (Choi and Han 2001; Mujica-Paz and Gontard, 1997; Gontard et al., 1996). Disadvantages include their higher cost and potential allergenicity (Choi and Han 2002; Han 2002).
Starch is the major constituent of legumes and cereals such as pea and rice flours, respectively. Most starches contain about 25% amylose and 75% amylopectin (Bemiller and Whistler, 1996; Haase, 1993). Starch is an attractive raw material for edible packaging because of its low cost, renewability and biodegradability (Guilbert, 2000). Furthermore starch is abundant and non-allergic, and as well it possesses good mechanical strength in films and gas barrier properties which may allow for wider food applications (Rindlav-Westling et al., 1998; Lloyd and Kirst, 1962; Gujska et al., 1994; Pagella et al., 2002). Films made of corn starches with high-amylose content showed excellent oxygen barrier properties, low water solubility, lower retrogradation temperature and more stable mechanical properties at high RH values compared to those made from other starches (Stading et al., 2001; Rindlav-Westling et al., 1998; Lawton, 1996). High-amylose starch is a very useful film-forming material due to its strong gelation properties and helical linear polymer structure (Juliano, 1985; Eliasson and Tatham, 2001). High-amylose corn starch that contains over 50% of amylose requires a very high temperature (> 130 °C) to be gelatinized (Case et al., 1998; Fredriksson et al., 1998). Therefore, it is essential to use a specially designed high pressure vessel with continuous agitation for starch gelatinization. Compared to high-amylose corn starch, high-amylose rice and pea starches contain 30% and 40% amylose, respectively, and accordingly only a low temperature is required (< 90 °C) for gelation. Therefore, simple water boiling units can produce gelatinized rice and pea starch dispersions.

Compared to the large amount of research and development studies in the area of edible packaging, commercialization studies are still few in number even though the potential
for huge markets exist (Anonymous, 2003). Starch-based films are sensitive to ambient relative humidity which may affect film barrier and mechanical properties when they are used on food with high water activity (Forssell et al., 2002). The usefulness of edible films and coatings on foods depends on film barrier properties and their ability to maintain the mechanical integrity of packaged foods. Since high-amylose starch films have properties superior to those made from other starches, the objectives of this work were to determine the basic physical and mechanical properties of high-amylose rice and pea starch films and characterize the effect of a plasticizer on these properties. The primary goal of the work was to identify the mechanism of starch film formation during dehydration processes.

3.3. Materials and Methods

3.3.1. Materials

High-amylose rice starch (RS) and pea starch (PS) was supplied by A & B ingredients (NJ, USA) and Parrheim Foods Co. (Portage-la-Prairie, MB, Canada), respectively. The rice and pea starch contained approximately 30 and 40% amylose, respectively. Glycerol from Sigma Chemical Co. (St. Louis, MO) was used as the plasticizer.

3.3.2. Film preparation

Aqueous dispersions of 3% (w/w) RS or PS were prepared and glycerol was added at a ratio of 1:2 (w/w) of glycerol:starch. For reference tests, pure rice and pea starch films were produced without glycerol. The solutions were heated with continuous mixing until boiled and were kept at boiling temperature for 15 min to allow complete gelatinization
of the starch granules. The film-forming solutions were cooled to ~ 60 °C then cast into 10 cm diameter polystyrene Petri dishes by pouring 12 g into each dish positioned on a smooth level surface. The Petri dishes were kept at room temperature for 24 h to allow the film-forming solution to be dried thoroughly. The thickness of the films was measured with a caliper micrometer (B.C. Ames Co., Waltham, MA) at 5 random film positions after the films were peeled from the plates. The average thickness was used for subsequent mechanical, water vapor permeability and oxygen permeability determinations. The films were transferred into large plastic bags (lubricant-free LDPE sandwich bags) and arranged side by side in a single layer. The filled bags were then gently pressed on each side of the films which allowed both film surfaces to stick to the bag and minimized the access of air or moisture to the films. The bags were then sealed and stored at room temperature for < 7d until tested.

3.3.3. Photomicrography

After drying for 24 h in air at ambient RH, the whole cast films in Petri dishes were conditioned for 5 d at 51% or 90% RH by storage over saturated salt solutions of Ca (NO₃)₂·4H₂O or Zn SO₄·7H₂O, respectively, at 25 °C. Petri dishes containing the films were then placed on an inverted phase-contrast microscope (PCM, Nikon Diaphot) equipped with a TV camera (Panasonic WV-1550) and the surface structure of the films was observed. With 4X and 40X power lenses, the image of film surfaces was recorded by a personal computer equipped with a video capture card. The image resolution was set at 640×480 pixels.

3.3.4. Tensile tests
The films stored in plastic bags were cut in 1 cm \times 8 \text{ cm} strips and conditioned in a desiccator for 3 d at 51 or 90\% RH. In the case of the non-plasticized starch films, the pure rice and pea starch films were humidity-conditioned before being peeled from the Petri dishes. Tensile strength (TS) and elongation-at-break (E) were determined from a stress-strain curve using a texture analyzing instrument (Texture Analyzer, TA-XT2, Texture Technologies, Corp., NY, USA) following the procedure outlined in ASTM method D 82-91 (ASTM, 1991). Initial grip distance and crosshead speed were 5 cm and 100 mm/min, respectively. TS was calculated by dividing the peak load by the cross-section area (thickness \times 1 \text{ cm}) of the initial specimen. E was expressed as the percentage of change in the length of the specimen to the original length between the grips (5 cm). TS and E were obtained from 3 replicate measurements. After RH conditioning, the moisture content of films was determined by measuring the weight difference of film samples before and after dehydration at 105\^\circ\text{C} for 24 h.

3.3.5. Water vapor permeability (WVP)

The Choi and Han (2001) procedure taken from the modified ASTM E96-92 gravimetric method of McHugh et al. (1993) was used to determine the WVP and the RH under the film. Distilled water (10 mL) was dispensed into 50 ml flat-bottom acrylic (Plexiglas\textsuperscript{®}) cups with wide rims. The openings of the cups were covered with the films, sealed with a ring and silicon sealant (High Vacuum Grease, Dow Corning, Midland, MI), and placed in a desiccator cabinet equipped with fans and a digital hygrometer, where they were held a constant humidity (RH 30\%) at 25 \text{ \degree C}. Weight changes were measured periodically, and after the steady state of weight loss was achieved were used to
calculate the water vapor transmission rate (WVTR). Relative humidity underneath the film was calculated from free diffusion of moisture in air inside the cup and the distance from the water surface to the test film. The procedure of McHugh et al. (1993) was followed which uses a visual basic program. The WVP was calculated as follows:

\[
WVP = \frac{WVTR \times \text{Thickness}}{\Delta P_{H2O}}
\]

The term \(\Delta P_{H2O}\) is the partial pressure difference of water vapor between the two sides of the film.

3.3.6. Oxygen permeability (OP)

The oxygen transmission rate (OTR) was measured using OX-TRAN 2/61 (Mocon, Minneapolis, MN). The measurements were done at 23 °C and the films were equilibrated at RH values of 30, 35 and 45% during the experiment. The OP was calculated by the following equation:

\[
OP = \frac{OTR \times \text{Thickness}}{\Delta P_{O2}}
\]

The term \(\Delta P_{O2}\) is the partial pressure difference of oxygen between the two sides of the film, which was 1 atm during the experiment.

3.3.7. Percentage of soluble matter (%SM)

Film specimens (1.5 cm × 1.5 cm) were dried for 3 d in a desiccator over anhydrous calcium sulphate. The specimens were weighed to obtain the initial dry weight \((W_i)\). The dried specimens were immersed in 5 mL distilled water in test tubes. The tubes were covered with aluminium foil and incubated at ambient temperature for 24 h with occasional gentle agitation. The films then were dried in an oven at 70 °C for 24 h and
weighed to obtain the final dry weight \((W_f)\). The percentage of soluble matter (% SM) of the films was calculated using the following equation:

\[
\% \text{SM of the films} = \left[\frac{(W_i - W_f)}{W_i}\right] \times 100
\]

3.3.8. Statistical Analysis

Data obtained were the average values of three replicates for treatments which were compared by the statistical analytical system (Version 8.2, SAS Institute Inc., Cary, NC.) for each treatment. A significance level of 5 % was used for all analyses.

3.4. Results and Discussion

3.4.1. Film preparation

After drying the film-forming solutions, the glycerol-plasticized films were easily peeled from the Petri dishes, whereas without glycerol, RS films were much stickier than PS films. Both films were translucent, strong and flexible enough to be easily handled. Non-plasticized films containing pure rice and pea starch were too brittle to be peeled from the petri dishes. However, the films conditioned in the 90 % RH chamber were flexible and easily peeled from the dishes. Fig. 3.1 shows the visual appearance of rice and pea starch films with and without glycerol at 51 % and 90 % humidity.

3.4.2. Photomicrography

At 4X power, regardless of the incorporation of glycerol, RS and PS films possessed two phases: one was the starch gel matrix and the other was a black porous structure. The RS film had bigger gel matrix particles and pores than those of PS films. In particular, the RS film containing glycerol had smaller gel matrix particles than the non-plasticized films.
Figure 3.1. Rice and pea starch films after RH conditioning.
The large-size gel matrix particles were formed from dried swollen particles of starch granules. Amylopectin (a major polymer of starch), forms gel particles during gelatinization from which amylose is excluded (Goodfellow and Wilson, 1990). Therefore, the large gel particles that appeared to be swollen starch granules in 4X images were probably an amylopectin-rich phase. At 40X power it was observed that the black pores of non-plasticized RS and PS films were filled with very small gel granules estimated to be < 2 μm diameter. When amylose is excluded from starch granules during gelatinization of amylopectin it forms small granular aggregations (Zobel, 1984). Thus, the small granules in 40X images were assumed to be amylose-rich aggregations. When RS films were plasticized by glycerol, the small granules in the amylose-rich phase (RG-40X, Fig. 3.2) were swollen and formed an opaque matrix (RG+40X in Fig. 3.2). Therefore, the structure of plasticized films became more continuous in the amylose-rich phase. In PS films, the large gel matrix (amylopectin-rich phase) and the small granules (amylose-rich aggregations) in treatment PG-40X (Fig. 3.2) disappeared and formed a more homogeneous structure, when the PS films were plasticized (PG+40X, Fig. 3.2). It was concluded that the addition of plasticizers to starch films interferes with the self-aggregation of amylopectin, and amylose reduces the phase separation of both polymers and forms a more homogeneous mixed amylose and amylopectin structure.

3.4.3. Moisture content (MC)

Plasticized films contained more moisture than non-plasticized films, and the films conditioned at 90% RH possessed a higher MC than films in 51% RH (Table 3.1).
Figure 3.2. Photomicrography (4X and 40X magnitude) of rice (R) starch and pea (P) starch films with (G+) and without (G-) glycerol at 51% RH. Scale bars in 4X and 40X are 100 μm and 10 μm, respectively.
The MC of plasticized RS and PS films conditioned at 51% RH (i.e., 27% MC) had the same MC as non-plasticized RS and PS films at 90% RH, while they contained 11.4-11.6% MC at 51% RH. The plasticizer (glycerol) in RS and PS films held 15.4-15.6% more water than non-plasticized films after conditioning at 90% RH. Glycerol is a good water-holding agent and was probably responsible for greater moisture retention by the films. This maybe related to its function as a plasticizer.

Table 3.1. Moisture content (MC), tensile strength (TS) and elongation-at-break (E) of starch films stored at 51% and 90% relative humidity.

<table>
<thead>
<tr>
<th>Film</th>
<th>RH (%)</th>
<th>MC (%)</th>
<th>TS (MPa)</th>
<th>E (%)</th>
<th>Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice starch</td>
<td>51</td>
<td>27</td>
<td>3.2 ± 0.3</td>
<td>265.6 ± 13.6</td>
<td>0.071</td>
</tr>
<tr>
<td>Pea starch</td>
<td>51</td>
<td>27</td>
<td>4.2 ± 0.4</td>
<td>323.7 ± 12.0</td>
<td>0.072</td>
</tr>
<tr>
<td>Rice starch</td>
<td>90</td>
<td>43</td>
<td>1.9 ± 0.2</td>
<td>751.4 ± 20.6</td>
<td>0.077</td>
</tr>
<tr>
<td>Pea starch</td>
<td>90</td>
<td>44</td>
<td>2.8 ± 0.2</td>
<td>420.9 ± 93.2</td>
<td>0.075</td>
</tr>
</tbody>
</table>

1 With glycerol (plasticizer).
2 Without glycerol.
3 Means within the same column with different letters are significantly (p<0.05) different, n=3.

3.4.4. Tensile tests
The tensile strength (TS) and elongation-at-break (E) of plasticized high-amylose RS and PS films stored at two RH values are shown in Table 3.1. At the same RH, plasticized PS films had higher TS than plasticized RS films. The TS of both films decreased at the higher RH, while the E of both films increased with increasing RH. The E of PS at 90% had very large standard deviation compared to other films. When RS and PS were not plasticized by glycerol, free, stand-alone RS and PS films were not produced at 51% RH. At 90% RH non-plasticized RS films was stronger and more extendable (in TS and E, respectively) than non-plasticized PS films (Table 3.1). Comparing TS and E values of 90% RH conditioned non-plasticized pure starch films to glycerol-plasticized films, the addition of plasticizer decreased the TS of RS films and the E of both RS and PS films. The TS of PS films were not affected by plasticizers at 90% RH. Additional moisture retained by PS films stored at 90% RH may be involved in the greater plasticity of these films.

Rindlav-Westling et al. (1998) found that stress at break decreased and strain at break increased when storage RH of amylose films plasticized with glycerol (2.5:1 of polymer:glycerol) was increased from 20 to 70%. The elastic modulus of both amylose and amylopectin films decreased when RH was increased from 20 to 80% (Stading et al., 2001). With corn starch films, increasing the RH from 35 to 65% resulted in a reduction in strength of about 43% (Lloyd and Kirst, 1962). Increasing the plasticizer levels in edible films was generally found to increase E and decrease TS (Choi and Han 2001). As with other starch (Rindlav-Westling et al., 1998; Stading et al., 2001; Lloyd and Kirst, 1962) and protein films (Choi and Han, 2001), RS and PS films conditioned at high RH (90%) were weaker but more flexible than those conditioned at low RH (51%)
as shown in Table 3.1. While the greater moisture contributed to this flexibility, too much moisture in the starch films could cause a more loose and heterogeneous structure that may weaken film strength (Stading et al., 2001). Regardless of RH, MC and film thickness, plasticized PS films overall had stronger mechanical properties than plasticized RS films (Table 3.1) and this was probably because of its higher amylose content (Lloyd and Kirst, 1962). The changes in TS and E of PS films in response to changes in RH was less significant than those of RS films. This may have been due to the greater stability of high-amylose starch films in terms of mechanical properties (Lawton, 1996).

The RS films contained lower concentrations of amylose than PS films. Amylose is a helical linear polymer and is responsible for the elongation properties of high-amylose starch films. Lloyd and Kirst (1962) found that the TS of corn starch films increased with increasing content of amylose at the same RH values. High amylose films were the most resistant to changes in E following change in RH from 20 to 90% when compared to films containing normal starches (Lawton 1996). Lourdin et al. (1995) found that pure amylopectin films showed elongation behaviour similar to that of ductile material, while that of pure amylose films was similar to brittle material. They related this to possible differences in glass transition temperature (Tg) values at room temperature.

Amylopectin films have a Tg value slightly lower than 25 °C while it is much higher than 25 °C for amylose films. Amylose-based films have more stable microstructure at higher RH values (> 50%) with stronger molecular orientation than those of amylopectin films (Rindlav-Westling et al., 1998; Stading et al., 2001). The higher concentration of
amylose in PS films may allow for higher stability and subsequently greater extensibility at higher RH than RS films (Table 3.1).

3.4.5. Water vapour permeability

The WVPs of plasticized RS and PS films ranged between 130 – 150 g mm m$^{-2}$ d$^{-1}$ KPa$^{-1}$ (Table 3.2). With 21 - 45 g mm m$^{-2}$ d$^{-1}$ kPa$^{-1}$ of standard deviation, there was not a large difference in WVP between RS and PS films (131 vs. 150 g mm m$^{-2}$ d$^{-1}$ kPa$^{-1}$). The hydrophilic nature of both starch molecules made the films easily penetrable by water. Water molecules can more easily diffuse through amylopectin films than through amylose films (Rindlav-Westling et al., 1998). The RS and PS films prepared here contained higher proportions of amylopectin (approximately 70% and 60%, respectively) which made them poor barriers for water. Although there was a 10% difference in amylose/amylopectin content between the RS and PS films, the WVP of both films did not show a significant difference. The WVP of PS had a coefficient of variance (C.V. = 29.9%) twice as large as the C.V. of RS (16.1%). It was found that inclusion of hydrophobic materials such as edible fatty acids, protein-based materials or waxes in hydrophilic edible films can retard water diffusivity through films and improve their water barrier properties (Krochta and De Mulder-Johnston, 1997; Ayranci and Tunc, 2001). Chemical modification of polymer structure by increasing crosslinking showed promise in improving film water barrier properties (Sebti et al., 2002).
Table 3.2. Water vapor permeability (WVP) and water solubility (soluble matter, SM) of starch films containing glycerol.

