

Microbiological characteristics of pork carcasses and vacuum packaged blade steaks
during storage at $0\pm 1^{\circ}\text{C}$

By

Francisco Najar-Villarreal

B.S., University of Nuevo León, 2014

A THESIS

submitted in partial fulfillment of the requirements for the degree

Food Science

KANSAS STATE UNIVERSITY

Manhattan, Kansas

2018

Approved by:

Major Professor
Dr. Elizabeth Boyle

Copyright

© Francisco Najar-Villarreal 2018.

Abstract

To evaluate the effect of extended post-harvest hanging time on pork sides and the microbial shelf-life of fabricated steaks, aerobic plate count (APC), *Enterobacteriaceae* (EB), yeast and mold populations, pH, and moisture content of pork sides hung for 21 days at $0\pm 1^{\circ}\text{C}$ was determined. After hanging, blade steaks fabricated from these sides were vacuum packaged, stored up to 35 days at $0\pm 1^{\circ}\text{C}$, and evaluated for APC, EB, yeast and mold populations, and pH. Carcass cooler temperature and percent relative humidity, measured every hour during storage, averaged $0\pm 1^{\circ}\text{C}$ and 87.3% (66-100%), respectively. Pork carcass surface moisture content declined ($P \leq 0.05$) from 65.1% on day 1 to 50.5% on day 21. The carcass pH was similar ($P \geq 0.05$) at 5.88 on days 1, 7, and 14; however, by day 21 the pH declined ($P \leq 0.05$) to 5.72. The flank, shoulder, and jowl were sampled on pork carcasses. There was no day effect for carcass APC; however, there was a location effect. The jowl had the highest ($P \leq 0.05$) APC with 1.21 log CFU/cm². There was no carcass location or day effect for EB or mold populations, but there was a carcass location and day effect for yeast populations. The detection limit (DL) for EB and yeast and mold populations on pork carcass samples ($n = 240$) was 0.06 and 0.41 log CFU/cm² for the shoulder and flank, and jowl, respectively. For EB and mold populations, 98.8 and 97.9% of carcass samples, respectively, were below the DL. For yeast populations, 37.5, 45.0, and 63.8% were above the DL for flank, shoulder, and jowl, respectively. On days 1, 7, 14, and 21, 60.0, 55.0, 53.3 and 26.6% were above the DL, respectively, for carcass yeast populations; however, none were greater than 2.83 log CFU/cm². The pH of pork steaks was 5.69-5.89. There was a day effect for APC on pork steaks. The initial APC of steaks was 1.61 log CFU/g, increasing ($P \leq 0.05$) to 5.06 log CFU/g on day 35. There was a day

effect for EB and mold populations; but not for yeast populations. The DL for EB and yeast and mold populations on pork steaks ($n = 102$) was 0.70 log CFU/g. On days 0, 7, 14, 21, 28, and 35, the percent of EB populations above the DL on pork steaks were 0.0, 0.0, 23.5, 41.2, 94.1, 41.2%, respectively, however, none of the steak samples exceeded 4.40 log CFU/g. For mold populations, 100.0% of steaks were below the DL on days 0, 7, and 14. On days 21, 28, and 35, 18, 24, and 12%, respectively, were above the DL. None of the steak samples exceeded 2.68 log CFU/g for mold populations. For yeast populations, 56.8% of pork steaks samples were above the DL, but none were greater than 3.69 log CFU/g. These results indicate that pork carcasses and vacuum packaged shoulder blade steaks fabricated from these carcasses have acceptable quality for 21 and 35 days, respectively, when stored at $0\pm 1^{\circ}\text{C}$ after harvest.

Keywords: blade steaks, microbial, pork carcass, storage

Table of Contents

Table of Contents	v
List of Figures	vii
List of Tables	viii
Acknowledgements	x
Chapter 1 - Introduction	1
Review of Literature	3
Meat Refrigeration and Pork History	3
Storage Temperature	4
Shelf-life of Meat	6
Meat Spoilage	7
Anaerobic Packaging	9
Legislation	10
Microbiological Background	10
pH	12
Moisture	12
Sampling Method	13
Materials and Methods	15
Study Design	15
Microbiological Sampling of Pork Carcasses	15
Microbiological Analysis of Pork Shoulder Blade Steaks	17
Determination of pH	19
Moisture Analysis	19
Carcass and Steak Sample Preparation and Bacterial Enumeration	20
Statistical Design	21
Results and Discussion	22
Pork Carcass	22
pH	22
Moisture Content	22
Microbiological	23

Pork Shoulder Blade Steaks.....	27
pH.....	27
Microbiological.....	27
Harvest Day	32
Conclusions.....	34
References.....	36
Appendix A - Statistical Codes.....	46
Pork Carcass	46
pH.....	46
Moisture Content	46
Microbiological.....	46
APC.....	46
EB, Yeast and Mold.....	47
EB	47
Yeast	47
Mold.....	48
Pork Shoulder Blade Steaks.....	49
pH.....	49
APC.....	49
EB, Yeast and Mold.....	49
Harvest Day	50
Pork Carcass APC.....	50
Pork Blade Steaks APC	50
Appendix B - Microbiological and pH tables	51

List of Figures

Figure 1. Carcass sampling locations on pork carcass.....	17
Figure 2. Pork shoulders were cut into six 2.54 cm thick steaks and randomly assigned for each vacuum packaged storage day.	18
Figure 3. Hanging pork carcass side (n = 12) pH during cooler storage at $0\pm 1^{\circ}\text{C}$ and 87.3% relative humidity for up to 21 days.	24
Figure 4. Hanging pork carcass side (n = 20) moisture content during cooler storage at $0\pm 1^{\circ}\text{C}$ and 87.3% relative humidity for up to 21 days.	25
Figure 5. Aerobic plate counts population by sample location of hanging pork carcass sides (n = 20) during cooler storage at $0\pm 1^{\circ}\text{C}$ and 87.3% relative humidity for up to 21 days.	25
Figure 6. Pork shoulder blade steak (n = 12) pH during cooler storage at $0\pm 1^{\circ}\text{C}$ for up to 35 days.	30
Figure 7. Aerobic plate count populations of pork shoulder blade steaks (n = 17) during cooler storage at $0\pm 1^{\circ}\text{C}$ for up to 35 days.....	30
Figure 8. Aerobic plate count populations pooled from the flank, shoulder, and jowl of pork carcasses harvested on two separate production days and hung up to 21 days at $0\pm 1^{\circ}\text{C}$ and 87.3% relative humidity.....	33
Figure 9. Aerobic plate count populations of pork shoulder blade steaks fabricated from hogs harvested on two separate production days [October (n = 5) ¹ and February (n = 12) ¹] and stored up to 35 days at $0\pm 1^{\circ}\text{C}$	33

