FUNCTIONAL PROPERTIES AND UTILIZATION OF HIGH pH BEEF

A Dissertation

by

LYDA GUADALUPE GARCIA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2009

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Jeffrey W. Savell
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ABSTRACT

Functional Properties and Utilization of High pH Beef.

(August 2009)

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Chair of Advisory Committee: Dr. Jeffrey W. Savell

Two Texas fed beef and cow/bull packing plants were surveyed for high pH beef carcasses as well as the evaluation of functional properties of high pH beef in whole muscle beef jerky, frankfurters, and snack stick production. An estimated 42% of cow carcasses were of 6.0 muscle pH or greater as well as exhibiting darker, less red colored lean. Jerky high in pH from cow inside rounds resulted in the greatest percent moisture and least percent protein compared to other treatments. High pH cooked jerky were higher in ($P < 0.05$) pH and water activity and lower in percent fat and shear force values. In cooked jerky slices, fed-high resulted in the highest percent MPR and cook yield, but was the most tender. In contrast, fed-normal resulted in the least water activity, MPR and toughest jerky slices. In frankfurter production, emulsion stability and hydration values was highest for C (1.98mL) followed by 100H (3.37mL) that decreased as percent high pH decreased. As storage day increased, frankfurter pH decreased, especially by day 56 (5.67) where LAB and APC counts had reached a log of 6.0 indicative of spoilage by day 28 and became lighter and less red in color with minimal lipid oxidation. 100H was harder and less cohesive with trained panelists reported containing at least 50% high pH meat was harder. The pH and internal color of cooked snack sticks significantly increased

and became lighter and redder as percent high pH meat increased. Even though water activity compared to a whole muscle dry product or an emulsified, water added product ranged from 0.85 to 0.86 ($P > 0.05$), minimal ($P > 0.05$) lipid oxidation occurred. 100N resulted in the least percentage of fat but 3% more ($P < 0.05$) protein and highest shear force values. Overall, beef raw materials high in pH may be better suited in a semi-dry fermented product.

DEDICATION

I dedicate this dissertation to all of my family who has supported me throughout my journey:

- To Tia Aida who always reminded me to always do what was right and never look back.
- To Tio Marcos who reinforced my inner strength and helped me put things in perspective.
- To mom and dad who were always there to pick up the phone to listen and guide me through every step of the way and reminded me to always keep God close. No matter how bad it was, both of you were right behind me pushing me forward.
- To my sister and brother-in-law, Stephen. Thank you for keeping me calm and helping me think things through. Without you guys I am not sure how I would have kept my sanity.
- To my nephews, Trevor Dillon and Ethan Jordan Young who always reminded me that their Tia Lyda can conquer all.

ACKNOWLEDGEMENTS

A project of this magnitude represents the combined efforts of many individuals. I thank my advisor Dr. Jeffrey Savell for his guidance and knowledge he has provided me. I would like to express my sincere appreciation to Dr. Dan Hale for his patience, guidance, friendship, but most importantly his positive attitude. Also, Dr. Wes Osburn who took me under his wing and challenged me in every which way. Thanks also to Dr. Joe Townsend who kept me positive and who supported me throughout my journey.

I would particularly like to thank the graduate students at Texas A&M University who made the impossible happen when I was in a jam. Justice would not be served if I did not mention my fellow graduate students: Diana Huerta, Danielle Espitia, Ashley Haneklaus, James Dillon, Jarrett Hudek, Tiffany Muras, Melissa Davidson, Dawna Winkler, Amanda King, Will Wiederhold, Laura May, Scott Langley, Robert Merrill, and Daniel Genho. You guys made my time in Aggieland worth while. I would especially like to thank Andy King, Kristin V. Nicholson, Brad Kim, and Austin Lowder. I especially want to say thank you to Brad Kim for continuously trying to keep me positive and tapping my shoulder reminding me that the world was not ending.

I thank my 2007 Fighting Texas Aggie Meats Judging team. Coaching you guys was one of the best things I could have ever done. Each and everyone one of you taught me something about life, but most importantly, you taught me things about myself. I will forever hold each and every one of you close to me. I can only hope you learned from me as much as I learned from you.

Finally, thanks to my family who was always willing to lend a shoulder, share a smile, and check up on me. I love you all with all my heart.

TABLE OF CONTENTS

		Page
ABSTRACT		iii
DEDICATION		v
ACKNOWLEDGEMENTS		vi
TABLE OF CONTENTS		vii
LIST OF FIGURES		xii
LIST OF TABLES		xiii
CHAPTER		
I	INTRODUCTION	1
II	LITERATURE REVIEW	3
	2.1. Conversion of muscle to meat	3
	2.1.1. Glycolysis	3
	2.1.1.1. Aerobic glycolysis	4
	2.1.1.2. Anaerobic glycolysis	5
	2.2. Quality defects	6
	2.2.1. Pale, soft, and exudative	6
	2.2.2. Dark, firm, and dry	8
	2.3. Impact on meat quality	10
	2.3.1. pH	11
	2.3.2. Muscle color	11
	2.3.3. Water-holding capacity	14
	2.4. Nonconforming meat	16
	2.4.1. Factors affecting muscle quality	16
	2.4.1.1. Stress effects	17
	2.4.1.2. Evaluating feeding regime on meat color	18
	2.4.1.3. Advanced maturity	19
	2.4.1.4. Cold shortening	20
	2.4.1.5. Electrical stimulation	21
	2.5. Meat processing	22
	2.5.1. Functional properties of raw materials	22
	2.5.1.1. Protein solubility	23
	2.5.1.2. Factors impacting protein solubility	24

CHAPTER Page

2.5.1.3. Water-holding capacity 24
2.5.1.4. Factors impacting water-holding capacity. 25
2.5.2. Protein gelation 25
2.5.2.1. Factors impacting protein gelation 26
2.5.3. Functional properties of nonconforming raw materials 27
2.5.3.1. Pale, soft, and exudative 27
2.5.3.2. Dark, firm, and dry 28
2.5.4. Use of nonmeat ingredients to improve the functional properties of nonconforming raw materials 28
2.5.4.1. Sodium chloride 28
2.5.4.2. Phosphates 30
2.5.4.3. Nitrite 32
2.5.4.4. Starter cultures 33
2.6. Manufacturing of products with nonconforming meat 35
2.6.1. Beef jerky 36
2.6.2. Beef snack sticks 37
2.6.3. Challenges 38
2.6.3.1. Beef jerky 38
2.6.3.2. Beef snack sticks 38
2.6.3.3. *Listeria monocytogenes* 38
2.6.3.4. *Escherichia coli 0157:H7* 40
2.6.3.5. *Staphylcoccus aureus* 41
2.6.4. Opportunities 43
2.6.5. Beef frankfurters 43
2.6.6. Challenges 43
2.6.7. Opportunities 44

III A SURVEY OF NORMAL AND HIGH pH BEEF CARCASSES IN FED BEEF AND COW CARCASSES IN TEXAS 45

3.1. Introduction 45
3.2. Materials and methods 47
3.2.1. Statistical analysis 48
3.3. Results and discussion 48
3.3.1. Arithmetic means 50
3.3.2. Correlations 52
3.3.3. Muscle pH 55
3.3.4. Hunter mini-scan (color space values) 56
3.3.5. RMS beef cam 60

IV EVALUATION OF FUNCTIONAL PROPERTIES IN NORMAL AND HIGH pH INSIDE ROUNDS FROM FED AND COW BEEF FOR JERKY PRODUCTION 63

CHAPTER Page

4.1 Introduction 63
4.2. Materials and methods.................... 65
4.2.1. Jerky manufacturing.................... 66
4.2.2. Sample preparation 68
4.2.3. pH.................... 68
4.2.4. Water activity 70
4.2.5. Color 70
4.2.6. Percent moisture and fat.................... 70
4.2.7. Percent protein 71
4.2.8. Moisture:protein ratio 71
4.2.9. Cook yield.................... 71
4.2.10. Warner Bratzler shear force determination.................... 71
4.2.11. Targeted processing parameters.................... 72
4.2.12. Statistical analysis.................... 72
4.3. Results and discussion.................... 72
4.3.1. Raw beef jerky slices.................... 72
4.3.2. Cooked beef jerky slices.................... 74

V EVALUATION OF FRANKFURTERS MANUFACTURED FROM HIGH AND NORMAL pH BEEF.................... 79

5.1. Introduction 79
5.2. Materials and methods.................... 82
5.2.1. Frankfurter manufacture.................... 82
5.2.2. Analysis of raw emulsion - day 0.................... 83
5.2.2.1. Emulsion stability 83
5.2.2.2. Hydration 84
5.2.2.3. pH 84
5.2.2.4. Percent moisture and fat. 84
5.2.2.5. Percent protein 86
5.2.3. Analysis of cooked frankfurters – days 0, 7, 14, 28, and 56.................... 87
5.2.3.1. pH. 87
5.2.3.2. Color 87
5.2.3.3. Purge.................... 87
5.2.3.4. Chemical analysis.................... 87
5.2.3.5. Lipid oxidation.................... 87
5.2.3.6. Sodium analysis.................... 88
5.2.3.7. Nitrite analysis.................... 89
5.2.3.8. Microbiological analysis.................... 89
5.2.4. Sensory and textural analysis – day 28.................... 90
5.2.4.1. Torsion analysis.................... 90
5.2.4.2. Textural profile analysis (TPA) 90

CHAPTER Page

5.2.4.3. Sensory analysis 91
5.2.5. Statistical analysis 91
5.3. Results and discussion 92
5.3.1. Raw emulsion analysis - day 0 92
5.3.1.1. Raw batter pH, hydration, emulsion stability, and proximate analysis 92
5.3.2. Frankfurter analysis – days 0, 7, 14, 28, and 56 93
5.3.2.1. Proximate analysis, purge, sodium, and cook yield 93
5.3.2.2. Frankfurter pH, color, nitrite, lipid oxidation, and aerobic plate count 94
5.3.2.3. Lactic acid bacteria 94
5.3.2.4. Analysis of textural and sensory attributes 98

VI FUNCTIONAL PROPERTIES OF BEEF SNACK STICKS MANUFACTURED WITH VARIOUS LEVELS OF HIGH pH COW MEAT 102

6.1. Introduction 102
6.2. Materials and methods 104
6.2.1. Snack stick manufacture 104
6.2.2.Analysis of raw meat mixer – day 0 107
6.2.2.1. pH 107
6.2.2.2. Percent moisture and fat 108
6.2.2.3. Percent protein 108
6.2.2.4. Water activity 108
6.2.3. Analysis of cooked snack sticks – day 0, 7, 14, 28, and 56 108
6.2.3.1. pH 108
6.2.3.2. Color 108
6.2.3.3. Purge 109
6.2.3.4. Chemical analysis 109
6.2.3.5. Moisture:protein ratio 109
6.2.3.6. Lipid oxidation 109
6.2.3.7. Sodium analysis 110
6.2.3.8. Nitrite analysis 110
6.2.3.9. Microbiological anlaysis 111
6.2.4. Allo-Kramer shear force and sensory analyses for snack sticks - day 28 112
6.2.4.1. Allo-Kramer shear force determination 112
6.2.4.2. Sensory analysis 112
6.2.5. Statistical analysis 113
6.3. Results and discussion 113
6.3.1. Raw meat mixture analysis - day 0 113

CHAPTER Page

6.3.2. Snack stick analyses - days 0, 7, 14, 28, and 56 114

VII CONCLUSIONS 120

7.1. Survey 120
7.2. Beef jerky 120
7.3. Beef frankfurters 121
7.4. Beef snack sticks 122
7.5. Overall conclusions 123

REFERENCES 124

APPENDICES 138

VITA 169

LIST OF FIGURES

FIGURE Page

1 Frequency diagram of pH values observed in the *M. longissimus lumborum* of fed beef and cull cow carcasses from fed beef and cull cow plants in Texas 56

2 Frequency distribution of L* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas 57

3 Frequency distribution of L* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of cow carcasses from two cow plants in Texas 57

4 Frequency distribution of a* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas 58

5 Frequency distribution of a* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of cow carcasses from two cow plants in Texas 58

6 Frequency distribution of b* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas 59

7 Frequency distribution of b* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of cow carcasses from two cow plants in Texas 59

8 Frequency distribution of L* color space values using the automated system (Beef Cam) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas 60

9 Frequency distribution of a* color space values using the automated system (Beef Cam) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas 62

10 Frequency distribution of b* color space values using the automated system (Beef Cam) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas 62

LIST OF TABLES

TABLE Page

1 Arithmetic means of fed beef and cow carcasses selected randomly in Texas 49

2 Arithmetic means of fed beef carcasses selected randomly in Texas 50

3 Arithmetic means of cow carcasses selected randomly in Texas 52

4 Pearson correlation coefficients (P values) for pH and color space values using the Hunter miniscan (D65 illuminant) of fed beef carcasses (Plant 1 – Fed) 53

5 Pearson correlation coefficients (P values) for pH and color space values using the Hunter miniscan (A°10 illuminant) of fed beef carcasses (Plant 2 – Fed) 53

6 Pearson correlation coefficients (P values) for pH and color space values using the Hunter miniscan (D65 illuminant) of cow carcasses (Plant 3 –Cow)........ 54

7 Pearson correlation coefficients (P values) for pH and color space values using the Hunter miniscan (A°10 illuminant) of cow carcasses (Plant 4 – Cow) 54

8 Pearson correlation coefficients (P values) for pH and color space values using an automated camera system (RMS Beef Cam) of fed beef carcasses 55

9 LSMeans (SEM) for pH by plant 55

10 Commercial brine formulation used for the manufacture of jerky from inside rounds obtained from fed and cow, normal and high pH carcasses. 67

11 Thermal processing schedule used for the manufacture of jerky from inside rounds obtained from fed and cow, normal and high pH carcasses........ 69

12 Main effects of carcass class (Fed and Cow) and for pH type (Normal and High) for pH and fat of raw beef jerk slices 73

13 LSMeans of carcass class (Fed and Cow) and pH type (Normal and High) for moisture and protein of raw beef jerky 74

14 Main effect means of carcass class (Fed and Cow) for pH type (Normal and High) for pH, water activity, percent fat, redness, and shear force

TABLE Page

values of cooked beef jerky. 74

15 Two-way of carcass class (Fed and Cow) and pH type (Normal and High) for moisture, protein, moisture:protein ratio, cook yield, yellowness (b*), and thickness values of cooked beef jerky.. 77

16 LSMeans (SEM) and main contrasts for pH, Aw, proximate composition moisture:protein ratio, and color space values from high (6.0-6.4) and..... extremely high (6.5) inside rounds 78

17 Frankfurter formulations (kilogram and percent basis) for a 14.97 kg batch weight at a 10% fat level... 85

18 Thermal processing schedule used for manufacture of beef frankfurters... 86

19 LSMeans for the main effect of treatment on raw emulsion pH, hydration, emulsion stability, and proximate composition 95

20 LSMeans for the main effect of treatment for proximate composition, purge, sodium content, and cook yield of frankfurters containing varying levels of high pH trimmings 96

21 LSMeans for the main effect of storage day for pH, external and internal color space values, nitrite, lipid oxidation, and aerobic plate counts of frankfurters containing varying levels of high pH trimmings 97

22 Two-way interaction LSMeans (treatment x storage) for frankfurters of lactic acid bacteria (log/CFU) growth and storage day 97

23 LSMeans for the main effect of treatment for stress, strain, and texture of frankfurters containing varying levels of high pH trimmings................ 100

24 LSMeans for sensory attributes for beef frankfurters by treatment........... 101

25 Beef snack stick formulations (kilogram and percent basis) for an 11.98 kg batch weight at a 10% fat level. 106

26 Thermal processing schedule used for manufacture of beef snack sticks .. 107

27 LSMeans for the main effect of treatment for raw pH, water activity, and proximate composition of snack sticks containing varying levels of high pH trimmings 114

TABLE Page

28 LSMeans for the main effect of treatment for pH, water activity, color space values (L*, a*, b*), lipid oxidation (TBARS), proximate analysis, purge, and allo-kramer shears of snack sticks containing various levels of high pH trimmings 115

29 LSMeans for sensory attributes for beef snack sticks by treatment........... 119

CHAPTER I

INTRODUCTION

The beef industry relies heavily on producing a beef product that is superior in palatability and quality (Wulf, O'Connor, Tatum, & Smith, 1997). The physical state of an animal, at time of slaughter, plays a significant role on beef quality as the muscle undergoes a series of mechanical and chemical events. Ultimate pH and overall maturity in beef are known to affect palatability and overall appearance of end products.

Normal pH from fed beef (pH between 5.4 and 5.7) results in good chilling rates, a bright, cherry red lean color, and good flavor (Gill and Newton, 1981). Carcasses harvested at advanced maturity, such as non-fed beef (cows), are not comparable in these traits to young beef carcasses even at a normal pH. Muscles from cow carcasses are more distinct than muscles from young beef carcasses primarily due to greater concentrations of myoglobin (Mb).

Cows that are generally grass fed, do not incorporate much carbohydrates in their diet. It is these carbohydrates that help produce glycogen in the muscle. Cow beef tend to have a higher prevalence of resulting in muscle high in pH, compared to young beef cattle, because they contain a lower level of glycogen (at slaughter) due to feeding regime.

The onset of dark cutting beef results from muscle glycogen depletion at time of slaughter due to long-term stress. Low levels of glycogen produce low levels of lactic acid, which result in an overall pH closer to a neutral pH (7.0). Hence, higher pH (> 6.0) meat results in a darker red lean color and exhibits higher water-holding capacity, both of

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This dissertation follows the style of *Meat Science.*

which could be advantageous to processors of value added meat products.

Today, beef is used in a variety of further processed meat products to meet consumer demand (for convenience, palatability and appearance). As we continue to increase the quantity of enhanced and further processed beef products, muscle pH and its affects on functional properties of beef such as water-holding-capacity, protein extraction and gelation have become an important consideration in maximizing consumer satisfaction.

In the raw state of beef, functional properties affecting meat quality include water-holding capacity, protein extractability and solubility; all impact the manufacturing quality in meat. Raw material selection may have a direct impact on the ability of processors to achieve both targeted safety and product standards. In the case of dark cutting beef, water activity and pH are higher than normal, which can be a food safety concern in "shelf-stable" products.

In summary, these functional properties impact the suitability of beef muscles for processing applications in "shelf stable" products. Hence, a greater understanding of functional properties in fed beef, cow beef and dark cutting beef should be further investigated.

CHAPTER II

LITERATURE REVIEW

Muscle is a highly well organized group of proteins that function in locomotion and thermoregulation. Muscle predominantly consists of highly specialized contractile cells that are either classified as skeletal muscle, cardiac muscle, or smooth muscle. Muscle undergoes glycolytic metabolism during movement (i.e, exercise) and/or contraction as well as during emotional stress. Antemortem stress has been linked to meat quality defects such as the dark, firm and dry (Apple et al., 1995) and pale, soft, and exudative (Rosenvold & Anderson, 2003). These defects are accredited to abnormal muscle pH decline caused by stress. Because meat producing animals are intentionally raised on the basis of providing food, it is important that marketed livestock are treated humanely and are provided with a well-balanced diet. Several factors can easily influence the production efficiency of lean muscle that will in turn influence food quality.

2.1. Conversion of muscle to meat

2.1.1. Glycolysis

Glycogen is an essential substrate in energy metabolism in living and postmortem muscle and is used as fuel in exercise skeletal muscle. Glycogen concentration, at time of slaughter, is one of the most important factors in ultimate muscle quality. McVeigh and Tarrant (1982) noted that skeletal muscle contains approximately 1.5-1.8% glycogen. Increased circulating epinephrine or strenuous muscular activity triggers the breakdown of glycogen. It is the last few days of a steer's physical life that affects tenderness, juiciness, and flavor, the consumer's biggest interest in meat.

Glycolysis involves the breakdown of glucose and other simple sugars producing energy for the body. "Glycolysis is regulated by insulin, calcium release, induced exercise, or epinephrine induced by stress and increased phosphorylase. However, insulin is the major factor responsible for regulating glycolysis" (Hocquette, Ortigues-Marty, Pethick, Herpin, & Fernandez, 1998). Glucose uptake, storage and oxidation is activated by insulin that then stimulates glucotransporter No. 4 (GLUT4), glycogen synthase, and pyruvate dehydrogenase (PDH) (Hocquette, et al., 1996). Glycogen can be mobilized either for glycolysis or for oxidative metabolism." (Hocquette et al., 1996). Glycogen is destined to undergo one of two pathways: 1) aerobic (presence of oxygen) metabolism or 2) anaerobic (without oxygen).

2.1.1.1. Aerobic glycolysis

In the living animal, glycolysis occurs under aerobic conditions providing a continuous supply of energy molecules, known as adenosine triphosphate (ATP). During glycolysis, these high-energy molecules are produced to transport chemical energy within cells for metabolism as part of aerobic and also for anaerobic respiration (Voet & Voet, 2004).

In the cytoplasm, under aerobic conditions, muscle glycogen is first broken down by the key regulatory enzyme *glycogen phosphoryalse.* Upon the phosphorylation of glycogen, glucose-1-phosphate is developed and then rapidly converts to glucose-6-phosphate (G-6-P) by *phosphoglucomutase* where it then undergoes a series of enzymatic conversions to produce pyruvic acid, the principle end product of glycolysis (Voet & Voet, 2004). Enzyme, phosphofructokinase (PFK) is the rate limiting step controlling glycolysis (Hocquette et al., 1998; Voet & Voet, 2004). Pyruvic acid then is converted to

acetyl-coenzyme A, where it passes into the mitochondria to begin the Kreb's cycle. The Kreb's cycle is responsible for converting carbohydrates, fats, and proteins into carbon dioxide and water to generate usable energy (Aberle, Forrest, Gerrard, & Mills, 2001). During the Kreb's Cycle, electron donors such as Nicotinamide Adenine Dinucleotide (NADH) and Flavin Adenine Dinucleotide (FADH) are produced to carry high energy electrons into the electron transport chain (Voet & Voet, 2004). The electron transport chain involves the transfer of H^+ ions across the mitochondrial cell membrane by way of utilizing electron donors and acceptors to produce ATP from ADP (Adenosine diphosphate) and Pi (inorganic phosphate). The ATP can be utilized by the muscle as energy with one molecule of glucose producing approximately 36 ATP. Unutilized ATP is redirected back to the liver to regenerate glucose back to the muscle tissue to provide glycogen availability for its next use.

2.1.1.2. Anaerobic glycolysis

During harvest, it is after exsanguination that blood circulation ceases and causes a shift from aerobic to anaerobic metabolism (Voet, Voet, & Pratt, 1999; Voet & Voet, 2004).

Under anaerobic conditions, glycogen is converted to lactic acid through the glycolytic pathway. During anaerobic glycolysis, hydrogen ions are produced causing lactic acid to accumulate in the muscle causing the muscle pH to decrease. When the glycogen stored in muscle has been depleted, muscle pH begins to deviate from physiological pH of 7.0 (Young, West, Hart, & van Otterdijk, 2004). Sellier and Monin (1994) reported that the glycolytic potential (GP) is defined as the sum of glycogen, glucose, glucose-6-phosphate, and lactate responsible for lactic acid production in

postmortem muscle. Once oxygen is depleted and the making of ATP has ceased, calcium ions in the sarcolpasmic reticulum, diffuse from a higher concentration area (terminal cisternae) to a lower concentrated area (sarcomere) binding with a regulatory protein, Troponin causing a cross-bridge to occur between contractile proteins, Myosin and Actin proteins that result in the actomyosin complex creating a permanent bond referred to as rigor mortis.

Hocquette et al. (1998) stated that glycogen levels that are too high or too low may decrease meat quality. A decrease in meat quality may result in quality defects known as pale, soft, and exudative that is caused by low pH meat and/or rapid pH decline or dark, firm, and dry caused by high pH meat caused by low levels of glycogen at slaughter.

2.2. Quality defects

Two most common quality defects (stress induced) resulting from postmortem glycogen metabolism are: Pale, Soft, and Exudative (PSE), and Dark, Firm, and Dry (DFD). Quality defects, such as PSE and DFD, represent lost revenue opportunities for the meat industry (Cannon et al., 1996).

2.2.1. Pale, soft, and exudative

Pale, soft, and exudative (PSE) is a quality defect commonly found in fresh pork muscle between 10%-40% (Cannon, 1996). PSE is characterized as having a pale or light color, soft in texture, and exudative (loses fluid from the muscle) that is commonly found in hogs because of their higher rates of post mortem glycolysis (Offer, 1991). During aerobic conditions, glycolysis occurs at a rapid pace. Postmortem muscle pH decline of PSE meat has been known to be three times faster than normal muscle (Penny, 1969).

Rapid pH decline, as well as elevated body temperatures, causes protein denaturation, particularly myosin, that results in the loss of free water from muscle (Bendall & Swatland, 1988). In 1996, quality defects were estimated to $10.10 per market hog slaughtered. However, of the $10.10, $0.35 made up for muscle and water-holding capacity issues associated with PSE and DFD muscle (Cannon et al., 1996). In 2002, A National Pork Quality Audit reported 15.5% of pork surveyed had a PSE characteristic that was costing the pork industry $0.90 per head. Moreover, in 2005, 3.34% of surveyed hogs from a similar study revealed PSE characteristics (National Pork Board, 2006).

Ultimate pH for the PSE condition is about 5.4 or lower (Camou & Sebranek, 1991) that results in the vicinity of muscle's isoelectric point. A major issue with muscle pH approaching the isoelectric point is the occurrence of protein to protein interaction versus protein to water interaction. According to Bertram, Kristensen, and Anderson (2004), myofibrillar protein reaches its isoelectric point around a pH of 5. "Muscle pH increasing or decreasing from 5 results in more negative or positive charges that causes an increased repulsion of the myofilaments." Protein denaturation is important in determining functional properties, textural strength, and cook loss in meat products. Functional properties include: water holding capacity, protein extraction and gelation.

Penny (1969) confirmed that protein denaturation is a determinant factor of water holding capacity (WHC) in muscle. In investigating pH at two different times: 90 min. and 24 h after death, it was concluded that the rate of pH decline (at 90 min.) was most critical in effecting WHC. Penny concluded that it was the high rate of pH falling and high body temperatures that created an ideal condition for protein denaturation. Loss of protein functionality due to protein denaturation is considered the primary factor of PSE

(Alvarado & McKee, 2007). Denatured protein, such as myosin and actomyosin, make it impossible to be extracted and utilized in a processed meat product. Pre-rigor muscle contains more free myosin compared to actomyosin from rigor meat. Extraction and solubilization of myosin contributes to favorable functional properties (Xiang & Kenney, 1999). It has been demonstrated that ionic strength also has an effect on myofibrillar swelling.

Bendall and Swatland (1988) explained that both pH and ionic strength affect WHC and water binding capacity (WBC) that is due to the effect of pH on the net charge of myofibrillar proteins that therefore affects the degree of electrostatic repulsion between myofilaments. Bertram et al. (2004) observed effects of different ionic strengths (0.29M, 0.41M, and 0.71M) and different levels of pH (5.4, 6.2, and 7.0). The authors found that there was no effect ($P < 0.05$) on water content with a pH of 5.4 and either of the ionic strengths (0.29M, 0.41M, and 0.71M). Water content seemed to be affected ($P < 0.05$) at a pH of 6.2 and 7.0 regardless of ionic strength. However, a more drastic effect was seen on water content when pH increased from 5.4 to 6.2 and an ionic strength (0.46M to 0.71M). The authors theorized that this may be due to myofibrillar swelling which may be restricted due to structural constraints, is eliminated at ionic strengths between 0.29M and 0.46M. Also, myofibrils may have a swelling saturation level that may be reached when ionic strengths of 0.46M and 0.71M are in effect.

2.2.2. Dark, firm, and dry

Dark, Firm, and Dry (DFD) muscle refers to a condition in which the muscle appears dark in color, firm in texture, and dry, also known as dark cutting. In sufficient amounts of glycogen storage at times at harvest indicates that reduced glycogen will not

produce sufficient amounts of lactic acid that is required to reduce muscle pH, which therefore, muscle pH remains closer to physiological levels (pH 7.0). Because the pH does not fall below 6.0, the meat possesses a dark red color, that is classified as dark, firm and dry (DFD). Because the pH has not declined enough, water holding capacity is greater than normal pH meat. Insufficient amounts of glycogen may be due to stress (whether physical, mental or a combination of the two). It is recommended that an effort be made in securing high glycogen stores in animals prior to slaughter (Immonen et al., 2000). Grandin (1992) reported that DFD beef is most prevalent during cold times of the year coupled with precipitation and also during extreme warm temperatures over short periods of time (Grandin, 1992; Scanga, Belk, Tatum, Grandin, & Smith, 1998). In addition, it is advisable to avoid unfamiliar environments, close confinement, extreme temperatures, and reduction in noise iss also recommended (Warris, 1990).

When beef carcasses are graded, specifically for quality, trained USDA personnel evaluate muscle color as it relates to skeletal maturity and muscle pH (USDA, 1997). Dark cutting carcasses are classified as either 1/3, 1/2, 2/3 or full dark cutter. Carcasses classified as a full dark cutter are discounted a full quality grade because of its low consumer appeal due to its dark colored lean (Hedrick, 1965). It has been suggested that physical stress and emotional stress combined may be the main factor responsible for glycogen depletion (Tarrant, 1989; Sanz, verde, Sáenz, & San do, 1996). In a study by Lacourt and Tarrant (1985) using young bulls subjected to mix penning, authors found that glycogenolysis was increased in cattle due to dynamic muscle contraction. Davey and Gilbert (1976) reported that about 100 mmol/kg of lactate is produced in normal muscle pH (5.5), whereas about 40 mmol/kg would be expected in DFD.

Meat high in pH would be "better suited to manufacturing purposes," but would still be worth less in dollar values than normal pH meat (Young, West, Hart, & van Otterdijk, 2004). In 2000, the Agricultural Marketing Service (AMS) reported a discount for dark cutting carcasses was $30.00 per 45 kg of carcass weight (NBQA, 2000). Specifically, the week of April 6, 2009, the USDA Market News Service reported, on the average slaughter cattle were discounted $29.72 / 45 kg with a range of $15.00 to $55.00. Of the total beef carcasses surveyed in previous audits (NBQA, 1995 and 2005), only 2.7% were of dark cutting in 1995 (Smith, Savell, Dolezal, Field, Gill, Griffin, Hale, Morgan, Northcutt, & Tatum, 1995) whereas only 1.9% was found in 2005 (Garcia, Nicholson, Hoffman, Lawrence, Hale, Griffin, Savell, VanOverbeke, Morgan, Belk, Field, Scanga, Tatum, & Smith). Both quality audits (1995 and 2005) reported 95% and 97% of total fed beef carcasses surveyed were of A maturity (30 months of age or younger).

2.3. Impact on meat quality

Postmortem glycolysis and muscle pH decline, under normal carcass chilling ceases when one of two events occur: 1) "muscle glycogen stores are depleted or 2) muscle pH declines to approximately 5.4 inhibiting the activity of glycolytic enzymes" (Wulf, Emnet, Leheska, & Moeller, 2002). Low levels of glycogen at the time of harvest can be detrimental to the overall end product of a carcass in regards to muscle quality. A primary factor involved in the amount of glycogen at time of harvest is stress. Long and short term stress plays a critical role on the quality of the lean from a beef carcass. Muscle pH can greatly affect muscle color and water holding capacity that overall determines meat quality.

2.3.1. pH

Measuring muscle pH determines its acidity or alkalinity. The pH of meat product is "the negative logarithm of the hydrogen ion concentration and is indicative of the acid concentration" of the product (FSIS, 2005). The acidity or alkalinity is determined using a scale from 0 (acidic conditions) to 14 (basic conditions), with the pH of living muscle at 7.0 (neutral) (Voet & Voet, 2004). Meat quality is greatly influenced by muscle pH and it is strongly affected by pre-slaughter conditions such as long and short term stress imposed on the animal. With short-term stress, muscle pH becomes more acidic due to the higher concentration of hydrogen ions. In contrast, long-term stress results in products more basic due to the lack of hydrogen ions. It is not uncommon for the meat industry to measure muscle pH in carcasses as a way of estimating meat quality. Kivarki (1996) reported because one molecule of glucose yields two molecules of lactic acid, 43 mmol/kg of expressed glucose is required to drop muscle pH from 7.0 to 5.5. Whether muscle pH rapidly declines or not, muscle color is negatively affected.

2.3.2. Muscle color

Changes in meat color from normal pose a risk of consumer dissatisfaction. Kropf (1980) reported that color was a critical factor on consumer purchasing decisions. Meat (lean) color is attributed by a globular protein known as Myoglobin (Mb) found in all muscles of vertebrate organisms which is needed to store and facilitate oxygen diffusion in rapidly contracting muscle (Renerre, 1990; Voet & Voet, 2004).

