AN ABSTRACT OF THE DISSERTATION OF

Austin Cole Lowder for the degree of Doctor of Philosophy in Food Science and Technology presented on June 3, 2013

Title: Addressing Sodium Reduction and Pathogen Internalization in Non-Intact Whole Muscle Beef: Evaluation of Dehydrated Collagen and Hydrostatic Pressure as Impact Technologies

Abstract approved:

____________________________________________________________________

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These studies focused on maximizing the nutrition and safety of raw whole muscle beef while minimizing quality and shelf-life alteration. The first study used response surface methodology to predict fluid loss from injected beef strip loins and steaks as influenced by levels of salt and sodium phosphates (SP) in the injection brine. Also, a beef-sourced dehydrated beef protein (DBP) water binding ingredient was evaluated. Paired U.S. select beef strip loins were quartered before being injected to 110% of initial weight with brines containing various concentrations of salt and SP (CON) or salt, SP and 5% DBP. Steaks were sliced, overwrapped and stored in the dark for 4 d. Purge values ranged from 0.6% to 4.6% for CON and 0.3% to 2.1% for DBP. Fluid losses when accounting for the fluid lost from injection to slicing were as high as 6.8% for CON brines, but only 2.8% for DBP brines. The models on
fluid loss generated from the study and the DBP product could help processors achieve acceptable purge while reducing sodium use.

The second study sought to determine the viability of DBP as a replacement for SP in beef injection brines. U.S. Select strip loins (n=20) were injected to 110% of their initial weight with a brine containing 3.6% salt and 4.5% sodium phosphate (CON) or 3.6% salt and 5% dehydrated beef protein (DBP). DBP loins had less fluid loss after 30 min. Steaks from both treatments lost similar amounts of fluid during storage. Total fluid loss was lower for DBP injected product. Lipid oxidation (TBARS) products were 0.23 – 0.60 mg/Kg higher for DBP steaks, but still within acceptable limits (<1.0 mg/Kg). DBP steaks were slightly less red than CON steaks according to instrumental measurements. Sensory panel evaluation, however, indicated no differences in redness. DBP steaks were less tender according to trained sensory panel. Results indicated the DBP to be effective in increasing brine retention and a suitable alternative to phosphates when used in brines injected into beef strip loins.

The third study aimed to determine the interactions between salt (NaCl), sodium phosphate (SP) and mild HPP in brine injected beef, as previous studies on comminuted products have shown the action of hydrostatic pressure to increase the effectiveness of salt and phosphates on protein functionality. Beef strip loin segments were injected to 10% over initial weight with solutions containing water and various levels of salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution). Pieces from the loin sections were exposed to varying pressure levels (0.1, 152 or 303 MPa) and evaluated for selected quality and biochemical characteristics. Use of SP and pressure application increased pH additively. $L^*$ values were increased by pressure and decreased by SP. Redness ($a^*$) increased at 303 MPa. Purge increases due to
pressure were mitigated by SP. Pressure application at 303 MPa reduced total and sarcoplasmic protein solubility. This study determined there was no interaction between salt or SP and mild HPP. However, results indicate SP may have use in preventing yield loss due to HPP.

The final study attempted to use high pressure-low temperature (HPLT) processing to achieve *E. coli* O157:H7 inactivation in non-intact, whole muscle roasts while maintaining acceptable quality characteristics. Beef *semitendinosus* was internally inoculated with a four strain *E. coli* O157:H7 cocktail and frozen at -30 °C, then subjected to 550 MPa for 4 min (HPLT). Compared to frozen, untreated control (F), HPLT reduced microbial population by 1.7 log colony forming unit (CFU)/g on selective media (Cefixime-Tellurite Sorbitol MacConkey agar) and 1.4 log on non-selective media (Trypticase soy agar). High pressure without freezing (550 MPa/4 min/3 °C) increased pH and lightness while decreasing redness, cook yield, tenderness, and protein solubility. High pressure low temperature, aside from a 4% decrease in cook yield, had no significant effects on quality relative to the control. High pressure low temperature is an effective way to subject red meat to high pressures without compromising quality. However, its diminished effect on internalized *E. coli* limits its effectiveness as a food safety intervention.
Addressing Sodium Reduction and Pathogen Internalization in Non-Intact Whole Muscle Beef: Evaluation of Dehydrated Collagen and Hydrostatic Pressure as Impact Technologies

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Dr. Christina Mireles DeWitt was involved with the conception, experimental design and writing of all chapters. Dr. Carla Goad was the primary statistician for chapters 2 and 3. Dr. Xingqiu Lou and Dr. J. Brad Morgan were involved with the conception of chapters 2 and 3. Chern Lin Koh and Alisha Parsons Deakins assisted with gathering experimental data for chapters 2 and 3. Dr. Joy Waite-Cusic helped to design, interpret data for, and review chapter 5.
# TABLE OF CONTENTS

1. Review of Literature ............................................................................................................. 1
   1.1 Introduction .................................................................................................................. 1
   1.2 Effect of sodium compounds on myofibrillar proteins ................................................. 2
      1.2.1 Sodium chloride .................................................................................................. 2
      1.2.2 Sodium polyphosphates ...................................................................................... 3
      1.2.3 Injection of sodium compounds into whole muscle cuts ..................................... 7
      1.2.4 Effects of salt and sodium phosphate injection on shelf life and sensory characteristics ......................................................................................................................... 11
   1.3 Comparison of sodium free water binding adjuncts to salt and sodium phosphates .......................................................................................................................... 14
      1.3.1 Alternative ingredients and pH adjustment .......................................................... 14
      1.3.2 Collagen extracts as water binding adjuncts ......................................................... 17
   1.4 Use of high pressure processing (HPP) on whole muscle beef ..................................... 20
      1.4.1 Effects of HPP on muscle proteins ...................................................................... 21
      1.4.2 pH ...................................................................................................................... 24
      1.4.3 Texture ................................................................................................................ 24
      1.4.4 Color ................................................................................................................... 27
      1.4.5 Water holding capacity and cook loss ................................................................... 28
      1.4.6 Lipid oxidation .................................................................................................... 30
      1.4.7 The case for HPP of raw beef .............................................................................. 31
      1.4.8 High pressure-low temperature treatment of raw beef ....................................... 38
   1.8 Conclusions .................................................................................................................... 39

2. Response surface modeling to predict fluid loss from beef strip loins and steaks injected with salt and phosphate with or without a dehydrated beef protein water binding adjunct ......................................................................................................................... 41
   2.1 Abstract ....................................................................................................................... 42
   2.2 Introduction .................................................................................................................. 42
   2.3 Materials and Methods ............................................................................................... 44
      2.3.1 Collection of select beef strip loins ..................................................................... 44
      2.3.2 Brines .................................................................................................................. 44
      2.3.3 Injection ............................................................................................................... 45
TABLE OF CONTENTS (Continued)

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3.4 Equilibration, slicing, and packaging</td>
<td>45</td>
</tr>
<tr>
<td>2.3.5 Storage</td>
<td>47</td>
</tr>
<tr>
<td>2.3.6 Purge</td>
<td>47</td>
</tr>
<tr>
<td>2.3.7 Experimental design</td>
<td>48</td>
</tr>
<tr>
<td>2.3.8 Statistical analysis</td>
<td>49</td>
</tr>
<tr>
<td>2.4 Results</td>
<td>50</td>
</tr>
<tr>
<td>2.4.1 Loin and Steak pH</td>
<td>50</td>
</tr>
<tr>
<td>2.4.2 Fluid loss</td>
<td>51</td>
</tr>
<tr>
<td>2.4.3 Purge and ( \text{Purge}_{\text{total}} )</td>
<td>52</td>
</tr>
<tr>
<td>2.4.4 Brine loss total</td>
<td>54</td>
</tr>
<tr>
<td>2.5 Discussion</td>
<td>55</td>
</tr>
<tr>
<td>2.6 Conclusions</td>
<td>64</td>
</tr>
<tr>
<td>3. Evaluation of a dehydrated beef protein to replace sodium-based phosphates in injected beef strip loins</td>
<td>65</td>
</tr>
<tr>
<td>3.1 Abstract</td>
<td>66</td>
</tr>
<tr>
<td>3.2 Introduction</td>
<td>66</td>
</tr>
<tr>
<td>3.3 Materials and Methods</td>
<td>67</td>
</tr>
<tr>
<td>3.3.1 Collection of beef strip loins</td>
<td>67</td>
</tr>
<tr>
<td>3.3.2 Brines</td>
<td>68</td>
</tr>
<tr>
<td>3.3.3 Injection</td>
<td>68</td>
</tr>
<tr>
<td>3.3.4 Equilibration, slicing, packaging and storage</td>
<td>69</td>
</tr>
<tr>
<td>3.3.5 Headspace analysis, proximate composition and pH</td>
<td>70</td>
</tr>
<tr>
<td>3.3.6 Purge</td>
<td>70</td>
</tr>
<tr>
<td>3.3.7 Lipid oxidation</td>
<td>72</td>
</tr>
<tr>
<td>3.3.8 Microbial analysis</td>
<td>73</td>
</tr>
<tr>
<td>3.3.9 Shear force</td>
<td>73</td>
</tr>
<tr>
<td>3.3.11 Instrumental color</td>
<td>74</td>
</tr>
<tr>
<td>3.3.12 Cook yield</td>
<td>75</td>
</tr>
<tr>
<td>3.3.13 Trained sensory analysis</td>
<td>75</td>
</tr>
<tr>
<td>3.3.14 Statistical analysis</td>
<td>75</td>
</tr>
<tr>
<td>3.4 Results and Discussion</td>
<td>76</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS (Continued)

5. High pressure-low temperature processing of beef: effects on survival of internalized *E. coli* O157:H7 and quality characteristics ..................................................119

5.1 Abstract ........................................................................................................120

5.2 Introduction ....................................................................................................120

5.3 Materials and methods ..................................................................................123

5.3.1 Inoculum preparation ..............................................................................123

5.3.2 Raw material preparation and inoculation .............................................124

5.3.3 High pressure processing .......................................................................125

5.3.4 Microbiological sampling and plating ..................................................125

5.3.5 Purge and color analysis ........................................................................126

5.3.6 Cooking characteristics and tenderness .............................................127

5.3.7 Protein solubility ....................................................................................127

5.3.8 Sulfhydryl content ...............................................................................128

5.3.9 Experimental design and statistical analysis .......................................128

5.4 Results and discussion .................................................................................129

5.4.1 Microbiological data ..............................................................................129

5.4.2 Quality ....................................................................................................131

5.5 Conclusions ..................................................................................................142

6. General Conclusions ......................................................................................143

7. References ......................................................................................................144
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>Response surfaces of predicted $Brine\ loss_{30\ min}$ (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Contour plots of predicted $Purge$ (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Contour plots of predicted $Purge_{total}$ (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Response surfaces of predicted $Brine\ loss_{total}$ (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>CIE $a^*$ values of steaks (n = 10/treatment) injected with a salt/phosphate brine (Control) or a brine containing salt and a dehydrated beef protein (DBP). Day = day of retail display following 4 d of dark storage.</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Trained color panel ratings for muscle color of steaks (n = 10/treatment) injected with a salt/phosphate brine (Control) or a brine containing salt and a dehydrated beef protein (DBP).</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>Trained color panel ratings for overall color of steaks (n = 10/treatment) injected with a salt/phosphate brine (Control) or a brine containing salt and a dehydrated beef protein (DBP).</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Pressure x sodium phosphate (SP) interaction pH values for beef from strip loins injected to 110% of initial weight with solutions containing salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature).</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>$L^*$ (lightness) values of beef from strip loins injected to 110% of initial weight with solutions containing salt at (a) 0, (b) 2 or (c) 4% of solution and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature).</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES (Continued)**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 4.3</td>
<td>109</td>
</tr>
<tr>
<td>Pressure x sodium phosphate (SP) interaction for $a^*$ (redness) values of beef from strip loins injected to 110% of initial weight with solutions containing salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature).</td>
<td></td>
</tr>
</tbody>
</table>

| Figure 4.4  | 110 |
| Pressure x sodium phosphate (SP) interaction for Purge values of beef from strip loins injected to 110% of initial weight with solutions containing salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature). |

| Figure 4.5  | 111 |
| (a) Pressure x sodium phosphate (SP) and (b) salt x SP interactions for Purgetotal values of beef from strip loins injected to 110% of initial weight with solutions containing salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature). |

| Figure 4.6  | 117 |
| Thiobarbituric reactive substances (TBARS) values of beef from strip loins injected to 110% of initial weight with solutions containing salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature). |

| Figure 5.1  | 138 |
| Cook yield, peak shear force and moisture content following cooking of beef semitendinosus steaks subjected to freezing (F; -25 °C), high pressure low temperature treatment (HPLT; <-30 °C/550 MPa/4 min) or high pressure processing (HPP; 3 °C/550 MPa/4 min) |

| Figure 5.2  | 140 |
| Protein solubility of beef semitendinosus subjected to freezing (F; -25 °C), high pressure low temperature treatment (HPLT; <-30 °C/550 MPa/4 min) or high pressure processing (HPP; 3 °C/550 MPa/4 min) following extraction in high (total soluble; 0.6 M) and low (water soluble; 0.03 M) ionic strength buffer solutions |

| Figure 5.3  | 141 |
| Reactive/total sulfhydryl (SH) ratio of beef semitendinosus subjected to freezing (F; -25 °C), high pressure low temperature treatment (HPLT; <-30 °C/550 MPa/4 min) or high pressure processing (HPP; 3 °C/550 MPa/4 min) following extraction in high (total soluble; 0.6 M) ionic strength buffer solution |
LIST OF TABLES

Table 2.1  Least squares means of fluid loss variables of loins and steaks injected with various brines containing salt and sodium phosphate with or without a dehydrated beef protein.................................................................46

Table 2.2  Least squares means of pH for the main effect of brine combination before and after injection and on d 5 of storage of loins and steaks injected with varying levels of salt and sodium phosphates with or without a dehydrated beef protein......51

Table 2.3  Coefficients for substitution into Eq. (1)$^a$ for Purge and Brine loss measurements of loins and steaks injected with a final concentration of up to 0.36% salt and up to 0.45% sodium phosphates with or without a dehydrated beef protein (DBP)$^b$ ..............................................................................................................................................55

Table 3.1  Main effect least squares means of moisture, fat, ash, protein and pH of beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein.................................................................................................................................78

Table 3.2  Least squares means of Brine Loss$_{30\text{ min}}$ and Purge$_{30\text{ min}}$ of beef strip loins injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein......................................................................................................................................................80

Table 3.3  Main effect least squares means of Purge, Purge$_{\text{total}}$, Brine Loss$_{\text{total}}$ of beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein......................................................................................................................................................81

Table 3.4  Least squares means for the two way interaction of treatment x storage day of TBARS$^c$ values, aerobic plate count (APC) and Warner Bratzler shear force (WBSF) values$^d$ of beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein......................................................................................................................................................83

Table 3.5  Main effect least squares means of CIE $L^*$ and $b^*$ values of beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein......................................................................................................................................................85

Table 3.6  Main effect least squares means of Cook Yield and Cook Yield$_{\text{adj}}$ for beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein......................................................................................................................................................91
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 3.7</td>
<td>92</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>103</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>108</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>115</td>
</tr>
<tr>
<td>Table 5.1</td>
<td>131</td>
</tr>
<tr>
<td>Table 5.2</td>
<td>133</td>
</tr>
<tr>
<td>Table 5.3</td>
<td>137</td>
</tr>
</tbody>
</table>

Table 3.7  Main effect least squares means of sensory characteristics of beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein

Table 4.1  Main effect LS means for $Purge_{inj}$ and $Brine Loss_{inj}$ of loin segments injected to 10% over initial weight with varying levels of salt and/or sodium phosphates (SP)

Table 4.2  Redness ($a^*$ values) of beef pieces from loins injected to 10% over initial weight with varying levels of salt and/or sodium phosphates (SP) and subjected to high pressure processing at 0.1, 152 or 303 MPa as affected by salt/SP levels

Table 4.3  Sarcoplasmic, myofibrillar and total protein solubility and reactive, total and reactive/total sulfhydryl (SH) ratio of beef pieces from loins injected to 10% over initial weight with varying levels of salt and/or sodium phosphates (SP) as affected by applied high pressure processing (HPP)

Table 5.1  *E. coli* O157:H7 populations (mean ± standard deviation) recovered on selective (CT-SMAC$^c$) and non-selective (TSA$^c$) media from frozen (F; -30 °C) or frozen, high pressure-low temperature (HPLT; 550 MPa/-30 °C/4 min) treated beef pieces inoculated internally

Table 5.2  Least squares means for $L^*$ (lightness), $a^*$ (redness), $b^*$ (yellowness), hue angle, chroma (saturation), ratio of reflectance spectra at 630 and 580 nm and total color difference ($\Delta E$) of beef semitendinosus steaks subjected to freezing (F; -25 °C), high pressure low temperature treatment (HPLT; <-30 °C/550 MPa/4 min) or high pressure processing (HPP; 3 °C/550 MPa/4 min)

Table 5.3  Least squares means for pH of beef semitendinosus subjected to freezing (F; -25 °C), high pressure low temperature treatment (HPLT; <-30 °C/550 MPa/4 min) or high pressure processing (HPP; 3 °C/550 MPa/4 min)
1. Review of Literature

1.1 Introduction

Meat is composed of approximately 75% water. This water is held in three distinct phases. Free water, which is held entirely by capillary action, will flow freely out of muscle upon physical action or heating. Immobilized (sometimes referred to as entrapped) water, is attracted by the hydrophilic side groups of amino acids, but not actually bound to them. This attraction becomes weaker as the distance of the water from the charged side group increases. Bound water is bound tightly to hydrophilic amino acid side groups and is difficult to remove using physical force or conventional heating. While bound water will vary little from slaughter to consumption, the amount of water held as either immobilized or free can be altered by various factors. The ability of muscle to bind an increasing amount of water as immobilized water as opposed to free water will determine its retention of water during processing and cooking. This aspect of muscle is termed its water holding capacity. Water holding capacity is very important for both economic impact and consumer appeal; it will vary greatly depending on a number of factors, including: species, breed, sex, genetics, diet, temperament, stress, post-slaughter chilling and meat ageing. Additionally, various non-meat ingredients may be added during processing that alter the functional properties of muscle proteins and affect their ability to bind water. Most common among these ingredients are sodium based compounds, including sodium chloride (NaCl) and sodium phosphates (SP). These compounds are powerful tools for processors to use to increase yield and lengthen shelf-life, but they are detrimental to the nutritional value of processed meat products. Epidemiological studies have linked excessive dietary sodium to an increased risk of cardiovascular disease; both salt and SP contain an appreciable amount of sodium (39.4 and ~31%, respectively) and are mainly responsible for processed meat’s contribution of about 21% of the US dietary sodium intake. Because of this, many food companies,
including many meat focused companies, have attempted wholesale reductions of the sodium content of their processed products. This prompts a balancing act between the sodium content of the product and the potential detriments to flavor, juiciness, tenderness, color, oxidative stability and food safety that result from reducing sodium based non-meat ingredient usage. A number of alternative ingredients and processing techniques have been investigated for their ability to replace the functional attributes of salt and SP in meat systems. In this text, the actions of salt and SP, as well as their impact on quality, shelf-life and yield of whole muscle roasts will be discussed. The use of potential sodium reduction strategies, including non-meat ingredients like collagen extracts and the process of high pressure processing (HPP), will also be reviewed.

1.2 Effect of sodium compounds on myofibrillar proteins

1.2.1 Sodium chloride

Sodium chloride is an ion consisting of one atom of sodium (Na\(^+\)) and one atom of chlorine (Cl\(^-\)) and is commonly referred to as salt. Although the noun “salt” can refer to a number of charged ions, its use in common vernacular, as well as any reference in this text, will refer to the sodium chloride ion. While salt has been used for thousands of years as a preservative, its effects on myofibrillar proteins and, in turn, water holding capacity, are a more recent discovery. When salt solutions of varying concentrations are introduced into post rigor muscle a swelling is observed, especially at salt concentrations of 0.6 M and above (Offer & Trinick, 1983). Water molecules, attracted by the hydrophilic side chains of amino acids are pulled into the spaces opened by the swelling. This phenomenon, known as hydration, is responsible for the increase in water holding capacity seen by salt infused muscle. The earliest description of this mechanism
(Hamm, 1960) asserted that electrostatic repulsion was responsible. That is to say, the negatively charged chloride ion would preferentially bind to the positively charged amino acid side groups. As the number of chloride anions bound to positively charged side groups increases, so does the electrostatic repulsion, expanding the filament lattice to allow swelling and hydration. This type of expansion is limited by the number and strength of the actomyosin cross-bridges remaining after rigor-mortis. Offer and Knight (1988) present their own theory of the lattice expansion mechanism. According to their theory, salt acts as a “structure-breaker”, depolymerising myosin and dissociating the thick filament. The myosin molecules from the H zone, which were not bound to actin, are free to either diffuse out of the myofibril or attach to unbound sections of the actin filaments. Actin bound myosin molecules, however, stay attached to the actin filaments, their tales free to range between neighboring cross-bridges. As the salt solution becomes more concentrated, more of the thick filament dissociates until it is completely gone. At this point the myosin tails have a larger range of movement and the actomyosin cross-bridges that restricted swelling are gone. Osmotic pressure is increased, pulling water into the system.

Also of importance are the actions of individual Na$^+$ ions, which are pulled close to the filament surfaces. The unequal distribution of ions within the system is another means to create osmotic pressure, which acts to pull water molecules into the filament lattice.

1.2.2 Sodium polyphosphates

There are numerous phosphate manufacturers, each producing their own proprietary blends of different chain-length phosphates, along with the commonly used pure sodium tripolyphosphate. Chain length of phosphates will determine their
functional characteristics, including solubility, solution pH and ionic strength, as well as their ability to solubilize acto-myosin and chelate divalent cations (Trout & Schmidt, 1986b; Knipe, 2003, 2004). Blends are generally application specific. Pure tripolyphosphate, which is a common solution for processors because of its relatively low cost and ease of solubility, can be reduced to the active diphosphate by myosin-ATPase, but lacks the robust functionality of blended phosphates. The use of the term sodium phosphates in this text will be a blanket reference to food grade sodium phosphates, including proprietary blends and pure tripolyphosphate.

1.2.2.1 pH effects

The first of these is pH alteration. Typically, brines containing sodium phosphates are highly alkaline (pH 6.5 – 9.0 depending on chain length composition) and will slightly increase the pH of meat, adding more negatively charged amino acid side groups and increasing electrostatic repulsion. Although this will have an effect on water holding capacity, it is only part of the mechanism employed, because, as pointed out by Offer and Knight (1988), different polyphosphates at a constant pH exert varied effects on water holding capacity.

1.2.2.2 Myosin solubilization

Phosphates, like salt, have the ability to depolymerise the thick filament. Pyrophosphate and triphosphate will bind to myosin tails, promoting depolymerisation. However, phosphates also cleave actomyosin bonds. Sodium pyrophosphate and triphosphate bind to myosin heads and, acting as an ATP analogue, cleave the rigor-formed actomyosin bond. This mechanism seems to be rather weak in a solution of phosphates alone, with most of the actomyosin crossbridges remaining attached. The addition of salt, however, is believed to weaken the actomyosin bond, causing a greater
number of crossbridges to relax as the salt concentration is increased. The observed effects of the presence of salt on swelling by sodium phosphate suggest a synergistic interaction. Triphosphate’s role in myosin solubilization is unclear, as it may have to undergo enzymatic hydrolysis to pyrophosphate for it to take an active role. The complete solubilization of myosin as studied using individual myofibrils produces little effect on swelling and hydration, as the free myosin molecules tend to leach out into the irrigating solution. In whole muscle cuts of meat, however, intact cell membranes and the presence of endomysial connective tissue sheaths will hold the free myosin molecules inside the muscle fibers (Knight & Elsley, 1989; Wilding, Hedges, & Lillford, 1986). The effects of polyphosphates on swelling and hydration are only seen at pH 5.5 or greater and the most significant increases only occur within a certain range of salt concentrations.

The instrumental role of myosin solubilization by phosphate in hydration as described by Offer and Knight (1988) is disputed by Trout and Schmidt (1986a) based on two reasons: (i) the occasionally poor relationship between solubilized myosin and meat protein functionality and (ii) the poor functionality of extracted myosin under heating. They also dispute the role of phosphate in actomyosin dissociation, stating that the degree of phosphate hydrolyzation in meat products is not sufficient to indicate any great amount of actomyosin dissociation. An alternate specific effect of sodium phosphates is offered by Trout and Schmidt (1986a). They state that the alteration of hydrophobic interactions by phosphates is responsible for at least a part of the observed increases in water binding.
1.2.2.3 Ionic strength

While Offer and Knight (1988) offer the above mechanisms as the main actions of phosphates in hydration, there are other hypotheses. Perhaps the most common mechanism discussed is increasing ionic strength. Because phosphates are polyvalent anionic compounds, they have a much greater ionic strength than NaCl, allowing for greater electrostatic repulsion within the filament lattice. Trout and Schmidt (1984) suggest that most of the changes in water binding and hydration can be explained by ionic strength, with as much as 96% of the effects of phosphates being due to a combination of ionic strength and pH effects. A later manuscript by Trout and Schmidt (1986a) would revise this hypothesis, allowing for a roughly equal three-way mechanism involving ionic strength, pH effects and specific polyphosphate effects.

1.2.2.4 Other mechanisms

Lewis, Groves and Holgate (1986) suggested that high pH brines could induce resolubilization of sarcoplasmic proteins, thereby increasing cooked yield. They did not offer a specific mechanism for this action. The binding of divalent cations Ca$^{2+}$ and Mg$^{2+}$, which compromise muscle proteins’ ability to bind water, has long been described as a mechanism of phosphate for increasing water hydration (Hamm, 1960). Offer and Knight (1988) state that this is not a principle mechanism as EDTA, a powerful chelating antioxidant, was less effective at stimulating water uptake when compared to pyrophosphate. Whiting (1987) also observed EDTA to be less effective at limiting water exudation in meat batters that salt. These comparisons only demonstrate that metal and cation chelation is not the principal mode of action for phosphates. Work by Lawrence, Dikeman, Hunt, Kastner and Johnson (2003) has shown that beef muscles, when subjected to increased calcium concentrations, can have some of their water holding
capacity restored by treatment with sodium phosphates. Although the amount of cations present in the rounds in this study were well above endogenous levels, it can be inferred that at least a small portion of the hydration activity of phosphates is due to chelation.

1.2.3 Injection of sodium compounds into whole muscle cuts

1.2.3.1 Fluid retention in beef and pork

While there are a multitude of benefits to shelf-life and quality due to salt and sodium phosphates, they’re first and most important function to meat processors is to increase product yield and retain injected fluid. The following studies describe the use of salt and/or sodium phosphates to increase the yield of beef and pork products.

Top sirloin steaks marinated in 2.5% sodium phosphate plus 10% beef seasoning (containing salt) were higher for marinade uptake and final yield than other treatments not marinated with phosphate or salt (Scanga, Delmore Jr., Ames, Belk, Tatum, & Smith, 2000). A treatment containing 150 mM CaCl with a pH of 7.26, compared with the phosphates solution’s pH of 6.79, actually lost weight during the brine uptake period. This reinforces the point that there are many factors to consider other than the effects of pH increase alone. Introducing divalent cations, such as Ca$^{2+}$ or Mg$^{2+}$, into a muscle protein system greatly reduces ionic strength and water binding ability is lost as a result (Poulanne & Halonen, 2010).

Beef rounds (Robbins, Jensen, Ryan, Homco-Ryan, McKeith, & Brewer, 2002) injected to include 0.4% phosphate and 0.4% salt on a finished weight basis had higher cook losses (32.10%) than those injected with water alone (28.53%). The authors attributed this to the higher amount of water held by the enhanced rounds although the measurements necessary to confirm this were not included in the study. This effect was also seen by Boles and Shand (2001) who saw reduced cook yields in roasts injected to
150% initial weight with a brine formulated to give 1.8% salt and 0.3% phosphate in the final product compared to those injected to 125% or 110%.

Lawrence, Dikeman, Hunt, Kastner and Johnson (2004) injected select strip loins to 111.5% of green weight using 4.4% phosphate and 2.2% salt or 2.4% calcium lactate with various combinations of non-phosphate water binders, beef broth and natural flavorings. The phosphate/salt combination treatments had much higher final pumped yields (108 – 109.48%) compared to the treatments without phosphate (103.12 – 105.51%). The authors of this study point out that increasing amounts of cations in a meat system, such as the ones provided by the calcium (Ca\(^{2+}\)) lactate, decrease water binding, though they are important for activating calcium dependant protease tenderizers. Because phosphates are believed to chelate the calcium ions needed to increase tenderness, non-phosphate water binders were included. While the non-phosphate water binders increased water holding capacity within the calcium lactate treatments, the phosphate treatments still held more water at injection and during storage.

A study (Baublits, Pohlman, Brown Jr., & Johnson, 2005a) to determine the effects of various phosphates (tetrasodium pyro-, sodium tripoly-, and sodium hexametaphosphate) at 0.2 or 0.4% with a constant 2.2% salt level was carried out on bottom round steaks. Tetrasodium pyrophosphate at both concentrations and all phosphate solutions at 0.4% bound more water than the control treatment. Cooking losses were reduced by tetrasodium pyrophosphate and sodium tripolyphosphate, while sodium hexametaphosphate had no effect relative to the control. The authors of this study agree with the assertion of Trout and Schmidt (1986a; 1986b) that longer chain phosphates, like hexametaphosphate, have a lower degree of dissociation and,
therefore, a lower ionic strength than shorter chain phosphates; this would provide for less electrostatic repulsion and a lower hydration capacity. The same phosphate solutions were injected into bottom rounds without sodium chloride (Baublits, Pohlman, Brown Jr., & Johnson, 2005b). Utilization of sodium tripolyphosphate and trisodium phosphate gave similar free water binding and cook yields as the control, but was unable to increase them as the phosphate salt combinations had in Baublits et al. (2005a).