<table>
<thead>
<tr>
<th>Film</th>
<th>WVP (RH) (^{1}) (g mm m(^{-2}) d(^{-1}) kPa(^{-1}))</th>
<th>SM (%) with glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice starch</td>
<td>130.93 ± 21.08(^{b2})</td>
<td>44.4 ± 4.39(^{a})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.48 ± 1.001)(^{3})</td>
</tr>
<tr>
<td>Pea starch</td>
<td>150.31 ± 44.93(^{a})</td>
<td>32.0 ± 3.35(^{b})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.50 ± 0.832)(^{3})</td>
</tr>
</tbody>
</table>

\(^{1}\) RH values above both films with glycerol were 30.0% and RH values below RS and PS films were 72.9% and 72.0%, respectively.
\(^{2}\) Means within the same column with different letters are significantly (p<0.05) different, n=3.
\(^{3}\) Reference SM of non-plasticized pure starch film without glycerol.

3.4.6. Oxygen Permeability

Plasticized RS and PS films were excellent oxygen barriers (OP < 0.5 cm\(^{3}\) μm m\(^{-2}\) d\(^{-1}\) kPa\(^{-1}\)) at RH below 35% (Fig. 3.3). At the RH values above 40%, the oxygen permeability of both films was increased to 1.2-1.4 cm\(^{3}\) μm m\(^{-2}\) d\(^{-1}\) kPa\(^{-1}\) which still represented superior oxygen barrier capability. However, at < 40% RH, RS and PS films also showed larger standard deviation than those at low RH (Fig. 3.3). Although OPs of RS film and PS film did not show significant differences (p > 0.01) in the experimental ranges of RH value tested, the PS films were slightly better oxygen barriers at high RH (45%). Forssell et al. (2002) found that amylose films plasticized with glycerol had good oxygen barrier properties at RH values below 50% at glycerol content up to 30%.

Increasing the surrounding RH from 50 to 90% resulted in increased oxygen permeability of both amylose and amylopectin films. However, amylose films were
Figure 3.3. Oxygen permeability of rice and pea starch films containing glycerol at different relative humidities. Bars represent standard deviation.
better oxygen barriers than amylopectin films. Similar findings were obtained by Rindlav-Westling et al. (1998) when they found that amylose films had lower OP than amylopectin films at RH values between 35-75%. The network structure of amylose films is very stable up to 50% RH. Increasing the RH above 50% resulted in increased mobility of oxygen molecules in the polymeric network structure and increased permeability through the loosened structure (Rindlav-Westling et al., 1998; Gontard et al., 1996). The higher concentration of amylose in RS and PS films used in the present study may be responsible for the excellent oxygen barrier properties observed especially at 45 % RH.

3.4.7. Total soluble matter

Plasticized RS and PS films had moderate solubility of 44% and 32%, respectively (Table 3.2). This may have been due to the incorporated soluble component, glycerol. The glycerol:starch ratio in these films was 1:2. Therefore, total loss of glycerol from the films may account for 33% of the soluble matter. The PS film showed 32% solubility which was very close to its glycerol content, but the RS film had higher solubility (44%). This may have occurred because the starch network of the RS film was partially soluble. From these solubility results, it was concluded that the PS network interacted more strongly with glycerol molecules and had higher starch-starch intermolecular forces than the RS structure. Considering that PS possessed 10% more amylose than RS, the greater intermolecular forces within PS would be caused by the interaction between amylose molecules, which is hypothesized as a major film-forming mechanism for starch films. For the non-plasticized pure starch films, SM ranged from 1.48 to 1.50 % and there was no difference in SM values between RS and PS films. Since non-plasticized starch films
did not exhibit any difference in SM, but plasticized films had a different SM, it is
concluded that the glycerol contributes to the increased SM of RS films.

Table 3.3 shows results from a comparison of the physical and mechanical properties of
RS and PS films to other carbohydrate and protein films at approximately 25 °C and
50 % RH. Compared to other modified cellulose edible films, RS and PS films
possessed 1.9 – 7.8 times higher TS, 2.4 – 4.3 times higher E, 2.2 – 7 times lower OP,
and 1.7 – 3.5 times lower WVP. Compared to edible protein films, the RS and PS films
had 2 - 16 times higher E, 2 - 7 times lower OP and 1.1 – 1.6 times higher WVP,
whereas their solubility and TS were comparable to other protein films. These properties
suggest that the RS and PS films should find use where higher elongation, intermediate
water vapour permeability and excellent oxygen barrier characteristics are required such
as in the packaging of intermediate or dried foods which are sensitive to oxidation.
Table 3.3. Comparison of the properties of edible films.

<table>
<thead>
<tr>
<th>Film</th>
<th>TS (MPa)</th>
<th>E (%)</th>
<th>WVP</th>
<th>OP</th>
<th>SM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice starch</td>
<td>3.2</td>
<td>265.6</td>
<td>130.9</td>
<td>2.0-2.2</td>
<td>44.4</td>
</tr>
<tr>
<td>Pea starch</td>
<td>4.2</td>
<td>323.7</td>
<td>150.3</td>
<td>1.5-1.8</td>
<td>32.0</td>
</tr>
<tr>
<td>Methylcellulose⁴</td>
<td>20-25</td>
<td>75-80</td>
<td>270-450</td>
<td>7.2-10.5</td>
<td>82.8</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose⁴</td>
<td>8-10</td>
<td>101-110</td>
<td>250-320</td>
<td>4.8-5.7</td>
<td>40</td>
</tr>
<tr>
<td>Whey protein⁵</td>
<td>6.9</td>
<td>199.0</td>
<td>119.0</td>
<td>1.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Soy protein⁵</td>
<td>5.0</td>
<td>86.0</td>
<td>91.2</td>
<td>6.1</td>
<td>35.1</td>
</tr>
<tr>
<td>Pea protein⁶</td>
<td>7.3</td>
<td>31.9</td>
<td>98.0</td>
<td>-</td>
<td>38.7</td>
</tr>
<tr>
<td>Wheat gluten⁵</td>
<td>2.0</td>
<td>170.0</td>
<td>108.0</td>
<td>6.7</td>
<td>-</td>
</tr>
</tbody>
</table>

¹ Edible films contained glycerol as a plasticizer at a ratio 2:1 of biopolymer:glycerol.
² Conditions for TS and OP were approximately 25 °C, 50% RH. OP data for rice and pea starch films were estimated by extrapolation from Fig. 3.3.
³ Units are g mm m⁻² d⁻¹ kPa⁻¹ for WVP and cm³ μm m⁻² d⁻¹ kPa⁻¹ for OP.
⁴ Data from Park et al. (1993) for TS, E, WVP and OP, and Song and Kim (1999) for SM. WVP was determined at very low RH (0%/11%), while WVP of other starch and protein films were determined at higher RH (approximately 10%/70%).
⁵ Data from Krochta (2002)
⁶ Choi and Han (2001).

3.5. Conclusions

The low cost, ease of preparation, non-allergenicity and the superior mechanical and barrier properties of the high-amylose PS and RS films suggest these films may be valuable for food packaging. PS films were strong and elastic and possessed good oxygen barrier properties and physical integrity. RS films showed similar properties at low RH values but they were weaker and more stretchable than PS films. Both films lost
some of their oxygen barrier properties with increasing RH. The addition of glycerol increased solubility and moisture content of starch films. The content of amylose in starch was used to explain the strong film-forming properties of the pea and rice starch-based edible films at different RH values. With the exception of WVP, RS and PS films were either comparable or superior to other edible films such as those made from whey protein, soy protein, pea protein and wheat gluten. Substitution of protein-based packaging materials with high-amylose starches in food applications is suggested. Development of composite packaging materials containing high-amylose rice or pea starches with other biopolymers may be useful in understanding the functional properties and roles of the other constituents in film formulations. This may allow wider application of films and coatings made of high-amylose rice and pea starches.

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CHAPTER 4

Effectiveness of trisodium phosphate, lactic acid and commercial antimicrobials against pathogenic bacteria on chicken skin
4.1. Summary

Four experiments were used to evaluate the effectiveness of treatments to reduce the numbers of inoculated zoonotic bacteria on the skin of chicken drumettes. Antimicrobial treatments were applied to chilled and unchilled drumettes singly or in a dual sequence by dipping before or after bacterial inoculation. First, 10 % (w/v) trisodium phosphate (TSP) or 3 % (v/v) lactic acid (LA) with or without 2 % (w/v) NaCl were used against *Escherichia coli* O157:H7. Second, TSP and proprietary products Sanova (acidified sodium chlorite), Safe2O (acidified calcium sulfate), Cecure (cetylpyridinium chloride, CPC) and Inspexx 100 (peroxyacetic acid) were used on drumettes against *E. coli* O157:H7, *Campylobacter jejuni* or a cocktail containing two strains of *Salmonella* Typhimurium and one strain of *S. Heidelberg*. Third, selected antimicrobials were evaluated for their ability to control growth of the naturally occurring psychrotrophs on chicken skin and extend shelf-life at 7°C. Fourth, food grade carbohydrate biopolymers were used as carriers to enhance activity of TSP and Sanova on chicken skin. TSP was twice as effective as LA in reducing the numbers of viable *E. coli* O157:H7 on chicken skin (≥ 1 log cfu/g reduction by TSP) with exposure of 1 min when treated drumettes were stored 24h at 4°C. Exposures > 20 min did not increase reductions with either treatment. TSP alone or LA followed by TSP (which maintained the skin pH ≥ 10) gave greater reductions of *E. coli* O157:H7. Antimicrobial treatments (TSP, Sanova, Safe2O, Cecure and Inspexx 100) were more effective against tested bacteria when applied on warm (unchilled) drumettes before bacteria were added. The reductions in numbers of *Salmonella, C. jejuni* and *E. coli* O157:H7 were not significantly different among antimicrobials but reductions with TSP, Sanova and Safe2O were generally greater. Dual treatments of Cecure with other antimicrobials against the *Salmonella* cocktail increased
bacterial reductions, particularly when Cecure was used last, however, reductions were not significantly different from dual treatment with Cecure. Bacterial reductions following treatment with TSP and Sanova appeared related to the high or low pH values these generated. All treatments delayed the growth of pseudomonads and psychrotrophs naturally present on chicken skin when stored aerobically at 7 °C. Sanova, 10 % TSP and Safe2O delayed growth slightly better than Cecure or Inspexx 100 but all antimicrobials extended product shelf-life by about 3 d. Carbohydrate biopolymers (TSP in guar or locust bean gum and Sanova in pectin or carboxymethyl cellulose, CMC) did not enhance antimicrobial action against the Salmonella cocktail inoculated on chicken skin drumettes stored 72h at 4 °C.

4.2. Introduction

The microbiological quality of fresh eviscerated chickens has become a major concern because fresh poultry is frequently involved in foodborne illness outbreaks caused by Salmonella and Campylobacter. These organisms are the predominant pathogens found on raw chicken (Jones et al., 1991; Waldrup, 1996). E. coli O157:H7 is not presently associated with poultry. Salmonella and Campylobacter can survive the conditions used for marketing fresh poultry and cause illness, if adequate handling procedures are not followed. Pseudomonads are the major psychrotrophic spoilage bacteria found on fresh chicken (Gallo et al., 1988; Regez et al., 1988). They can grow quickly at low temperature and cause early termination of refrigerated shelf-life. Several commercial antimicrobial products have been evaluated for their ability to reduce pathogen contamination on chicken carcasses such as cetylpyridinium chloride, CPC (Breen et al.,
1997; Wang et al., 1997), trisodium phosphate, TSP (Coppen et al., 1998; Slavik et al., 1994), acidified sodium chlorite (Kemp et al., 2000, 2002; Mullerat et al., 1994) and organic acids (Tamblyn and Conner, 1997), but little comparative information is available (Hwang and Beuchat, 1995). Antimicrobial films and coatings are recognized as useful vehicles for extended delivery of inhibitory agents under a variety of conditions (Han, 2003). The aim of this study was to compare the antimicrobial activity of commercial antimicrobials against Salmonella spp., Campylobacter jejuni and E. coli O157:H7 and from the group tested choose those most active for incorporation in a biopolymer coating to increase their stability and bioactivity. The study examined the effect of processing variables such as contact time and sequence of treatment application to maximize antimicrobial activity. The effect of treatments upon the appearance of fresh chicken was also informally determined.

4.3. Materials and Methods

4.3.1. Cultures and inoculation of chicken drumettes

Chilled drumettes (proximal portion of wing including the humerus) were obtained from a retail outlet and kept at 4°C ≤ 2 h before use. Unchilled drumettes were also obtained from a local poultry processing plant directly after the defeathering stage, transported to the laboratory in a thermally insulated container and used within 1 h. The temperature of the unchilled drumettes, measured by inserting a thermometer under the skin, ranged between 38-40 °C. Chilled drumettes were used in experiments reported in Table 4.1 and Figs. 4.1 - 4.3 Unchilled drumettes were used to obtain results presented in Tables 4.2 - 4.3 and Figs. 4.5, 4.8 and 4.9. Both chilled and unchilled drumettes were used in tests reported in Figs. 4.4, 4.6 and 4.7. Bacterial cultures used to inoculate drumettes were: a
nalidixic acid resistant strain of *E. coli* O157:H7 (# E318N, R. Johnson, Health Canada, Guelph, ON), a strain of *Campylobacter jejuni* (# SH26 J. Farber, Health Canada, Ottawa, ON) and a cocktail of three ampicillin resistant *Salmonella enterica* serovars which included strains of *S. Typhimurium* (# 02-8425 and # 02-8421) and *S. Heidelberg* (# 271) from R. Ahmed, Canadian Science Centre for Human and Animal Health, Winnipeg MB. All test cultures were human clinical isolates except *S. Heidelberg* which was isolated from chicken liver. Salmonellae and *E. coli* O157:H7 were grown in tryptic soy broth (TSB, Difco division of Becton Dickinson, Sparks, MD) for 24 h at 37°C. Cultures were separately standardized to an OD600 of 0.80 using sterile TSB (corresponding to approximately 9 log cfu/ml). In the case of salmonellae, the three strains were grown separately and following standardization were combined in equal portions. *C. jejuni* was grown in brain heart infusion broth (Difco) containing 0.5 % yeast extract and 10 % lysed horse blood (Oxoid, Ltd., Nepean, ON). The culture was incubated anaerobically (CampyPak Plus™, Becton Dickinson Co., Cockeysville, MD) for 48 h at 42°C. Prior to use all cultures were further diluted 1:100 using sterile 0.85% (w/v) saline. Inoculations were performed by dipping triplicate drumettes into 300 ml cell suspension containing 7 log cfu/ml for ≤ 15 sec. In order to facilitate handling of drumettes, sterilized metal loops made from commercial paper clamps were attached to the proximal joint of each animal limb. Following dipping, the drumettes were hung for 15 min in an enclosed empty glass tank to allow draining and bacterial attachment (Conner and Bilgili, 1994).

4.3.2. Treatment of drumettes with antimicrobials
Trisodium phosphate (TSP, 10 % w/v; Sigma Chemical Corp., St. Louis, MO), lactic acid (LA, 3 % v/v; Sigma) and sodium chloride (NaCl, 2 % w/v, Sigma) were prepared using potable tap water. Commercial antimicrobials used were: Sanova containing 0.12 % acidified sodium chlorite (Alcide Corp., Redmond, WA), SafezO containing a proprietary amount (≤ 10 %) of acidified calcium sulfate (Mionix, Naperville, IL), Cecure containing 0.5 % cetylpyridinium chloride, CPC (Safe Foods Corp., North Little Rock, AR) and Inspexx 100 containing peroxyacetic acid (0.2 %), plus hydrogen peroxide, octanoic and acetic acids (Ecolab, Saint Paul, MN) were prepared according to the manufacturer’s directions. Each solution (100 ml) was transferred to polystyrene cups and within 30 min groups of three inoculated drumettes were dipped for specified times in antimicrobials or water (which served as a negative control). Both the control and treated samples were allowed to drain for 30 sec before storage at 4 ºC in plastic bags (16 × 15 cm; Ziploc, S. C. Johnson & Son Ltd., Brantford, ON). Bacterial numbers were determined following prescribed incubation periods. In some cases a combination antimicrobial treatment was used and consisted of two 30 sec dips in different solutions, separated by an interval of 1 min. In other tests the antimicrobial was applied using one min dips before or after bacterial inoculation and results compared. Drumettes were not rinsed after treatments and were kept at 4 ºC for 2 h before microbiological evaluation. The shelf-life extension of unchilled drumettes by these antimicrobials was also investigated using naturally present pseudomonads and psychrotrophs as index microorganisms and 6 log cfu bacteria/g was used as the shelf-life endpoint. Following a one min dip in each antimicrobial and draining 30 sec, the drumettes were transferred to
plastic bags and held at 7 °C. Microbial evaluations were performed at intervals from 2 to 120 h.

4.3.3. Coating of drumettes with biopolymer films

In these tests guar and locust bean gums were used with TSP while pectin and carboxymethyl cellulose (CMC) were used with Sanova. Locust bean gum solutions of 1 and 2 % (w/v) (Sigma–Aldrich Canada Ltd., Oakville, ON) were prepared using cold water. TSP was added to achieve a final concentration of 10 % (w/v). A similar protocol was used for guar gum (Sigma). Pectin (1 % w/v) and carboxymethyl cellulose (2 % w/v) base solutions (Sigma-Aldrich) were separately prepared in the sodium chlorite solution (Sanova base) supplied by Alcide Corp. Citric acid solution (Sanova activator) was added according to the manufacturer’s instructions to give a final concentration of 0.12 % (w/v) acidified sodium chlorite. Unchilled drumettes inoculated as previously described were dipped ≤ 15 sec in the biopolymer solutions or in solutions containing 10% TSP, 0.12 % Sanova, or tap water (controls) and allowed to drain as previously described.