Myoglobin contains 153 amino acids in a single polypeptide chain and a single iron protoporphyrin, or heme group (Voet & Voet, 1999). "The Mb contains a heme portion consisting of iron (Fe) in a tetrapyrollic ring structure of which four of the six

binding sites are bound by the heme group" (Renerre, 1990). The fifth ligand of the heme is a histidine amino acid with the remaining sixth binding site having the ability to bind to "high-field ligands" that includes oxygen (Hood, 1984; Voet & Voet, 2004).

Lean color is dependent on two factors: 1) The chemical state of iron and 2) the molecule bound to the sixth binding site. Iron can be found in two states: 1) Ferrous (Fe^{++}) or 2) Ferric (Fe^{+++}). Ferrous state of iron is the non-oxidized form whereas as ferric is oxidized containing one more positive electron. Muscle color can be attributed to the concentration of myoglobin. Pearson and Young (1989) noted that greater amounts of myoglobin in different muscle fiber types that has been associated with increased oxidative metabolism and a greater supply of blood to the muscle fibers. Myoglobin has three forms: deoxymyoglobin (absence of oxygen; purplish-red; Fe^{++}), oxymyoglobin (presence of oxygen; cherry red), and metmyoglobin (oxidized iron, $Fe^{+++,}$ brownish-red).

Lawrie (1958) explained that cytochrome oxidase was probably the main enzyme responsible for oxygen uptake in meat that is pH dependent. If the ultimate pH is high, Lawrie (1958) theorized that the rate of oxygen would considerably rise and would contribute to a dark color. Protein under high ultimate pH is "associated with more water and there is a consequent diminution in the fluid phase of the muscle." (Lawrie, 1958). This leads to a more "close" structure in muscle that more than likely lowers the inward diffusion of oxygen to the intracellular proteins that imposes an additional limit on oxymyoglobin formation on the surface of muscle (Lawrie, 1958). Lawrie (1958) noted that Bate-Smith (1948) previously explained that lean surface from muscle high in pH will scatter light less than normal beef in which muscle fibers will have shrunken apart,

whereas in high pH, the muscle fibers will be swollen and tightly packed together. Additionally, Hunt, Sørheim and Slinde (1999) theorized that because dark cutting beef contains a higher amount of water, myoglobin may become centralized making Mb denser that would in turn have an effect on degree of doneness. Myoglobin begins to denature when exposed to "heat between 55°C (131°F) and 65°C (149°F) in meat, with most denaturation occurring between 75°C (167°F) and 80°C (176°F)" (Hunt et al.,1999). Muscle high in pH has been attributed to persistent pinking in beef patties, which inhibits myoglobin denaturation (Berry, 1998). Berry (1998) investigated how the color of beef patties influenced by formulation and final internal temperature, and found that high pH (> 6.2) beef internally produced more red color. Also, high pH beef patties had the highest saturation index and CIE a* values (at 71.1°C), but still required the longest cooking time to reach final internal temperatures.

Research in evaluating lean color has been used to predict quality factors in meat using a colorimeter. A colorimeter is "an instrument that provides measurement that correlates with the human eye-brain perception. It compares similar colors and adjustments of small color differences under constant conditions" (Hunterlab, 2008). In 1999, Wulf and Wise showed the benefits of utilizing a colorimeter, specifically a Minolta Chromameter CIE LAB color space, on beef longissimus muscle and its ability to predict lean maturity and muscle pH. Wulf and Wise (1999) found that bloom time, which is the time of development of the bright red color (conversion of deoxymyoglobin to oxymyoglobin) on the surface of meat cuts when exposed to oxygen (Aberle et al., 2001), had a lesser effect on L* (lightness) values obtained following 30 minutes after ribbing ($P < 0.05$). Redness (a*) and yellowness (b*) values continued to change after 75

min of bloom time. In addition, one day's postmortem did not affect colorimeter readings ($P > 0.05$) on lean color. Authors (Wulf and Wise, 1999) also found that colorimeter readings were not affected by position of measurement (ribeye versus loineye and medial versus lateral) ($P > 0.05$). In 1993, Hoffman-LaRoche Inc. noted that 4-5 percent of wholesale price was lost value in beef primarily due to meat discoloration.

2.3.3. Water-holding capacity

The properties of muscle (water holding capacity, protein extraction and gelation) determine how useful a meat product will be during fabrication, processing, and cooking. Water binding capacity (WBC) is the ability of muscle proteins to retain water from external forces, such as injection or pumping (Aberle et al., 2001). The ability of muscle tissue binding to water during pre-rigor and post- rigor is known as water-holding capacity (WHC) (Aberle et al., 2001). Water-holding capacity determines how much water will be lost during storage and handling of product. Water lost during storage and handling is known as purge (NPPC, 2000). Skeletal muscle normally consists of 75% water, 20% protein, approximately 3% fat, and about 2% ash (Aberle et al., 2001). Water present in muscle tissue can be classified as bound, immobilized or free. Bound water (0.8 – 2.0%) is tightly held (postmortem) to muscle protein molecules. In layer form, bound water sits closest to muscle proteins followed by "immobilized" water that makes up four to twelve percent. Sixty to seventy percent of remaining water classified as free water is held by capillary forces (Aberle et al., 2001). Wierbicki and Deatherage (1958) stated that binding of water to proteins is related to the polar hydrophilic groups and the capability of the proteins to retain water depends on the number of polar groups. Of the three types of water, free water shares the weakest association with proteins. This results

in the first to be lost during storage, cooking, mishandling, etc. Muscle proteins carry a charge that either bonds or repels added electrons/protons. Offer and Trinick (1983) noted that water lost or gained is due to the shrinking and swelling of myofibrils causes by the filament lattice expanding or shrinking of the filament lattice.

In the case of PSE, water is unable to bind to proteins because of the denaturation caused by acidic conditions during glycolysis (under anaerobic conditions) followed by increased body temperature antemortem. Denatured proteins negatively affect meat color (pinkish-red to pale), texture (firm to soft) and low water holding capacity. Water loss from meat products is termed as either drip loss, expressible water, cook loss, or cooling loss (Bond, Can, and Warner, 2004). According to Bond et al. (2004), terminology used to describe the type of water loss is dependent upon the stage of processing at which water loss is measured. Cooked PSE meat naturally has a low water holding capacity. PSE has shown to be less tender when compared to normal lean. Dildey, Aberle, Forrest, and Judge (1976) found PSE pork had higher shear force values ($P < 0.05$) and lower myoglobin concentrations (3.22 mg/g) than normal (3.48mg/g). Shear values continued to increase for PSE pork than in normal pH pork. The quality defect, PSE, is of most concern because of its low consumer appeal and low water holding capacity.

Muscle proteins of high pH meat posses a net negative charge that causes repulsion between myofilaments thereby increasing filament spacing. This increased inner spacing is due to an increase of OH ions and a higher muscle pH due to depleted glycogen stores resulting in minimal production of lactic acid. A correlation exists between pH and water holding capacity. As pH increases or decreases from the isoelectric point, water holding capacity increases. Muscles high in pH tend to retain

more water due to a higher number of hydrogen bound to the oxygen molecule in water. Final sensory attributes of cooked meat can be contributed to post-mortem meat quality. Many factors play a role in meat palatability. For instance, some factors would include quality of raw materials, heat treatment and cooking (Risvik, 1994). Studies have investigated the effects of meat quality defects such as PSE and DFD on flavor and juiciness.

In 1960, Purchas correlated sarcomere length to tenderness and found that a negative correlation existed (Warner-Bratzler Shears) up to a pH of 6.0. This lead to Tornberg (1996) who reported that PSE meat was significantly less tender than DFD due to large variations in sarcomere lengths caused by reduced shortening (due to protein denaturation of sarcoplasmic proteins during rigor).

2.4. Nonconforming meat

Because pH has such an influence on meat quality and it functional properties, the overall product may be difficult to utilize and may not conform to specific requirements. A product not in agreement with requirements or meeting product specifications is known as nonconforming product. Some factors affecting muscle quality include: stress, feeding regime, age, cold shortening and electrical stimulation.

2.4.1. Factors affecting muscle quality

An animal removed from its comfort zone and placed in a loud, crowded, unfamiliar environment will tend to stress and increase its heart rate, which will increase the breakdown of glycogen. Stress is a common factor that affects meat quality. Feeding regime as well as the age of an animal also has shown to influence meat quality which in

turn, affects the dollar value of the carcass. In beef cattle, stress is the major contributor to meat quality rather than exercise.

2.4.1.1. Stress effects

Stress and prolonged physical activity are the most common causes of variation in muscle color. Short term stressors can be in the form of: loud noises, unfamiliar environment, or fighting. These can be responsible for PSE. Long term stressors would include: sickness, dehydration, or malnourishment, responsible for DFD.

Stress and prolonged physical activity have similar effects across species. Short term stress (measured in minutes) has more of an effect on pork and poultry while long term (measured in hours) stress primarily affects beef and lamb. The body itself, under normal conditions, is maintained via homeostasis (Aberle et al., 2001). Homeostasis is "the maintenance of a relatively constant internal physiological environment" (Aberle et al., 2001). "When a deviation occurs, such as stress, the body uses a system responsible for creating a response to these deviations known as the Autonomic Nervous System (ANS)" (Esler, Jennings, Korner, Willet, Dudley, Hasking, Anderson, & Lambert, 1988). The ANS has two components responsible for "response and recovery": sympathetic (mediated arousal and activation) and parasympathetic (responsible for relaxation) (Esler et al., 1988). These two systems work in balance of each other with the sympathetic nervous system responsible of speeding up heart rate whereas the parasympathetic slows it down. Immonen, Ruusunen, Hissa, and Puolanne (2000) noted that stress is the muscle glycogen's worst enemy. The level of stress has been known to have a major impact on lean color.

2.4.1.2. Evaluating feeding regime on meat color

Prior to slaughter, fed beef cattle (steers and heifers) spend their last 100 days in a feedlot where they are fed a high concentrated diet with the intent of depositing fat, specifically marbling, before harvest. Cows, in contrast, are primarily fed a forage diet the majority of their life. Studies have shown cattle fed a high concentrate diet tend to exhibit meat products that are more palatable (more flavorful, juicier, and more tender).

Craig, Blummer, Smart, and Wise (1966) evaluated thirty four Hereford steers assigned to either ladino clover-orchard grass pasture or fed a concentrated grain in a drylot. Authors (Craig et al., 1966) reported that myoglobin content of the *longissimus dorsi* muscle from pasture fed steers had slightly higher myoglobin content and were tougher than steers fed in a dry lot (3.25 mg/gm vs. 3.10 mg/gm). In another study, Bowling, Smith, Carpenter, Dutson, and Oliver (1977) reported forage grain cattle (n= 30) exhibited darker colored lean of the *longissumus muscle* compared to grain-finished beef (n = 30). Bowling et al. (1977) noted Cross and Smith (1976) and Schupp et al. (1976) suggested that "forage-finished cattle are more susceptible to pre-slaughter stress and to the dark cutting beef syndrome than grain-finished cattle." This may be due to grain finished cattle are more accustomed to the pressure and noise created by people in conjunction to a high concentrate diet, which results in these type of cattle being less susceptible to stress.

Priolo, Micola, and Agabriel (2001) examined data between 1977 and 2000 involving the effects of production systems (100% pasture versus concentrate diets) on meat color of the *longissimus dorsi* muscle. Muscles from pasture fed cattle exhibited a darker red color when compared to animals finished on concentrate. There was a 5%

decrease in lightness (L*) values for pasture fed cattle using objective measurements. Moreover, using subjective measures, pasture fed cattle were 10% lower in L* values. After compiling data from experiments conducted between 1977 and 2000, Priolo et al. (2001) concluded that meat color may also be influenced by factors such as ultimate pH, animal age, carcass weight and intramuscular fat.

2.4.1.3. Advanced maturity

Advanced skeletal cattle resulting in skeletal maturity of 42 months and older, such as cows are not as comparable to young (skeletal maturity of less than 30 months of age) fed beef carcasses. Cow muscles differ from young beef muscles primarily due to greater concentrations of muscle myoglobin, the muscle pigment responsible for meat color. As an animal advances in age, myoglobin concentrations increase because of its decreased affinity to bind with oxygen (Voet & Voet, 2004) thus a darker lean color is observed.

In addition to a darker lean, studies (Judge and Aberle, 1982; Shorthose & Harris, 1990; and Moon, Yang, Park, & Joo, 2006) have shown that meat tenderness is affected with increased chronological age. Goll, Bray, & Hoekstra (1963) noted that structural changes occur in collagen as age increases that in turn affect meat tenderness. Hill (1966) cited Harding (1965) who reported that the changes in collagen was due to the ester linking in collagen that take part in the intramolecular cross linking of the polypeptide chains. Hill (1966) investigated percent total collagen and collagen solubility in various ages of livestock. After 1 h of heating at 77°C, male calves ranging from eight to eighteen weeks of age resulted in the highest percentage total collagen (20.66% - 24.59%) followed by steers ranging from four to twenty two months of age

(8.47%-21.13%) second and finally cows who ranged from two and a half years of age to four and a half years of age with the least (3.71%-4.51%) total collagen. Hill (1966) also found that little variation existed in collagen solubility in muscles from different anatomical locations of the same carcass.

Tuma, Henrickson, Stephens and Moore (1962) evaluated tenderness in twenty four Hereford females at 18, 42, and 90 months of age. Authors found that tenderness significantly decreased with age in evaluating Warner-Bratzler Shear force 10.56kg, 18.18kg, and 19.09kg) and taste panel (6.50, 4.80, and 4.88). Tuma et al. (1962) found there was a greater difference in tenderness between the ages of 18 months and 42 months of age.

2.4.1.4. Cold shortening

During harvest, it is the packer's intent to chill the beef carcasses as soon as possible. There are two reasons for this: 1) to inhibit microbial growth and 2) slows down rigor mortis. Beef carcasses with less than two-tenths of external fat can result in a quality defect known as cold shortening. Cold-shortening occurs when lean carcasses are chilled before the onset of rigor mortis, which causes muscle fibers to shorten (contract) during this process (Aberle et al., 2001). A carcass chilled below 15°C (59°F) before rigor mortis sets in, causes severe muscle contraction to occur damaging the sarcoplasm reticulum (SR). A damaged SR is unable to regulate the influx of calcium which then triggers muscle contraction. Muscle fibers that have undergone cold-shortening tend to be tougher compared to muscle fibers that have not cold-shortened (Stiffler et al., 1983; Aberle et al., 2001). Marsh and Leet (1966) stated that the "ultimate structure of meat is determined partly by the rate and extent of the series of biochemical changes" muscle

undergoes on its way to rigor. Therefore, during rigor mortis contractile proteins, myosin and actin, bind resulting in actomyosin cross-bridges. Tenderness is a major concern in whole muscle because of the actomysoin cross-bridge. Thaw rigor also results from pre rigor meat exposed to low temperatures that then is thawed. Upon thawing, juices and calcium are released uncontrollably due severe contraction.

2.4.1.5. Electrical stimulation

Electrical stimulation (ES) has been used in the meat industry since in 1951, but was not utilized in the packing industry until 1976 (Stiffler, Savell, Smith, Dutson, & Carpenter, 1983). Electrical stimulation was first introduced in 1749 when Benjamin Franklin discovered that turkeys electrically killed resulted in more tender meat. The majority of research conducted involving ES was first conducted in Australia, New Zealand, Great Britain and the United States in 1976. Muscle electrically stimulated undergoes a series of biochemical, histological and physical events during postmortem chilling. The manipulation of these events has a profound influence on meat palatability. Three factors are hypothesized to be major contributors to meat tenderness: (1) prevention of cold shortening through accelerated glycolysis and onset of rigor mortis before cold shortening can occur; (2) accelerated proteolytic activity through enhanced calcium release; and (3) fiber structure disruption via extreme muscle contractions (Stiffler et al., 1983). Research has shown positive effects of ES in livestock involving accelerated pH decline, hastening of rigor development, and improving palatability, specifically tenderness.

Savell, Smith, and Carpenter (1978) showed that ES (Electrically Stimulated) exhibited an advantage in meat quality attributes compared to the NON-ES (Not

Electrically Stimulated) beef carcass sides. Beef carcass sides electrically stimulated resulted in a positive effect in lean color ($P < 0.01$) with beef steaks (*longissimus muscle*) being more tender ($P < 0.01$), with less organoleptically detectable connective tissue ($P < 0.05$ to $P < 0.01$) than the steaks from control sides. Additionally, ES steaks were more satisfactory in overall palatability. In a similar study regarding ES, Solomon and Lynch (1988) found that carcasses exposed to ES significantly ($P < 0.05$) exhibited a brighter cherry red colored lean that was more tender which was determined by a sensory panel and shear force values. Additionally, pH was significantly lower at 1 (6.47 vs. 6.67 pH), 3 (6.13 vs. 6.43 pH), 5 (6.02 vs. 6.29 pH), and 24 (5.72 vs. 5.81 pH) h postmortem compared to NON-ES.

Bendall, Ketteridge, and George (1976) reported a major advantage to electrical stimulation is it effect in hastening muscle pH to fall and loss of ATP, allowing rapid cooling or freezing to be carried out after slaughter avoiding cold shortening or thaw rigor from occurring.

2.5. Meat processing

Today, beef is used in a variety of further processed meat products to meet consumer demand (for convenience, palatability and appearance). As we continue to increase the quantity of enhanced and further processed beef products, functional properties of beef such as water holding capacity, protein gelation and emulsion have become an important consideration in maximizing consumer acceptability.

2.5.1. Functional properties of raw materials

Functional properties in meat refer to attributes that give meat products their characteristics. Quality defects that are pH induced, such as PSE and DFD, have a

significant effect on these functional properties that can result in food safety concerns. These quality defects can have an effect on protein extraction and solubility. In processed meats, protein solubility is the most critical factor in the overall binding ability of a product. Other factors affecting these functional properties are water-holding capacity and protein gelation.

2.5.1.1. Protein solubility

In processed meats, one of the most important factors in producing a high quality product is the raw material, in this case, skeletal muscle. Skeletal muscle can vary in level of fat, protein and moisture, which can either have a high, intermediate, or low binding ability. Raw materials of high binding ability are bull and cow meat because of its high lean and low connective tissue content; intermediate, beef cheek meat and beef briskets; and low, beef hanging tenders and tongue trimmings (Aberle et al., 2001). Binding ability involves extraction of salt soluble proteins with the intent for protein to protein interaction. Salt soluble proteins consist of myosin (making up 50%) and actin (making up 20%) that is found in the myofibrillar structure (Pearson & Young, 1989). Protein to protein interaction affects the products ability to hold more water and pieces together, which in turn has positive effects on texture and desired fat (Clause, Colby, & Flick, 1994). The solubility of a protein is based on the physiochemical state of the protein molecule that also plays a role on the effectiveness of protein extraction (Zayas, 1997).

Generally, skeletal muscle must first be comminuted (reduced in size) by way of a grinder or bowl chopper to assist in extracting protein. It then goes through a type of mixer with added non-meat ingredients (Aberle et al., 2001). Examples of basic non

meat ingredients are phosphates, salt, nitrite, and sodium erythorbates/ascorbate. However, the phosphates and salt are primarily responsible for protein extraction.

2.5.1.2. Factors impacting protein solubility

Sodium chloride, also known as salt, is a very common non meat ingredient used in processed meats that is multi-functional. Salt is used as a flavor enhancer, preservative, and protein extractor. The level of salt used in a food product is one of the determining factors on the level of protein extracted. On average, formulations consist of 2% - 3% salt; however, salt levels can range from 1.5% to 5% (Claus et al., 1994; Aberle et al., 2001).

In postmortem meat, actin and myosin (contractile proteins) form cross-bridges that give meat its firm texture. In a comminuted product, skeletal muscle is reduced in size via grinder or bowl chopper, and then placed in a mixer where salt is added. During the mixing process, salt begins to detach myosin heads from actin allowing myosin and actin to be extracted. Upon extraction, proteins open due to the electrical charge of the chloride element (Cl^-) making it more easily dissolved in an aqueous solution.

Gadea De Lopez and Hand (1993) noted that as fat level decreased, textural properties become more dependent upon the lean of low fat and low salt meat products. Protein binding to added water was reduced when fat was replaced with water in comminuted products. Therefore, Gadea De Lopez and Hand (1993) noted that proteins in low fat products play a critical part in comminuted product.

2.5.1.3. Water- holding capacity

Water-holding capacity is a critical aspect in whole muscle foods and processed meats. The level of moisture lost during thermal processing has an effect on overall

product yield, which affects quality and dollar value. The level of moisture lost is dependent upon several factors that will influence the ability of muscle proteins to bind to water. Factors include: pH, steric effect, cold shortening/thaw rigor, electrical stimulation, and stress.

2.5.1.4. Factors impacting water-holding capacity

A factor affecting water-holding capacity in processed meat is pH. Muscle pH accounts for 33% of water holding capacity (Aberle et al., 2001). Of the three forms of water, bound, immobilized, and free, the level of pH affects the free water form the most. Muscle pH that has rapidly decreased in pre-rigor muscle rapidly produces an abundance of lactic acid simultaneously, body heat is escalated. With this combination, some of the muscle proteins become denatured causing a decrease in the availability of reactive groups binding to water.

When divalent metal ions such as calcium (Ca^{+}) and magnesium (Mg^{+}) are present, the negative muscle protein reactants become neutralized by the positive charge which then prevents water from binding. The lack of myofibrillar space to bind within protein structures is known as the steric effect. In addition, during the conversion of pre-rigor to post-rigor muscle steric effect accounts for 66% of water-holding capacity (Aberle et al., 2001). In addition, when these divalent metal ions neutralize muscle protein reactants, repulsive forces are minimized which allows proteins to pack closely together preventing the available reactants to bind with water.

2.5.2. Protein gelation

Protein gelation is the key factor required to occur for binding of meat pieces in processed meats. Aggregation of proteins is induced in a pre-heating step that makes it

possible to fully denature the protein ingredient before gelation sets in. Muscle proteins, myosin, and actomyosin play a very important role in protein gelation and the binding of muscle pieces. Proteins undergo a physical change that starts with a tertiary structure that eventually breaks down to secondary structure, then primary structures. Unfolding of protein structures is caused be the application of heat during thermal processing.

Low muscle pH, in the case of PSE, has resulted in low extractabile proteins because of the denaturation of proteins caused by pH and elevated temperatures. Trautman (1964) found that decreasing pH was linear on the solubility of water and salt soluble proteins.

2.5.2.1. Factors impacting protein gelation

The gelatin of meat proteins plays an essential role in the development of binding properties of comminuted meat products, such as sausage type (Samejima, Hashimote, Yasui, & Fukazawa, 1969; Ishioroshi, Samejima, & Yasui, 1983). Protein gelation is the process of the globular myosin heads aggregating upon heating (30–50°C). Temperature rising between 50–70°C causes a structural change in myosin helical rods that results in an increase in cross-linking formation of myosin. Increased temperature causes hydrogen to decrease. However, when cooled hydrogen bonds are able to reform and gain strength producing a glue-type substance, known as gel. Mysoin and actomyosin not only contribute to the firmness of heat induced meat gels, but also affect water holding capacity, which affects cooking yields of comminuted meat products. (Hamann, 1988 & Smith, 1988). The protein network physically and chemically enhances the water retention by their capillarity and non-covalent bonding (Acton & Dick, 1984). Cooking loss directly impacts both economic and palatable value of processed meats.

2.5.3. Functional properties of nonconforming raw materials

Raw material selection may have a direct impact on the ability of processors to achieve both targeted food safety and product standards. In most cases of dark cutting beef, pH and water activity are higher than normal, which can be a food safety concern in "shelf-stable products." A "shelf-stable product" refers to products that do not require freezing or refrigeration for safety and are of acceptable characteristics (FSIS, 2005). According to the Principles of Preservation of Shelf-Stable Dried Meat Products (FSIS, 2005) the two most important factors in the safety/stability of shelf-stable dried meat products are water activity and pH.

Water activity is probably the most important factor contributing to shelf-stability as it refers to the amount of water available for microbiological activity. Microbial growth is dependent primarily on water activity and pH. A product that is high in pH will contain higher water activity levels. In general, products considered to be "shelf-stable," must achieve a pH of less than 5.0; or water activity of less than 0.91; or a combination of a pH of less than 5.2 and a water activity for dried products such as beef jerky and beef sticks (FSIS, 2005). For example, the Standards of Identity U.S. Safety (2003) recommends that jerky products must have a water activity less than 0.85 to be considered microbially safe. Shelf stable dry sausage must have a moisture to protein ratio of less than or equal to 1.9:1 (FSIS, 2005).

2.5.3.1. Pale, soft, and exudative

Lost protein functionality is known to be the main cause of PSE in meat (Alvarado & Sams, 2004). Alvarado and Sams (2004) investigated normal and pale broiler breasts and brining effects. Authors reported that color and pH of broiler breast

fillets were highly correlated to water-holding capacity and brining pick up. Broilers pale in color were lower in pH, water-holding capacity, and brine pick up.

Joo, Kaufaman, Kim, and Kim (1994) investigated percent drip loss in different levels of quality defects: Pale, Soft and Exudative (PSE), Red, Soft, and Exudative (RSE), Red, Firm and Normal (RFN), and Dark, Firm, and Dry (DFD). They (Joo et al., 1994) found PSE (10.4%) to have the greatest percent drip loss, followed by RSE (7.4%), RFN (3.3%), and DFD (1.2%). In 2000, the National Pork Producers Council reported a similar trend to Joo et al. (1994) with PSE (> 4.7%) resulting in the greatest drip loss followed by RFN (1.7% to 4.7%), and DFD (< 1.7%) (NPPC, 2000).

This indicates that the level of pH does have a major affect on water holding capacity in muscle, which supports Bendall and Swatland (1988) who found that pH was an acceptable factor to test a muscles ability to hold water.

2.5.3.2. Dark, firm, and dry

As mentioned, dark cutting results in a darker colored lean, which results in a low consumer appeal. Hence, in order to avoid losing complete dollar value, dark cutting products are commonly prepared as pre-cooked products (i.e. jerky) or ground and combined with other ground processed meat products, such as beef sticks.

2.5.4. Use of nonmeat ingredients to improve the functional properties of nonconforming raw materials

2.5.4.1. Sodium chloride

Salt has been used for centuries as a preservative. Salt is included in food products to: enhance flavor, inhibit bacterial growth, increase moisture retention and serve as a preservative (Aberle et al., 2001; Alvarado & McKee, 2007). Sodium chloride

has been reported to increase cook yields, increase water binding, and decrease post mortem pH decline (Boles & Swan, 1997). However, salt is one of the most relied-on non-meat ingredients to assist with the extraction of salt-soluble protein (SSP) and water binding ability. Salt-soluble proteins (SSP) are the proteins easiest to extract starting with the most abundant, myosin, followed by actin and actomysoin. Myosin, found in the thick filament of a myofilament and actin found in the thin filament and their combined structure, actomysoin, are found in the salt soluble fraction that determines meat characteristics (texture and firmness) based on their state during extraction (Alvarado & McKee, 2007). The initiation of SSP extractions begins with the unfolding of proteins followed by solubilization in salt water solution. The unfolding of the proteins is due to the electrostatic repulsion from the chloride of $Na^{+}Cl^{-}$. Repulsion of proteins exposes more free space between myosin and actin that allows water to bind increasing water retention. Heat solubilizes the SSP into a gel that works like glue keeping sectioned muscles bound and assists in food particle definition by surrounding fat particles from melting and smearing out (Colmenero, 2002). Salt is a self-limiting product that if overly used, can have a negative impact on palatability. Depending on the product, salt levels can range from 1.5% to 3.0%. In addition to NaCl, potassium chloride has been used as a salt substitute. It is not readily used in further comminuted products because it contributes to a stronger flavor that requires use in lower amounts (Montana Meat Processors, 2001). In regard to protein functionality, sodium chloride is more effective than potassium chloride (Lamkey, 1998).

2.5.4.2. Phosphates

Phosphates are the second most common non-meat ingredient used in processed meats to increase water-holding capacity. By increasing water-holding capacity, products exhibit higher cook yield percentages. In the processed meat, depending on the product, alkali and acid type phosphates are used. Alkali phosphates are used to increase muscle pH and water-holding capacity. Acid phosphates, commonly used in marinades, lower pH and water-holding capacity, which in turn drives the pH closer to the isoelectric point (Alvarado & McKee, 2007). The United States Department of Agriculture (USDA) requires that phosphate concentrations cannot exceed 0.5% of finished product weight when it comes to enhancing water holding capacity (Alvardo & McKee, 2007). Four predominate phosphates commonly used in meat processing are: Orthophosphate (P) {least functional} (shortest chained), Tetrasodium Pyrophoshate (PP) {most effective}, Sodium Tripolyphosphate (STPP) {most commonly utilized}, and Sodium Hexametaphosphate (SHMP) {harder to dissolve} (longest chained) (Alvardo & McKee, 2007). Trout and Schmidt (1986) found that with increased chain length, a reduction in degree dissociation resulted. Of the four phosphates, short-chain phosphates (P and PP) are considered to be the best in buffering capacity. Like salt, alkali phosphates cause repulsion allowing more myofilament spacing for water binding, however this is due to positive charges. Dominant positive charges contributed by alkali phosphates causes a dramatic increase of the proteins net charge to be more basic. Therefore, pH is driven further away from the isoelectric point increases water-holding capacity.

Utilizing salt and phosphates simultaneously has been shown to work synergistically in extracting myofibrillar proteins that increase water-holding capacity.

Phosphates alone are unable to increase ionic strength however, in conjunction with salt (1-2%) the ionic strength is maximized (Trout & Schmidt, 1984). Phosphates initiate the extracting process by dissociating actomyosin which makes it easier for salt to solubilize (Clause et al., 1994). Phosphates are the most difficult ingredients to dissolve, hence, it is recommended to add phosphate first followed by salt during manufacturing of brine. In another study conducted by Detienne and Wicker (1999), interaction of salt and phosphate decreased percent purge, cook loss, and expressible moisture in injected pork loins. Trout and Schmidt (1983) found that approximately 4.6-5.8% (0.08M-1.0M) salt was needed to achieve maximum water holding capacity, but only 0.4-0.6M was needed to obtain good functionality. Additionally, phosphates have been shown to influence product texture such as shear strain and fracture as well as cook loss. Torley, D'Arcy, and Trout (2000) found that gel strength increased as ionic strength increased due to the use of phosphate. According to Park, Lanier, and Pilkington (1993), it is the un-denatured proteins available to form the protein network structure that is responsible for gel strength. Therefore, in the case of PSE, it is the because of the amount of denatured proteins that result in weak gels (shear stress values). McKeith, Holmer, Killefer, and Jones (2008) investigated the effects of pH on color, textural properties, shelf life, and consumer acceptability. Utilizing three different phosphates (acidic, neutral, and alkaline) in frankfurters of normal pH, authors targeted four pH levels: low (pH = 5.60), intermediate (pH=6.00), high (pH= 6.30), and control (pH=5.90). Results showed that frankfurters with alkaline phosphate were harder in texture, ligher in color, and more acceptable by consumer panelists.

2.5.4.3. Nitrite

The preservation of meat first began with the use of salt and their natural contaminants. Nitrates, an impurity of salt, affects meat color by providing a cured-reddish-pink color (Sofos, 1981). During the 1970's, extensive research determined that nitrate was broken down to nitrite by bacteria which lead to the discovery that nitrite was responsible for the cured pink color (Sofos, 1981). It was soon discovered that nitrates and nitrites were poisonous if used in excessive amounts. According to the National Research Council (1981), the government has been regulating the use of nitrate and nitrites since the 1900's. In the United States, nitrite levels have been reduced over the years and are closely regulated. Excessive use of nitrates/nitrites results in nitrite burn that exhibits a green discoloration in cured meat (Montana Meat Processors Convention, 2001) that results in a low consumer appeal. Today, nitrite is commonly used in place of nitrates because of the quicker conversion of nitric oxide myoglobin to nitrosylhemochromogen, which means less cooking time.