List of Tables

Table 1. The number of yeast populations by sample location above the detection limit (DL) ¹ of hanging pork carcass sides (n = 20) stored at 0±1°C and 87.3% relative humidity for up to 21 days.	26
Table 2. The number of yeast populations above the detection limit (DL) ¹ of hanging pork carcass sides (n = 20) stored at 0±1°C and 87.3% relative humidity for up to 21 days.	26
Table 3. The number of presumptive positive Enterobacteriaceae (EB) populations above the detection limit (DL) ¹ of pork shoulder blade steaks (n = 17) stored at 0±1°C for up to 35 days.	31
Table 4. The number of mold populations above the detection limit (DL) ¹ of pork shoulder blade steaks (n = 17) stored at 0±1°C for up to 35 days.	31
Table B.1. Least squares means and standard error ¹ for pH and moisture content of right pork carcass sides (n = 20) measured on four dates and stored at 0±1°C and 87.3% relative humidity for up to 21 days.	51
Table B.2. Aerobic plate count (APC) ¹ populations of right pork carcass sides (n = 20) stored at 0±1°C and 87.3% relative humidity for up to 21 days.	51
Table B.3. Aerobic plate count (APC) ¹ populations of right pork carcass sides (n = 20) stored at 0±1°C and 87.3% relative humidity for up to 21 days.	52
Table B.4. Least squares means and standard error ¹ for pH of pork shoulder blade steaks (n = 17) stored at 0±1°C for up to 35 days.	52
Table B.5. Aerobic plate count (APC) ¹ populations of pork shoulder blade steaks (n = 17) stored at 0±1°C for up to 35 days.	53
Table B.6. Mold populations of pork shoulder blade steaks (n = 17) stored at 0±1°C for up to 35 days.	53
Table B.7. Yeast populations of pork shoulder blade steaks (n = 17) stored at 0±1°C for up to 35 days.	53
Table B.8. Aerobic plate count (APC) ¹ populations pooled from the flank, shoulder, and jowl of pork carcasses (n = 20) harvested on two separate production days and hung up to 21 days at 0±1°C and 87.3% relative humidity.	54

Table B.9. Aerobic plate count (APC)¹ populations of pork shoulder blade steaks fabricated from hogs harvested on two separate production days October (n = 5)¹ and February (n = 12)¹ and stored up to 35 days at 0±1°C..... 54

Acknowledgements

The completion of this work would not have been possible without the generous guidance and support of my major professor, Dr. Elizabeth Boyle. I am hugely indebted to you for suggesting the topic of this project, and for providing your expertise and advice when I needed it. I would also like to express my deepest gratitude to my committee members Dr. Terry Houser and Dr. Randall Phebus. To Dr. Houser, thank you for being ever so encouraging at the right time and for being a source of motivation.

I would like to show my appreciation to my statistician Dr. Christopher Vahl for always finding the time to reply to emails and for helping with the statistical analysis; I will forever be thankful.

I am deeply grateful to Dr. Justin Kastner, who has been an excellent guide throughout my master's program. I wish to express my sincere thanks to the meat science faculty members, my fellow graduate students, John Wolf, and Sally Stroda for your kind help and support during these two years.

To my lovely wife Daniela, thank you for being by my side even at the roughest times of graduate school. This accomplishment could not possibly be achieved without your love and patience. We are grateful to God for giving us the blessing of our new baby Paulina.

I would also like to take this opportunity to acknowledge my family for all the love and support you have given me throughout my professional career. Thank you, Francisco, Adriana, and Diana.

Chapter 1 - Introduction

In the U.S., pork production has increased 60% during the last two decades (USDA-NASS, 2016). In 2016, the number of hogs slaughtered in the U.S. averaged 118.2 million head (USDA-NASS, 2017). In 2015, 11.1 billion kg of pork were commercially produced in the U.S. This number was 7% higher compared to pork production in 2014 (NASS-USDA, 2016). The 2015 per capita consumption of pork was estimated to be 22.4 kg in the U.S. (USDA-ERS, 2016). Moreover, pork accounts for 40% of meat consumed around the world (USDA-FAS, 2015).

Meat is a highly perishable food due to its chemical structure that enhances bacterial growth to significant levels, resulting in meat spoilage (Doulgeraki et al., 2012). Refrigeration is used for preservation in the meat industry, and increases fresh meat shelf-life by depressing molecular activity as well as slowing down chemical and biological processes (Lovatt, 2014). The meat industry employs refrigeration to maintain the integrity of fresh meat and/or to reduce the rate of microbial growth on the surfaces of pork carcasses (Cano-Muñoz, 1991). James (2002) stated that storing meat at $-1.5 \pm 0.5^{\circ}\text{C}$ would prolong fresh meat shelf-life the longest. At -1°C and 5°C , microbial spoilage activity is minimized, but spoilage microorganisms continue to grow at a slow rate (Gill, 1997). Sebranek (2008) suggested pork carcasses be stored in chill rooms with 85-90% relative humidity at $0-1^{\circ}\text{C}$.

Scientists define shelf-life as the length of time that a food product can be stored, with the quality attributes remaining unchanged before deterioration occurs (Lulietto et al., 2015). Some of the most common aerobic bacteria known to cause meat spoilage are *Pseudomonas* spp., *Acinetobacter* spp., and *Moraxella* spp. (Rossaint et al., 2015). Due to the ability of *Pseudomonas* spp. to grow at a high rate under aerobic conditions, Gill and Mollin (1991)

identified this organism as the most significant spoilage organism in non-vacuum packaged meat products. Furthermore, lactic acid bacteria grow under anaerobic conditions such as those found with vacuum packaged meat (Gill, 1997). Jeremiah and Gibson (1997) demonstrated that pork can be stored up to 9 weeks at -1.5°C when vacuum packaged. Pork is typically held at 4°C throughout the marketing channels and some psychrophilic bacteria including *Lactobacillus* spp., *Pseudomonas* spp., *Alcaligenes-Enterobacter* spp., *Flavobacterium* spp., *Micrococcus* spp., and *Moraxella-Acinetobacter* spp. can be present on pork (Kotula, 1987). The majority of bacterial contamination in the slaughterhouse is derived from fecal material and animal hides (Sofos, 1994; Gill, 1998). In addition, Lee et al. (2016) studied the effect of extended hanging time on pork carcasses stored at $2\pm 1^{\circ}\text{C}$ and 80% relative humidity, and they concluded that after 40 d of hanging the quality of pork carcasses is unharmed. The objective of this study was to examine the microbial quality of pork carcasses and vacuum packaged pork blade steaks fabricated from these carcasses during extended post-harvest cold storage.

Review of Literature

Meat Refrigeration and Pork History

Throughout America's history, several preservation methods have been used to store meat. For example, salt was added to pork in barrels to increase fresh pork life (Aberle et al., 2003). The use of ice cut from frozen ponds and rivers in New England was used to maintain the freshness of meat which was stored underneath sawdust to prevent melting (Briley, 2004). In the late 19th century, the use of compressors and refrigerants began to be used, and is considered the beginning of mechanical refrigeration (USDA-FSIS, 2010).

Refrigeration is defined as "the process of removing heat from an object" and chilling as "cooling an object to a temperature above its freezing temperature" (Lovatt, 2014). These terms are commonly used for preservation in the meat industry, and increase fresh meat shelf-life by depressing molecular activity as well as slowing down chemical and biological processes (Lovatt, 2014). In addition, refrigeration is used as the air-chilling system in meat plants to help maintain processing areas at low temperatures to delay microbial growth (Lovatt, 2014). Several authors have recommended temperatures to maintain refrigerated meat. For example, Gill and Newton (1978) reported that keeping meat between -1°C and 5°C can slow down bacterial proliferation. James (2002) stated that storing meat at $-1.5 \pm 0.5^{\circ}\text{C}$ would prolong meat shelf-life the longest. The USDA-FSIS (2010) published a food safety information guide indicating that most foods will be protected if maintained at temperatures of 4.4°C or less. Berk (2013) suggested using chilling in the range of $0-8^{\circ}\text{C}$. Alternatively, pork carcasses and cuts are to be stored at -1.5 to 0°C and 90-95% relative humidity for up to 10-14 days (International Institute of Refrigeration, 1967 and 1971).