Pietrasik, Jagvinder, Shand and Pegg (2006) observed that beef strip loins injected to contain 0.5% salt and 0.3% sodium tripolyphosphate in the finished product had less purge loss than a non-injected control (2.69% vs 3.86%). Molina, Johnson, West and Gwartney (2005) enhanced various muscles from the chuck to include 0.5% salt and 0.4% sodium tripolyphosphate in the final product using three application methods: marination, needle injection and vacuum tumbling. The researchers found that the muscles tested did not react uniformly to salt and phosphate enhancement. For example, the complexus, latissimus dorsi, rhomboideus, subscapularis and supraspinatus saw less thaw loss compared to an untreated control, while the serratus ventralis, splenius and triceps brachii only saw less thaw loss in some samples specific to application treatment. Similarly, 0.5% sodium chloride and 0.4% sodium tripolyphosphate were more effective at increasing cook yields in the supraspinatus than in the triceps brachii (Walsh, Martins, O’Neill, Kerry, Kenny, & Ward, 2010). Differences were also seen in brine effectiveness according to application method (Molina et al., 2005). Vacuum tumbling, which works to spread brine pockets and release myofibrillar proteins, had the greatest effect in reducing thaw loss in the complexus, latissimus dorsi, splenius and subscapularis. Marinating was observed to be the most effective method in reducing cook loss in the latissimus dorsi, rhomboideus, splenius, subscapularis and
serratus ventralis. Boles and Shand (2001) also saw differences in functionality between muscles injected to 1.8% salt and 0.3% phosphate in the final product. The clod, chuck roll and knuckle, for example, had higher brine retention and cook yields than the brisket, inside round, eye of round and outside round. It is likely that the ability of myofibrillar proteins to act on water is affected by variation of intrinsic factors such as amount of fat and connective tissue due to muscle location.

Wynveen et al. (2001) investigated the use of sodium polyphosphates to improve the functionality of pale, soft and exudative (PSE) pork. Injections of 0.2%, 0.3%, 0.4% or 0.5% sodium phosphate with 0.3% sodium chloride 15 minutes postmortem were observed to increase the final pH of pork carcasses and decrease drip, thaw and cook losses over PSE controls. The authors suggested that the pH buffering effect of phosphate, rather than the specific and ionic strength effects, were responsible for curbing the pH decline and increasing functionality of the PSE pork carcasses.

Pork loins injected with a 5.5% salt and 3.3% sodium phosphate solution to 110% of their original weight did not significantly differ from the control as far as cook loss and purge loss (Hayes, Desmond, Troy, Buckley, & Mehra, 2006). As with the beef rounds in Robbins et al. (2002) this was most likely due to the extra water held by the enhancement brine. Results seen in Brashear, Brewer, Meisenger and McKeith (2002) are similar in that increasing pump levels (6, 12 and 18%) with constant phosphate levels of 0.4% (final product) without salt caused increased purge and cook loss of pork chops from injected loins compared to non-injected control loins. However, Brasher et al. (2002) also observed that chops from pork loins injected to contain 0.4% phosphate and 0.4% salt had lower purge loss and greater cook yields compared to non-injected control loins at the 6 and 12% injection level. Detienne and Wicker (1999) saw differing
results from those of Hayes et al. (2006) in pork loins injected with varying levels of salt (0, 0.5, 1, or 1.5%) and sodium tripolyphosphate (0, 0.15, 0.3 or 0.45%). A phosphate and salt solution similar to the one used in Hayes et al. (2006) produced 57.8% lower purge losses and 6.8% lower cook losses compared to an untreated control. These differing results are seen because the control loin in Detienne and Wicker (1999), while containing no added salt or phosphate, is injected with 10% water to simulate the added moisture in the treatment loins. The control loins in Hayes et al. (2006) are uninjected. Injecting the control loins with water allows for a more appropriate comparison.

Detienne and Wicker (1999) establish optimal levels of salt and phosphate for increasing yields in enhanced pork loins. Maximum final product yield and minimum cook loss were seen at 1% salt and 0.45% phosphate. Minimal purge losses were seen with either 1% salt or 0.5% salt and 0.3% phosphate, once again showing the ability of phosphate to reduce the need for salt. Decreases in purge and cook loss compared to the control were seen at the lowest combined levels of salt (0.5%) and phosphate (0.15%).

1.2.4 Effects of salt and sodium phosphate injection on shelf life and sensory characteristics

1.2.4.1 Sensory and tenderness

Previous research has established the role salt and SP play in enhancing the eating quality of meat products. Vote, Platter, Tatum, Schmidt, Belk, & Smith. (2000) observed no differences in tenderness, juiciness or flavor between steaks enhanced to 0.25% SP and those injected with 10% water alone, as determined by trained sensory panel. Warner-Bratzler shear values were also not different between the two treatments. McGee, Henry, Brooks, Ray and Morgan (2003), however, saw higher ratings for
appearance, juiciness, flavor, tenderness and acceptability for steaks injected to 0.25% SP and 0.35% salt compared to non-injected controls. In addition, WBS values were higher (less tender) for the control steaks (4.53 Kg) than the injected steaks (3.64 Kg). Baublits, Pohlman, Brown, Jr., Yancey and Johnson (2006a) injected steaks to either 0.4% SP or 0.4% SP and 0.5%, 1% or 1.5% salt. All treatments were more tender than an untreated control, as determined by Warner-Bratzler shear. A trained sensory panel also rated all treatments containing both SP and salt as being more tender than the control and phosphate only treatment. Also, there was a linear increase in salt intensity as the salt concentration increased, but no other differences in flavor were observed. A comparison of different phosphate types by Baublits et al. (2005a) revealed similar WBS values for steaks injected with tripolyphosphates or tetrasodiumphosphates compared to those that were untreated or injected with hexametaphosphates. Steaks enhanced with 0.4% SP, 0.3% salt and 0.1% natural flavoring were more tender, juicier and saltier than unenhanced steaks, according to trained sensory panel (Wicklund, Homco-Ryan, Ryan, McKeith, McFarlane & Brewer, 2005). Lower WBS values were also observed for enhanced steaks. Rowe, Pohlman, Brown, Jr., Johnson, Whiting and Galloway (2009) noted that steaks injected with 0.4% SP or 0.4% SP and 0.5% salt were more tender according to both trained sensory panelists and WBS values.

While there were some exceptions, the reviewed literature showed enhancement with salt and sodium phosphates to be effective in increasing juiciness and tenderness. The mechanism by which they increase juiciness is to simply allow the meat proteins to retain more free water through cooking. Increases in tenderness are theorized to be caused by two mechanisms: (1) increases in held water dilute out load resisting material
and (2) protein solubilization destabilizes the structure of the myofibers (Offer & Knight, 1988).

1.2.4.2 Shelf life

The actions of salt and SP to stabilize shelf life have been extensively studied. Lawrence et al. (2004) noted that steaks enhanced with 0.44% SP and 0.22% salt were darker and less red than steaks injected with calcium lactate. Steaks injected with 0.4% SP and 0.5 - 1.5% salt were darker than untreated steaks or phosphate only treated steaks (Baublits et al., 2006a). All treatments were similar in redness except SP/1.5% salt, which was less red than the others.

Baublits, Pohlman, Brown, Jr., & Johnson (2006b) evaluated steaks enhanced with 0.2% and 0.4% tripolyphosphate, tetrasodium phosphate or hexametaphosphate without salt. All SP treated steaks had higher $L^*$ values (lighter) through 5 d of display, although the steaks injected at 0.4% were no different at d 3 and 7. All SP treated steaks had higher $a^*$ values (more red) than steaks treated with salt alone. Additionally, steaks treated with SP at 0.4% were more effective at maintaining oxymyoglobin concentrations through display than those treated with 0.2%. Beef strip steaks injected to 0.3% SP and 0.5% salt were less red than uninjected steaks at packaging, but there were no differences in redness after 3 wk of storage (Pietrasik et al., 2006). Rowe et al. (2009) observed $a^*$ values in 0.4% SP injected steaks that were numerically greater than non-injected control steaks and significantly greater than those injected with 0.5% salt. Typically SP and salt injected meat will be darker (lower $L^*$ values) than non-injected meat due to decreased oxygen penetration and lower amount of reflected light from the surface (Abril, Campo, Önenç, Sañudo, Albertí, & Negueruela, 2001; Baublits et al., 2006a). The effects of salt and SP on $a^*$ (redness) differ, as salt commonly decreases
redness and SP generally maintains redness over time. This is due to the pro-oxidative nature of salt, which speeds the conversion of the red oxymyoglobin pigment to the brown metmyoglobin pigment responsible for discoloration of fresh meats; sodium phosphates act as an antioxidant, due to their ability to chelate the metal ions responsible for oxidation in fresh meats (Akamittath, Brekke & Schanus, 1990; Torres, Pearson, Gray, Booren & Shimokomaki, 1988).

Steaks injected with 0.25% SP and 0.35% salt underwent less oxidation than those without SP, as indicated by thiobarbituric acid reactive substances (TBARS; McGee et al., 2003). Pietrasik et al. (2006) saw no differences in TBARS between beef strip steaks injected with 0.3% SP and 0.5% salt and non-injected control steaks. Rowe et al. (2009) saw higher TBARS values in steaks injected to 0.5% salt than those injected to 0.4% SP. Steaks containing both salt and SP were no higher than the control for lipid oxidation products. The effects of salt and SP on lipid oxidation are similar to their effects on pigment oxidation. Enhancement with salt alone will increase the rate of oxidation relative to untreated meat while SP alone will reduce it; it appears injecting with comparable levels of both compounds stabilizes the rate of oxidation.

1.3 Comparison of sodium free water binding adjuncts to salt and sodium phosphates

1.3.1 Alternative ingredients and pH adjustment

The reduction of sodium usage has been an industry goal for nearly thirty years and, as such, many research studies have focused on the use of sodium-free alternative ingredients. Lawrence et al. (2004) compared steaks injected with 0.44% SP, 0.22% salt and 1% or 2% commercial beef broth or carrageenan to those with SP and salt alone. Adding beef broth or carrageenan did not increase pumped yields or reduce
purge losses compared to salt and phosphate only. Cooking losses, however, were reduced by 3.85% by beef broth and 3.98% by carrageenan compared to SP/salt only. Trained sensory flavor and tenderness attributes and instrumental tenderness attributes were not affected. Redness (a*) was increased by beef broth and carrageenan treatments and metmyoglobin (brown pigment) formation was reduced. The beef broth and carrageenan treatments, however, also included a natural flavoring compound with rosemary extract, a commonly used antioxidant, that was absent from the SP/salt only treatment and more likely to be responsible for the increased color stability than either water binding adjunct.

Walsh et al. (2010) injected steaks with combinations of 0.5% salt, 0.4% SP, 1.5% whey protein concentrate (WPC) and 1% carrageenan. All treatments had higher cook yields than a non-injected control. The 0.5% salt, 0.4% SP treatments had a higher cook yield (89.6%) than all other treatments, including 0.5% salt only (74.0%), 1.5% WPC and 0.5% salt (81.6%), and 1.0% carrageenan/0.5% salt (78.2%). No differences were observed among the treatments for sensory or instrumental (Warner-Bratzler Shear) tenderness, but all treatments were more tender than the non-injected control.

The efficacy of a solubilized protein solution to increase water holding capacity and tenderness and maintain color of beef strip loin steaks was investigated by Vann and Mireles DeWitt (2007). A 10% beef/90% water solution was acidified to pH 2.5 and injected into beef loins along with salt to a final concentration of 0.5% solubilized protein and 0.18% salt. Control loins were injected to 0.45% SP and 0.36% salt. Protein treated steaks had a 6.64% higher purge value and 2.74% higher cook losses after 5 d of storage. The authors theorized that the lower salt content of the protein treatment was partially responsible for the diminished water binding ability. The protein treated
steaks were shown to be less tender by trained sensory panel and Warner-Bratzler shear force testing. Lipid oxidation products, as determined by TBARS, were higher for protein injected steaks at d 5 (1.48 mg/Kg) and 9 (1.54 mg/Kg) than phosphate steaks (0.68 and 0.76 mg/Kg, respectively). The researchers found that a trained color panel could not detect differences in lean color between the two treatments, indicating an ability to maintain color stability even in the presence of accelerated lipid oxidation.

A high pH solution (pH 10) using 0.1% ammonium hydroxide (AH) and 3.6% salt was compared to a 3.6% salt, 4.5% phosphate solution in beef strip steaks injected 110% of green weight (Cerruto-Noya, VanOverbeke & Mireles DeWitt, 2009). Steaks treated with AH had purge and cook losses that were higher than SP steaks by 3.16 and 6.64%, respectively on d 5 and 2.90 and 6.15%, respectively on d 12. The AH steaks were also rated as being less tender and juicy by a trained sensory panel and were less tender according to WBS values. Furthermore, AH steaks had lower a* (redness) values across all days of testing and had a higher rate of discoloration up to 14 d. The authors hypothesized that the buffering capacity of meat proteins limited the pH increase gained from AH injection and that a greater concentration of AH in the brine would increase meat pH further and, subsequently, increase protein functionality with regards to binding water.

Further work in this area was carried out by Parsons, VanOverbeke and Mireles Dewitt (2011a) and Parsons, VanOverbeke and Mireles DeWitt (2011b). The authors added 1% ammonium hydroxide (AH) to a brine containing 3.6% salt and compared beef strip steaks injected with the brine to those injected with a brine containing 4.5% SP and 3.6% salt. Loins were injected to 110% of green weight. Purge values were slightly higher for the AH treatment on d 0 (0.57% higher), but were 1.27% higher by d 14.
Differences in tenderness were not detected between the AH and SP steaks by either trained sensory panel or Warner-Bratzler shear force testing. Additionally, trained sensory panelists could not detect differences in flavor or juiciness between the two treatments (Parsons et al., 2011b). Parsons et al. (2011a) also determined that AH treated steaks had higher $a^*$ (redness) values and maintained lean color better than SP steaks. The lower degree of discoloration (metmyoglobin formation) seen in the AH steaks is likely related to the lower lipid oxidation values, as determined by TBARS.

1.3.2 Collagen extracts as water binding adjuncts

Connective tissue, which permeates the structure of most living organisms, is predominantly comprised of collagen, a family of proteins containing approximately 27 different isoforms (Strasburg, Xiong, & Chiang, 2008). Collagen proteins are present in and around bone, tendons, blood vessels, skin, teeth and muscle and are responsible for maintaining the toughness and stability of these structures. The morphology, chemistry, structure and composition of various types of collagen have been thoroughly reviewed by Asghar and Henrickson (1982). Various forms of isolated and extracted collagen exist which may be used to alter the functional properties of various food systems. Animal skins, which contain the bulk of the collagen deposits, or individual pieces of connective tissue, may be processed by means of chemical treatments, enzyme hydrolysis, physical comminution or a variety of other methods to obtain unique fractions of food-grade collagen or gelatin, a collection of peptide fragments derived from collagen, for use in food systems (Asghar & Henrickson, 1982).

Use of meats that are naturally high in connective tissues, including tripe, hearts, lips, skins and other animal byproducts termed 'short meats', at excessive levels is
known to reduce emulsion stability and cook yields and generate a less acceptable product (Whiting, 1989; Wiley, Reagan, Carpenter, & Campion, 1979). Many researchers have characterized the effects of various collagen and gelatin fractions on batter stability in comminuted meat products, such as frankfurters and bologna. Pork and beef skin hydrolysates were effective in reducing fat and fluid release from a beef/pork sausage blend when replacing non-fat dry milk at a 2.3% usage level; however, the texture of the cooked sausage was significantly weakened (Satterlee & Zachariah, 1973). Use of 10% native or preheated connective tissue reduced hardness and cohesiveness of restructured beef containing 0.2% salt and 0.5% trisodium phosphate, while addition of 1% gelatin reduced cook yields by 1.9% (Kenney, Kastner, & Kropf, 1992). Sensory testing in the previously mentioned study showed higher levels of collagen usage, regardless of fraction, reduced juiciness and beefy flavor. Chicken skin connective tissue gels containing 100, 200 or 300% added water were included in a 20% fat bologna at levels of 10, 20 or 30% (Osburn & Mandigo, 1998). The level of added water in the gels had little effect on the characteristics of the bologna, but juiciness increased and hardness and shear force decreased with increasing levels of collagen addition. The addition of collagen had the curious effect of reducing emulsion temperature and extrusion force, indicating a decrease in viscosity; this is contrary to other published data using collagen in processed meats and is likely due to the use of gels with high amounts of added water (Ladwig, Kниpe, & Sebranek, 1989; Whiting, 1989). Calhoun, Eilert and Mandigo (1996) observed that a connective tissue/sodium acid pyrophosphate pre-blend caused extensive emulsion breakdown when included at 2% in 10% and 30% fat frankfurters while the use of the connective tissue alone had no detrimental effects. Bologna made with 20% lean replacement by a wet fibrous collagen
did not impact emulsion stability or cook yields, but did increase textural characteristics (Rao & Henrickson, 1983). Use of a similar collagen fraction in replacing lean of pork sausages reduced cook yields while fat replacement with the collagen reduced texture and increased yields (Arganosa, Henrickson, & Rao, 1988). This collagen fiber fraction is one of two materials derived from animal hides and other low-cost byproducts, the other being collagen powder. Collagen powder and collagen fiber are quite similar in their physicochemical make-up, composed of ~85% protein, but quite different in their microstructure, with the fiber displaying filament type structure and the powder presenting a more dispersed arrangement, as the names suggest (Wolf, Sobral, & Telis, 2009). Both fractions are known to have superior gelling and emulsification characteristics to gelatin and, thus, are considered a desirable functional protein source for food products (Neklyudov, 2003).

Dried, purified collagen powders sourced from fresh pork trimmings and turkey skins are currently commercially available for pork and turkey, respectively, which offer enhanced water binding and emulsification capabilities, as well as the prospect of a more consumer friendly label (Prabhu, Doerscher, Hull, & Schoenberg, 2000; Prabhu, Doerscher, Hull, & Nnanna, 2002). These have been investigated in several meat products. A model gel system of pork myofibrillar proteins with pork collagen (PC) substituted at up to 50% was created by Doerscher, Briggs and Lonergan (2003). Adding PC to the gels increased water holding capacity by between 1.57 and 2.57% over the collagen free control, but it was thought that substitution levels of over 10% disrupted the gel matrix and caused a weaker gel. Adding between 1.5 and 3.5% collagen to pork frankfurters increased cook yields and decreased purge losses through 8 wk of storage (Prabhu, Doerscher, & Hull, 2004). Hams in the same study injected
with at least 1% PC had higher cook yields and less purge loss after 8 wk of storage. The color of hams or frankfurters in Prabhu et al. (2004) did not seem to be affected by the inclusion of PC. Cooked restructured hams with 3% PC had 5.67 – 11.6% lower expressible moisture than control hams without PC, depending on the level of pale, soft and exudative (PSE) meat incorporated (0, 50 or 100%; Schilling, Mink, Gochenour, Marriott, & Alvarado, 2003). Cook losses were lower for PC hams with 0% PSE, but no significant differences were observed when PSE meat was incorporated into the formulation. Protein bind was numerically, but not significantly, increased by PC in 0 and 100% PSE hams. Inclusion of 1.5% turkey collagen (TC) in chunked and formed turkey breasts made with PSE-like raw materials decreased cooking losses and purge losses by 1.19 and 1.15%, respectively, over controls without TC (Daigle, Schilling, Marriott, Wang, Barbeau, & Williams, 2005). Additionally, protein bind of PSE-like meat was increased to the level of non-PSE-like meat by using TC. Use of up to 1% collagen fiber from bovine hide was investigated in pork frankfurters with up to 100% replacement of pork meat with mechanically deboned poultry meat (MDPM; Pereira, Ramos, Teixeira, Cardoso, Ramos, & Fontes, 2011). Collagen fiber proved effective in decreasing cook losses regardless of MDPM content and was able to offset the negative texture and color effects of MDPM inclusion, increasing hardness and chroma, or color saturation, values.

1.4 Use of high pressure processing (HPP) on whole muscle beef

Though the concept of high hydrostatic pressure treatment, or high pressure processing (HPP), has been known for over a hundred years, its popularity has grown in the past few decades as technological barriers that prevent its commercial viability are broken. HPP is renowned for to its ability to allow extended shelf life and enhance
microbial stability of fruits and vegetables, meat, poultry and seafood, juices and a variety of other products (Campus, 2010; Torres & Velazquez, 2005). However, the same principles that make HPP such an effective pasteurization treatment cause it to exert a variety of other effects on food systems as well. This is especially evident in protein heavy systems like muscle foods, as each type of protein is quite unique, ruled by a complex set of chemical interactions that are susceptible to very high pressures in different ways. A brief overview of the effects of high hydrostatic pressures specific to proteins will be given here, though more comprehensive reviews are available (Balny & Masson, 1993; Messens, Van Camp, & Huyghebaert, 1997; Mozhaev, Heremans, Frank, Masson, & Balny, 1996).

1.4.1 Effects of HPP on muscle proteins

High pressure treatment affects the fundamental physicochemical properties that govern interactions of constituents in food systems. Of specific importance to muscle foods are proteins, especially their interactions with water and other proteins. There are several chemical bonds that are responsible for the structure and behavior of proteins in any given system, including steric strains, van der Waals interactions, hydrogen bonds, electrostatic interactions, hydrophobic interactions and disulfide bonds (Damodaran, 2008). Under very high pressures, events associated with a decrease in volume are enhanced and vice versa (Cheftel & Culioli, 1997). Since the stated interactions all constitute a change in volume to some degree they are all affected by HPP, especially hydrophobic (disrupted) and electrostatic (disrupted) interactions, which are important in determining the structure and functionality of processed meats (Cheftel & Culioli, 1997; Jimenez-Colmenero, 2002; Messens et al., 1997). Hydrogen bonds, also important in meat products because of their role in stabilizing collagen, are thought to be slightly
strengthened under very high pressures (Messens et al., 1997). Molecules which are
ordered around charged groups by electrostriction, such as water molecules around an
ion or charged protein group, may be compacted (Cheftel & Culioli, 1997; Messens et
al., 1997). The complex relationships of these interactions make prediction of the effects
of HPP on muscle systems difficult, but there has been research to elucidate said
effects.

Solubility of proteins from bovine *semitendinosus* muscle extracted under low
ionic strength (sarcoplasmic) increased more than two-fold with pressure treatment up to
500 MPa (10 s), while that of high ionic strength extractable (myofibrillar) proteins
(2010) alternatively observed increased myofibrillar protein solubility up to 200 MPa (10
min), then a reduction up to 600 MPa; sarcoplasmic protein solubility was not affected.
Turkey meat in a model batter system (0.5% salt) increased in total protein solubility in a
pressure range of 50-200 MPa (5 min), but sarcoplasmic protein solubility was reduced
in the same range (Chan, Omana, & Betti, 2011). Chicken breast batters experienced
reduced total and sarcoplasmic protein solubility at 400 and 600 MPa when compared to
200 MPa (30 min); inclusion of 0.3% phosphate mitigated the reduction in total solubility
(Omana, Plastow, & Betti, 2011). Reduced solubility of myofibrillar and sarcoplasmic
proteins was also seen in pressurized muscle by Chapleau and de Lamballerie-Anton
(2003a) and Marcos, Kerry and Mullen (2010) and Souza et al. (2011). Macfarlane and
McKenzie (1976) showed pressure induced solubility of proteins to be dependent on
cationic and anionic species of salt. Conclusions on the nature of protein solubility are
difficult to draw due to the inconsistent experimental conditions of the available studies
and the contradictory nature of results seen. In most of these cases, a batter or a salt
extract was pressurized instead of whole muscle. Extrapolation of these results to a whole muscle product is inappropriate, as can be seen in the cases where whole muscle products pressurized and, subsequently, solubility in salt solution decreased (Marcos et al., 2010; Souza et al., 2011).

Some protein fractions released following pressurization have been identified. The dissociation of myosin light and heavy chain has been reported by several researchers to begin around 140-200 MPa in cod, beef, chicken, pork and sheep muscle (Angsupanich & Ledward, 1998; Iwasaki, Noshiroya, Saitoh, Okano & Yamamoto, 2006; Macfarlane & MacKenzie, 1976; Sikes, Tobin, & Tume, 2009). Likewise, actin was observed to begin dissociation from F-actin to G-actin in a similar pressure range (Angsupanich & Ledward, 1998; Iwasaki et al., 2006; Sikes et al., 2009). Other notable protein fractions extracted under very high pressures include myomesin (M-line protein), C-protein (myosin bundling protein), α-actinin (binds actin to the Z-disk), troponin and tropomyosin (Iwasaki et al., 2006; Sikes et al., 2009).

Response surface studies on extracted myofibrillar proteins revealed that surface exposure of hydrophobic side groups is intensified up to three-fold at 440 MPa and reactive sulfhydryls are predicted to reached a maximum of 69% of total at 452 MPa (Chapleau, Delepine, & Lambellerie-Anton, 2002; Chapleau, Mangavel, Compoint, & Lamballerie-Anton, 2003b). Yamamoto, Hasi and Yasui (1993) showed that myosin molecules tend to aggregate, forming a shared head with multiple tails following high pressure treatment. These observations support the aggregation and arrangement of myofibrillar proteins in a ‘structure-forming’ phase after pressurization, partially explaining the formation of high pressure set gels. At least a partial denaturation of myosin and actin in pork batters and whole muscle beef are supported by differential
scanning calorimetry (DSC) thermograms (Fernández-Martín, Fernández, Carballo & Jiménez Colmenero, 1997; Ma & Ledward, 2004) which show the disappearance of thermal denaturation enthalpy peaks normally associated with actin and myosin around 200 MPa.

Denaturation/aggregation of these proteins results in a loss of integrity within the sarcomeric structure, with the severity dependent on the pressure level used. Iwasaki et al. (2006), using both electron and light microscopy on isolated chicken myofibrils, observed the destruction of the Z-disk at 100 MPa along with the formation of protein aggregates. At 200 MPa the M-line was almost completely dissolved and more protein aggregates were formed, but the thick and thin filaments seemed to retain a portion of their conformation. Pressurization at 300 MPa brought about a total loss of a recognizable sarcomere along with increased formation of aforementioned protein aggregates.

1.4.2 pH

Post-rigor *Longissimus dorsi* samples (2.5 × 2.5 × 6 cm) were pressurized at 200 – 800 MPa at ambient temperature, 40, 60 and 70 °C (Ma & Ledward, 2004). Pressure treatment tended to increase sample pH by 0.10 – 0.19 units. This increase, much like the one seen after cooking meat, is likely caused by the conformational changes and denaturation of proteins which may mask or bury acidic side groups. This same phenomenon was seen by Ansupanich & Ledward (1998) in cod filets and by McArdle, Marcos, Kerry and Mullen (2010; 2011) in beef briskets exposed to HPP. Combination thermal (70 °C) and high pressure treatments revealed that this pH increase is not additive (Ma & Ledward, 2004).

1.4.3 Texture
Texture profile analysis (TPA) of beef *longissimus* muscle showed a softer texture (more tender) for combination treatment at high temperatures (60 – 70 °C) and moderate (200 MPa) pressures, while pressures of 200-600 at ambient temperature induced harder textures (Ma & Ledward, 2004). McArdle et al. (2011) saw similar results in heat (35, 45 or 55 °C) and pressure treated (400, 600 MPa) beef *pectoralis profundus*. Warner-Bratzler shear force values were higher for low temperature (35 °C) high pressure (600 MPa) treated beef (83.68 N) than non-treated (61.98 N) while that treated at higher temperature and milder pressure (55 °C; 400 MPa) had lower values (51.36 N). The increases in toughness induced by pressurization of post-rigor muscle are well established but perplexing as high pressure treatment in known to make drastic changes to muscle structure, removing the M-line, depolymerizing myosin filaments and fragmenting the Z-disk (Jung, Lamballerie-Anton, & Ghoul, 2000a; Suzuki, Watanabe, Iwamura, Ikeuchi, & Saito, 1990). Jung, Ghoul and Lamballerie-Anton (2000b) however, observed reduced sarcomere length, as well as greater myofibril diameter, in beef *biceps femoris* and *longissimus dorsi* muscles pressurized at 520 MPa for 260 s. Reduced sarcomere length minimizes the width of the I-band, which is a point of weakness within the protein structure (Koohmaraie, 1996). The softened texture of meat processed with concurrent pressure and thermal treatment is, by now, well established, but the specific mechanisms that augment texture are poorly understood (Jiménez Colmenero, 2002; Sun & Holley, 2010). Beilken, Macfarlane and Jones (1990), as well as Bouton, Ford, Harris, Macfarlane and O’Shea (1977) found that beef treated at temperatures of 60 or 80 °C and 150 MPa of pressure were more tender compared to beef heated or pressure treated alone. The authors in both cases believed that this effect was entirely due to changes in the order of dissociation/aggregation of myofibrillar
proteins and not changes in stromal protein structure or behavior. This is supported by Suzuki, Watanabe and Ikeuchi (1993) who observed no changes in beef collagen ultrastructure or denaturation temperature and enthalpy due to pressure treatment up to 300 MPa. Bouton et al. (1977) provided a generalized scheme for the mechanism of myofibrillar rearrangement due to pressure-heat treatment. The ‘dissociation’ of proteins posited in this scheme would undoubtedly be the disruption of electrostatic and hydrophobic interactions that result in the depolymerization of myosin and actin (at this point it is worth reiterating that the disruptions induced under pressure are not permanent; hydrophobic and electrostatic interactions may reform after pressure is released but pressurization has irreversibly altered the structure of intact muscle fibers). Heating under pressure would give rise to a structure predominantly stabilized by heat-labile hydrogen bonds and disulfide bridges rather than the hydrophobic interactions that would normally occur with heat alone (Angsupanich, Edde, & Ledward, 1999). Ma and Ledward (2004) suggest that accelerated proteolysis is responsible for the softened texture of pressurized meat. Activities of cathepsins B, D and L are known to increase at pressures up to 400 MPa (Homma, Ikeuchi & Suzuki, 1994). Jung, Ghoul and Lamballerie-Anton (2000b) found increased cathepsin D activity in beef pressure treated at 520 MPa for 260 s which was likely caused by the breakdown of the lysosomes that house the enzyme. However, Warner-Bratzler shear testing done in this study revealed that the toughness induced by pressurization was not overcome by the increased catheptic activity and, in fact, the pressurized meat seemed to have a reduced rate of tenderization during 17 d of aging compared to non-pressurized beef. This reduced rate of tenderization is likely due to the fact that μ- and m-calpains, which are the predominant proteolytic enzymes in postmortem muscle (Aberle, Forrest, Gerrard, &
Mills, 2001), experience reduced activity above 100 MPa and near complete inactivation at or above 300 MPa (Homma, Ikeuchi, & Suzuki, 1995). The softening effect of heat/pressure combinations seen by Ma and Ledward (2004) was consistent over a wide range of pressures (200-800 MPa), even at levels above those that are known to inactivate most proteolytic enzymes (500 MPa; Homma et al., 1994). In light of this it is more plausible that the protein rearrangement/restructuring scheme suggested by Bouton et al. (1977) is the primary mechanism responsible for altering the texture of meat products subjected to combination treatments of heat and pressure.