The conversion process of nitrate to nitrosylhemochromogen is as follows: nitrate is converted to nitrite by bacteria (*Lactobacillus* or a *Micrococcus*). Nitrite is then converted to nitrous oxide by way of the *Micrococcus* bacteria followed by nitrous oxide converting to nitric oxide. Toldrá (2007) noted that the micrococcus group (composed primarily of *Staphylococcus* and *Kocuria*) has proteolytic and lipolytic activity that is responsible for the nitrate reductase activity that reduces nitrate to nitrite that also contributes to flavor, color, and safety. The micrococcus bacteria group is also responsible in preventing lipid oxidation because of its catalase activty. At this point the nitric oxide binds with myoglobin at the sixth binding site known as

nitricoxidemyoglobin. This nitricoxidemyoglobin results in a metmyoglobin (brown) color. Heat is then applied by way of thermal process that converts the nitric myoglobin to nitrosylhemochromegen that is responsible for the cured pink color. Currently, sodium nitrite is allowed in maximum levels depending on the product: bacon, 120 mg/kg; comminuted cured products, 156 mg/kg; and 200 mg/kg for pickled-cured products (NRC, 1981). Nitrite is mainly used in meat processing to assist in increasing the conversion to the cured pink color. However, nitrate is solely used in long curing processes such as dry-cured hams.

2.5.4.4. Starter cultures

Starter cultures are microorganisms used in food products that function in lowering pH that leads to flavor adjustment and texture. Food products would include: dairy (yogurt and cheese) and fermented meat products (snack sticks) to help lower pH, adjust flavor, and texture. Fermentation is the production of lactic acid by microbial metabolism of proteins and sugars to yield specific flavors, aroma, and product texture that are "cured and shelf stable (without cooling) and are commonly consumed without application of any heating process." (Hamees, Bangleton, and Seunghwa, 1990; Aberle et al., 2001). Lücke (2000b) noted that the addition of starter cultures may have four different purposes: "1) – to improve food safety; 2) – to improve stability (increase shelf life); 3) provide diversification (modify raw materials to add sensory attributes); and 4) – provide health benefits (positive effects on the intestinal flora." Starter cultures are chosen on the basis of their stability and ability to produce desired products. Tuldrá (2007) defined stability as "a microbial starter capable of resisting acidic pH, tolerate the presence of salt, low water activity, ability to grow in fermentation temperatures (35-

40°C) and must also have a good enzyme profile for the generation of desired products, such as lactic acid." Starter cultures can be distributed in a frozen state in freeze dried carriers. According to Lücke (2000b) an even distribution of culture in the mix is important to ensure homogenous fermentation.

Everson, Danner, and Hammes (1970) discovered that fermented sausage, also known as dry or semi-dry sausage, had the ability to be eaten during warm times of the year when refrigeration was a concern. Various bacteria are used either in single form or in combination as starter cultures. In traditional fermentation process bacteria, yeasts and fungi are known to work synergistically, however, lactic acid bacteria (LAB) was determined to contribute to all aims of the ripening portion of fermentation (Hammes et al., 1990). Of the bacteria used as starter cultures, different strains are used for different products: In the case of meat, lactic acid bacterium is commonly used in fermented sausage such as salami, and beef snack sticks to drop pH. Examples of lactic acid bacteria are: *Lactobacillus acidophilus* (used in probiotics), *L. sake, Lactococcus loactis, Pediococcus acidilactici*, and *P. pentosaceus* (Hammes & Hertel, 1998). In lactic acid bacteria, the production of lactic acid reduces pH that aids in preservation and inhibits bacteria growth. The production of lactic acid is solely responsible for the pH decreasing in products (Demeyer & Vandekerckhove, 1979). It is critical to keep starter cultures pure and active. Activity is based off of growth rate of the bacteria followed by production of products. For instance, thawed starter cultures refrozen before use may not have the same effects compared to starter cultures kept frozen prior to use. Lactic acid bacteria function to improve safety, stability, and diversification, whereas catalase-positive cocci (*Staphyloccoccus*), yeasts (Debaryomyces), and molds (Penicillium)

stabilize sensory attributes (Lücke, 2000a). Hammes and Knauf (1994) stated that in order to obtain products of good quality, starter cultures must "contribute to hygienic safety, sensory attractiveness, high and constant levels of quality and shelf-life."

2.6. Manufacturing of products with nonconforming meat

It is not uncommon for US beef packing plants to do one of two things: fabricate dark cutting beef carcasses and sell them as boxed beef to be shipped to food services and/or sell as export. In a phone interview with assistant professor Dr. Ty Lawrence from West Texas A&M University, he stated that some of the beef packing plants were grinding up a whole dark cutting beef carcass and selling it as ground beef. According to the National Beef Quality Audit-2005, only 1.9% of the beef carcasses surveyed were dark cutting. Even though this is a small percentage, this type of product will eventually be utilized. Perhaps taking what was once a discounted beef carcasses and utilizing some of its subprimals as a whole muscle snack food or in a comminuted product may be an alternative source to utilizing an inexpensive, low quality product. Beef jerky and beef snack sticks are convenient snack products that do not require refrigeration that conveniently have an extensive shelf life. Beef frankfurters are also a popular food product in the United States that is commonly enjoyed in the United States, especially around the July 4th time where fifty million were consumed in one day (National Hotdog and Sausage Council, 2007). Using raw materials high in pH as either a whole muscle or a comminuted product may or may not exhibit textural or meet safety standards which therefore can lead to problems.

Shelf-stable meat products are commonly found throughout the United States that offer consumers the convenience of flavorful products not requiring refrigeration. A

processing endpoint in such products is obtained by monitoring pH, water activity, or moisture content (Aberle et al., 2001).

In general, products considered to be “shelf-stable” must achieve a pH of less than 5.0; or water activity of less than 0.91; or a combination of a pH of less than 5.2 and a water activity of 0.91 in dried products such as beef jerky and beef sticks (FSIS, 2005). Water activity is the most important factor contributing to shelf-stability as it refers to the amount of water available for microbiological activity. Depending on the type of product and associated bacteria, a certain level of water activity should be achieved. For instance, inhibition of *L. monocytogenes* requires an A_w level of 0.91 or less whereas *Staphylococcus* requires an A_w of 0.85 or less. A raw product high in pH will naturally contain higher water holding capacity, which may require longer drying times compared to raw materials normal in pH. Products classified as shelf-stable include beef jerky and fermented sausages.

2.6.1. Beef jerky

Beef jerky is an extremely popular high protein processed snack that is consumed in the United States. FSIS (2006) defines jerky as “a nutrient-dense meat that has been made lightweight by drying.” It can be made from whole lean muscle that is sliced into thin strips, marinated in a salt/sodium nitrite solution then thermally processed (cooked/drying procedures). The word "jerky" came from the Spanish word "charque" that has been in existence since ancient Egypt (FSIS, 2006). Because of jerky’s low moisture content and lack of refrigeration requirements, it is able to have a long shelf life and therefore, is classified as a shelf-stable product.

2.6.2. Beef snack sticks

Beef sticks are fermented snack sticks that can be made from coarse ground beef that is shelf stable due to its low moisture content. Beef sticks are considered a semi-dry, fermented product that requires a critical fermentation step to drop pH below 5.3, creating the distinct tangy flavor. This tangy flavor is favorable as long as a sufficient amount of carbohydrates (i.e. dextrose) is available for the production of acid by added bacteria. According to Zapsalis and Beck (1985), fermentation is generally described as the “oxidative decomposition of organic compounds by enzymes that produce simpler by-products.” Fermentation involves lowering product pH followed with decreasing water activity during drying. The addition of salt is applied to assist in inhibiting the growth of undesirable bacteria. Favorable, salt-tolerant bacteria are utilized in converting carbohydrates to lactic acid. Utilizing raw materials high in pH for beef sticks production may pose a problem in water activity reduction. In the case of dark cutting beef, moisture levels are higher than normal which poses a food safety and standard of identity concern in both beef jerky and snack sticks.

Pearson and Gillett (1999) classified fermented meat products into two groups: 1) low acid or 2) high acid products. Low acid products would involve products of a final pH of 6.0, whereas high acid products have a final pH of 5.3 or lower. Most high acid products include long cured hams and sausages specifically beef sticks. Low pH of high acid products is contributed by a fermentation step in thermal processing. Most fermented products rely heavily on reducing water activity and pH to prevent microbial growth (Pearson & Gillett, 1999).

2.6.3. Challenges

2.6.3.1. Beef jerky

According to the Standards of Identity U.S. Safety (2005) recommendations, jerky products must have a water activity less than 0.85 to control bacterial growth. Water activity is used as a measurement of available water for microbial growth. Therefore, it has become the primary measurement used to attain control of pathogen growth (Daigle, 2005). Because jerky is such a popular, easy to make food product, it is commonly manufactured at home, especially during hunting season. Home dehydrators are the most common home use equipment used in manufacturing jerky at home. Because some dehydrators do not allow the temperature of the dehydrators to be altered, food safety concerns have surfaced by the government (Harrison & Harrison, 1996).

2.6.3.2. Beef snack sticks

Fermented semi-dry sausages, such as beef sticks, should attain a pH of 5.3 or lower (FSIS, 2005). During fermentation, lactic acid bacteria utilize carbohydrates reducing them to lactic acid, which leads to a drop in pH. End product of beef sticks may result in a water activity as low as 0.65 after heat has been applied or by slower drying times at lower temperatures (Pearson & Gilltett, 1999). However, if pH and water activity are not reduced via fermentation and drying, then bacteria is a primary concern.

Bacteria of concern most commonly associated with beef jerky and beef snack sticks include: *Listeria monocytogenes*, *Escherichia coli O157:H7and Staphylcoccus aureus* .

2.6.3.3. Listeria monocytogenes

Listeria monocytogenes (L. monocytogenes) is a Gram-positive, lacultatively anaerobic organism that is capable of survival and growth under refrigerated conditions (at

5°C or below) (Jay, 1996). This bacteria gained attention in the 1980's when several listeriosis outbreaks occurred in the United States (AMIF, 1995). This outbreak was traced to coleslaw, raw vegetables and soft cheeses. *L. monocytogenes* is a ubiquitous microorganism commonly found in plants, soil, animals, condensed or stagnant water, dirt, dust, floors, drains, and silage (AMIF, 1995; USDA, 2006). A main concern of *L. monocytogenes* is its ability to survive in extended shelf-life products as well as Ready to Eat (RTE) and its tolerance to heat and salt (Farber & Peterkin, 2000). This bacterium has a wide growth range. This range includes temperatures of 2.5-44°C (36.5-112°F) (AMIF, 1995). Because *L. monocytogenes* may be present in slaughter animals and subsequently in raw meat, it can be continuously introduced into the processing environment through cross-contamination of fabrication equipment and employees. A major concern about this bacterium is its ability to grow in damp environments and establish itself well enough creating a "biofilm" making it difficult to eliminate during cleaning and sanitizing (USDA, 2006). A biofilm is formed when microorganisms attach themselves to solid surfaces creating microbiological communities where they multiply and grow. Multiple microorganisms following this process eventually grow cells that attach to one another creating a film type substance termed "biofilm" (Zottola, 1994). Growth of *L. monocytogenes* requires a minimal water activity of 0.92 (USDA, 2006), therefore, it is recommended a water activity of 0.90 or less as be used as the target (AMIF, 1995). If *L. monocytogenes* is ingested, listeriosis may occur, which can lead to serious illness and death (FSIS, 2005). In addition to the previous concerns mentioned, *L. monocytogenes* is also known to cause abortions in pregnant women. "*L. monocytogenes* is second to only the staphylococci as food-borne pathogens in being able to growth at A_w values < 0.92" (Jay, 1996). According to the American Meat Institute Foundation (AMIF) (1995), *L. monocytogenes* can be contaminated in a variety of meat and

meat products, including fermented sausages. Control of *L. monocytogenes* begins at the raw stage. It is important to avoid cross contamination between the raw and finished product.

2.6.3.4. Escherichia coli *O157:H7*

Escherichia coli O157:H7 (*E. coli* O157:H7) is a Gram-positive, pathogenic strain of *Escherichia coli* that is commonly found in the gastro intestinal tract of warm-blooded animals that is shed in feces (AMIF, 1995). *E. coli* O157:H7 was recognized in the 1980's as a pathogen in hamburger meat and first quickly gained attention in 1993 when it was identified as the cause of a large outbreak of food-borne illness associated with ground beef in Washington State (Ingraham & Ingraham, 2000). *E. coli* O157:H7 can grow in the presence or absence of oxygen at a minimum temperature of 7°C - 8°C (44.6°F – 46.4°F) and an optimum temperature of 37°C (67.2°F), maximum 46°C (114.8° F), an optimum water activity growth of 0.950 (New Zealand Food Safety Authority, 2001). Any age group can be affected, however, it is commonly found in infants (< 4 years) and the elderly (>65 years). Severe complications and illnesses caused by *E. coli O157:H7* is manifested in three ways: hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TPP). Hemorrahagic colitis will cause abdominal pain and watery, bloody diarrhea that is known for the recovery period to range from two to nine days after the onset of illness (AMIF, 1995). Hemolytic Uremic Syndrome (HUS) can occur over long term. HUS is a life-threatening condition that produces severe kidney problems, including renal kidney failure that may lead to death that is also associated with children. There has been a 5% death occurrence in HUS cases (AMIF, 1995). *Thrombocytopaenic purpura (TTP)*, is a version of HUS, also a life threatening condition, often found in the elderly that involves loss of platelets, skin

coloration, nervous system disorder (seizures and strokes), and blood clots in the brain usually resulting in death (AMIF, 1995; New Zealand Food Safety Authority, 2001).

Listeria monocytogenes and *E. coli O157:H7* are two bacteria of concern during thermal processing. However, *Staphylcoccus aureus* is known to produce toxins in RTE products. Cross contamination of *Staphylcoccus aureus* infected humans can pose a higher risk because they are usually held in a non vacuumed package under room temperature (USDA, 2004).

2.6.3.5. Staphylcoccus aureus

USDA (2004) determined that *Staphylcoccus aureus (S. aureus)* is of concern with post processing contamination because of its inability to grow in aerobic conditions at a water activity level of 0.85 or anaerobically at a water activity of 0.88 or less. However, Baird-Parker (2000) reported that *S. aureus* survived better under anaerobic conditions. No one factor contributes to the growth of *S. aureus*. Several factors such as pH, water activity, and temperature are involved in development of bacterial growth. Whting, Sackitey, Calderone, Morely, and Phillips (1996) stated that in order to prevent *S. aureus* from growing, intrinsic factors of the food, such as pH and salt, and temperature must interact. *S. aureus* is one of the most tolerant opportunistic food-borne illness causing bacterium that is known to cause a range of diseases and intoxications in warm-blooded mammals (Baird-Parker, 2000). It is commonly found on hair and skin of healthy humans which is associated with skin infections such as ulcers, impetigo, lesions, and wounds, (FSIS, 2005; Baird-Parker, 2000). Typically, raw foods are not a source to find staphylococcus (AMIF, 1995). It is found in meat products by way of cross contamination. Humans carrying *S. aureus* pose the most risk of reintroducing it to

cooked products that may lead to food poisoning when consumed. Staphlyococci produces enterotoxins, which are produced by growth of the bacteria as a bi-product of their metabolism (Baird-Parker, 2000). The Food Safety Inspection Service (2005) has determined that *S. aureus* is not a good competitor with other microorganisms, such as *E.coli* O157:H7 and *Listeria*. Eliminating competing microbes by heat and/or salt cause problems in cooked meat products regarding *S. aureus.* In the presence of competing bacteria such as *E. coli O157:H7* and *L. monocytogenes*, *S. aureus* is over-powered inhibiting S. *aureus* from growing. It is thought that if *S. aureus* is controlled by reducing water activity levels (0.85), then *L. monocytogenes* would be accounted for and both of these bacteria would be less of a concern. According to Baird-Parker (2000), *S. aureus* survives well in the environment and is commonly found in food facilities. Bolton, Dodd, Means, and Waites (1988) found that strains of *S. aureus* should be of great concern as they found these strains were resistant to chlorine, during harvesting, when testing the defeathering machine in a poultry plant under normal cleaning and disinfecting process. They further found that *S. aureus* also survived well in dust, which implicates additional contamination of birds prior to harvest. Therefore, *S. aureus* may be found in ventilations, which can be an additional hazard. In the case of fermented foods, pH is the most important in controlling *S. auerus*. A rapid pH below 5.3 prevents the growth of this bacterium and toxin production as well as a water activity of 0.85 or less (AMIF, 1995). According to Holley (1985), drying at a temperature of 52.9°C for 2.5 – 3 hours is needed to reduce water activity levels below 0.86, which will reduce contamination or reduce bacterial growth.

2.6.4. Opportunities

Utilizing beef high in pH as a precooked product is one way to increase dollar value by masking its dark color. Utilizing high pH beef as a precooked product such as jerky and/or beef snack stick is one way to add value to a once discounted beef carcass. With modifying cooking/drying times during thermal processing and the addition of enough carbohydrates for starter culture to produce lactic acid, what was once a low quality product can soon be converted into an enjoyable convenient snack.

2.6.5. Beef frankfurters

According to the National Hotdog and Sausage Council (2006), U.S. consumers spent more than $3.9 billion on hot dogs and sausages. One hundred and fifty million hot dogs were consumed on Independence Day alone. To date, the hot dog market trend remains strong proving to be a popular American food item. Frankfurters can be made of beef, pork, chicken, and/or poultry. Beef frankfurters consist of beef trimmings from fed and cow beef carcasses. According to the 2007 National Beef Market Cow and Bull Quality Audit, fifty one percent of a carcass is used as trim with the remaining forty nine percent left to be used as steaks and roasts. Because of the physical makeup of dark cutting beef (dark in color and high in pH), this can pose a problem with textural and sensory characteristics in comminuted products such as frankfurters.

2.6.6. *Challenges*

As mentioned, dark cutting results in a darker colored lean, which results in a low consumer appeal. Hence, in order to avoid losing complete dollar value, dark cutting products are commonly prepared as pre-cooked products (i.e., jerky and beef sticks) or ground and combined with other ground processed meat products, such as beef

frankfurters. An advantage of using high pH in a comminuted product, specifically in an emulsified product, is its ability to bind. However, in benefiting from binding effects, color and texture may be sacrificed.

2.6.7. Opportunities

A major advantage of high pH is its ability of holding water and binding effects. In utilizing high pH beef containing higher moisture when compared to normal pH beef, less water may be added to an emulsion when manufacturing beef frankfurters. For consumers who are not key on additional added water to their products, this may an advantage of pleasing the consumer.

CHAPTER III

A SURVEY OF NORMAL AND HIGH pH BEEF CARCASSES IN FED BEEF AND COW CARCASSES IN TEXAS

3.1 Introduction

The beef industry relies heavily on producing beef products that are superior in quality and palatability (Wulf, O'Connor, Tatum, & Smith, 1997). The physical state of an animal, at time of slaughter, plays a significant role on beef quality as the muscle undergoes a series of bio-chemical changes. In beef, muscle pH and physiological maturity are known to affect palatability and overall appearance of end products.

Muscle pH of fed beef carcasses normally falls between 5.4 and 5.7, resulting in bright, cherry red lean color, and good flavor (Gill and Newton, 1981). Carcasses harvested at advanced maturity, such as cows, are not comparable in these traits to young beef carcasses, even at a normal pH. Cow muscles are more distinct than muscles from young beef carcasses primarily due to greater concentrations of myoglobin (Mb), the water-soluble protein responsible for meat color. As an animal advances in age, myoglobin concentration increases. Three most common forms of Mb exist resulting in different muscle color: deoxymyoglobin (absence of oxygen; purplish-red), oxymyoglobin (presence of oxygen; cherry red), and metmyoglobin (brownish-red). The three forms of Mb are sensitive to heat, but all vary. One factor attributing to the quantity of myoglobin in beef muscle at time of harvest is prior stressors.

A common factor involved in the amount of glycogen at time of harvet is stress. Long and short-term stresses on cattle play a critical role on the overall quality of beef carcass. As an animal is transported from their comfort zone to an unfamiliar location,

the onset of panic (stress) occurs. Glycogen, an energy source for muscle, begins to be utilized under stressful conditions and is converted to lactic acid in an anaerobic environment.

The glycogen content of muscle in meat animals at time of harvest plays a critical role on the overall quality of the meat, by way of glycolysis. Low levels of glycogen at the time of harvest can be detrimental to the overall end product of a carcass. Low levels of glycogen usually results in a high ultimate pH of 6.0 or greater that is often associated with the quality defect, Dark, Firm, and Dry (DFD), which is also known as dark cutting. Hydrogen ions are produced simultaneously, causing the pH to decrease from physiological level (pH 7.4) (Young, West, Hart, & van Otterdijk, 2004).

Postmortem glycolysis and muscle pH decline during normal carcass chilling. Postmortem glycolysis and subsequent pH decline ceases when one of two events occur: 1) muscle glycogen stores are depleted or 2) muscle pH declines to approximately 5.4 inhibiting the activity of glycolytic enzymes (Wulf, Emnett, Leheska, & Moeller, 2002). Prolonged physical activity and stress have similar effects across species. Short-term stress has a greater effect on pork and poultry, while long-term stress primarily affects beef and lamb. Short-term stressors include loud noises, unfamiliar environments, and fighting. Long-term stressors typically involve sickness or weather. The body itself, under normal conditions, is maintained via homeostasis. But when a deviation occurs, such as stress, the body uses the autonomic nervous system (ANS). The autonomic nervous system has two components responsible for “response and recovery:” sympathetic (mediated arousal and activation) and parasympathetic (responsible for relaxation) (Esler, Jennings, Korner, Willet, Dudley, Hasking, Anderson, & Lambert,

1988).

As seasons change, both temperature and daylight changes are known to affect livestock performance. Areas with high ambient temperatures during summer can cause heat stress, which can negatively affect beef cattle performance during the finishing phase. Mitlöhner, Galyean, and McGlone (2002), compared beef quality attributes in beef cattle under shaded and unshaded conditions. The authors found that dark cutting carcasses occurred less frequently in shaded (8.3%) cattle versus non-shaded (19.8%) ($P < 0.004$).

Currently, beef carcasses exhibiting dark cutting conditions are relatively low. For instance, the 2005 National Beef Quality Audit found that an estimated 2.0 % of the 9, 475 beef carcasses surveyed were dark cutters. In 2007, the National Cow and Bull Quality Audit identified 2.0% of the estimated 1,900 carcasses were of dark cutting. An interest was taken in knowing the prevalence of high pH beef carcasses in the state of Texas. Also, are cow and bull carcasses more likely to result in high pH.

The objective of the current study was to survey the variability in muscle pH and color from fed and cow beef.

3.2. Materials and methods

Beef carcasses (n = 2,324) were selected randomly from two fed beef processors (Plants 1 and 2) and two cull cow processors (Plants 3 and 4) in Texas. Plants were surveyed three times throughout a year to account for seasonal differences.

Ten percent of individual production lots were audited in a single day's production for each processor. Muscle pH was measured using a hand-held pH meter probe (3.5 mm OD; Meat Probes, Inc., Topeka, KS) inserted in the center of *M.*

longissimus lumborum between the $12^{th}/13^{th}$ rib cross section of the beef carcasses. Muscle color was measured using a Hunter Miniscan XE (Hunter Labs, Inc., Reston VA; Illuminant A, 10° observer and Illuminant D65, 10° observer). CIE L*(lightness) a* (redness), and b* (yellowness) values were reported as the average of two readings taken on the posterior surface of the *M. longissimus thoracis*, where the carcass had been ribbed. Additionally, the RMS Beef CAM was utilized, when available, for additional data. Readings were taken at the $12^{th}/13^{th}$ rib intersection on the *M. longissumus thoracis* and CIE L*(lightness) a* (redness), and b* (yellowness) were reported as single readings taken on the posterior surface of the *M. longissimus thoracis.* Finally, hot carcass weight, adjusted fat thickness, ribeye area, kidney pelvic, and heart fat, lean maturity scores, skeletal maturity scores, marbling, and fat color were determined by trained personnel. *M. longissimus* (ribeye) area was assessed using a dot grid.

3.2.1. Statistical analysis

Significant main effect (muscle pH and color) means ($P < 0.05$) were separated using the pdiff option of SAS using $P < 0.05$. Mean, standard deviation, minimum and maximum values were generated using the PROC MEANS procedure of SAS (SAS Inst. Inc., Cary, NC). Frequency distributions were analyzed using the PROC FREQ procedure of SAS. Pearson's CORR procedure was used to analyze correlations of the data using SAS.

3.3. Results and discussion

Descriptive statistics for quality and yield traits, pH, and dark cutter are represented for overall data (Table 1). Tables 2 and 3 contain all data based on each

Table 1.
Arithmetic means of fed beef and cow carcasses selected randomly in Texas (n = 2,322).

Trait	Mean[a]	SD[b]	Min[c]	Max[d]
Hot carcass wt, kg	335.9	59.7	95.7	587.9
Ph	5.6	0.2	5.0	7.4
Fat thickness, cm	0.9	0.3	0.0	4.9
Longissimus muscle area, cm^2	82.5	2.5	23.2	131.6
KPH, %[e]	1.7	0.9	0.0	4.5
USDA Yield Grade	2.8	0.9	0.2	7.1
Lean Maturity[f]	199	94	0	499
Skeletal Maturity[g]	129	139	100	560
Overall Maturity[h]	129	126	125	499
Marbling[i]	347	102	100	800
USDA Quality Grade[j]	351	138	10	810

[a]Mean = arithmetic mean
[b]SD = standard deviation
[c]Min = minimum
[d]Max = maximum
[e]KPH = Kidney, pelvic and heart fat
[f]Lean Maturity: 100 = A; 200 = B; etc.
[g]Skeletal Maturity:100 = A; 200 = B; etc.
[h]Overall Maturity: 100 = A; 200 = B; etc.
[i]Marbling: 400 = Small; 500 = Average Choice; 600 = High Choice, etc.
[j]USDA Quality Grade: 10 = Cutter; 100 = Utility; 200 = Commercial; 300 = Standard; 400 = Select; 500-700 = Choice; 800-900 = Prime.

category of fed beef packing plants (n = 2) and cow/bull packing plants (n = 2). Because of confidentiality, individual plants were given anonymous identification (Plant 1 - Fed, 2 - Fed, 3 - Cow, and 4 - Cow). Color values were not included in all tables because different illuminants (A°10 and D65) were used when using the Hunter miniscan during evaluation. However, two fed beef plants utilizing the RMS Beef CAM were reported (Table 2).

Table 2.
Arithmetic means of fed beef carcasses selected randomly in Texas (n = 1,887).

Trait	Mean[a]	SD[b]	Min[c]	Max[d]
Hot carcass wt, kg	350.6	44.0	204.1	544.8
L* (lightness)[e]	39.6	0.2	24.9	54.2
A* (redness)[f]	27.9	2.9	-6.4	40.3
B* (yellowness)[g]	12.8	4.4	4.2	36.8
pH	5.6	0.2	5.0	6.6
Fat thickness, cm	1.1	0.2	0	4.9
Longissimus muscle area, cm^2	87.7	1.6	30.9	127.7
KPH, %[h]	2.1	0.5	0	4.5
USDA Yield Grade	2.9	0.9	0.2	7.1
Lean Maturity[i]	161	16	100	170
Skeletal Maturity[j]	167	23	100	270
Overall Maturity[k]	165	17	125	200
Marbling[l]	373	81	200	800
USDA Quality Grade[m]	403	83	100	810

[a]Mean = arithmetic mean
[b]SD = standard deviation
[c]Min = minimum
[d]Max = maximum
[e]L* = values using the BeefCAM
[f]a* = values using the BeefCAM
[g]b* = values using the BeefCAM
[h]KPH = Kidney, pelvic and heart fat
[i]Lean Maturity: 100 = A; 200 = B; etc.
[j]Skeletal Maturity:100 = A; 200 = B; etc.
kOverall Maturity: 100 = A; 200 = B; etc.
[l]Marbling: 400 = Small; 500 = Average Choice; 600 = High Choice, etc.
[m]USDA Quality Grade: 10 = Cutter; 100 = Utility; 200 = Commercial; 300 = Standard; 400 = Select; 500-700 = Choice; 800-900 = Prime.

3.3.1. Arithmetic means

Overall, mean hot carcass weight was 335.9 kg, however, hot carcass weight ranged from 95.7 to 587.9 kg. Carcass pH data indicated that, on the average, muscle pH was normal. However, the range extended from very acidic and analogous RSE pork (5.0) to a physiological pH of 7.0 (7.4). The median marbling score was equivalent to USDA Select, but the range varied from Commercial to Prime. In data not reported in

tabular form, 96% of the carcasses were native beef type, 2.1% were Brahman, and 2% were dairy type cattle. Additionally, 50% were steers, 31.4% were heifers, 15.2% were cows, and 3% were bulls.

Tables 2 and 3 display arithmetic means of fed (n=2) and cow (n=2) processors in Texas. In Table 2, the mean hot carcass weight (350.6 kg) and fat thickness (1.1 cm) values for fed beef carcasses were similar to the average reported in the 2005 National Beef Quality Audit (Garcia, Nicholson, Hoffman, Lawrence, Hale, Griffin, Savell, VanOverbeke, Morgan, Belk, Field, Scanga, Tatum, & Smith, 2008). On the average, color space values and pH were similar to Wulf and Wise (1999) who reported surveyed beef carcasses (n = 145) exhibited L* (lightness) values of 38.3; a* (redness) values of 23.4; and b* (yellowness) values of 12.8. The average carcass pH (5.6) was identical to Wulf and Wise (1999) but the current study had greater variation.

In Table 3, arithmetic means of cow carcasses indicate that mean muscle pH was 5.8, but ranged from 5.0 to 7.4. Cow carcasses had a slightly higher mean pH than fed carcasses as well as a slightly greater range. Mean fat thickness of cow carcasses was 0.1 cm that ranged from 0.0 to 0.8 cm. This is much lower than the mean reported in the 2007 National Market Cow and Bull Beef Quality Audit which reported a mean fat thickness of 0.56 cm. On the average, lean maturity resulted in “B” maturity, but ranged from “A to E,” which is expected amongst cow carcasses that contain more myoglobin. This also differed from the 2007 National Market Cow and Bull Beef Quality Audit which reported average lean maturity scores equivalent to “C” maturity.

Table 3.
Arithmetic means of cow carcasses selected randomly in Texas (n = 435).

Trait	Mean[a]	SD[b]	Min[c]	Max[d]
Hot carcass wt, kg	268.8	74.5	95.7	587.9
pH	5.8	0.3	5.0	7.4
Fat thickness, cm	0.1	0.1	0.0	0.8
Longissimus muscle area, cm^2	9.1	2.5	3.6	20.4
KPH, %[e]	0.0	0.0	0.0	0.0
USDA Yield Grade	2.1	0.6	0.0	4.2
Lean Maturity[f]	278	104	180	499
Skeletal Maturity[g]	399	106	180	560
Overall Maturity[h]	349	84	100	499
Marbling[i]	232	102	100	590
USDA Quality Grade[j]	116	86	10	380

[a]Mean = arithmetic mean
[b]SD = standard deviation
[c]Min = minimum
[d]Max = maximum
[e]KPH = Kidney, pelvic and heart fat
[f]Lean Maturity: 100 = A; 200 = B; etc.
[g]Skeletal Maturity:100 = A; 200 = B; etc.
[h]Overall Maturity: 100 = A; 200 = B; etc.
[i]Marbling: 400 = Small; 500 = Average Choice; 600 = High Choice, etc.
[j]USDA Quality Grade: 10 = Cutter; 100 = Utility; 200 = Commercial; 300 = Standard; 400 = Select; 500-700 = Choice; 800- 900 = Prime.

3.3.2. Correlations

Tables 4 through 8 present simple correlation coefficients for the relationships between muscle pH and color values. Tables are presented by individual processors and illuminant used. In Table 4, muscle pH appears to be moderately correlated to lightness (L*) ($P < 0.0001$). However, with the exception of Table 6 (Plant 3 – Cow), which only showed correlations for redness and (a*) and yellowness (b*), the remaining Tables 5 and 7 though 9, revealed that muscle pH was moderately correlated with all three color space values ($P < 0.0001$). Studies have shown that muscle pH was correlated to all three color

Table 4.
Pearson correlation coefficients (*P* values) for pH and color space values using the Hunter miniscan (D65 illuminant) of fed beef carcasses (Plant 1 - Fed).

Trait	pH	L*	a*	b*
pH	--	-0.19 (<0.0001)	0.06 (0.06)	-0.04 (0.21)
L* (lightness)		--	-0.35 (<0.0001)	0.24 (<0.0001)
a* (redness)			--	0.67 (< 0.0001)
b* (yellowness)				--

Table 5.
Pearson correlation coefficients (*P* values) for pH and color space values using the Hunter miniscan (A°10 illuminant) of fed beef carcasses (Plant 2 - Fed).