Hernando de Soto is credited with bringing the first 13 hogs to the U.S., introducing them in Tampa Bay, Florida in 1539, which subsequently led to a rapid spread of hogs across the country (USDA-FSIS, 2013). In the 1800's, the city of Cincinnati, Ohio, also known as "Porkopolis", became the center for slaughtering and packing pigs each year (Hurley, 1981). In the U.S., pork production has increased 60% during the last two decades (USDA-NASS, 2016). In 2016, the number of hogs slaughtered in the U.S. averaged 118.2 million head (USDA-NASS, 2017). In 2015, 11.1 billion kg of pork were commercially produced in the U.S. This number was 7% higher compared to pork production in 2014 (USDA-NASS, 2016). In 2016, the livestock slaughter summary reported that 60% of pork establishments had a production capacity of up to 999 head of hogs per year, representing less than 1% of the total hogs harvested in the U.S. (USDA-NASS, 2017). Pork, after poultry and beef, is the third largest meat commodity produced in the U.S. (USDA-ERS, 2017). The 2015 per capita consumption of pork was estimated to be 22.4 kg in the U.S. (USDA-ERS, 2016). Moreover, pork accounts for 40% of meat consumed around the world (USDA-FAS, 2015). Fresh pork, at the retail level, represents 25% of meat sales; however, a majority of the commercial pork supply is used by the meat industry for processing meats (Wright et al., 2005).

Storage Temperature

Chilling can be described as the application of temperatures in the range of 0-8°C to meat (Berk, 2013). The meat industry employs refrigeration to maintain the integrity of fresh meat and/or to prevent microorganisms from growing at a high growth rate on the surface of pork carcasses (Cano-Muñoz, 1991). At all stages of distribution and at retail, maintaining meat at a low temperature is necessary to prolong shelf-life. The muscles of healthy animals are naturally

sterile; however, carcass meat may be in contact with bacteria during the harvest process, resulting in cross-contamination (Chung et al., 1989; Gill et al., 1998). After harvest, carcasses must be chilled rapidly to prevent the growth of spoilage and pathogenic microorganisms (Gill, 2000). According to Mallikarjunan and Mittal (1995), there are four factors that meat processors should take into consideration to efficiently manage carcass chilling: 1) following required regulations; 2) diminishing carcass mass loss; 3) prevent cold-shortening; and 4) control chilling time to maximize throughput. Ultimately, these criteria can help to control bacterial growth.

In the U.S., there are no official regulations specifying the time/temperature that pork carcasses must undergo after harvest; however, the USDA-FSIS (1995) recommends the deep muscle (approx. 15 cm) reach an internal temperature of 10°C and 7.2°C at 24 h and 36 h after slaughter, respectively, and that the carcass surface be cooled down to 10°C in 5 h and 4.4°C in 24 h. As a result, the lag phase of bacteria can be extended, thus reducing the microbial population on the carcass (Vanderzant et al., 1985). This is important because an abuse of temperature can lead to an increase of the internal temperature of the carcass due to pre-rigor activity (Gill, 2000). Post-harvest chilling procedures are important to minimize growth of spoilage and pathogenic bacteria (Gill, 1998). At -1°C and 5°C, microbial spoilage activity is minimized, but spoilage microorganisms continue to grow at a slow rate. (Gill, 1997). Nevertheless, temperature abuse occurs even if the initial bacterial load on meat is low, and off-odor development could occur (Kotula, 1987). Temperatures near the freezing point have the potential to kill bacteria because of the effect of ice crystal formation on the cell structure resulting in damage to the bacterial cell membrane (Mazur, 1966).

Bacteria cells are damaged by the cold shock promoted by accelerated chilling (El-Kest and Marth, 1992). Freezing temperatures favors pork carcass quality because of the carcass size

which can undergo rigor faster, resulting in no negative impact due to cold shortening or toughening of muscle (Murray, 1995). To date, the pork industry employs various chilling systems to commercially chill pork carcasses: conventional (1° C), spray-chilling (1-5 ° C), and blast-freezing (-20° C to -40° C) (Huff-Lonergan and Page, 2010). Nychas et al. (1988) stated that 10% of the bacteria population adapt to changing conditions (temperature) and can adapt their metabolism to survive. Pork carcass temperature is reduced to avoid pale, soft, and exudative (PSE) product (Milligan et al., 1998; Savell et al., 2005). Chilling decelerates the growth of bacteria; however, it cannot ameliorate product quality, thus, the integrity of raw material during chilling depends on the initial microbial quality (Berk, 2013). Refrigeration can restrict approximately 10% of the total microflora growth during storage (Lulietto et al., 2015).

Sebranek (2008) suggested pork carcasses be stored in chill rooms with 85-90% relative humidity at 0-1°C. These ranges are usually employed in the pork industry to hold carcasses prior to fabrication. Lee et al. (1985) found that pork held at 0°C had a shorter shelf-life (14 to 28 days) than pork held at -4°C which had less microbial growth and maintained its quality attributes during 49 days of storage. Lee et al. (1985) concluded that lower storage temperatures are more efficacious.

Shelf-life of Meat

Meat is a highly-perishable food due to its chemical structure that enhances bacterial growth to significant levels, resulting in meat spoilage (Doulgeraki et al., 2012). Scientists define shelf-life as the length of time that a food product can be stored, with the quality attributes remaining unchanged before deterioration occurs (Lulietto et al., 2015). There are a number of intrinsic and extrinsic elements that have an impact on the shelf-life of meat. Intrinsic factors

include pH, nutrient content of the meat, redox potential, naturally occurring and added antimicrobials, water activity and moisture and fat content. Extrinsic factors include storage temperature, type of packaging, relative humidity, light intensity and wavelength, atmospheric gas composition, and processing, storage, distribution, and display characteristics. (Nychas et al., 2008; Remenant et al., 2015).

In addition, meat has a high moisture content at 75%, and a protein and lipid content of 19 and 3%, respectively (Davies and Board, 1998). Meat is also a source of low molecular weight components such as carbohydrates, lactate, and amino acids (Dainty et al., 1975; Dave and Ghaly, 2011). Carbohydrates are found in low concentrations in meat around 1.2% (Nychas et al., 1998). A review published by Nychas et al. (1988) discussed the importance of the role of glucose, which increases the metabolism of spoilage bacteria primarily on fresh meats held at 2-7°C under aerobic conditions.

Meat Spoilage

Despite the use of refrigeration, preservatives or innovative technologies, 25% of the food produced worldwide is discarded because of spoilage (Gram et al., 2002; Cenci-Goga et al., 2014). Aerobic bacteria are considered the primary group of organisms responsible for spoilage (Adams and Huffman, 1972). Spoilage is present when organoleptic properties are lost and bacterial degradation of amino acids triggers slime formation and off-flavor development on the meat surface (Gill, 1997). Off-odors are formed when growth reaches 10^8 log CFU/cm², resulting in an unmarketable and unacceptable product for consumers (Gill, 1982; Ellis and Goodacre, 2001). An article published by the USDA-FSIS (2010) stated that low levels of spoilage bacteria will not cause an acute illness. In other words, consumers may not find spoiled food very

palatable; however, if consumed, consumers will likely not get sick. Meat spoilage represents primarily a quality concern rather than a safety issue. However, one study reported that protein and lipid decomposition may lead to health issues (Shamberger et al., 1974). Kotula (1987) stated that spoilage bacteria can be a useful tool for consumers to help them identify off-odors or slime formation which can be used as quality indicators of fresh pork. Otherwise, consumers would be susceptible to consuming meat undergoing a process of decomposition produced by spoilage bacteria.