4.3.4. Microbial analysis and surface pH determination

For sampling, the whole skin from each treated drumette was aseptically removed using a scalpel and pummeled in a Stomacher Lab-Blender 400, (Seward Medical, London, UK) for 2 min in 45 ml sterile, buffered peptone water (10 g peptone, 5 g NaCl, 3.5 g Na₂HPO₄, 1.5 g KH₂PO₄ per liter) according to Kim and Slavik (1996). Following serial dilution with 0.1 % peptone water, the samples were surface-plated on prepoured agar. Salmonellae were selected using XLD agar (Difco) containing 100 ppm ampicillin
(Sigma-Aldrich) while \textit{E. coli} O157:H7 was enumerated on MacConkey agar (Difco) containing 40 ppm nalidixic acid (Sigma-Aldrich). XLD plates were incubated for 24h at 37 °C while MacConkey agar plates were incubated for 48h at 37 °C. Karmali agar base (Oxoid Ltd., Basingstoke, England) containing a growth supplement (Oxoid, SR 139) was used for enumerating \textit{C. jejuni}. Plates were incubated for 48 h at 42 °C under microaerophilic conditions. Reductions in viable bacteria were calculated as log cfu/g control – log cfu/g treated sample.

Pseudomonads were counted on \textit{Pseudomonas} agar base (Oxoid) containing an antibiotic supplement (Oxoid, SR103) following incubation for 48 h at 25 °C. Psychrotrophic bacteria were enumerated on plate count agar (Difco) after 10 d incubation at 7 °C. The surface pH of each treated sample was measured at 3 locations and average values were reported (pH meter IQ240, IQ Scientific Instruments Inc., San Diego, CA.).

4.3.5. \textit{Statistical analysis}

Results are presented as means ± standard deviation (SD). The statistical analytical system (SAS Institute Inc., Cary, N.C.) package (Version 8.2) was used to compare means of microbial numbers for each treatment at each sampling time. A significance level of 5 % was used for all analyses. Means were calculated from one (Appendix 1, Figs. 4.1-4.3) or two (Tables 4.2-4.3, Figs. 4.4-4.9) experiments where triplicate samples per treatment (n=3 or 6, respectively) were plated in duplicate.

4.4. \textit{Results}
4.4.1. *Trisodium phosphate and lactic acid treatments*

The initial *E. coli* O157:H7 levels on the drumettes following dipping in the inoculum ranged between 4-5 log cfu/g. All data are presented as log cfu/g. Although there was some variability in skin thickness, based on 10 measurements, the conversion of data to log cfu/cm² drumette skin can be calculated as 0.18 × log cfu/g for comparative purposes. No significant differences were observed in the reduction of *E. coli* O157:H7 on drumettes stored for 2 h following any of the 30 sec dip treatments (Fig. 4.1). Regardless of antimicrobial and application protocol, the maximum reduction was < 1 log cfu/g even when stored 120 h at 4 °C. Increasing the drumette dipping time from 1 to 20 min significantly reduced numbers of *E. coli* O157:H7 following either 10 % TSP or 3 % LA use, however, further increases in contact time did not cause any additional reduction regardless of treatment (Fig. 4.2). Among the treatments tested 10% TSP was the most effective and caused a 1.6 log reduction in *E. coli* O157:H7 at 2 h which was maintained throughout 120 h of storage (Fig. 4.3).
Figure 4.1. Reduction of *E. coli* O157:H7 on chilled drumettes held at 4 °C for up to 120 h following 30 sec dipping in trisodium phosphate (TSP), lactic acid (LA) or NaCl singly or sequentially. Drumettes were inoculated before antimicrobial treatment and were not rinsed between or after treatment. Means at the same storage time with different letters are significantly (p < 0.05) different. * Negative values were obtained at 120 h when NaCl was followed by LA treatment.
Figure 4.2. Reduction of *E. coli* O157:H7 on chilled drumettes inoculated before antimicrobial treatment and held at 4°C for 2h (a) and 24 h (b) following dipping for 1, 20, 40 or 60 min with trisodium phosphate (TSP), lactic acid (LA) or NaCl. Means at the same storage time with different letters are significantly (p < 0.05) different.*Negative values were obtained with NaCl.
**Figure 4.3.** Reduction of *E. coli* O157:H7 on chilled drumettes inoculated before antimicrobial treatment held for up to 120 h at 4 °C following the various treatments. Contact times were 20 min for trisodium phosphate (TSP) and 10 min for lactic acid (LA) treatments. Means at the same storage time with different letters are significantly (p<0.05) different. * Negative values were obtained with NaCl.
Drumettes treated with 10% TSP or 3% LA exhibited the highest and lowest pH, respectively, immediately after treatment. The pH of treated drumettes tended to return toward normal (which is ~ 6.4, Capita et al., 2000a) during the initial 24h then remained relatively constant throughout the study period (Appendix 1). Alkaline treatments were approximately twice as effective as acidic treatments in causing bacterial reductions (Fig. 4.3). Skin softening and bleaching occurred where treatments were >30 sec with LA alone, when TSP treatment was followed by LA, and when LA was dissolved in 2% NaCl. These effects were probably the result of the low pH (Appendix 1).

4.4.2. Commercial antimicrobial treatments

The antimicrobial activity of most of the commercial agents also appeared related to their ability to raise or lower the pH. Sanova caused the largest initial decrease in skin pH after treatment while TSP had the opposite effect (Appendix 2). Initial reductions in pH caused by Safe2O and Inspexx 100 were also significant but were relatively small. Cecure had little effect on pH. TSP caused the largest reduction in numbers of the three bacterial genera tested. Sanova was less effective than TSP but reductions in numbers of bacteria were similar and slightly > 1 log cfu/g. While effective in causing ≥ 1 log cfu/g reductions in numbers of Salmonella spp. and C. jejuni, Safe2O was not effective against E. coli O157:H7 (Table 4.1). Increasing the exposure time to Sanova, Safe2O, Cecure and Inspexx 100 solutions from 1 to 10 min also increased the reductions of Salmonella strains (Figs. 4.4 and 4.5). The largest increase in reduction resulting from the longer exposure was shown by Sanova with the total reduction reaching 3.5 log cfu/g. Double treatments of antimicrobials applied before and after inoculation were significantly more effective in reducing salmonellae than when applied either before or
after inoculation, and with Safe\textsubscript{2}O, Sanova and 10\% TSP reductions ranged from 2.0 to 2.5 log cfu/g (Fig. 4.4). Treatment of drumettes before inoculation rather than after also gave larger microbial reductions (Figs. 4.4, 4.6, 4.7). Results with \textit{C. jejuni} were similar to those with salmonellae and while the extent of the reductions was smaller than with salmonellae, a double treatment with either Sanova or Safe\textsubscript{2}O was the most effective and yielded about 2.5 log cfu/g reductions (Fig. 4.6).
Table 4.1. Reductions\(^1\) in numbers of inoculated bacteria on unchilled drumettes treated\(^2\) 1 min with commercial antimicrobials and stored at 4 °C for 2 h.

<table>
<thead>
<tr>
<th></th>
<th>Reductions (log(_{10}) cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salmonella cocktail(^3)</td>
</tr>
<tr>
<td>TSP (10%)</td>
<td>1.56(^{a})</td>
</tr>
<tr>
<td>Sanova</td>
<td>1.11(^{b})</td>
</tr>
<tr>
<td>Safe2O</td>
<td>1.20(^{a})</td>
</tr>
<tr>
<td>Cecure</td>
<td>1.36(^{a})</td>
</tr>
<tr>
<td>Inspexx 100</td>
<td>0.04(^{c})</td>
</tr>
</tbody>
</table>

\(^1\) Reduction = log\(_{10}\) cfu/g of control − log\(_{10}\) cfu/g treated sample.
\(^2\) Drumettes were treated before bacterial inoculation. Initial inoculated number was 4-5 log cfu/g.
\(^3\) Cocktail consisting of one strain of S. Heidelberg and two strains of S. Typhimurium.
\(^4\) Means within the same column with different letters are significantly (p<0.05) different, n=6.
Figure 4.4. Reduction of *Salmonella* cocktail (S. Heidelberg plus two strains of *S*. Typhimurium) on unchilled or chilled drumettes treated 1 min and held at 4 °C for 2 h. Means within the same antimicrobial treatment with different letters are significantly (*p*<0.05) different.
Figure 4.5. Reduction of *Salmonella* cocktail (as in Fig. 4.4) on unchilled drumettes treated 10 min and held at 4 °C for 2 h. Means within the same antimicrobial treatment with different letters are significantly (p<0.05) different.
Figure 4.6. Reduction of *Campylobacter jejuni* on unchilled or chilled drumettes treated 1 min and held at 4 °C for 2 h. Means within the same antimicrobial treatment with different letters are significantly (p<0.05) different.
Figure 4.7. Reduction of *E. coli* O157:H7 on chilled or unchilled drumettes treated 1 min and held at 4 °C for 2 h. Means within the same antimicrobial treatment with different letters are significantly (p<0.05) different. SA: single antimicrobial treatment.
The effectiveness of using a combination of Cecure plus one other antimicrobial for reducing numbers of salmonellae is shown in Fig. 4.8 (where bacterial inoculation took place between the two antimicrobial treatments). Except with Sanova, use of Cecure as the second of the two antimicrobial treatments yielded significantly greater reductions in Salmonella numbers. This effect was more pronounced in TSP treatments where differences in reductions reached 2.4 log cfu/g.

4.4.3. Psychrotrophs and shelf-life

Exposure of drumettes to the antimicrobials prolonged the lag phase and reduced the maximum numbers of pseudomonads and psychrotrophic bacteria reached within 5d on drumettes stored at 7°C. The bacteria in control samples slowly increased from the first day of storage, while in samples treated with TSP their numbers were significantly reduced (≤ 2.5 log cfu/g) 2h after treatment. Numbers of bacteria remained relatively constant up to 72h before substantial growth occurred (Fig. 4.9). Similar trends were found with Sanova and Safe2O (Appendices 3-6). With Cecure and Inspexx 100 there was no significant reduction in bacterial numbers initially, but they were reduced at 24h, and thereafter numbers increased at about the same rate as shown in Appendices 3-6. Shelf-life defined as time to reach 6 log cfu/g pseudomonads or psychrotropic bacteria was 42-44h for untreated controls and 112-123h for treatments. TSP, Sanova and Safe2O gave greater reductions in numbers and consequent shelf-life extensions (Table 4.2).
Table 4.2. Reductions\(^1\) in numbers of pseudomonads (pseudo) and psychrotrophic (psychro) bacteria present on unchilled drumettes\(^2\) treated with commercial antimicrobials, then stored at 7 °C for 5 days.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Storage period (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>pseudo</td>
</tr>
<tr>
<td>TSP</td>
<td>2.60(^{a2})</td>
</tr>
<tr>
<td>Sanova</td>
<td>2.52(^{a})</td>
</tr>
<tr>
<td>Safe2O</td>
<td>1.64(^{a})</td>
</tr>
<tr>
<td>Cecure</td>
<td>0.26(^{b})</td>
</tr>
<tr>
<td>Inspexx100</td>
<td>0.05(^{b})</td>
</tr>
</tbody>
</table>

\(^1\)Reduction = \(\log_{10}\) cfu/g of control – \(\log_{10}\) cfu/g treated sample.
\(^2\)Means within the same column with different letters are significantly (p<0.05) different, n=6.
Table 4.3. Reductions\(^1\) of a salmonellae cocktail inoculated on chicken drumettes then treated with TSP (10\%) or Sanova (0.12\%) held within polymers and stored at 4 °C.

<table>
<thead>
<tr>
<th>Storage period (h)</th>
<th>Polymer concentration (%) (w/v)</th>
<th>TSP held in guar gum</th>
<th>TSP held in locust gum</th>
<th>Sanova held in pectin</th>
<th>Sanova held in CMC</th>
<th>10 % TSP solution</th>
<th>0.12 % Sanova solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>1</td>
<td>0.27 ± 0.02 (^a)</td>
<td>0.37 ± 0.04 (^c)</td>
<td>0.11 ± 0.02 (^d)</td>
<td>23 ± 0.02 (^d)</td>
<td>0.32 ± 0.09 (^c)</td>
<td>0.28 ± 0.02 (^d)</td>
</tr>
<tr>
<td>48</td>
<td>1</td>
<td>0.10 ± 0.01 (^b)</td>
<td>0.39 ± 0.12 (^c)</td>
<td>0.12 ± 0.01 (^d)</td>
<td>0.1 ± 0.00 (^c)</td>
<td>0.82 ± 0.06 (^b)</td>
<td>0.50 ± 0.06 (^b)</td>
</tr>
<tr>
<td>72</td>
<td>1</td>
<td>0.08 ± 0.00 (^b)</td>
<td>0.10 ± 0.02 (^c)</td>
<td>0.07 ± 0.00 (^c)</td>
<td>0.07 ± 0.00 (^f)</td>
<td>0.12 ± 0.08 (^e)</td>
<td>0.38 ± 0.06 (^a)</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>-0.66</td>
<td>0.17 ± 0.06 (^f)</td>
<td>0.58 ± 0.10 (^a)</td>
<td>0.70 ± 0.09 (^a)</td>
<td>0.95 ± 0.09 (^a)</td>
<td>0.50 ± 0.07 (^b)</td>
</tr>
<tr>
<td>48</td>
<td>2</td>
<td>0.06 ± 0.00 (^c)</td>
<td>0.63 ± 0.08 (^b)</td>
<td>0.33 ± 0.05 (^b)</td>
<td>0.30 ± 0.12 (^c)</td>
<td>0.28 ± 0.02 (^d)</td>
<td>0.61 ± 0.08 (^a)</td>
</tr>
<tr>
<td>72</td>
<td>2</td>
<td>0.10 ± 0.02 (^b)</td>
<td>0.68 ± 0.02 (^a)</td>
<td>0.23 ± 0.08 (^c)</td>
<td>0.45 ± 0.11 (^b)</td>
<td>-0.01</td>
<td>0.35 ± 0.02 (^c)</td>
</tr>
</tbody>
</table>

\(^1\) Reductions = log\(_{10}\) cfu/g of control – log\(_{10}\) cfu/g treated sample; dipping time for all samples < 15 sec, n=6.  
\(^2,3\) TSP or Sanova solutions without stabilizer.  
\(^4\) Means within the same column with different letters are significantly (p<0.05) different, n=6.
Figure 4.8. Reduction in viability of the *Salmonella* cocktail (as in Fig. 4.4) following dual 1 min treatments with Cecure (0.5%) and other commercial antimicrobials on unchilled drumettes (38-40 °C) held at 4 °C for 2 h. Inoculation was done between the two treatments. Means within the same treatment with different letters are significantly (p<0.05) different.
Figure 4.9. Growth of naturally present pseudomonads and psychrotrophs on unchilled drumettes (38-40 °C) during storage at 7 °C for up to 120 h following dipping in 10 %TSP for 1 min. Means at the same incubation time with different letters are significantly (p < 0.05) different.
4.4.4. Biopolymer films

Suspending TSP in 1% locust bean or guar gum and Sanova in 1% pectin or carboxymethyl cellulose when applied to drumettes reduced overall effectiveness of the antimicrobials, and reductions in Salmonella numbers were < 0.4 log cfu/g (Table 4.3). Increasing the concentration of polymers to 2% slightly enhanced Salmonella reduction to 0.70 log cfu/g, but greater reductions were obtained using the identical antimicrobials without polymer.

4.5. Discussion

Antimicrobials were different in their effectiveness against bacterial strains inoculated on drumettes with alkaline treatments being found more effective than acidic treatments. Skin surface pH was highest 2h after treatment with TSP alone (>10) before dropping to 8-9. Capita et al. (2002b) measured the surface pH of chicken skin following dipping in 10% TSP and holding for 15 min. The pH of the treated samples remained relatively constant between 8 and 9 directly after the treatment and for up to 5d. The buffering capacity of the skin and meat tissue is likely responsible for movement of the pH to ≤ 9 after 72h of treatment. In addition to causing high pH, it has been suggested that TSP may have other modes of action including high ionic strength and solubilizing the lipid bilayer outer cell of gram negative bacteria since TSP and NaOH solutions with same pH possessed different levels of antimicrobial effectiveness (Capita et al., 2002a,b). The reductions in numbers of Salmonella and Campylobacter shown by TSP in the present study would contribute to a noticeable reduction in the frequency of carcasses contaminated by these pathogens (Mead 2004). TSP has been shown to be more effective against gram negative than gram positive bacteria on chicken (Capita et al.,...
This was believed related to its ability to dissolve the outer membrane of gram negative bacteria and subsequently increase trans-membrane permeability. The strong antimicrobial activity of TSP found against gram negative spoilage and pathogenic bacteria is consistent with other work (Capita et al., 2000a, 2002a; Kim et al., 1994; Slavik et al., 1994), but gram positive bacteria are also susceptible (Capita et al., 2002a). It has been suggested that TSP is able to remove a thin layer of lipids from chicken skin and thus expose any attached bacteria which may be otherwise protected in crevices and feather follicles, to the high pH. In contrast with TSP, the inhibitory activity of LA against E. coli O157:H7 and activities of Sanova and Safe2O against Salmonella spp, C. jejuni and E. coli O157:H7 are all likely due to pH reduction.

The antimicrobial activities of Cecure and Inspexx 100 were different from the other agents used. Neither caused substantial change in surface pH at the concentrations used. The activity of Inspexx 100 is largely oxidative and due to the presence of peroxycetic acid and hydrogen peroxide. At the concentration used, Inspexx 100 was not strongly antimicrobial. In this study, we combined Cecure with acid or alkaline treatments to expose bacterial cells to two antimicrobials with different modes of action.

Cetylpyridinium chloride, the active component of Cecure, is a cationic surfactant which has a neutral pH. Its antimicrobial activity results from its interaction with acidic groups at the surface or within bacteria to form weakly ionized compounds that inhibit bacterial metabolism (Kim and Slavik, 1996). The cumulative effect of the mixed treatments with Cecure was, however, not as effective as treatment with TSP. Reduction in Salmonella
from combinations involving Inspexx 100 were significantly lower than from other treatments.