Trait	pH	L*	a*	b*
pH	--	0.41 (<0.0001)	0.46 (<0.0001)	0.41 (<0.0001)
L* (lightness)		--	0.13 (0.01)	0.03 (0.51)
a* (redness)			--	0.89 (< 0.0001)
b* (yellowness)				--

Table 6.
Pearson correlation coefficients (*P* values) for pH and color space values using the Hunter miniscan (D65 illuminant) of cow carcasses (Plant 3 - Cow).

Trait	pH	L*	a*	b*
pH	--	-0.07 (0.31)	-0.20 (0.003)	-0.22 (0.0001)
L* (lightness)		--	-0.41 (<0.0001)	-0.38 (<0.0001)
a* (redness)			--	0.99 (<0.0001)
b* (yellowness)				--

Table 7.
Pearson correlation coefficients (*P* values) for pH and color space values using the Hunter minican (A°10 illuminant) of cow carcasses (Plant 4 - Cow).

Trait	pH	L*	a*	b*
pH	--	0.26 (0.0001)	-0.43 (<0.0001)	-0.47 (<0.0001)
L* (lightness)		--	-0.62 (<0.0001)	-0.47 (<0.0001)
a* (redness)			--	0.89 (<0.0001)
b* (yellowness)				--

Table 8.
Pearson correlation coefficients (*P* values) for pH and color space values using an automated camera system (RMS Beef CAM) of fed beef carcasses.

Trait	pH	L*	a*	b*
pH	--	-0.33 (<0.0001)	0.34 (<0.0001)	-0.27 (<0.0001)
L* (lightness)		--	0.50 (<0.0001)	0.45 (<0.0001)
a* (redness)			--	0.66 (<0.0001)
b* (yellowness)				--

Table 9.
LSMeans (SEM)[d] for pH by plant.

Trait	n	1 - Fed	n	2 - Fed	n	3 - Cow	n	4 - Cow
pH	1,495	5.69[b] (0.29)	392	5.40[c] (0.06)	224	5.74[ab] (0.07)	211	5.94[a] (0.08)

[a,b,c] Means in a row with different letter are different ($P < 0.05$).
[d]SEM = standard error of the mean.

space values (L*, a*, and b*) with a* and b* values being highly correlated (Wulf, O'Connor, Tatum, & Smith, 1997; Page et al. 2001). Effect of processor on muscle pH is shown in Table 9. The second fed beef plant (Plant 2 -Fed) in Table 10 had the lowest muscle pH (5.40), which significantly differed from the other plants. However, both cow plants (Plants 3 and 4) exhibited a slightly higher pH overall.

3.3.3. Muscle pH

Frequency diagrams of muscle pH for all carcasses (fed and cow) surveyed are shown in Figure 1. The majority of the fed beef carcasses (approximately 82%) fell within a range of 5.5 – 5.89. About 4% of the fed beef population resulted in a pH of 5.9

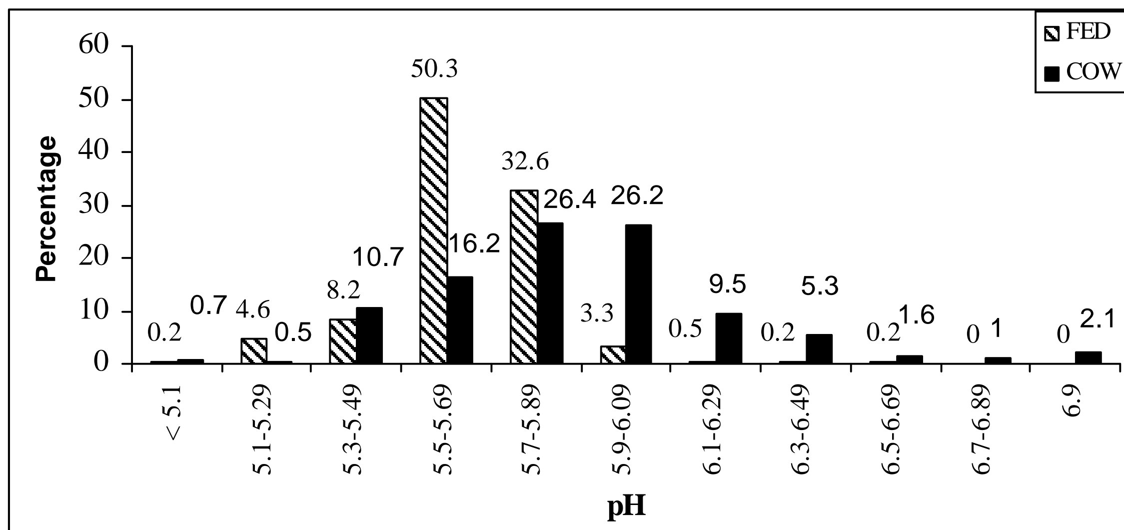

Figure 1. Frequency diagram of pH values observed in the *M. longissimus lumborum* of fed beef (n = 1,887) and cull cow carcasses (n = 435) from fed beef and cull cow plants in Texas.

or greater, but only 0.5% had a pH of 6.1 to 6.29. The low percentage of dark cutting carcasses is similar to results found by Garcia et al. (2008) who reported that only 1.9% of the beef carcasses were dark cutting. Of the cow carcasses surveyed, about half (52%) fell between a pH of 5.7 to 6.09. However, 19.4% resulted in a muscle pH of 6.1 or higher.

3.3.4. Hunter mini-scan (color space values)

The frequency distribution of color space values are shown in Figures 2 through 7. Of the two fed beef carcass plants surveyed (Figure 2), regardless of illuminant used, carcasses fell between a range of lightness (L*) values of 35 to 44. A small percentage (~6%) had a low lightness (L*) value (<35). This corresponds to the fed beef carcasses resulting in a muscle pH of 6.1 or higher. Page, Wulf, and Schowtzer (2001) reported a

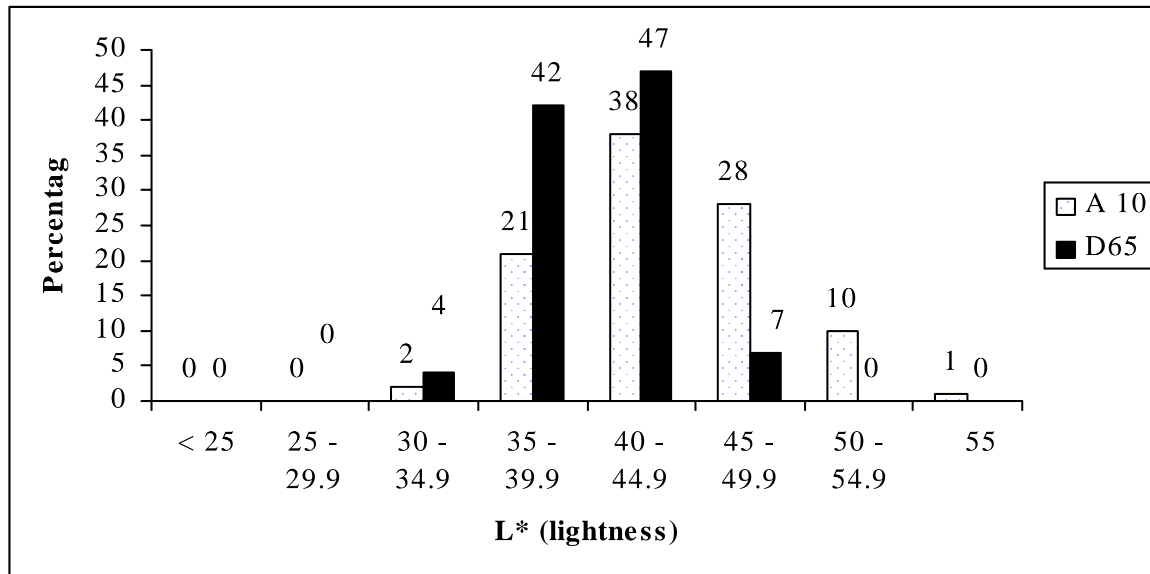

Figure 2. Frequency distribution of L* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas.

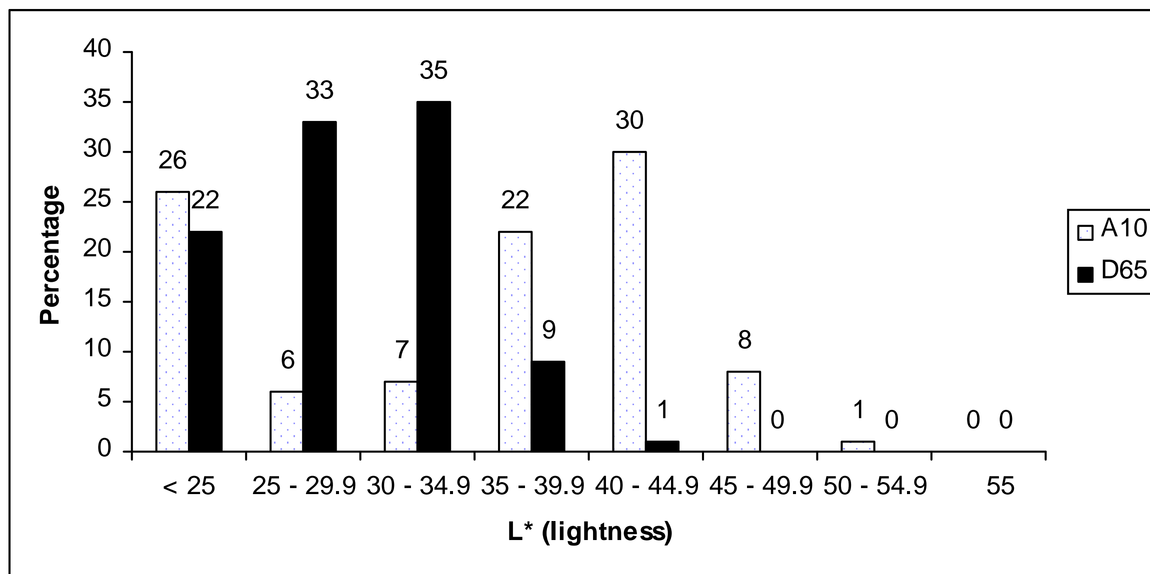

Figure 3. Frequency distribution of L* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of cow carcasses from two cow plants in Texas.

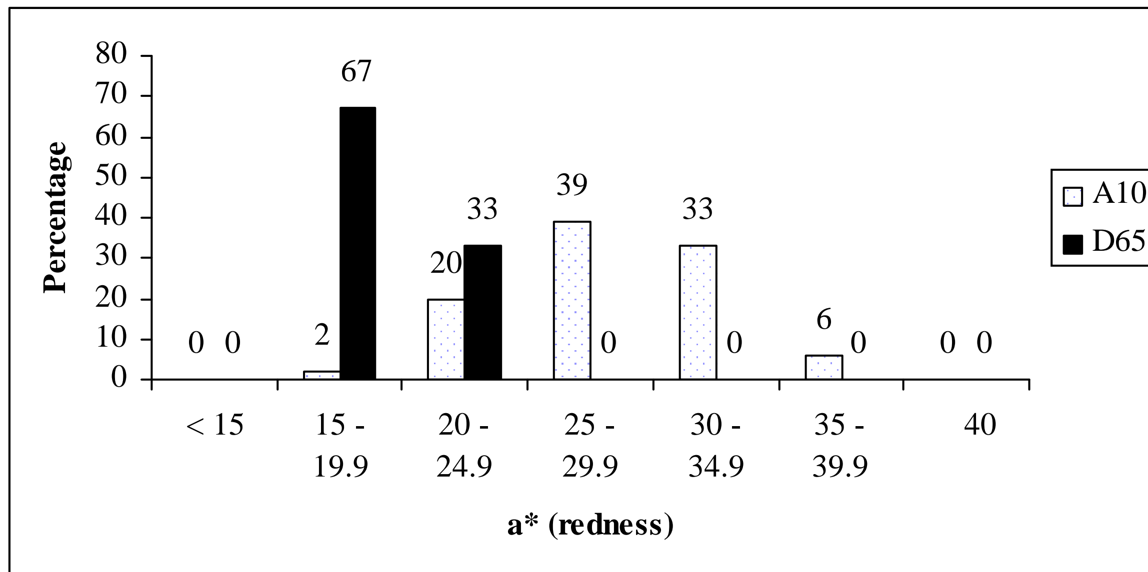

Figure 4. Frequency distribution of a* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas

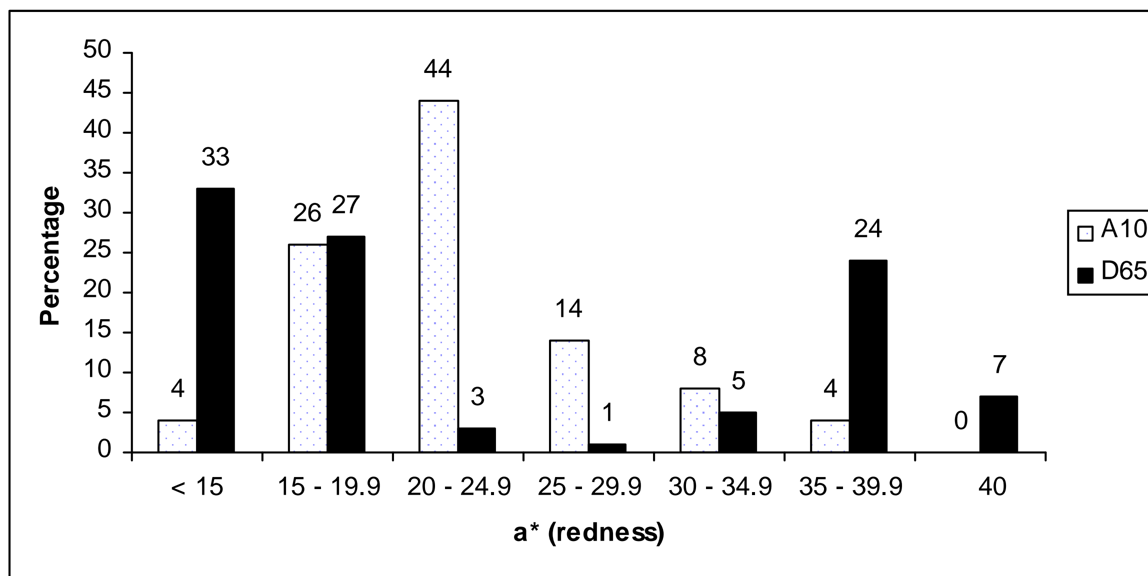

Figure 5. Frequency distribution of a* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of cow carcasses from two cow plants in Texas.

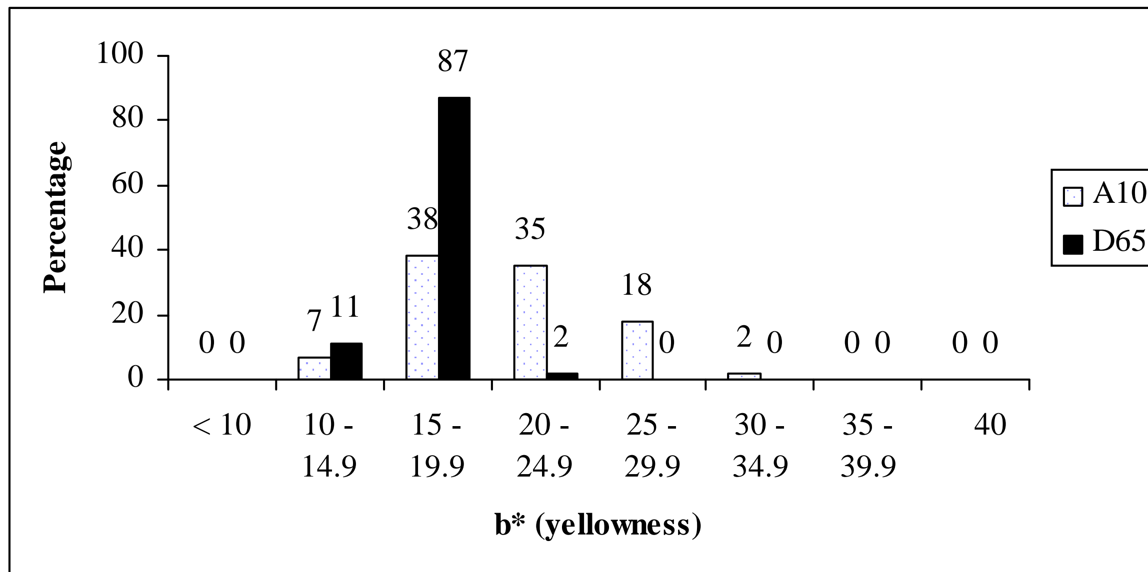

Figure 6. Frequency distribution of b* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas.

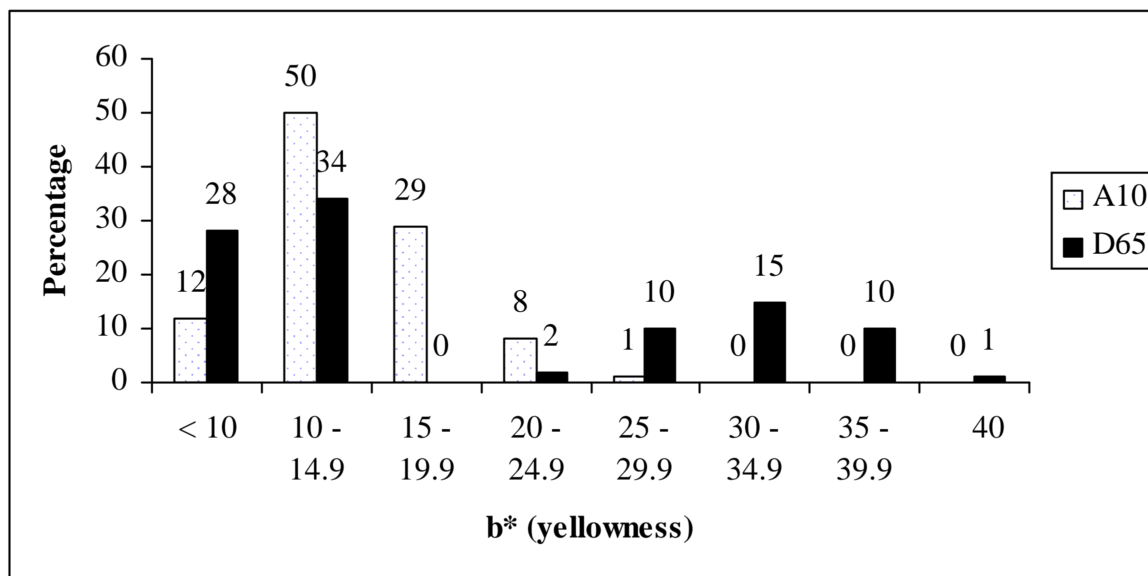

Figure 7. Frequency distribution of b* color space values using the Hunter mini-scan (A°10 and D65 illuminant) observed in the *M. longissimus thoracis* of cow carcasses from two cow plants in Texas.

mean lightness value of 39 in cattle surveyed (n = 1,000). The same authors reported that lightness values varied between 31 and 47. This is consistent for both illuminants for fed beef carcasses. A higher percentage of cow carcasses were darker (< 35) compared to fed beef carcasses, which correlated to the muscle pH of 6.1 and greater. Studies have shown that lean maturity was correlated with L*, a*, and b* but was mostly correlated with L* (Orcutt et al., 1984; Wulf & Wise, 1999; Page et al., 2001). This supports our findings with cow carcasses who exhibited a greater variation resulting in lower values.

Percentage of redness (a*) values (Figure 4) revealed that illuminant is critical when measuring redness values. A normal distribution was seen for illuminant A°10 starting at a value of 15 to 39 with 82% of the carcasses falling between 20 and 34, whereas values of 30 to 34 are typical in beef products. The frequency distribution in table 5 revealed great variation for both illuminants. Regardless of illuminant, the majority of the values were less than 30. Figure 6 showed the frequency distribution of yellowness (b*) for both illuminants. The figure showed that regardless of illuminant, the majority of the carcasses fell within a range of 15 - 24. These values are much lower from Wulf et al. (1997) and Page et al. (2001). Yellowness values for both cow plants revealed great variation between illuminants. The majority of the cow carcasses were on the lower end of the range (< 20). This would explain the higher percentage of dark cutters in comparison to fed beef carcasses.

3.3.5. RMS beef cam

Of the 2,324 carcasses surveyed, 85% were of fed beef carcasses where we were able to capture color values (L*, a*, and b*) using the Beef Cam. Figures 8 through 10 display frequency distributions of muscle lightness, redness, and yellowness for both fed

beef plants (Plants 1 and 2 - Fed). About 90% of the fed beef carcasses fell between the values of 35 and 44 for lightness (L*) which is consistent with Page et al. (2001). As for redness (a*) values, 76% of the fed beef carcasses alone were between values of 25 - 29.

The discrepancy of our results is likely due to the different illuminants used during evaluation. The frequency distribution of b* (yellowness) in Figure 10 resulted in vast majority (approximately 79%) of beef carcasses that fell between the values of 10 to 14. Color space values found that using the Beef Cam showed that the large proportion of fed beef carcasses is correlated to the pH values of 5.4 to 5.8.

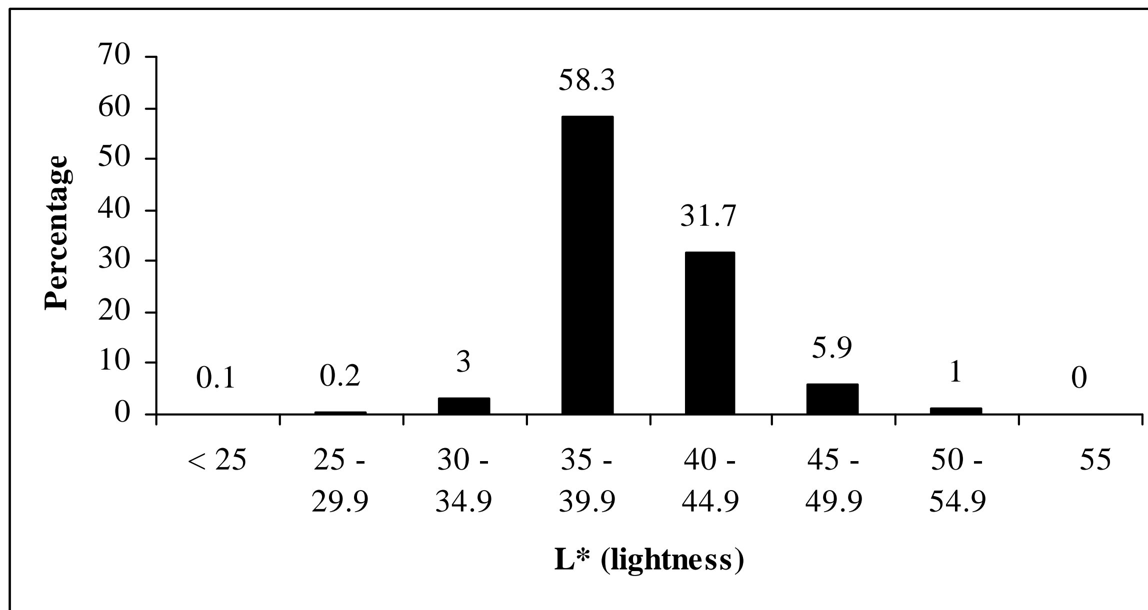

Figure 8. Frequency distribution of L* color space values using the automated system (Beef Cam) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas.

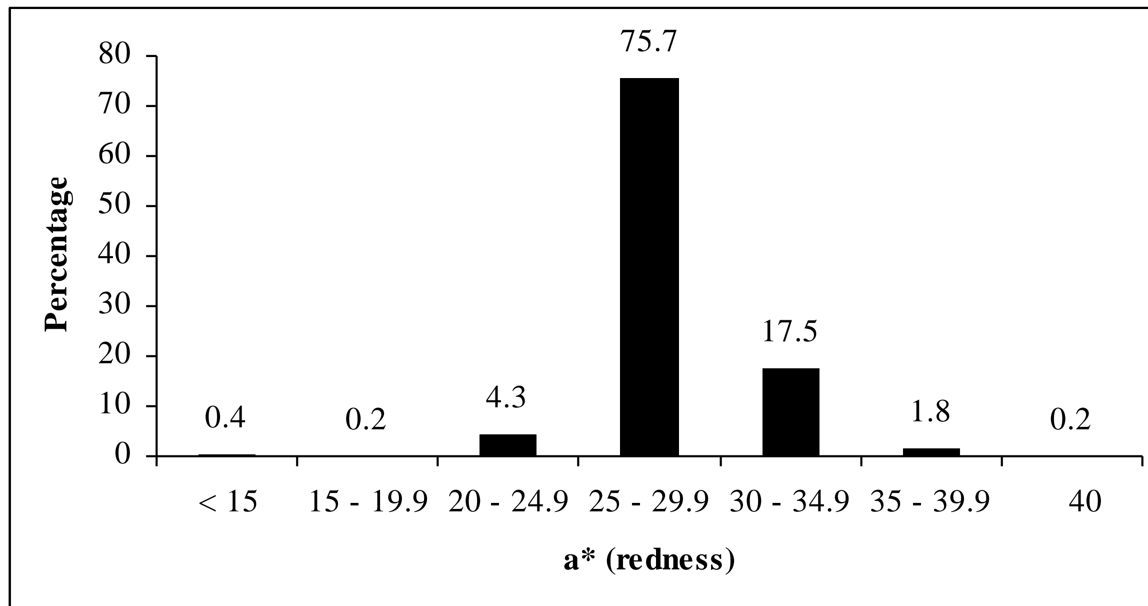

Figure 9. Frequency distribution of a* color space values using the automated system (Beef Cam) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas.

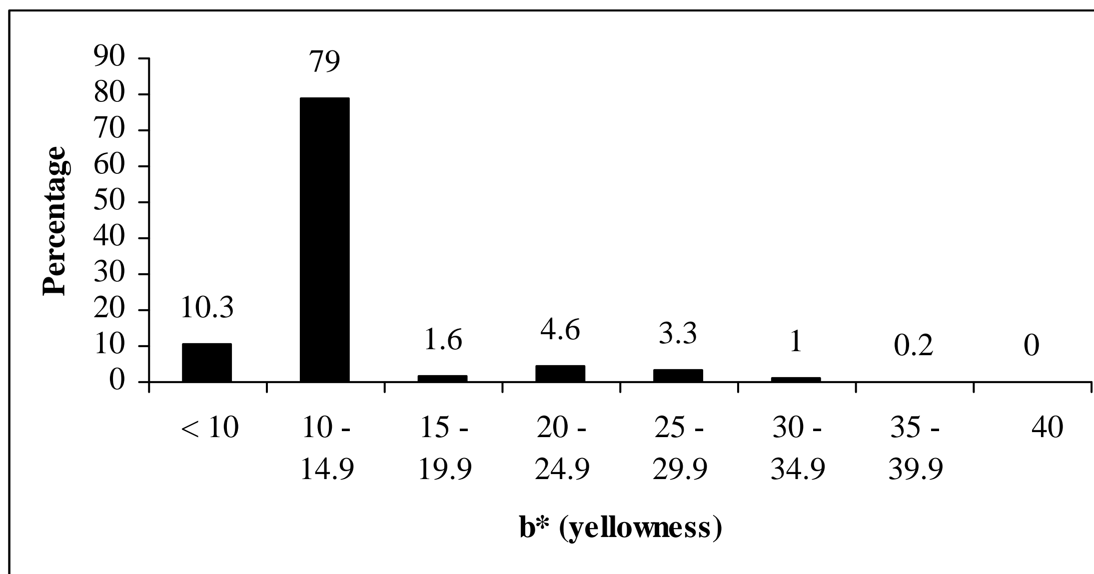

Figure 10. Frequency distribution of b* color space values using the automated system (Beef Cam) observed in the *M. longissimus thoracis* of fed beef carcasses from two fed beef plants in Texas.

CHAPTER IV

EVALUATION OF FUNCTIONAL PROPERTIES IN NORMAL AND HIGH pH INSIDE ROUNDS FROM FED AND COW BEEF FOR JERKY PRODUCTION

4.1. Introduction

The physical state of a live animal, at time of slaughter, significantly impacts meat quality as the muscle undergoes a series of mechanical and chemical events. Ultimate pH and overall maturity in beef are known to affect palatability and overall appearance of end products.

Beef carcasses harvested from advanced maturity cattle usually result in tougher meat and darker lean color even at a normal pH (5.3 – 5.7). Cow muscles are more distinct than muscles from young beef carcasses primarily due to greater concentrations of myoglobin (Mb) and decreased connective tissue solubility. As animals increase in age, connective tissue matrix increases resulting in less solubility, which results in tougher meat. Reagan, Carpenter and Smith (1976) showed that muscle from animals with increased maturity are less tender due to increased collagen matrix that results in less soluble collagen.

Myoglobin is the oxidative state responsible for meat color that increases in concentration with increased age. Breidenstein, Cooper, Cassens, Evans, and Bray (1968) illustrated that cattle of advanced maturity tend to result in higher Mb content compared to young cattle, resulting in darker colored lean. Wulf and Wise (1999) noted that “blooming” (conversion of deoxymyoglobin to oxymyoglobin) has an effect on color having more of an effect on a* and b* values than L*. However, stress, a major component of muscle color, has a negative affect on overall quality.

Long-term stress prior to harvest results in glycogen depletion of energy stores within the muscles. Depletion of glycogen energy alters the postmortem conversion of muscle to meat. Low glucose levels prior to harvest lead to low levels of lactic acid production and therefore, the meat pH remains closer to physiological levels (pH 7.4). Since the pH does not decline below 6.0, the meat possesses a dark red color, that is classified as dark, firm and dry (DFD), also known as "dark cutting." Because the pH has not significantly decreased from physiological pH, water-holding capacity is greater than normal pH meat, which would be "better suited for manufacturing purposes," but would still be worth less in dollar values than normal pH meat (Young et al., 2004). However, because of its dark appearance, DFD product results in a lower consumer appeal that would be difficult to sell. Additionally, dark cutting beef costs the industry millions of dollars annually (Grandin, 1992). In 2000, dark cutters were discounted as much as $30.00 per one hundred pounds, thus fed cattle were discounted $5.43 per one hundred pounds per carcass (Roeber, Morgan, Belk, Brooks, and Bates, 2000). Meat color is a major driver of retail meat case sales and profitability (McKenna, Mies, Baird, Pfeiffer, Ellebracht, & Savell, 2005). Hence, dark cutting beef might be better utilized in an alternative meat product, such as jerky.

Beef jerky is an extremely popular high protein processed snack product. It can be made from whole lean muscle that is sliced into thin strips, marinated in a salt/sodium nitrite solution then thermally processed (cooked/drying procedures). Jerky is a dried meat snack that is shelf stable due to its low moisture content. However, in the case of dark cutting beef, moisture levels are higher than normal. According to the Standards of Identity U.S. Safety (2003) recommendations, jerky products must have moisture to

protein ratio (MPR) of 0.75:1 or less or water activity less than 0.85 to ensure the product is microbially safe.

Raw material selection may have a direct impact on the ability of processors to achieve both targeted safety and product standards. Because pH can have a detrimental affect on functional properties (water activity, color, muscle protein, etc.), inside rounds manufactured from two carcass classes (fed and cow) and two pH types (normal and high) were utilized to investigate the overall effect of water holding capacity of high pH on beef jerky.

Therefore, the objective of this study was to manufacture beef jerky, under uniform processing conditions, from inside rounds obtained from two carcass classes (Fed and Cow) and two pH types (Normal (<5.3 to 5.7) and High (>6.0) and determine finished product attributes with a primary interest in finished product pH, water activity, proximate composition, and moisture:protein ratio, and a secondary interest in color (L*, a*, b*) and percent cook yields.

4.2. Materials and methods

Beef inside round subprimals (IMPS #168) were obtained from two classes (fed or cow) and two types (normal or high pH) of beef carcasses (n=24). The four beef carcass categories were identified as: 1) normal (pH 5.3 to 5.7) fed beef carcasses (n=6); 2) dark cutting (pH > 6.0) fed beef carcasses (n=6); 3) normal (pH 5.3 to 5.7) cow carcasses (n=6), and 4) dark cutting (pH > 6.0) cow carcasses (n=6). Classification of beef carcasses as normal or high pH was conducting by determining the pH value of the posterior side of the $12^{th}/13^{th}$ rib interface of each carcass via a hand held pH probe. For this study, a total of 48 (n = 12 per carcass class and pH type) beef inside rounds were

obtained from the four beef carcass categories and transported to the Texas A&M University (TAMU) Rosenthal Meat Science and Technology Center (RMSTC). It was noted that pH evaluated at the *m. longissimus lumborum* of utilized carcasses, was not always the same for inside rounds. Therefore, the number of high pH dark cutting inside rounds differed from the number of carcasses.