Some of the most common aerobic bacteria known to cause meat spoilage are *Pseudomonas* spp., *Acinetobacter* spp., and *Moraxella* spp. (Rossaint et al., 2015). Due to the ability of *Pseudomonas* spp. to grow at a high rate under aerobic conditions, Gill and Mollin (1991) identified this organism as the most significant spoilage organism. Nychas et al. (1988) indicated that green discoloration in meat results from sulfide produced by *Shewanella putrefaciens* at 2-7°C under aerobic conditions. Additionally, *S. putrefaciens* is one of the microorganisms responsible for spoilage affecting chill-stored and vacuum packaged meat (Doulgeraki et al., 2012). Psychotropic *Pseudomonas* survive at temperatures below 0°C and are competitive due to a high growth rate that overgrows other bacteria in fresh meat under aerobic conditions (Egan and Roberts, 1987). Storage conditions dictate the number of days of shelf-life before meat exhibits spoilage signs (Garcia-Lopez et al., 1998). Spoilage bacteria are normally found on the carcass surface (Gill et al., 1976). Gill and Newton (1980) demonstrated that spoilage bacteria grow similarly on adipose tissue and muscle tissue. However, Blickstad and Molin (1983) demonstrated that fat surfaces on meat have a higher pH than muscle tissue, increasing the likelihood for some microorganisms (*Alteromonas putrefaciens*, *B. thermosphacta* and *Aeromonas*) to grow. Low molecular weight soluble components such as glucose and amino

acids are depleted by aerobic and anaerobic bacteria. Spoilage bacteria have the ability to metabolize glucose ($\leq 1\text{mg/g}$), a low molecular weight component of meat, for growth.

When *Pseudomonas* reaches $10^8 \text{ log CFU/cm}^2$ and depletes glucose, the breakdown of amino acids increases leading to ammonia production (Ingram and Dainty, 1971). Additionally, some types of psychotrophic *Enterobacteriaceae* can grow at $\leq 4^\circ\text{C}$ under aerobic conditions on pork and lamb with a $\text{pH} \geq 6$ (Grau, 1981; Blickstad and Molin, 1983; Dainty and Mackey, 1992).

Anaerobic Packaging

In the meat industry, packaging is primarily used to prolong product shelf-life by removing oxygen, that consequently slows chemical oxidation and bacterial growth. Jeremiah and Gibson (1997) demonstrated that pork can be stored up to 9 weeks at -1.5°C when vacuum packaged. Furthermore, lactic acid bacteria grow under anaerobic conditions such as those found with vacuum packaged meat (Gill, 1997). A study in Finland reported that psychrotrophic strains of lactic acid bacteria were responsible for producing ropy filaments, or slime in vacuum packaged meats (Korkeala et al., 1988). Additionally, the surface of under vacuum packaged pork will turn into a faded green caused by *Leuconostoc*, *Lactobacillus* spp., and *Alteromonas*, which can occur at low bacterial numbers (Kotula, 1987). Gram-positive bacteria can resist the heat impact when chilling due to a thick bylayer that surrounds them as opposed to the thin membrane found in gram-negative bacteria (Breidenstein et al., 1994).

Legislation

According to the Livestock Slaughter Summary, 99.3% of the total of hogs produced in the U.S. were harvested in establishments overseen by the USDA (USDA-NASS, 2016). The USDA-FSIS is the government agency responsible for overseeing that establishments follow Good Manufacturing Practices (GMP's) and Hazard Analysis and Critical Control Point (HACCP) plans. The USDA-FSIS pathogen reduction rule (USDA-FSIS, 1996) states that pork products must be tested for *Salmonella* with no more than 6 positive samples out of 55. Additionally, Sanitation Standard Operating Procedures (SSOP's) must be in place to ensure the cleanliness of the establishments. Having good hygiene practices can help prevent foodborne illness (USDA-FSIS, 1996). Scallan et al. (2011) reported 47.8 million people suffer from foodborne illness each year. There are numerous interventions used in the pork packing industry to reduce pathogen contamination on pork carcasses. One example is using a hot water wash cabinet that sprays carcasses with hot water (Jensen and Unnevehr, 1999).

Microbiological Background

Pork is normally held at $\leq 4^{\circ}\text{C}$ throughout marketing channels. Psychrotrophic bacteria including *Lactobacillus* spp., *Pseudomonas* spp., *Alcaligenes-Enterobacter* spp., *Flavobacterium* spp., *Micrococcus* spp., and *Moraxella-Acinetobacter* spp., can be present on pork (Kotula, 1987). Moreover, pathogenic bacteria such as *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium prefringens*, *Salmonella* spp., *Staphylococcus aureus*, and *Yersinia enterocolitica* could be present on meat (Kotula, 1987), as well as *Aeromonas hydrophila* and *Aeromonas sobria* (Buchanan and Palumba, 1985). Ray and Bhunia (2013) reported that meat products may have an initial bacterial population of approximately 10^2 to 10^3 log CFU/g,

which is made up of a diversity of microbial species. Similarly, Mackey and Roberts (1993) found a bacterial population of 10^2 to 10^4 log CFU/cm² on pork carcasses prior to entering the hotbox, with *Enterobacteriaceae*, coliforms, and *E. coli* being present. Initially, meat carries two types of microorganisms: mesophilic and cold-tolerant bacteria. Cold-tolerant bacteria are capable of growing at low temperatures and are broken up into two groups, psychrophiles and psychrotrophs (Eddy, 1960). Psychrotrophs are of great interest in fresh meats due to their ability to grow at 7°C or less (Kraft, 1992).

The majority of bacterial contamination at harvest is derived from fecal material and animal hides (Sofos, 1994; Gill, 1998). Jensen and Unnevehr (1999) indicated that carcass contamination occurs during pork processing at the evisceration step and during chilling. As a result, bacteria can enter the food supply chain, resulting in economic losses. For this reason, effective sanitation procedures are of utmost importance to minimize pathogenic microorganisms in food (Buzby et al., 1996).

Some studies have shown the prevalence of *Salmonella* on hogs entering the slaughter facility (Wilkins et al., 2010; Blagojevic et al., 2011). Additionally, Rossel (2009) stated that the skin of hogs is an important source of contamination at the stunning step. Blagojevic et al. (2011) reported a 28–40% incidence level of *Salmonella* on the skin of hogs. Another study indicated that sows, nursery swine, and grower-finisher hogs were positive for *Salmonella* with an incidence level of 51, 32, and 38%, respectively (Wilkins et al., 2010).