1.4.4 Color

The color changes induced by HPP in comminuted or minced meat systems are discussed elsewhere in this text. However, color in fresh meat products, such as whole muscle, uncooked roasts or steaks which are not cured or smoked, is an important factor in the consumer purchasing decision (Mancini & Hunt, 2005). A discolored product will be deemed not fresh or unwholesome by consumers. Therefore, attention must be paid to the potential effects of high pressure on meat appearance during storage and retail display. Beef *biceps femoris* were studied at pressure levels ranging from 0-600 MPa and pressure duration in a range of 0-300 s at 10 °C (Jung, Ghoul, & Lamballerie-Anton, 2003). The roasts were estimated to have maximum redness (a* of 17-19) values after pressurization at 300-400 MPa for 100-200 s. This was also seen in beef *longissimus* muscle pieces which were redder after pressurization at 400 MPa than 600 MPa (Marcos et al., 2010). Heat treatment during pressurization likely increases the loss of redness, as McArdle et al. (2010) reported a* values that were slightly lower than control for beef briskets subjected to pressures of 200, 300 or 400 MPa at 40 °C or 400 MPa at 20 °C. Similarly, McArdle et al. (2011), also using beef briskets, found reductions in
redness ($a^*$) of 5-10 units at 400 and 600 MPa and 35, 45 or 55 °C, with all pressure treatments being demonstrably lower even after 15 and 30 days of storage. Lightness ($L^*$) is also affected by pressurization. Marcos et al. (2010) and McArdle et al. (2010) reported similar trends in the lightness ($L^*$) of pressurized whole muscle beef cuts. The authors of both studies reported increases (<10 units) of muscle pieces pressurized at 200 MPa at temperatures of less than 40 °C. However, when the pressure was increased to 300-600 MPa, $L^*$ values of 50.3-55.34 were reported, constituting an increase of more than 20 units in both cases. McArdle et al. (2011) showed that pressurized beef brisket pieces (400 or 600 MPa; 35, 45 or 55 °C) had both lightness and redness values similar to that of unpressurized, oven cooked (70 °C) beef, supporting the oft-reported fact that muscle pieces subjected to sufficient pressure or pressure/heat combination treatment have an appearance similar to that of cooked meat (Cheftel & Culioli, 1997). Carlez, Veciana-Nogues and Cheftel (1995) suggested two mechanisms for the color changes of pressurized meat, (1) whitening due to globin denaturation and/or release of heme iron, and (2) oxidation of ferrous myoglobin to ferric metmyoglobin. The first mechanism, at least, would seem to be supported by previous work observing the decreased solubility of sarcoplasmic proteins at pressure levels as low as 100 MPa, denoting denaturation or aggregation of these proteins (Chan et al., 2011; Lee, Kim, Lee, Hong, Yamamoto, & Kim, 2011; Marcos et al., 2010).

1.4.5 Water holding capacity and cook loss

Beef *semitendinosus* steaks pressurized to 100 – 500 MPa for 5 min at 15 °C experienced reduced water holding capacity at 200 MPa and increased cook losses at 100 – 300 MPa when compared to an unpressurized control (Kim, Lee, Lee, Kim, & Yamamoto, 2007). These increases in fluid loss likely correlate to the loss of protein
solubility seen by the authors at 200 MPa and above, specifically the reduction in extractable myosin. The authors also observed that the muscle fibers were more compact when pressurized to 200 MPa or above, possibly limiting space available to retain bound water. Increased cook losses were likewise reported by McArdle et al. (2010) in beef briskets pressurized to 400 MPa at 20 °C. Higher pressure (600 MPa) was necessary to increase losses during cooking of briskets that were heat treated at 35 and 45 °C during pressurization as those treated at 400 MPa were similar to the control (McArdle et al., 2011). Those treated at 55 °C did not experience increased cook losses at any pressure level. Thermal treatment during pressurization will cause mild denaturation of native proteins, reducing their ability to bind water and giving them less inherent moisture to expunge during cooking. A measure of expressible moisture on beef longissimus steaks pressurized at 0, 200, 400 or 600 MPa for 20 min at 10 – 30 °C was determined (Marcos et al., 2010). Steaks treated at 200 MPa were not significantly different from non-treated controls in their ability to bind water, while 400 or 600 MPa increased expressible moisture values by 8.15 – 11.6%. In this study, sarcoplasmic protein solubility was decreased at 400 and 600 MPa regardless of processing temperature and was highly negatively correlated with expressible moisture. Several major protein fractions separated by gel electrophoresis were reduced at higher pressurization levels, which could indicate pressure induced polymerization of protein fractions. Contrary to most published literature on the subject, Liu et al. (2010) observed decreased cook and drip losses compared to controls for beef round muscle pieces pressurized at 300 or 400 MPa for 10 min. The 12% and 2% reductions in cook loss and drip loss, respectively, were attributed to the increase in pH (5.8 vs 5.6) caused by pressurization. This is an unlikely explanation as the stated shift in pH would probably
not be responsible for a significant increase in the water binding ability of beef (Silva, Patarata, & Martins, 1999). Also, pressures of 500 and 600 MPa caused similar or greater cook losses compared to control despite having significantly higher pH values than the control. The current body of work on water holding in pressurized meat is far from conclusive. However, the myriad changes brought about by pressurization, most notably the condensation of fibril structure, reduction of protein solubility and formation of agglomerated protein complexes, are not conducive to a strong water binding environment.

1.4.6 Lipid oxidation

Beef briskets exposed to 200 – 400 MPa pressure treatments experienced decreased oxidative stability, according to thiobarbituric acid reactive substances (TBARS) measurements (McArdle et al., 2010). All pressurized samples treated at 40 °C and the 300 and 400 MPa samples treated at 20 °C were significantly higher than a non-pressurized control, with values ranging from 0.79 to 2.143 mg/Kg malonaldehyde. Similar results were seen by McArdle et al. (2011) in beef briskets exposed to combination thermal/pressure treatments, as 400 and 600 MPa were sufficient to produce significantly increased oxidation products at most temperature treatments. Additionally, the steaks in this study were analyzed during refrigerated storage, revealing reduced oxidative stability over 30 d, especially at 600 MPa. Cheah and Ledward (1996) noted that minced pork was oxidatively stable given pressures up to 300 MPa, but that stability was lost with more intense treatment. Metal chelating antioxidants, such as citric acid and ethylenediamenonetetraacetic acid (EDTA) have been effective in reducing TBARS values of pork, beef and chicken muscle exposed to pressure levels up to 800 MPa (Beltran, Pla, Yuste, & Mor-Mur, 2003; Cheah & Ledward, 1997; Ma, Ledward,
This suggests an oxidative process primarily catalyzed by free transition metal ions such as iron (Fe) or copper (Cu). This position is supported by the work of Carlez et al. (1995), who observed an increased presence of non-heme iron in beef exposed to 500 MPa of pressure. This could be caused by destruction or denaturation of the porphyrin ring of the heme protein during pressurization. Exposure of the heme group to unsaturated fatty acids because of pressure induced conformational changes is also likely (Cheah & Ledward, 1997). This view is disputed by Orlien, Hansen and Skibsted (2000) who observed no catalytic activity of metmyoglobin or increase in free iron up to 800 MPa in chicken breast meat. These mechanisms, however, do not provide for conditions which would accelerate lipid oxidation following pressurization under 500 MPa, as seen by McArdle et al. (2010, 2011) and Cheah and Ledward (1996). Many other oxidative mechanisms exist that could be exacerbated by the effects of high pressure. For example, disruption of cell membranes could have a two-fold effect, causing the release of highly unsaturated fatty acids and the penetration of free radical molecules into the membrane where they may catalyze lipid peroxidation (Kanner, 1994). Very high pressures may also inactivate antioxidant enzyme systems inherent to meat products that maintain oxidative stability, such as catalase, glutathione peroxidase or superoxide dismutase; though this has not been investigated (Mei, Crum, & Decker, 1994).

1.4.7 The case for HPP of raw beef

The previous sections make a very good argument against the use of HPP in raw meat, especially beef, simply by describing its effects on various quality factors. In fact, the use of HPP of raw beef at pressures similar to those used in the ready-to-eat (RTE) meat sector (600 MPa; 5 min) would yield a product that would most likely be deemed
unacceptable by consumers immediately upon depressurization based on color alone. The concurrent detriments to tenderness, water holding capacity and oxidative stability only add to the argument. However, if these effects could be eliminated, or at least curbed, it could be of great benefit to the fresh meat sector, as there is always a legitimate need for alternative food safety interventions (Aymerich, Picouet & Monfort, 2008; Chen, Ren, Seow, Liu, Bang & Yuk, 2012; Zhou, Xu & Liu, 2010). There are two areas where food safety is of greatest concern in fresh meats.

The first is in raw ground beef products, which include raw ground or chopped beef or veal, hamburger, and trimmings commonly used as components of raw ground beef and are routinely tested for the presence of Escherichia coli serotype O157:H7 by the Food Safety and Inspection Service (FSIS, 1998). The concern about this pathogen goes back to 1993, when a multi-state outbreak of this pathogen linked to undercooked fast food hamburgers sickened over 500 people and killed four children in the Pacific Northwest United States (CDC, 1993). Although that was not the first incidence of foodborne O157, it was the most significant seen at the time, and soon after E. coli O157:H7 was declared an adulterant in raw ground beef (FSIS, 1998). The incident also prompted the FSIS to require mandatory implementation of Hazard Analysis and Critical Control Point (HACCP) programs for meat plants and processors (FSIS, 1996). HACCP is a food safety program that uses science based preventative measures to control hazards specific to a given food production system if those hazards are deemed to be of significant risk for chemical, physical and/or microbiological contamination or proliferation. In September of 2012 six other pathogenic serotypes of shiga toxin-producing E. coli (non-O157 STEC; O26, O45, O103, O111, O121 and O145) were given adulterant status and included in the FSIS testing program (FSIS, 2012a).
The second area is raw non-intact products which can be restructured, as well as whole muscle cuts, such as those that undergo mechanical tenderization or brine enhancement (Sofos & Geornaras, 2010). In mechanical, or blade, tenderization, small sharp blades are forced through whole muscle roasts, tearing muscle fibers and connective tissue to increase tenderness. This process can translocate surface bound contaminants, including E. coli O157:H7 into muscle tissues (Hajmeer, Ceylan, Marsden & Phebus, 2000). Sporing (1999) found that 10³ CFU/g E. coli O157:H7 had been translocated 6 cm into deep muscle tissue of beef steaks from a surface inoculation of 10⁶ CFU/cm² during blade tenderization. Luchansky, Phebus, Thippareddi and Call (2008), in a similar experiment, observed up to 41 and 6% of surface inoculation levels of E. coli O157:H7 were transferred within 1 and 2 cm of the meat surface, respectively. Although transfer reduced sharply after the first 2 cm, E. coli O157:H7 was still recovered at 8 cm, the deepest level tested. Similarly, 10³ CFU/g E. coli O157:H7 and 10² CFU/g non-O157 STEC were recovered 10 cm within the tissue of surface inoculated (10⁵) blade tenderized top butt roasts (Luchansky et al., 2012). Bacteria on the surface of a steak or roast are expected to be inactivated due to the high external temperatures employed during most cooking processes, but those that are internalized may be protected (FSIS, 2002a,b). E. coli O157:H7 internally inoculated in 2.4 cm thick steaks at 3.7 log CFU/g were still detected between 2.1 and 3.1 log CFU/g at 0.3 – 1.2 cm depth when roasted to 60 °C (Adler, Geornaras, Belk, Smith & Sofos, 2012). Pan broiling, in the same study, reduced counts to less than 2 log CFU/g except at 0.9 – 1.2 cm depth, where 2.5 log CFU/g still remained. Luchansky et al. (2012) observed 1.5 – 4.5 log reductions of O157 and non-O157 STEC E. coli in beef steaks inoculated with up to 5.5 log CFU/g when cooked to internal temperatures of 48.9 – 71.1 °C. Thicker
steaks consistently saw greater reductions, presumably because of longer cook times. However, recovery of 1 – 2 log CFU/g was still possible at nearly all endpoint temperatures. The recovery of any surviving pathogenic *E. coli* is worrisome because the infectious dose is believed to be very low, possibly less than 100 cells (Doyle, Archer, Kaspar, & Weiss, 2006).

Products that have been brine enhanced, often referred to as needle injected or moisture enhanced, share similar concerns with internalizing pathogens as blade tenderized cuts with additional complications. During brine injection, excess spray will run off the steak surface or purge out of the interior where it is recycled into the brine well and back through the injector. This can cause pathogenic and spoilage bacteria to build up within the brine solution and be injected throughout the interior of subsequently processed product (Adler, Geornaras, Byelashov, Belk, Smith & Sofos, 2011; Paulson, Wicklund, Rojas & Brewer, 2010; Sofos & Geornaras, 2010; Wicklund, Paulson, Rojas & Brewer, 2007). Wicklund et al. (2007) was able to recover at least 5.28 log CFU/g of *E. coli* K12 after 14 d of storage (4 °C) when steaks were surface inoculated with 4 log CFU/cm². In needle injected beef top rounds and sirloins surface inoculated with 6.4 – 6.7 log CFU/cm² *E. coli* O157:H7 recovery of at least 3.74 log CFU/g throughout the muscle was possible after enhancement (Ponrajan et al., 2011). The thermal inactivation of *E. coli* O157:H7 in moisture enhanced product has been studied as well. A model system (ground beef knuckle) containing salt/SP and inoculated with 10⁷ CFU/g O157 was only reduced to 5.4 log CFU/g after cooking to 65 °C (Byelashov et al., 2010). Yoon et al. (2009, 2011) also used ground meat with added water and salt or salt/sp combinations inoculated with 6 – 7 log CFU/g *E. coli* O157:H7. When cooked to 60 °C, microbial reductions were only ~1 log CFU/g, regardless of the presence or absence of
salt and SP. Cooking to 65 °C showed salt and SP to actually protect *E. coli* O157:H7, allowing about 1 – 2 log greater recovery compared to control or water-only added.

Current FSIS regulations regard *E. coli* O157:H7 as an adulterant in raw non-intact whole muscle beef products, just as in ground beef (FSIS, 1999). The regulation expansion which included the aforementioned non-O157:H7 STECs on the adulterant list also included non-intact whole muscle products (FSIS, 2012b). However, unlike ground beef, there is currently no FSIS mandated testing program for *E. coli* in whole muscle products. It has been maintained that the incidence of pathogenic surface contamination on beef cuts is low, and, therefore, incidence of mechanically induced subsurface translocation should be low and current recommended best practices should eliminate any significant public health threat (NCBA, 2006). In fact, *E. coli* O157:H7 was found on only 0.2% of sampled beef cuts intended for tenderization and currently available surface antimicrobials were sufficient to prevent their intrusion into muscle tissue after tenderization (Heller et al., 2007). These findings do not guarantee the safety of non-intact whole muscle raw beef. Non-O157 STECs may be present where O157:H7 is not and not all processors use surface antimicrobial treatments (FSIS, 2012c). It was also noted previously that heat inactivation resistance may be significantly higher in products containing commonly used ingredients such as salt or SP, a fact that has come to light since the 2006 NCBA report that declared pathogen internalization to be a non-issue. The most compelling reason to take this issue seriously is the record of outbreak and recall associated with these products (CDC, 2010; FSIS, 2005; Laine et al., 2005). *E. coli* O157:H7 outbreaks from mechanically tenderized steaks in 2000 and 2003 prompted FSIS to require that processors of these products account for the risk of pathogen internalization in their HACCP plans (FSIS,
2005). The most recent outbreak occurred in 2009, sickening at least 21 people in 16 states (CDC, 2010). There have also been two recalls associated with mechanically tenderized beef in 2012 alone, one from a processor in Maine and another associated with a much larger instance of product recall from a large beef plant in Canada (FSIS, 2012d; FSIS, 2012e).

There has been an increased amount of research conducted on ensuring the safety of non-intact whole muscle products in recent years, much of it on the use of chemical antimicrobials on blade tenderized/brine enhanced meat and within injection brines. Use of lactic acid bacteria (LAB; $10^7$ CFU/ml), acidified sodium chlorite (ASC; ~1000 ppm) and lactic acid (LA; 3%) reduced internal loads of *E. coli* O157:H7 from 3 – 4 log CFU/g in controls to 1 – 2 logs depending on treatment and depth (Echeverry, Brooks, Miller, Collins, Loneragan, & Brashears, 2009). Wellings (2011) found that 6% sodium metasilicate and 175ppm buffered sulfuric acid reduced surface loads of *E. coli* O157:H7 by 2 log CFU/g each 24 hours post treatment, while 5% lactic acid and 1100 ppm ASC only reduced counts by <1 log CFU/g. Ponrajan et al. (2011) investigated the use of 1% sodium citrate/diacetate and 2% buffered vinegar in enhancement brines. Internalized *E. coli* O157:H7 counts were reduced by <1 log CFU/g relative to a salt/SP (0.5/0.4%) control, leaving over 3 log CFU/g within the meat. Ground beef knuckles inoculated with $10^7$ CFU/g *E. coli* O157:H7 and mixed with various brine formulations and antimicrobial substances were analyzed after 24 hr storage at 4 °C (Byelashov et al., 2010). Only cetylpyridinium chloride (0.5%), which is not currently approved for meat brine usage, was effective in reducing pathogen counts; the reduction was still less than 1 log CFU/g (7.1 vs 6.3). Other commonly used antimicrobials, including potassium lactate, sodium diacetate, lactic acid, acetic acid, citric acid, nisin, pediocin, sodium
metasilicate and hops beta acids, were wholly ineffective during the limited storage in this study. Use of 3% sodium lactate reduced the growth rate of *E. coli* K12 by $1 – 1.5 \log \text{CFU/g}$ in brine enhanced steaks compared to salt/SP (0.3/0.3%) control while lactate/diacetate (3/0.25%) inclusion reduced populations below the detection limit (Wicklund et al., 2007). *E. coli* O157:H7 within model enhancement brines containing various ingredients, including antimicrobials, was investigated by Adler et al. (2011). In this application, 1.65% lactic acid, 2.2% sodium metasilicate, and 5.5% cetylperidinium chloride, the latter two of which are not currently approved brine ingredients, were effective in reducing *E. coli* populations below the detection limit ($1.3 \log \text{CFU/g}$) within 4 hr. Nisin and acetic acid showed inactivation after 8 and 48 hr, respectively, while lactate/diacetate combinations were completely ineffective. In the cited studies, the most consistently effective treatments have been processing aids (CPC and SM) common to the poultry industry but not widely used in the beef sector. Unfortunately, the beef industry currently has a desire to move away from processing aids and antimicrobials that may have a heavy label footprint, due, in no small part, to the BPI fiasco. This makes continued research into alternative food safety interventions for non-intact raw beef a worthwhile endeavor.

Of the two areas discussed, ground vs. whole muscle, the latter, especially brine injected products, presents a much more challenging case for the use of high pressure processing for several reasons: 1) the tendency for use of lower final internal temperatures during cooking, 2) the observed protective effect of very common brine ingredients salt and SP on pathogen inactivation during cooking, and 3) the added quality concerns when whole muscle, as opposed to ground product, is HPP treated, including reduced tenderness and water holding capacity. It stands to reason that a
solution to the problems of HPP treatment of whole muscle would also be compatible with ground product.

1.4.8 High pressure-low temperature treatment of raw beef

One possible solution, high pressure-low temperature (HPLT) treatment of meat, has been presented and undergone cursory investigation on quality and microbial effects (Fernández, Sanz, Molina-García, Otero, Guignon & Vaudagna, 2007; Realini, Guàrdia, Garriga, Pérez-Juan, & Arnau, 2011; Vaudagna, Gonzalez, Guignon, Aparicio, Otero, & Sanz, 2012). HPLT treatment involves keeping meat at subfreezing temperatures during the entirety of the HPP process. Fernandez et al. (2007) investigated the effects of HPLT (650 MPa; 10 min) on meat previously air-blast frozen to -35 °C. When compared to HPP treated and, even untreated, raw beef, the expressible moisture of HPLT beef was 16 and 10% lower, respectively. Similarly drip losses were lower for HPLT than raw HPP beef (4.8 vs 6.4%, respectively). The redness of HPLT beef was numerically, but not significantly higher than the raw HPP treatment; while it was lower than the raw untreated sample, it was similar to the air-blast frozen sample with no HPP treatment. The lightness increase seen in the raw HPP sample (~20 L units) was not seen when comparing the raw and frozen samples to the HPLT samples. Total aerobic counts and lactic acid bacteria counts were both reduced by at least 2 logs CFU/g to below the detection limits by both HPLT and non-frozen HPP treatment.

The lightness increase was also prevented by HPLT treatment of raw beef carpaccio (-30 °C) at 650 MPa (Vaudagna et al., 2012). Redness was numerically decreased when processed at 20 °C compared to -30 °C (2.47 vs. 4.04 a* units, respectively). However, HPLT treatment resulted in significantly higher expressible moisture. Treatment at 20 °C decreased tenderness compared to HPLT treatment.
Microbial enumeration showed that 20 °C treatment was far more effective in reducing total plate count, psychrotrophs and lactic acid bacteria than HPLT. According to Realini et al. (2011) HPLT treatment (400 or 600 MPa; 6 min) of pork carpaccio at -15 or -35 °C reduced lactic acid bacteria and psychrotrophs by ~1 log 1 d after pressurization and promoted increased shelf life, with 3 – 4 log CFU/g lower counts of each at 41 d of storage. Clearly HPLT treatment shows promise as an alternative food safety treatment for raw beef, but there is necessary information lacking from the established literature. Published accounts of HPLT treatment on *E. coli* O157:H7, or any other Shiga toxin producing serotype, in any food system are non-existent; it is essential that the effectiveness against this pathogen be established before any raw beef application is viable. Likewise, there is no established description of the effects on the shelf life of beef. The cooking characteristics, including cook loss, cooked color development and cooked tenderness of HPLT treated beef have not been investigated at all. The color changes, or lack thereof, due to HPLT treatment have been inconsistent as observed in the published literature. All of these factors must be characterized clearly before the use of HPLT as a food safety intervention against *E. coli* in raw beef is a viable option for processors, food service and consumers.

### 1.8 Conclusions

The compounds sodium chloride and sodium phosphate are used widely in the meat industry to increase the functionality of myofibrillar proteins and increase water holding capacity. Although it is generally accepted that phosphates act by altering pH and ionic strength, binding divalent cations and dissolving actomyosin bonds, the exact contributions of these mechanisms to meat hydration are unknown. Sodium
polyphosphate and sodium chloride are known to impact the water holding capacity of meats at high levels (>0.3 and 0.5%, respectively), however, there is a need for more research on the effects and ability of these compounds to act synergistically at lower levels in beef.

There are a great number of food grade ingredients that may serve as alternatives to salt and phosphates. They vary in their functionality, effectiveness, cost and impact on quality. However, due to the consistent public demand for wholesome, high quality meat products with a minimum of added ingredients it is necessary to continue to investigate new products that may be more robust or better accepted by consumers than existing solutions.

High pressure processing is known to have positive effects on the microbial quality of meat products while also contributing to extreme quality detriment of raw meat; this makes HPP as it is currently practiced in the commercial sector untenable for fresh, whole muscle beef and pork. However, commercialization of HPLT processes may provide an avenue for the use of high hydrostatic pressure in raw meat, providing a safe, wholesome and effective food safety alternative with no label footprint. The use of HPLT has only been casually examined in meat products, so more research is needed to establish its effectiveness against *E. coli* O157:H7 and provide more detail as to its effects on raw and cooked product quality.
2. Response surface modeling to predict fluid loss from beef strip loins and steaks injected with salt and phosphate with or without a dehydrated beef protein water binding adjunct

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2.1 Abstract
This study was conducted using response surface methodology to predict fluid loss from injected beef strip steaks as influenced by levels of salt and sodium phosphates (SP) in the injection brine. Also, a beef-based dehydrated beef protein (DBP) water binding ingredient was evaluated. Paired U.S. select beef strip loins were quartered before being injected to 110% of initial weight with brines containing various concentrations of salt and SP (CON) or salt, SP and 5% DBP. Steaks were sliced, overwrapped and stored in the dark for 4 d. Purge values ranged from 0.6% to 4.6% for CON and 0.3% to 2.1% for DBP. Fluid losses when accounting for the fluid lost from injection to slicing were as high as 6.8% for CON brines, but only 2.8% for DBP brines. The equations generated here and the DBP product could help producers achieve acceptable purge while reducing sodium use.

2.2 Introduction
Meat products are commonly injected with brines containing sodium chloride (NaCl; salt) and sodium phosphates (SP) in an effort to improve product quality and consistency. The combined use of salt and sodium phosphates alter protein functionality in such a way that the water holding capacity of the product is increased, causing greater retention of injected and natural fluids (Offer & Knight, 1988). This improves sensory attributes such as tenderness and juiciness (Vote, Platter, Tatum, Schmidt, Belk, Smith & Speer, 2000) and offsets the loss of fluids during storage and display (purge). As a result, the use of salt and sodium phosphate based brines has become economically important to processors.
Currently, however, the industry is looking to scale back the use of sodium in its products. Processed meats contribute an estimated 21% of the sodium in the typical U.S. diet (Engstrom, Tobelmann & Albertson, 1997). This is largely because of salt and sodium phosphates, as they are the most common ingredients added to brine injected meats besides water and both contain a high amount of sodium (39.3 and 31.2%, respectively; Ruusunen & Puolanne, 2005). Since these ingredients are directly responsible for the increased functionality seen in brine injected meats, removing them entirely is not an attractive option. It is likely possible to make minor, or even significant, reductions in salt and sodium phosphates and still retain enough functionality to keep water holding capacity and fluid loss at a minimum. There is very little research in this area with respect to brine injected beef. Additionally, it is important to research the use of alternative ingredients that act as water binders and could reduce, or potentially replace, the use of salt or sodium phosphates in brine injected meats. In support of these objectives a study was conducted using response surface experimental methodology to model the changes in purge of beef strip loin steaks according to the amount of salt and phosphate included in the injection brine. A second model was also created which included the use of a dehydrated beef protein powder (DBP; Proliant Meat Ingredients, Inc.), a newly developed beef collagen based water binding adjunct. This product was previously evaluated for its impact on fluid loss, sensory, microbial stability, color and tenderness characteristics when used to replace SP in a 3.6% salt brine used to inject beef strip loins at 10% over initial weight (Lowder, Goad, Lou, Morgan, & Mireles DeWitt, 2011). The authors determined 5% DBP in the brine was a suitable replacement for 4.5% SP. The current study investigates the use of DBP with SP at various levels.
2.3 Materials and Methods

2.3.1 Collection of select beef strip loins

Paried U.S. Select beef strip loins (Institutional Meat Purchase Specifications 169a) were collected at a processing facility at the time of carcass fabrication. Carcasses were aged 48 hours prior to fabrication. Loins were vacuum packaged at the processing facility and placed on ice in a cooler for transportation. Upon arrival, loins were transferred to a 4°C cooler and stored overnight. All subsequent preparation of brines and raw materials was conducted in a processing facility with a constantly maintained temperature of 4°C.