4.2.1. Jerky manufacturing

Upon arrival at TAMU RMSTC, beef inside rounds were trimmed of excess fat and connective tissue. Additional pH determinations were made on the beef inside rounds by inserting a probe into the left, center, and right sections of the muscle and recording the pH value of each section (three readings total per inside round) which were averaged to obtain an overall pH reading for each inside round. The inside rounds were then vacuum packaged, placed in cardboard boxes and frozen (-20°C) for two weeks. Each jerky manufacturing replication (4) included slicing frozen inside rounds (3 inside rounds per carcass class and pH type; n=12) on a commercial band saw to produce 0.25 cm thick slices which were placed on plastic trays, allowed to temper at 2°C for 4 hours, cut into uniform strips (~15 cm x 7.5 cm), placed on plastic trays and allowed to thaw for 12 hours (2°C). The uniform strip sizing was conducted to attempt to minimize any potential drying differences due to non-uniform product surface area. Excess trimmings generated from fabricating uniform jerky slices were used to determine the raw proximate composition of each individual inside round. Jerky slices from each carcass class and pH type were placed in a vacuum bag, weighed (weight of bag not included) and then a commercial jerky brine solution (Table 10) was added at 15% of the sliced jerky raw

Table 10.
Commercial brine formulation used for the manufacture of jerky from inside rounds obtained from fed and cow, normal and high pH carcasses.

Dry Ingredients	% in Brine	Pounds	Ounces	Grams	% in Product	pH/PPM[a]
Commercial Jerky Seasoning	20.00	3.00	48.00	1360.80	3.00	
Restricted Ingredients						
Prague Powder (6.25% sodium nitrite)	1.67	0.25	4.00	113.40	0.25	5.6/156
Liquid Ingredients						
Water	78.33	11.75	188.00	5329.80	11.75	
Total Brine	100.00	15.00	240.00	6804.00	15.00	

PPM[a] = parts per million of sodium nitrite.

weight. The bagged jerky slices were sealed while drawing a slight vacuum then rebagged and sealed again ("double bagged and tagged").

All twelve bagged jerky treatments were placed in a horizontal Leland vacuum tumbler and tumbled continuously for 15 min at 4 rpm and 25 PSI vacuum. After tumbling the bagged jerky slices were removed from the tumbler and allowed to equilibrate for 24 hr (2°C). After equilibration, each bag of marinated jerky strips were placed flat on horizontal stainless steel racks (one treatment per rack), weighed (stainless steel rack weight accounted for), the racks placed on a smoke truck and the smoke truck placed in an Alkar single-truck smokehouse. The marinated slices were thermally

processed (cooking and drying; Table 11) until a water activity (A_w) value of <0.85 was reached for the fed, normal pH jerky strips (considered the normal raw material for jerky production). Temperature was also monitored by wrapping a single jerky slice (fed, normal pH) around each smokehouse internal temperature probe (2 probes). No smoke was used so that unbiased color differences due to carcass type and muscle pH could be determined. Samples of fed, normal pH jerky were removed during the last 30 min of thermal processing to monitor A_w and thermal processing stopped once the A_w reached <0.85.

After thermal processing the jerky slices were removed from the smokehouse and chilled to 2^oC, weighed (weight of racks accounted for), removed from the stainless steel racks, analyzed for cooked jerky color (L*, a* and b* values), vacuum packaged (5 slices per bag) and refrigerated (2^oC), until analyzed for water activity, pH, cooked moisture and protein percent, moisture:protein ratio, and cook yields.

4.2.2. Sample preparation

Raw and cooked jerky samples were finely cut, immersed in liquid nitrogen, powdered using a Waring blender, placed in Whirl-pak bags and stored frozen until subsequent analyses were conducted.

4.2.3. pH

The pH of raw beef inside rounds was determined at 2^oC using an Acumet pH Meter calibrated with phosphate buffers 4.0 and 7.0. Duplicate, 10 g powdered cooked jerky samples were placed in a plastic containers with 90 mL of distilled and deionized water, mixed with a Polytron homogenizer and measured by the slurry method adapted for meat products using an Orion^{TM} (Model 720A Orion Research, Inc.) pH meter

Table 11.
Thermal processing schedule used for the manufacture of jerky from inside rounds obtained from fed and cow, normal and high pH carcasses.

Step	**Time**	**Dry Bulb(C)**	**Wet Bulb(C)**	**Relative humidity**	**Blower Speed**	**Smoke**	**Internal Temperature(F)**	**A_w[a]**
1	15 min	63	0		40	OFF		
2	15 min	77	0		40	OFF	39	
3	60 min	77	57	42	40	OFF	52	
4	60 min	77	0	0	40	OFF	59	
5	---	77	0		40	OFF	70	< 0.85

[a] A_w = water activity

calibrated with phosphate buffers 4.0 and 7.0.

4.2.4. Water activity

Approximately 8 g of powdered raw and cooked jerky samples were placed into plastic water activity disposable cups, placed into the water activity chamber, sealed and sample water activity was measured with an Aqualab meter (Model 3, Decagon Devices, Inc., Pullman, WA.) calibrated at ambient temperature (20°C) with distilled water (a_w = 0.999) and saturate solutions of NaCl (a_w = 0.756) and NaCl (a_w =0.984). Triplicate samples of each round assigned to a carcass class and pH type were taken.

4.2.5. Color

Color measurements were taken on randomly selected raw and cooked jerky slices. Color was measured using a color Hunter Miniscan XE (Hunter Labs, Inc. Reston, VA; Illuminant A, 10° observer) set for L* (lightness), a* (redness), and b* (yellowness). Four readings per slice were taken.

4.2.6. Percent moisture and fat

Proximate analysis for fat and moisture was performed in triplicate within 48 hr as described by AOAC (2005a) air-dry oven and soxhlet ether extraction methods. Powdered raw and cooked jerky samples (~ 2.5 g) were placed in pre-weighed, pre-dried paper thimbles (Whatman #2) re-weighed, and recorded. Samples were dried for 16h at 100°C, cooled to room temperature in a dessicator, and weights were recorded. Percent moisture was calculated as the difference between wet weight and dried sample weight divided by sample weight. Oven dried samples (3 samples per treatment)) were placed in a soxhlet apparatus and fat extracted using petroleum ether for approximately 12 h. with an ether drip rate of 3 drops per second and a reflux rate of 1 per 5 min. Percent fat was

determined by calculating the difference between dried sample weight and extracted sample weight divided by sample weight.

4.2.7. Percent protein

Protein was determined using the LECO Protein/Nitrogen Analyzer (Model FP 528, LECO Corporation, St. Joseph, MI) to determine nitrogen content as described by AOAC (2005b). The percent protein was be calculated by multiplying the percent nitrogen times 6.25, the protein conversion factor for meat and poultry. Each raw and cooked sample moisture and protein measurement was performed in triplicate.

4.2.8. Moisture: protein ratio

After determining the percent moisture and protein of cooked beef jerky samples, the moisture protein ratio was determined for all jerky manufactured from each round assigned to carcass class and pH type by dividing the percent moisture by the percent protein. The USDA standard of identity for beef jerky is 0.75:1 moisture:protein ratio.

4.2.9. Cook yield

Cook yields were determined for each carcass class and pH type per replication. The following formula was used: [(marinated raw weight – cooked weight/marinated raw weight) x 100].

4.2.10. Warner Bratzler shear force determination

Jerky samples were analyzed for shear force measurements using the Universal Machine (Model No. SSTM-500; Huntington Beach, CA). Three samples (2.54 cm in length x 1.27 cm in width) for each treatment was used in triplicate. Shear force was conducted using a slice shear blade at a cross speed of 200 mm/min with a 500 load cell.

4.2.11. Targeted processing parameters

The goal of beef jerky manufacture was to attain a A_w of <0.85, a moisture:protein ratio of 0.75:1 and a cook yield of 40% for the fed, normal pH beef jerky treatments (baseline jerky). These targeted parameters served as the basis to determine differences between carcass class and pH type among jerky treatments.

4.2.12. Statistical analysis

Data were analyzed using PROC GLM of SAS (SAS Institute; Cary, NC). A 2 x 2 factorial design was used with beef carcass class (fed or cow) and type (normal or high pH) as the main effects. A total of three rounds per class and type category (total of n =12) were manufactured on each production day. Significant main effect means ($P<0.05$) were separated using Tukey's Honest Significant Difference. Based on the pH of M. *longissimus lumborum,* muscle pH varied from inside rounds. Upon determining the pH of the inside rounds it was observed that the inside round high in pH ranged from 5.5 to 7.2. Therefore, further statistical analyses were conducted to further compare differences among high pH (6.0 – 6.4) and extremely high pH (6.5. Differences within high pH were determined using nonorthogonal contrasts.

4.3. Results and discussion

4.3.1. Raw beef jerky slices

In comparing raw beef jerky slices from fed and cow carcasses (Table 12) inside rounds originating from fed beef carcasses had greater percent fat which would be due to feeding regime (grain vs. forage fed). The pH values for raw beef jerky slices (representing normal and high pH, Table 12) were higher for high pH jerky slices. Wide

ranges existed for pH: 5.4 – 6.9 for fed inside rounds and 5.4 – 7.2 for cow inside rounds, respectively. However, regardless of the variation in pH, water activity was not impacted

Table 12.
Main effect means of carcass class (Fed and Cow) and for pH type (Normal and High) for pH and fat of raw beef jerky slices.

Traits	**Class**		
	Fed	Cow	SEM[c]
Fat, %	2.08[a]	1.34[b]	0.08
	Type		
	Normal pH	High pH	SEM[c]
pH	5.57[b]	6.08[a]	0.07
Fat, %	1.83[a]	1.44[a]	0.08

[a,b] Means within class and type rows lacking a common superscript letter differ ($P < 0.05$) using Tukey
[c]SEM = standard error of the means

($P > 0.05$) by either the main effects (carcass class and pH type) or their interaction. Table 13 shows a two-way interaction of carcass class (fed and cow) by pH type (normal and high) for percent moisture and protein. Within fed and cow class, jerky slices high in pH contained 1.83% and 3.25%, respectively greater moisture compared to normal pH jerky slices. High pH values in meat products are associated with higher-water holding capacity, which would explain slightly higher percent moisture in both high fed and cow jerky slices. Percent protein did not increase for inside rounds originating from fed beef carcasses nor did color space values differ ($P > 0.05$) for lightness (L*) redness (a*), and yellowness (b*).

4.3.2. Cooked beef jerky slices

Table 14 showed that fed beef jerky slices contained slightly greater percent fat. This could be in part of the different marbling scores between fed and cow carcasses. Overall, fed beef carcasses averaged Select marbling at the $12^{th}/13^{th}$ rib interface whereas

Table 13.
LSMeans of carcass class (Fed and Cow) and pH type (Normal and High) for moisture and protein of raw beef jerky.

Trait	**Fed-Normal**	**Fed-High**	**Cow-Normal**	**Cow-High**	**SEM**[d]
Moisture, %	73.34^{c}	75.17^{b}	74.64^{b}	77.89^{a}	0.23
Protein, %	22.64^{ab}	22.11^{b}	23.14^{a}	21.45^{c}	0.22

[a,b,c] Means within same row lacking a common superscript letter differ ($P < 0.05$) using Tukey
[d]SEM = standard error of the means

Table 14.
Main effect means of carcass class (Fed and Cow) and pH type (Normal and High) for pH, water activity, percent fat, redness, and shear force values of cooked beef jerky.

Traits	**CLASS**		
	Fed	Cow	SEM[c]
Fat, %	4.86^{a}	3.46^{b}	0.32
a*(redness)[d]	11.88^{a}	9.24^{a}	0.93
	TYPE		
	Normal	High	SEM[c]
pH	5.57^{a}	6.07^{b}	0.07
Aw	0.83^{b}	0.85^{a}	0.01
Fat, %	4.88^{a}	3.48^{b}	0.31
Shear Force, kg / sample	29.90^{a}	23.43^{b}	2.12

[a,b] Means within class and type row lacking a common superscript letter differ ($P < 0.05$) using Tukey
[c]SEM = standard error of the means
[d]a* = redness values

cow carcasses averaged Practically Devoid. Although not statistically different, redness (a*) values for cooked beef jerky slices obtained from fed and cow inside rounds were slightly higher (11.88 vs. 9.24).

Cooked beef jerky pH values (Table 14) obtained from normal and high pH rounds were higher ($P < 0.05$) than normal pH jerky slices that resulted in a slightly higher water activity level (0.85 vs. 0.83). Water activity values indicate that under uniform beef jerky processing conditions raw material variation (i.e., high pH) can result in jerky slices within a batch failing to meet food safety requirements ($A_w < 0.85$). Shear force values between pH types significantly showed high pH was more tender than normal, which may be related to higher moisture and water activity.

During thermal processing, high pH jerky slices (Table 15) retained more moisture upon cooking/drying compared to normal cooked beef jerky. However, jerky of the fed high category retained the greatest percent moisture that led to higher M:P ratio exceeding the standard of identity requirements. Additionally, jerky from fed high pH inside rounds resulted in the highest percent cook yield followed by cow normal then fed normal. On the other hand, jerky manufactured from cow high in pH resulted in the lowest percent cook yield. Regardless of level of pH, cow inside rounds were thinner and smaller in size compared to fed inside rounds, which may have contributed to the lowest percent cook yield and the least in thickness.

In order to determine the true effects of high pH on jerky attributes, the high pH type was broken down into two categories: 1) high (6.0 – 6.4) and 2) extremely high (6.5) (Table 16). Significant differences existed for pH, redness (a*), yellowness (b*), and shear force values. Overall, inside rounds in the extremely high category had an overall

pH ($P < 0.05$) of 6.78 compared to high pH jerky that had 6.17 (Table 16). However, even with pH differing ($P < 0.05$), water activity was not statistically different ($P > 0.05$). Jerky slices of the extremely high category were slightly higher than high pH jerky slices. Interestingly, the high pH category was well below food safety requirements whereas jerky extremely high in pH was not (0.69 vs. 0.88). It is possible that jerky having a pH between 6.0 and 6.4, is more likely of meeting a water activity level of < 0.85 without additional drying times. Jerky in the extremely high category resulted in a higher percent moisture (42.32 vs. 35.13%) but slightly lower protein content (50.36% vs. 51.94%). With a 7% percent protein difference, it is to no surprise that inside rounds representing extremely high pH resulted in a higher moisture to protein ratio of 0.88. Naturally, beef products manufactured from high pH raw materials would be expected to appear darker and less red in color. Within both high pH categories, color values (L*, a*, and b*) were lower than normal pH jerky slices. Jerky slices in the extremely high category were interestingly slightly higher (23.95) in L* (lightness) values compared to the high (18.90) category. It was also observed, though not measured, that high pH jerky slices had a shiny (glossy) exterior, which may explain high L* values caused by light reflection. Additionally, extremely high pH jerky slices resulted in a less red color with a lower degree of yellowness. These findings confirm what Page, Wulf, and Schwotzer (2001) reported that a* (redness) and b* (yellowness) were more closely associated with muscle pH than L*. This apparently holds true for jerky manufactured form high pH inside rounds used in this study. Gault (1985) noted that muscles high in pH were indirectly shown to be more tender than normal pH muscles. Tenderness increased as water-holding capacity increased due to the swelling characteristics of the muscle fibers, especially with

swelling occurring at a little more than 50% of muscle fiber size (Gault, 1985). With a pH 6.5 and 42% moisture content, the same results were observed in that shear force values were much lower in the extremely high jerky category.

Table 15.
Two-way interaction of carcass class (Fed and Cow) and pH type (Normal and High) for moisture, protein, moisture:protein ratio, cook yield, yellowness (b*), and thickness values of cooked beef jerky.

Trait	**Fed-Normal**	**Fed-High**	**Cow-Normal**	**Cow-High**	**SEM[d]**
Moisture, %	29.57[b]	40.42[a]	32.40[b]	33.77[ab]	1.85
Protein, %	56.32[a]	49.50[b]	54.45[ab]	54.81[ab]	1.66
M:P[e]	0.54[b]	0.83[a]	0.61[b]	0.65[ab]	0.05
Cook Yield, %	37.60[b]	42.95[a]	38.48[b]	34.91[c]	1.21
b* (yellowness)[f]	5.92[a]	3.71[ab]	3.10[b]	3.71[ab]	0.69
Thickness, mm	3.38[a]	3.17[a]	3.00[a]	2.08[b]	0.18
Kg/g sample	26.63[ab]	16.28[b]	26.61[ab]	33.61[a]	4.00

[a,b,c] Means within same row lacking a common superscript letter differ ($P < 0.05$) using Tukey
[d]SEM = standard error of the means
[e]M:P = moisture to protein ratio
[f]b* = yellowness values

Table 16.
LSMeans (SEM)[a] and main contrasts for pH, Aw, proximate composition, moisture:protein ratio, and color space values from high (6.0 – 6.4) and extremely high (6.5) inside rounds.

	pH Type		
Trait	High	Extremely High	P - value
pH	6.13 (0.13)	6.78 (0.10)	0.0104
Aw	0.82 (0.04)	0.86 (0.03)	0.5008
Moisture, %	35.13 (3.52)	42.32 (2.78)	0.1762
Protein, %	51.94 (3.59)	50.36 (2.84)	0.9598
M:P[b]	0.69 (0.11)	0.88 (0.09)	0.3185
Fat,%	3.89 (1.30)	2.58 (1.03)	0.1465
L*[c]	18.90 (4.07)	23.95 (3.21)	0.5123
a*[d]	12.04 (1.04)	10.23 (0.82)	0.0432
b*[e]	3.89 (0.41)	2.97 (0.32)	0.0296
Shear force, kg/sample	24.67 (2.18)	14.95 (1.99)	0.0109

[a]SEM = standard error of the means
[b]M:P = moisture to protein ratio
[c] L* = lightness color values
[d]a* = redness color values
[e]b* = yellowness color values

CHAPTER V

EVALUATION OF FRANKFURTERS MANUFACTURED FROM HIGH AND NORMAL pH BEEF

5.1. Introduction

According to the National Hotdog and Sausage Council (2007), consumers spent more than $4.1 billion on hot dogs and sausages with one hundred and fifty million hot dogs consumed on Independence Day alone. Frankfurters can be made of beef, pork, and/or chicken. Beef frankfurters can consist of beef trimmings from either fed and/or cow trimmings. According to the 2007 National Beef Market Cow and Bull Quality Audit, fifty one percent of a carcass (cow or bull) is used as trim with the remaining forty nine percent left to be used as whole muscle products. However, there is a muscle quality discrepancy in utilizing trimmings or whole muscle products originating from bull or cow carcasses. Cows usually are fed a forage diet most of their lifetime. In comparison to grain-based diets, forage-based (grass) diets do not contain as much starch that can be broken down into glycogen for muscle energy. Therefore, an insufficient amount of muscle energy (glycogen) is available at time of slaughter. If the animals underwent some type of long term stress (extreme weather conditions, long transportation, reduced access to food and/or water) prior to slaughter these two factors (low muscle energy and stress) increases the likelihood of creating a higher ultimate muscle pH (6.0) (Warris, 1990; Immonen, Ruusunen, Hissa, & Puolanne, 2000).

Garcia, Lawrence, Hale and Savell (2009) investigated the prevalence of high muscle pH (6.0) in fed and cow carcasses in Texas. Of the cow carcasses surveyed (n=435) ~41% exhibited ultimate muscle pH (in the *M. longissimus lumborum*) of 6.0 or

greater while ~90% of the fed beef carcasses (n=1,885) were within a normal ultimate muscle pH range (5.4 – 5.8).

Muscles high in pH are often associated with the quality defect known as dark, firm, and dry (DFD). DFD muscles appear dark in color, firm in texture and dry at the muscle surface. Despite the darker colored lean appearance, muscles high in pH do exhibit higher water holding capacity compared to muscles normal (5.4 – 5.8) in pH (Joo, Kaufmann, Kim, & Kim, 1995). One study reported that appearance and palatability can be sacrificed in DFD muscle (Wulf, Emnett, Lehska, & Moeller, 2002). In 2002, Wulf et al. found that DFD *M. longissimus lumborum* steaks had substantially lower tenderness scores (via sensory panel) than normal steaks and were tougher compared to *M. gluteus medieus* (33%); and *M. semimebranosus* (36%). However, the sensory panel noted that neither juiciness nor flavor was significantly ($P > 0.05$) affected by DFD meat. Extensive research (Egbert & Cornforth, 1986; Wulf et al, 1999; Wulf, Emnett, Lehska, & Moeller, 2002; Viljoen, Knock, & Webb, 2002) has been conducted to investigate the functional (water holding capacity) and sensory (flavor, juiciness, and tenderness) attributes of DFD beef in whole muscle products, however, limited research has been investigated regarding comminuted products. Water holding capacity (WHC) is the ability of meat to retain its moisture during application of external forces (Aberle, Forrest, Gerrard, & Mills2001). Raw materials (muscle) with acceptable WHC will result in less purge (moisture loss) in fresh muscle products, and an increase in cook yields and juiciness (cooked product).

In a comminuted product, such as low-fat frankfurters, water holding capacity is critical. Alkaline phosphates are a common non-meat ingredient used to increase WHC

by initially increasing muscle pH and ionic strength (Hamm, 1970). In 2008, McKeith, Holmer, Boler, Killefer, and Jones investigated the affects of pH in frankfurters. Raw beef materials normal (5.4 - 5.8) in pH was altered using an acidic, neutral, and alkaline phosphates. Frankfurters with alkaline phosphates had higher water-holding capacity that resulted in frankfurters that were harder in texture, lighter (L* lightness) in color, and were more acceptable by consumers.

Currently, consumer demand in the United States for organic foods is increasing as shown in a 2008 survey conducted by the organic industry (Organic Trade Association, 2009). The survey showed that purchases of U.S. organic products rose 17% from 2007 to 2008 with 3.5% of all food product sales accounted for in the United States. According to the Food Standards and Labeling handbook, synthetic ingredients cannot be used in a product that is labeled "natural." Consequently there is a trend in reducing and/or eliminating phosphates in "natural" products (Food Standards and Labeling, 2005).

Since 51% of all beef/cow carcasses generated trimmings for further processing (Nicholson, 2008) and a large percentage of these carcasses exhibit high muscle pH (Garica, Lawrence, Hale, & Savell, 2009) there may be an opportunity to utilize these higher pH trimmings as a potential "phosphate replacement" in a comminuted product such as a frankfurter. We hypothesized that high pH cow trimmings may be a potential substitute for alkaline phosphates. This study focused on investigating the effects of high pH cow trimmings on water holding capacity of frankfurters.

The objective of the current study was to evaluate the effect of high pH cow trimmings on emulsion stability, cooked yield, color, lipid oxidation, textural and sensory attributes of beef frankfurters.

5.2. Materials and methods

Shoulder clods (IMPS #114) and short plates (IMPS# 121) were obtained from cow carcasses representing two pH levels: 1) normal pH (5.4 to 5.8), and 2) high pH (6.2). Classification of carcasses in these pH categories was conducted by averaging three pH readings from three muscles: *M. Teres major*, *M. Triceps brachii*, and *M. Infraspinatus* via a hand held pH probe. Shoulder clods and short plates were obtained from a commercial cow/bull plant and transported to the Texas A&M University (TAMU) Rosenthal Meat Science and Technology Center (RMSTC) vacuum packaged and stored (4°C) for two weeks.

5.2.1. Frankfurter manufacture

Shoulder clods were trimmed of excess fat and connective tissue. Additional pH determinations were made on the shoulder clods by inserting a hand held pH probe (3.5 mm OD; Meat Probes, Inc., Topeka, KS) into the three main muscles (*M. Teres major*, *M. Triceps brachii*, and *M. Infraspinatus)* and recording the pH value of each muscle. The pH values were averaged to obtain an overall pH value. Shoulder clods (lean component) and short plates (fat compoenent) were ground separately through a 1.27 cm plate, followed by a second grind using 0.48 cm plate. High pH shoulder clods and short plates were ground first followed by normal pH clods and short plates.

Frankfurters were formulated using the appropriate amounts of normal and/or high pH clods and short plates to achieve a 13.6 kg meat block at a 10% fat content (raw

basis). Six treatments were formulated: 1) 100% high pH (100H); 2) 75% high pH/25% normal pH (75H/25N); 3) 50% high pH/ 50% normal pH (50H/50N); 4) 25% high pH/75% normal pH (25H/75N); and 5) 100% normal pH (100N), and 6) Control (normal pH with phosphate; C) (Table 17). Meat trimmings and non-meat ingredients were added (Table 14) then chopped in a bowl chopper (Type K64V-VA, Seydelman, Germany), then stuffed into 30 mm dia collagen casing (Dewied International Inc., San Antonio, TX) to produce links weighing approximately 86 g each. Frankfurter batches were weighed individually, hung on stainless steel rods were then placed on a smoketruck and thermally processed in a single truck smokehouse (Model 1000, Alkar, Lodi, WI, U.S.A.) until an internal temperature of 71°C was reached (Table 18). Upon cooking, frankfurters were showered with water to reduce product temperature to 37°C and placed in a 4°C cooler for 12 h. Frankfurter batches were reweighed, hand peeled, vacuum packaged (Grip & Tear Easy Open CRYOVAC, Sealed Air Corporation, Duncan, SC 29334), labeled and stored at 4°C until further analysis (Day 0, 7, 14, 28, 56).

5.2.2. Analysis of raw emulsion – day 0

5.2.2.1. Emulsion stability

Emulsion stability was measured as described by Townsend, Witnauer, Riloff, and Swift (1968). Thirty-four grams of raw emulsion from each treatment was stuffed in a 50cc polycarbonate syringe tube, weighed and covered. Tubes were placed in a 48.8°C water bath until 68.8°C internal temperature (1.25 to 1.5 h) was reached. Water bath temperatures automatically increased 4 degrees every 15 min. until 68.8°C internal temperature was reached. Temperature was monitored by placing a thermocouple in the geometric center of one “test” sample. Once internal temperature has been reached, the

liquid released of test tube frankfurters during cooking was decanted into15 mL graduated centrifuge tubes and centrifuged at 5,500 rpm (3,659 g's) for 10 min. After centrifugation, total volume, fat volume, volume of gel water, and proteinaceous solids was recorded. Samples were in triplicates where averages were taken per treatment and reported as volume lost per 100 g of test tube frankfurters.

5.2.2.2. Hydration

Thirty three grams of raw emulsion was placed into a 50 cc centrifuge tubes and placed in a pre-chilled (2°C) Sorvall SA-600 Rotor and a Sorvall RC 5B centrifuge (Du Pont Company, Wilmington, DE) and centrifuged at 15,000 rpm (27,000 x g) for 15 min. The supernatant was decanted through one layer of cheese cloth into pre-weighed 50 cc polycarbonate tubes followed by hydration determination. Hydration was expressed as g/water held/ g wet tissue.

5.2.2.3. pH

Raw emulsion pH (2°C) was determined using a pH meter (IQ Scientific Instruments, Inc. Reston, VA) and internal probe (piercing tip micro probe with heavy duty handle, Model PH57-SS, IQ Scientific Instruments, Inc. Reston, VA) calibrated with buffers 4.01 and 7.0. Three readings were taken and averaged per treatment.

5.2.2.4. Percent moisture and fat

Proximate analysis for fat and moisture was performed in triplicate within 48 hr as described by AOAC (2000a) air-dry oven and soxhlet ether extraction methods. Powdered raw and cooked frankfurters samples (~ 2.5 g) were placed in pre-weighed, pre-dried paper thimbles (Whatman #2) re-weighed, and recorded. Samples were dried for 16h at 100°C, cooled to room temperature in a dessicator, then weights were

Table 17.
Frankfurter formulations (kilogram and percent basis) for a 14.97 kg batch weight at a 10% fat level.

Ingredient	Teatment[d]					
	100H	75H/25N	50H/50N	25H/75N	100N	C
Ingredients						
Lean Source, kg	10.21	7.62	5.13	2.54	0.00	0.00
High pH , 3% fat	0.00	3.40	6.80	10.21	13.61	13.61
Normal pH , 3% fat	3.40	2.59	1.68	0.82	0.00	0.00
Fat Source , kg	0.00	0.00	0.00	0.00	0.00	0.00
High pH , 24 % fat						
Normal pH, 24 % fat						
Non-meat Ingredients, g (%)[e]						
Phosphate [a]	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	39.73 (0.35)
Salt	204.3 (1.80)	204.3 (1.80)	204.3 (1.80)	204.3 (1.80)	204.3 (1.80)	204.3 (1.80)
Spice (no salt added)[b]	56.75 (0.50)	56.75 (0.50)	56.75 (0.50)	56.75 (0.50)	56.75 (0.50)	56.75 (0.50)
Cure Salt[c]	28.38 (0.25)	28.38 (0.25)	28.38 (0.25)	28.38 (0.25)	28.38 (0.25)	28.38 (0.25)
Ice/Water	3405 (30.00)	3405 (30.00)	3405 (30.00)	3405 (30.00)	3405 (30.00)	3405 (30.00)

[a] Brifisol 450 (BK Guilini; Simi Valley, CA)
[b] Bolonga/Frank Seasoning (Blend 125), with 0.03% included Na Erythorbate. A.C., Legg, Inc., Calera, AL, U.S.A.
[c] D.Q. Curing Salt, Butcher & Packer Supply Company, Detroit, MI., U.S.A.
[d]Treatment: 100H = 100% high pH; 75H/25N = 75% high pH / 25% normal pH; 50H/50N = 50% high pH/ 50% normal pH; 25H/75N = 25% high pH / 75% normal pH; 100N = 100% normal pH; and C = 100% normal pH with added 0.35% phosphate.
[e] Non-meat ingredients (g, %) were calculated using weight of the meat block (13.61 kg).

Table 18.
Thermal processing schedule used for manufacture of beef frankfurters.

Step	Dry Bulb (°C)	Wet Bulb (°C)	Time	Damper	% Relative Humidity
1	54	41	30 min	Auto	45.6
2	60	46	15 min	Auto	45.5
3	66	49	15 min	Closed	40.0
4	71	54	15 min	Closed	42.1
5	77	63	15 min	Closed	51.8
6	82	71	71C Int.	Closed	61.5

recorded. Percent moisture was calculated as the difference between wet weight and dried sample weight divided by sample weight. Oven dried samples (3 samples per treatment)) were placed in a soxhlet apparatus and fat extracted using petroleum ether for approximately 12 h. with an ether drip rate of 3 drops per second and a reflux rate of 1 per 5 min. Percent fat was determined by calculating the difference between dried sample weight and extracted sample weight divided by sample weight.

5.2.2.5. Percent protein

Protein was determined using the LECO Protein/Nitrogen Analyzer (Model FP 528, LECO Corporation, St. Joseph, MI) to determine nitrogen content as described by AOAC (2000b). The percent protein was be calculated by multiplying the percent nitrogen times 6.25, the protein conversion factor for meat and poultry. Each raw and cooked sample moisture and protein measurement was performed in triplicate.

5.2.3. Analysis of cooked frankfurters – days 0, 7, 14, 28, and 56

5.2.3.1. pH

The pH of frankfurters was conducted as previously described.

5.2.3.2. Color

Individual frankfurters were randomly selected for external and internal color measurements that was taken in triplicate. Color was measured using a color Hunter Miniscan XE (Hunter Labs, Inc. Reston, VA; Illuminant A, 2° observer) CIE color space values set for L* (lightness), a* (redness), and b* (yellowness). Six readings per treatment were taken.

5.2.3.3. Purge

On each storage day, packaged frankfurters (4 frankfurters per package) from each treatment were weighed (total packaged weight). Frankfurters were removed from the package and a paper towel used to remove excess moisture from both the individual frankfurters and the empty pouch. The frankfurters and pouch were reweighed separately and their weights recorded to calculate percent purge.

Purge, % = (Weight of dry frankfurters and bag / Initial total weight) * 100.

5.2.3.4. Chemical analysis

Proximate analysis for frankfurters (moisture, fat and protein) were conducted as previously described.

5.2.3.5. Lipid oxidation

Lipid oxidation was determined on frankfurter samples using the 2-thiobarbituruc acid reactive substances (TBARS) method of Tarladgis et al. (1960) as modified by Rhee (1978) for cured meat products. Thirty grams of sample was blended with 43.5 ml of

distilled water, 1.5 ml of sulfanilamide reagent and 15 ml of 0.5% solution of propyl gallate (PG) and ethylenediamine tetraacetic acid for 2 min. A 30 g slurry was transferred to a 500 ml Kjeldahl flask using 78 ml of 50°C distilled water. The Kjeldahl flask containing slurry was placed on a distillation unit and heated until 50 ml of distillate was collected. Five ml of distillate and 5 ml of thiobarbituric acid (TBA) reagent was added to a glass (x CC) screw cap test tube. The tubes were capped, placed in a large beaker containing water and boiled on a hot plate for 35 min. to develop color. Test tubes were removed from the beaker, cooled in tap water for 10 min., vortexed and approximately one mL of solution placed in a cuvettte. Absorbance was measured at 530 nm using an UV-violet spectrophometer (Model Cary 300 BIO-UV- Visible Spectrophometer, Varian Instruments, Walnut Creek, CA 94598). Absorbance readings were multiplied by a factor of 7.8 and the results were reported as mg of malonaldehyde per kilogram of sample.