Microorganisms can produce biofilms to use as a protective layer on joints, walls, floors surfaces, or on equipment. Biofilms protect bacteria to be resistant to washing and cold environments (Fenlon et al., 1996; Wong, 1998). *Pseudomonas* have been shown to develop a strong attachment to equipment surfaces in refrigerated rooms (Newton et al., 1978; Nortje et al.,

1990). The composition of psychrotrophic flora exhibits seasonal variation (Blaise and Armstrong, 1973). According to Hald and Andersen (2001), the microbial population on pork carcasses may increase if the outdoor ambient temperature becomes warmer, leading to a higher risk of contamination on pork carcass during processing. Yeast and mold populations can survive on the dried surface of a carcass at ≤ 0.95 water activity under aerobic conditions (Van-Netten et al., 1995).

pH

The measurement of hydrogen ions in a meat product is defined as pH (Heinz and Hautzinger, 2007). After harvest, muscle is converted to meat and lactic acid is built up leading to a pH decline from 7.0-7.2 to 5.5-5.7 (Voyle, 1974; Heinz and Hautzinger, 2007). Muscle pH can vary from carcass to carcass as well as in different muscles within the same carcass (Gill and Newton, 1978). Meat surface pH may differ from muscle pH due to microbial growth (Leet and Locker, 1973). The optimal pH range for microbial growth is 5.5-6.5 (Buncic et al., 2014). Rey et al. (1976) demonstrated that psychrotrophic bacteria could grow on pork at pH 5.8 and 6.2. During cold storage, bacterial populations may increase on meat, producing proteases or proteinases that will consequently degrade protein, which can result in pH decline (Agunbiade et al., 2010). The pH on pork loins was 5.66 after an extended hanging period for up to 40 days at $2\pm 1^{\circ}\text{C}$ and 80% relative humidity (Lee et al., 2016).

Moisture

During the initial hours of chilling after harvest, the pork carcass surface starts to dry off, resulting in a water activity decline to ≤ 0.95 (Grau, 1979). Scott (1936) demonstrated that water

evaporation from the meat surface is critical in inhibiting bacterial growth. Gill (2000) indicated that a carcass surface can lose water content during storage at chilling temperatures, which contributes to the safety and shelf-life of a carcass. However, Nottingham (1982) reported that the composition of aerobic bacteria on carcasses during chilling did not vary due to dehydration on meat surfaces. Drying can reduce carcass weight, resulting in economic losses (Bailey, 1986). A relative humidity range between 85 and 95% is recommended in chilling rooms (Heinz and Hautzinger, 2007; Sebranek, 2008).

Sampling Method

The International Organization for Standardization (ISO-17604, 2015) defines the microbiological methods used at harvest establishments to detect and enumerate microorganisms on the surface of harvested animal carcasses. Meat processing establishments have two sampling methods available: swabbing and excision. Several authors have worked together to compare these two methods for carcass sampling (Gill et al., 1988; Dorsa et al., 1997; Bolton, 2003; Pepperell et al., 2005). Gill et al. (1988) found that the excision method obtained significantly higher bacterial counts from the surface of the carcasses in comparison to the other nondestructive method. The swab method has been scientifically validated for microbiological work on meat; however, when using the excision method some pathogenic microorganisms such as *E. coli* O157:H7 and *Salmonella* spp., can be difficult to detect on the carcass surface. This is due to non-uniform distribution of these microorganisms and the small size sampling area, typically about 20 cm² per carcass, which is an important limitation (Bolton, 2003). Overall, the excision method is used by the meat industry as the reference method (Dorsa et al., 1997; Pepperell et al., 2005).

The objective of this study was to evaluate the effect of extended post-harvest hanging time on pork sides and the subsequent microbial shelf-life of pork shoulder steaks fabricated from these pork carcasses.

Materials and Methods

Study Design

This experiment consisted of evaluating the microbial quality of pork carcass sides ($n = 20$), harvested on two different days, during extended post-harvest hanging time of 21 d at $0\pm 1^\circ\text{C}$ and 87.3% relative humidity. These chilled carcasses were fabricated and the microbial shelf-life of pork shoulder blade steaks ($n = 17$) was evaluated during vacuum packaged storage for up to 35 d at $0\pm 1^\circ\text{C}$. Pork carcasses were sampled to determine aerobic plate count (APC), *Enterobacteriaceae* (EB), and yeast and mold populations on d 1, 7, 14, and 21 post-harvest. In addition, surface pH and surface moisture content of pork sides while hanging were determined at each storage time. Each pork carcass surface pH was taken from the carcass shoulder muscle in two different spots as duplicate readings. After 21 d of hanging, because three pork carcasses were used for another project, only 17 pork shoulders were available to fabricate into six 2.54 cm thick pork shoulder blade steaks. Vacuum packaged pork blade steaks were evaluated for APC, EB, and yeast and mold populations and plated in duplicate on 0, 7, 14, 21, 28, and 35 d of refrigerated storage. Additionally, surface pH was determined at each sampling time.

Microbiological Sampling of Pork Carcasses

Hogs, weighing approximately 130 kg ($n = 20$), were obtained from the Kansas State University (KSU) Research Swine Center, and harvested on two different days. Hogs were harvested at the KSU Meats Laboratory (Manhattan, KS) in October ($n = 8$) and February ($n = 12$) using an approved USDA protocol. Hogs were rendered unconscious using electrical stunning (400 V for 15 s), and were immediately exsanguinated. The processing of hogs included scalding, dehairing, singeing, lymph node removal, bunning, evisceration, and carcass splitting.

After splitting, carcass sides were trimmed for zero tolerance (free of feces and ingesta). In addition, a hot water intervention ranging from 77 to 94°C was applied using a hose nozzle held 20 cm away from the pork carcasses and were sprayed for approximately 23 s at 35 PSI. Before entering the carcass cooler, the right sides (skin-on) were weighed and labeled (left sides were fabricated and used for other research project), then hung in a carcass cooler for up to 21 d. The carcass cooler temperature and percent relative humidity were measured every hour during the hanging period using a data logger (Model TM320, DicksonTM, Addison, IL), and averaged $0\pm 1^\circ\text{C}$ and 87.3% (range 66-100%), respectively.

Pork carcass sampling took place after 24 h (1 d), 7, 14, and 21 d after harvest. Aerobic plate count, EB populations, yeast and mold populations, pH, and moisture content were determined on each pork side. Three anatomical locations (flank, shoulder, and jowl) were selected for microbiological sampling. At each location, four coring areas were randomly assigned for each sampling day. Two sterilized stainless-steel meat coring devices were used to delineate the sampling area: a 21.6 cm² core for flank and shoulder locations and a 9.6 cm² core for the jowl location (Figure 1). Microbiological samples ($n = 240$) were excised from the surface of pork carcasses. After coring, the outlined piece of pork was aseptically excised to a depth of 1.5 ± 0.5 mm, using a sterile scalpel. After excising each sample, sterilization of scalpel and tweezers occurred using 70% alcohol and then flaming the implements. Following excision, meat sample cores were placed into sterile plastic bags (Whirl-Pak® bags, Nasco®, Fort Atkinson, WI) containing 50 ml of sterile 0.1% peptone water (Bacto; Flankin Lakes, NJ). At the end of each sampling occasion, samples were immediately transported to the KSU Meat Microbiology Laboratory for microbiological analysis.