2.3.2 Brines

Table 2.1 provides the concentration by weight of salt and a sodium phosphate (SP) blend (Brifisol® 85 Instant; BK Giulini Corporation, Simi Valley, CA), containing di-, tri-, and long chain polyphosphates, in each of the brines that were evaluated. For brines containing the dehydrated beef protein (DBP; Proliant Meat Ingredients, Inc., Ankeny, IA), the level utilized was 5%. All brines contained 1% Herbalox seasoning HTS (Kalsec, Kalamazoo, MI). When DBP was used in the formulation it was first mixed with 9.07 Kg of water at 30°C. A separate ice water slurry was created using 13.61 Kg of ice water (1:1) solution containing required levels of salt and SP. This ice slurry was then added to the DBP mixture. Herbalox was then added and followed by the balance of water at 4°C. The technique and amounts used for making the DBP containing brines was following the supplier’s recommendations. Brines without DBP were formulated with 4°C water. All brines were injected at 4°C. Individual brine batches (45.45 Kg) were prepped immediately prior to injection.
2.3.3 Injection

Brine injection was conducted on three separate days. Paired loins were each quartered. A sample was removed for initial pH and proximate composition. The quarters from one side of each pair were randomly assigned to brines with DBP. The other side of the pair was assigned to brines without DBP. Strip loin quarters were weighed and then injected with brine at 4 °C using a 20 needle (interior bore size of 2.5 mm, Model # 2 – 1 – 4 x 0.6 x 272 – H x 2 x 2.5 x 5, Fomaco Food Machinery Co., Copenhagen, Denmark) automatic brine injector (Fomaco Model FGM 20/20S, Fomaco Food Machinery Co., Copenhagen, Denmark) calibrated to inject at ~110% of the recorded initial meat weight. Needles penetrated meat to 0.64 cm above the bottom of the meat surface at a rate of 40 strokes/min and a pressure of 179 kPa. Needles were approximately 2.54 cm apart. Loins were re-weighed immediately after injection.

2.3.4 Equilibration, slicing, and packaging

Injected loin quarters were allowed to equilibrate 30 min on cutting tables and then re-weighed prior to slicing into 2.54 cm steaks using a standard 33.02 cm manual slicer (Model 3600P, Globe Food Equipment Co., Ohio, U.S.A.). Three steaks were collected from each quarter, weighed and packaged by overwrapping. Overwrapped steaks were placed individually onto an absorbent pad (Pad-Loc Super Absorbent Pads, Sealed Air, Duncan, SC) in a 21.75 x 16.51 x 2.78 cm white tray (Cryovac 3 Processor Trays, Sealed Air, Duncan, SC). Trays were then overwrapped with oxygen permeable film (Oxygen transmission rate = 23,250 cc/m²(24h), OmniFilm, Pliant Corp., Schaumburg, IL) and sealed using a film wrapper (Model WHSS-1, Win-HOLT Equipment Group, Syosset, NY). Overwrapped steaks were placed in 63.5 x 76.5 cm bags (“motherbags”; OTR: <0.1 cc/645 cm²(24 h) @ 23°C
Table 2.1 Least squares means of fluid loss variables of loins and steaks injected with various brines containing salt and sodium phosphate with or without a dehydrated beef protein.

<table>
<thead>
<tr>
<th>NaCl&lt;sup&gt;a&lt;/sup&gt; %</th>
<th>SP&lt;sup&gt;b&lt;/sup&gt; %</th>
<th>DBP&lt;sup&gt;c&lt;/sup&gt; %</th>
<th>Brine loss&lt;sub&gt;30min&lt;/sub&gt; %</th>
<th>Purge %</th>
<th>Purge&lt;sub&gt;total&lt;/sub&gt; %</th>
<th>Brine loss&lt;sub&gt;total&lt;/sub&gt; %</th>
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<td>6.1</td>
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<td>4.5</td>
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<td>1.2</td>
<td>12.5</td>
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<td>&lt;0.01</td>
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<td>&lt;0.01</td>
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<td></td>
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<td>8.2</td>
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<td>&lt;0.01</td>
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<td>P - value&lt;sup&gt;d&lt;/sup&gt;</td>
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<td></td>
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</table>

<sup>a</sup>Sodium chloride (as a percentage of the brine)

<sup>b</sup>Sodium phosphates (as a percentage of the brine; Brifisol® 85 Instant; BK Giulini Corp., Simi Valley, CA, USA)

<sup>c</sup>Dehydrated beef protein (Proliant Meat Ingredients, Ankeny, IA, USA)

<sup>d</sup>P-value for the comparison of each brine combination with or without DBP present

<sup>e</sup>Standard error of the mean
and 0% RH), each containing 4 overwrapped steaks. The air was evacuated and replaced with 35% CO₂, 0.4% CO and 64.6% N₂ gas using a MultiVac C500 (MultiVac, In., Wolfertschwenden, Germany). The final atmosphere in the motherbag was evaluated with a headspace analyzer (CheckMate 9900 O₂/CO₂, PBI Dansensor, Denmark). The motherbags were filled using the MultiVac C500 with a gas inlet pressure at 241 kPa, gas flushing at 60 kPa and seal at 25 kPa.

2.3.5 Storage

Motherbags were stored for 4 days at 4 °C in the dark to simulate transportation conditions. On d 5, the atmosphere in the motherbag was evaluated and then overwrapped packages were removed from the bag and steaks were evaluated.

2.3.6 Purge

Purge was measured and calculated as described previously by Lowder et al. (2011). Purge measurements included Brine loss_{30min}, Brine Loss_{total}, Purge, and Purge_{total}. Briefly, Brine loss_{30min} represents the injection fluid lost during the 30 min equilibration period. Brine Loss_{total} represents the total amount of fluid lost from injection through storage. Both are calculated as a percentage of the total fluid injected. Purge represents the fluid lost from the steak during storage and is calculated as percentage of initial steak weight. Purge_{total} represents the fluid lost from the steak from injection through storage. It back calculates the theoretical weight of the steak if it had been cut immediately after injection instead of after the 30 min loin equilibration period.

**Measurements taken:**

A = initial weight of loin

B = weight of loin after injection
Calculations:

\[ \% \text{ Brine loss}_{30\text{min}} = \frac{B - C}{B - A} \times 100 \]

\[ \% \text{ Brine Loss}_{\text{total}} = \frac{\frac{D}{C} \times (B - C) + (D - E)}{(B - A) \times \frac{D}{C}} \times 100 \]

\[ \% \text{ Purge} = \frac{D - E}{D} \times 100 \]

\[ \% \text{ Purge}_{\text{total}} = \frac{\frac{D}{C} \times (B - C) + (D - E)}{(\frac{D}{C} \times (B - C) + D)} \times 100 \]

2.3.7 Experimental design

In order to develop a response surface model, a central composite design was applied. Variables were concentration of sodium chloride (salt, NaCl) and concentration of sodium phosphate (SP) applied in the combinations shown in Table 2.1. The combinations were assigned to experimental units (loin quarter) in a randomized incomplete block design with animal (loin pairing) designated as the block. Four replications were analyzed for each combination except 9 (1.8% salt, 2.25% SP) which had six replications and was present within each block.
2.3.8 Statistical analysis

PROC GLIMMIX of SAS (Version 9.2, Cary, NC) was used to generate the least squares estimators that created the response surfaces for each treatment (CON, DBP). The following saturated model was assumed for all response variables:

\[ \bar{Y} = \beta_0 + \beta_1 \text{NaCl} + \beta_2 \text{SP} + \beta_{11} \text{NaCl}^2 + \beta_{22} \text{SP}^2 + \beta_{12} \text{NaCl} \text{SP} \]  

A DBP interaction term was included with each individual term. Non-significant quadratic and interaction terms, including the DBP interaction, were removed after consulting the fit statistics in the GLIMMIX package as well as the Type I and III tests of effects. NaCl and SP were left in the model regardless of significance.

Loin pairing was designated as a random effect. Separate equations were created for the control and DBP treatment for each response variable. Purge and Purge\text{total} were modeled using a normal distribution and a linear mixed model. Brine Loss\text{30 min} and Brine Loss\text{total} were converted to proportions by dividing the values by 100, and analyzed using a generalized linear mixed model (GLMM) with a logit link function since responses followed a beta distribution. The following equation is used to invert obtained predicted values to proportions, which can then be multiplied by 100 to produce percentages:

\[ \% \text{Brine loss}_x = \left( \frac{\exp(\text{logit}\bar{Y})}{1+\exp(\text{logit}\bar{Y})} \right) \times 100 \]  

where \( \% \text{Brine loss}_x \) is the respective response variable and \( \text{logit}\bar{Y} \) is the predicted value obtained from the GLMM analysis.

Data for these two variables were converted back to percentages for visual representations. Three dimensional response surface graphs and contour plots were generated by SigmaPlot (Version 12.0, Systat Software, Inc., Chicago, IL). Comparisons among brine combinations (Table 2.1) and treatments (CON, DBP) were
made, where necessary, using Tukey’s honestly significant difference method with a predetermined significance level of $P = 0.05$. For pH, differences between brine combinations at different time periods (before injection, after injection and at 5 d) and vice versa were made using the SLICEDIFF option in SAS and Tukey’s honestly significant difference.

### 2.4 Results

#### 2.4.1 Loin and Steak pH

The pH of loins measured prior to injection was between 5.39 and 5.7 (Table 2.2). There were differences in initial loin pH amongst loins ($P < 0.05$), however, there were no differences between loins within a pair. The pH of loins after injection was affected by brine only. The brines containing 0.66% SP or less had lower pH. The maximum pH occurred with the brine containing 4.5% SP. On d 5, loins injected with brines with at least 3.84% SP had higher ($P < 0.05$) pH than brines which contained 0.66% SP or less. An increase in pH due to injection was observed when the brine contained 2.25% SP or greater ($P < 0.001$; data not shown in tabular form). Regardless of brine injection treatment, pH was increased by d 5 compared to initial levels ($P < 0.01$). Observed pH values for injected and non-injected steaks are similar to those reported by Baublits et al. (2006a,b) and Knock, Seyfert, Hunt, Dikeman, Mancini, Unruh, Higgins & Monderen (2006).
Table 2.2 Least squares means of pH for the main effect of brine combination before and after injection and on d 5 of storage of loins and steaks injected with varying levels of salt and sodium phosphates with or without a dehydrated beef protein

<table>
<thead>
<tr>
<th>NaCl%</th>
<th>SP%</th>
<th>Before Injection</th>
<th>SEMg</th>
<th>After Injection</th>
<th>SEMg</th>
<th>5 d</th>
<th>SEMg</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.02</td>
<td>5.69c</td>
<td>0.04</td>
<td>5.75abc</td>
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</tr>
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</table>

Means within a column with different superscripts are significantly different (P < 0.05)

Sodium Chloride

Sodium Phosphate (Brifisol® 85 Instant; BK Giulini Corp., Simi Valley, CA, USA)

Standard error of the mean

2.4.2 Fluid loss

2.4.2.1 Brine loss<sub>30 min</sub>

The targeted injection level was 110% of initial meat weight; actual injections ranged from 106.6-116.6% with a mean of 111.4 ± 1.9%. After the 30 minute equilibration time, the injections ranged from 105.6-114% with a mean of 109.7 ± 1.9%.

As expected, SP was predicted to have a greater impact in improving fluid retention during the equilibration period than salt (Table 2.3). When brines did not contain DBP, maximum observed values for Brine loss<sub>30min</sub> (brine lost during the equilibration period, the time between injection and slicing) were in excess of 30%, while minimums were observed at slightly less than 5% (Table 2.1; Figure 2.1a). This is contrasted with the addition of DBP (Figure 2.1b) which significantly reduced (P < 0.05) Brine loss<sub>30 min</sub> during the equilibration period in all but three of the brine combinations tested (Table
2.1. The decreased DBP effectiveness with respect to fluid retention was observed for brines containing high levels of SP (4.5%, 3.84%, and 2.25%) in combination with high levels of NaCl (1.8%, 3.07%, and 3.6%, respectively). The numeric reduction in Brine loss as a result of DBP addition ranged from 3.7% to 19.2%. Adding DBP to the brine is predicted to effectively reduce Brine loss during equilibration; however, this impact is minimal at high SP and salt levels.

2.4.3 Purge and Purge total

Contour plots for Purge (this represents brine loss from the steaks during storage) are shown in Figure 2.2. The plots were generated using the coefficients given in Table 2.3. Observed Purge values ranged from 0.6% to 4.6% for CON and 0.3% to 2.1% for DBP (Table 1). For both equations, the factors of salt level, SP level, quadratic SP level and the salt x SP interaction were significant. Predicted values suggest that, at the levels investigated, using SP alone would be more effective in reducing Purge values than salt alone. Predicted values for Purge from steaks demonstrate that if processors are willing to accept a fluid loss minimum at around 2.0% as opposed to <1%, both SP and salt can be reduced by ~50%. However, by including DBP in the brine a 50% reduction in SP and salt would result in a Purge of <1%. The Purge intercept (Table 2.3), which denotes the predicted Purge level if no salt or SP had been added, of 2.7% in the DBP equation as compared to 6.0% for the control equation reinforces this observation. The range of Purge values for the control steaks seen in this study are similar to those seen by Lawrence, Dikeman, Hunt,
Figure 2.1 Response surfaces of predicted $Brine\ loss_{30\ min}$ (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).
Kastner and Johnson (2004) and Rowe, Pohlman, Brown, Johnson, Whiting and Galloway (2009) when using similar brines. Contour plots are shown for the predicted $\text{Purge}_{\text{total}}$ of brines without and with DBP (Figure 2.3). Prediction equation coefficients for the control and DBP brines are given in Table 2.3. The $\text{Purge}_{\text{total}}$ calculation takes into account not only the fluid lost after the steak was cut, but it back calculates to account for the fluid that was originally in the steak at the time of injection. Observed $\text{Purge}_{\text{total}}$ values were as high as 6.8% for CON brines, but only 2.8% for DBP brines. The contour plot for control brines is similar to the one seen for $\text{Purge}$, showing decreased values as the levels of both salt and SP in the brine increase. As expected, inclusion of phosphates reduced fluid loss. As with $\text{Purge}$, addition of DBP can significantly reduce $\text{Purge}_{\text{total}}$. There are a broad range of values of salt or SP or combinations of both ingredients that produce predicted $\text{Purge}_{\text{total}}$ values of less than 1.5% or less when DBP is present. Another interesting observation is the use of DBP with high levels of salt and SP in combination can be detrimental to fluid retention, as indicated by the much higher positive interaction coefficient in the DBP equation (0.3068) as compared to the control equation (0.059).

2.4.4 $\text{Brine loss}_{\text{total}}$

Response surfaces are shown for the predicted $\text{Brine loss}_{\text{total}}$ of control brines (Figure 2.4a) and DBP brines (Figure 2.4b). Without DBP in the brine, predicted $\text{Brine loss}_{\text{total}}$ from injection to d 5 of storage can be reduced to just under 20% of the total amount of injected fluid when both salt and SP are maximized. Reductions in salt and/or SP are predicted to increase that value, reaching near 80% at minimum levels of both ingredients. Adding DBP to the brine reduces the maximum amount of fluid loss to just under 40% given minimum levels of salt and SP. Using median to high levels of SP
Table 2.3 Coefficients for substitution into Eq. (1)\(^a\) for Purge and Brine loss measurements of loins and steaks injected with a final concentration of up to 0.36% salt and up to 0.45% sodium phosphates with or without a dehydrated beef protein (DBP)\(^b\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intercept ((\beta_0))</th>
<th>NaCl ((\beta_1))</th>
<th>SP ((\beta_2))</th>
<th>NaCl(^2) ((\beta_{11}))</th>
<th>SP(^2) ((\beta_{22}))</th>
<th>NaCl*SP ((\beta_{12}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.0004</td>
<td>-0.8357</td>
<td>-1.5130</td>
<td>-</td>
<td>0.0880</td>
<td>0.1164</td>
</tr>
<tr>
<td>DBP</td>
<td>2.7088</td>
<td>-0.5869</td>
<td>-0.9263</td>
<td>-</td>
<td>0.0880</td>
<td>0.1534</td>
</tr>
<tr>
<td><strong>Purge(_{total})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.9270</td>
<td>-0.8750</td>
<td>-1.9351</td>
<td>-</td>
<td>0.1559</td>
<td>0.0585</td>
</tr>
<tr>
<td>DBP</td>
<td>4.1695</td>
<td>-0.8750</td>
<td>-1.5148</td>
<td>-</td>
<td>0.1559</td>
<td>0.3068</td>
</tr>
<tr>
<td><strong>Brine loss(_{30min})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-0.8801</td>
<td>0.2767</td>
<td>-0.3538</td>
<td>-0.1606</td>
<td>-</td>
<td>0.0961</td>
</tr>
<tr>
<td>DBP</td>
<td>-2.2946</td>
<td>0.2767</td>
<td>-0.3538</td>
<td>-0.1606</td>
<td>-</td>
<td>0.1690</td>
</tr>
<tr>
<td><strong>Brine loss(_{total})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.9265</td>
<td>-0.2477</td>
<td>-1.2268</td>
<td>-0.1286</td>
<td>0.0852</td>
<td>0.1688</td>
</tr>
<tr>
<td>DBP</td>
<td>0.0259</td>
<td>-0.2477</td>
<td>-1.2268</td>
<td>-0.1286</td>
<td>0.0852</td>
<td>0.3032</td>
</tr>
</tbody>
</table>

\(^a\) \(Y = \beta_0 + \beta_1\text{NaCl} + \beta_2\text{SP} + \beta_{11}\text{NaCl}^2\text{NaCl} + \beta_{22}\text{SP}^2\text{SP} + \beta_{12}\text{NaCl}^2\text{SP}\) where NaCl is the level of salt in the brine and SP is the level of sodium phosphate in the brine

\(^b\) Proliant Meat Ingredients, Ankeny, Iowa, USA

\(^c\) Variables were converted to proportions and fit to a beta distribution

(>2.25%) with minimum levels of salt (<1.5%) with DBP is predicted to decrease total injected fluid loss to less than 10%.

### 2.5 Discussion

The higher pH values seen in steaks injected with sodium phosphates is expected (Lawrence et al., 2004; Baublits et al., 2006a, b; Cerruto-Noya, VanOverbeke & Mireles DeWitt, 2009). Increased pH values are often considered important in injected or marinated meat products because as the pH shifts away from the isoelectric point of muscle proteins, their ability to bind water increases (Oreskovich, Bechtel, McKeith, Novakofski, & Basgall, 1992). However, there are other factors to consider with regard to the water binding abilities of meat proteins.
Figure 2.2 Contour plots of predicted Purge (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).
Figure 2.3 Contour plots of predicted $P_{\text{urgetotal}}$ (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).
Figure 2.4. Response surfaces of predicted Brine loss$_{total}$ (%) of overwrapped steaks as salt (NaCl) and sodium phosphate (SP) are varied in brines (a) without dehydrated beef protein (CON) and (b) with dehydrated beef protein (DBP).
Trout and Schmidt (1986a) observed increased cook yields and tensile strengths of beef rolls when increasing ionic strength at a constant pH. While sodium phosphates increase both pH and ionic strength, much of their functionality is due to their ability to dissociate the acto-myosin bond (Trout & Schmidt, 1986a; Offer & Knight, 1988). Additionally, sodium chloride (NaCl) generally does not increase pH, but it is effective in increasing the water binding ability of meat by increasing ionic strength, dissolving myosin filaments and reducing the isoelectric point (Offer & Knight, 1988).

Salt and SP have long been known to work synergistically to affect the functional attributes of meat proteins (Offer & Trinick, 1983; Trout & Schmidt, 1986a). Since SP is restricted to no more than 0.5% in the final product, its effects at or near that level combined with various levels of salt have been thoroughly researched and are well known. The efficacy of salt/phosphate combinations using amounts of SP closer to the minimum is less clear, however. Also, since the use of salt is not restricted, amounts used in previous research of whole muscle injected beef can differ greatly. Salt levels targeted for the final injected product are typically 0.5% or lower (Cerruto-Noya et al., 2009; Lawrence et al., 2004; McGee et al., 2003; Robbins et al., 2002; Rowe et al., 2009; Vote et al., 2000; Wicklund et al., 2005;). The maximum level of salt used for this study was 0.36% in the final product; extrapolation beyond that amount is not statistically appropriate. While it is possible that further increases in salt concentration could have functional benefits, many of the surface maps and contour plots show a plateau near their maximum evaluated levels. This suggests that the functional value gained from further increases beyond this point would be greatly diminished, especially when DBP and/or SP are included in the brine. This plateau effect at higher levels of NaCl has
been demonstrated previously with response surface modeling using posterior pork loin sections injected with salted marinade (Detienne & Wicker, 1999).

Detienne and Wicker (1999) performed a response surface study on pork loins, with level of salt (0 – 1.5%) and sodium tripolyphosphate (STPP; 0 – 0.45%) in the final product as the variables. The purge calculation described by the authors is similar to the Brine loss_{total} calculation performed in this study. The authors saw maximum predicted values of greater than 90% purge loss for tail loin sections and just over 70% for head sections of the loin when salt and SP were at their minimum levels and minimum predicted values near 0% for both sections when salt and SP were at their maximum. The predictive equations for Detienne and Wicker (1999) included the significant effects of salt concentration, quadratic salt concentration and the salt x phosphate interaction for the head section, whereas, similar to the present study, all effects were significant for the tail section.

The reduction of salt and SP can also be achieved by the addition of non-meat ingredients that act as alternative alkaline pH substitutes or water binding agents. The use of a high pH brine using 0.1% ammonium hydroxide (AH) as the alternative alkaline pH substitute and 3.6% salt was compared to a 4.5% STPP, 3.6% salt brine in beef strip steaks injected to 110% green weight (Cerruto-Noya et al., 2009). The AH injected steaks had 3.16% higher purge values after 4 d of dark storage and 2.9% higher values purge values after an additional 7 d of retail display. Authors concluded higher levels of AH would be needed to completely replace phosphates in the brine and produce a comparable quality product. A follow-up study by Parsons et al., 2011a demonstrated that 1% AH could successfully replace 4.5% SP (Brifisol 85® Instant) in a meat injection brine. Purge from beef loin steaks was reported as being an average of 1.31% from SP
injected beef loins, while AH injected steaks had a purge of 2.07%. Complete replacement of the SP by an alternative alkaline agent such as AH was predicted to reduce final sodium in the product by almost 50%. However, there are concerns with the use of alkaline agents. Although this ingredient has been determined to be GRAS by USDA when used to increase meat brines to a final pH ≤11.6, there has been misguided consumer concern with this ingredient as the media has associated it with toxic levels of ammonia. As a result, water binding agents such as the dehydrated beef protein described in this study may offer more acceptable alternatives from a consumer perspective as they are derived from their destination protein source.

Studies evaluating same source meat binding ingredients have been previously reported. Lawrence et al. (2004) compared a commercial beef broth and carrageenan in their ability to prevent purge losses in strip steaks injected to a final concentration of 0.44% SP (Curavis® 250 blend) and 0.22% salt. After 5 d of retail display the authors saw no significant differences in purge values between the steaks injected with salt and SP alone, and steaks injected with 1 or 2% beef broth or carrageenan in addition to the salt and SP. A brine containing acid solubilized beef protein with 1.8% salt was evaluated compared to a 3.6% salt, 4.5% phosphate brine injected into strip loins at 10% over initial weight (Vann & Mireles DeWitt, 2007). Protein injected steaks had 6.64% higher (P < 0.05) purge values than phosphate injected steaks after 5 d of storage. A pork collagen protein powder, similar to the one used in this study, was evaluated by Schilling, Mink, Gochenour, Marriott and Alvarado (2003) and Prabhu, Doerscher and Hull (2004). Schilling et al. (2003) found that restructured hams formulated with 3% collagen protein powder had lower expressible moisture than those formulated without it. Frankfurters formulated with at least 1.5% collagen and hams including 3% collagen
experienced significantly less purge losses than those formulated without it (Prabhu et al., 2004). Lowder et al. (2011) evaluated fluid loss and quality attributes of steaks from brine injected beef loins in which the SP (Brifisol® 85 Instant) in the brine was completely replaced with 5% dehydrated beef protein (DBP). The Brine loss of DBP loins was 2.99% compared to 13.35% for SP treated loins while DBP steaks evaluated over 6 d of display had 2.9% less brine loss. Steak color as determined by a trained panel was no different after 6 d and sensory flavors and juiciness were also found to be similar between the DBP and SP treatments. However, the DBP steaks were found to be slightly less tender than SP by a trained panel and Warner-Bratzler shear force.

There are several possible mechanisms that allow collagen protein powders to increase water holding capacity of raw and cooked meat, including: (1) hydration of hydrophilic moieties; (2) increase in viscosity of the brine dispersion due to thickening of collagenous proteins at low temperatures, (3) formation of a progressive gel network after injection into the meat product, (4) possible interactions with myofibrillar proteins (Schilling et al., 2003; Lowder et al., 2011). The calculated Brine loss value represents the percentage of fluid injected into the meat product that is lost between injection and slicing/packaging. This variable is typically not reported in scientific studies. However, as shown here, the losses during this short equilibration time can be significant. The ability to hold more injected fluid during this time period is important to processors because it represents an ability to increase the weight of packaged product and reduce plant generated waste. The Brine loss values seen during this study suggest the equilibration time is a major point of fluid loss for processors. As a collective, the eighteen brines tested in this study suffered ~48% of their Brine loss in the first thirty minutes after injection (data not shown). This time period seems to be
when the DBP has the greatest effect in reducing fluid loss. The increase in viscosity and ability to form a gel network at low temperatures act to restrict free water within the muscle structure immediately after injection. With salt and SP, the benefits of ionic strength and pH increases may be seen right away, but effects of reorganizing the myofibrillar protein structure to increase water binding may take more time.

It should be noted that the phosphate blend used in this study differs from some of the other studies discussed and differs from those used by some processors. There are numerous phosphate manufacturers, each producing their own proprietary blends of different chain-length phosphates, along with the commonly used pure STPP. Chain length of phosphates will determine their functional characteristics, including solubility, solution pH and ionic strength, as well as their ability to solubilize acto-myosin and chelate divalent cations (Knipe, 2003, 2004; Trout and Schmidt, 1986b). The Brifisol® 85 Instant blend used in this research is rich in diphosphate, the active form in dissociating the acto-myosin complex, but also contains STPP and long chain phosphates, which are effective chelators and may bind water molecules directly. Pure STPP, which is a common solution for processors because of its relatively low cost and ease of solubility, can be reduced to the active diphosphate by myosin-ATPase, but lacks the robust functionality of blended phosphates.

Finally, data demonstrates that minor compromises in the loss of brine from the product can produce significant reductions in the sodium content regardless of whether a water binding agent (DBP) is included or not. The prediction equations such as those generated by this study allow processors to predict the fluid loss that will occur when salt or phosphates are reduced or a binding agent such as DBP is added to beef strip loins. In the past, salt and phosphate incorporation has been focused on maximizing the
quality attributes of injected products. The impact on nutritional quality of the product was thought to be minimal as levels of salt and phosphate incorporated in injected meat products are much lower than levels used in comminuted products. While the levels of sodium are much lower than those we find in products such as frankfurters, they are at least 4x higher than the levels in natural meat (Parsons et al., 2011b). Due to the increasing concern over the level of sodium consumption by consumers, the meat industry should be looking to carefully scrutinize and justify the level of sodium formulated into all products, not just comminuted meat products. Results from this study demonstrate that an increased understanding of the dynamic between salt and phosphate in injected beef products can produce opportunities for sodium reduction.

2.6 Conclusions

Use of salt and SP in injection brines decreased observed and predicted purge losses in beef strip loins and steaks. At the levels tested in this study, SP appears to have a stronger effect than salt on reducing purge losses when used alone. The use of DBP reduced observed and predicted purge losses at most levels of salt and SP, especially when used with a single ingredient or at low levels of both. The functional benefits of adding DBP are predicted to be strongest when either salt or SP are eliminated or included at only minimal levels. The equations generated by these results could help processors to achieve acceptable purge losses while reducing sodium use.
3. Evaluation of a dehydrated beef protein to replace sodium-based phosphates in injected beef strip loins

Austin C. Lowder, Carla L. Goad, Xingqiu Lou, J.Brad Morgan, Christina A. Mireles DeWitt

Published in Meat Science
3.1 Abstract

A dehydrated beef protein (DBP) was evaluated as a replacement for the phosphate added to beef injection brines. U.S. Select strip loins (n=20) were injected to 110% of their initial weight with a brine containing 3.6% salt and 4.5% sodium phosphate (CON) or 3.6% salt and 5% dehydrated beef protein (DBP). DBP loins had less fluid loss after 30 min. Steaks from both treatments lost similar amounts of fluid during storage. Total fluid loss was lower for DBP injected product. Lipid oxidation (TBARS) products were 0.23 – 0.60 mg/Kg higher for DBP steaks. DBP steaks were slightly less red than CON steaks according to instrumental measurements. Sensory panel evaluation, however, indicated no differences in redness. DBP steaks were less tender according to trained sensory panel. Results indicated the DBP to be effective in increasing brine retention and a viable alternative to phosphates when used in brines injected into beef strip steaks.

3.2 Introduction

It is common for meat products to be injected with a brine containing water, sodium chloride (NaCl; salt) and sodium phosphates (SP). Salt and SP are important ingredients in enhanced meats because they act synergistically to alter myofibrillar protein to improve the water holding capacity of the product (Offer & Trinick, 1983; Offer & Knight, 1988). This effect is important to processors because it compensates for the natural loss of fluid (purge) from meat in storage and retail display and retains fluid through cooking (Baublits, Pohlman, Brown, & Johnson, 2005a). Brine injection with sodium phosphates can also provide the benefits of increased oxidative and microbial stability (Lamkey, Mandigo, & Calkins, 1986; Pohlman, Stivarius, McElyea, Johnson,
Johnson, 2002), increased tenderness (Baublits et al., 2005a; Vote et al., 2000), and improved juiciness (McGee et al., 2003). However, color stability can be adversely affected by brine injection (Lawrence et al., 2004; Stetzer, Wicklund, Paulson, Tucker, Macfarlane, & Brewer, 2007). The high sodium content of salt (39.3%) and sodium phosphates (30±1%; Brifisol® 85 Instant) can negatively affect the nutritional value of meat. Sodium reduction has long been advocated by government and consumer groups because of its association with hypertension, cardiovascular disease and stroke (Desmond, 2006; Meneton, Jeunemaitre, de Wardener, & MacGregor, 2005).