5.2.3.6. Sodium analysis

Sodium analysis was conducted on days 0 and 56. Frankfurter samples (10 g) and 90 ml of distilled water were blended for 30 sec. using a stainless steel hand-held blender (Cuisinart, Model CSb-77, East Windsor, NJ). The diluted sample was transferred to a 150 ml beaker with 10 ml of ISA (Sodium Ionic Strength Adjuster) and continuously stirred. Readings were measured using a ROSS® Sodium Ion Selective Electrode (Model 8611BN). Concentration values were recorded in triplicate as ppm then converted as a percentage. Percent sodium was calculated as the follows:

% Sodium = (sodium ppm * 0.0001)

5.2.3.7. Nitrite analysis

Residual nitrite (AOAC, 2000) was determined by combining 5 g samples with 40 ml of distilled water and heated to 80°C in a 100 mL beaker. The samples were transferred to a 500 ml volumetric flask and 300 ml of hot distilled water added. The flasks were placed in a water bath and boiled for 2 h. Sample flasks were cooled to room temperature then filtered through two Whatman No. 2 filter papers into a into 25 ml flask. Twenty five ml of the filtered sample was transferred into a 50 ml volumetric flask with 2.5 ml sulfanilamide reagent and allowed to stand for 5 min. Afterwards, 2.5 ml N-(1-naphthyl) ethylene diamine (NED) was added and the sample diluted to volume and allowed to stand for an additional 15 min. to allow color development. Absorbance was measured at 540 nm using an UV-visible spectrophotometer (Model Cary 300 Bio, Varian Instruments, Walnut Creek, CA 94598). Residual nitrite was then converted to parts per million (ppm) by multiplying by 200 (200 dilutions).

Nitrite ppm = (residual nitrite * 200)

5.2.3.8. Microbiological analysis

Vacuum packaged frankfurters (3 frankfurters / package) were sterilized by spraying the exterior top edge of the packaging with 70% ethanol and then wiped with a paper towel. The packages were then opened aseptically using flame sterilized forceps and scalpels to which 99 ml of 0.1% peptone diluents were added. Each package was then hand massaged for one min. Aerobic plate counts were determined by plating 1 ml of the sample rinse and 1 ml of the appropriate 10-fold dilutions of the same on Petrifilm™ aerobic count plates (3M, St. Paul, MN). Lactic acid bacteria counts were determined simultaneously by dispensing 1 ml of the sample rinse and the appropriate

10-fold dilutions of the same into sterile petri dishes to which Lactobacilli MRS Agar (Difco, Detroit, MI) was added and allowed to solidify. An additional MRS overlay was added to each plate. Both Petrifilm™ and MRS plates were incubated at 30°C for 72 h before counting and reporting CFU per package.

5.2.4. Sensory and textural analysis - day 28

5.2.4.1. Torsion analysis

Torsion analysis was performed following methods performed by Bourne (1978) using a digital viscometer modified with a torsion fixture (Brookfield viscometer, model 5XHBTD, London, England). Samples were removed from vacuum package bag and allowed to sit at room temperature for at least 30 min (Kim, Hamann, Lanier, and Wu, 1986). Samples were cut into 5 – 28 mm disks. Plastic torsion disks, assigned for torsion testing, were affixed to the and of each sample by way of cyanoacrylate glue, milled into a dumbbell shape with a midsection diameter of 100 mm, and placed on the torsion apparatus. Shear stress (kPa) and strain at gel failure was determined as a mean of five samples.

5.2.4.2. Textural profile analysis (TPA)

Packaged frankfurters were removed from the cooler and allowed to equilibrate to room temperature. Frankfurters were removed from the package and cut into 14 mm disks. Four disks were randomly chosen per treatment which was then compressed to 75% of their original height using the Instron Universal Testing Machine (Model 1130, Instron, Corp., Canton, MA 02021) Force-time deformation curves were recorded at a crosshead speed of 50 mm/min and full-scale 200 N load cell. The first compression peak value was reported as Hardness-1. The second peak value represents Hardness-2.

Cohesiveness was determined as the ratio of the area under the first compression curve to the area under the second compression curve. Gumminess was calculated as the product of hardness x cohesivenss. Chewiness was calculated as the product of gumminess × springiness.

5.2.4.3. Sensory analysis

A 5-member, trained, descriptive attribute panel evaluated samples defined by AMSA (1995). Texture attributes were evaluated by trained panelists using the Spectrum Intensity Scale (Meilgaard, Civille, & Carr, 2007). Ballot development sessions were conducted by trained panelists.

On the day of testing, frankfurters were removed from their packages and placed in boiling water for 5 min. Frankfurters then were removed from the water and cut into 1.27 cm slices. Three slices were immediately served to trained panelists. Six samples were evaluated per day with a 5 min. gap between samples. Treatment samples were assigned a randomized three digit code and a randomized order on testing day. Panelists were placed in individual booths separated from preparation area. A warm-up sample was provided to panelists to standardize and calibrate the panelists each day. In order for panelists to cleanse their palettes, fat-free ricotta cheese, salt-less saltine crackers and double distilled water was provided.

5.2.5. Statistical analysis

Data were analyzed using PROC GLM of SAS (SAS, version 9.1.3., SAS Institute; Cary, NC) as a randomized complete block with treatment as the main effects and processing day (replicated three times) as the block. Treatment by storage day interaction was included in the model. Differences between significant main effect means

($P < 0.05$) were determined using Tukey's Studentized Range Test at $P < 0.05$. The experiment was replicated three times.

Trained descriptive attribute texture sensory frankfurter data were analyzed as previously described; however, panelist was included in the model. Initially, the analysis was conducted to determine the efficacy of the panelist. The data were analyzed as a split-plot design. For the split plot, the effect of panelist and two-way interactions with the main effects were included in the model. The residual error was used as the error term for the split plot. When no panelist interactions were found, data within an attribute were averaged across panelists and data were analyzed as previously described. When the analysis of variance indicated significance ($P < 0.05$) differences least squares means were separated using the pdiff procedure of SAS (SAS, version 9.1.3., SAS Institute; Cary, NC).

5.3. Results and discussion

5.3.1. Raw emulsion analysis – day 0

5.3.1.1. Raw batter pH, hydration, emulsion stability, and proximate analysis

Table 19 shows treatment effects for the raw emulsion pH, hydration, and emulsion stability. Even though raw batter pH ranged from 5.61 to 5.88, treatments did not differ ($P>0.05$). The control (C, w/ added phosphate) exhibited greater emulsion stability as indicated by the least volume of fluid released after heating (1.41 mL lost), whereas 100N exhibited the poorest emulsion stability (6.50 mL lost).

Raw frankfurter emulsions formulated with at least 50% high pH trimming exhibited greater water holding capacity due to lower hydration values and were not different than C. As the percent of high pH increased, less water, fat, and solids were

lost. These results indicate that pH is the main influence on emulsion stability. Regardless of treatment and of some statistical difference, proximate analysis was similar for percent moisture, protein, and fat. A similar study was conducted by Young, Zhang, Faroouk, and Podmore (2005) regarding different levels of muscle pH (*M. longissimus lumborum* originating from bull) and its affects on functional properties before and after adjusting muscle pH by the addition of an acidic and alkaline pyrophosphate. Four treatments were prepared: 1) high was adjusted to high; 2) high was adjusted to normal; 3) normal was adjusted to normal; and 4) normal was adjusted to high. The authors found that emulsion stability was greatest for highest pH (high – high) ($P < 0.01$). Also, by adding an alkaline phosphate, water-holding capacity had increased from 71.3% in the initial high pH to 100% in the final high pH. Also, meat originally normal in pH had increased by ~ 56% after final pH had been adjusted to high (41.3% to 98.9%). Table 16 clearly shows that hydration and emulsion stability was affected by pH and an added phosphate.

5.3.2. Frankfurter analysis – days 0, 7, 14, 28, and 56

5.3.2.1. Proximate anlaysis, purge, sodium, and cook yields

No differences were found for treatment effects (Table 20) on the proximate composition or percent sodium content of frankfurters. Percent cook yield was significantly lower for 100N but were similar across treatments. These results were expected based on raw emulsion stability and hydration analyses. Young et al. (2005) did show that percent cook yield was significantly highest in bull minced meat that was adjusted (addition of an alkaline phosphate) to a high pH (high to high, 99.1%; high to normal, 95.2%, normal to normal, 94.2; and normal to high, 97.4%). Additionally, the

authors noted that final pH had more of an impact on cook yield when compared to initial pH.

5.3.2.2. Frankfurter pH, color, nitrite, lipid oxidation, and aerobic plate count

Day of storage (Table 21) affected frankfurter pH, external and internal color, lipid oxidation and aerobic plate counts. Frankfurter pH remained stable until Day 28 then significantly decreased by Day 56. External and internal lightness (L*) values increased as length of storage increased while redness (a*) values decreased. Additionally, residual nitrite values significantly decreased as length of storage increased. Ferreira, Tavares, and Ferreira (1999) noted that nitrite decreases after cooking due to the conversion of nitrite to nitrosating agents (added nitrite that reacts with amines) that then reacts with amines and amides of the meat to form N-nitroso compounds. Cooking temperatures assist in speeding up these reactions. Nitrite may have been depleted during processing especially when subjected to high temperature. Ockerman (1986) noted that as conditions become more acidic due to pH declining with storage day, residual nitrite will be reduced. The current study agrees with Ockerman's (1986) statement showing how the pH of frankfurters significantly decreased (~6.18 vs. 5.72) due to the increase of lactic acid production. All treatments indicated little oxidation had occurred with TBARS values ranging from 0.13 to 0.25 g/kg. According to Ockerman (1986), a value of 1.0 g/kg is the beginning of noticeable oxidation.

5.3.2.3. Lactic acid bacteria

Table 22 shows a two-way interaction of day by treatment of frankfurters for lactic acid bacteria. Lactic acid bacteria significantly grew by day 28 reaching a log/CFU of 6. A log of 6 is commonly associated with spoilage, however the production of slime is

Table 19.
LSMeans for the main effect of treatment on raw emulsion pH, hydration, emulsion stability, and proximate composition.

	Treatment[e]						
Trait	100H	75H/25N	50H/50N	25H/75N	100N	C	SEM[d]
pH	5.83[a]	5.88[a]	5.65[a]	5.78[a]	5.61[a]	5.66[a]	0.09
Hydration (mL)	0.10[ab]	0.07[ab]	0.00[b]	0.71[a]	0.44[ab]	0.00[b]	0.22
Emulsion stability (mL)							
Total Loss, mL	2.51[bc]	2.20[bc]	2.68[bc]	4.29[b]	6.50[a]	1.41[c]	0.92
Water lost, mL	2.19[c]	1.97[c]	2.42[bc]	3.90[b]	6.18[a]	1.13[c]	0.54
Fat lost, mL	0.20[a]	0.12[a]	0.12[a]	0.18[a]	0.19[a]	0.20[a]	0.07
Solid lost, mL	0.14[bc]	0.11[c]	0.16[abc]	0.21[ab]	0.23[a]	0.08[c]	0.03
Proximate composition							
Moisture, %	75.83[ab]	76.73[a]	76.17[ab]	75.31[b]	75.98[ab]	75.36[b]	0.28
Fat, %	8.67[a]	8.09[a]	7.43[a]	8.05[a]	7.04[a]	8.41[a]	0.43
Protein, %	13.98[a]	14.36[a]	14.59[a]	14.36[a]	14.86[a]	14.56[a]	0.27

[a,b,c] Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).
[d]SEM = standard error of the means
[e]Treatment: 100H = 100% high pH; 75H/25N = 75% high pH / 25% normal pH; 50H/50N = 50% high pH/ 50% normal pH; 25H/75N = 25% high pH / 75% normal pH; 100N = 100% normal pH; and C = 100% normal pH with added phosphate (0.35%).

Table 20.
LSmeans for the main effect of treatment for proximate composition, purge, sodium content, and cook yield of frankfurters containing varying levels of high pH trimmings.

	Treatment[e]						
Trait	100H	75H/25N	50H/50N	25H/75N	100N	C	SEM[d]
Moisture, %	73.50[a]	73.78[a]	73.45[a]	73.44[a]	74.19[a]	73.98[a]	0.57
Fat, %	8.98[a]	8.95[a]	9.37[a]	9.01[a]	8.27[a]	8.58[a]	0.42
Protein, %	15.69[a]	15.86[a]	16.24[a]	15.90[a]	16.45[a]	16.26[a]	0.23
Purge, %	1.45[b]	1.59[b]	2.06[a]	1.54[b]	1.72[ab]	1.64[b]	0.13
Sodium, %							
Raw	0.18[a]	0.19[a]	0.19[a]	0.17[a]	0.18[a]	0.20[a]	0.01
Cooked	0.17[b]	0.16[b]	0.16[b]	0.17[b]	0.17[b]	0.19[a]	0.00
Cook Yield, %	87.94[bc]	90.25[ab]	91.13[a]	90.45[ab]	87.83[c]	90.40[ab]	0.82

[a,b,c] Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).
[d]SEM = standard error of the means
[e]Treatment: 100H = 100% high pH; 75H/25N = 75% high pH / 25% normal pH; 50H/50N = 50% high pH/ 50% normal pH; 25H/75N = 25% high pH / 75% normal pH; 100N = 100% normal pH; and C = 100% normal pH with added phosphate (0.35%).

Table 21.
LSMeans for the main effect of storage day for pH, external and internal color space values, nitrite, lipid oxidation, and aerobic plate counts of frankfurters containing varying levels of high pH trimmings.

Trait	Day					
	0	7	14	28	56	SEM^{f}
pH	6.17^{a}	6.18^{a}	6.20^{a}	6.18^{a}	5.72^{b}	0.03
External color						
L* (lightness)	-na-	-na-	55.41^{c}	57.27^{b}	3.89^{a}	0.45
a* (redness)	-na-	-na-	25.70^{a}	24.80^{a}	20.63^{b}	0.89
B* (yellowness)	-na-	-na-	15.78^{a}	15.63^{a}	14.35^{a}	0.29
Internal color						
L* (lightness)	57.72^{b}	56.99^{b}	55.89^{b}	56.41^{b}	62.37^{a}	0.49
a* (redness)	23.00^{ab}	24.28^{a}	24.77^{a}	24.45^{a}	20.25^{b}	0.73
B* (yellowness)	12.32^{b}	12.55^{ab}	13.23^{ab}	13.42^{a}	12.39^{b}	0.24
TBARS	0 .17^{bc}	0.25^{a}	0.21^{ab}	0.13^{c}	0.14^{bc}	0.20
APC, log/CFU	1.06^{d}	3.38^{c}	5.35^{b}	5.76^{b}	7.31^{a}	0.17

$^{a\text{-}e}$ Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).
^{f}SEM = standard error of the means
-na- = data values were not measured (not available)

Table 22.
Two-way interaction LSMeans (treatment x storage) of frankfurters of lactic acid bacteria (log/CFU) growth and storage day.

LAB, log/CFU	DAY					
Treatment^{g}	0	7	14	28	56	SEM^{f}
100H	0.7^{e}	2.9^{d}	5.2^{c}	6.4^{b,}	7.5^{a,}	0.35
75H/25N	0.7^{e}	2.6^{d}	5.0^{c,}	6.7^{a}	6.5^{a,}	0.35
50H/50N	0.9^{e}	2.6^{e}	4.9^{c}	7.4^{b}	7.9^{a,}	0.35
25H/75N	1.2^{d,}	2.7^{c}	5.5^{b}	7.3^{a}	7.8^{a}	0.35
100N	0.6^{c}	1.4^{e}	3.2^{b}	7.9^{a}	8.2^{a}	0.35
C	0.8^{d}	1.8^{c}	5.3^{b}	7.0^{a}	7.7^{a}	0.35

$^{a\text{-}e}$ Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).
^{f}SEM = standard error of the means.
^{g}Treatment: 100H = 100% high pH; 75H/25N = 75% high pH / 25% normal pH; 50H/50N = 50% high pH/ 50% normal pH; 25H/75N = 25% high pH / 75% normal pH; 100N = 100% normal pH; and C = 100% normal pH with added 0.35% phosphate.

indicative of product spoilage. In the current study, the production of slime was noticed as packages were opened by day 28 that explains log counts ranging from 6.2 to7.9.

Zurera-Cosano, Rincon-Leon, Moreono-Rojas, and Pozo-Lora (1998) the formation of slime after 28 days of storage with counts of 8.0 (log cfu g-l). Lactic acid bacteria utilize sugar for growth producing lactic acid as a by-product. This may explain why the frankfurter pH significantly decreased by day 56.

5.3.2.4. Analysis of textural and sensory attributes

The texture of meat gels was affected by treatment ($P < 0.05$) during torsion testing (Table 20). Treatments C, 100N, and 25H/75N meat gels required the greatest force (28.28 kPa, 30.79 kPa, and 32.11 kPa) to break compared to other treatments. Treatments containing a higher percentage of normal pH lean resulted in greater strain or deformation of meat gels before failure. Control and 100N treatments had similar strain force compared to treatments containing high pH trimmings.

Attributes for TPA was conducted by treatment (Table 23) and showed within treatment, Hardness-1st bite and hardness-2 had similar trends as affected by 100H, 100N and C. Hardness-1 is defined as the amount of force of gel failure during the first compression whereas hardness 2 is the amount of force for gel failure for the second compression (Bourne, 1982). On the Cohesiveness was defined as the degree of the deformation the gel can withstand before failure (Bourne, 1982). And the magnitude of hardness-1 times cohesiveness is known as Gumminess (Bourne, 1982). According to Bourne (1978) it is not uncommon for frankfurters to have questionable fracturability using the TPA curve. Frankfurters in the current study did not show a fracturability point when viewed on the TPA recorder chart. Therefore, fracturability was not recorded. Treatments consisting of at least 50% high pH meat were similar to C (added phosphate) in TPA values that are indicative of being harder in texture when compared to 100N. Young et al. (2005) noted that sarcoplasmic protein solubility is a good indicator of functional properties,

particularly for shear strain of cooked batter. Results of their study showed that protein solubility was highest for meat derived from high pH that resulted in the highest stress to fracture and strain to fracture values with initial pH having the greatest impact. Minced bull meat that was initially high in pH that was adjusted to high (high – high) resulted in a higher percentage of sarcoplasmic protein solubility when compared to normal pH adjusted to high (7.54% vs. 6.29%). This may be due to the muscle (*M. longissimus lumborum)* undergoing more protein denaturation (during pH decline) during rigor mortis compared to high pH meat. hydration and emulsion stability values showed that high pH lean is most likely to hold more water and solids that led to higher percent cook yields.

Trained descriptive panel (Table 24) did not differentiate in fracturability for treatments 100H, 50H/ 50N, and C. Overall, high pH beef did not negatively impact sensory attributes and texture. Trained panelists did find that frankfurters containing at least 50% high pH were harder. These results are indicative that as percent high pH increases, firmness is increased due to greater moisture and protein retention. These results are similar to Keeton et al (1984) who showed that a frankfurters consisting of an added phosphate increased sensory firmness (4.03 to 4.40) and TPA fracturability (56.39N to 62.86N) and hardness (Hardness-1, 79.53N to 87.07N; and hardness-2, 57.18N to 63.53N). The current data clearly showed that frankfurters manufactured with varying levels of high pH trimmings possess similar characteristics compared to a frankfurter manufactured with normal pH trimmings and added phosphate.

Table 23.
LSMeans for the main effect of treatment for stress, strain and texture of frankfurters containing varying levels of high pH trimmings.

	Treatment[f]						
	100H	75H/25N	50H/50N	25H/75N	100N	C	SEM[e]
Torsion test							
Stress (kPa)	15.96[a]	18.40[a]	18.04[a]	32.11[a]	30.79[a]	28.28[a]	7.79
Strain	1.20[a]	1.28[a]	1.32[a]	1.33[a]	1.41[a]	1.66[a]	0.12
Texture Profile Analysis							
Hardness, 1st Bite, N	191.80[a]	168.00[b]	155.33[b]	140.50[cd]	128.83[d]	163.00[b]	6.05
Hardness, 2nd Bite, N	151.17[a]	130.50[b]	130.50[b]	126.00[b]	108.67[c]	111.83[c]	5.02
Cohesiveness	0.24[a]	0.29[a]	0.29[a]	0.30[a]	0.27[a]	0.28[a]	0.02
Gumminess	46.76[a]	47.55[a]	43.84[a]	41.32[ab]	35.07[b]	43.48[a]	3.06

[a,b,c,d] Means within same row with same letter are not significantly different ($P > 0.05$).
[e] = standard error of the mean.
[f]Treatment: 100H = 100% high pH; 75H/25N = 75% high pH / 25% normal pH; 50H/50N = 50% high pH/ 50% normal pH; 25H/75N = 25% high pH / 75% normal pH; 100N = 100% normal pH; and C = 100% normal pH with added 0.35% phosphate.

Table 24.
LSMeans for sensory attributes for beef frankfurters by treatment.

Attributes	Treatment^{d} 100H	75H/25N	50H/50N	25H/75N	100N	C	SEM^{c}
TEXTURE							
Springiness	6.08^{a}	6.08^{a}	5.93^{a}	5.90^{a}	5.55^{a}	5.93^{a}	0.16
Fracturability	4.66^{a}	4.65^{ab}	4.73^{a}	4.37^{ab}	4.22^{b}	4.73^{a}	0.09
Hardness	6.10^{a}	6.07^{ab}	5.93^{ab}	5.63^{ab}	5.25^{b}	6.28^{a}	0.17
Cohesiveness	6.15^{abc}	6.27^{ab}	6.28^{b}	5.55^{bc}	5.45^{c}	6.42^{a}	0.16
Juiciness	4.58^{a}	4.48^{a}	4.62^{a}	4.87^{a}	5.12^{a}	4.37^{a}	0.18
AROMATICS							
Ck Beef Lean	3.58^{a}	3.78^{a}	3.73^{a}	3.97^{a}	3.97^{a}	3.97^{a}	0.21
Ck Beef Fat	3.07^{a}	3.23^{a}	3.10^{a}	3.55^{a}	3.72^{a}	3.02^{a}	0.17
Spice Complex	3.55^{a}	3.52^{a}	3.98^{a}	4.08^{a}	4.13^{a}	3.95^{a}	0.15
Cardboard	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Painty	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fishy	0.88^{a}	0.40^{a}	0.13^{a}	0.00^{a}	0.07^{a}	0.07^{a}	0.24
Cowy	1.40^{a}	1.23^{a}	0.83^{a}	0.52^{a}	0.73^{a}	0.73^{a}	0.43
Soda	0.17^{a}	0.13^{a}	0.00^{a}	0.07^{a}	0.07^{a}	0.13^{a}	0.10
Chemical	0.00^{a}	0.00^{a}	0.00^{a}	0.08^{a}	0.00^{a}	0.00^{a}	0.03
TASTES							
Salt	5.22^{a}	5.18^{a}	5.03^{a}	4.93^{a}	5.80^{a}	5.12^{a}	0.29
Sweet	0.93^{a}	0.95^{a}	1.07^{a}	1.07^{a}	1.02^{a}	1.12^{a}	0.16
Bitter	1.83^{ab}	1.57^{ab}	1.42^{b}	1.78^{ab}	1.92^{a}	1.72^{ab}	0.10
MOUTHFEELS							
Soapy	1.02^{a}	1.10^{a}	0.87^{a}	0.93^{a}	1.45^{a}	0.90^{a}	0.28
Metallic	1.68^{a}	1.78^{a}	2.00^{a}	1.85^{a}	1.92^{a}	1.85^{a}	0.09
Chemical Burn	0.48^{a}	0.55^{a}	0.63^{a}	0.65^{a}	0.75^{a}	0.48^{a}	0.13

Intensity scale: 0 = None; 15 Extremely intense

^{a,b} Means within same row with same letter are not significantly different ($P > 0.05$).

^{c} = standard error of the mean.

^{d}Treatment: 100H = 100% high pH; 75H/25N = 75% high pH / 25% normal pH; 50H/50N = 50% high pH/ 50% normal pH; 25H/75N = 25% high pH / 75% normal pH; 100N = 100% normal pH; and C = 100% normal pH with added 0.35% phosphate.

CHAPTER VI

FUNCTIONAL PROPERTIES OF BEEF SNACK STICKS MANUFACTURED WITH VARIOUS LEVELS OF HIGH pH COW MEAT

6.1. Introduction

The beef industry is continuously exploring alternatives to add value to beef cuts. Between the years of 1993 to 1998, it was observed that the value of beef chuck and round muscles had dropped 25% - 26% (Von Seggern. Calkins, Johnson, Bruckler, & Gwartney, 2005). This discovery led to an emphasis on “value added technology” for underutilized value cuts. Since this development, Von Seggern et al. (2005) reported that the price for steers and heifers increased $50 to $70 per head partially attributed to “value added technology.” Cow muscles have been investigated as an alternative source to be utilized in meat products. Even though muscles from cow carcasses are not as comparable to fed beef carcasses on a quality standpoint, the industry continues to investigate how to further improve undervalues beef cuts.

At time of slaughter, fed beef carcasses average less than 30 months of age (Garcia, et al., 2008) whereas cow carcasses average 72 months of age (Nicholson, 2008). Cow carcasses are often discriminated against because of their darker lean color as a result of increased myoglobin concentrations (Craig, Blummer, Smart, & Wise, 1966; and Bowling, Smith, Carpenter, Dutson, & Oliver, 1977) and poor tenderness due to increased collagen crosslinking (Goll, Bray, & Huekstra, 1963; Hill, 1966; Tuma, Henrickson, Stephens, & Moore, 1962).

Furthermore, feeding regime (grain vs. forage) has had an effect on muscle pH which ultimately affects muscle quality. Bowling et al. (1977) noted Cross and Smith (1976) and Schupp et al. (1976) had suggested that "forage-finished cattle are more susceptible to pre-slaughter stress and to the dark cutting beef syndrome than grain-finished cattle." This may be because grain finished cattle are more accustomed to the pressure and noise created by people in conjunction to a high concentrate diet, which results in these type of cattle being less susceptible to stress. Dark cutting or dark, firm, and dry is a quality defect that is often associated with high pH meat caused by long term stress. At time of slaughter, muscle containing insufficient amounts of glycogen lead to insufficient amounts of lactic acid production (a by-product of glycogen under anaerobic conditions) that causes muscle pH to slowly deviate from physiological pH (7.0) resulting in an ultimate pH of 6.0 or greater.

In addition to exhibiting lean that is dark in color, firm in texture, and dry on the surface, muscles high in pH tend to exhibit greater water-holding capacity (WHC) (Egbert & Cornforth, 1986; Wulf et al, 1999; Wulf, Emnett, Lehska, & Moeller, 2002; Viljoen, Knock, & Webb, 2002). Improved muscle WHC can be beneficial in an emulsified product such as beef frankfurters (Young, Zhang, Farouk, & Podmore, 2005; Garcia, Osburn, & Savell, 2009) however, WHC can be detrimental to the production of a dried product, such as beef snack sticks where pH decline, moisture loss and final water activity is critical.

Therefore, the question was asked, would utilizing raw materials that are high in pH be used as an alternative source in a fermented, dry product such as beef snack sticks while adding value? The current study focused on investigating the effects of high pH

beef on pH decline and water holding capacity in snack sticks. The objective of the current study was to evaluate textural, sensory, and shelf-life attributes in snack sticks from various percentages of high pH beef compared to a normal pH beef snack stick.

6.2. Materials and methods

Shoulder clods (IMPS #114) and short plates (IMPS# 121) were obtained from cow carcasses representing two pH levels: 1) normal pH (5.4 to 5.8) and 2) high pH (6.2). Classification of carcasses was determined by averaging three pH readings from three muscles: *M. Teres major*, *M. Triceps brachii*, and *M. Infraspinatus* via a hand-held pH probe. Shoulder clods and short plates were obtained from a commercial cow/bull plant and transported to the Texas A&M University (TAMU) Rosenthal Meat Science and Technology Center (RMSTC) vacuum packaged and stored (4°C) for two weeks.

6.2.1. Snack stick manufacture

Shoulder clods were trimmed of excess fat and connective tissue. Additional pH determinations were made on the shoulder clods by inserting a hand-held pH probe (3.5 mm OD; Meat Probe, Inc., Topeka, KS) into three main muscles (*M. Teres major*, *M. Triceps brachii*, and *M. Infraspinatus)* and recorded the pH value of each muscle. The pH values were averaged to obtain an overall pH value and separated into normal and high pH types. Shoulder clods (lean component) and short plates (fat component) were ground separately through a 1.27 cm plate, followed by a second grind using a 0.48 cm plate. High pH shoulder clods and short plates were ground first followed by normal pH clods and short plates.

Snack sticks were formulated using the appropriate amounts of normal and/or high pH clods and short plates to achieve a 11.34 kg meat block at a 10% fat content (raw

basis). Five treatments were formulated: 1) 100% high pH (100H); 2) 75% high pH/25% normal pH (75H/25N); 3) 50% high pH/ 50% normal pH (50H/50N); 4) 25% high pH/75% normal pH (25H/75N); and 5) 100% normal pH (100N) (Table 22). Meat trimmings and non-meat ingredients (Table 25) were added and mixed using a paddle mixer (Butcher Boy Model 150, Lasar MFG Inc., Los Angeles, CA). Non-meat ingredients were: Prague powder, seasoning (containing 0.03% sodium erythorbate), dextrose (0.25%), and starter culture (Table 25). After mixing, pH and temperature were recorded in triplicate.

After mixing, the meat mixture was stuffed into 19 mm dia edible cellulosic casings (Dewied International Inc., San Antonio, TX) using a vacuum stuffer (Handtmann VF-612) to produce links weighing approximately 56 g each. Snack stick batches were weighed individually, hung on stainless steel rods that were then placed on a smoketruck. Snack sticks were thermally processed (cooked/dried, Table 26) in a single truck smokehouse (Model 1000, Alkar, Lodi, WI, U.S.A.) until a water activity (A_w) of < 0.85 was reached for the normal pH snack stick. Samples of normal pH snack sticks were removed every hour of thermal processing (cooking/drying) to monitor A_w. Temperature was monitored by inserting two internal temperature probes in two randomly selected snack stick links. Upon reaching the desired water activity, snack sticks were chilled to 2°C, weighed, and vacuum packaged (Grip & Tear Easy Open CROVAC, Sealed Air Corporation, Duncan, SC 29334), labeled, and stored at 4°C until further analysis (Day 0, 7, 14, 28, and 56).

Table 25.
Beef snack stick formulations (kilogram and percent basis) for an 11.98 kg batch weight at a 10% fat level.

	Treatment[e]									
Ingredients	100H		75H/25N		50H/50N		25H/75N		100N	
Lean Source, kg										
High pH , 3% fat	8.29		6.21		4.14		2.07		0.00	
Normal pH , 7% fat	0.00		14.95		5.21		7.82		10.42	
Fat Source , kg										
High pH , 29 % fat	3.02		2.29		1.53		0.76		0.00	
Normal pH, 44 % fat	0.00		0.23		0.46		0.69		0.92	
Non-meat Ingredients, g (%)[a]										
Cure Salt[b]	113.50	(0.25)	113.50	(0.25)	113.50	(0.25)	113.50	(0.25)	113.50	(0.25)
Spice[c]	526.64	(4.64)	526.64	(4.64)	526.64	(4.64)	526.64	(4.64)	526.64	(4.64)
Dextrose	113.50	(0.25)	113.50	(0.25)	113.50	(0.25)	113.50	(0.25)	113.50	(0.25)
Lactic Acid Starter Culture[d]	18.50	(0.16)	18.50	(0.16)	18.50	(0.16)	18.50	(0.16)	18.50	(0.16)

[a]Non-meat ingredients (g, %) were calculated using weight of the meat block (11.34 kg).
[b] D.Q. Curing Salt, Butcher & Packer Supply Company, Detroit, MI., U.S.A.
[c] Snack Stick Seasoning (OPS 116), with 0.03% included Na Erythorbate, 16.22% dextrose. A.C., Legg, Inc., Calera, AL, U.S.A.
[d] Bactoferm™ Lactic Acid Starter Culture HPS 70mL (Chr Hansen, Hoersholm, Denmark)
[e]Treatment: 100H = 100% high pH; 75H/25N = 75% high pH / 25% normal pH; 50H/50N = 50% high pH/ 50% normal pH; 25H/75N = 25% high pH / 75% normal pH; 100N = 100% normal pH; and C = 100% normal pH with added 0.35% phosphate.