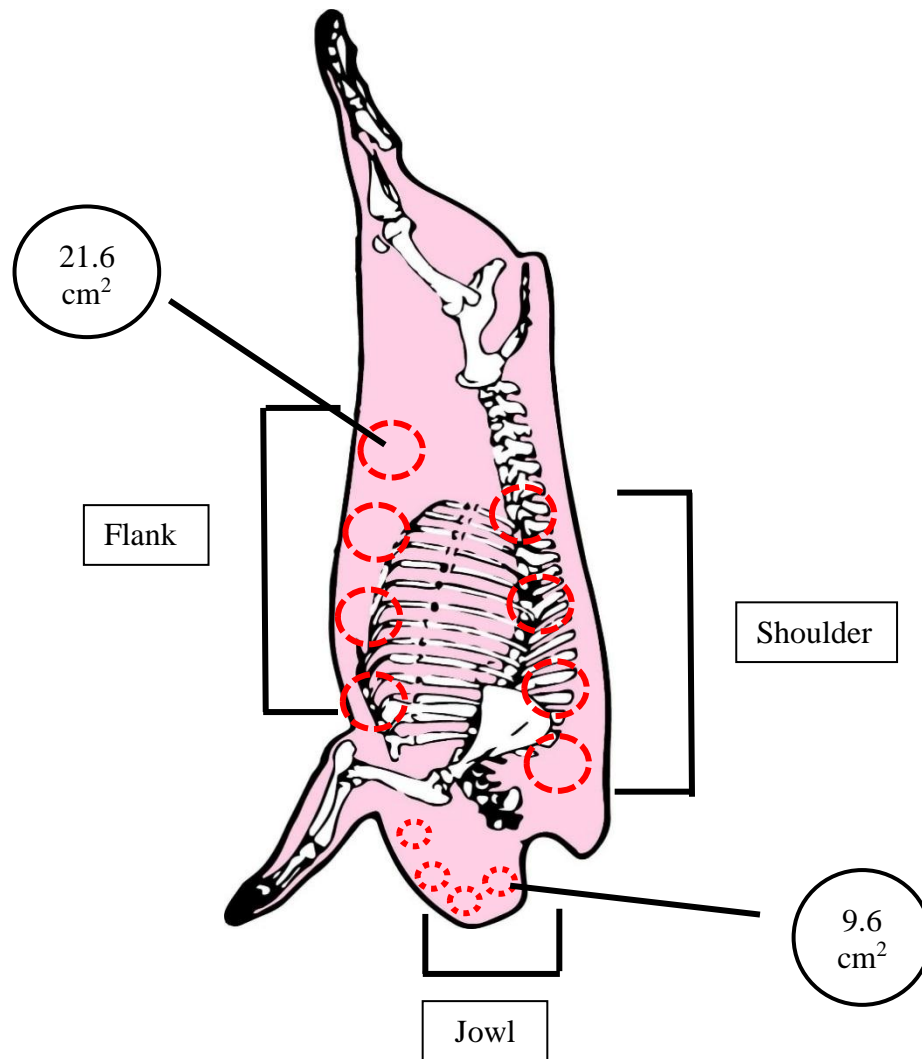


Figure 1. Carcass sampling locations on a pork carcass and core size used at each location.

Microbiological Analysis of Pork Shoulder Blade Steaks

After the 20 right sides of pork carcasses had been sampled over the 21-day hanging period, pork butt shoulders ($n = 17$) were separated from the carcasses (carcass numbers 6, 7, and 8 were removed from the vacuum packaged steak portion of the study and utilized for another research project). Pork shoulders (Figure 2) were cut into six 2.54 cm thick blade steaks using a Biro™ saw (Model 3334, Marblehead, Ohio) that was sterilized between samples using a hot

water (82°C) wash. Pork shoulder blade steaks from each carcass were vacuum packaged in 36x41 cm pouches (Prime Source Vacuum Pouches, 3 mil Nylon/PE, Koch Supplies, Kansas City, MO), which had an oxygen transmission rate of 3.5 g/645.16 cm²/24 h at 21°C and 0.6 g/645.16 cm²/24 h at 0°C, and a water vapor transmission rate of 0.6 cc /645.16 cm²/24 h at 37.8 °C. The samples were vacuum packaged using a Multivac Vacuum Package Machine (Model C 500, Lagny Sur Marne, France), vertically stacked back to back into a corrugated box and then stored at 0±1°C for up to 35 d. Blade steaks were analyzed on 0, 7, 14, 21, 28, and 35 d and were randomly assigned within each shoulder unit for each sampling day. On each sampling occasion, vacuum bags were aseptically opened. A sterile scalpel excised 25 g of surface meat from each pork blade steak, which was placed into a sterile plastic bag (Whirl-Pak® bags, Nasco®, Fort Atkinson, WI) with 225 ml of 0.1% peptone water (Bacto; Flankin Lakes, NJ).

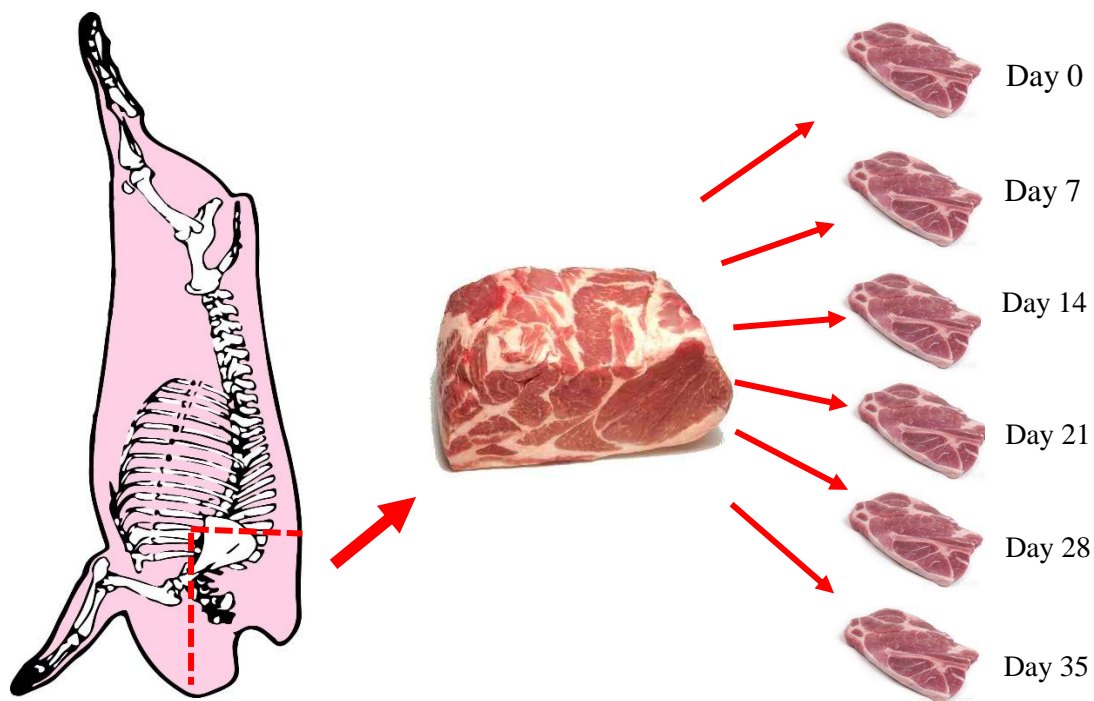


Figure 2. Pork shoulders were cut into six 2.54 cm thick steaks and randomly assigned for each vacuum packaged storage day.

Determination of pH

To determine the pH of pork carcasses and pork shoulder blade steaks, a calibrated pH probe (Model FC232, Hanna Instruments Inc.TM, Woonsocket, RI) with a pH meter (Model HI 99163, Hanna Instruments Inc.TM, Woonsocket, RI) was calibrated using pH 4.0 and pH 7.0 buffer solutions (Hanna Instruments Inc.TM, Woonsocket, RI).