Approximately 21% of the sodium in the U.S. diet is supplied by processed meats (Engstrom, Toblemann, & Albertson, 1997). In light of this, it would be advantageous to research novel ingredient technologies that can increase water binding while allowing for an overall reduction of sodium. One such ingredient, a dehydrated beef protein (DBP), is a purified beef collagen protein powder. A similar product manufactured from pork collagen has previously been successful in reducing purge and cook loss in hams and pork frankfurter-type sausages (Prabhu, Doerscher, & Hull, 2004; Schilling, Mink, Gochenour, Marriott, & Alvarado, 2003). The objective of this study was to investigate the effects of DBP, on shelf life and palatability characteristics, such as purge, color, lipid oxidation and microbial stability, cook yield, shear force and sensory attributes of beef strip steaks.

3.3 Materials and Methods

3.3.1 Collection of beef strip loins

Paired USDA select beef strip loins (IMPS 180; n = 20) were collected at a processing facility at the time of carcass fabrication. Paired loins were vacuum
packaged at the processing facility, transported to Oklahoma State University and stored at 4 ºC overnight. All subsequent preparation of brines and raw materials was conducted in a processing facility with a constantly maintained temperature of 4 ºC.

3.3.2 Brines

The control brine (CON) consisted of 3.6% salt and 4.5% sodium phosphate (SP) blend (Brifisol® 85 Instant; BK Giulini Corporation, Simi Valley, CA) and 1% Herbalox seasoning type HT-S wt/wt (Kalsec, Kalamazoo, MI), which has antioxidant capacity due to the presence of rosemary. The brine containing dehydrated beef protein (DBP) consisted of 3.6% salt, 5% dehydrated beef protein (Proliant Meat Ingredients, Inc., Ankeny, IA) and 1% Herbalox seasoning type HT-S wt/wt. The dehydrated beef protein was prepared in 9.07 Kg of 30 ºC water before being mixed with a 13.61 Kg ice/water (1:1) solution containing the salt. The 30 ºC water was necessary to properly disperse the DBP powder. The balance of water was then added at 4 ºC. The CON and DBP brines had pH of 8.44 and 7.49, respectively. Both brines were injected at 4 ºC. Individual brine batches (45.45 Kg) were prepped immediately before injection.

3.3.3 Injection

Strip loins were trimmed, a sample (~30 g) was collected from each loin for initial pH and proximate composition and then loins were weighed. Injection took place approximately 24 hr postmortem. Loins within pairs were assigned to CON and DBP treatments. Weights were taken immediately after injection. Loins were injected with an automatic brine injector (Fomaco Model FGM 20/20S, Fomaco Food Machinery Co., Copenhagen, Denmark) calibrated to inject at 110% of initial weight was used. The needles (n = 20; Model #6, Fomaco Food Machinery Co., Copenhagen, Denmark)
penetrated the meat to 0.64 cm above the lower meat surface at a rate of 40 strokes/min and a pressure of 26 psi and were spaced 2.54 cm apart.

3.3.4 Equilibration, slicing, packaging and storage

Injected loins were allowed to equilibrate 30 min before slicing into 2.54 cm steaks using a standard 33.02 cm manual slicer (Model 3600P, Globe Food Equipment Co., OH). Twelve steaks from each loin were weighed individually and packaged on trays (Cryovac 3 Processor Trays, Sealed Air, Duncan, SC) with absorbent pads (Pad-Loc Super Absorbent Pads (PLS), Sealed Air, Duncan, SC) overwrapped with oxygen permeable film (Oxygen transmission rate = 23,250cc/m²(24 h), OmniFilm, Pliant Corp., Schaumburg, IL). Overwrapped steaks were placed in 63.5 x 76.5 cm bags ("motherbags"; Oxygen transmission rate = <0.1 cc per 645 cm²/24 h @ 23°C and 0% RH), each containing 4 randomly selected steaks. The air was evacuated and replaced with 35% CO₂, 0.4% CO and 64.6% N₂ gas using a MultiVac C500 (MultiVac, In., Wolfertschwenden, Germany). Motherbags containing the steaks were stored for 4 d at 4 °C in the dark to simulate transportation. After storage, steaks were removed from their bags and either taken for analyses (d 0) or placed in retail display until needed (d 2, 4, 6). Steaks were randomly assigned to their respective analyses and day before the initial dark storage period. Retail display was conducted in a cooler at 4 °C under 40 watt Rapid Start T12 Flourescent Platinum lights (Promolux, B.C., Canada; 1600 – 1900 lux). Lights were arranged to deliver 807 to 1614 lux (75-150 ft-candles) of continuous intensity on the meat surface (AMSA, 1991). A GE Triple Range Light Meter (Model 217, GE Lighting, Cleveland, OH) was utilized to verify light intensity. Steaks were randomly placed in the display and were rotated each day to negate any inconsistencies in light intensity among different display areas. Three steaks from each loin were
randomly collected from retail display on d 0, 2, 4 and 6 for further analyses. The first and second steaks were used for purge and pH. The first steak would also be used for cook yield and Warner Bratzler shear force. The second steak would be used for cook yield and sensory. The second steak designated for d 6 was also used for color panel and instrumental color. The third steak was used for thiobarbituric acid reactive substances (TBARS), aerobic (APC) and anaerobic plate counts (AnPC) and proximate compositional analyses. All analyses were conducted on d 0, 2, 4 and 6 except for Warner Bratzler shear force, which was not measured on d 6.

3.3.5 Headspace analysis, proximate composition and pH

Headspace composition of each motherbag was determined after the 4 d storage period using a headspace analyzer (CheckMate 9900 O₂/CO₂, PBI Dansensor, Denmark). After sampling for lipid oxidation, each steak was cut into cubes (~1 cm) and frozen using liquid nitrogen. Frozen cubes were blended into a fine powder using a Waring blender. The powder was placed in a Whirl-Pak® bag and stored at -20 °C until used for proximate analyses. Moisture (AOAC, 2003; method 950.46), crude fat (AOAC, 2003; method 960.39), ash (AOAC, 2003; method 920.153) and protein (AOAC, 2003; method 928.08) were determined. The DBP powder was also analyzed for moisture and protein using the previously mentioned methods. Crude fat (AOAC, 2003; method 991.36) was also determined on the DBP powder.

3.3.6 Purge

Measurements taken:

A = initial weight of loin

B = weight of loin after injection

C = weight of loin 30 min after injection
D = initial weight of steak

E = weight of steak on day 5

B – A = brine added to the loin

B – C = fluid loss 30 min after injection

D – E = fluid loss from time steak was cut until day 5.

\[
\frac{D}{C} = \text{proportion of steak from loin}
\]

\(Brine\ loss_{30\ min}\) (%) represents the fluid lost during the 30 min equilibration period as a percentage of the total fluid injected. It was measured by taking the difference between the weights of the loin immediately after and 30 minutes after injection. This difference is the fluid loss during the 30 min equilibration and it was then divided by the difference between the weight of the loin prior to and immediately after injection and multiplied by 100.

\[
Brine\ loss_{30\ min} = \frac{B - C}{B - A} \times 100
\]

\(Purge_{30\ min}\) (%) represents the fluid lost during the 30 min equilibration period as a percentage of the total loin weight. It was calculated by subtracting the weight of the loin 30 min after injection from the weight of the loin immediately after injection and dividing it by the immediate pumped weight. It represents the weight of fluid lost during equilibration as a percentage of the total weight of the loin.

\[
Purge_{30\ min} = \frac{B - C}{B} \times 100
\]

\(Purge\) (%) reflects the amount of fluid that collects in the trays as the steak sits in retail display. \(Purge\) was measured by taking the weight of the steak after storage and subtracting that weight from the initial weight of the steak at the time it was sliced. This difference was then divided by the initial weight of the steak and multiplied by 100.
\[ \text{Purge} = \frac{D-E}{D} \times 100 \]

\( \text{Purge}_{\text{total}} \) (%) represents the amount of weight lost from injection through display time as a percentage of the total steak weight. It was calculated by determining the proportional weight of a steak and multiplying the ratio by the water loss during the 30 min equilibration. This was the calculated steak fluid loss attributed to each steak 30 min after injection. The fluid loss from the steak 5 days after it was cut was then added to the calculated steak fluid loss. This gives the total calculated fluid loss for the steak from the time of injection. The total water loss is then divided by the calculated steak weight. Calculated steak weight is the weight of the steak at the time it was cut plus the proportion of water loss during the 30 min equilibration that is attributable to the steak.

\[ \text{Purge}_{\text{total}} = \frac{\frac{D}{C} \times (B-C) + (D-E)}{\frac{D}{C} \times (B-C) + D} \times 100 \]

\( \text{Brine loss}_{\text{total}} \) (%) represents the total amount of fluid lost from injection through display expressed as a percentage of the total fluid injected. It was calculated by dividing the total calculated steak fluid loss from the total calculated brine added to the steak at the time of injection. The total calculated water added to the steak is calculated by multiplying the proportional weight of the steak by the total water added to the loin.

\[ \text{Brine Loss}_{\text{total}} = \frac{\frac{D}{C} \times (B-C) + (D-E)}{(B-A) \times \frac{D}{C}} \times 100 \]

3.3.7 Lipid oxidation

Frozen steaks were placed in a 4 °C cooler the night before sampling to thaw slightly. Lipid oxidation analysis was conducted on a 10 g sample cut approximately 2mm deep from the surface of the steak. A modified method of Buege and Aust (1978) as described by Cerruto-Noya, VanOverbeke and Mireles-DeWitt (2009) was used to
analyze the sample for thiobarbituric acid reactive substances (TBARS). Results were reported as mg malondialdehyde per kg of meat (mg/kg).

3.3.8 Microbial analysis

Microbial analysis was conducted using the pour plate method (Morton, 2001). Steaks designated for microbial analysis were aseptically transferred from the original package to a sterile cutting board, where they were cut into small pieces (< 0.5 cm²). A 50 g of sample was then placed into a stomacher bag (Nasco Whirl-Pak® filter bag, model B01318, Fort Atkinson, WI) and 450 ml of 0.1% peptone water solution was added. Sample was pummeled for 2 min at normal speed, and then appropriate dilutions were prepared from the stomacher slurry (Morton, 2001). Duplicate samples were pour-plated using Plate Count Agar (BD Difco™, Sparks, MD) media containing 0.5% of 2,3,5-Triphenyl tetrazolium chloride (TTC; BioChemika, Sigma-Aldrich, St. Louis, MO). Two sets of plates were pour-plated; one set was aerobically incubated at 35 °C for 48 h. While the second set of plates was placed into an anaerobic jar containing GasPak Plus with Palladium Catalyst (BD BBL™, Sparks, MD) to produce anaerobic conditions and then incubated for 48 h at 35 °C. Both aerobic (APC) and anaerobic (AnPC) plate counts were reported as colony forming units per gram (cfu/g).

3.3.9 Shear force

Steaks used for shear force evaluation were cooked in the same manner as the steaks used for cook yield. They were subsequently allowed to cool to approximately 21 °C before six cores per steak were excised parallel to the direction of the muscle fibers to be used to determine shear force according to AMSA (1995). An Instron Universal Testing Machine with a Warner-Bratzler shear head attachment (Model 4502, Instron, Grove City, PA) was used for testing. Shear force values for each steak are the average
of six cores and are reported as peak force (N). Steaks to be used for shear force determination on d 6 were compromised before they could be analyzed; their results were not included in the data analysis and presentation.

3.3.10 Subjective color score

Color scoring was conducted on steaks in retail display at 12 hour intervals (am and pm). Six panelists were selected and trained according to AMSA (1991). Muscle color (brightness and color of non-discolored lean; 8 = very bright cherry red, 7 = moderately bright cherry red, 6 slightly cherry red, 5 = red, 4 = slightly dark red, 3 = moderately dark red or brown, 2 = dark red or brown and 1 = extremely dark brown), fat color (5 = white, 4 = creamy white, 3 = slightly yellow, 2 = moderately yellow and 1 = yellow), percent discoloration (7 = none, 6 = 1 – 20%, 5 = 21 – 40%, 4 = 41 – 60%, 3 = 61 – 80%, 2 = 81 – 99% and 1 = 100% or complete) and overall acceptability (7 = extremely desirable, 6 = desirable, 5 = slightly desirable, 4 = acceptable, 3 = slightly undesirable, 2 = undesirable and 1 = extremely undesirable) which takes into consideration the three other factors, were evaluated. Panelist scores were averaged for each steak.

3.3.11 Instrumental color

CIE (1978) $L^*$, $a^*$ and $b^*$ color space values were determined with a Hunter Miniscan™ XE Plus (Model 45/0-L, HunterLab, Reston, Va, U.S.A.) using illuminant $D_{65}$ and a $10^\circ$ observer and a 2.54 cm aperture. Three measurements every 12 h were taken at random locations on each steak through the overwrap film and averaged for each steak at each evaluation period, avoiding any seam fat. Black and white tiles covered with the overwrap film were used for calibration.
3.3.12 Cook yield

Steaks were weighed, cooked to an internal temperature of 70 °C using an impingement oven (Lincoln Model 1022, Lincoln Food Service Products Ind., Fort Wayne, IN, U.S.A.), then reweighed after being allowed to cool to approximately 40 °C. *Cook Yield* was calculated by dividing the final cooked weight by the weight of the steak after removal from retail display and multiplying by 100. *Cook Yield*\textsubscript{adj} takes into account the fluid loss that occurred during the equilibration period. *Cook Yield*\textsubscript{adj} was calculated by dividing the final cooked weight of the steak by the calculated initial weight of the steak (*Calc Steak Weight*\textsubscript{total}), and multiplying by 100.

3.3.13 Trained sensory analysis

A six member panel was selected and trained according to Meilgaard, Civille and Carr (2007) and AMSA (1995). There were five training sessions of approximately one hour each. Steaks were cooked in the same manner as those used for cook yield. Cooked steak cubes (1 cm × 1 cm; two per panelist) were evaluated for initial juiciness (8 = extremely juicy and 1 = extremely dry), sustained juiciness (8 = extremely juicy and 1 = extremely dry), initial tenderness (8 = extremely tender and 1 = extremely tough), connective tissue amount (8 = none and 1 = extremely abundant) and overall tenderness (8 = extremely tender and 1 = extremely tough) using an 8 point scale. Cooked beef flavor and salty flavors were evaluated on a 3 point scale (3 = strong and 1 = not detectable). Panelists participated in 2 separate sessions on each day of evaluation (d 0, 2, 4, 6). Samples were cut into approximately 1 cm cubes, which were held in insulated containers that the panelists would receive in a completely random order. Panelists were placed in separate booths with red lighting during testing.

3.3.14 Statistical analysis
Data were analyzed as a randomized complete block design with treatment (CON vs. DBP) as the independent variable. The experimental unit was loin for all response variables. Paired loin (animal) was defined as a random block. Day of display (0, 2, 4, 6) was defined as a repeated measure where appropriate, with covariance structures modeled in PROC MIXED. An analysis of variance (ANOVA) was conducted for all response variables. Pair-wise comparisons of least squares means were conducted, when appropriate, using unadjusted t-tests. PROC MIXED of SAS (Version 9.2, SAS Institute, Inc., Cary, NC, U.S.A.) was used for all analyses. All tests were conducted at a significance level of 0.05.

3.4 Results and Discussion

3.4.1 Enhancement

The injected loins were targeted for a 10% increase over initial weight. However, final loin weights after equilibration were slightly higher than their targets. Loins injected with the DBP brines averaged an 11.48% increase over initial weight while CON loins averaged an 11.78% increase. Although the injected weights were slightly over their targets, they were similar between treatments ($P = 0.636$) and provide a good basis for treatment comparison.

3.4.2 Loin and steak pH

Initial (pre-injection) pH values for the loins ranged from 5.38 to 5.58 (data not shown in tabular form). For injected steaks, the main effects of treatment and storage day were significant. Average pH values for DBP and CON steaks were 5.45 and 5.81, respectively. The higher ($P < 0.001$) pH values seen for the control steaks are to be expected as the control injection brine had a pH of 8.44 due to the presence of 4.5% SP.
The steak pH values were greatest \((P < 0.05)\) for both treatments at d 0 of display (Table 3.1). They then fluctuated, decreasing \((P < 0.001)\) from d 0 to 2 and increasing \((P < 0.001)\) from d 2 to 4. Although this fluctuation was minor \(<0.2\) it was statistically significant \((P < 0.05)\). The higher meat pH of phosphate containing products compared to non-phosphate containing products is quite common, as observed by previous researchers (Baublits et al., 2005a; Baublits, Pohlman, Brown Jr, & Johnson, 2006a; Boles & Shand, 2001; Lawrence et al., 2004). Our observed pH values for phosphate enhanced steaks were similar to those previously reported by Baublits, Pohlman, Brown Jr., Yancey, & Johnson (2006b) and Knock, Seyfert, Hunt, Dikeman, Mancini, Unruh, Higgings, & Monderen (2006). While raising the pH of meat products does increase water binding ability, there are other factors to consider. Trout and Schmidt (1986a) note that increasing ionic strength while holding pH steady increases cook yields and tensile strength of beef rolls. Also, Offer and Knight (1988) maintain that, in addition to altering ionic strength and pH, salt and SP cause the solubilization of myosin bands and the dissociation of actomyosin, respectively. Because of these mechanisms hydrophilic sites are exposed for water binding and myofibrillar structure is weakened, allowing for an expansion of available space for fluid entrapment.

3.4.3 Compositional analysis

The dried beef protein consisted of 85.4% protein, 8.6% moisture and 0.4% fat (data not shown in tabular form). Addition of DBP should increase the protein content, by calculation, slightly less than 0.5%. The increase was observed in the present study, but it was not sufficient enough to make a significant difference in protein content (Table 3.1). There was no (treatment x storage day) interaction for percentage fat, moisture,
Table 3.1  Main effect least squares means of moisture, fat, ash, protein and pH of beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>74.7</td>
<td>3.76</td>
<td>1.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.4</td>
<td>5.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DBP</td>
<td>74.4</td>
<td>3.86</td>
<td>1.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.9</td>
<td>5.45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4</td>
<td>0.28</td>
<td>0.03</td>
<td>0.3</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Day

<table>
<thead>
<tr>
<th>Day</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>75.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.61</td>
<td>1.51</td>
<td>20.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>75.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.70</td>
<td>1.62</td>
<td>20.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.54&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>74.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.96</td>
<td>1.55</td>
<td>21.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.65&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>74.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.97</td>
<td>1.51</td>
<td>21.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4</td>
<td>0.38</td>
<td>0.04</td>
<td>0.3</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means within a column and main effect with different superscripts are significantly different (<i>P</i> < 0.05)

<sup>c</sup>Pooled standard error of the mean

protein or ash (<i>P</i> > 0.05). The main effect of storage day was significant for moisture (<i>P</i> = 0.0082) and protein (<i>P</i> = 0.0003). No differences in fat, protein or moisture were seen due to treatment effects (<i>P</i> > 0.05). Percent ash was greater (<i>P</i> = 0.0001) in CON steaks, which is to be expected because the CON brine contained 4.5% SP and the DBP brine contained none. Cerruto-Noya et al. (2009) saw similar results when comparing beef strip loins injected with a high pH ammonium hydroxide brine to those injected with a brine containing phosphates.

3.4.4 Fluid loss

3.4.4.1 Fluid loss from intact loins

The ability of the injected loins to retain fluid immediately after injection is explained by the Brine Loss<sub>30 min</sub> and Purge<sub>30 min</sub> calculations (Table 3.2). The fluid lost as a percentage of the fluid injected (Brine Loss<sub>30 min</sub>) was greater (<i>P</i> < 0.001) for loins injected with the control brine than those injected with a brine containing DBP. When compared as a percentage of the total weight of the loin (Purge<sub>30 min</sub>), the DBP injected
loins lost less ($P < 0.001$) fluid in the first 30 min than the CON injected loins (0.31 vs 1.59%, respectively).

3.4.4.2 Fluid loss from cut steaks

There were no interactions (treatment x storage day) for Purge, $Purge_{total}$ or Brine Loss$_{total}$ (Table 3.3). Purge was not affected by treatment ($P = 0.705$). However, this value does not consider the fluid loss prior to loins being cut into steaks. When calculated to account for all the brine lost, the CON brine steaks actually had greater ($P < 0.001$; Table 3.3) $Purge_{total}$ values over the entire display time than the DBP treatment. As expected, steaks from both groups had increased ($P < 0.05$) $Purge_{total}$ values as display time increased. The amount of weight lost during storage as a percentage of the amount of brine injected is calculated as Brine Loss$_{total}$. Throughout storage the CON steaks lost more ($P = 0.008$) of the injected brine than the DBP treatment steaks. These results are similar to the $Purge_{total}$ results, which show an increase in fluid loss over display time. If only the traditional purge calculation ($Purge$) had been used to evaluate fluid retention over the duration of the study, it would appear that the brine containing phosphate (CON) was retaining the brine over time similar to that of the DBP treatment. However, comparing the Brine Loss$_{total}$ and $Purge_{total}$ calculations demonstrate that the DBP steaks retained a greater amount of injected brine from the time of enhancement until the end of retail display. Various approaches have been investigated to replace phosphates in beef injection brines. Addition of functional ingredients to whole muscle beef cuts was attempted by Lawrence et al. (2004) and Vann & Mireles DeWitt (2007). Lawrence et al. (2004) saw similar weight losses and expressible moisture after 5 d of retail display when evaluating the use of 1 or 2% beef broth or carrageenan with phosphate and salt in injected (11.5%) longissimus beef steaks. When Lawrence et al.
Table 3.2 Least squares means of \( \text{Brine Loss}_{30 \ min} \) and \( \text{Purge}_{30 \ min} \) of beef strip loins injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( \text{Brine Loss}_{30 \ min} )</th>
<th>( \text{Purge}_{30 \ min} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>13.35(^{a})</td>
<td>1.60(^{a})</td>
</tr>
<tr>
<td>DBP</td>
<td>2.99(^{b})</td>
<td>0.32(^{b})</td>
</tr>
<tr>
<td>SEM(^{c})</td>
<td>1.94</td>
<td>0.24</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Means within a column with different superscripts are significantly different \((P < 0.05)\)

\(^{c}\) Pooled standard error of the mean

(2004) added beef broth or carrageenan to the phosphate/salt formulation, there was no significant decrease in fluid lost during retail display compared to phosphate/salt only injected steaks. Vann & Mireles DeWitt (2007) replaced 4.5% sodium phosphate with an acid solubilized protein solution in beef strip steaks injected 10% over green weight. The authors saw purge values that were between 3.9% and 6.5% lower for the sodium phosphate containing steaks. Use of a pork collagen protein powder was investigated in cured hams (Prabhu, et al., 2004; Schilling et al., 2003) and pork frankfurters (Prabhu et al., 2004). Prabhu et al. (2004) found that restructured, cooked hams containing 2% or 3% pork protein had lower purge values after 4 and 8 wk of storage than those not containing pork protein. Pork frankfurters from the same study had lower purge values with increasing amounts of pork protein \((0 – 3.5\%)\) after 4 wk. Schilling et al. (2003) observed lower expressible moisture from restructured hams formulated with 3% pork protein than those without. The authors concluded that, upon cooking and cooling, the collagen protein formed a gel matrix that helped entrap the loosely held free water. In this case, however, the water binding benefits were realized before cooking. We believe the efficacy of the protein powder to depend on several actions, including (1) hydration of the protein powder upon introduction into the brine due to hydrophilic interactions, (2) subsequent increase in viscosity of the brine dispersion due to the thickening effect of
Table 3.3 Main effect least squares means of Purge, Purge$_{total}$, Brine Loss$_{total}$ of beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Purge</th>
<th>Purge$_{total}$</th>
<th>Brine Loss$_{total}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>4.77 ± 0.14</td>
<td>6.05$^a$ ± 0.15</td>
<td>54.4$^a$ ± 1.79</td>
</tr>
<tr>
<td>DBP</td>
<td>4.80 ± 0.14</td>
<td>5.15$^b$ ± 0.15</td>
<td>51.5$^b$ ± 1.86</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>Purge</th>
<th>Purge$_{total}$</th>
<th>Brine Loss$_{total}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.21$^a$ ± 0.14</td>
<td>5.05$^a$ ± 0.15</td>
<td>48.0$^a$ ± 1.82</td>
</tr>
<tr>
<td>2</td>
<td>4.67$^b$ ± 0.14</td>
<td>5.47$^b$ ± 0.15</td>
<td>51.8$^b$ ± 1.82</td>
</tr>
<tr>
<td>4</td>
<td>5.03$^c$ ± 0.14</td>
<td>5.85$^c$ ± 0.15</td>
<td>55.2$^c$ ± 1.82</td>
</tr>
<tr>
<td>6</td>
<td>5.22$^d$ ± 0.16</td>
<td>6.03$^d$ ± 0.16</td>
<td>56.7$^d$ ± 1.94</td>
</tr>
</tbody>
</table>

$^a$-$^d$Means within a column and main effect with different superscripts are significantly different ($P < 0.05$; mean ± standard error of the mean)

collagenous proteins at low temperatures and (3) progressive formation of a collagenous gel network upon injection into the meat product.

3.4.5 Lipid oxidation

There was a treatment x storage day interaction for TBARS values of steaks in retail display (Table 3.4). Initial TBARS values for loins following injection were similar ($P > 0.05$) for control and DBP treatment. Treatment (DBP) steaks had greater ($P = 0.0037$) TBARS values on d 0 (initial removal from dark storage) than CON steaks. The DBP treatment steaks continued to have increased ($P < 0.05$) TBARS values through day 6. The threshold value for oxidized flavor has been reported to be 2.0 mg/Kg in beef steaks (Campo, Nute, Hughes, Enser, Wood, & Richardson, 2006). Steaks containing DBP did not get above the lower end of the threshold range (0.6) until day 4 and were still below 1.0 mg/Kg by day 6.

The greater TBARS values in the DBP steaks are not surprising, as there was no sodium phosphates in the formulation to counteract the pro-oxidant activity of salt. Rowe et al. (2009) observed increased TBARS values in brine injected steaks formulated without phosphates compared to those with phosphates. Our observations of lipid
oxidation over time are also consistent with other researchers (Cerruto-Noya et al., 2009; Djenane, Sanchez-Escalante, Beltran, & Roncales, 2003; Knock et al., 2006; Rowe et al., 2009; Seyfert et al., 2005) who displayed enhanced beef steaks under retail lighting in high oxygen environments. The intense lighting conditions common to retail display in concert with high oxygen environments (overwrap or high oxygen modified atmosphere packaging) provide ideal conditions for conventional free radical oxidation and photosensitized singlet oxygen oxidation (Frankel, 1980).

3.4.6 Aerobic and anaerobic plate counts

There was a significant treatment x storage day interaction for aerobic plate count (Table 3.4). Aerobic plate counts for treated and control steaks were similar on d 0. Control steaks had greater \( P < 0.05 \) APC counts on d 2 and d 4, whereas treatment steaks were higher \( P = 0.039 \) on d 6. While these differences were statistically significant, from a practical standpoint they are negligible with respect to display in the retail market. As expected, aerobic plate counts increased \( P < 0.05 \) for both treatments as storage time increased. Anaerobic plate count (data not shown in tabular form) was only affected by storage day \( P < 0.001 \), increasing as time in display increased. While the greater pH values of the CON steaks might have been expected to produce increased plate counts, that was not seen consistently in the present study; possibly due to the antimicrobial nature of sodium phosphates (Pohlman et al., 2002). It is also possible that the presence of the rosemary (Herbalox) and its action as an antimicrobial agent minimized any large microbial differences that might have been seen otherwise (Djenane et al., 2003).
Table 3.4 Least squares means for the two way interaction of treatment x storage day of TBARS' values, aerobic plate count (APC) and Warner Bratzler shear force (WBSF) values\(^g\) of beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>TBARS</th>
<th>APC</th>
<th>WBSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>CON</td>
<td>0.09(^d)</td>
<td>3.50(^e)</td>
<td>39.77(^a)</td>
</tr>
<tr>
<td></td>
<td>DBP</td>
<td>0.32(^c)</td>
<td>3.44(^e)</td>
<td>47.41(^a)</td>
</tr>
<tr>
<td>2</td>
<td>CON</td>
<td>0.13(^d)</td>
<td>3.89(^a)</td>
<td>37.64(^b)</td>
</tr>
<tr>
<td></td>
<td>DBP</td>
<td>0.49(^b)</td>
<td>3.50(^e)</td>
<td>37.87(^b)</td>
</tr>
<tr>
<td>4</td>
<td>CON</td>
<td>0.07(^d)</td>
<td>4.55(^c)</td>
<td>32.80(^b)</td>
</tr>
<tr>
<td></td>
<td>DBP</td>
<td>0.65(^a)</td>
<td>4.05(^d)</td>
<td>38.56(^b)</td>
</tr>
<tr>
<td>6</td>
<td>CON</td>
<td>0.17(^cd)</td>
<td>6.91(^b)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>DBP</td>
<td>0.77(^a)</td>
<td>7.17(^a)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SEM(^h)</td>
<td>0.06</td>
<td>0.09</td>
<td>2.80</td>
</tr>
</tbody>
</table>

\(^a\)Means within a column with different superscripts are significantly different \((P < 0.05)\)
\(^b\)Thiobarbituric acid reactive substances (TBARS)
\(^c\)Reported as Newtons (N)
\(^d\)Pooled standard error of the mean

3.4.7 Warner-Bratzler shear force

There was a significant treatment x storage day interaction for shear force values (Table 3.4). Warner-Bratzler shear force values were greater \((P < 0.001)\) for the DBP treatment steaks on day 0 compared to CON steaks. Both groups had similar \((P > 0.05)\) shear values on d 2 and 4, although the d 4 comparison was approaching significance \((P = 0.054)\). The decrease in tenderness experienced by the DBP steaks on d 0 could be explained by their lower \(Cook\ Yield_{adj}\) values. Having less free water held after cooking can decrease tenderness because, as Offer and Knight (1988) explain, “the swelling of muscle fibers and connective tissues dilutes out the amount of load resisting material”. It is also thought that the action of phosphate in dissociating actomyosin and dissolving myosin filaments increases tenderness (Offer & Knight, 1988). However, as the CON treated steaks continued to lose more fluid as purge during retail display than the DBP treated steaks, the difference in tenderness decreased.
Rowe et al. (2009) found that uninjected steaks were less tender according to Warner-Bratzler shear force than those injected with 0.4% STPP and 0.5% salt. Baublits et al. (2006b) found that steaks injected with 0.4% STPP and varying levels of salt (0 – 1.5%) were more tender than untreated controls. Cerruto-Noya et al. (2009) saw that steaks injected with 0.36% salt and 0.01% ammonium hydroxide instead of 0.36% salt and 0.45% STPP had higher Warner-Bratzler shear force values. In each of these cases the treatments that held less water through cooking produced the tougher steaks; this supports our results. Walsh et al. (2010) saw no differences in tenderness when comparing steaks injected with 1.5% WPC and 0.5% salt or 1% carrageenan and 0.5% salt to those injected with 0.4% STPP and 0.5% salt, even though both treatments including alternative ingredients had lower water retention through cooking than the STPP treatment. Szerman et al. (2007) observed lower Warner-Bratzler shear values for semitendinosus roasts injected to contain 3.5% whey protein concentrate and 0.7% salt compared to non-injected control roasts.