Exposure time was another significant factor in determining antimicrobial effectiveness. Twenty min was more effective than 1 min in causing significant reductions in salmonellae on chicken skin. Dipping for > 20 min in the commercial antimicrobials did not cause any further reductions. Treatment of post-chill chicken carcasses with 10 % TSP at 10 or 50 °C for 15 sec failed to cause significant reductions in *Salmonella* inoculated on carcasses (Kim *et al.*, 1994). Breen *et al.* (1997) found that the reductions of *S*. Typhimurium inoculated on chicken skin were dependent on both the CPC concentration and treatment time. Increasing the treatment time > 3 min at 4 mg/ml CPC was enough for complete elimination of *S*. Typhimurium inoculated on chicken skin with initial numbers of 4 log cfu/cm² skin.

Exposures of chicken skin to lower pH during acidic treatments caused skin discoloration which may be controlled by limiting exposure times to < 30 sec. Similar results were obtained Kemp *et al.* (2000) where exposure of chicken to acidified sodium chlorite at 1200 ppm for 5 sec caused transient mild whitening of the skin. However, Schneider *et al.* (2002b) did not find changes in chicken skin color under similar conditions with acidified sodium chlorite. In other work, concentrations ≥ 2 % of organic acids were enough to cause bleaching of the skin and off odor (Tamblyn and Conner, 1997).
Treatment of unchilled drumettes before inoculation was the most effective antimicrobial protocol. This approach may facilitate penetration of the antimicrobial into empty follicles and may change the availability of binding sites for the bacteria, reduce their ability to attach and increase sensitivity of any attached bacteria to subsequent treatments. When antimicrobial treatment and then inoculation was followed by a second antimicrobial treatment, increased reductions were obtained. When treatments after inoculation with unchilled and chilled drumettes were compared, the reductions were higher in chilled drumettes. During chilling the skin pores and feather follicles may undergo reduction in size and reduce the capacity of follicles and crevices to adsorb antimicrobial solutions or harbor bacteria in the skin (Kemp and Schneider, 2002) and expose more bacteria at the skin surface to the treatments (Kim et al., 1994; Lillard, 1986; Thomas and McMeekin, 1984). While this may be important, in other work the effectiveness of antimicrobials was found to be influenced by poultry carcass sampling site with reductions being significantly larger in the leg and dorsal areas. This was believed related to differences in feather follicle size in different areas of the chicken carcasses. Follicles are generally larger in the dorsal and leg areas (Capita et al., 2002c) and have the potential to afford protection to bacteria present, but may also more easily “pick-up” antimicrobial solutions. Sizes of follicles in drumette skins do not appear in the literature, however, they are probably small since primary (large) feathers are mainly located on the distal portion of the wing.

The antimicrobial activity of TSP against naturally present psychrotrophic bacteria was found similar to that reported by Capita et al. (2000a). They found that the reductions of psychrotrophic bacteria were 0.92 and 1.94 log cfu/g immediately following 8 or 12 %
TSP treatment, respectively. These reductions increased to 2.79 and 4.09 log cfu/g, respectively, after 5d of refrigerated storage. Our results did not generate such large reductions and showed that psychrotrophic bacteria were less sensitive than pseudomonads. It is well known that pseudomonads are very sensitive to conditions which are acidic or highly alkaline. Gram positive psychrotrophs like *Listeria monocytogenes* and *Brochothrix thermosphacta* could be a part of the psychrotrophic bacteria counted on drumettes. They are normally present on raw chicken and might be expected to be less sensitive to decontamination procedures used. A study involving the effect of these treatments on the proliferation of gram positive psychrotrophs on chicken should be done to clarify this issue.

Cecure was found to have lower antimicrobial activity than shown by others (Breen *et al.*, 1997; Kim and Slavik, 1996; Wang *et al.*, 1997; Xiong *et al.*, 1998a,b; Yang *et al.*, 1998). In the present study 5 mg/ml CPC was used and it was not as effective as 10% TSP or Sanova. None of the treatments reported here gave reductions as large as those reported by Breen *et al.* (1997). Although this is unexplained since concentrations used were similar, it is possible that the use of pressure during spray application and longer exposure time in other work may have influenced the outcome (Breen *et al.*, 1995, 1997; Xiong *et al.*, 1998a; Yang *et al.*, 1998). In the present study biopolymer application was made using dip application. Spraying was tried but the high viscosity of solutions prevented polymer application evenly (Izydorczyk and Wang, 2005). Further development of the system is underway because the opportunity for sustained reduction of viable pathogens is apparent using this approach.
4.6. Conclusion

The antimicrobial effectiveness of TSP and LA against *E. coli* O157:H7 inoculated on drumettes was greatest (1.6 log cfu/g) when TSP exposures were 20 min. TSP activity was compared with other commercial antimicrobial agents that were either acidic (Sanova and Safe2O) or almost neutral in pH reaction (Cecure and Inspexx 100). Acidic treatments were less effective than TSP, and Inspexx 100 was least effective. A similar trend was found when the treatments were tested against salmonellae and *C. jejuni*. Applying a dual treatment, both before and after inoculation, was the most effective protocol against all the microorganisms tested. When a single treatment was used on unchilled drumettes, it was found that treatment before inoculation was more effective than after bacterial addition. However, antimicrobial treatment after inoculation was more effective in some tests on chilled than on unchilled drumettes. All 5 treatments were effective against pseudomonads and psychrotrophic bacteria and increased the shelf-life of drumettes stored at 7° C from 2d to 5d. TSP held in guar or locust bean gums and Sanova in CMC or pectin failed to reduce salmonellae > 0.7 logs. Since results in general indicated that antimicrobial treatments generated greater reductions when applied before bacteria contaminated the skin surface, the application of treatments as soon after defeathering as possible is recommended to optimize antimicrobial effectiveness.

Acknowledgements

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following companies provided proprietary antimicrobials: Alcide Corp, Redmond, WA (Sanova); Mionix, Naperville, IL (Safe2O); Safe Foods Corp, North Little Rock, AR (Cecure); and Ecolab, Saint Paul MN (Inspexx 100). Dunrite Poultry (Winnipeg, MB) supplied unchilled broiler drumettes. Excellent technical assistance was provided by George Romanovich.
CHAPTER 5

Suitability of Pea Starch and Calcium Alginate as Antimicrobial Coatings on Chicken Skin
5.1. Abstract

The effect of incorporating trisodium phosphate (TSP) in pea starch (PS) and acidified sodium chlorite (ASC) in calcium alginate upon the antimicrobial activity of TSP and ASC was studied against a 3 strain cocktail of Salmonella inoculated on chicken skin. The influence of polymer coating concentration on skin pH, coating-skin adhesion and coating absorption upon antimicrobial performance were investigated. Aqueous solutions of 0.5, 1.5, 2.0, 3.5, 4.0 or 4.8 % (w/v) PS were prepared with 10 % (w/v) TSP (PS+TSP coating), while alginate+ASC coatings contained 1 % (w/v) calcium chloride in 1200 ppm ASC mixed with an aqueous solution of 0.5, 1.0 or 1.5 % (w/v) sodium alginate. Coating drops (10 μl) were placed on chicken skin thighs and their contact angles were measured using a digital camera in order to assess coating-skin adhesion. Excised skins were mounted in a ring holder and 5 ml of the coatings were applied to the skin. Weight changes in the skins which represented coating absorptiveness were recorded. TSP stabilized in 3.5 % PS and ASC in 1 % alginate reduced Salmonella by 1.6 log cfu/g and 1.4 log cfu/g, respectively within 24 h. These reductions were significantly greater than those caused by TSP or ASC alone in water for up to 120 h. Stabilized TSP and ASC caused significant elevation and reduction of skin surface pH for up to 120 h, respectively. TSP destabilized PS with 88 % of the coating having dripped from the skin one hour later. Coatings with 0.5 % PS were absorbed quickly by the skin and had high skin adhesion, while those with > 3.5 % PS had low skin adhesion, and slow absorption. Alginate coatings with or without ASC were stable, and about 50 % of the coating weight was retained at 120 h. The latter coatings appeared to have low absorptiveness since the skin gained approximately 1.0 % of its weight within 60 min following application. These findings indicate that effects of the agents in coatings
on skin pH, the extent of coating adhesion and absorption may contribute to overall antimicrobial behaviors.

Key words: antimicrobial coating, broiler, *Salmonella*, calcium alginate, pea starch

5.2. Introduction

Raw poultry products can serve as a source of human pathogens such as *Salmonella* and *Campylobacter* that may cross-contaminate other foods. When appropriate rearing and shipping practices are followed, most poultry contamination by these organisms may occur during or after slaughtering and processing (Slader *et al.*, 2002; Zhao *et al.*, 2001). Carcass washing with approved antimicrobials (AMs) has had limited success because many microorganisms are physically hidden in the feather follicles and skin folds which protect them from the action of AMs (Mehyar *et al.*, 2005; Schneider *et al.*, 2002a; Wang *et al.*, 1997; Xiong *et al.*, 1998a). Furthermore, increased line speed reduces the antimicrobial contact time with target microorganisms, and the moisture on chicken skin surface can act as a diluent, reducing antimicrobial effectiveness (Oyarzabal *et al.*, 2004). An alternative approach that would extend the contact time, would increase the effectiveness of AMs. To obtain improved effectiveness without changing process speeds in the plant, edible gels containing AMs could be sprayed on chicken surfaces. In theory, the agents would gradually diffuse from the gels or coating material into skin irregularities and if applied early (after defeathering), provide increased contact time with target microorganisms and yield improved effectiveness. Most food-related antimicrobial coatings have been tested only for their quantitative antimicrobial effectiveness (Janes *et al.*, 2002; Natrajan and Sheldon, 2000a,b; Siragusa and Dickson, 1992). No report has been found to relate the antimicrobial activity of the coatings to
their surface properties or absorption into contaminated foods. Studying these physio-chemical properties will help in determining the minimum quantities of AMs required to eliminate pathogens from foods using methods which have beneficial economic and environmental consequences.

Chicken skin consists of two layers, the upper layer called the epidermis and the lower layer called the dermis (Lucas and Stettenheim, 1972). The epidermis is divided into the Stratum corneum (cuticle) and Stratum germinativum. The cuticle of the epidermis consists of waxy material which covers the skin surface, whereas the lower region is composed of cell layers that can be differentiated to become a part of the cuticle in response to damage. Scalding at high temperature removes the cuticle layer from the skin which will affect skin adhesiveness characteristics (Lucas and Stettenheim, 1972). Indeed, a thinner cuticle layer increases skin hydrophilicity and makes microbial contamination more likely whereby organisms may be deposited within the skin and its folds (Suderman and Cunningham, 1980). The contact angle of a liquid drop on a smooth surface has been used to characterize the surface energies of solids (Choi and Han, 2002; Han and Krochta, 1999). In this study, this physical characteristic has been used to measure the adhesion force of coatings to the skin. In addition, it is also known that the contact angle of a liquid drop is affected by the extent of roughness of the target surface, and such effects could be substantial on a rough surface like chicken skin. The determination of contact angles can be used to explain solid surface properties in terms of both surface energy and roughness (Han and Krochta, 1999). The dermal layer of chicken skin contains collagen which readily absorbs water from the skin surface and swells, causing changes in skin microtopography (Thomas and McMeekin, 1982).
Liquid absorption rate and maximum absorptiveness can be measured to reflect how fast and how much of an applied liquid penetrates and is absorbed by the skin.

Consumer interest in unprocessed foods preserved with natural ingredients has significantly increased recently (Cagri et al., 2004; Debeaufort et al., 1998). Development of edible films and coatings which have comparable properties with synthetic preservative ingredients is an approach taken to satisfy this interest (Mehyar and Han, 2004). Both starch and alginate have been shown to be structurally compatible with alkaline and acidic agents (Siragusa and Dickson, 1992; Ratnayake et al., 2002). The goal of the present work was to model the effectiveness of trisodium phosphate (TSP) and acidified sodium chlorite (ASC) in pea starch (PS) and alginate coatings, when applied to broiler carcasses during processing for their ability to reduce surface contamination by Salmonella. Since current standards require that carcasses should be free of any residual additives before shipping from the processing plant, the effect of these chemical applications on skin pH and persistence of coatings on the chicken skin were also determined, targeting 60 min for completion of carcass chilling and neutralization of the additives.

5.3. Materials and Methods

5.3.1. Antimicrobial coatings

A 100 ml dispersion of 3.5 % (w/v) pea starch (Nutri-Pea Ltd., Portage-la-Prairie, MB, Canada) was prepared in cold water. The mixture was heated to boiling with mixing and held for 5 min to complete starch gelatinization. The solution was then cooled to room temperature and trisodium phosphate (TSP) (Sigma Chemical Co., St.
Louis, MO) was added (10 % w/v), mixed and homogenized by a Powergen-700 homogenizer (Model GLH 115, PG700, Fisher Scientific International Inc., Hampton, NH) for 5 s at 20 000 rpm. This yielded PS+TSP coating solution.

Calcium alginate coating (alginate+ASC) consisted of two solutions of 100 ml each. Solution (a) was 1 % (w/v) calcium chloride (Sigma Chemical Co., St. Louis, MO) in acidified sodium chlorite (ASC, 1200 ppm) prepared by mixing equal portions of the acid and salt parts of Sanova provided by Alcide Corp. (Redmond, WA.). This solution was used within 30 min as recommended by Alcide Corp. Solution (b) contained 1 % (w/v) sodium alginate (Sigma Chemical Co., St. Louis, MO) dissolved in water and mixed. Coatings free of AMs were prepared following the same procedures but without TSP addition to PS and without ASC addition to alginate. PS+TSP solutions containing 0.5, 1.5, 2.0, 3.5, 4.0 or 4.8 % (w/v) PS, and alginate+ASC with 0.5, 1.0 or 1.5 % (w/v) alginate were prepared as outlined above. These solutions were used for absorptiveness, initial contact angle and viscosity measurements.

5.3.2. Chicken treatment

Unchilled chicken thighs and drumettes (Mehyar et al., 2005) were obtained from a local processing plant immediately after slaughtering and used within 30 min after their arrival. The warm thighs were used for contact angle tests. The drumettes were inoculated with an ampicillin-resistant Salmonella cocktail. Bacterial cultures used to inoculate drumettes were: Salmonella enterica serovars Typhimurium (# 02-8425 and # 02-8421) and Heidelberg (# 271) provided by R. Ahmed (Canadian Science Centre for
Human and Animal Health, Winnipeg, MB, Canada). The three strains were grown separately in tryptic soy broth (TSB) (Difco division of Becton Dickinson, Sparks, MD.) for 24 h at 37°C. Cultures were standardized to an OD$_{600}$ of 0.80 using sterile TSB to yield about 9 log cfu/ml and were combined in equal portions. Inoculations were performed by dipping drumettes in triplicate into 300 ml bacterial suspension containing 7 log cfu/ml for ≤ 15 sec. The drumettes were hung for 10 min to allow bacterial attachment before being dipped for 0.25 min in one of the following solutions: (1) TSP (10% w/v); (2) ASC (1200 ppm); (3) PS+TSP coating; (4) calcium chloride in ASC (solution a) then dipped in sodium alginate solution (solution b) to form the alginate+ASC coating; (5) coatings of 3.5 % (w/v) PS without AMs; or (6) 1 % (w/v) calcium alginate without AMs. Drumettes were weighed before and directly after dipping using a digital balance (Model TR-203, Denver Instrument Co., Denver, CO.) (± 0.05 mg). The drumettes were hung inside a covered glass chamber maintained at 85 % relative humidity with a saturated ZnSO$_4$ solution, and incubated at 4 °C for 120 h. Samples were withdrawn in triplicate for testing after 1, 24, 72 and 120 h incubation.

5.3.3. Changes in drumette pH, weight and viable Salmonella after coating

At each sampling day, the surface pH of the coated drumettes was measured at three different locations using a pH meter equipped with an Isfet surface probe (Type Titan, Sentron Europe B. V., Roden, The Netherlands.) and their average values were recorded. Drumettes were then weighed and their skins were excised and placed in stomacher bags with buffered peptone water (10 g peptone, 5 g NaCl, 3.5 g Na$_2$HPO$_4$, 1.5 g KH$_2$PO$_4$ per liter) and pummeled in a stomacher 400 (A. J. Seward, London, UK) for 3 min to
prepare $10^{-1}$ dilutions. The samples were then serially diluted and plated on pre-poured XLD agar (Oxoid Ltd., Nepean, ON, Canada) containing 100 ppm ampicillin (Sigma Chemical Co., St. Louis, MO), and *Salmonella* were counted after 24 h at 35 °C. Logarithmic reductions were determined by calculating the differences in *Salmonella* numbers between the control and the treated samples.