An attempt was made to measure the pH of eight pork carcasses using the slurry prepared for microbial sampling with 0.1% peptone water. Due to the buffering characteristics of the peptone water solution, the resulting pH values were 6.5-7.0. A direct pH probe method was used instead to measure the pH of the remaining carcasses. Only pH readings made using the direct pH probe ($n = 12$) are reported. These pH readings were taken by inserting the pH probe 1.5 cm deep at a 45° angle into the shoulder muscle of the pork carcasses. The pH probe was inserted at two locations, one on each side of core taken for microbial sampling, to obtain duplicate readings. To measure the pH of pork blade steaks, the pH probe was inserted into the steak in two locations on the side not used for microbiological sampling.

Moisture Analysis

The moisture content of the pork carcass surface was determined using a 9.6 cm² stainless-steel corer to excise approximately 5 g of meat surface adjacent to the microbiological sample core taken from the shoulder of the carcass. Moisture content was measured at each storage time. Due to the small size of sample, the meat core was manually chopped using a scalpel for 2 min for moisture determination. After chopping the sample thoroughly, moisture content was determined in the KSU Analytical Laboratory using a SMART system 5 (CEM Corp., NC) procedure for moisture analysis (AOAC Official Method 2008.06).

Carcass and Steak Sample Preparation and Bacterial Enumeration

Excised pork carcass and blade steak sample tissue in 50 and 225 ml of 0.1% peptone water, respectively, were homogenized for 60 s using a blender (AES Chemunex™, Model AESAP1064, Bruz, France). Serial dilutions of this homogenate were prepared using 9 ml of 0.1% peptone water, and plated in duplicate on Petrifilm™ (3M, St. Paul, MN, USA) to enumerate APC, EB, and yeast and mold populations. Aerobic plate count plates were incubated at 35±2°C for 48±2 h, EB plates at 35±2°C for 24±2 h, and yeast and mold plates at 25±2°C for 3-5 d. After incubation, colonies were enumerated according to manufacturer's instructions, and then counts were calculated and transformed logarithmically to be reported as log CFU/cm² for carcass data and log CFU/g for blade steak data. Additionally, the detection limit of the microbiological samples was calculated as follows:

$$\frac{(\text{Solvent})}{(\text{Sampling Area})} \div 2 (\text{Duplicate}) = x = \log(x) = \text{Detection Limit}$$

1. Flank and shoulder:

$$\frac{50 \text{ ml}}{21.64 \text{ cm}^2} \div 2 \text{ CFU/ml} = 1.15 = \log(1.15) = 0.06 \text{ log CFU/cm}^2$$

2. Jowl:

$$\frac{50 \text{ ml}}{9.6 \text{ cm}^2} \div 2 \text{ CFU/ml} = 2.60 = \log(2.60) = 0.41 \text{ log CFU/cm}^2$$

3. Blade Steaks:

$$\frac{250 \text{ ml}}{25 \text{ g}} \div 2 \text{ CFU/ml} = 5.00 = \log(5.00) = 0.70 \text{ log CFU/g}$$

Statistical Design

For this study, all data were analyzed using SAS version 9.4 (SAS Inst. Inc., Cary, NC), and all pairwise treatment comparisons were performed using Fisher's protected least significant difference (LSD). For pork carcass sides, APC was analyzed using a randomized complete block design in Proc MIXED. Carcass was considered to be the blocking factor in the model. Location, day, and location by day were used as fixed effects and day was treated as a repeated measure. Similarly, moisture and pH were analyzed as repeated measures over time. Because the majority of observations for EB, and yeast and mold were below the detection limit (DL) in the pork carcass and steak data sets, these variables were analyzed as binary responses (1 = above DL and 0 = below DL) in Proc FREQ. For the pork blade steaks, APC was analyzed in Proc MIXED and day was analyzed as a repeated measure and used as a fixed effect.

For the harvest day analyses, APC was analyzed assuming a split-plot design with harvest day as the whole-plot treatment factor, carcass sampling location as the subplot treatment factor, and day of sampling treated as a repeated measure. Carcass was considered the whole-plot experimental unit and an individual location within a carcass was assumed to a sub-plot experimental unit. The corresponding linear mixed model was fit in Proc MIXED with harvest day, carcass sampling location, day of sampling, and all two- and three-way interactions as fixed effects, carcass nested within harvest day as a random effect. The SAS codes are presented in Appendix A.

Results and Discussion

Pork Carcass

pH

There was a sampling day main effect ($P = 0.0257$) for pork carcass pH. As shown in Figure 3 and Appendix Table B.1, carcass pH was similar ($P \geq 0.05$) at 5.84-5.88 on d 1, 7, and 14; however, by d 21 the pH declined ($P \leq 0.05$) to 5.72. A statistical difference for pH for d 21 was found; however, a minor difference of ≤ 0.12 units may not have much effect on microbial growth (DeGeer et al., 2009). The recommended pH in pork carcasses at 24 h after slaughter is 5.7 to 6.1 (PIC, 2016). In this study the pH ranged from 5.72 to 5.88. Very few studies have evaluated the extended hanging time on pork carcasses. Recently, Lee et al. (2016) found a final pH of 5.66 on the left sides of pork carcasses after being hung for 40 d at $2 \pm 1^\circ\text{C}$ and 80% relative humidity.

Moisture Content

There was a sampling day main effect ($P \leq 0.01$) on pork carcass moisture content (Figure 4 and Appendix Table B.1). The initial moisture content of the pork carcasses was 65.1%. On d 7, the moisture content decreased ($P \leq 0.05$) by 6.5%. On d 14, the moisture content was 6.6% dryer ($P \leq 0.05$) than on d 7. The moisture content of the surface of pork carcasses declined ($P \leq 0.05$) to 50.5% on d 21, which was the lowest ($P \leq 0.05$) moisture content. As expected, air exposure on a carcass in a cooler may result in a dried-meat surface (Campbell et al., 2001).