3.4.8 Instrumental Color

There was no interaction (treatment x storage day) for CIE (1978) $L^*$ values (Table 3.5). $L^*$ values (lightness) pooled across day were greater ($P < 0.0001$) for DBP treatment steaks than control steaks. After 5 days in display the $L^*$ values for either group were not different ($P > 0.05$) from the day 0 value. There were, however, large fluctuations above and below the starting value for both the control and DBP treatment during the display time. Rowe et al. (2009) found lower $L^*$ values from steaks injected with 0.4% STPP and 0.5% salt than those that were injected with either salt or phosphate alone, or neither. Cerruto-Noya et al. (2009) also observed lower $L^*$ values in steaks injected to 0.4% STPP and 0.36% salt compared to steaks containing 0.36% salt
Table 3.5  Main effect least squares means of CIE L* and b* values of beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>35.3\textsuperscript{b}</td>
<td>18.8\textsuperscript{b}</td>
</tr>
<tr>
<td>DBP</td>
<td>38.4\textsuperscript{a}</td>
<td>20.7\textsuperscript{a}</td>
</tr>
<tr>
<td>SEM\textsuperscript{e}</td>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Day\textsuperscript{f}

<table>
<thead>
<tr>
<th></th>
<th>L*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>36.6\textsuperscript{abc}</td>
<td>19.5\textsuperscript{bc}</td>
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<tr>
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<td>20.7\textsuperscript{a}</td>
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<td>21.2\textsuperscript{a}</td>
</tr>
<tr>
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<td>36.6\textsuperscript{abc}</td>
<td>20.7\textsuperscript{a}</td>
</tr>
<tr>
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<td>37.8\textsuperscript{a}</td>
<td>19.7\textsuperscript{b}</td>
</tr>
<tr>
<td>3.5</td>
<td>36.7\textsuperscript{ab}</td>
<td>20.5\textsuperscript{a}</td>
</tr>
<tr>
<td>4</td>
<td>37.6\textsuperscript{a}</td>
<td>18.9\textsuperscript{cd}</td>
</tr>
<tr>
<td>4.5</td>
<td>37.5\textsuperscript{a}</td>
<td>18.9\textsuperscript{cd}</td>
</tr>
<tr>
<td>5</td>
<td>37.4\textsuperscript{a}</td>
<td>18.6\textsuperscript{d}</td>
</tr>
<tr>
<td>5.5</td>
<td>37.8\textsuperscript{a}</td>
<td>18.4\textsuperscript{d}</td>
</tr>
<tr>
<td>SEM\textsuperscript{e}</td>
<td>0.9</td>
<td>0.7</td>
</tr>
</tbody>
</table>

\textsuperscript{a-d}Means within a column and main effect with different superscripts are significantly different (P < 0.05)

\textsuperscript{e}Pooled standard error of the mean

\textsuperscript{f}Readings were taken at 12 h increments beginning at the time of placement in retail display

and 0.01% ammonium hydroxide. Baublits et al. (2006b) can also corroborate the results of the previously mentioned studies, as they saw darker muscle color in meat with 0.4% STPP as levels of salt increased (0 – 1.5%). In each of the previously mentioned cases, the authors attributed the lower $L^*$ values to higher water holding capacity. In this case, however, our DBP treatment, which had higher $L^*$ values, was shown to be retaining more water throughout the study. The greater pH seen in the CON steaks was likely responsible for the darker color.

There was a treatment x storage day interaction for $a^*$ values (Figure 3.1). The $a^*$ (redness) values were higher (P < 0.05) for CON steaks at d 0 and after d 3.

Redness seemed to fluctuate for both groups before establishing a definite decrease
Figure 3.1 CIE $a^*$ values of steaks ($n = 10$/treatment) injected with a salt/phosphate brine (Control) or a brine containing salt and a dehydrated beef protein (DBP). Day = day of retail display following 4 d of dark storage. Color evaluation was conducted every 12 hours for 5 d. A * indicates a significant difference ($P < 0.05$) between CON and DBP for that time period. Standard error of the mean = 0.73.

after d 3. Final $a^*$ values on day 6 were 1.95 and 4.45 units lower ($P < 0.001$) than the respective starting values for CON and DBP steaks. These results suggest that the presence of the dehydrated beef protein product caused a slightly less red color than phosphate. Sodium phosphate injected products have been shown by other studies (Baublits et al., 2006b; Cerruto-Noya et al., 2009; Rowe et al., 2009) to be superior to non-injected products and ammonium hydroxide injected products at maintaining red color under retail display. The pro-oxidant activity of salt likely sped
the oxidation of oxymyoglobin (red) to metmyoglobin (brown) without phosphate present to counteract it (Akamittath, Brekke, & Schanus, 1990; Torres, Pearson, Gray, Booren, & Shimokomaki, 1988).

There was no two way (treatment x storage day) interaction for $b^*$ (yellow) values (Table 3.5). The $b^*$ values fluctuated during display before settling 1.9 units lower ($P = 0.006$) than the d 0 value (19.48). Pooled across storage day, DBP steaks had higher ($P < 0.001$) $b^*$ (yellowness) values (20.69) than CON steaks (18.76). Utilization of a similar, pork collagen based, protein ingredient caused higher ($P < 0.05$) $b^*$ values in cooked hams (Schilling et al., 2003).

3.4.9 Color Panel

No interactions (treatment x storage day) were found for muscle color, fat color or discoloration. In addition, fat color was not affected by either treatment or storage day individually. There was a treatment x storage day interaction for overall color. Muscle color score ratings for the DBP treatment were slightly greater ($P = 0.014$) than the control throughout the study. Ratings for muscle color (Figure 3.2) decreased ($P < 0.001$) in tandem for both groups as display time increased, with a rating of 3.84 or “Moderately dark red or brown” at the final evaluation period compared to the initial rating of 4.6 or “Slightly dark red”. Discoloration, which is accumulation of the metmyoglobin (brown) pigment on the steak surface, was affected by storage day only ($P < 0.001$; data not shown in tabular form). Scores for discoloration averaged 6.67 initially, and decreased ($P < 0.001$) to 6.10 at the end of retail display; both scores correlate to 1 – 20% discoloration. Overall color (Figure 3.3) was rated much the same as muscle color, with very minor differences between the DBP treatment and the control and a decrease ($P < 0.05$) in acceptability as time in display went on (data not shown in
Figure 3.2 Trained color panel ratings for muscle color of steaks (n = 10/treatment) injected with a salt/phosphate brine (Control) or a brine containing salt and a dehydrated beef protein (DBP). Day = day of retail display following 4 d of dark storage. Color evaluation was conducted by a 7 member trained panel every 12 hours for 5 d. Muscle color is the brightness and color of non-discolored lean, where 8 = very bright cherry red, 7 = moderately bright cherry red, 6 slightly cherry red, 5 = red, 4 = slightly dark red, 3 = moderately dark red or brown, 2 = dark red or brown and 1 = extremely dark brown. A * indicates a significant difference (P < 0.05) between CON and DBP for that time period. Standard error of the mean = 0.21.

DBP treatment steaks were rated higher (P = 0.006) at d 0 than CON steaks (5.04 or “Slightly desirable” vs. 4.57 or “Acceptable”). Both CON and DBP were given a rating of “Slightly undesirable” by 3 d of retail display, and at the end of retail...
Figure 3.3 Trained color panel ratings for overall color of steaks (n = 10/treatment) injected with a salt/phosphate brine (Control) or a brine containing salt and a dehydrated beef protein (DBP). Day = day of retail display following 4 d of dark storage. Color evaluation was conducted by a 7 member trained panel every 12 hours for 5 d. Overall acceptability takes muscle color, percent discoloration and fat color into account, where 7 = extremely desirable, 6 = desirable, 5 = slightly desirable, 4 = acceptable, 3 = slightly undesirable, 2 = undesirable and 1 = extremely undesirable. A * indicates a significant difference (P < 0.05) between CON and DBP for that time period. Standard error or the mean = 0.23.

display, both CON and DBP steaks were rated as “Slightly undesirable”. The subjective color results suggest that DBP treated steaks maintain color stability at least as well as CON steaks. This is in contrast to our own instrumental color data, which identify the CON steaks as having a redder color. It is possible that the human panelists were
responding favorably to a “brighter” cherry red color produced by the greater lightness values of the DBP steaks.

3.4.10 Cooked product yield

There was no interaction (treatment x storage day) for Cook Yield or Cook Yield_{adj} (Table 3.6). Control steaks had greater ($P < 0.001$) Cook Yield values than DBP treatment steaks across all days. Cook Yield values increased ($P < 0.001$) from d 0 to d 2, most likely because of a reduction of free water due to purge during display. Cook Yield_{adj} represents the ability of the steak to retain its weight as well as the weight of the added brine from injection through cooking. This calculation adjusts for the inherent bias seen in the traditional cook yield calculation which can cause a steak with more brine retained through injection and display to have a lower cook yield while still maintaining a greater proportion of its weight. Across all days, CON steaks had greater ($P = 0.008$) Cook Yield_{adj} values. However, the differences were numerically lower than the 3.5% difference seen with the traditional calculation. By taking into account total brine loss prior to and after cooking the differences are lessened in cook yields between to the two groups.

Once again, greater cook yields were seen as display time increased. Walsh et al. (2010) replaced 0.4% STPP in injection brines with 1% carrageenan or 1.5% whey protein concentrate (WPC). Supraspinatus roasts injected with WPC and 0.5% NaCl had greater cook yields than those injected with carrageenan and 0.5% NaCl, but both were lower than those injected with 0.4% STPP and 0.5% salt. The results from Walsh et al. (2010) are similar to the present study in that alternative ingredients were not as successful as phosphates in retaining fluid through cooking. Schilling et al. (2003) reported that inclusion of a pork collagen based
Table 3.6 Main effect least squares means of Cook Yield and Cook Yield_{adj} for beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cook Yield</th>
<th>Cook Yield_{adj}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>80.2^a ± 0.42</td>
<td>69.0^a ± 0.51</td>
</tr>
<tr>
<td>DBP</td>
<td>76.7^b ± 0.38</td>
<td>67.0^a ± 0.42</td>
</tr>
<tr>
<td>Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>75.5^b ± 0.47</td>
<td>64.5^b ± 0.51</td>
</tr>
<tr>
<td>2</td>
<td>80.0^a ± 0.47</td>
<td>69.3^a ± 0.51</td>
</tr>
<tr>
<td>4</td>
<td>79.9^a ± 0.47</td>
<td>70.2^a ± 0.51</td>
</tr>
</tbody>
</table>

^a,b Means within a column and main effect with different superscripts are significantly different (P < 0.05; means ± standard error of the mean)

protein reduced cooking loss in non-pale, soft and exudative (PSE) restructured hams, while Prabhu et al. (2004) reported no differences between hams formulated with or without the protein. The latter study did report greater cook yields for frankfurters containing at least 1% pork protein than those formulated without it.

3.4.11 Sensory panel

There were no interactions (treatment x storage day) for initial juiciness, sustained juiciness, initial tenderness, connective tissue, overall tenderness, cooked beef flavor or saltiness (Table 3.7). Storage day was the only significant effect for initial juiciness and sustained juiciness. Steaks were rated most juicy (P < 0.05) for both juiciness indicators on d 0. This was expected as steaks continue to purge fluid during storage or display. The lack of a treatment effect suggests that the slightly lower Cook Yield_{adj} values of the DBP steaks did not produce a detectable loss of juiciness to trained sensory panelists. Rowe et al. (2009) found that phosphate injected steaks were more juicy than non-injected control steaks as determined by a trained sensory panel. Lawrence et al. (2004) found that steaks were juicier when injected with sodium phosphate than when injected with calcium lactate. Control steaks were more tender.
Table 3.7 Main effect least squares means of sensory characteristics of beef strip steaks injected with a salt/phosphate brine or a brine containing salt and a dehydrated beef protein

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial Juiciness</th>
<th>Sustained Juiciness</th>
<th>Initial Tenderness</th>
<th>Connective Tissue</th>
<th>Overall Tenderness</th>
<th>Cooked Beef Flavor</th>
<th>Salty</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>5.84</td>
<td>5.41</td>
<td>6.08</td>
<td>6.46</td>
<td>6.04</td>
<td>1.90</td>
<td>2.23</td>
</tr>
<tr>
<td>DBP</td>
<td>5.76</td>
<td>5.32</td>
<td>5.82</td>
<td>6.11</td>
<td>5.75</td>
<td>2.02</td>
<td>1.92</td>
</tr>
<tr>
<td>SEM</td>
<td>0.13</td>
<td>0.12</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Day
- 0: 5.97<sup>a</sup> 5.68<sup>a</sup> 6.05 6.48<sup>a</sup> 6.03 2.17<sup>a</sup> 2.24<sup>a</sup>
- 2: 5.74<sup>b</sup> 5.15<sup>b</sup> 5.89 5.95<sup>b</sup> 5.81 1.78<sup>c</sup> 2.03<sup>b</sup>
- 4: 5.69<sup>b</sup> 5.26<sup>b</sup> 5.91 6.43<sup>a</sup> 5.84 1.93<sup>b</sup> 1.95<sup>b</sup>

SEM<sup>f</sup> 0.14 0.13 0.18 0.18 0.18 0.07 0.07

**Means with different superscripts within a column and main effect are significantly different (*P* < 0.05)

<sup>d</sup>Attributes were evaluated on an 8-point scale

<sup>e</sup>Attributes were evaluated on a 3-point scale

<sup>f</sup>Standard error the mean

more tender than DBP steaks over all days as indicated by initial tenderness and overall tenderness.

These findings support our own instrumental tenderness data. Cerruto-Noya et al. (2009) saw similar results comparing steaks injected with a high pH ammonium hydroxide brine to those injected with STPP. Their STPP injected steaks were determined to be more tender according to a trained sensory panel. Steaks injected with phosphate and/or salt by Rowe et al. (2009) were more tender than non-injected steaks.

It is probable that the differences in tenderness were due to an increase in protein solubility (Offer & Knight, 1988) because of the action of phosphate and not increased fluid retention since there were no discernible differences in juiciness. Although CON steaks were rated significantly greater (*P* = 0.001) for connective tissue than DBP steaks, the ratings were consistently in the same range (6.46 vs. 6.11 where 8 = none and 1 = abundant). Cooked beef flavor was not different between the treatments. It was affected by storage day, displaying the greatest (*P* < 0.05) value on d 0 before decreasing (*P* < 0.05) on d 2 and increasing again on d 4. As expected, steaks from the
CON group were rated more salty ($P < 0.001$) due to a higher amount of sodium from the sodium phosphate.

3.5 Conclusions

Results of this study indicate that replacement of sodium phosphates by dehydrated beef protein in injection brines allowed beef strip loin steaks to retain a similar amount of inherent and added fluid through cooking while maintaining similar sensory characteristics, color stability and microbial stability. While the DBP steaks displayed greater lipid oxidation, it was still within acceptable levels over the course of retail display. Injection with DBP resulted in a slightly less tender product. However, the potential to reduce sodium use by approximately half, thereby creating a healthier product, will likely mitigate any negative impact this may have. This suggests that the dehydrated beef protein would be useful in replacing phosphates and reducing overall sodium content in enhanced beef steaks.
4. Impact of High Pressure Processing on the Functional Aspects of Beef Muscle Injected with Salt and/or Sodium Phosphates

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4.1 Abstract
This study aimed to determine the interactions among salt (NaCl), sodium phosphate (SP) and mild HPP in brine-injected beef. Beef strip loin segments were injected to 10% over initial weight with solutions containing water and various levels of salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution). Pieces from the loin sections were exposed to varying pressure levels (0.1, 152 or 303 MPa) and evaluated for selected quality and biochemical characteristics. Use of SP and pressure application increased pH by ~0.2 units. \( L^* \) values were increased by pressure and decreased by SP. Redness \( (a^*) \) increased at 303 MPa. Purge increases due to pressure were mitigated by SP. Pressure application at 303 MPa reduced total and sarcoplasmic protein solubility by 24 and 32%, respectively. There were no beneficial interactions among salt or SP and HPP. However, results indicate SP may prevent yield loss due to HPP.

4.2 Introduction
It is common practice to inject whole muscle, fresh meat cuts with a brine containing sodium chloride (salt; NaCl) and sodium phosphates (SP), as they act synergistically through several mechanisms to increase the water binding ability of meat proteins. Salt causes depolymerization of myosin as well as a downward shift in the protein’s isoelectric point (Offer & Knight, 1988). Sodium phosphates dissociate the acto-myosin bond, further increasing solubilization and relaxation of the protein structure (Offer & Knight, 1988). These effects translate to a significantly heightened ability of salt/SP injected meat to retain injected fluid throughout storage and display (Baublits, Pohlman, Brown, Jr., Yancey, & Johnson, 2006a; Lawrence, Dikeman, Hunt, Kastner, & Johnson, 2004; McGee, Henry, Brooks, Ray, & Morgan, 2003). Sodium phosphates
also improve color stability (Baublits, Pohlman, Brown, Jr., & Johnson, 2006b) and inhibit lipid oxidation (McGee et al., 2003; Rowe, Pohlman, Brown, Jr., Johnson, Whiting, & Galloway, 2009).

High pressure processing (HPP) is a non-thermal, non-chemical treatment which subjects materials to very high hydrostatic pressures (100-1000 MPa). Observations in comminuted products suggest the potential for HPP to be used as a way to enhance the functionality of salt and SP, possibly allowing for usage reductions of these sodium heavy ingredients. Low-salt (1%) beef hot dogs had similar cook losses and improved texture characteristics compared to controls (2%) when subjected to 200 MPa for 2 min (Sikes, Tobin, & Tume, 2009). Water holding capacity and protein solubility of 0.5% salt restructured turkey rolls were increased when subjected to 50 – 200 MPa HPP. However, whole muscle products are very different from comminuted products in their character; instead of an amorphous protein network there is the ordered structure of myofibers and connective tissue layers. Duranton, Simonin, Cheret, Guillou and de Lamballarie (2012) noted that making inferences on the effects of HPP on whole muscle products based on results from restructured or comminuted food matrices is ill-advised. In their work it was found that 1.5 or 3% salt injection increased tenderness and water holding capacity of HPP (500 MPa; 6 min) whole muscle hams compared to no salt. Beef loin muscle injected with 1% salt and HPP treated (650 MPa; 10 min) had lower expressible moisture than raw meat without salt with or without high pressure treatment (Fernández, Sanz, Molina-García, Otero, Guignon, & Vaudagna, 2007). However, these salt levels are much higher than what is commonly used in brine enhanced beef (<0.5%). Additionally, sodium phosphates, commonly used concurrently with salt, have not been investigated in whole muscle HPP-treated product. This study aims to
establish what, if any, interactions exist between salt, phosphates and high pressure in brine injected beef. Common indicators of protein functionality, like protein solubility, and quality aspects, like color and purge loss, were investigated.

4.3 Methods and Materials

4.3.1 Raw materials and injection

Beef strip loins (IMPS 180; n = 10) were purchased from a local processor. Loins were trimmed of all excess fat and connective tissue, cut into thirds and assigned to injection treatments. Loin sections were injected to 110% of initial weight with a solution containing 0, 2 or 4% salt with or without 4% sodium phosphate (Brifisol® 85 Instant; BK Giulini Corp., Simi Valley, CA, USA) using a single-needle hand-operated injector (Koch, Kansas City, MO, USA). The loins identified as 0% salt and 0% SP were injected with water targeting 110% of initial weight. Pieces were then weighed and allowed to rest for 20 min after injection before being cut into 2.5 cm x 2.5 cm x ~8 cm pieces, re-weighed, vacuum packaged in 15.5 x 22 cm oxygen impermeable bags and transported to the pressurization facility in a cooler on ice. Fluid loss during the rest period was determined using the \( \text{Purge}_{\text{inj}} \) and \( \text{Brine Loss}_{\text{inj}} \) variables, calculated as described in Lowder, Goad, Lou, Morgan and Mireles DeWitt (2011).

4.3.2 Pressure treatment

Pressurization took place in a 22-L chamber (National Forge Company, Andover, MA, USA) using a pressurization medium of soluble oil (Hydrolubric® 123-B, Houghton International, Valley Forge, PA, USA) in water (5% w/w). Packaged beef pieces were subjected to either 0.1 (atmospheric pressure), 152 or 303 MPa for 1 minute. The samples were submerged in ice-water within a nylon bag placed in the chamber during
pressurization to deter temperature-induced denaturation. The pressure ramp-up rate was approximately 4 MPa/sec. Depressurization time was ~30 sec regardless of final pressure.

4.3.3 Processing and sampling

After pressurization beef pieces were either frozen for further analyses or removed from their packages, pat dry with a paper towel and reweighed to determine purge due to pressurization. Subsequently, meat used for purge was allowed to bloom for 30 min and used for color and pH analysis and the rest were frozen and stored at -10 °C to be used for further analyses.

4.3.4 pH and color

A pH meter (pH 3210, WTW GmbH, Weilheim, Germany) equipped with a piercing probe was used to determine pH. Instrumental color was determined using a Minolta CM – 600 (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) with a 10° observer and illuminant A. The device was calibrated using a white tile. CIE L* and a* values (CIE, 1978) were recorded with the spectral component excluded, to more closely simulate the color as perceived by the human eye. Average measurements were taken from two readings on the surface of each beef piece perpendicular to muscle fiber direction, as if they were taken on the sliced surface of a steak.

4.3.5 Purge

Multiple variables were used to detail the fluid loss from beef loins and pieces. The calculations used to generate these variables are presented in detail in Lowder et al. (2011, 2013). They will be described here briefly.

\( Purge_{nj} \) represents the fluid lost during the equilibration period as a percentage of the total loin segment weight. It was calculated by taking the difference between the loin
immediately after injection and after the rest period, dividing it by the weight of the freshly injected loin and multiplying it by 100.

*Brine Loss* reports the fluid lost during equilibration as a percentage of the total fluid injected. To calculate the difference between loin segment weight immediately after injection and 20 min after is divided by the difference between loin weight immediately after injection and initial loin weight. The resulting value is multiplied by 100 to get a percentage.

*Purge* represents the fluid lost from the beef strip during pressurization. It is calculated using the following equation: \( \text{Purge(\%) = } \frac{(S_0 - S_t)}{S_0} \times 100 \), where \( S_0 \) = the weight of the beef piece at slicing and \( S_t \) = the weight of the beef piece after storage.

*Purge* total represents the weight lost from injection through pressurization as a percentage of the total weight of the strip.

### 4.3.6 Protein solubility

Sarcoplasmic (water soluble) and total (salt and water soluble) protein solubility was determined using the Bradford (1976) method with premixed reagents (Bio-Rad Laboratories, Hercules, CA, USA). For sarcoplasmic fraction, two g of sample were homogenized (Polytron PT10-35, Kinematica, Inc., Bohemia, NY) in 10 volumes of a low ionic strength buffer (30 mM sodium phosphate, pH 7.4) then incubated on a rocker for 2 h. After centrifugation at 5000g and 4 °C for 15 min, supernatant was decanted, reacted with Bradford reagent and read on a spectrophotometer (Shimadzu UV-2400, Shimadzu Scientific Instruments, Inc, Columbia, MD, USA) at 595 nm. For total protein solubility 2 g of sample was homogenized in 10 volumes of a high ionic strength buffer (0.6 M KCl, 50 mM sodium phosphate, pH 7.4), incubated on a rocker for 2 h and clarified at 5000g and 4 °C for 15 min. The supernatant was used in the same manner as the
sarcoplasmic fraction to determine total protein solubility. Myofibrillar (salt soluble) solubility was estimated by subtracting the water soluble fraction from the total fraction for a given sample. Protein solubility was reported as mg/g sample.

4.3.7 Total and reactive sulfhydryls (SH)

Total and reactive sulfhydryl groups were determined in the presence and absence of urea by a modification of the procedures described by Hamada, Ishizaki and Nagai (1994). Samples (1 mg protein/ml) were retained from the protein solubility test. Adjusted sample (0.5 mL) was mixed with 2 mL 8M urea in 0.2 M Tris (pH 7.0) and 50 μL of 10 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) with 0.1 M sodium phosphate and 0.2 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.2) and incubated in a 40 °C water bath for 15 min. Reacted samples were measured on a spectrophotometer (UV 2401, Shimadzu Corporation, Kyoto, Japan) at 412 nm. Total SH content was determined as μMol/mg protein using a molar extinction coefficient of 14,150 M⁻¹ cm⁻¹ (Riddles, Blakeley, & Zerner, 1979). The adjusted protein sample was incubated at 5 °C for 1 hr in the absence of urea to determine reactive SH content.

4.3.8 Lipid oxidation

Lipid oxidation was determined using the 2-thiobarbituric acid reactive substances (TBARS) method of Buege and Aust (1978) with slight modifications. Briefly, 10 g of sample was homogenized in 3 volumes of distilled water and centrifuged at 3000g for 10 min at 4 °C. Supernatant (2 mL) was mixed with 4 mL 15% trichloroacetic acid/20 mM thiobarbituric acid and 100 μL 10% butylated hydroxyanisole and vortexed thoroughly before being placed in a boiling water bath for 15 min. Subsequently, samples were cooled in an ice water bath for 10 min, vortexed again and centrifuged as above. Absorbance of the supernatant was read on a spectrophotometer.
at 532 nm and compared to a malondialdehyde standard constructed using tetra-ethoxypropane. Results are reported as mg malondialdehyde/kg of meat.

4.3.9 Experimental design and statistical analysis

The experiment was arranged as a split plot with the whole plot being a 3 x 2 factorial (3 salt levels; 2 SP levels) in a balanced incomplete block design with loin as the block. The whole plot was replicated 5 times and each treatment appeared in a block twice with any other given treatment. The split plot factor was pressure level (0.1, 152, 303 MPa) with a replication of 30.

Data were analyzed in PROC GLIMMIX of SAS Version 9.2 with block (loin) defined as a random variable. The whole plot error term was defined as block x salt level x phosphate level. Significance was pre-determined at α = 0.05. Treatment effects were subjected to analysis of variance (ANOVA) and, where applicable, means were separated using t-tests. In order to protect experiment-wise error while maintaining power, mean separations within two-way interactions were performed only across certain levels of the other main effect. The $L^*$ data, due to a non-normal distribution, were transformed using the procedure of Box and Cox (1964) as implemented by SAS for ANOVA and mean comparisons. Because of a three-way interaction, the $L^*$ data were sliced by SP level and subjected to two-way ANOVA. Figures and reported means for $L^*$ are based on the reverse-transformed geometric means; standard errors are approximations from those means.
4.4 Results and Discussion

4.4.1 Enhancement

Final gains after the equilibration period ranged between 5.3 and 7.7% and were significantly greater ($P = 0.006$) when SP was included in the brine (data not shown in tabular form). Means for the $\text{Purge}_{\text{inj}}$ and $\text{Brine Loss}_{\text{inj}}$ variables are given in Table 4.1. Curiously, $\text{Purge}_{\text{inj}}$ was not significantly affected ($P > 0.05$) by either salt or phosphate, though it was numerically reduced by the presence of each. $\text{Brine Loss}_{\text{inj}}$ was reduced ($P < 0.05$) by 15.13 and 16.38% by using 4% of salt or phosphate in the brine. The fluid retention during the rest period was lower than that seen in previous studies using similar ingredients and raw materials (Lowder et al., 2011). Fluid loss may have been encouraged by segmenting the loins, which increases surface area compared to internal area of the muscle, or the use of a manual, as opposed to automated, injection system.