5.3.4. *Coating absorptiveness*

The method of Han and Krochta (1999) was modified to measure the coating absorption into chicken skin. A plastic ring specimen holder with four screws, similar to that used by Han and Krochta (1999), was used to fix skin samples. Skins of unchilled chicken thighs were excised and used within 10 min. The outer surface of the skin was placed between the base and the ring (diameter 5.8 cm) facing upward in the holder and the ring was secured with screws. The holder with the skin was then weighed ($W_o$) and 5 ml of the PS+TSP coating solution, or 2.5 ml of 1 % (w/v) calcium chloride in ASC (solution a) and 2.5 ml of solution b were applied on the top of the skin. Nine samples were prepared for each coating and the holding units were placed on a flat plate at room temperature to allow the skin samples to absorb the coating solutions. Samples were withdrawn in triplicate at 10, 30 and 60 min after application. Absorption was terminated by wiping away the excess coating solutions which remained on the skin surface with a tissue at each sampling time. The weights of the apparatus holding the skin were recorded before ($W_{wet}$) and after drying ($W_{dry}$). The absorptiveness ($% A_t$) was defined as:

$$% A_t = \frac{(W_{wet} - W_{dry})(W_o - W_e)}{W_o - W_e} \times 100$$

Where $W_e$ is the weight of an empty apparatus without skin.
5.3.5. Contact angle and skin wetting properties

The initial contact angles for the various probe liquids and the coating solutions on the skin were used to determine critical surface energy of skin and absorption profile of coating solutions, respectively. Fresh, unchilled chicken thighs were used and their surfaces were wiped with a dry tissue to remove any residual water. The thighs were cut on one side lengthwise to the bone with a razor blade and a portion of the skin and flesh was removed from the thigh. For testing, the specimens were placed on a rack with adjustable height, and attached to the rack using plastic putty (Play Dough®, Hasbro Canada, Longueuil, QC, Canada). A digital microscope (Intel play QX3 computer microscope, Santa Clara, CA) (10 X magnification) was aimed horizontally to observe the cut chicken surface. Drops of 10 μL of the probe liquids or coating solutions were placed on the skin surface using a microsyringe and the side images of the liquid drops were recorded by a computer after confirming the horizontal level position of samples. In order to account for any asymmetry of the image caused from improper leveling, the contact angles of both sides of each liquid drop were measured and the average values were recorded. All measurements were done inside a closed chamber equipped with an electric fan to circulate the internal air which was equilibrated to 85 % relative humidity with a saturated solution of zinc sulfate (Sigma Chemical Co., St. Louis, MO). The probe liquids used were HPLC grade water (Fisher Scientific International Inc., Hampton, NH), glycerol (Sigma Chemical Co., St. Louis, MO), ethylene glycol (Fisher Scientific International Inc., Hampton, NH) and dimethyl sulfoxide (Sigma Chemical Co., St. Louis, MO). In order to study the effect of PS viscosity on the contact angle, the dynamic viscosity of PS+TSP solutions with different PS concentrations was determined.
using a rheometer (AR 1000, TA instruments, Inc., New Castle, DE). The instrument was operated with parallel plate geometry (plate diameter = 20 mm and gap = 1 mm). Samples were placed in the apparatus and allowed to equilibrate at 25 °C prior to analysis. Measurements were conducted at 3 Pa shear stress and 1 Hz frequency. The relationships between the initial contact angle and PS concentration of PS+TSP coating solution, and between the initial contact angle and the PS+TSP coating solution viscosity were determined.

5.3.6. *Statistical Analysis*

Data obtained were the average values of three replicates for treatments. Each treatment was conducted twice in separate experiments. The statistical analytical system (Version 8.2, SAS Institute Inc., Cary, NC.) was used to compare means of the replicates at each sampling time. A significance level of 5% was used for all analyses. Linear regression analysis for absorption rate was conducted using the data analysis option of a spreadsheet (Microsoft® Excel 2002, Redmond, WA 98052-6399) for the absorption curves (weight vs. time).

5.4. *Results and Discussion*

5.4.1. *Antimicrobial effectiveness, drumette weight and surface pH changes*

PS+TSP and alginate+ASC coatings on chicken appeared clear, continuous and homogenous (Fig. 5.1). Alginate+ASC coating imparted a pale yellowish color to the drumettes while the PS+TSP coating did not induce any noticeable visual changes. Fig. 5.2 shows the reduction in *Salmonella* on drumettes over 120 h at 4 °C. PS not only
maintained the antimicrobial activity of TSP longer but also increased its antimicrobial activity compared to the TSP treatment without PS. Enhanced antimicrobial activity was also exhibited in the alginate+ASC coating. Coatings with TSP and ASC had significantly ($P \leq 0.05$) greater antimicrobial activity than the corresponding solutions without polymers after 24 h. AMs in aqueous solution and in antimicrobial-free coatings were unable to cause $>1.0$ log cfu/g reductions. Previously, Mehyar et al. (2005) reported greater reductions of Salmonella using similar experimental conditions, however, a longer dipping time (1.0 min) was used than in the present study. Results obtained in this study were similar to those reported by Oyarzabal et al., (2004), Schneider et al. (2002b) and Wang et al. (1997).
Figure 5.1. Application of coatings to chicken drumettes: A) 3.5 % (w/v) pea starch (PS) containing 10 % (w/v) trisodium phosphate (TSP); B) 1 % (w/v) alginate containing 1200 ppm acidified sodium chlorite (ASC). Solution (a) contained 1 (w/v) % CaCl$_2$ plus ASC; solution (b) contained 1 % (w/v) sodium alginate.
Figure 5.2. Effect of inclusion of commercial AMs in polymeric coatings on survival of inoculated *Salmonella* on chicken skin during storage at 4 °C for 5 d. TSP = trisodium phosphate, ASC = acidified sodium chlorite, PS = pea starch. Columns with different letters at the same sampling time are significantly (*P* ≤ 0.05) different.
Most (88%) of the PS+TSP coating containing TSP appeared to drip from the skin within 1 h (Table 5.1), whereas the coating without TSP was better retained on the surface for 24h. This suggests that TSP may have reduced the viscosity of the PS coatings and accelerated its drip from the skin, which could have occurred as a result of starch degradation under alkaline conditions (BeMiller, 1965). Calcium alginate coatings with and without ASC were more stable throughout incubation. Initial weight gains of 7.9 and 6.9% during alginate treatments were also greater than that of untreated controls (water) at the end of the tests (Table 5.1). Weights of the drumettes (g) before and after the application of antimicrobial coatings are shown in appendix 7. It was suggested that the acidic nature (pH 5.0) of ASC increased the viscosity of the alginate matrix by enhanced charging of calcium ions and protonation of carboxyl groups (King, 1982). Under these conditions calcium ions can more readily form bridges with the negatively-charged alginate matrix and the repulsion between protonated carboxyl groups of alginate is lowered, which promotes the formation of cross-linked networks (King, 1982).
Table 5.1. Weight changes\(^1\) of chicken drumettes dipped in 10 % (w/v) trisodium phosphate (TSP) with or without 3 % (w/v) pea starch (PS), or in 1200 ppm acidified sodium chlorite (ASC) with or without 1 % (w/v) calcium alginate during storage at 4 °C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% weight change during storage (means ± SD)</th>
<th>0h</th>
<th>1h</th>
<th>24h</th>
<th>72h</th>
<th>120h</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS+TSP</td>
<td></td>
<td>5.12 ± 0.48(^a)</td>
<td>0.62 ± 0.2(^b)</td>
<td>0.12 ± 0.19(^c)</td>
<td>0.35 ± 0.14(^c)</td>
<td>-0.68 ± 0.13</td>
</tr>
<tr>
<td>PS</td>
<td></td>
<td>4.84 ± 0.49(^a)</td>
<td>3.86 ± 0.37(^b)</td>
<td>3.64 ± 0.36(^b)</td>
<td>0.91 ± 0.31(^c)</td>
<td>0.89 ± 0.31(^c)</td>
</tr>
<tr>
<td>TSP</td>
<td></td>
<td>1.49 ± 0.18(^a)</td>
<td>0.52 ± 0.21(^b)</td>
<td>-0.89 ± 0.19(^b)</td>
<td>ND (^2)</td>
<td>ND</td>
</tr>
<tr>
<td>Alginate+ASC</td>
<td></td>
<td>7.86 ± 0.84(^a)</td>
<td>5.32 ± 1.0(^b)</td>
<td>5.25 ± 1.07(^b)</td>
<td>3.98 ± 0.84(^c)</td>
<td>4.05 ± 1.2(^c)</td>
</tr>
<tr>
<td>Calcium alginate</td>
<td></td>
<td>6.88 ± 0.47(^a)</td>
<td>4.98 ± 0.29(^b)</td>
<td>4.1 ± 0.30(^b)</td>
<td>2.5 ± 0.19(^c)</td>
<td>2.6 ± 0.11(^c)</td>
</tr>
<tr>
<td>ASC</td>
<td></td>
<td>1.43 ± 0.15(^a)</td>
<td>1.25 ± 0.16(^a)</td>
<td>-0.55 ± 0.13(^b)</td>
<td>ND (^2)</td>
<td>ND</td>
</tr>
<tr>
<td>Water (control)</td>
<td></td>
<td>1.47 ± 0.04(^a)</td>
<td>1.66 ± 1.1(^a)</td>
<td>-1.93 ± 0.60(^a)</td>
<td>ND (^2)</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^1\)Weight gained or lost/ initial weight × 100.

\(^2\)Not determined.

\(^3\)Means within the same raw with different letters are significantly (\(P \leq 0.05\)) different.

Fig. 5.3 shows that TSP increased and ASC decreased the initial pH of the chicken skin. Although AMs in solution caused significant (\(P \leq 0.05\)) initial changes in the skin pH, the effects were transient and did not last more than 24 h. TSP and ASC in coatings significantly changed the surface pH which was maintained up to 120 h and 72 h, respectively (Fig. 5.3). Gelatinized starch is soluble in aqueous environments (Ratnayake et al., 2002). It slowly dissolves within the pores and follicles of the skin and ostensibly releases TSP into skin, which improves its antimicrobial action. The alginate matrix seemed to be more stable but chlorous acid (HClO\(_2\)) which is formed by sodium chlorite acidification during ASC formulation, may gradually diffuse inside the matrix. As it reaches the higher pH of the skin, chlorous acid is dissolved into the skin structure.
Figure 5.3. Surface pH of chicken drumettes dipped in 10 % (w/v) trisodium phosphate (TSP) and 1200 ppm acidified sodium chlorite (ASC) with and without inclusion in 3.5 % (w/v) pea starch (PS) or 1.0 % (w/v) calcium alginate (Algn).
(King 1982; Oyarzabal et al., 2004; Schneider et al., 2002a). From the results of this study, it is shown that the PS and alginate coatings can prolong the exposure of surface bacteria to the TSP and ASC at high and low pH, respectively, thereby enhancing interference with cell metabolic activity (Siragusa and Dickson, 1992).

5.4.2. Coating absorptiveness

Both the rate and amount of PS+TSP and alginate+ASC coating absorption to the skin depended on the polymer content of the coatings (Table 5.2, 5.3). At concentrations > 3.5 % PS and > 0.5 % alginate, the absorptiveness was significantly ($P \leq 0.05$) reduced during 60 min. At the lowest PS concentration (0.5 %), the amount of coating absorbed by the skin was higher than that of water (Table 5.2). In addition, these values are comparable to the amounts of absorbed water during commercial immersion chilling for 30 min (Thomas and McMeekin, 1984). Retention of residual polymers inside skin crevices, folds and follicles which would not be removed by surface wiping may have contributed to the weight gain. PS+TSP coatings were absorbed quicker than alginate+ASC coating as indicated by the higher absorption rate values (i.e., the slope of the absorption curve) shown in Table 5.3. Both the rate and quantity of PS absorbed was higher compared to alginate at concentrations that were antimicrobial (3.5 % and 1.0 %, respectively) (Table 5.2, 5.3). This may explain the higher and more prolonged (120 h) antimicrobial effectiveness of the PS+TSP coating compared to the alginate+ASC coating (Fig. 5.2). This may also explain the greater antimicrobial activity of TSP in aqueous media against Salmonella on chicken skin (Mehyar et al., 2005). In addition, gelatinized PS at low viscosity may more easily fill skin follicles and pores, bringing TSP directly in contact with more surface bacteria that may have been protected by
irregularities in skin surface topography. Alginate+ASC exhibited higher antimicrobial activity than ASC alone only at \( \leq 72 \) h of treatment (Fig. 5.2B). This could have been due to the method of its application where the skin was first dipped in calcium chloride solution with ASC followed by dipping in an aqueous solution of sodium alginate. The formation of an ASC gradient in the alginate coating may have occurred which altered the amount of ASC exposed to targeted bacteria.

**Table 5.2.** Percentage absorptiveness (\( \% A_t \))\(^1\) of antimicrobial pea starch (PS+TSP) and calcium alginate (alginate+ASC) coatings containing different polymer concentrations applied to chicken skin and held at room temperature for \( \leq 60 \) min

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Polymer concentration (% w/v)</th>
<th>( % A_t ) (means ± SD) after holding (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>PS+TSP</td>
<td>0.5</td>
<td>2.40 ± 0.61(^ a )</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>1.98 ± 0.23(^ a )</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>0.93 ± 0.20(^ b )</td>
</tr>
<tr>
<td>Alginate+ASC</td>
<td>0.5</td>
<td>0.98 ± 0.26(^ b )</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.62 ± 0.15(^ c )</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.45 ± 0.21(^ c )</td>
</tr>
<tr>
<td>Water (control)</td>
<td>0.0</td>
<td>1.8 ± 0.20(^ a )</td>
</tr>
</tbody>
</table>

\(^{a-c}\) Means within the same column with common letters are not significantly \((P > 0.05)\) different.

\(^1\)%\( A_t \) = \((W_{wet}-W_{dry})/(W_0-W_e)\) \times 100; \( W_{wet} \) and \( W_{dry} \) are weights of the absorptiveness apparatus holding the skin before and after drying, respectively; \( W_0 \) initial weight of the skin; \( W_e \) weight of the empty apparatus.
Table 5.3. Linear regression analysis$^1$ of time (from 1 to 120h) and changes in weight of chicken skin coated with antimicrobial pea starch (PS+TSP) and calcium alginate (alginate+ASC) coatings containing different polymer concentrations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Polymer concentration (% w/v)</th>
<th>Absorption rate (g/min)</th>
<th>Y-intercept (initial weight g)</th>
<th>R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS+TSP</td>
<td>0.5</td>
<td>0.066</td>
<td>2.00</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>0.053</td>
<td>1.74</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>0.013</td>
<td>0.81</td>
<td>0.99</td>
</tr>
<tr>
<td>Alginate+ASC</td>
<td>0.5</td>
<td>0.005</td>
<td>0.96</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.006</td>
<td>0.56</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.001</td>
<td>0.46</td>
<td>0.51</td>
</tr>
<tr>
<td>Water (control)</td>
<td>0.0</td>
<td>0.060</td>
<td>1.10</td>
<td>0.98</td>
</tr>
</tbody>
</table>

$^1$ equation: $Y = ax + b$; $Y$ is weight of sample; $x$ is time in min.; $a$ is absorption rate; $b$ is initial absorption.

5.4.3. Coating adhesion and skin wetting properties

Although the contact angle technique was successfully used to determine the critical surface energy of solids such as coated paper surfaces using probe liquids (Han and Krochta, 2001), the method was less successful on chicken skin. None of the probe solutions formed drops on the skin regardless of their surface tension values, which indicates that other factors beside surface energy, such as surface roughness, affected the initial contact angle. Nonetheless, measurements of initial contact angle were successfully used to determine adhesion of liquid materials to food surfaces (Michalski et al., 1997). In the present tests, the formation of discrete drops by the PS+TSP coating solution enabled the contact angle measurement. However, stable drops with measurable angles were unobtainable from alginate+ASC coatings. Due to low viscosity, calcium chloride and sodium alginate solutions diffused over the skin and yielded a thin film.
PS+TSP coating at low viscosity (below 0.37 N s m$^{-2}$) linearly affected the contact angle. At higher viscosity PS+TSP formed a gel at room temperature and the contact angle was no longer dependent on the viscosity (Fig. 5.4). The effect of PS concentrations on the contact angle as an indicator of coating adhesiveness to the skin is shown in Fig. 5.5. In general, increasing the PS concentration increased coating adhesion to the skin. At < 0.5 % PS measurement of the contact angle was not possible, but between 0.5 and 1.5 % PS, the contact angle increased with concentration. At PS levels ranging from 1.5 % through 3.5 %, the contact angle was not affected ($P > 0.05$). At 4.0 %, the contact angle increased to 70 °, whereas at higher concentrations the solutions began to gelatinize to form a soft solid, which invalidated estimation of adhesion by contact angle measurement. Several factors could influence the changes in the initial contact angles shown in Figs. 5.4 and 5.5. Skin roughness was believed responsible for generating unstable liquid drops of the PS+TSP coating solution at low PS concentrations (< 0.5 %). Under these conditions the drops were quickly absorbed and disappeared in the skin. Increasing the PS concentration from 0.5 % to 1.5 % increased the coating viscosity from 0.004 to 0.37 N s m$^{-2}$, which resulted in proportional increases in the initial contact angle. The increase in viscosity gave the coating drops the strength to overcome the effects of skin roughness and become stabilized on the surface. At 1.5 % to 3.5 % PS the initial contact angle was not affected by the increases in viscosity (from 0.37 to 1.0 N s m$^{-2}$) and the resulting contact angle could account for the difference in the surface energies between the skin and the coating solution. In order for the probe solutions to accurately measure critical surface energy of the skin, they should have a viscosity in the range of 0.37 to 1.0 N s m$^{-2}$. At high levels of PS (> 4.0 %) the solutions started to gelatinize and the initial contact angle measured
Figure 5.4. Effect of antimicrobial pea starch (PS+TSP) coating viscosity (0.5 to 4.8 % w/v) on the initial contact angle of coating drops applied to the chicken skin surface.
Figure 5.5. Effect of pea starch (PS) concentration change in antimicrobial pea starch (PS+TSP) coatings on the initial contact angle of the coating drops on the chicken skin surface.
was independent of the surface energy difference. Overall, the adhesion of the coating to
the skin depended on PS concentration and solution viscosity.