Table 26.
Thermal processing schedule used for manufacture of beef snack sticks.

Step	Type	Dry Bulb (°C)	Wet Bulb (°C)	Time Hr	Damper	Relative Humidity %
1	Ferment	37.3	36.7	12	Auto	96
2	Cook	60.0	51.7	1	Open	65
3	Cook	71.2	60.0	1	Open	57
4	Cook	79.5	71.2	1	Open	69
5	Dry	76.7	60.0	$A_w < 0.85$	Open	44

6.2.2. Analysis of raw meat mixer – day 0

6.2.2.1. pH

The pH of the raw meat mixer (2°C) was determined using a pH meter (IQ Scientific Instruments, Inc. Reston, VA) and internal probe (piercing tip micro probe with heavy duty handle, Model PH57-SS, IQ Scientific Instruments, Inc. Reston, VA) calibrated with buffers 4.01 and 7.0. Three readings were taken and averaged per treatment.

6.2.2.2. Percent moisture and fat

Proximate analysis for fat and moisture was performed in triplicate within 48 hr as described by AOAC (2005a) air-dry oven and soxhlet ether extraction methods. Powdered raw and cooked frankfurters samples (~ 2.5 g) were placed in pre-weighed, pre-dried paper thimbles (Whatman #2) re-weighed, and recorded. Samples were dried for 16h at 100°C, cooled to room temperature in a dessicator, and then weights were recorded. Percent moisture was calculated as the difference between wet weight and dried

sample weight divided by sample weight. Oven dried samples (3 samples per treatment)) were placed in a soxhlet apparatus and fat extracted using petroleum ether for approximately 12 h with an ether drip rate of 3 drops per second and a reflux rate of 1 per 5 min. Percent fat was determined by calculating the difference between dried sample weight and extracted sample weight divided by sample weight.

6.2.2.3. Percent protein

Protein was determined using the LECO Protein/Nitrogen Analyzer (Model FP 528, LECO Corporation, St. Joseph, MI) to determine nitrogen content as described by AOAC (2005b). The percent protein was be calculated by multiplying the percent nitrogen times 6.25, the protein conversion factor for meat and poultry. Each raw and cooked sample moisture and protein measurement was performed in triplicate.

6.2.2.4. Water activity

Approximately 8 g of powdered raw and cooked snack stick samples were placed into plastic water activity disposable cups, placed into the water activity chamber, sealed and sample water activity was measured with an Aqualab meter (Model 3, Decagon Devices, Inc., Pullman, WA.) calibrated at ambient temperature (20°C) with distilled water (a_w = 0.999) and saturate solutions of NaCl (a_w = 0.756) and NaCl (a_w =0.984). Triplicate samples of each treatment were taken.

6.2.3. Analysis of cooked snack sticks – days 0, 7, 14, 28, and 56

6.2.3.1. pH

The pH of snack sticks was conducted as previously described.

6.2.3.2. Color

Individual snack sticks were randomly selected for internal color measurements

that were taken in triplicate. Color was measured using a color Hunter Miniscan XE (Hunter Labs, Inc. Reston, VA; Illuminant A, 2° observer) set for L* (lightness), a* (redness), and b* (yellowness). Three readings per link per treatment were taken.

6.2.3.3. Purge

On each storage day, packaged snack sticks (4 snack sticks per package) from each treatment were weighed (total packaged weight). Snack sticks were removed from the package and a paper towel was used to remove excess moisture from both the individual snack sticks and the empty pouch. The snack sticks and pouch were reweighed separately and recorded to calculate percent purge.

Purge, % = (Weight of dry frankfurters and bag / Initial total weight) * 100.

6.2.3.4. Chemical analysis

Proximate analysis for snack sticks (moisture, protein, and fat) were conducted as previously described.

6.2.3.5. Moisture: protein ratio

After determining the percent moisture and protein of cooked beef snack stick samples, the moisture to protein ratio was determined for all snack sticks by dividing the percent moisture by the percent protein.

6.2.3.6. Lipid oxidation

Lipid oxidation was determined on snack stick samples using the 2-thiobarbituruc acid reactive substances (TBARS) method of Tarladgis et al. (1960) as modified by Rhee (1978) for cured meat products. Thirty grams of sample was blended with 43.5 ml of distilled water, 1.5 ml of sulfanilamide reagent and 15 ml of 0.5% solution of propyl gallate (PG) and ethylenediamine tetraacetic acid for 2 min. Thirty grams of slurry was

transferred to a 500 ml Kjeldahl flask using 78 ml of 50°C distilled water. The Kjeldahl flask containing slurry was placed on a distillation unit and heated until 50 ml of distillate was collected. Five ml of distillate and 5 ml of thiobarbituric acid (TBA) reagent was added to a glass (x CC) screw cap test tube. The tubes were capped, placed in a large beaker containing water and boiled on a hot plate for 35 min. to develop color. Test tubes were removed from the beaker, cooled in tap water for 10 min., vortexed and approximately one mL of solution placed in a cuvettte. Absorbance was measured at 530 nm using an UV-violet spectrophometer (Model Cary 300 BIO-UV- Visible Spectrophometer, Varian Instruments, Walnut Creek, CA 94598). Absorbance readings were multiplied by a factor of 7.8 and the rResults were reported as mg of malonaldehyde per kilogram of sample.

6.2.3.7. Sodium analysis

Sodium analysis was conducted on days 0 and 56. Snack stick samples (10 g) and 90 ml of distilled water were blended for 30 sec. using a stainless steel hand-held blender (Cuisinart, Model CSb-77, East Windsor, NJ). The diluted sample was transferred to a 150 ml beaker with 10 ml of ISA (Sodium Ionic Strength Adjuster) and continuously stirred. Readings were measured using a ROSS® Sodium Ion Selective Electrode (Model 8611BN). Concentration values were recorded in triplicate as ppm then converted as a percentage. Percent sodium was calculated as the follows:

% Sodium = (sodium ppm * 0.0001)

6.2.3.8. Nitrite analysis

Residual nitrite (AOAC, 2005c) was determined by combining 5 g samples with 40 ml of distilled water and heated to 80°C in a 100 mL beaker. The samples were

transferred to a 500 ml volumetric flask and 300 ml of hot distilled water added. The flasks were placed in a water bath and boiled for 2 h. Sample flasks were cooled to room temperature then filtered through two Whatman No. 2 filter papers into a into 25 ml flask. Twenty five ml of the filtered sample was transferred into a 50 ml volumetric flask with 2.5 ml sulfanilamide reagent and allowed to stand for 5 min. Afterwards, 2.5 ml N-(1-naphthyl) ethylene diamine (NED) was added and the sample diluted to volume and allowed to stand for an additional 15 min. to allow color development. Absorbance was measured at 540 nm using an UV-visible spectrophotometer (Model Cary 300 Bio, Varian Instruments, Walnut Creek, CA 94598). Residual nitrite was then converted to parts per million (ppm) by multiplying by 200 (200 dilutions).

Nitrite ppm = (residual nitrite * 200)

6.2.3.9. Microbiological analysis

Vacuum packaged snack sticks (3 snack sticks / package) were sterilized by spraying the exterior top edge of the packaging with 70% ethanol and then wiped with a paper towel. The packages were then opened aseptically using flame sterilized forceps and scalpels to which 99 ml of 0.1% peptone diluents were added. Each package was then hand massaged for one min. Aerobic plate counts were determined by plating 1 ml of the sample rinse and 1 ml of the appropriate 10-fold dilutions of the same on Petrifilm™ aerobic count plates (3M, St. Paul, MN). Lactic acid bacteria counts were determined simultaneously by dispensing 1 ml of the sample rinse and the appropriate 10-fold dilutions of the same into sterile petri dishes to which Lactobacilli MRS Agar (Difco, Detroit, MI) was added and allowed to solidify. An additional MRS overlay was

added to each plate. Both Petrifilm™ and MRS plates were incubated at 30°C for 72 h before counting and reporting CFU per package.

6.2.4. Allo-Kramer shear force and sensory analyses for snack sticks- day 28

6.2.4.1. Allo-Kramer shear force determination

After 28 days of vacuum packaged refrigerated storage beef snack sticks were removed froom refrigeration (4°C) and allowed to sit for 30 min at room temperature before being cut into 1.27 cm in length. Samples were weighed and shear values were recorded using an Instron Universal Testing Machine (Instron Corp., Canton, Mass., U.S.A.) equipped with a 10-blade Allo-Kramer shear compression cell using a 500-kg load cell with a load range of 500 kg and a crosshead speed of 200 mm/min. Shear values were reported as Newtons/gram.

6.2.4.2. Sensory analysis

A 5-member, trained, descriptive attribute panel evaluated samples defined by AMSA (1995). Texture attributes were evaluated by trained panelists using the Spectrum Intensity Scale (Meilgaard, Civille, & Carr, 2007). Ballot development sessions were conducted by trained panelists.

On the day of testing, snack sticks were removed from their vacuum package one hour before testing and cut into 1.27 cm disks. Three disks were immediately served to trained panelists. Five samples were evaluated per day with a 5 min. gap between samples. Treatment samples were assigned a randomized three digit code and a randomized order on testing day. Panelists were placed in individual booths separated from preparation area. A warm-up sample was provided to panelists to standardize and

calibrate the panelists each day. In order for panelists to cleanse their palettes, fat-free ricotta cheese, salt-less saltine crackers and double distilled water was provided.

6.2.5. Statistical analysis

Data for beef snack sticks were analyzed using PROC GLM of SAS (SAS, version 9.1.3., SAS Institute; Cary, NC) as a randomized complete block design with day and treatment as main effects that were blocked by processing day. Treatment by storage day interaction was included in the model. Significant main effect means ($P < 0.05$) were determined using Tukey's Studentized Range Test at $P < 0.05$. The experiment was replicated three times.

Trained descriptive attribute texture sensory frankfurter data were analyzed as previously described; however, panelist was included in the model. Initially, the analysis was conducted to determine the efficacy of the panelist. The data were analyzed as a split-plot design. For the split plot, the effect of panelist and two-way interactions with the main effects were included in the model. The residual error was used as the error term for the split plot. When no panelist interactions were found, data within an attribute were averaged across panelists and data were analyzed as previously described. When the analysis of variance indicated significance ($P < 0.05$) differences least squares means were separated using the pdiff procedure of SAS (SAS, version 9.1.3., SAS Institute; Cary, NC).

6.3. Results and discussion

6.3.1. Raw meat mixture analysis – day 0

Table 27 shows the effect of varying levels of high pH trimmings on raw meat mixture pH, water activity, and proximate composition. The pH of the meat mixture

showed that as the percentage of high pH trimmings increased, the pH increased. The 100N beef snack sticks was significantly lower in pH (5.67) ($P < 0.05$) from 50H/50N (5.75), 75H/25N (5.89), and 100H (5.87). Even though water activity ranged from 0.95 to 0.98, treatments did not differ ($P > 0.05$). Regardless of treatments and some statistical differences proximate analysis was similar for moisture, protein, and fat.

6.3.2. Snack stick analyses – days 0, 7, 14, 28, and 56

No day by treatment interactions existed ($P < 0.05$) for the overall data thus only significant main effects are reported ($P < 0.05$). Table 28 shows treatment effects of beef snack sticks for pH, A_w, internal color (L*,a*,b*), lipid oxidation, proximate composition, percent purge, and shear force values. Romans, Costello, Carlson, Greaser,

Table 27.
LSMeans for the main effect of treatment for raw pH, water activity, and proximate composition of snack sticks containing varying levels of high pH trimmings.

Trait	Treatment[e]					
	100H	75H/25N	50H/50N	25H/75N	100N	SEM[d]
pH	5.87[a]	5.89[a]	5.75[b]	5.72[bc]	5.67[c]	0.03
A_w	0.95[a]	0.96[a]	0.98[a]	0.98[a]	0.97[a]	0.01
Proximate composition						
Moisture, %	67.21[c]	70.21[ab]	69.22[b]	69.41[ab]	70.23[a]	0.35
Fat, %	10.05[a]	8.52[a]	9.90[a]	9.04[a]	8.73[a]	0.59
Protein, %	16.83[a]	15.95[a]	15.83[a]	16.73[a]	16.89[a]	0.68

[a,b,c] Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).
[d]SEM = standard error of the means
[e]Treatment: 100H = 100% high pH; 75H/25N = 75% high pH / 25% normal pH; 50H/50N = 50% high pH/ 50% normal pH; 25H/75N = 25% high pH / 75% normal pH; 100N = 100% normal pH; and C = 100% normal pH with added 0.35% phosphate.

& Junes (1994) noted that a fermented sausage would typically exhibit a pH ranging from 4.7 to 5.3. Snack stick pH ranged from 5.04 to 5.12 that significantly differed among

treatments however, still resulted below the recommended pH of 5.3 by FSIS (2005). However, in the current study, a pH of 5.0 was used as the target to determine the true affect of pH on pH decline. It was noticed (Table 28) that as the percent of high pH decreased in the formulations, snack sticks were closer to the targeted pH of 5.0 (100H, 5.12; 75H.25N, 5.10; 50H/50N, 5.06; 25H/75N, 5.04; and 100N, 5.06). Even though not significantly different ($P > 0.05$), A_w levels did slightly vary among treatments, particularly with two treatments (50H/50N, 0.86 and 25H/75N, 0.86) that resulted in a water activity level above 0.85. According to the FSIS, water activity levels must result in 0.91 or less to

Table 28.
LSMeans for the main effect of treatment for pH, water activity, color space values (L*, a*, b*), lipid oxidation (TBARS), proximate analysis, purge, and allo-kramer shears of snack sticks containing various levels of high pH trimmings.

Trait	Treatment[e]					
	100H	75H/25N	50H/50N	25H/75N	100N	SEM[d]
pH	5.12[a]	5.10[b]	5.06[c]	5.04[d]	5.06[c]	0.01
A_w	0.85[a]	0.85[a]	0.86[a]	0.86[a]	0.85[a]	0.01
L* (lightness)	42.26[b]	42.98[ab]	43.68[a]	50.01[a]	44.59[a]	2.66
a* (redness)	17.22[c]	16.78[c]	17.32[bc]	18.28[ab]	18.41[a]	0.37
b*(yellowness)	22.63[a]	10.24[a]	10.58[a]	10.89[a]	10.98[a]	5.57
TBARS	0.16[a]	0.15[a]	0.19[a]	0.18[a]	0.19[a]	0.01
Proximate analysis						
Moisture, %	41.56[a]	40.44[a]	40.46[a]	41.78[a]	42.27[a]	3.62
Protein, %	32.62[c]	32.72[c]	33.58[bc]	34.48[b]	35.89[a]	0.46
M:P ratio	1.28:1[a]	1.23:1[a]	1.20:1[a]	1.21:1[a]	1.18:1[a]	0.10
Fat, %	23.75[a]	23.77[a]	22.28[ab]	20.60[b]	16.81[c]	0.81
Sodium, %	0.24[c]	0.24[bc]	0.24[c]	0.25[b]	0.26[a]	0.00
Purge, %	1.00[ab]	0.99[ab]	0.99[ab]	0.99[ab]	1.00[b]	0.002
Allo-Kramer (N/g)	132.27[b]	132.23[b]	131.66[b]	133.31[b]	144.65[a]	0.19

[a,b,c] Mean values within a row followed by the same letter are not significantly different ($P > 0.05$).
[d]SEM = standard error of the means
[e]Treatment: 100H = 100% high pH; 75H/25N = 75% high pH / 25% normal pH; 50H/50N = 50% high pH/ 50% normal pH; 25H/75N = 25% high pH / 75% normal pH; 100N = 100% normal pH; and C = 100% normal pH with added 0.35% phosphate.

ensure the product is microbialy safe from *L. monocytogenes*. However, in order to control *S. aureus*, a water activity level 0.85 or less must be achieved. The current study showed that raw materials high in pH may result in variations in water activity even under uniform processing conditions that may require additional drying times. Even though water activity values did not result in less than 0.85, these values may still be low enough, in combination with pH and refrigeration, to have led to extremely low lactic acid bacteria (LAB) and aerobic plate counts (APC) counts. There were a large number of samples with no detectable colonies and the counts within detectable levels were very close to the minimum detection level of 25/ (unit). Detectable LAB counts ranged between 0.3 and 0.7 log CFU/ package that comprised 13% of the total analysis (n = 150). Detectable APC counts ranged from 0.3 and 1.1 log CFU/package that comprised of 24% of the total analysis (n = 150). The fact that the bacterial counts was so infrequent and at such low levels statistical analysis would not be reliable and therefore indicative of shelf-life not being an issue in the current study.

Mean objective color values are reported in Table 28. 100H and 75H/25N were lower and thus darker in color than other treatments. There were L* (lightness) value differences ($P < 0.05$) that ranged from 42.26 to 50.01 with 100H and 75/25N resulting in the lowest values. However, a* values slightly differed among treatments displaying a range of 16.78 to 18.41, but were still lower for 100H and 75H/25N that resulted in a less red color. Yellowness (b*) values indicated small differences except for 100H that resulted in the highest degree (22.63) of yellowness among treatments. Page, Wulf, and Schwotzer (2001) reported that a* (redness) and b* (yellowness) were more associated

with muscle pH than L* (lightness). The authors stated that these findings indicate that muscle pH affects muscle color by altering hue (red, yellow, green, blue and intermediate). This would explain why 100H resulted in the highest yellowness values.

Proximate composition (Table 28) did not differ ($P > 0.05$) in percent moisture (among treatments), yet differed ($P < 0.05$) in protein content, specifically 100N resulting in the highest percentage. According to Acton and Keller (1974), a typical fermented sausage would have a composition of 41% moisture, 25% protein, 28% fat and 6% ash. In comparing the beef snack sticks of the current study to Acton and Keller (1974), the main differences were protein (7 to 10% greater) and fat (5 to 12% lower) content, which would explain such low moisture:protein ratio (MPR). Campbell-Platt and Cook (1995) noted that a typical MPR for a semi-dry sausage is from 2.3 to 3.7:1, even though, FSIS (2005) recommends semi-dry sausages must have a MPR 3.1:1. The MPR for this study was well below the targeted MPR value with percent protein increasing as percent fat content decreased while percent moisture was stabilized. Treatments obviously contained less fat than the typical snack stick mentioned by Acton and Keller (1974). Treatment 100N had the greatest percent protein and lowest percent fat content. It appeared that the low fat content for 100N contributed to a non-linear relationship between fat and protein during thermal processing (cooking/drying). Because fat is less dense than coagulated protein (Smith & Carpenter, 1976), this would explain shear force values for 100N resulting in the highest Allo-Kramer shear force values.

Among all treatments, TBARS values (Table 28) were well below the threshold of 1.0 mg malonaldehyde/kg sample associated with rancidity (Ockerman, 1981). According to Toldrá (2007), fatty acids with double bonds (mono- and polyunsaturated)

are more susceptible to oxidation, especially with external lighting, heating, and the presence of moisture and/or metallic cations. The current study used raw materials from beef that primarily contained a high proportion of saturated fat with single bonds. Also, cooked beef snack sticks were vacuum packaged and placed in a cardboard box and kept in a cool, dry environment. Therefore, the combination of the factors mentioned above, pH and the use of nitrite explains the low oxidation values.

Trained panelists evaluated the beef snack sticks on a 15-point scale for texture, aromatics, taste, and mouthfeels (Table 29). Treatments did not affect ($P > 0.05$) sensory attributes of beef snack sticks (Table 29). Overall, hardness and cohesiveness were the strongest texture attributes, respectively. Even though values were not significantly different, 100N resulted in the hardest (8.47) and most cohesive (8.93) in texture that reveals a mirror image of shear force values. Wulf, Emnett, Leheska, and Moeller (2002) investigated effects of dark, firm, and dry (pH > 6.00) on the palatability in cooked beef. Their study found that DFD muscles were substantially lower in palatability and had resulted in more off-flavors (84%) compared to muscles normal in pH. Not only did our study show there were no differences in sensory attributes, but no documented comments was reported by panelists regarding any off-flavors of beef snack sticks. Since Wulf et al. (2002) noted that a high percentage of off-flavors were found in high pH beef, then perhaps utilizing raw materials high in pH may be ideal in a dry fermented product, such as snack sticks, to help mask these off-flavors due to the fermentation and drying steps of thermal processing.

Table 29.
LSMeans for sensory attributes for beef snack sticks by treatment.

	Treatment[d]					
Attributes	100H	75 H/25N	50H/50N	25H/75N	100N	SEM[c]
TEXTURE						
Fracturability	4.13	4.00	4.20	4.13	4.13	0.10
Hardness	8.20	8.00	8.07	8.20	8.47	0.17
Cohesiveness	8.73	8.67	8.60	8.67	8.93	0.14
Juiciness	0.00	0.00	0.00	0.00	0.00	0.00
AROMATICS						
Cook Beef Lean	4.07	4.00	4.00	4.07	4.20	0.13
Cook Beef Fat	1.67	1.53	1.60	1.53	1.53	0.06
Spice Complex	4.60	4.87	4.20	4.40	4.40	0.12
Chemical	0.00	0.00	0.00	0.00	0.00	0.00
Cardboard	0.00	0.00	0.00	0.00	0.00	0.00
Painty	0.00	0.00	0.00	0.00	0.00	0.00
Fishy	0.00	0.00	0.00	0.00	0.00	0.00
TASTES						
Salt	3.80	3.87	3.60	4.07	3.80	0.17
Sweet	0.00	0.00	0.00	0.00	0.00	0.00
Bitter	2.13	2.07	2.07	2.20	2.00	0.08
Sour	3.00	3.13	3.00	3.33	3.33	0.09
Metallic	2.60	2.47	2.47	2.60	2.47	0.12
MOUTHFEELS						
Sour	3.00	3.13	3.00	3.33	3.33	0.09
Burn	3.73	3.67	3.67	4.07	3.60	0.24
Acid	2.40	2.33	2.27	2.40	2.33	0.07
Spice	3.40	3.40	3.13	2.53	3.33	0.11

Intensity scale: 0 = None; 15 Extremely intense
[a,b] Means within same row with same letter are not significantly different (P > 0.05).
[c] = standard error of the mean.
[d]Treatment: 100H = 100% high pH; 75H/25N = 75% high pH / 25% normal pH; 50H/50N = 50% high pH/ 50% normal pH; 25H/75N = 25% high pH / 75% normal pH; 100N = 100% normal pH; and C = 100% normal pH with added 0.35% phosphate.

CHAPTER VII

CONCLUSIONS

7.1. Survey

Results of our study indicate that muscle pH and color did vary between fed beef and cow carcasses. Fed beef carcasses fell between the normal ranges as compared to previous studies. A large portion of the fed beef carcasses had values within acceptable ranges. Cow carcasses revealed a higher prevalence of resulting in higher pH values and displaying darker, red colored lean. Perhaps sorting beef carcasses using muscle pH color space values, specifically L* may be useful in reducing the variation in processing functionality.

7.2. Beef jerky

Variability among cooked jerky attributes from normal and high pH inside rounds from fed or cow carcasses were observed. Variations in raw material source (carcass class and pH type) can impact the water activity and moisture:protein ratio of finished jerky that may cause the product to fail to meet food safety requirements (<0.85 Aw) and the standard of identity for beef jerky (M:P ratio of 0.75:1). Naturally, meat originating from cow contains a higher level of myoglobin and is higher in pH (in comparison to fed beef) appearing darker in color (less red) and containing much more moisture. In the current study, fed high pH jerky slices was of most concern with exhibiting the highest water activity, percent moisture as well as appearing slightly darker in color. Even though they did not meet a water activity level of less than 0.85,

extended drying period would be recommended. However, drying for extended periods of time may result in sacrificing texture (Konieczny, Stangierski, and Kijowski; 2007).

In contrast, jerky products exceeding the recommended water activity and MPR standards appear to be somewhat of an advantage in tenderness, especially in jerky slices with a pH of 6.5 or greater. Even though color is naturally impacted by carcass class (cow vs. fed) and/or muscle high in pH, beef jerky color may not be a critical attribute to the average consumer. Konieczny, Stangierski and Kijowski (2007) reported that mean sensory scores in home style beef jerky regarding color preference fell within a wide sensory range, confirming the differences of consumer color preference.

Overall, pH affects more than one functional property in jerky production: water activity, proximate composition, color parameters, and shear force. Therefore, it is recommended that whole muscle beef jerky manufacturers check the pH of raw materials to determine if changes in drying times are warranted to meet the critical food safety target of $\leq 0.85\ A_w$ and product standard of identity requirements.

7.3. Beef frankfurters

These results indicate that frankfurters with varying levels of high pH cow trimmings were similar in raw emulsion stability and hydration, as well as cooked frankfurter color, lipid oxidation, sensory, and textural attributes compared to an emulsion/frankfurter containing 0.35% phosphate. Young, et al. (2005) noted that by increasing the pH of raw materials, high emulsion stability, strong gels and high cook yields can be achieved. This study confirms what Young et al. (2005) noted. Even though lipid oxidation and textural attributes (sensory and instrumental) did not differ

from the phosphate C, the rapid growth of spoilage organisms resulting in a short shelf life was of concern. This study explored the potential of frankfurters manufactured with varying levels of high pH cow trimmings as a potential phosphate replacement When compared to a phosphate added frankfurter, similar results were noted between treatment and control frankfurters for many analyzed attributes.

However, even though there were no differences among treatments for lipid oxidation, the growth of lactic acid bacteria significantly increased between 14 and 28 days of vacuum packaged refrigerated storage, and slime had been produced. Therefore, further investigations that incorporate an antimicrobial should be conducted before high pH cow trimmings can be considered as a phosphate replacement for the manufacture of frankfurters.

7.4. Beef snack sticks

The current study indicates that cow trimmings high in pH have potential to be used as an alternative source in beef snack stick production. The most pronounced differences among treatments were color; percent protein, percent fat, and Allo-Kramer shear force values. Regardless of treatment, snack stick pH resulted in 5.1 or less. On the other hand, snack sticks consisting of a higher percent of normal pH managed to reach the targeted pH of 5.0. With this, perhaps adding a greater amount of dextrose is or adding a longer more controlled fermentation time is necessary to reach the targeted pH of 5.0. Also, with water activity values ranging between 0.85 and 0.86, additional drying times would be necessary. Perhaps checking the pH of raw material can help in adjusting the thermal processing program to help achieve food safety requirements (A_w

< 0.85) and in reducing variability occurring in beef snack sticks due to various levels of high pH trimmings. As the percent of lean high in pH increased, snack sticks became darker in color. Lipid oxidation and microbial analysis indicated that shelf-life was not of concern confirming to be a very shelf-stable product. Even though tenderness was affected by a low fat content, sensory attributes was not affected among treatments which was shown by panelists unable to detect differences in texture, aromatics, taste, and mouthfeels. Therefore, this study shows that processors can utilize high pH fed and cow trimmings to manufacture an acceptable fermented snack stick product. Although the current study investigated snack sticks stored under refrigerated conditions, it would be beneficial to investigate the affect of storage conditions (ambient vs. refrigeration) on textural, sensory, and shelf-life attributes in vacuum packaged beef snack sticks.

7.5. Overall conclusions

Even though a small percentage of fed beef and cow carcasses exhibited high muscle pH, however, there is a possibility of processors receiving raw materials high in pH. If raw materials from cow carcasses are used by the processor, the it may be important to check pH. Beef raw materials with high in pH may be better suited in a dry fermented product compared to a whole muscle dry product or an emulsified product.

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APPENDICES

APPENDIX A

THERMAL EMULSION STABILITY PROCEDURE

(Townsend, W.E., Witnauer, L.P., Riloff, J.A. and Swift, C.E. 1968. Comminuted meat emulsions: Differential thermal analysis of fat transitions. Food Tech. 22:319-323)

EQUIPMENT:

50 ml polycarbonate centrifuge tubes
Tube rack
Scale
Centrifuge with rotor
Graduated Conical tubes
Thermometer
Thermocouples
Water bath
Funnels

PROCEDURE:

1. Stuff 34 g of emulsion into three pre-weighed 50ml polycarbonate centrifuge tubes. Eliminate all air pockets in the batter. Record the weight of the batter.
2. Place tubes in test tube rack into a 48.8°C water bath.
3. Add two extra tubes stuffed with emulsion to monitor temperature with a thermocouple during cooking. Seal the top of the tubes with a cover that has an opening for the thermocouple probe.
4. Raise temperature intermittently until product reaches 68.8°C in approximately 1.25 to 1.5 hour.
5. Remove samples of cooked emulsion from the water bath and immediately decant liquid released during cooking into funnels allowing liquid to drain into graduated conical tubes. Centrifuge tubes with decanted liquid at 5,500 rpm for 10 min.
6. After centrifugation, record total volume of fat, volume of gel-water and proteinaceous solids released during cooking.
7. Averages of triplicate determinations are calculated and reported as volume lost per 100 g batter.
8. Record the final weight of the decanted liquid for the triplicate samples to determine the average weight of the liquid lost per 100 g batter.

APPENDIX B

DETERMINATION OF HYDRATION VALUES FOR MEAT BATTERS

EQUIPMENT:

Scale	Funnels
50 ml polycarbonate tubes	Cheesecloth
Tube rack	Centrifuge with rotor

PROCEDURE:

1. Weigh 33 g (+/- 1 g) of meat batter into pre-weighed 50 ml polycarbonate tubes (in duplicate).
2. Pre-chill (2°C) centrifuge and rotor.
3. Place tubes in rotor and centrifuge at 15,000 rpm (27,000 x g) for 15 min.
4. Decant free liquid from t he tube through one layer of cheesecloth. Weigh supernatant into pre-weighed 50ml polycarbonate tubes.
5. Determine hydration (g water held/g wet tissue):

   HYDRATION = (Sample wt – supernatant wt) – (

APPENDIX C

pH MEASUREMENT PROCEDURE

EQUIPMENT:
Food processor or homogenizer
pH meter with pH electrode
Waring® Blender
Stir plate
Magnetic stir bars
Thermometer

REAGENTS:
Distilled water
Buffer, pH 4.0 and pH 7.0

PROCEDURE:

1. Obtain 50-100 g of product or tissue sample and grind 3 to 4 times or homogenize in a food processor. For pre-rigor sausage it is important to take the pH reading immediately after slaughter and/or use iodoacetate to halt the pH decline prior to rigor mortis resolution.
2. Place approximately 30 g of the finely chopped, minced or ground sample into a blender.
3. Add 90 g distilled water (at least three times the weight of the sample) to the blender and blend on high speed for 15 to 20 seconds to make a smooth slurry.
4. Measure the pH of this slurry with a pH meter that has been calibrated with two standard buffer solutions. One buffer at pH = 7.0 and the other having a pH value near that of the final sausage pH (range 4.0 to 4.5).
5. The electrode should be placed in the stirred slurry for about 30 to 60 seconds to allow the electrode to equilibrate. Record the pH of the slurry after the electrode has stabilized. The temperature of the solution being measured should be close to the temperature calibration of the pH meter, otherwise the pH determination will not be accurate.
6. Do **NOT** leave the pH probe in the meat slurry. Remove the pH probe from the slurry and wash it thoroughly with distilled water. Be sure to gently wipe all fat and connective tissue from the probe.
7. Always store the pH probe in **CLEAN** distilled water or pH 7 buffer. **NEVER** let the bulb dry out.

APPENDIX D

HUNTER LAB MINI SCAN XE PLUS STANDARD OPERATING PROCEDURES

** Always handle the black and white standardization plates with care. Do not scratch or chip them.

Plug Mini Scan into electrical outlet.

Wrap PVC overwrap over aperture insuring a smooth, tight fit. Also wrap the black and white standardization plates with PVC overwrap. Make sure there are no air bubbles or wrinkles on the surface of the plates where the readings will be taken.

Wipe the black plate with a Kimwipe to insure it is clean and place the black plate on the circle of the calibration tile holder.

Place the Mini Scan on the calibration tile holder so the two rubber feet are in the two holes of the holder and the aperture is centered on the black plate. The aperture should fit flatly on the black plate to insure that there is no interference when taking readings.

Push the lightning bolt key on the Mini Scan to turn the unit on.

Make sure that the XYZ values on the screen correspond to the XYZ values listed on the back of the white plate.

You are now ready to standardize the unit. Press the lightning bolt key and the Mini Scan will read the black plate.

When the reading is complete, the screen will indicate that the machine is ready to read the white plate.

Remove the black plate from the calibration tile holder and replace it with the white plate. Wipe the white plate with a Kimwipe. Make sure that the aperture of the Mini Scan sits flatly on the white plate.