4.4.2 pH

The two way interaction between pressure level and SP was significant ($P = 0.013$) for pH (Figure 4.1). Phosphate increased ($P < 0.05$) pH at all pressure levels. Pressure treatment at 152 MPa increased ($P = 0.014$) the pH of samples with SP but not those without it. 303 MPa increased pH of non-SP samples and showed a possible trend of increasing ($P = 0.07$) SP pH above non-pressurized. Both SP and pressure treatment as low as 200 MPa have been shown to raise the pH of muscle foods by 0.1 - 0.2 units (Lawrence et al., 2004; Ma & Ledward, 2004). The two effects are seen here to be additive, with 4% SP not only increasing the final pH of 303 MPa pressurized meat, but reducing the pressure needed to cause the increase. The changes in pH seen here are similar to those seen by other researchers (Lawrence et al., 2004; Ma & Ledward, 2004) even though the values of the non-pressurized meat are lower than those.
Table 4.1  Main effect LS means for \( \text{Purge}_{\text{inj}} \) and \( \text{Brine Loss}_{\text{inj}} \) of loin segments injected to 10% over initial weight with varying levels of salt and/or sodium phosphates (SP)

<table>
<thead>
<tr>
<th>Salt (%)</th>
<th>( \text{Purge}_{\text{inj}} )</th>
<th>SEM</th>
<th>( \text{Brine Loss}_{\text{inj}} )</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.66</td>
<td>0.31</td>
<td>66.67(^a)</td>
<td>3.75</td>
</tr>
<tr>
<td>2</td>
<td>3.55</td>
<td>0.31</td>
<td>56.59(^{ab})</td>
<td>3.92</td>
</tr>
<tr>
<td>4</td>
<td>3.77</td>
<td>0.27</td>
<td>51.54(^b)</td>
<td>3.45</td>
</tr>
<tr>
<td>SP (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.19</td>
<td>0.25</td>
<td>66.46(^a)</td>
<td>3.19</td>
</tr>
<tr>
<td>4</td>
<td>3.79</td>
<td>0.23</td>
<td>50.08(^b)</td>
<td>2.87</td>
</tr>
</tbody>
</table>

\(^a,b\)Means within a column and main effect with differing superscripts are significantly different (\( P < 0.05 \))

\( ^c \) As a percentage of the brine

\( ^d \) Standard error of the mean

frequently observed (Lowder et al., 2011; Parsons, VanOverbeke, Goad, Mireles DeWitt, 2011). Increases in meat pH due to pressure have been attributed to increased ionization, which may sequester free hydrogen ions, and protein denaturation, which can bury acidic side groups (Macfarlane, McKenzie, & Turner, 1980; Ma & Ledward, 2004).

4.4.3 Color

The \( L^* \) (lightness) values are shown visually in Figure 4.2. Data were sliced by salt level and analyzed using the SP and pressure variables to explain the three-way interaction. This, unfortunately, limits the inferences that can be made on the impact of salt level. When salt was absent from the brine, SP inclusion reduced (\( P < 0.01 \)) lightness at the 0.1 and 152 MPa pressure levels, but not at 303 MPa (\( P = 0.98 \)). Sodium phosphate reduced (\( P < 0.017 \)) lightness at all pressure levels at 2% salt but had no significant effect (\( P > 0.05 \)) at 4% salt. In addition to increasing water binding ability, SP increases the pH of meat which increases mitochondrial oxygen consumption rate, causing competition with myoglobin and resulting in darker muscle appearance (Faustman & Cassens, 1990). Absent salt, 152 MPa decreased (\( P = 0.021 \)) lightness of beef without SP but did not affect (\( P = 0.28 \)) beef with SP. Lightness was decreased
Figure 4.1 Pressure x sodium phosphate (SP) interaction pH values for beef from strip loins injected to 110% of initial weight with solutions containing salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature). Data points with differing letters within an SP level are significantly different ($P < 0.05$); an ‘*’ denotes a significant difference ($P < 0.05$) between SP levels at that pressure.

($P < 0.05$) by 152 MPa regardless of SP presence at 2% salt, but at 4% salt it was the SP treated beef that was darker ($P < 0.01$). At 303 MPa, all beef was significantly lighter ($P < 0.05$) than atmospheric pressure or 152 MPa except the salt and phosphate free treatment which was almost significantly higher ($P = 0.052$). Previous studies (Carlez et al., 1995; Hong, Park, Kim, Lee & Min, 2005) on minced beef and pork loin found numerical but non-significant increases in $L^*$ values upon pressurization at 150 MPa, whereas the current data show a minor (1 – 4 units) decrease at 152 MPa. Higher pressures are more commonly examined and the current study is in agreement with the
Figure 4.2  $L^*$ (lightness) values of beef from strip loins injected to 110% of initial weight with solutions containing salt at (a) 0, (b) 2 or (c) 4% of solution and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature). Data points with differing letters within an SP level are significantly different ($P < 0.05$); an "*" denotes a significant difference between SP levels at that pressure.
The majority of the literature. At pressures in the range of 200 – 400 MPa with as little as 15 s dwell time a major (5 – 15 units) increase in lightness is frequently observed in beef and pork (Carlez et al., 1995; Cheftel & Culioli, 1997; Hong et al., 2005; McArdle et al., 2010, 2011; Souza et al., 2011). The increase in lightness, believed to be caused by globin denaturation or protein coagulation, is often cited as a negative effect of HPP on beef and, in this case, the darkening effect of SP should be considered a benefit (Carlez et al., 1995; Simonin, Duranton & Lamballarie Anton, 2012).

The values for significant $a^*$ (redness) two-way interactions are shown in Table 4.2 (salt/SP means) and Figure 4.3 (pressure/SP). Redness of injected beef was 2.3 units higher ($P = 0.005$) due to SP use when salt was not included. It was unaffected ($P > 0.515$), however, when salt was used at 2 or 4%. Presence of 4% salt increased the redness of non-SP beef by 1.7 units while SP samples were unaffected ($P > 0.13$) by salt level. Increasing salt level is not typically associated with an increase in $a^*$ values, as salt is pro-oxidative and has been shown to contribute to myoglobin oxidation (Baublits et al., 2006a; Knock et al., 2006; Lawrence et al., 2004). The pressure/SP interaction (Figure 4.3) shows an increase ($P < 0.001$) in redness upon pressurization to 303 MPa by 2 units over non-pressurized for both 0 and 4% SP. An increase of redness ($P = 0.044$) at 303 MPa by SP was also observed. Much like salt, the observed increases of $a^*$ due to SP use are curious and not common in the literature, though it is known to increase color stability over time by acting as an antioxidant (Baublits, et al., 2006a; Rowe et al., 2009). The increase in redness by HPP at 303 MPa range is supported by other work. Hong et al. (2005) noticed an increase in redness of pork longissimus dorsi at 200 MPa and Jung, Ghoul and Lamballerie-Anton (2003)
Table 4.2  Redness (a* values) of beef pieces from loins injected to 10% over initial weight with varying levels of salt and/or sodium phosphates (SP) and subjected to high pressure processing at 0.1, 152 or 303 MPa as affected by salt/SP levels

<table>
<thead>
<tr>
<th>SP (%)</th>
<th>Salt (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>0</td>
<td>20.72 ± 0.63</td>
<td>22.23 ± 0.61</td>
<td>22.46 ± 0.56</td>
</tr>
<tr>
<td>4</td>
<td>23.07 ± 0.56</td>
<td>22.02 ± 0.61</td>
<td>22.01 ± 0.56</td>
</tr>
</tbody>
</table>


\[ P \text{ value} = 0.005 \]

\[ P \text{ value} = 0.795 \]

\[ P \text{ value} = 0.515 \]

a,b\(^c\)Means (± standard error of the mean) within a row with differing superscripts are significantly different \((P < 0.05)\)

\(^c\)As a percentage of the brine

\(^d\)P value of the comparison of SP levels at a given salt level

observed increased redness between 130 and 350 MPa in beef \textit{biceps femoris}. The mechanism of this redness increase is not well understood and ambiguously attributed to the activation of enzyme systems with metmyoglobin reducing activity (MRA; Jung et al., 2003; Simonin et al., 2012). The observation by Cheah and Ledward (1997) that this phenomenon is more apparent in the \textit{longissimus dorsi}, which is considered color stable with a surplus of MRA species, than in the \textit{psoas major}, which lacks in color stability, lends some credence to this idea (Joseph, Suman, Rentfrow, Li, & Beach, 2012).

4.4.4 Purge

\textit{Purge}, describing the fluid loss due to pressurization, is represented visually by Figure 4.4 (pressure/SP interaction). \textit{Purge} in non-SP treated beef increased \((P = 0.003)\) by 0.80% when exposed to 152 MPa but was only numerically greater \((P = 0.279)\) when exposed to 303 MPa. In the SP meat, \textit{Purge} values ranged from 2.94 to 3.17%, were unaffected \((P = 0.725)\) by HPP and were 1.35 – 2.31% lower \((P < 0.001)\) than their non-SP counterparts. The \textit{Purge}_{\text{total}} variable, which estimates fluid loss from the meat from injection through pressurization, is shown in Figure 4.5. The pattern seen in the
pressure/SP interaction for Purge\textsubscript{total} is exactly the same as that seen for Purge, 152 MPa, but not 303 MPa increased ($P = 0.033$) fluid loss from 0% SP meat while having no effect ($P = 0.487$) on SP meat. The salt/SP interaction showed a synergistic cooperation, with at least 2% salt reducing ($P = 0.035$) Purge\textsubscript{total} of SP beef by an additional 1.5 – 2.5% over that without salt. The cooperative effects of salt and SP on fluid retention properties of muscle foods are well documented and understood (Lawrence et al., 2004; Lowder et al., 2013; Offer & Trinick, 1983; Offer & Knight, 1988); observations of these actions here are expected.
Figure 4.4 Pressure x sodium phosphate (SP) interaction for Purge values of beef from strip loins injected to 110% of initial weight with solutions containing salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature). Data points with differing letters within an SP level are significantly different ($P < 0.05$); an ‘*’ denotes a significant difference ($P < 0.05$) between SP levels at that pressure.

Pressurization has been previously documented as negatively affecting fluid retention variables in whole muscle meat. 200 MPa of pressure (5 min) reduced WHC of beef *semitendinosus* steaks (Kim et al., 2007). Hong et al. (2005) and Marcos et al. (2010) investigated pressurization on pork loin muscle. The former noted reduced WHC at 150 – 200 MPa when exposed for long periods of time (15 – 60 min) while the latter observed increased expressible moisture at 400 MPa, but not 200 MPa. Pork *biceps femoris* was reduced in WHC upon pressurization to 500 MPa for 6 min (Duranton et al.,
Figure 4.5  (a) Pressure x sodium phosphate (SP) and (b) salt x SP interactions for \( \text{Purge}_{\text{total}} \) values of beef from strip loins injected to 110% of initial weight with solutions containing salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature). Data points with differing letters within an SP level are significantly different (\( P < 0.05 \)); an “*” denotes a significant difference between SP (a) or salt (b) levels at that pressure.
Observations of cross-sectional microstructure through electron microscopy revealed contracted myofibril structure and increased extracellular channels, which are known to reduce water holding capacity by allowing diffusion of water out of the myofibrils (Kim et al., 2007; Liu, Xiong & Chen, 2010; Duranton et al., 2012). Reductions in protein solubility, which is correlated with water binding characteristics, were also reported concurrently with the rises in fluid loss in cases where it was investigated (Joo, Kauffman, Kim & Park, 1999; Kim et al., 2007; Marcos et al., 2010); it is likely that the two phenomena are related. Duranton et al. (2012) and Fernandez et al. (2007) showed that salt injection of pork and beef, respectively, prevented the detrimental effects of pressurization on fluid retention. These effects were not seen in the present work, most likely because the salt level (0.4% final target) was much lower than the other studies (1.5 and 1% final target, respectively). Use of sodium phosphate, however, which was targeted (0.4%) near the legal limit of 0.5% final weight, was able to negate the water loss induced by HPP.

4.4.5 Protein solubility and sulfhydryls

Protein solubility, whether measured as sarcoplasmic or total, or estimated as myofibrillar, was only affected ($P < 0.002$) by pressure level (Table 4.3). In all cases, solubility was decreased when HPP was applied at 353, but not 152 MPa. No tested factor influenced reactive or total sulfhydryls (SH) or the reactive/total SH ratio. Pressure level showed a tendency to increase ($P = 0.078$) reactive SH content, but, curiously, the reactive/total SH ratio did not seem to be influenced by this ($P = 0.520$). Varying observations on protein solubility of HPP meats have been reported in the literature. Lee, Kim, Lee, Hong and Yamamoto (2007) reported decreased solubility of beef *semitendinosus* in 0.6 M KCl at pressures of 400 MPa or greater; low ionic strength
buffer (0.1 M KCl), however, solubilized greater amounts of protein at 200 MPa. Solubility of beef *biceps femoris* myofibrils was increased by pressurization at 300 – 600 MPa while in 0.1 M KCl buffer (Jung, Lamballerie-Anton, & Ghoul, 2000a), while that of chicken breast myofibrils was increased at 100 – 300 MPa in a similar solution (Iwasaki et al., 2006). Marcos et al. (2010) noted stepwise decreases in solubility of sarcoplasmic protein fractions (extracted under very low ionic strength) when subjected to 200 and 400 MPa for 20 min. Total protein extraction (0.55 M KI) from minced chicken breast containing 0 or 2.5% salt was decreased when 400 or 600 MPa pressure was applied when compared to 200 MPa, but this decrease was almost completely counteracted when 0.3% sodium tripolyphosphate (STPP) and 1% salt were used (Omana, Plastow, & Betti, 2011). The same study reported decreased solubility of the sarcoplasmic fraction regardless of included non-meat ingredients. It is difficult to discuss the implications of protein solubility in such complex systems as muscle foods. Indeed, there are numerous proteins with various biochemical functions in the living animal, many of which have subsequent contributions to meat quality factors, such as color, texture and water holding capacity, post-rigor. The use of HPP subjects these molecules to far more extreme conditions than they might have become acclimated to through evolutionary processes, especially in the case of land mammals. While it is possible to broadly characterize the effects of hydrostatic pressure on protein-water and protein-protein interactions due to Le Chatelier’s principle (Messens, Van Camp, & Huyghebaert, 1997) and observations of specific proteins in pure systems (Mozhaev, Heremans, Frank, Masson, & Balny, 1996), predictions of behavior in systems containing various proteinaceous species along with other organic compounds (fats, sugars) become uncertain. The presence of other common food ingredients, most notably salt and
phosphate in meats, further confuses matters as these compounds have their own stabilizing/destabilizing effects on proteins. Decreases in soluble protein, regardless of fraction (myofibrillar/sarcoplasmic) are associated with both protein denaturation and aggregation, both of which inhibit functionality of proteins important to meat quality (Joo et al., 1999). Denaturation of proteins, specifically a folded → unfolded transition, with exposure of hydrophobic side groups, is expected when proteins are subjected to high hydrostatic pressure (Messens et al., 1997; Mozhaev et al., 1996). Observations in pressurized myofibrillar suspensions support this assertion (Chapleau, Delepine, & Lamballerie-Anton, 2002; Chapleau & Lamballerie-Anton 2003a; Chapleau, Mangavel, Compoint & Lamballerie-Anton, 2003b). The cited studies characterized many of the changes within myofibrillar proteins, including a three-fold increase in surface hydrophobicity, which represents significant denaturation, and protein aggregation. Greater exposure of sulfhydryl groups due to unfolding may encourage this aggregation, as the ratio of reactive to total SH groups has been seen to increase upon pressurization in myofibrillar suspensions (Chapleau et al., 2003b) and in model meat systems (Chan et al., 2011; Omana et al., 2011). While reactive SH group exposure showed some amenability to pressure application in the current work, true significant effects on SH characteristics may have been difficult to discern from analysis of the total extractible protein as opposed to a more pure fraction. The lack of influence of salt and SP on solubility suggests that, at the tested levels, they were unable to significantly alter how hydrostatic pressure affects muscle proteins. The fact still remains, however, that SP use was able to completely counteract the purge increase seen as a result of pressurization. Given the evidence from this study, we assert that the additive pH increase from combined SP/pressure treatment was primarily responsible.
<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Sarcoplasmic Solubility(^c)</th>
<th>Myofibrillar Solubility(^c)</th>
<th>Total Solubility(^c)</th>
<th>Reactive SH(^d)</th>
<th>Total SH(^d)</th>
<th>Reactive/Total SH Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1.64(^a) ± 0.07</td>
<td>1.64(^a) ± 0.11</td>
<td>3.28(^a) ± 0.15</td>
<td>106 ± 4.7</td>
<td>150 ± 6.6</td>
<td>0.74 ± 0.03</td>
</tr>
<tr>
<td>152</td>
<td>1.58(^a) ± 0.07</td>
<td>1.69(^a) ± 0.11</td>
<td>3.26(^a) ± 0.15</td>
<td>113 ± 4.7</td>
<td>155 ± 6.3</td>
<td>0.76 ± 0.03</td>
</tr>
<tr>
<td>303</td>
<td>1.37(^a) ± 0.07</td>
<td>1.11(^b) ± 0.12</td>
<td>2.48(^b) ± 0.15</td>
<td>120 ± 4.6</td>
<td>156 ± 6.7</td>
<td>0.77 ± 0.03</td>
</tr>
<tr>
<td>(P) value(^e)</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.078</td>
<td>0.576</td>
<td>0.52</td>
</tr>
</tbody>
</table>

\(^a,b\) Means within a column with differing superscripts are significantly different (\(P < 0.05\))

\(^c\) Protein solubility means are expressed as mg protein/g sample ± standard error of the mean

\(^d\) Sulfhydryl means are expressed as μMol SH/mg protein ± standard error of the mean

\(^e\) \(P\) value for the main effect of pressure level
4.4.6 Lipid oxidation

Oxidation, measured by the TBARS method of malondialdehyde (MDA) quantification, was not induced ($P = 0.865$) by pressure treatment and no significant interactions were observed. The only significant effect ($P = 0.028$) was the presence of SP, which decreased MDA when used at 4% of the brine (Figure 4.6). The antioxidative activity of phosphates has been documented previously in injected beef (Rowe et al., 2009; Lowder et al., 2011), however, the values reported here for both non-SP and SP treatments (0.25 and 0.17 mg/kg MDA, respectively) are inconsequential in practice (Campo, Nute, Hughes, Enser, Wood & Richardson, 2006). There are numerous examples of pressure induced oxidation at pressures at or above 400 MPa in minced pork (Cheah & Ledward, 1996), chicken (Beltran, Pla, Yuste & Mor-Mur, 2003; Bolumar, Andersen, Orlien, 2011; Orlien, Hansen & Skibsted, 2000) and beef (Ma et al., 2007; McArdle et al., 2011). McArdle et al. 2011 saw increased TBARS values in beef brisket pressurized as low as 300 MPa. Oxidative acceleration due to pressure is largely thought to be catalyzed by transition metal exposure/oxidation (Angsupanich & Ledward, 1997; Carlez et al., 1995), though membrane disruption to expose unsaturated fatty acids and the inactivation of antioxidant enzyme systems may also be implicated (Kanner, 1994; Mei, Krum, & Decker, 1994). Bolumar, Skibsted and Orlien (2012) have presented evidence that free radical species are directly formed as a result of pressurization at 400 – 500 MPa. The low level of detectable oxidation products in the current work could be due to a number of factors, including the low pressures used, short dwell time of pressurization and the lack of a storage/display period.
Figure 4.6 Thiobarbituric reactive substances (TBARS) values of beef from strip loins injected to 110% of initial weight with solutions containing salt (0, 2 or 4% of solution) and/or SP (0 or 4% of solution) and exposed to high pressure (0.1, 152 or 303 MPa; 1 min at ambient temperature). Values are expressed as mg malondialdehyde (MDA) per kg of meat. Only the SP main effect is presented; different letters indicate a significant difference ($P < 0.05$) between treatments.

4.5 Conclusions

In the current study, no evidence of interactions between high hydrostatic pressure and the ingredients salt or sodium phosphate were seen. It is likely that levels in this study, while appropriate for a similar commercial product, where too low to elicit any changes due to pressure. Additionally, the highly ordered character of intact muscle prevents the degree of ingredient-protein interaction achievable in comminuted and restructured products. However, SP addition was able to lessen the
whitening effect to some degree and completely counteract the purge losses incurred upon pressurization. Since protein solubility and SH content were not affected and salt was unable to achieve the same result, the additive SP/pressure induced pH increase is implicated in both cases rather than retention of protein functionality. This assertion is not definitive, however, as only basic protein characterization was carried out in this study. Current results suggest SP may play a role in producing acceptable fresh HPP whole muscle beef, but further experimentation at higher pressures with longer dwell times is needed to confirm this. Independent of pressure application, the ability of salt and SP to retain fluid in whole muscle injected beef seen here further supports the body of work already done on the subject.
5. High pressure-low temperature processing of beef: effects on survival of internalized *E. coli* O157:H7 and quality characteristics

Austin C. Lowder, Joy G. Waite-Cusic, Christina A. Mireles DeWitt
5.1 Abstract

This study attempted to use high pressure-low temperature (HPLT) processing to achieve *E. coli* O157:H7 inactivation in non-intact, whole muscle roasts while maintaining acceptable quality characteristics. Beef *semitendinosus* was internally inoculated with a four strain *E. coli* O157:H7 cocktail and frozen at -30 °C, then subjected to 550 MPa for 4 min (HPLT). Compared to frozen, untreated control (F), HPLT reduced microbial population by 1.7 log colony forming unit (CFU)/g on selective media (Cefixime-Tellurite Sorbitol MacConkey agar) and 1.4 log on non-selective media (Trypticase soy agar). High pressure without freezing (550 MPa/4 min/3 °C) increased pH and lightness while decreasing redness, cook yield, tenderness, and protein solubility. High pressure low temperature, aside from a 4% decrease in cook yield, had no significant effects relative to the control. High pressure low temperature is an effective way to subject red meat to high pressures without compromising quality. However, its diminished effect on internalized *E. coli* suggests limited effectiveness as a food safety intervention.

5.2 Introduction

Internal muscle tissue is generally free of pathogenic and spoilage microorganisms. However, microbiological contaminants located on the surface of intact cuts can be internalized by common meat industry practices, such as mechanical tenderization or brine injection/enhancement (Gupta, 2012; Luchansky, Phebus, Thippareddi & Call, 2008). Additionally, during brine injection, excess solution that runs off or is purged from the meat is recycled back into the brine well and through the injector; this creates opportunity for bacteria to be harbored within
the brine and introduced within the muscle tissue on subsequent injections (Adler, Geornaras, Byelashov, Belk, Smith & Sofos, 2011; Paulson, Wicklund, Rojas & Brewer, 2010). The internalization of pathogenic bacteria, such as *Escherichia coli* O157:H7, can protect them from thermal inactivation or destruction during cooking. This is especially a concern if the product is undercooked by the consumer or foodservice outlet, whether because it was unknown that the cut was non-intact or unknown that non-intact cuts pose increased risk (FSIS, 2002a,b; Sofos & Geornaras, 2010). In fact, there have been multiple *E. coli* O157:H7 outbreaks and associated product recalls in the US associated with whole muscle, non-intact meat over the past decade, the most recent in 2009 affected at least 21 people in 16 states (CDC, 2010; FSIS, 2005; Laine et al., 2005). The Food Safety and Inspection Service (FSIS) has considered *E. coli* O157:H7 an adulterant in blade tenderized and brine enhanced beef since 1999 and in 2005 required processors of these products to reassess their HACCP plans due to increased risk of pathogenic internalization (FSIS, 1999; FSIS, 2005). It has been maintained that the incidence of pathogenic surface contamination on beef cuts is low, and, therefore, incidence of mechanically induced subsurface translocation should be low and current recommended best practices should eliminate any significant public health threat (Heller et al., 2007; NCBA, 2006; Sofos & Geornaras, 2010). However, as noted previously, recalls and outbreaks still occur. Investigation into prevention of subsurface contamination of non-intact whole muscle products has recently focused on use of antimicrobials on product surfaces (Eager, 2011; Echeverry, Brooks, Miller, Collins, Loneragan & Brashears, 2009; Heller et al., 2007; Wellings, 2011) and in enhancement brines (Adler et al., 2011; Byelashov et al., 2010; Wicklund, Paulson, Rojas & Brewer, 2006;
Wicklund, Paulson, Rojas & Brewer, 2007). Hydrodynamic pressure as an additional intervention process has also been discussed (Patel, Williams-Campbell, Liu & Solomon, 2005).

A technology that has not been mentioned as a possible intervention in the literature is high hydrostatic pressure processing (HPP), a non-thermal, non-chemical, pasteurization treatment with established efficacy against *E. coli* O157:H7 (Campus, 2010; Simonin, Duranton & Lamballerie, 2012). Commercial HPP application within the meat industry has gained popularity in recent years, mainly as a post-processing pasteurization tool to eliminate *Listeria* in cooked, ready-to-eat (RTE) products (Campus, 2010; Torres & Velasquez, 2005). The lack of interest in using HPP on fresh, uncooked beef is due to the marked detrimental changes in quality caused by treatments at pressures relevant to food safety (400 – 600 MPa). When exposed to >200 MPa fresh beef greatly increases in lightness, taking on an appearance similar to pork; pressures above 350 MPa cause browning, resulting in a grayish hue (Carlez, Veciana-Nogues, & Cheftel, 1995; Cheftel & Culioli, 1997). Greater purge and cook losses, decreased tenderness and increased lipid oxidation rates also occur, with severity depending on pressure level and dwell time (Bolumar, Skibsted & Orlien, 2012; Cheftel & Culioli, 1997; Ma & Ledward, 2004; Ma, Ledward, Zamri, Frazier & Zhou, 2007; McArdle, Marcos, Kerry & Mullen, 2010, 2011; Suzuki, Watanabe, Iwamura, Ikeuchi, & Saito, 1990). However, there is evidence that freezing meat before exposure to high pressure application will help protect color and fluid retention while still inactivating microorganisms (Fernández, Sanz, Molina-García, Otero, Guignon & Vaudagna, 2007; Realini, Guàrdia, Garriga, Pérez-Juan & Arnau, 2011; Vaugadna, Gonzalez, Guignon, Aparicio, Otero & Sanz, 2012). There
is little research in the area of high pressure-low temperature (HPLT) treatment of meat. Because of this, the effects on multiple quality indicators affected by HPP, including tenderness and cooking characteristics, as well as the ability to inactivate of the pathogen of concern, *E. coli* O157:H7, are unknown. The advent of commercially viable HPP treatments for raw beef would allow flexibility in dealing with subsurface translocation of microorganisms in non-intact products, possibly even eliminating the need for chemical antimicrobials. The goal of this research is to elucidate the effects of HPLT processing on *E. coli* O157:H7 inactivation and various shelf life and cooking characteristics of whole muscle beef.

### 5.3 Materials and methods

#### 5.3.1 Inoculum preparation

The inoculum was composed of four *E. coli* O157:H7 strains (ATCC 43894, ATCC 43895, an isolate from alfalfa sprouts and an isolate from an outbreak associated with an Oregon steakhouse). Strains were cultured individually in 10 mL tryptic soy broth (TSB; BD Bacto™, Becton, Dickinson and Company, Sparks, MD, USA) at 37 °C for 24 hr from working cultures maintained on tryptic soy agar (TSA; BD BBL™ TSA II, Becton, Dickinson and Company, Sparks, MD, USA). The cultures were combined and centrifuged (4000 x g; 10 min; 4 °C) to harvest cells. The pellet was washed with 10 mL Butterfield’s phosphate buffer (BPB; 6.8% KH₂PO₄; pH 7.2) once and re-suspended to obtain ~10⁹ CFU/mL.
5.3.2 Raw material preparation and inoculation

5.3.2.1 Microbiological processing

Beef *semitendinosus* (IMPS 171C; ~5% fat) was obtained vacuum packaged from a local retail outlet and aseptically trimmed of visible fat and connective tissue. Initial beef pH was obtained using a piercing probe (SenTix SP, WTW GmbH, Weilheim, Germany) attached to a pH meter (pH 3210, WTW GmbH, Weilheim, Germany). The beef was portioned into 25 g pieces. Two uninoculated samples from each round were taken for background microbiota enumeration. The other pieces were aseptically sliced in the center and 0.25 mL of inoculum was applied, giving an inoculation concentration of approximately $10^7$ CFU/g meat. Once the inoculum was absorbed, individual pieces were placed into sterile vacuum packages (15.5 x 22 cm) and vacuum sealed. All vacuum sealed pieces were then frozen (-30 °C) and randomly assigned to one of two treatments: (1) frozen (-30 °C; 0.1 MPa; F) or (2) HPLT treated (-30 °C; 551 MPa, 4 min; HPLT). To monitor temperature before and after pressurization, a type T thermocouple with a 1.8 m lead was inserted into the center of beef pieces or steaks that were packaged as the other samples; these were used only for monitoring temperature.

5.3.2.2 Quality processing

For quality tests, 2.54 cm thick steaks were sliced from trimmed *semitendinosus*, weighed and packaged as in the microbiological testing. All steaks were frozen at -25 °C. Another control treatment was added for quality comparisons: (3) thawed, HPP treated (3 °C; 551 MPa, 4 min; HPP). All HPLT samples were pressurized within two weeks of freezing, held in storage (-25 °C) and analyzed after one month. Thawed HPP samples were pressurized after 1 month of frozen storage.
and were not refrozen between pressurization and analysis, which took place within 48 hr of pressurization. All treatments were analyzed at the same time.

5.3.3 High pressure processing

Pressurization took place in a 22 L chamber (National Forge Company, Andover, MA, USA) using 5% soluble oil (Hydrolubric® 123-B, Houghton International, Valley Forge, PA, USA) in water as the pressurization medium. The HPLT samples were held in a cooler with dry ice for transport to the pressurization facility; temperature was maintained at -30 to -40 °C before pressurization. Samples were taken from the cooler and put directly into a nylon bag filled with ice/water, which was placed into the pressurization chamber. After pressurization HPLT samples were put back into frozen (-25 °C) storage. Pressure was applied at 551 MPa for 4 min. Ramp up rate was ~5 MPa/s with 31 s depressurization. Pressurization was applied in duplicate (microbiological) and triplicate (quality) due to space constraints with each block and treatment represented equally in pressure runs.