Stabilizing TSP and ASC in PS and alginate coatings, respectively, enhanced their
antimicrobial activity against *Salmonella* on chicken skin. PS+TSP caused significant
reductions in bacterial numbers for longer periods than alginate+ASC. This could have
been caused by several factors including: distribution of the AMs within the coatings;
prolonged effects of the treatments on skin pH; coating absorptiveness, and coating
adhesion to the skin. Although PS+TSP was more effective, it was less stable on the skin.
The coating tended to drip from the skin but also absorbed quicker than the
alginate+ASC coating. Since they had transient (≤ 60 min) stability on the skin surface,
but had good skin adhesion, with low absorption and significant antimicrobial activity,
10 % TSP in coatings of 2-4 % (w/v) PS may be of industrial value in applications to
reduce numbers of *Salmonella* on poultry skin.

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CHAPTER 6

Characterization of mechanical properties and release of antimicrobial agents from hydrogels into physiological saline
6.1 Abstract

Mechanical properties of antimicrobial hydrogels and the release of antimicrobial agents to a physiological saline solution was investigated. Pea starch (3 % w/v) with trisodium phosphate (10 % w/v) (PS+TSP) formed a firmer hydrogel (higher storage modulus $G'$) than alginate (0.5 % w/v) with acidified sodium chlorite (1200 ppm) (alginate+ASC). The storage modulus of both hydrogels decreased when placed in the physiological solution and the decrease was faster in the alginate+ASC hydrogel presumably as a result of polymer swelling. The release of TSP from the PS+TSP hydrogel was slower ($D = 2.72 \times 10^9$ m$^2$/s) than the release of ASC from the alginate hydrogel ($D = 6.58 \times 10^9$ m$^2$/s). Addition of TSP to the PS hydrogel increased the dissolution rate and lower $G'/G'_o$ values were recorded during dissolution than from coating without TSP. In contrast, with ASC stabilized in the alginate hydrogel, higher $G'/G'_o$ values were recorded. The gel-sol transition, identified by the cross-over point where $G' = G''$ (the loss modulus) occurred after about 4.1 and 1.4h of the dissolution period for the PS+TSP and alginate+ASC coatings, respectively. Changes in hydrogel mechanical properties and rates of antimicrobial release into the physiological saline fit well with models established and had a correlation coefficient $> 0.95$. These results showed that PS+TSP coatings may be useful for the food industry since they slowly dissolve upon exposure to animal tissue and allow increased contact time with target microorganisms. Performance of the PS and alginate hydrogels on chicken skin could be optimized by using the models established.

Key words: Hydrogel; antimicrobial coating; gel dissolution; antimicrobial release
6.2. Introduction
Hydrogels are a crosslinked network of water soluble polymer chains. To form a hydrogel, it is necessary that polymers be soluble in water or contain abundant hydrophilic groups (e.g. hydroxyl or carboxyl). Reversible transition from polymer solution to a gel, or the sol-gel transition occurs when a critical polymer concentration is surpassed. The critical gel concentration depends primarily on the nature of both the polymer (e.g., its molecular weight, and electrostatic charge) and solvent characteristics (e.g., pH, ions). Due to their ability to be swollen by water, hydrogels are useful in medicine for drug release (Bodmeier et al., 1989, Rajaonarivony et al., 1993), and in biotechnology for tissue engineering (Ahearne et al., 2005).

As a major storage polysaccharide found in cereals, legumes and tubers, starch is comprised of amylose and amylopectin, with its exact composition depending on the plant of its origin. Amylose is a nearly linear polymer of \( \alpha-1,4 \) anhydroglucose units, with molecular weight of \( 10^5-10^6 \) (Ahearne et al., 2005; Galliard and Bowler, 1987). In contrast, amylopectin is a highly branched polymer consisting of short \( \alpha-1,4 \) chains linked by \( \alpha-1,6 \) glucosidic branch points occurring every 25-30 glucose units, with a molecular weight of \( 10^7-10^9 \) (Ahearne et al., 2005; Galliard and Bowler, 1987). When heated in water, generally at 60 °C or above, starch granules gelatinize, and this is characterized by granular swelling, amylose exudation and disruption of the linear-order crystalline structure (Liu, 2005b). Conversion of granular starch to the gel form upon cooling is due to a structural coil-to-helix transition. During this transition, starch polymers are cross-linked by inter- and/or intra-molecular hydrogen bonding of amylose and linear branches of amylopectin (Goodfellow and Wilson, 1990, Liu and Han, 2005,
Liu, 2005a). Basically, a starch gel is a three-dimensional network (Fig. 6.1a) constructed mainly of spring-like strands of chains associated with hydrogen bonds (Ring, 1987).

Alginates can also form hydrogels. Alginate (in the form of a free acid or sodium salt) is a collective term used for a family of polysaccharides prepared mostly from brown algae (Smidsrød and Grasdalen, 1984). Chemically, alginate is a mixture of poly(β-D-mannuronate), poly(α-L-glucuronate), and poly(β-D-mannuronate plus α-L-glucuronate), with its exact composition depending on the algal source. Alginate forms a hydrogel when polymeric chains interact with Ca$^{2+}$ and other divalent as well as trivalent metal ions (Donati, 2005; Rees and Samuel, 1967; Seely and Hart, 1974), according to the so-called “egg-box” model (Grant et al., 1973). The presence of multivalent cations creates ionic associations of alginate chains and causes formation of junction zones (Fig. 6.1b) between helical chains of glucuronate blocks, as well as mannuronic blocks, and those of mannuronic- glucuronate blocks (Grant et al., 1973).
Figure 6.1. Schematic diagram of (a) starch and (b) calcium alginate gel structures (adapted from Schacht, 2004).
The transition from a solid to a liquid phase in food occurs as a result of an increase in molecular mobility (Roos, 1995). Water acts as a plasticizer in hydrophilic polymers and makes the system softer by interfering with intermolecular bonding and causing polymer swelling (Gontard, 1996). The storage modulus ($G'$) is an indicator of network elasticity and the loss modulus ($G''$) reflects the energy dissipated in the system, and the latter increases with increasing network viscosity (Ross-Murphy, 1995). Water diffusion in hydrogels causes changes in their rheological properties; a decrease in $G'$ and an increase in $G''$ occur during polymer swelling (Fennema et al., 2003; Roos, 1995).

Coatings consisting of 3 % pea starch (PS) with 10 % trisodium phosphate (TSP) and 0.5 % alginate with 1200 ppm acidified sodium chlorite (ASC) were significantly more effective in reducing *Salmonella* numbers during 72 and 120h exposure, respectively than when the same antimicrobials were used as aqueous solutions (Chapter 5).

Absorptiveness and physical interactions of these coatings with the skin decreased with increasing polymer concentration (Chapter 5). The improved antimicrobial activity of these coatings could be related the slower release of antimicrobials and the increased contact time of the antimicrobial agents in polymers with the target microorganisms (Han, 2004). The present study was undertaken to find an explanation for the improved antimicrobial activity of the above coatings on animal tissue and to establish models that best describe the changes in properties of the coatings.

Experiments were designed to examine and model coating material mechanical properties and antimicrobial release from hydrogels (antimicrobial coatings) in
physiological saline. Physiological saline was chosen because it is simple environment and is the solute in chicken skin, pork and beef tissue. Most importantly, this paper will illustrate the potential effect of entrapped bioactive molecules on mechanical properties of host hydrogel matrices, which is often an issue overlooked in the design of drug delivery systems. The model is critically important for designing antimicrobial release kinetics from hydrogels so that release matches the growth rate of target microorganisms in a physiologically active environment.

6.3. Materials and Methods

2.3.1 Preparation of starch and alginate hydrogels

Pea starch (PS, 37-40 % amylose, Nutri-Pea Ltd., Portage-la-Prairie, MB) was dispersed in cold water at 3 % (w/v). The dispersion was heated with mixing to boiling and held for 5 min to allow starch granule gelatinization. The solution was then cooled to room temperature (23°C), and 10 g trisodium phosphate (TSP, Sigma Chemical Co., St. Louis, MO) was added, followed by homogenization with a blade-homogenizer (Powergen-700, Fisher Scientific International Inc., Whitby, ON) for 5 s at 20,000 rpm. Fifty ml of the solution was then poured into each of two 200 ml beakers, and left overnight at room temperature to allow the stabilization of gel structure. PS hydrogel without TSP was also prepared and used as a control.

Two solutions were used to prepare the calcium alginate hydrogel. Solution (a) was acidified sodium chlorite (1200 ppm) (ASC, Sanova, Alcide Corp., Redmond, WA) solution containing 1% (w/v) calcium chloride (CaCl₂, Sigma Chemical Co.). The ASC solution was prepared by mixing equal portions of citric acid solution (900 ppm) and
sodium chlorite solution (1100 ppm), and used within 30 min after preparation. Solution (b) contained 0.5% (w/v) sodium alginate (Sigma Chemicals Co.) dissolved in water at room temperature. An assembly (Fig. 6.2) made of polymethyl methacrylate was used to prepare the calcium alginate gel. The assembly consisted of a mould (internal diameter 80 mm), and two fixative rings. A piece of CaCl₂ permeable membrane (dialysis tubing, regenerated cellulose, Fisher Scientific, Nepean, ON) was first attached to the mould by one fixative ring. Solution (b) 50 ml, was poured into the mold, with the membrane side on a flat support. Then the mould was covered by another piece of CaCl₂-permeable membrane, which was fixed onto the mould using the second ring. Two assemblies containing the sodium alginate solution were then immersed in 500 ml solution (a) and taken out after 24h. Self-standing calcium alginate gels containing ASC were obtained after the removal of the rings and membranes. Calcium alginate gels without ASC were also prepared by the same procedure with a 1% (w/v) CaCl₂ solution instead of solution (a).

6.3.2. Mechanical properties of hydrogels in air and in saline

The mechanical properties of the hydrogels were analyzed using an advanced rheometer (AR 1000, TA Instruments, Inc., New Castle, DE) operating in a parallel-plate configuration, at an oscillation frequency of 1 Hz and at 25°C. An upper plate with a diameter of 20 mm was chosen for the analysis. Freshly prepared hydrogel was cut into a cylindrical shape with a height of 10 mm and internal diameter of 20 mm using a cylindrical plastic borer. The gel cylinder was then sliced into specimens with a thickness of 5 mm and was placed centrally on the base plate of the rheometer.
Figure 6.2 Schematic of assembly used for preparing the calcium alginate gel
Then the upper plate was moved by computer guidance towards the top surface of the specimen at decelerating speed in order to avoid any pre-loading deformation. A stress sweep from 0.1 Pa to 10 Pa was used to determine the viscoelastic region of the antimicrobial hydrogel in air.

To study the mechanical properties of hydrogels in vitro, a physiological saline solution was prepared by dissolving 0.86 g of NaCl (Sigma Chemical Co.) in 100 ml tap water. The solution was then adjusted to pH 6.8 with 0.01 N HCl, in order to mimic the conditions of animal tissue. A short tube (height 30 mm, inner diameter 34 mm, outer diameter 64 mm) glued to the base plate of the rheometer was used to hold the solution. After a gel specimen was placed centrally in the tube, saline solution having twice the volume of the specimen was placed inside the tube while the upper plate was in touch with the specimen. A time sweep at a constant shear stress of 1 Pa was run for all specimens in saline.

6.3.3. Determination of TSP and ASC concentrations in saline

After the rheometer started loading, 0.5 ml saline solution was withdrawn periodically and collected in a vial. After all solution samples were collected, the concentrations of phosphate and chlorite anions were determined by an ion chromatography system (IC-1000, Dionex, Sunnyvale, CA), equipped with anion separation column, guard column, and anion suppressor device. A 30 mM NaOH solution was used as the eluent for all samples. The original sample solution was diluted 1000 times prior to injection in the chromatograph for analysis. Throughout the analysis, the injection volume and flow rate were maintained at 50 µl and 1 ml/min, respectively. The peak area for each elution was
calculated by Chromeleo software (Denville, NJ) installed on a computer connected to the chromatograph. External standards (0, 5, 25, 50, 75 and 100 μg/ml) for both anions were used in calibration. It should be noted that the concentration of chlorite anions was determined by subtracting the peak area of chlorine anions in the 1000 times diluted pre-release saline solution from the peak area of both chlorine and chlorite anions in the sample containing ASC. Preliminary results showed that both chlorite anions in ASC and chlorine anions in NaCl eluted at the same time (3 min).

6.3.4. The hydrogel swelling ratio

The swelling ratio of PS and alginate hydrogels with and without the antimicrobial agents was determined after 15 min ($S_o$) and 18 h ($S_\infty$) of immersion in the physiological solution. Specimens of 5 mm thickness and 20 mm diameter were prepared as outlined above. The specimens (6 pieces) were placed in 100 ml saline solution and removed in triplicate at 15 min and 18 h. The weight of each sample was taken directly after removing the sample from the solution-which represented the wet weight ($W_w$), and after drying in an oven at 103 °C for 3h to obtain the dry weight ($W_d$). The swelling ratio (S) was calculated as follows

$$S = \frac{(W_w - W_d)}{W_d} \times 100$$

6.3.5. Modeling mechanical properties and antimicrobial release of hydrogels in saline

Since hydrogels immersed in water often swell until reaching equilibrium (Makino, 1996), it is plausible that the change in mechanical properties of a hydrogel is related to the diffusion of water into the hydrogel. On this basis, it was presumed that the storage
modulus ($G'$) of a hydrogel in saline is a function of time ($t$) according to a Fickian diffusion process. The function can be described by:

$$\frac{G' - G'_{\infty}}{G'_0 - G'_{\infty}} = \sum_{n=1}^{\infty} c_n \exp\left(-q_n^2 \frac{Dt}{l^2}\right)$$  \hspace{1cm} (1)

where $G'_0$ is the initial storage modulus, $G'_{\infty}$ is storage modulus at equilibrium, $c_n$ and $q_n$ are parameters associated with diffusion conditions, $D$ is the diffusivity of water, and $l$ is the radius of the cylindrical specimen (10 mm). Since the top and bottom surfaces of the specimen were not exposed to the saline solutions, the following relationship between $c_n$ and $q_n$ for an infinite cylinder was used:

$$c_n = \frac{4\alpha(\alpha + 1)}{4(\alpha + 1) + (\alpha q_n)^2}$$  \hspace{1cm} (2)

where $\alpha$ is a constant stripping factor, which is equal to the volume ratio of the saline solution to the sample ($\alpha = 2$). Often, $n = 1$ can be taken from Equation 1 without creating significant regression error. Rearranging Equation 1 leads to:

$$\frac{G'}{G'_0} = a + b \exp(-kt)$$

$$a = \frac{G'_{\infty}}{G'_0}, \quad b = c_1(1 - \frac{G'_{\infty}}{G'_0}), \quad k = \frac{q_1^2D}{l^2}$$  \hspace{1cm} (3)

Plots of storage modulus ($G'$) against time ($t$) were averaged in triplicate. The initial storage modulus ($G'_0$) was calculated by linear extrapolation to zero time based on the first three data points. SigmaPlot (v9.01, Systat Software Inc., Point Richmond, CA) was used for nonlinear regression of $G'/G'_0$ vs. $t$ based on Equation 3. From the obtained parameters $a$, $b$, and $k$, the values of interest ($G'_{\infty}$, $\tau$ and $D$) were calculated:
\[ G_\infty = aG_0 \]
\[ c_1 = \frac{b}{1 - a} \]
\[ q_1 = \frac{1}{\alpha} \sqrt{\frac{4(\alpha + 1)(\alpha - c_1)}{c_1}} \quad (4) \]
\[ \tau = \frac{1 - \ln(a/b)}{k} \]
\[ D = \frac{k l^2}{q_1^2} \]

It should be noted that the constant \( \tau \) denotes the time when \( G' / G'_0 \) drops to \([1 + \text{Exp}(-1)]\)
\( G' / G'_0 \), or simply the time when \( G' / G'_0 \) is about to reach equilibrium.

A diffusion model similar to Equation 3 was used for release of antimicrobial agents from hydrogels:

\[ \frac{Y}{Y_\infty} = 1 - b \text{Exp}(-kt) \quad (5) \]
\[ b = c_1, \quad k = \frac{q_1^2 D}{l^2} \]

where \( Y \) and \( Y_\infty \) are the concentration in ppm of antimicrobial agents in solution at time \( t \) and at equilibrium, respectively; \( c_1 \) and \( q_1 \) are parameters associated with the diffusion conditions, \( D \) is the apparent diffusivity of antimicrobial agents into the solution, and \( l \) is the radius of the gel specimen (10 mm). From the parameters \( b \) and \( k \) obtained from nonlinear regression of \( Y/Y_\infty \) vs. \( t \) data averaged from triplicate measurements, mass transfer constants were calculated:
\[ c_1 = b \]
\[ q_1 = \frac{1}{\alpha \sqrt{\frac{4(\alpha + 1)(\alpha - c_1)}{c_1}}} \]
\[ \tau = \frac{1 + \ln(b)}{k} \]
\[ D = \frac{\kappa t^2}{q_1^2} \]

Similarly, the constant \( \tau \) denotes the time when \( Y/Y_\infty \) jumps to \( 1-\exp(-1) \), or the time when \( Y/Y_\infty \) is about to reach equilibrium.

### 6.4. Results and Discussion

In general, a polymeric hydrogel is viscoelastic, and its mechanical properties depend chiefly on chemical structure, concentration, and molecular weight of the polymer, and components in the solvent (Crank, 1975). Pea starch and calcium alginate gels in air exhibited linear elastic regions at a shear stress below 2 Pa (Fig. 6.3). A shear stress of 1 Pa was therefore chosen for all time-sweep experiments. An elastic-to-viscous transition, which reflects the breakdown or disintegration of the 3-dimensional gel structure (Ring et al., 1987; Schacht, 2004) was observed for both hydrogels at a shear stress of 4 Pa.