Press the lightning bolt key to read the white plate.

Press the lightning bolt key three times and the MiniScan will be ready to read the first sample.

The Tristimulus values L*a*b* will be recorded.

Position the aperture of the Mini Scan on the part of the meat sample to be tested. Be sure that the aperture fits flatly on the meat but do not apply pressure. The spot to be tested should be representative of the steak **muscle** tissue. There should not be a lot of connective tissue, seam fat or subcutaneous fat where the color reading is taken.

To take a reading, press the lightning bolt key.

Record the L*a*b* values and press the lightning bolt key again to take a second reading of exactly the same spot on the meat sample.

Record the L*a*b* values and take a third reading by pressing the lightning bolt key.

Record the L*a*b* values and press the lightning bolt key to display the average values. Record these values.

Press the lightning bolt key again to display the standard deviations. Record these values.

The Mini Scan is now ready to read the next sample. Repeat the process. Before taking readings on the second meat sample, make sure that the PVC overwrap covering the aperture is clean and free of fat or anything that might interfere with a clean reading.

When all readings are complete, unplug it from the electrical source.

Be sure that the Mini Scan is clean and that the aperture is clean before putting the machine away.

APPENDIX E

LIPID OXIDATION ANALYSIS PROCEDURE

FOR CURED MEATS

Apparatus:

500 or 800 ml Kjeldahl flasks
Spectrophotometer with 1 cm cells
Hot plate or Bunsen burner
Waring Blender
Boiling chips
250 ml beakers
Balance / Scale
400 ml beakers
Screw cap test tubes
Test tube rack
Graduated cylinder
Timer
Pipette

Reagents:

0.02 M 2-Thiobarbituric Acid (1.442 g 2-Thiobarbituric acid in 500 ml distilled water). Heat just enough to dissolve, DO NOT BOIL.
0.5% Propyl gallate (PG) and 0.5% ethylenediamine tetraacetic acid (EDTA) solution (5g PG + 5 g EDTA in 1 liter distilled water, heat just enough to dissolve, DO NOT BOIL).
Sulfanilamide Reagent (0.5% sulfanilamide in 20% HCL, v/v); For 500 ml: 20% HCL combine 100 ml conc. HCL + 400 ml dd-water
4 N HCL (1 volume concentrated HCL and 2 volumes of distilled water) or (384 g conc. HCL in 1 liter dd-water)
Slipicone® Spray (reduces foaming)

PROCEDURE:

Extraction Solution Combinations for Various Nitrite Levels in the Sample (For Step #1)
BLENDING SOLUTION

_

	Amount of Residual Nitrite (ppm)				
	0	**0-50**	**50-100**	**100-150**	**150-200**
Meat	**30**	**30.0**	**30**	**30.0**	**30**
Sulfa Reagent	**0**	**1.5**	**3**	**4.5**	**6**
dd-water	**45**	**43.5**	**42**	**40.5**	**39**
PG + EDTA	**15**	**15.0**	**15**	**15.0**	**15**

_

Transfer Solution Combinations for Various Nitrite Levels in the Sample
(For Step #2)
DISTILLATION SOLUTION

	Amount of Residual				
Nitrite (ppm)	**0**	**0-50**	**50-100**	**100-150**	**150-200**
dd-water	**77.5**	**78**	**78.5**	**79**	**79.5**
1:2 N HCL	**2.5**	**2**	**1.5**	**1**	**0.5**

1. Blend 30 g of meat with 43.5 ml of 50°C distilled water plus 1.5 ml of sulfanilamide reagent and 15 ml of 0.5% solution of PG and EDTA for 2 min. (Sulfanilamide forms a diazonium salt with nitrite and prevents interference).
2. Quantitatively transfer 30 g of the slurry into a 500 ml Kjeldahl flask using 78 ml of 50°C distilled water. Add 2 ml of HCL solution (1:2).
3. Spray Slipicone® into the neck of the flask and add 5 – 6 boiling chips.
4. Turn on Kjeldahl distillation hot plate and the condenser water.
5. After the heating element is hot, connect the flask and collect 50 ml of distillate (12 – 15 min).
6. Remove the distillate and replace with a beaker containing 400 ml distilled water. Turn off the heat and allow the water to be drawn back through the distillation apparatus. Then turn off the cooling water.
7. Add 5 ml of the distillate to a screw cap test tube along with 5 ml of the TBA reagent. Mix and heat in a boiling water bath for 35 min to develop the color. For the blank, use 5 ml distilled water + 5 ml TBA reagent and heat with the sample.
8. Cool in tap water for 10 min, place sample in a cuvette. Read the sample absorbance in the spectrophotometer at 530 nm. The blank should be read first and set at 0 absorbance.

NOTE: For accurate results, a standard curve should be run for quantities of malonaldehyde over the expected range of values.

CALCULATION OF TBA NUMBER:

TBA number = O.D. x K

Where K = 7.8, which was determined for the distillation set up in the lab.

Specifically,

TBA number = Abs 530 x 7.8 (conversion factor) mg malonaldehyde/kg sample

Standard deviations of the duplicates should be approximately ±0.2 TBA Value.

Slight changes occur in the K value from laboratory to laboratory. Therefore, the K value or standard curve for known dilutions of 1, 1, 3, 5 tetraethyoxypropane should be calculated in each laboratory. K=7.0 is an average value that can be used but may not be the most accurate (Tarladgis et al., 1960).

Expected TBA Values:

0.7 to 1.0	Fresh manufactured product (maximum)
1.0 or higher	Considered to be rancid by some processors
0.1 to 0.2	Reported rancidity for cooked pork
0.5 to 1.0	Considered the threshold level in pork by come researchers
0.3	Maximum allowed for rework material by some packers

1.0 mg malonaldehyde/1000g meat has been reported as the threshold for rancidity by organoleptic detection.

APPENDIX F

INSTRUCTIONS FOR OPERATING THE VARIAN SPECTROPHOTOMETER TO DETERMINE TBAR VALUES

Turn the spectrophotometer on at least 15 minutes prior to running samples. This allows the machine time to warm up.

The **ON** switch is located on the front of the spec in the lower left corner.

The computer should never be turned off.

With the computer mouse, double click on the icon '**CaryWinUV'**.

On the next screen, double click on the icon '**Advanced Reads'**.

A "Did you know" box will appear, click "**Close**".

Go to the left side of the screen and double click on the "**Setup**" button.

On the next screen, change the wavelength to "**530**" nm. Then click on the "**Samples**" tab and enter the number of samples that you will be running. You can also name the samples by typing each sample name. (If you don't want to do this, just keep track of what sample corresponds to sample 1, sample 2, etc. on you lab paperwork.) Click "**OK**" to close the setup screen.

**You should always wear disposable gloves to keep your hands and the cuvettes clean.

To run the blank, double click on the "Zero" button (which is located directly below the "Setup" key on the left side of the screen).

A box will appear prompting you to load the blank into the spec.

Open the green door on the top of the spec. Wipe the smooth sides of the cuvette with a Kimwipe to remove anything that could interfere with a clear reading. Insert the cuvette containing the sample blank into well #1 of the cell block. The smooth sides of the cuvette should be on the left and right. The grooved sides of the cuvette should face front and back.

Completely close the green door. You are now ready to read the blank.

Click "**OK**" on the computer screen to read the blank.

When the sample reading is complete, the “Zero” value will appear on the screen. Open the door and remove the cuvette.

To remove the cuvette, gently pull up on the silver knob that is located in front of the sample cell block. Using gloved hands or tweezers, pull the cuvette out of the cell block. **DO NOT** discard any samples until you are finished with the run, just in case any samples need to be rerun.

Take the next sample cuvette, wipe the sides clean with a Kimwipe and insert the cuvette into cell block well #1. Close the green door.

On the computer screen, click on the “**START”** traffic light key located toward the top of the screen.

A “Sample Collection” box will appear on the screen. In this box will be a list of your samples. If the list is correct, click “**OK**”. (If the list is not correct, go back to the “**Setup**” screen, “Samples” tab and enter the correct information.)

Then a “Save As” box will appear. Type in a file name for your sample run i.e. “Fat Blend Day 7 TBAR 081506”. Click “**Save**”.

A “Present Sample” box will appear on the screen. It is now ready to read the first sample, which you placed in the cell block.

Click “**OK**”.

When the first sample has been read, another box will appear on the screen prompting you to insert the second sample.

Remove sample 1 cuvette from the cell block. Wipe the sample 2 cuvette with a Kimwipe and insert the cuvette into well #1 of the cell block. Close the green door and click “**OK**”.

Continue this process until all samples have been read. When the last sample reading is complete, click on the “**Print**” button which is located toward the lower left corner of the screen.

Then click on the “**Clear Report**” button which is just above the “Print” button. This will clear the report from the screen but not from the computer files.

If you have more samples to run, you can start over again by clicking on “Setup” and check the wavelength and set up for the appropriate number of samples.

If you are finished running samples, click on the X in the upper right corner of the screen to escape from the program.

Click the X again and leave the computer turned ON.

Turn off the spec when you're finished.

APPENDIX G

NITRITE ANALYSIS IN CURED MEATS PROCEDURE

(AOAC Official Method 973.31, 2000, 39.1.21, PAGE 8)

EQUIPMENT:

100 ml beakers
1000 ml Volumetric flasks
50 ml Volumetric flasks
Spectrophotometer (UV/VIS 540 nm)
5 ml Pipettes
500 ml Erlenmeyer flasks
Heated Water Bath
Analytical balance
Homogenizer or food processor
Glass rods
500 ml Volumetric flasks
Hot Plate
Spec cuvettes
10 ml Pipettes
Whatman® No. 2 Filter paper

REAGENTS:

NED Reagent: Dissolve 0.2 g N-(1-naphthyl)ethylene diamine • 2HCl in 150 ml 15% (v/v) acetic acid. Store in a glass-stoppered brown glass bottle. If necessary, filter before use.

Sulfanilamide Reagent*: Dissolve 0.5 g sulfanilamide in 150 ml 15% (v/v) acetic acid. *Store in dark or brown glass bottle. If necessary, filter before use.

Standard Curve Preparation:
Nitrite Standard Solution

Stock solution (1,000 ppm $NaNO_2$): Dissolve 1 g (± 0.0001) $NaNO_2$ in distilled water and dilute to 1 L.
Intermediate Solution (100 ppm $NaNO_2$): Dilute 100 ml of Stock Solution to 1,000 ml with distilled water.
Working Solution (1 ppm $NaNO_2$): Dilute 10 ml of Intermediate Solution to 1,000 ml with distilled water.

Filter Paper:
Randomly select 3 to 4 sheets per box. Filter 40 ml water through each sheet.
Add 4 ml sulfanilamide reagent, mix and wait 15 min.
If any sheets are positive, discard entire box.

PROCEDURE:

1. Weigh 5 g (±0.01) of finely comminuted and thoroughly mixed sample into a 100 ml beaker.
2. Add approximately 40 ml distilled water and heat to $80^{o}C$. Use a glass rod to break up all lumps and mix thoroughly.
3. Transfer the heated solution to a 500 ml volumetric flask. Quantitatively wash the beaker and rod with successive portions of the hot distilled water, adding all washings to the flask (approximately 300 ml).
4. Transfer the flask to a steam bath (~$100^{o}C$) and shake occasionally for 2 hour. After cooling to room temperature, bring the volume to 500 ml with distilled water and remix. Filter through two Whatman No. 2 filter papers into flask and mix solution thoroughly (discard the residue). Then transfer 25 ml of the filtrate into a 50 ml volumetric flask then add 2.5 ml sulfanilamide reagent, mix thoroughly.
5. After setting for 5 min, add 2.5 ml NED reagent, mix. Dilute to volume with distilled water, mix and set for another 15 min to let the color develop.
6. Transfer a portion of the solution to the cuvette and read absorbance at 540 nm against a blank of 45 ml distilled water + 2.5 ml sulfanilamide reagent + 2.5 ml NED reagent.

Standard Curve Preparation:

Add 10, 20, 30 and 40 ml of nitrite working solution to individual 50 ml volumetric flasks. The nitrite concentration in each flask is 0.2, 0.4, 0.6 and 0.8 ppm, respectively. Add 2.5 ml of sulfanilamide reagent, mix and proceed as in steps 5 and 6. The standard curve is straight line to 1 µg/ml $NaNO_2$ in final solution.

CALCULATION:

Nitrite Residual (ppm or µg/g) = Absorbance x K x F

Where: K = Standard Curve Slope = 1.7438

F = Dilution Factor = 500 x 2 x 1/5 = 200

OR

The concentration may be read directly off of the spectrophotometer. Thus, K, Abs nor F are required in this case.

NITRITE DETERMINATION

Standard Curve: __________

Absorbance

Blank ___________

Sample ___________

Rep #1 ___________

Rep #2 ___________

Rep #3 ___________

Rep #4 ___________

Rep #5 ___________

Average ___________

Nitrite Residual (ppm or µg/g) = Absorbance x K x F

Absorbance: 540 nm (Spectrophotometer)

K: Standard Curve Slope ___________

F: Dilution Factor (500 x 2 x 0.20) ___________

Nitrite Residual:

Blank: ___________ Absorbance

Rep #1 ___________ Nitrite

Rep #2 ___________ Nitrite

Rep #3 ___________ Nitrite

Rep #4 ___________ Nitrite

Rep #5 ___________ Nitrite

Average Nitrite Concentration in the Product: ___________

APPENDIX H

SODIUM CONCENTRATION PROCEDURE

EQUIPMENT:

Orion Model 720A pH/ISE meter
Sodium Electrode
Scale
Stir Plate
Stir Bars
Food Processor or Homogenizer
Squeeze Bottle for Rinse Solution
Kim Wipes
Disposable pipettes
1000 ml volumetric flask
150 ml Glass Beakers

REAGENTS:

Sodium Known Standard (1,000 ppm Sodium, Orion 841108)
Sodium Ionic Strength Adjustor (Orion 841111)
Reference Electrode Filling Solution
Distilled, Deionized Water
Sodium Electrode Rinse Solution: Add 10 ml of ISA to volumetric flask. Bring to volume using distilled water.

Preparation of Standards:

Beaker ID	Standard (ml)	Water (ml)	Standard (ppm)
0	0	100	0
250	25	75	250
500	50	50	500
1000	100	0	1000

PROCEDURE:

Checking Electrode Operation (Slope):

1. If electrode has been stored dry, condition the electrode. Refer to electrode operating manual for this procedure.
2. Connect electrode to the meter.
3. Place 100 ml distilled water in to a 150 ml beaker. Add 10 ml ISA. Stir thoroughly. Set function switch of the meter to read in mV.
4. Rinse electrode with sodium electrode rinse solution and place in the solution prepared in step 3.
5. Select a sodium standard (1000 ppm Na). Pipet 1 ml of this standard solution into the beaker. Stir thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.
6. Pipet 10 ml of the same standard into the same beaker. Stir thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.

7. The difference between the first and the second potential readings is the slope of the electrode. The difference should be in the range of 54-60 mV, assuming the solution temperature is between 20 an 25°C.

Direct Calibration:

1. Prepare electrodes according to operating manual instructions.
2. Connect electrode to meter.
3. Electrode input will appear on the prompt line CH-1 or CH-2. Set channel to match input of electrode by pressing **2nd** then **Channel (5)** to change the channel is needed.
4. Press **Mode (1)** until concentration mode indicator CON appears.
5. Press **Calibrate (2)**. Calibrate will be displayed.
6. **ENTER NO. STDS** will appear on the display, enter **4** and press **Yes**.
7. Starting with the least concentrated standard, add 10 ml Sodium ISA solution and stir thoroughly.
8. Rinse the electrode with sodium electrode rinsing solution and place into beaker.
9. **READY ENTER VALUE** will appear (it takes a few minutes). Enter the concentration value standard **0** and press **Yes**.
10. Repeat steps 7, 8 & 9 for the 250, 500 and 1,000 ppm standards.
11. The electrode slope will be calculated and displayed.
12. The meter will then advance to the **MEASURE** mode.

Measurement of Samples:

1. Place 10 grams of sample in the blender and add 90 ml distilled water.
2. Blend for 30 sec on high.
3. Transfer 100 ml of diluted sample to a 150 ml beaker.
4. Add 10 ml Sodium ISA and stir thoroughly.
5. Rinse electrode in Sodium Electrode Rinse Solution and place into sample. Continue to stir the sample.
6. Record concentration directly from the meter display when **READY** appears.
7. When finished, rinse electrode thoroughly and store according to operating manual instructions.

Concentration Unit Conversion Factors:

To Convert From:	To:	Multiply By:
Moles/Liter NaCl or Moles/Liter Na	ppm Na	23,000
	ppm NaCl	58,500
	% Na	2.3

	%NaCl	5.85
	mg Na/100 g, mg Na/100ml	2,300
	mg NaCl/100 g, mg NaCl/100 ml	5,850
ppm Na	moles/liter Na	0.0000434
	moles/liter NaCl	0.0000171
	%Na	0.0001
	%NaCl	0.000254
	ppm NaCl	2.54
	mg Na/100 g, mg Na/100 ml	0.100
	mg NaCl/100g, mg NaCl/100 ml	0.254
%NaCl, % salt	moles/liter Na	0.434
	moles/liter NaCl	0.171
	ppm Na	3,932
	%Na	0.3932
	ppm NaCl	10,000
	mg Na/100 g, mg Na/100 ml	393
	mg NaCl/100 g, mg NaCl/100 ml	

APPENDIX I

CRUDE FAT DETERMINATION – AOAC PROCEDURE

EQUIPMENT:
Whatman® filter paper, 22 x 40 mm
Stapler
Aluminum dishes, 50 mm diameter x 40 mm deep
Desiccator with desiccant
Tongs
Analytical balance/ Scale
Convection oven
Soxhlet apparatus
Fume hood
Boiling chips

REAGENTS:
Ether (diethyl or petroleum)

PROCEDURE:
1. Construct thimbles from Whatman 22 x 40 mm filter paper folded into a sleeve open at one end and stapled at the other end. Dry thimbles overnight at 100°C using air dry oven. (Samples dried previously by the Air Oven method may be used.)
2. Cool thimbles in desiccator for 30 minutes.
3. Weigh thimble and record the weight (Beginning thimble weight). Put 2 to 3 grams of stirred sample into the thimble and seal. Record the weight to the nearest 0.0001 g (Beginning thimble and sample weight).
4. Dry overnight at 100°C.
5. Cool in desiccator for at least 30 minutes prior to reweighing.
6. Weigh the sample and record the weight (Dried thimble and sample weight).
7. Extract on the Soxhlet apparatus for 12 hours at an ether (diethyl or petroleum) drip rate of approximately 4 drops per second.
8. Allow sample to evaporate under the hood until thoroughly dry (no detectable ether odor) ** This is very important to avoid an explosion or flash fire**
9. Dry in the oven overnight at 100°C.
10. Cool in the desiccator of 30 minutes or until the sample cools to room temperature (this could be a long as one hour).
11. Weigh the sample and record (Fat free thimble and sample weight).

CALULATIONS:

$$\textbf{Percent Fat Content} = \frac{(B-C)}{A} \times 100$$

Where: **A = Sample Weight**
B = Dried thimble and sample weight
C = Fat free thimble and sample weigh

APPENDIX J

MOISTURE ANALYSIS – AOAC PROCEDURE

EQUIPMENT:

Whatman® filter paper, 22 x 40 mm
Stapler
Aluminum dishes, 50 mm diameter x 40 mm deep
Desiccator
Convection oven
Food Processor
Tongs
Analytical balance/ Scale

PROCEDURE:

1. Filter paper/extraction thimbles consisting of Whatman 22 x 40 mm filter paper folded in to a sleeve open at one end and stapled at the opposite end, or a covered aluminum dish at least 50 mm in diameter and not greater than 40 mm deep.
2. Mechanical convection oven, preferably one equipped with a booster heater.
3. Accurately weigh sample to the fourth decimal place (+/- 0.0001). Sample should weigh approximately 2 g. Then place sample into a previously dried and desiccated sleeve, paper thimble or a covered aluminum dish that has been dried and desiccated.

   Note: handle sample container with tongs to avoid moisture from your fingers. Never handle sample containers with gloved or ungloved hands for the most accurate results.

4. Dry sample for 16 to 18 hours at 100 to 102°C, or for four hours at 125°C, in the mechanical convection oven. Drying at higher temperature (125°C) may cause the fat to oxidize (vaporize) creating excessive fat loss and inaccurate fat percentages.
5. Cool the samples in a desiccator (with desiccant) to room temperature so that no additional moisture is absorbed by the sample. Reweigh the dried sample.

CALCULATIONS:

Percent Moisture $= \dfrac{100\,(B-C)}{A}$

Where: **A = Sample weight**
B = Weight of dish/thimble + sample before drying
C = Weight of dish/thimble + sample after drying

APPENDIX K

Directions for running protein analysis on LECO FP-528

1	Turn on all gases by opening the valve on the top of each tank. (Change to a new tank of gas when the regulator gauge reads 300 psi)
2	Press "EXIT"
3	Press "1 ANALYZE"
4	Press "MENU"
5	Press "6 INSERT BLANKS"
6	Press "5" Five blanks should be run before analyzing test samples. Protein results for blanks should be 0.0 (- 0.02).
7	Press "START" two times to start the blank sample run. It takes 167 seconds run time for each blank.
8	Weigh meat sample into gel capsule. Sample weight should not exceed 0.2 g.
9	Enter sample weight into LECO. The cursor will automatically begin at the right of the decimal point.
10	Press "START" to open sample reservoir.
11	Using tweezers, gently place sample capsule in opening insuring all of the sample remains in the capsule and doesn't spill.
12	Press "START" to begin analysis
13	When analysis is complete the percent protein will be displayed on the screen.

To change the ID code:

1	Press "SELECT"
2	Press "1" two times

P-Factor for meat is 6.25
To change the P-Factor press "SELECT". Highlight the P-Factor number and enter the new value by pushing the number keys.

Run EDTA Standards:
When change gas cylinders
When change filters, tube, etc.
Anytime the machine is opened and maintenance is performed

If the machine is turned off
If you think that there might be a problem
When you are starting a new study

Run standard once per month if running samples weekly.

Run EDTA Standard at least 3 times. Protein results should be very close to the values that are documented on the EDTA Certificate of Analysis. (59.75, 59.625, 59.875)
If the results are not very close to the certificate values, need to change program using the
three new numbers.

Press "EXIT"
Press "3 CALIBRATE"
Press "Previous" or "NEXT" to move cursor to number you want to select.
Press"SELECT" three times
Press "MENU"
Press "4 CALCULATE CALIBRATE"
The Nitrogen Standard should be 9.560
Press "SELECT"
Press "YES"
Press "YES" to return to the list of values
Press "EXIT" to return to the main menu
Press "1" to Analyze

APPENDIX L

CHILLED – MIRROR DEWPOINT DETERMINATION OF WATER ACTIVITY

(A_w)

EQUIPMENT:
Chilled Mirror Dewpoint Machine
Disposable sample cups
Homogenizer or Food Processor

REAGENTS:
Salt solution Standards
Distilled water

PROCEDURE:
Instrument Calibration
Linear Offset is a means of checking the calibration of the instrument and can be checked by using standardized salt solutions and distilled water. Verification standards are specially prepared salt solutions that have a specific molality and water activity that is constant and accurately measurable. Performance Verification Standards come in three water activity levels listed below.

Verification Standard @ 20°C	Water Activity
0.5 m KCl	0.983 ± 0.001
6.0 m NaCl	0.760 ± 0.003
8.5 m LiCl	0.500 ± 0.003
13.3 m LiCl	0.250 ± 0.003

Linear offset should never be verified against distilled water, but should be tested with a standard near the water activity level to be measured.

Verification for Linear Offset
1. Choose a verification standard that is close to the a_w of the sample you are measuring. Make sure that your standard is at ambient temperature before you load it into the sample drawer, and that the AquaLab has warmed up at least 15 minutes.
2. Empty the whole vial of solution into a sample cup and place it carefully into the sample drawer.
3. Carefully slide the drawer closed, being especially careful that the solution doesn't splash or spill and contaminate the chamber.

4. Turn the drawer knob to the READ position to make an a_w reading. Make two readings. The readings should be within ± 0.003 of the given value for the salt solution.
5. If the reading is within 0.003 of the salt solution, prepare a sample cup half full of distilled water and make two readings. The first reading may be low. The second reading should be 1.000 ± 0.003. If the salt reading is correct and the distilled water is not, it is probably due to contamination of the sensor chamber. For cleaning instructions, see Chapter 10 in the instruction manual.
6. If you consistently get readings that are outside of the a_w of the salt solution standard by more than ± 0.003, a linear offset has probably occurred. See Chapter 5 in the instruction manual for adjusting the linear offset.

Sample Preparation:
Make sure that the sample to be measured is **homogeneous**. For meat products, this requires homogenization of the sample in a food processor to create a homogeneous mass. Samples should be stored in a moisture proof container to avoid loss of moisture.

Place the sample in a disposable sample cup, **completely covering** the bottom of the cup if possible. Samples that contain propylene glycol in concentrations >10% will not damage the instrument, but a_x values for consecutive samples will not be accurate. Propylene glycol condenses on the mirror during the reading, but does not evaporate from the mirror as water does.

Do **NOT** fill the sample cup **more than half full**. Overfilled cups will contaminate the sensors in the sensor chamber. Make sure the rim and the outside of the sample cup are clean.

If a sample will be read at some other time, put the sample cup's disposable lid on the cup to restrict water transfer.

Taking a_w Readings:

1. Prepare the sample as described previously and place in the plastic sample cup. ALLOW THE SAMPLE TO EQUILIBRATE TO ROOM TEMPERATURE.
2. Turn the sample drawer knob to the OPEN/LOAD position and pull the drawer open.
3. Place the sample cup with sample in the drawer. CHECK THE TOP LIP OF THE CUP TO MAKE SURE IT IS FREE FROM SAMPLE RESIDUE.
4. Carefully slide the drawer closed, being especially careful if you have a liquid sample that may splash or spill and contaminate the chamber.
5. Turn the sample drawer knob to the READ position to seal the sample cup with the chamber. Readings normally take 5 minutes. Some extremely dry samples, highly viscous samples may require up to 10 minutes or more to reach an accurate measurement of a_w.

6. Take the a_w reading directly from the screen.

CAUTIONS:

Never leave a sample in the water activity meter after a reading has been taken. The sample may spill or contaminate the instrument's chamber.

Never try to move the instrument after a sample has been loaded. Same reason as above.

Take special care not to move the sample drawer too quickly when loading or unloading liquid samples.

If a sample has a temperature that is 4^oC higher than the sample chamber, the instrument will display "Sample too hot". Warm samples cause condensation on the mirror surface.

The operating temperature range of the instrument is 5° to 43^oC.

If a triangular warning symbol appears in the top right hand corner, this indicates that the mirror has become too dirty to give accurate measurements. Clean mirror.

If a sample has a lower a_w than about 0.08, a display message will indicate that the $a_w < 0.078$ at 24.7^oC.

APPENDIX M

Figure Ballot used for descriptive sensory analysis of beef frankfurters.

TEXTURE					
Sample	Springiness	Fracturability	Hardness	Cohesiveness	Juiceness
W/U					

Springiness
0.0 Cream Cheese
5.0 Frankfurter
9.5 Marshmallow
15.0 Jello

Fracturability
1.0 Corn Muffin
4.0 Graham Crackers
5.0 Rye Wafers
7.0 Ginger Snaps
9.0 Melba Toast
13.0 Peanut Brittle
15.0 Hard Candy

Hardness
1.0 Cream Cheese
3.0 American Processed
5.0 Frankfurter
7.0 Olives
9.0 Peanut
11.0 Carrots
14.5 Hard Candy-Lifesavers

Cohesiveness
1.0 Corn Muffin
4.0 American Processed Cheese
8.0 Dried fruit-Pretzel
10.0 Soft Pretzel
12.0 Candy Chews-
15.0 Chewing Gum

Juiciness
1.0 Banana
2.0 Carrot
4.0 Mushroom
8.0 Cucumber
10.0 Apple
12.0 Honeydew Melon
15.0 Watermelon

APPENDIX N

Table Ballot used for sensory analysis of beef frankfurters.

Sample	Ck Beef Lean	Ck Beef Fat	Smoke	Spice Complex	Cardboard	Painty	Fishy	Cowy	Soda	Salt	Chemical	Bitter	Soapy	Metallic	Chemical burn	OFF FLAVORS
1																
2																
3																
4																
5																

TERM	REFERENCE	VALUE
SODA	SALTINES (NABISCO	2
GRAPE	KOOL-AIDE	4.5
LEMON	LEMONADE (COUNTRY TIME)	5
COOKED APPLE	APPLE SAUCE (MOTT'S)	5
GRAPE	GRAPE JUICE (WELCH'S)	10
CINNAMON	BIG RED GUM (WRIGLEY'S)	12

OFF FLAVORS CHARACTERISTICS

X Other (describe)
A Acid
N Nutty
P Putrid
SD Soured
SO Sour
SW Sweet
SP Spice
SM Smoke
S Salty
SY Soapy
F Fishy

APPENDIX O

Table Ballot used for sensory analysis of beef frankfurters

SAMPLE ID	W/U	W/U	W/U	W/U	W/U	W/U
TEXTURE						
Fractuability						
Hardness						
Cohesiveness						
Juiciness						
AROMATICS						
Ck Beef Lean						
Ck Beef Fat						
Spice Complex						
Chemical						
Cardboard						
Painty						
Fishy						
Other						
TASTES						
Salt						
Sweet						
Bitter						
Sour						
MOUTHFEELS						
Metallic						
Sour						
Burn						
AFTER TASTES						
Acid						
Burn						
Sour						
Fishy						
Salt						
Spice						
Other (describe)						

APPENDIX P

RECOMMENDATIONS FOR FUTURE RESEARCH

The results of this study demonstrated that cow carcasses are more likely of producing high pH muscles at the same time proving to be beneficial in certain high protein products. Not only did products high in pH result in a higher water-holding capacity, but was also more tender. Cow trimmings high in pH proved to be advantageous in an emulsified and dry product.

Forty two percent of cow carcasses exhibited a pH of 6.0 or greater, whereas the majority of fed beef carcasses fell within acceptable ranges. When we investigated the effects of high pH beef in processed meat products, such as jerky, frankfurters, and snack sticks, it was soon realized that high ph beef may be an alternative source in different protein source products. In the future, identifying muscle pH with lean color (objectively and subjectively) of the same carcass may add more insight to understanding muscle pH with the quality defect dark, firm, and dry and additional correlations.

In the jerky study, whole muscle inside rounds were obtained from two different carcass classes (Fed and Cow) and two pH types (Normal and High). In a commercial setting, sliced inside rounds undergo a uniform thermal processing time. Therefore, inside rounds were uniformly cut and placed in a smokehouse for the same amount of time. Jerky slices then were removed from the smokehouse when normal jerky slices representing fed beef reached a water-activity of < 0.85. It was soon discovered that pH does have an affect on jerky attributes, but most importantly water-activity. The feasibility of investigating shelf-life and sensory attributes could be explored.

Beef frankfurters were manufactured consisting of various levels of high (100% High, 75%High/25Normal, 50% high/ 50% normal, 25% high/ 75% normal, 100% Normal, and Control, 100% normal with 0.35% added phosphate) pH cow trimmings that was compared to a normal pH frankfurter with 0.35% added phosphate. In commercial emulsified meat products, various trimmings are often used. If processors began to utilize cow trimmings to save cost then processors must familiarize themselves with cow carcasses. The current study showed that frankfurters consisting of at least 50% high pH were in fact firmer and harder than frankfurters that consisted of normal pH. Interestingly, they were similar to the added phosphate frankfurter in many attributes. However, regardless of pH, spoilage had set in earlier than expected with was indicative of a short-shelf life. Therefore, as a recommendation of future research, it is necessary of adding an antimicrobial if the vast majority of the lean trimmings are of cow carcasses.

Finally, similar to the frankfurter study, various levels of high pH was investigated in snack sticks (without a control). Similar to jerky, water activity is an important factor as well as pH decline. Regardless of percent high pH, all treatments reached a final pH < 5.3, however, did not meet food safety requirements which would require additional drying times. Unlike frankfurters, shelf-life was not an issue under refrigerated conditions. However, there was one discrepancy in this study. The discrepancy was that snack sticks were investigated under refrigerated conditions. Because snack sticks are known as a shelf-stable product, it is common to find this high protein snack stored under ambient temperatures. Therefore, for future research, investigating snack sticks containing various levels of high pH under room temperature and its affects on textural, sensory attributes, and shelf-life is need to be explored.

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