5.3.4 Microbiological sampling and plating

After pressurization, samples were held for 7 d at -30 °C, then allowed to thaw at 3 °C for 12 hr. They were then placed in sterile stomacher bags (Whirl-Pak, Nasco, Modesto, CA, USA) with 225 mL phosphate buffer and homogenized (Stomacher 400 Circulator, Seward, West Sussex, England) for 3 min. Following homogenization, samples were serially diluted with BPB and plated on TSA and Cefixime-Tellurite Sorbitol MacConkey agar (CT-SMAC; Becton, Dickinson and Company, Sparks, MD, USA). Duplicate plates for each sample and dilution were made. Plates were incubated for 24 - 48 hr at 37 °C prior to enumeration.
5.3.5 Purge and color analysis

Steaks were allowed to thaw for 24 hr at 3 °C, removed from their packages, pat dry with a paper towel and reweighed. Purge was determined using the equation: 

\[ \text{Purge} (\%) = \frac{(S_0 - S_1)}{S_0} \times 100 \]

where \( S_0 \) = the weight of the steak at slicing and \( S_1 \) = the weight of the steak after storage. Raw color was analyzed immediately after weighing for purge (packaged color) and after blooming for at least 30 min at 3 °C (display color). Instrumental color was determined using a portable spectrophotometer (CM-600; Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) with a 10° observer and illuminant A. The device was calibrated using a manufacturer supplied white tile. CIE \( L^* \) and \( a^* \) and \( b^* \) values (CIE, 1978) and reflectance spectra (400-700nm) were recorded (AMSA, 2012). Average measurements were taken from four readings on the surface of each steak. Total color difference (\( \Delta E \)) was calculated using the formula: 

\[ \Delta E = [(L^*o - L^*)^2 + (a^*o - a^*)^2 + (b^*o - b^*)^2]^{0.5} \]

where \( L^*o \), \( a^*o \) and \( b^*o \) are the averages for frozen samples at each respective time period. Hue angle was calculated as: \( \tan^{-1}(b^*/a^*) \) and chroma, or saturation index, as: \( \sqrt{(a'^2 + b'^2)} \). The ratio of reflectance spectra at 630 and 580 nm (R630/580) were used to estimate relative amounts of brown pigment (AMSA, 2012). After color measurement, samples were either cooked for Warner-Bratzler shear force or sent to biochemical analysis. The latter were probed for pH in the same manner as the raw materials, then frozen directly in liquid nitrogen and pulverized in a Waring blender. Sample powder was stored at -80 °C and used for all biochemical analyses.
5.3.6 Cooking characteristics and tenderness

Steaks were cooked on a grated, indoor electric grill (Hamilton Beach, Southern Pines, NC) preheated to 177 °C to an internal temperature of 35 °C and flipped once; cooking continued to 70 °C, as determined by a copper/constantan type T thermocouple inserted to the geometric center of the steak, before steaks were removed from the grill. Steaks were weighed immediately after cooking to determine cook yield using the formula: cooked weight/initial weight * 100. After cooking, steaks were covered with aluminum foil and allowed to equilibrate at ~23 °C for at least 4 h. Six cores of 1.3 cm diameter were taken from each steak, parallel to muscle fiber direction, and sheared using a Warner-Bratzler V-blade attachment on a TA.XT Plus (Texture Technologies Corp, Scarsdale, NY) testing machine with a crosshead speed of 200 mm/min. Peak shear force (N) was recorded. Moisture content of cooked steaks was determined using AOAC (2003) method 950.46.

5.3.7 Protein solubility

Sarcoplastic (water-soluble) and total (salt- and water-soluble) protein solubility was determined using the Bradford (1976) method with premixed reagents (Bio-Rad Laboratories, Hercules, CA, USA). For sarcoplastic, two g of powdered sample were vortexed for 15 s in 10 volumes of a low ionic strength buffer (30 mM sodium phosphate, pH 7.4) then incubated on a rocker on ice for 2 h with further mixing (15 s) every hour. Samples were kept on an ice bath or under refrigeration. After centrifugation at 5000 x g and 4 °C for 20 min, supernatant was decanted, reacted with Bradford reagent and read on a spectrophotometer (Shimadzu UV-2400, Shimadzu Scientific Instruments, Inc, Columbia, MD) at 595 nm. Total solubility used
the same procedures with a high ionic strength buffer (0.6 M KCl, 50 mM sodium phosphate, pH 7.4). Protein solubility was reported as mg/g sample.

5.3.8 Sulfhydryl content

Total and reactive sulfhydryl groups were determined in the presence and absence of urea by a modification of the procedures described by Hamada, Ishizaki and Nagai (1994). Samples (1 mg protein/ml) were retained from the protein solubility test. Adjusted sample (0.5 mL) was mixed with 2 mL 8M urea in 0.2 M Tris (pH 7.0) and 50 μL of 10 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) with 0.1 M sodium phosphate and 0.2 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.2) and incubated in a 40 °C water bath for 15 min. Reacted samples were measured on a spectrophotometer (UV 2401, Shimadzu Corporation, Kyoto, Japan) at 412 nm. Total SH content was determined as μMol/mg protein using a molar extinction coefficient of 14,150 M⁻¹ cm⁻¹ (Riddles et al. 1979). The adjusted protein sample was incubated at 5 °C for 1 hr without urea to determine reactive SH content. The reactive value was divided by the total value to get the reactive/total ratio.

5.3.9 Experimental design and statistical analysis

The experiment was designed as a randomized complete block with subprimal (n=4) as the random block. Beef pieces (micro) or steaks (quality) were the experimental unit. Treatment means from the microbiological data were compared using a t-test. Quality data were analyzed using PROC GLIMMIX of SAS. Analysis of variance (ANOVA) was conducted for each variable to determine whether differences existed. In the event of a significant (P < 0.05) ANOVA test, comparisons were made between the controls and treatment using t-tests. For quality analyses, the Bonferroni adjustment was applied when comparing treatment means. Time of
analysis (0 or 30 min) was treated as a repeated effect for the color variables and covariance structures were compared using the fit statistics generated by PROC GLIMMIX.

5.4 Results and discussion

5.4.1 Microbiological data

5.4.1.1 Pressurization

Due to the lack of temperature controls or monitoring on the high pressure unit it is impossible to determine the exact temperature path of the samples. Temperature would be expected to change for two reasons. The first is the temperature difference at the sample-fluid interface and the second is adiabatic heating. Adiabatic heating refers to the temperature increase that occurs as liquid or solid food components are compressed and intermolecular forces are strained. The heating rate is expected to be around 3 °C/100 MPa for lean muscle foods (Rasanayagam, Balasubramaniam, Ting, Sizer, Bush, & Anderson, 2003). When dealing with HPLT processes the effects of very high pressures on the phase transitions of ice, which induce temperature changes independent of adiabatic heating, must also be kept in mind. Although sample and chamber temperature could not be monitored, samples with detachable thermocouples were used to estimate temperature changes due to pressurization. Average temperatures before and after pressurization were -38.4 °C and -11 °C, respectively, denoting an average 27.5 °C change (data not shown in tabular form). The water present in the beef would have been in the state of ice I before and after pressurization and ice V during the dwell. It is only possible to estimate the phase transitions undergone during pressurization and depressurization
given the narrow boundaries between liquid water, ice III and ice II and variation in the temperature of the samples. It is highly unlikely that the samples were unfrozen during the dwell.

5.4.2.2 E. coli O157:H7 inactivation

Least squares means for microbial counts on both media are given in Table 5.1. Reductions ($P < 0.001$) of 1.7 and 1.4 log were achieved on CT-SMAC (selective) and TSA (non-selective) media by HPLT treatment. Previous reports on E. coli O157:H7 inactivation in a meat system due to HPLT treatment have not been published. However, general microbiological quality in HPLT beef and pork has been described. Beef treated at -35 °C for 10 min had total aerobic (ATC) and lactic acid bacteria (LAB) counts under the detection limit of 1 log CFU/g (Fernández et al., 2007). Untreated beef in that study had ATC and LAB counts of 3.7 and 2.7 log, respectively, indicating that a general reduction of at least 1.7 - 2.7 log had taken place. Reductions in ATC, psychrotrophs and LAB of 1-3 log were seen in beef carpaccio subjected to HPLT at -30 °C for 1 or 5 min at 400 or 650 MPa (Vaudagna et al., 2012). Treatment of pork carpaccio by HPLT (400 or 600 MPa, 6 min, -35 °C) caused initial reductions of <1 log of LAB and psychrotrophs (Realini et al., 2011). Microbiological quality was improved over 41 d of storage by HPLT treatment, but this may have been the result of the additional curing effects of salt and sodium nitrite included in that study.

The efficacy of high pressure treatment on microbial inactivation seems to be hindered greatly by the low water activity of frozen meat. However, HPLT treatments have demonstrated consistent effects on multiple microbial populations in several muscle food systems, as well as buffer solutions of Listeria innocua and E. coli
Table 5.1  *E. coli* O157:H7 populations (mean ± standard deviation) recovered on selective (CT-SMAC\textsuperscript{c}) and non-selective (TSA\textsuperscript{c}) media from frozen (F; -30 °C) or frozen, high pressure-low temperature (HPLT; 550 MPa/30 °C/4 min) treated beef pieces inoculated internally

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Media\textsuperscript{c}</th>
<th>CT-SMAC</th>
<th>TSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6.7 ± 0.1</td>
<td>6.8 ± 0.1</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>5.0 ± 0.2</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>HPLT</td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\textsuperscript{c}Cefixime-Tellurite Sorbitol MacConkey (CT-SMAC) is selective for *E. coli* O157:H7; Trypticase Soy Agar (TSA) is non-selective.  
\textsuperscript{d}P-value of the t-test for differences between treatment means

(Edebo & Hedén, 1960; Luscher, Balasa, Fröhling, Ananta, & Knorr, 2004). Ice form transitions due to pressure and temperature, which result in changes of sample density and volume, and likely cause some degree of mechanical stress to their surroundings, are implicated in reductions of microbial populations when treated by HPLT. This effect seems to be immediate and not dependent on pressure beyond certain form transition boundaries, making extended dwell times and extremely high pressures superfluous (Luscher et al., 2004; Realini et al., 2011).

5.4.2 Quality

5.4.2.1 Color

It is typical in color measurement of red meats to allow the sample to be exposed to air for at least 30 min (blooming) at refrigeration temperature (AMSA, 2012). This is enough time for the myoglobin on the surface of the meat to bind O\textsubscript{2} and switch from the deoxy- form to the oxymyoglobin form that gives the characteristic red color of beef. The measurement of color before any blooming could take place was carried out to gain information on the color of the treated meat as it would be in the vacuum package, which is the most common method of distribution for frozen beef. Least squares means for various color descriptors are given in Table
5.2. There was a significant two-way interaction \( (P < 0.05) \) between treatment and bloom time for all studied variables, save \( \Delta E \), which could not be compared between time periods. Lightness \( (L^*) \) at 0 min was not different \( (P = 0.924) \) between HPLT and F steaks, but was increased \( (P < 0.001) \) by ~14 units due to HPP. The frozen only (F) and HPLT steaks both saw slight increases \( (P < 0.001) \) in lightness after blooming, while HPP steaks did not change \( (P = 1.000) \). Redness \( (a^*) \) was greater \( (P < 0.040) \) in F and HPLT steaks than in HPP steaks by about 3 units at 0 min and 6 units at 30 min. The HPP steaks did not experience \( (P = 1.000) \) a redness increase due to bloom time as the F and HPLT treatments did \( (P < 0.001) \). The yellowness \( (b^*) \) of F and HPLT steaks was lower \( (P < 0.001) \) than that of HPP steaks by 5 – 6 \( b^* \) units at 0 min. However, they both increased \( (P < 0.001) \) to the 18 – 19 unit range upon blooming, putting them roughly equivalent \( (P = 1.000) \) to the HPP samples in yellowness. Hue angles did not change as a result of blooming for the HPLT or HPP samples, but increased \( (P < 0.001) \) marginally (37.21 vs. 40.35) in the frozen control. Conventional high pressure processed (HPP) beef had an average hue angle that was at least 10 degrees greater \( (P < 0.001) \) than the F and HPLT treatments, denoting a shift from true red hue toward a yellowish hue. Chroma, or saturation index, was initially similar \( (P = 0.199) \) between the HPLT and HPP steaks, while the F steaks were slightly lower \( (P = 0.040) \) than HPP. Blooming increased saturation of F and HPLT treatments, but not HPP. Ratio of reflectance at 630/580 nm was markedly higher \( (P < 0.001) \) in the F and HPLT steaks (2.56 and 2.39, respectively) than HPP (1.47), and further increased \( (P < 0.001) \) after blooming. Total color difference \( (\Delta E) \)
Table 5.2 Least squares means for $L^*$ (lightness), $a^*$ (redness), $b^*$ (yellowness), hue angle, chroma (saturation), ratio of reflectance spectra at 630 and 580 nm and total color difference ($\Delta E$) of beef semitendinosus steaks subjected to freezing (F; -25 °C), high pressure low temperature treatment (HPLT; <-30 °C/550 MPa/4 min) or high pressure processing (HPP; 3 °C/550 MPa/4 min)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time</th>
<th>F</th>
<th>SEM$^d$</th>
<th>HPLT</th>
<th>SEM$^d$</th>
<th>HPP</th>
<th>SEM$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L^*$</td>
<td>0</td>
<td>42.46$^b$</td>
<td>1.27</td>
<td>41.16$^b$</td>
<td>1.28</td>
<td>56.93$^a$</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>43.93$^b$</td>
<td>1.27</td>
<td>42.75$^b$</td>
<td>1.28</td>
<td>56.72$^a$</td>
<td>1.27</td>
</tr>
<tr>
<td>$P^e$</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>$a^*$</td>
<td>0</td>
<td>17.38$^a$</td>
<td>1.20</td>
<td>17.01$^a$</td>
<td>1.22</td>
<td>14.32$^b$</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>22.19$^a$</td>
<td>1.20</td>
<td>21.39$^a$</td>
<td>1.22</td>
<td>15.38$^b$</td>
<td>1.20</td>
</tr>
<tr>
<td>$P^e$</td>
<td></td>
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<td></td>
<td>&lt;0.001</td>
<td></td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>$b^*$</td>
<td>0</td>
<td>13.18$^b$</td>
<td>0.47</td>
<td>14.07$^b$</td>
<td>0.49</td>
<td>19.06$^a$</td>
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<tr>
<td></td>
<td>30</td>
<td>18.77$^a$</td>
<td>0.47</td>
<td>18.67$^a$</td>
<td>0.49</td>
<td>19.44$^a$</td>
<td>0.47</td>
</tr>
<tr>
<td>$P^e$</td>
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<td></td>
<td>&lt;0.001</td>
<td></td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Hue$^f$</td>
<td>0</td>
<td>37.21$^b$</td>
<td>1.45</td>
<td>39.70$^b$</td>
<td>1.50</td>
<td>52.96$^a$</td>
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<td></td>
<td>30</td>
<td>40.35$^b$</td>
<td>1.17</td>
<td>41.24$^b$</td>
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<td>51.69$^a$</td>
<td>1.17</td>
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<tr>
<td>$P^e$</td>
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<td></td>
<td>&lt;0.001</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Chroma$^g$</td>
<td>0</td>
<td>21.85$^b$</td>
<td>0.90</td>
<td>22.23$^{ab}$</td>
<td>0.92</td>
<td>23.82$^a$</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.04$^a$</td>
<td>1.08</td>
<td>28.51$^a$</td>
<td>1.10</td>
<td>24.78$^b$</td>
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<tr>
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<td></td>
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<td>&lt;0.001</td>
<td></td>
<td>0.879</td>
<td></td>
</tr>
<tr>
<td>630/580</td>
<td>0</td>
<td>2.56$^a$</td>
<td>0.20</td>
<td>2.39$^a$</td>
<td>0.20</td>
<td>1.47$^b$</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.54$^a$</td>
<td>0.26</td>
<td>3.28$^a$</td>
<td>0.27</td>
<td>1.59$^b$</td>
<td>0.26</td>
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<tr>
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<td></td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>$\Delta E^h$</td>
<td>0</td>
<td>-</td>
<td>4.16$^b$</td>
<td>0.84</td>
<td>15.99$^a$</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-</td>
<td>4.38$^b$</td>
<td>0.88</td>
<td>14.65$^a$</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Means within a row with differing superscripts differ significantly ($P < 0.05$)

$^b$Standard error of the mean

$^e$P values of the comparisons between time periods within a treatment and variable

$^f$Hue angle = tan$^{-1}$(b*/a*)

$^g$Chroma = $\sqrt{(a^2 + b^2)}$

$^h$$\Delta E = [(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2]^{0.5}$, where $L^*_0$, $a^*_0$ and $b^*_0$ are the averages for frozen samples at each respective time period
from the F steaks was far greater ($P < 0.001$) for the HPP steaks than HPLT steaks both before and after blooming.

The data presented here strongly indicate that the instrumentally determined color of high pressure low temperature treated beef does not differ from untreated, frozen beef. In this regard, the results seen here corroborate well with those seen in the previously mentioned experiments on beef muscle subjected to high pressure at subzero temperatures. Frozen beef studied by Fernández et al. (2007) did not differ from the frozen, pressurized samples in lightness, redness or yellowness. Similar results were seen in pork and beef carpaccio regardless of pressure and dwell time as long as sufficiently low temperatures ($\leq -30 \, ^\circ C$) were maintained (Realini et al., 2011; Vaudagna et al., 2012). Additionally, this study observes that the dynamics of myoglobin oxygenation seem to be unaffected, suggesting that the protein denaturation normally associated with high pressure is not of concern and any physical disruption caused by the ice form transitions has minimal effect on the integrity of myoglobin.

The effects of high pressure on the color of uncooked, uncured beef are well documented. The lightness increase upon pressurization is known to occur as low as 200 MPa and becomes especially severe in the 300 - 400 MPa range, depending on dwell time (Carlez et al., 1995; Lowder & Mireles DeWitt, 2013; McArdle et al., 2011). The exact mechanism behind this has not been clarified. Although myoglobin denaturation is often implicated, the cause is likely much less specific as all muscle foods, even fish with minimal myoglobin content, experience the same phenomenon after pressurization (Gómez-Estaca, López-Caballero, Gómez-Guillén, López de Lacey & Montero, 2009). The $a^*$ decrease and $b^*$ increase are commonly seen
above 400 MPa in beef (Carlez et al., 1995; Fernandez et al., 2007; Marcos, Kerry, & Mullen, 2010; McArdle et al., 2010, 2011; Vaudagna et al., 2012). These changes create drastic alterations in the hue angle, moving it more than 10 degrees away from true red in the current work. Unlike the lightness increase, these color differences are almost certainly related to the conformational, oxidation and ligand binding state of myoglobin. It has been confirmed by Raman spectroscopic examination that pressurization of oxymyoglobin results in heme exposure and oxygen release, catalyzing the oxidation and ferric (Fe$^{3+}$) transformation to metmyoglobin (Wackerbarth, Kuhlmann, Tintchev, Heinz, & Hildebrandt, 2009). Pressurization of deoxymyoglobin, which almost certainly accounted for the predominant myoglobin form in this work, was found to produce a ferrous (Fe$^{2+}$) six-coordinated low spin species of myoglobin which, according to the spectral observations of Bak, Lindahl, Karlsson and Orlien (2012), is transformed into conventional metmyoglobin within 24 h of pressurization. If the observations of Wackerbarth et al. (2009) and Bak et al. (2012), which were made on pork myoglobin, can be extrapolated to beef myoglobin, the ferric form would be expected to dominate at the time of analysis here. The reflectance ratio at 630/580 nm agrees with this, as it nears 1 for the HPP samples, suggesting high metmyoglobin content. An increase of this ratio upon blooming would be expected for samples with functional myoglobin, as the reflectance of deoxymyoglobin is both lower at 630 and higher at 580 than the oxy form (AMSA, 2012). The observation that sufficient pressure prevents meat from blooming was also recorded by Bak et al. (2012) in pork subjected to at least 400 MPa pressure for 10 min.
5.4.2.2 pH, purge and cooking characteristics

The pH values of the initial raw materials averaged 5.46 (data not shown in tabular form). Freezing, as expected, did not change pH; nor did treatment by HPLT as both averaged 5.47 (Table 5.3). Conventional high pressure (HPP), however, increased \( P < 0.001 \) pH by 0.15 units, which is a result noted in multiple published reports on HPP use in muscle foods (Lowder & Mireles DeWitt, 2013; Ma & Ledward, 2004; McArdle et al., 2010, 2011). While it is generally accepted that high pressure induces a shift in the pH equilibrium towards acidity (Mújica-Paz, Valdez-Fragoso, Samson, Welti-Chanes, & Torres, 2011), the mechanism behind the subsequent alkaline shift in muscle foods upon depressurization is not clear.

Purge values ranged from 4.70 to 5.35% and did not differ \( P = 0.666 \) between treatments (data not shown in tabular form). The beef *longissimus* subjected to HPLT treatment in Fernández et al. (2007) had expressible moisture values that were similar to those of frozen (-35 °C) beef and 15% lower than HPP (650 MPa/10 min), indicating an unchanged ability for fluid retention on its part. However, this result was not consistently seen in frozen (-30 °C) beef carpaccio treated at 650 MPa, which had higher expressible moisture values than HPP treated (650 MPa/20 °C) beef carpaccio (Vaudagna et al., 2012). Purge in whole muscle HPP treated beef has been seen to increase at pressure as low as 152 MPa (Lowder & Mireles DeWitt, 2013). Expressible moisture was increased by 9% upon pressurization of at least 400 MPa in beef *longissimus* and water holding capacity was reduced by only 200 MPa in beef semitendinosus (Kim, Lee, Lee, Kim, & Yamamoto, 2007; Marcos et al., 2010).
Cook yield followed a hierarchical arrangement with F having the highest ($P < 0.002$) at 79.18%, HPLT the second highest at 75.44% and HPP the lowest at 69.72% (Figure 5.1). Cook yield has not been previously investigated in HPLT meat products; here it is seen to be significantly lower than the control, but still at an acceptable level. Use of HPP Conversely, is known to decrease cook yield. Bovine semitendinosus experienced greater cook loss at just 100 MPa, further increasing to 300 MPa before hitting a plateau, while 600 MPa at $\leq 45$ °C increased cook losses by 4 – 9% in beef pectoralis (Kim et al., 2007; McArdle et al., 2010; 2011). Likewise, 520 MPa at 10 °C increased cooking losses by 7-9% in beef biceps femoris and longissimus (Jung, Ghoul, & Lamballerie-Anton, 2000a).

Peak force during shearing was numerically lowest for F at 29.64 N, which was not significantly different ($P = 0.557$) from the HPLT beef, but was lower ($P = 0.047$) than the HPP samples at 38.80 N (Figure 5.1). Like cook yield, shear force values have not been reported for cooked beef, although cured, uncooked beef carpaccio was found to have similar shear force and work of shear values whether treated by high pressure at -30 °C (HPLT) or 20 °C (HPP; Vaudagna et al., 2012). It is generally accepted that pressurization of muscle foods pre-rigor has a tenderizing effect, but application post-rigor lacks any beneficial effect and often induces toughness to some degree (Sun & Holley, 2010). Jung et al. (2000a) observed
Figure 5.1 Cook yield, peak shear force and moisture content following cooking of beef semitendinosus steaks subjected to freezing (F; -25 °C), high pressure low temperature treatment (HPLT; <-30 °C/550 MPa/4 min) or high pressure processing (HPP; 3 °C/550 MPa/4 min)

increased shear force values after cooking of *biceps femoris* and *longissimus* submitted to 520 MPa, especially after sufficient aging time. Hardness of meat increased after pressure application at 200 MPa and 20 °C for 20 min (Ma & Ledward, 2004). The application of 600 MPa to beef *pectoralis* caused only a numerical non-significant increase in cooked shear force when applied at 35 °C or greater (McArdle et al., 2011). The similarity between HPP and HPLT shear force values seen here may have been related to their low cook yields compared to the control, although only the former’s moisture after cooking was significantly lower ($P = 0.002$ vs 0.212) than F (Figure 5.1). In high pressure treated meat, extracellular
channel expansion has been observed by cross-sectional electron microscopy (Duranton, Simonin, Chéret, Guillou, & Lamballerie, 2012; Souza et al., 2011). In extracellular spaces water is loosely structured and weakly retained, especially when subjected to the heat of cooking; as the fluid discharges the muscle fibers become more compact, tighter bound and more resistant to mechanical forces such as chewing or shearing (Offer & Trinick, 1988). Although this effect would not be expected in HPLT due to the lack of protein denaturation, the retraction and expansion of ice during form transitions may be able to cause mechanical damage to muscle proteins in a similar fashion to microorganisms.

5.4.2.3 Protein characteristics

Protein solubility decreased \( P < 0.05 \) upon HPP treatment by 51.95 mg/g for total soluble protein and 13.36 mg/g for water soluble protein compared to the frozen control (Figure 5.2). Low temperature pressurization did not alter \( P > 0.23 \) protein solubility compared to freezing. The reactive/total sulfhydryl ratio of total soluble protein was not changed \( P = 0.242 \) by HPLT application, but was significantly greater \( P < 0.016 \) following HPP (Figure 5.3). The reactive/total SH ratio of the water soluble fraction was not affected by treatment \( P = 0.284; \) data not shown in tabular form). Solubility of beef protein in high (0.6 M) and low (0.05 M) ionic strength buffers was decreased following 303 MPa pressure application for 1 min (Lowder & Mireles DeWitt, 2013). Extraction of sarcoplasmic proteins (water soluble) was decreased in a stepwise fashion upon application of 200 or 400 MPa for 20 min (Marcos et al., 2010). Decreased protein recovery following homogenization and
Figure 5.2  Protein solubility of beef semitendinosus subjected to freezing (F; -25 °C), high pressure low temperature treatment (HPLT; <-30 °C/550 MPa/4 min) or high pressure processing (HPP; 3 °C/550 MPa/4 min) following extraction in high (total soluble; 0.6 M) and low (water soluble; 0.05 M) ionic strength buffer solutions centrifugation is typically a sign of denaturation, specifically an unfolding and exposure of hydrophobic amino acid side groups. Increased surface hydrophobicity is an established effect of pressurization on myofibrillar proteins (Chapleau, Delepine, & Lamballerie-Anton, 2002). The increased exposure of hydrophobic groups encourages proteins to aggregate, which limits the function of myofibrillar and sarcoplastic proteins (Joo, Kauffman, Kim, & Park, 1999). The unfolding induced
Figure 5.3 Reactive/total sulfhydryl (SH) ratio of beef semitendinosus subjected to freezing (F; -25 °C), high pressure low temperature treatment (HPLT; <-30 °C/550 MPa/4 min) or high pressure processing (HPP; 3 °C/550 MPa/4 min) following extraction in high (total soluble; 0.6 M) ionic strength buffer solution under high pressure is also responsible for the increased ratio of reactive SH groups seen in pressurized beef in this study. Similar results were observed by Chapleau et al. (2002) and Chapleau and Lamballerie-Anton (2003a) in myofibrillar suspensions. The additional exposed sulfhydryl groups may participate in cross-linking reactions which could directly decrease tenderness and contract intracellular spaces, increasing purge and cook losses. Because the highly abundant salt soluble protein myosin retains the bulk of its SH groups on its head (subfragment-1), which is largely
hydrophobic, its effect on altering the SH ratio is especially pronounced upon pressurization (Samejima, Ishioroshi, & Yasui, 1981). This explains why the total soluble fraction, which includes myosin, more readily experienced a SH ratio change upon pressurization. The lack of differences between the frozen control and HPLT treatment among any of the tested variables again supports the assertion that pressurization under frozen conditions prevents protein denaturation.

5.5 Conclusions

High pressure low temperature processing of uncooked beef under frozen conditions does not promote protein denaturation in the manner of conventional HPP treatments. This yields functional aspects of beef, such as color, purge and cooked tenderness that are similar to non-pressurized, frozen beef. While minimizing quality deterioration, prevention of protein denaturation most likely also limits HPLT’s ability to inactivate microorganisms, resulting in less than a 2 log reduction in the pathogenic organism *E. coli* O157:H7. Conventional high pressure processing caused extensive protein denaturation, promoting discoloration, reducing cook yields and decreasing tenderness upon cooking.
6. General Conclusions

Several potential strategies for reducing the sodium content and improving the safety of beef were examined in these studies. The use of response surface methodology allowed the fluid loss characteristics of beef strip loins and loin steaks to be modeled based on the levels of salt and sodium phosphates when brine injected at 10% of initial product weight. It was observed that a disproportionately high amount of fluid is lost in the first 30 minutes after injection compared to the following 4 days. The use of dehydrated collagen at 0.5% of initial product weight increases fluid retention as the sole ingredient in the brine and at low levels of salt and/or sodium phosphates. The use of high levels of salt and sodium phosphates with dehydrated collagen offers no further benefits with regards to fluid retention and yield. Dehydrated collagen a water binding adjunct offers superior performance to that of sodium phosphates for processors wanting to increase the yield of their products. Its lack of antioxidant capacity relative to sodium phosphates should be considered if total phosphate replacement is desired or the product is to be displayed aerobically in a retail setting.

The use of high hydrostatic pressure at mild intensity (150 – 300 MPa) does not offer any benefits to fluid retention in brine injected beef with or without sodium phosphates when ingredient levels are 0.4% of initial product weight or less. High pressure combined with low temperature (<-30 °C) preserves product quality characteristics, including color and tenderness, but the limited effect on pathogenic bacteria may constrain its viability for commercial scale use.
7. References


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