The starch gel was firmer than the alginate gel throughout the range of all shear stress applied (Fig. 6.3). This observation was presumably due to the high density of cross-links in the starch gel as a result of higher polymer concentration (3\% for the starch gel, compared to 0.5\% for the alginate gel) and its higher molecular weight (\( 10^5 - 10^9 \) g/mole for starch compared to \( 10^4 - 10^6 \) g/mole for alginate).
Figure 6.3. Storage moduli ($G'$) plotted against shear stress ($\tau$) for the pea starch gel with trisodium phosphate (PS+TSP) and the calcium alginate gel with acidified sodium chlorite (alginate+ASC) in air.
The storage modulus of hydrogels in saline decreased with soaking time (Fig. 6.4), following the proposed exponential decay function (Equation 3). The regression worked reasonably well, with all values of R-squared greater than 0.95. The observation of storage modulus decaying with time is not surprising, as many food materials in water show similar relationships between mechanical properties and soaking time (Crank, 1975). Water uptake during gel swelling is highly likely the main cause of gel softening, due to the plasticization effect of water (Makino, 1996; Donald, 2001; Tang and Chinachoti, 1996), by which inter- and/or intra-molecular cross-links loosen up. Sodium and chlorine ions in saline do not play a major role in weakening the cross-links of starch (Tang and Chinachoti, 1996) and alginate gels (Bodmeier and Wang, 1993; LeRoux, 1999; Walkenström, 2003) by electrostatic interaction.

It is of most interest to note that the addition of even a small amount antimicrobial agent may change the mechanical properties of hydrogels. As shown in Table 6.1, the presence of ASC decreased the \( G'_0 \) of the alginate gel from 188 Pa to 134 Pa, whereas the presence of TSP made \( G'_0 \) of pea starch gel drop from 321 Pa to 179 Pa. It is possible that the electrostatic repulsion between citrate anions and carboxyl groups on alginate and the electrostatic repulsion between phosphate anions and hydroxyl groups on starch (Tang and Chinachoti, 1996) prevented chain-chain association during gel formation, and thereby decreased the density of physical cross-links. The higher concentration of TSP (10%) in the starch gel presumably accounted for the sharper drop in \( G_0 \) of the PS+TSP gel. It was not unusual for the PS+TSP gel to exhibit weakness from the start to end of tests in contrast with the PS gel which had significantly strength (Table 6.1).
Figure 6.4 Dimensionless storage moduli ($G'/G_0$) plotted against soaking time ($t$) for (a) pea starch with and without trisodium phosphate, and (b) calcium alginate gel with and without acidified sodium chlorite. Solid lines represent estimates by models.
Accordingly, due to weakened cross-links, the storage modulus of PS+TSP dropped faster and took less time to approach equilibrium. However, this phenomenon was more complex for calcium alginate gels. Compared to alginate, alginate+ASC showed a smaller $G'_0$ but a greater $G'\infty$, smaller diffusivity and required a shorter time to approach equilibrium (Table 6.1). This complication could be the result of interactions involving water plasticization, electrostatic repulsion of citrate anions and negatively charged alginate moieties, and competition for $\text{Ca}^{2+}$ between citrate and alginate carboxyl groups. Due to weakening of the PS+TSP network, the gel-sol transition point ($G' = G''$) occurred at about 4.1 h while it was earlier for the alginate+ASC hydrogel (1.4 h, Fig. 6.5).

The antimicrobial release model proposed in this research worked well (Fig. 6.6), with all R-squared values higher than 0.95. Due to their high charge density, phosphate anions also tend to “structure” water by hydrogen bonding (Tang and Chinachoti, 1996), and facilitate water uptake by the starch gel. Since phosphate anions, together with structured or bound water are associated with starch molecules by hydrogen bonds, it would be difficult for phosphate anions to diffuse from the gel. This would account for the fact that TSP had much less diffusivity ($2.72 \times 10^{-9} \text{ m}^2/\text{s}$) than water uptake diffusivity ($9.58 \times 10^{-9} \text{ m}^2/\text{s}$), which here are equated to "mechanical" diffusivity. Consequently, it took much longer for TSP to reach an equilibrium concentration (Table 6.1). Therefore, PS+TSP would be of particular interest for applications where sustained release of the antimicrobial agent is required.
Figure 6.5 Changes in the storage ($G'$) and loss modulus ($G''$) of pea starch gel with trisodium phosphate (PS+TSP) (a) and calcium alginate gel with acidified sodium chlorite (alginate+ASC) (b) in the saline solution.
Table 6.1. Parameters for the storage modulus and antimicrobial release models of hydrogels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>alginate</th>
<th>alginate+ASC</th>
<th>PS</th>
<th>PS+TSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage Modulus G'₀ (Pa)</td>
<td>188</td>
<td>134</td>
<td>321</td>
<td>179</td>
</tr>
<tr>
<td>Models G'∞ (Pa)</td>
<td>4</td>
<td>32</td>
<td>173</td>
<td>54</td>
</tr>
<tr>
<td>τ (s)</td>
<td>19891</td>
<td>6431</td>
<td>13426</td>
<td>6117</td>
</tr>
<tr>
<td>$D_{\text{water}} \times 10^9$ (m²/s)</td>
<td>9.13</td>
<td>6.89</td>
<td>2.12</td>
<td>9.58</td>
</tr>
<tr>
<td>Antimicrobial Release Models τ (s)</td>
<td>-</td>
<td>5671</td>
<td>-</td>
<td>16329</td>
</tr>
<tr>
<td>$D_{\text{anion}} \times 10^9$ (m²/s)</td>
<td>-</td>
<td>6.58</td>
<td>-</td>
<td>2.72</td>
</tr>
</tbody>
</table>

1 Alginate-ASC: calcium alginate gel without acidified sodium chlorite.
2 Alginate+ASC: calcium alginate gel with acidified sodium chlorite.
3 PS: pea starch gel without trisodium phosphate.
4 PS+TSP: pea starch gel with trisodium phosphate.
5 $G'₀$: initial storage modulus; $G'∞$: storage modulus at equilibrium; τ: time when approaching equilibrium; $D$: diffusivity of water, ASC or TSP.

In contrast, since chlorite anions are present in a more free form in the alginate gel with higher diffusivity, it would be expected that chlorite should diffuse out in a similar manner as water diffuses in. Therefore, the apparent diffusivity ($6.58 \times 10^9$ m²/s) for ASC release would be close to the "mechanical" diffusivity value ($6.89 \times 10^9$ m²/s) and it took about the same time for these to reach equilibrium (Table 6.1). The transfer of ASC from the alginate gel was associated with the transfer of water which is its solvent.

The swelling ratio of the hydrogels in the saline solution is presented in Table 6.3. In both alginate and alginate+ASC hydrogels, water diffusion seemed to cause polymer swelling which was higher in the alginate than the alginate+ASC hydrogel (Tables 6.1, 6.2). This could have caused the greater changes in $G'₀$ observed in the alginate hydrogel than in the alginate+ASC hydrogel. The swelling ratio of alginate was higher than that recorded by Rhim (2004). It is possible that the higher alginate content
Figure 6.6 Concentration of antimicrobial agents in the saline solution for pea starch gel with trisodium phosphate (PS+TSP) and calcium alginate gel with acidified sodium chlorite (alginate+ASC)
and CaCl₂ contents in the networks decreased the intermolecular spaces and the polymer swelling ratio of gels. In PS, the smaller swelling ratio reflected the slower water diffusivity and therefore G' changed gradually during the soaking period (Table 6.1). PS+TSP had the lowest swelling ratio, the highest water diffusivity and changes in G' during the soaking period (Tables 6.1, 6.2). TSP which was present in higher concentration (10 %) than PS (3 %) in the hydrogel retarded PS swelling as the large amount of water which diffused into the gel was bound by both TSP and PS. This decreased the intermolecular interactions and caused a steeper reduction in G' during the soaking period.

Table 6.2. Swelling ratio of calcium alginate and pea starch gels with or without antimicrobials.

<table>
<thead>
<tr>
<th></th>
<th>alginate¹</th>
<th>alginate+ASC²</th>
<th>PS³</th>
<th>PS+TSP⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wₜ at 15min</td>
<td>1.99 ± 0.08 ⁹⁹</td>
<td>2.16 ± 0.15 ⁴</td>
<td>1.94 ± 0.9 ⁴</td>
<td>2.24 ± 0.6 ⁴</td>
</tr>
<tr>
<td>Wₜ at 15min</td>
<td>0.01 ± 0.008 ⁸</td>
<td>0.02 ± 0.001 ⁶</td>
<td>0.05 ± 0.01 ⁶</td>
<td>0.25 ± 0.09 ⁴</td>
</tr>
<tr>
<td>S₀ (%) ⁵</td>
<td>26168 ± 2643 ⁴</td>
<td>11126 ± 934 ⁴</td>
<td>3597 ± 549 ⁴</td>
<td>846 ± 72 ⁴</td>
</tr>
<tr>
<td>Wₜ (g) ⁶</td>
<td>2.27 ± 0.13 ⁴</td>
<td>2.43 ± 0.11 ⁴</td>
<td>2.14 ± 0.4 ⁴</td>
<td>2.48 ± 0.15 ⁴</td>
</tr>
<tr>
<td>Wₜ (g) ⁷</td>
<td>0.01 ± 0.00 ⁴</td>
<td>0.01 ± 0.00 ⁴</td>
<td>0.042 ± 0.01 ⁴</td>
<td>0.22 ± 0.03 ⁴</td>
</tr>
<tr>
<td>Sₐₜ (%) ⁸</td>
<td>29034 ± 941 ⁴</td>
<td>27473 ± 285 ⁴</td>
<td>5158 ± 275 ⁴</td>
<td>1066 ± 196 ⁴</td>
</tr>
</tbody>
</table>

¹ Calcium alginate without acidified sodium chlorite.
² Calcium alginate gel without acidified sodium chlorite.
³ Pea starch gel without trisodium phosphate.
⁴ Pea starch gel with trisodium phosphate.
⁵ S₀: initial (15 min.) swelling ratio.
⁶ Wₜ: Wet weight of the gel.
⁷ Wₜ: dry weight of the gel.
⁸ Sₐₜ: swelling ratio at equilibrium (18 h).
⁹ Means within the same row with common letters are not significantly (P > 0.05) different.
The diffusion/erosion ratio of PS+TSP and alginate+ASC hydrogels immersed in physiological saline is shown in Table 6.3. TSP release from the PS+TSP hydrogel was by erosion for up to 60 min; since relative changes in weight of the AM inside the gels were about the same as changes in the solid content. After 60 min, the release by diffusion increased compared to erosion. In the alginate+ASC hydrogel, the release of ASC was greater due to diffusion than erosion during the immersion period, and its contribution increased further after 8h. The latter gel may have had a more open structure than the PS gel and ASC probably moved more freely inside the alginate+ASC matrix. TSP was likely bound to PS, which resulted in a larger contribution of diffusion in the release of ASC from the alginate+ASC hydrogel than TSP from the PS+TSP hydrogel. The diffusion to erosion ratio for both gels increased as the immersion time increased. This could have been caused by polymer swelling which allowed the saline solution to reach the internal areas of the hydrogels and dissolve larger amounts of TSP and ASC.

Table 6.3. Diffusion/erosion ratio\(^1\) of PS+TSP and alginate+ASC hydrogels during immersion in physiological saline (0.8 %; w/v) for 8h.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>PS+TSP</th>
<th>Alginate+ASC</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.080 ± 0.441</td>
<td>1.291 ± 0.32</td>
</tr>
<tr>
<td>30</td>
<td>1.139 ± 0.481</td>
<td>2.171 ± 0.62</td>
</tr>
<tr>
<td>60</td>
<td>1.137 ± 0.821</td>
<td>1.384 ± 0.023</td>
</tr>
<tr>
<td>120</td>
<td>1.336 ± 0.880</td>
<td>3.434 ± 0.560</td>
</tr>
<tr>
<td>180</td>
<td>1.379 ± 0.019</td>
<td>2.097 ± 0.730</td>
</tr>
<tr>
<td>240</td>
<td>1.528 ± 0.423</td>
<td>5.095 ± 0.478</td>
</tr>
<tr>
<td>300</td>
<td>1.855 ± 0.042</td>
<td>3.711 ± 0.236</td>
</tr>
<tr>
<td>360</td>
<td>1.795 ± 0.051</td>
<td>3.337 ± 0.351</td>
</tr>
<tr>
<td>420</td>
<td>1.871 ± 0.184</td>
<td>3.093 ± 0.978</td>
</tr>
<tr>
<td>480</td>
<td>1.882 ± 0.431</td>
<td>4.114 ± 1.938</td>
</tr>
<tr>
<td>540</td>
<td>1.742 ± 0.374</td>
<td>4.978 ± 1.4813</td>
</tr>
</tbody>
</table>

\(^1\) Diffusion/erosion ratio = (AM content in the gel / initial AM content) / (Solids content of the gel / initial solids content).

\(^2\) Means within the same row with common letters are not significantly (\(P > 0.05\)) different.
Hydrogels of the same composition as those used in the present study had improved antimicrobial activity on chicken skin compared to antimicrobials in aqueous solution (Chapter 5). The observed delay in the release of the antimicrobials from the hydrogel could have been responsible for improved antimicrobial effectiveness because formulation in the gel can increase the contact time with target microorganisms (Han, 2000; Rwei, 2005; Natraj and Sheldon, 2000b). The PS+TSP coating prolonged changes in skin pH but dripped-off from the skin quicker than the alginate coating (Chapter 5). The stability of test gels differed when examined on chicken skin and in 0.86 % saline. Rheological measurements in 0.86 % saline showed that the alginate gel was less stable than the PS gel but the reverse was observed on the chicken surface. It is possible that a more open structure of the alginate gel permitted more rapid diffusion of ASC from the immersed gel than from the gel coating on the skin. The rheological study provided valuable information regarding mechanisms of gel disintegration which likely represent the situation on chicken skin, however, the kinetics of gel disintegration on poultry skin were different from those obtained from the study of gel cylinders in saline (Fig.6.4 and Table 6.2). The established model could be useful in designing antimicrobial coatings with antimicrobial release and disintegration rates that optimize antimicrobial actions.

6.4. Conclusions
Alginate+ASC and PS+TSP hydrogels exhibited different modes of disintegration and different rates of antimicrobial release. PS+TSP delivered TSP slower than ASC from alginate+ASC and had a more gradual disintegration rate. The PS+TSP hydrogel seems
more applicable for the food industry since it brought TSP in contact with the contaminated surface for a longer time and disappeared faster than alginate +ASC. Although PS+TSP had a rapid disintegration rate, it had a low swelling ratio presumably as a result of TSP binding water that would otherwise have been used for intermolecular bond disintegration and overall plasticization.

7.0 Discussion and Conclusions

Contamination of chicken skin by pathogenic and spoilage organisms is a natural result of raw chicken processing procedures (Jones et al., 1991; Gallo et al., 1988; Regez et al., 1988). Recent investigations have been initiated to determine the efficacy of the most commonly used and innovative antimicrobials (Mullerat et al., 1994; Oyarzabal, 2005; Rodriguez-de-Ledesma et al., 1996), but no comparative study has been done. Although commercial antimicrobials are varied in their activity, they have limited effectiveness on poultry skin. Results from Chapter 4 show that sequential treatments with acid and alkaline commercial agents did not add to the inhibition observed compared to single treatments. Increasing the antimicrobial concentration and exposure time had a limited effect on antimicrobial agent activity. This indicates that the resistance of microorganisms on chicken skin could be related to physical entrapment of the cells inside the skin and to biochemical factors associated with cell attachment to chicken skin (Kim et al., 1994; Conner and Bilgili, 1994). The lower effectiveness of antimicrobials on chicken skin compared to biofilms and on surfaces has previously been demonstrated (Tamblyn and Conner, 1997; Oyarzabal et al., 2004; Somers et al., 1994). Inclusion of antimicrobials within films or coatings was found to improve the effectiveness of antimicrobial agents on chicken skin (Natrajan and Sheldon, 2000a,b; Janes et al., 2002).
Interactions between the skin and applied antimicrobial films or coatings have not been investigated. Studying these interactions may explain the improved effectiveness of antimicrobial agents in coatings when used on chicken skin and enable selection of the most effective coating formulations. Ideal coatings should maintain their physical and mechanical properties at the higher relative humidity of the skin and increase contact between the microorganisms and the antimicrobials on the skin without altering skin appearance. Coatings or films should disappear from the surface before the carcass is shipped from the processing plant.

Water as a plasticizer increases molecular motility in hydrophilic polymers (Gontard et al., 1996) presumably by molecular swelling (Fennema et al., 2003) which reduces intermolecular interactions. Pea starch and rice starch films at high relative humidity had lower tensile strength, were capable of greater elongation and had higher moisture content than other similar films. This means that these two hydrophilic films can pick-up moisture from the surrounding environment at high relative humidity which causes polymer swelling and produces weaker but more extensible films (Chapter 3). Similarly, glycerol, a plasticizer used for PS and RS films, swelled amylose molecules present among the amylopectin network, decreased film tensile strength and increased film elongation (Chapter 3). Water diffusing from suspending saline solutions into the PS+TSP and alginate+ASC gels acted as a plasticizer, presumably reducing intermolecular interactions that lead to gel disintegration (Chapter 6). The rheological properties of a gel are a reflection of intermolecular interactions. Water diffusion into the gel causes matrix softening by swelling the polymer (Fennema et al., 2003), which can reduce gel mechanical strength during soaking in a solution (Chapter 6). It is also
seems that the presence of a plasticizer at a moderate concentration is necessary for formation of intermolecular bonding. In the absence of glycerol and at 51% RH, pea starch and rice starch films were very brittle and could not be removed intact from casting dishes, whereas the films were more flexible and easy to handle when glycerol was present. Results also showed that films with glycerol at 51% RH were more flexible and resistant to tearing than films stored at 90% RH (Chapter 3).

Commercial antimicrobials in aqueous solutions were varied in their antimicrobial effectiveness, and their activity was correlated with changes induced in the skin surface pH of chicken (Chapter 4). Alkaline treatment (TSP) was most effective for causing microbial reductions and this was followed by acidic (ASC and Safe2O) and neutral treatments (CPC and Inspexx100). The direct relationship between TSP treatment and skin pH response has been previously established (Capita et al., 2001). The antimicrobial effectiveness of alkaline TSP is well documented (Capita et al., 2002b; Hwang and Beuchat, 1995; Lillard, 1994; Somers et al., 1994), especially against gram negative bacteria, the only types of microorganisms investigated in this thesis. Although the mechanism of TSP action is not known, it is thought that the high pH of TSP dissolves the lipid components in the waxy surface layer of the poultry skin and the outer lipopolysaccharide layer of gram negative bacteria (Somers et al., 1994). It has been reported that physical entrapment rather than microbial attachment to the skin is responsible for the protective effect of the skin (Thomas and McMeekin, 1984; Kim et al., 1969). Dual combinations of tested antimicrobials with Cecure were the most effective antimicrobial combinations. When Cecure was combined with TSP, especially when treatment with TSP was followed by Cecure the greatest microbial reductions
were obtained (Chapter 4). This may indicate the greater ability of TSP to remove the waxy outer layer from the skin compared with the other antimicrobial agents, allowing better access by Cecure to the internal areas of the skin where most of the contaminating microorganisms are located (Thomas and McMeekin, 1984). The improved efficacy of TSP by pre-treatment before use of other antimicrobials on raw chicken has been previously reported and was correlated with the removal of lipid materials (Carneiro de Melo et al., 1998).

TSP followed by LA and LA followed by TSP were less effective than single treatments which indicate involvement of pH in inhibition and that the final pH of the skin surface was a major factor in determining antimicrobial activity (Chapter 4). The effect of TSP on pH was suggested as an important characteristic influencing antimicrobial action (Sampathkumar et al., 2003; Capita et al., 2002b). The surface pH of drumettes treated by the commercial antimicrobial agents tended to return toward normal (pH 6-7) during the initial 24h period and then remained relatively constant throughout the rest of the 5d study (Chapter 4). This neutralization of the antimicrobial treatments on chicken skin was previously reported (Capita et al., 2001) and was suggested to be the result of interaction of the antimicrobial treatments with skin components with high buffering capacity (such as skin proteins) as well as with compounds produced by the metabolic activity of psychrotrophic microorganisms (Capita et al., 2001; Kanellos and Burriel 2005b) such as Brochothrix thermosphacta and TSP tolerant microorganisms. B. thermosphacta was able to grow to $10^6$ cfu/g on TSP-treated chicken during 7 days of refrigerated storage (Salvat et al., 1997). Stabilizing TSP and Sanova in edible coatings was a successful approach to reduce these interactions and prolong the pH effect and
antimicrobial activity on the skin (Chapter 5). Adding substances with greater buffering capacity into the coatings may also increase antimicrobial effectiveness.

Results from the present work showed that TSP at 5% (w/v) was significantly less effective than 10% TSP against a *Salmonella* cocktail (Chapter 4). Xiong et al. (1998b) found that there was no significant difference between TSP at 5 and 10% against *S. Typhimurium*. In addition, the ineffectiveness of CPC against a *Salmonella* cocktail in the current study (Chapter 4) is in contrast with what has been reported in other studies (Cutter et al., 2000; Breen et al., 1995). However, the activity of these agents is affected by the processing parameters used such as application temperature, pressure and contact time (Rodriguez-de-Lebesma et al., 1996; Xiong et al., 1998a; Kemp et al., 2000; Kim and Slavik, 1996). In addition, different bacterial species (Kanellos and Burriel, 2005b), the recovery procedure used (Lillard, 1994) and cell injury (Chantarapanont et al. 2004) affect the outcomes of the decontamination procedure used for chicken skin.

The improved effectiveness of commercial antimicrobial agents on unchilled carcasses which would have open feather follicles and pores and the greater efficacy of the treatments when they were used before inoculation (where skin pores and follicles would be charged with the antimicrobial solutions before inoculation) indicate that microorganisms were protected from the action of antimicrobials when located in skin pores, folds and crevices (Chapter 4). This could also indicate the validity of the assumption that coating-skin adhesion and coating absorption are critical factors in determining the coating antimicrobial activity (Chapter 5). Chicken skin has a rough surface (Thomas and McMeekin, 1982). The surface (critical) energy of chicken skin
could not be measured by the probe solution technique (Han and Krochta, 2001) because the probe solutions were unable to form stable drops on the rough skin due to their low viscosity. However, some coating solutions at relatively higher viscosity formed stable drops, presumably because the increased viscosity overcame the influence of skin roughness (Chapter 5). The increased resistance of pathogenic bacteria to antimicrobial treatments on rough surfaces has been demonstrated on surfaces with artificial crevices (Korber et al., 1997). Therefore, skin microtopography should be taken into consideration when studying the relationship between polymer concentration and desired antimicrobial effectiveness.

Including commercial antimicrobials in edible coatings to be used on raw chicken is innovative. TSP dissolved in pea starch and ASC incorporated in alginate were more effective than when solubilized as solutions (Chapter 5). The use of high amylose PS starch may have value for raw chicken treatment. This starch exhibited more stable mechanical and barrier properties at higher relative humidity than rice starch (which has a lower amylose content) (Chapter 3). The resistance of films that contain high amylose starches to high relative humidity was previously reported (Rindlav-Westling et al., 1998; Stading et al., 2001). Although tolerant to high moisture, PS coating was soluble in aqueous environments and slowly delivered the TSP it contained (Chapter 6). Presumably the increase in contact between target microorganisms and the antimicrobial agent was responsible for the improved antimicrobial activity observed in Chapter 5. The slow release of antimicrobial agents in concert with dissolution of whey protein isolate gels containing antimicrobials was reported earlier (Han, 2004; Xin et al., 2002). The direct relationship between antimicrobial containment in coatings/films and slow
release with improved effectiveness was previously demonstrated (Siragusa and Dickson, 1992; 1993). Additionally, TSP was more effective than ASC in aqueous solutions (Chapter 4), and this probably added to the greater effectiveness of the PS+TSP coating compared to the ASC coating.

The intermediate values of the contact angle for the PS coatings indicated there was good compatibility of the hydrophilic coating with the slightly hydrophobic skin surface (Chapter 5). It was previously reported that both the scalding temperature and defeathering procedure affect the amount of the residual waxy layer on the surface of the skin that determines skin hydrophobicity (Suderman and Cunningham, 1980). Therefore, applicability of the results of this study could be affected by the chicken processing procedure used. Coatings with low adhesion could theoretically flow inside the skin irregularities whereas coatings with a high surface energy difference may not interact or be absorbed inside the skin (Michalski et al., 1997). Similarly, coatings with intermediate absorptiveness should have the highest antimicrobial effectiveness because they could exert prolonged pH effects on the skin (Chapter 5). The improved effectiveness of antimicrobials having increased contact time with targeted microorganisms has been previously reported (Natraj and Sheldon, 2000a). Coatings that were quickly absorbed were rapidly inactivated by skin (by neutralization) components and antimicrobial activity profiles did not match the microbial growth rate. Coatings with very slow absorptiveness may not maintain minimum threshold antimicrobial concentrations (Han 2003). The ideal situation would be to design an antimicrobial coating with active agents that maintain concentrations adequate to eliminate pathogens and control spoilage bacterial growth. The choice of antimicrobial
agent for food use must also be compatible with the predominant chemical characteristics of the food on which it is used (Cagri et al., 2002, 2003) and be active against target microorganisms (Devlieghere et al., 2000).

Alkaline degradation of PS by TSP may have caused the rapid drip of the PS+TSP coating from the skin surface. This was not found with the alginate+ASC coating which had low pH (BeMiller, 1965 and Chapter 5). Inclusion of TSP in PS also reduced gel stability and lowered the initial storage modulus \( (G') \) and caused faster dissolution of this coating compared to PS coating without TSP (Chapter 6). PS+TSP coating also was more absorptive to chicken skin than alginate+ASC and this seemed to be related to the lower viscosity of the PS+TSP coating (Chapter 5). The liquification of PS by TSP addition was considered to be beneficial since the coating could more easily penetrate the skin. Furthermore, the concurrent TSP release with coating disintegration suggested that certain interactions between the TSP and coating matrix occurred, whereas in alginate, ASC was able to more freely move inside the alginate matrix and was released before gel disintegration (Chapter 6). Alginate+ASC disintegration could have been due to weakening of the ionic bonding in the matrix by diffusion of ASC to the skin which resulted in an increase in coating pH (King, 1982). Ionic interactions are reversible and quickly respond to environmental changes of pH and temperature, which influence the physical stability of gels (Ross-Murphy, 1995). The PS+TSP coating disintegrated by exchanges of water from the erosion (aqueous) solution with TSP from the coating, which were under the influence of osmotic gradients (Roos, 1995). The prolonged antimicrobial activity of the PS+TSP coating compared to the alginate+ASC coating
(Chapter 5) was explained by the release data presented in Chapter 6. These properties make the PS+TSP coating ideal for chicken skin application.

In the pea starch and alginate antimicrobial gels, polymer dissolution and antimicrobial agent diffusion occurred concurrently when these gels were exposed to saline solutions (Chapter 6). The dramatic changes in rheological properties and changes in gel size indicate that the coatings underwent changes by erosion kinetics (Chapter 6). The recorded release of antimicrobial agents to the surrounding solution indicates that the systems also underwent diffusion changes (Chapter 6). However, it is important when designing delivery systems to determine by which kinetic mode active agent release occurs or at least be able to determine the relative contribution of the different release mechanisms if more than one mode is involved. Mathematical models for erosion and diffusion release systems have been developed and used in the literature (Parker et al., 2000; Makino et al., 1996; Han and Floros, 1998; Han and Floros 2000a; Han et al., 2000b). These models can be used to predict release time under different processing conditions. In designing a delivery system for an antimicrobial, it is important that the rate of release occurs in a manner that provides sufficient active agent to control growth of spoilage organisms and kills pathogens (Han, 2003). There is no published study that directly relates the rheological properties of antimicrobial polymeric coatings with the release mechanisms or antimicrobial properties. A strong relationship between these variables is expected since the relationship between controlled-release plus antimicrobial effectiveness with polymer cross-linking (or rheological properties) is well documented (Han and Floros, 1998, 2000a; Han, 2004; Sebti et al., 2002). Studies on the relationship
between rheological properties of polymeric coatings with antimicrobial effectiveness are worth investigation.

Addition of commercial antimicrobials to pea starch and alginate coatings changed their mechanical and barrier properties and these changes can affect the quality and shelf-life of treated products (Gennadios et al., 1997; Guilbert et al., 1996). This was expected because of the very high and low pH of TSP and ASC, respectively. Changes in film mechanical properties as a result of antimicrobial agent addition was previously reported (Sebti et al., 2002). Further studies on the effect of antimicrobial agent addition on film physical and mechanical properties and treated film effects on product quality and safety should be further investigated. Case by case studies are recommended on perishable and high fat products such as chicken skin, red meat and nuts.

8.0 Overall Conclusion

From among a group of commercial antimicrobials available, TSP and ASC were found to be the most effective on poultry skin against Salmonella and Campylobacter. Studies showed that early application of antimicrobials during the poultry slaughter process yielded the greatest microbial reductions. The addition of TSP to PS generated an effective antimicrobial coating that dripped from the carcass surface in a timely manner. Incorporating the most effective antimicrobial treatments in coatings increased the effectiveness of agents against pathogens on chicken skin but incorporation of these agents changed the rheological properties of the coatings. It was found that formulations of coatings that had intermediate adhesion to the skin and slow absorptiveness resulted in the best antimicrobial activity. Addition of TSP to the PS destabilized the PS+TSP
coating and increased the dissolution rate of the coating material into a physiological saline solution. In contrast, the addition of ASC to alginate resulted in a coating more stable to dissolution. Water diffusion into the alginate+ASC coating had the same rate of diffusion as ASC out of the coating, and both water and ASC reached equilibrium concentrations inside the alginate+ASC coating at approximately the same time. In contrast, water diffusion into the PS+TSP coating was higher than the diffusion of TSP out of the coating. Water uptake by the PS+TSP and alginate+ASC coatings during dissolution induced polymer swelling. This was likely the cause of hydrogel softening and a decrease in G'. The ASC coating, which had a lower polymer concentration than the PS+TSP coating, exhibited greater water diffusion and had an earlier sol-gel transition time than PS+TSP. Both antimicrobial agent release and the change in G' during dissolution followed exponential functions. The models established fit very well with the experimental data. The slower interaction of the PS+TSP coating with the chicken skin could have been responsible for the higher antimicrobial activity of the PS+TSP coating than that of the ASC coating. This probably increased the contact time of the antimicrobial agent with target microorganisms. Among antimicrobial hydrogels tested, PS containing TSP was the most effective and should find application by the poultry industry to restrict bacterial contamination.
9.0 Literature cited


Rodriguez-de-Ledesma, A. M., Riemann, H. P. and Farver, T. B. 1996. Short-time treatment with alkali and/or hot water to remove common pathogenic and spoilage bacteria from chicken wing skin. J. Food Prot. 59(7):746-750.


Appendix 1 Effect of antimicrobial treatments on surface pH of chilled drumettes after being inoculated with *E. coli* O157:H7 and stored at 4 °C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH during storage (h)²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TSP (10 %)</td>
<td>11.33abcd</td>
</tr>
<tr>
<td>LA (3 %)</td>
<td>3.33e</td>
</tr>
<tr>
<td>TSP (10 %) followed by LA (3 %)</td>
<td>5.26d</td>
</tr>
<tr>
<td>LA (3 %) followed by TSP (10 %)</td>
<td>10.36b</td>
</tr>
<tr>
<td>TSP (10 %) dissolved in 2% NaCl</td>
<td>10.56b</td>
</tr>
<tr>
<td>LA (3 %) dissolved in 2 %NaCl</td>
<td>3.56e</td>
</tr>
<tr>
<td>Control (water)</td>
<td>6.36c</td>
</tr>
</tbody>
</table>

1️⃣ 20 and 10 min dips in solutions of trisodium phosphate (TSP) and lactic acid (LA), respectively.

2️⃣ Means within the same column with different letters are significantly (p < 0.05) different, n=3. Data above are for results presented in Figure 4.3.
Appendix 2 Surface pH of unchilled drumettes treated\(^1\) with commercial antimicrobials and stored at \(7^\circ\) C \(\leq\) 5 days.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Storage period (h) (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Water (control)</td>
<td>6.16(^b)</td>
</tr>
<tr>
<td>TSP (10%)</td>
<td>9.92(^a)</td>
</tr>
<tr>
<td>Sanova</td>
<td>3.86(^c)</td>
</tr>
<tr>
<td>Safe(_2)O</td>
<td>5.00(^d)</td>
</tr>
<tr>
<td>Cecure</td>
<td>6.03(^bc)</td>
</tr>
<tr>
<td>Inspexx100</td>
<td>5.94(^c)</td>
</tr>
</tbody>
</table>

\(^1\) By dipping in the antimicrobial for 1 min.
\(^2\) Means within the same column with different letters are significantly \((p<0.05)\) different, \(n=6\). Data above are for results presented in Figure 4.4.
Appendix 3  Growth of naturally present pseudomonads and psychrotrophs on unchilled drumettes (38-40 °C) during storage at 7 °C for up to 120 h following dipping in 0.12 % Acidified sodium chlorite (ASC) for 1 min. Means at the same incubation time with different letters are significantly (p < 0.05) different.
Appendix 4 Growth of naturally present pseudomonads and psychrotrophs on unchilled drumettes (38-40 °C) during storage at 7 °C for up to 120 h following dipping in Safe2O for 1 min. Means at the same incubation time with different letters are significantly (p < 0.05) different.
Appendix 5 Growth of naturally present pseudomonads and psychrotrophs on unchilled drumettes (38-40 °C) during storage at 7 °C for up to 120 h following dipping in Cecure for 1 min. Means at the same incubation time with different letters are significantly (p < 0.05) different.
Appendix 6 Growth of naturally present pseudomonads and psychrotrophs on unchilled drumettes (38-40 °C) during storage at 7 °C for up to 120 h following dipping in Inspexx 100 for 1 min. Means at the same incubation time with different letters are significantly (p < 0.05) different.
### Appendix 7. Weight of drumettes (g) before and after coating with PS+TSP (3 %; w/v) and alginate+ASC (1%; w/v) during storage at 4 °C for 120h.

<table>
<thead>
<tr>
<th>Coating</th>
<th>Replicate</th>
<th>Before coating</th>
<th>After coating</th>
<th>Gain/loss</th>
<th>Weight of drumettes (g) in grams at (h)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>1</td>
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<td>5.47</td>
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</tr>
<tr>
<td></td>
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<td>39.83</td>
<td>45.15</td>
<td>5.32</td>
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</tr>
<tr>
<td></td>
<td>III</td>
<td>35.12</td>
<td>39.69</td>
<td>4.57</td>
<td>35.7</td>
</tr>
<tr>
<td></td>
<td>Av</td>
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<td>SD</td>
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<tr>
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<tr>
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<td>I</td>
<td>37.98</td>
<td>44.93</td>
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