Microbiological

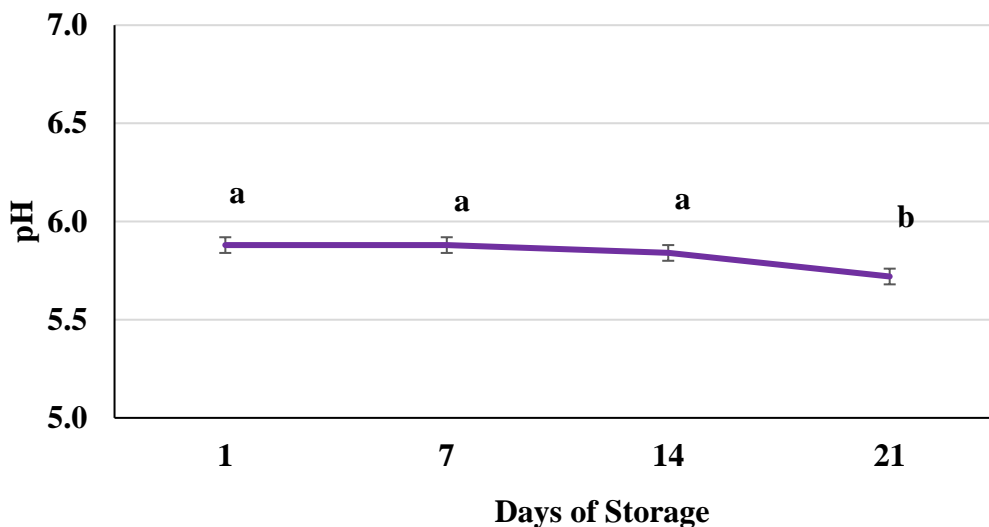
For APC, there was no carcass sampling location by day interaction ($P = 0.55$). There was no sampling day effect ($P = 0.55$); however, there were effects due to sample location ($P = 0.0055$). The jowl had the highest ($P \leq 0.05$) APC population with 1.21 log CFU/cm² compared to the flank and shoulder with 0.77 and 0.76 log CFU/cm², respectively (Figure 5 and Appendix B.2). The APC populations for each day are shown in Appendix B.3. Jowl had higher APC populations and a higher proportion of yeast populations above the DL than flank and shoulder likely due to water accumulation on the front quarter after the hot water wash, as well as the pork carcasses handling that generally occurs on the same area. Overall, APC populations remained very low throughout the post-harvest extended hanging time. One study found initial populations for APC in a range between 2.50 to 3.00 log CFU/cm² on pork carcasses before entering the cooler at the polishing step (Gill et al., 1995). Additionally, Lee et al. (2016) looked at the effect of extended hanging of pork carcasses stored at $2 \pm 1^\circ\text{C}$ after 40 days of cold storage and reported 4.96 log CFU/g for APC populations on the pork loin after fabrication on d 40.

The DL for EB and yeast and mold populations on pork carcass samples ($n = 240$) was 0.06 and 0.41 log CFU/cm² for the shoulder and flank, and jowl, respectively. The total number of samples from a carcass was 12 on each sampling day with 4 samples obtained from each location (flank, shoulder, and jowl). There was no carcass location ($P = 0.77$) or day ($P = 1.00$) effect for EB or mold populations. For EB and mold populations, 98.8 and 97.9% of carcass samples, respectively, were below the DL. There was a carcass location ($P = 0.0029$) and day ($P \leq 0.01$) effect for yeast populations. For all post-harvest extended hanging time points, 37.5% of pork carcass samples from the flank were above the DL for yeast populations (Table 1). The proportion of pork carcass samples above the DL for the shoulder was similar ($P = 0.42$) to the

flank with 45.0%. For the jowl, the percent of samples above the DL was the highest ($P \leq 0.05$) among all sampling locations with 63.8%, increasing by 41 and 31%, compared to the flank and shoulder, respectively. The proportion of pork carcass samples above the DL on d 1, 7, and 14 were similar ($P \geq 0.05$) with 60.0, 55.0, and 53.3% above the DL, respectively (Table 2).

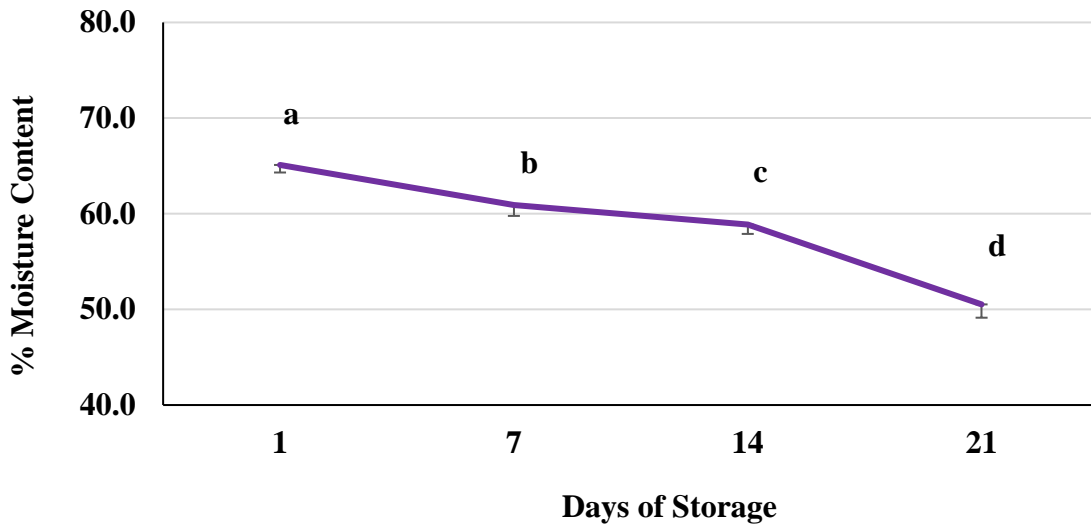
Conversely, on the last day of cooler storage, the percent of samples above the DL decreased by 51% on d 21 for yeast populations. Additionally, none of the carcass samples had yeast populations greater than 2.83 log CFU/cm². Ahnström et al. (2006) reported that yeast populations increased during extended cold storage. Yeast populations are known to survive on dried-carcasses surface at water activity values of ≤ 0.95 under aerobic conditions (Van-Netten et al., 1995). In this study, however, pork carcasses samples had fewer ($P \leq 0.05$) yeast populations below the DL on d 21 compared to days 1, 7, and 14.

Figure 3. Hanging pork carcass side ($n = 12$) pH during cooler storage at $0 \pm 1^\circ\text{C}$ and 87.3% relative humidity for up to 21 days.



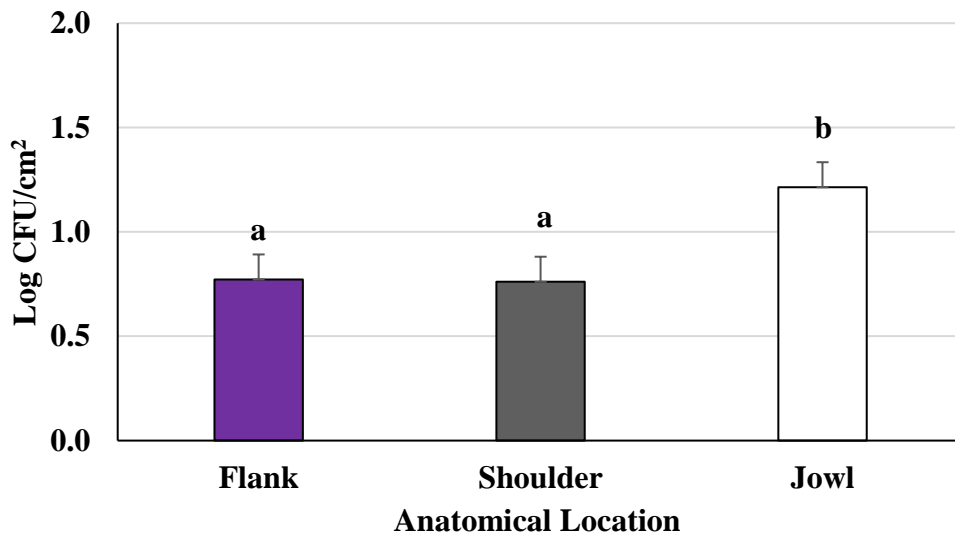
^{ab}Least squares means lacking a common superscript differ ($P \leq 0.05$).

Figure 4. Hanging pork carcass side ($n = 20$) moisture content during cooler storage at $0\pm 1^\circ\text{C}$ and 87.3% relative humidity for up to 21 days.



^{ab}Least squares means lacking a common superscript differ ($P \leq 0.05$).

Figure 5. Aerobic plate counts population by sample location of hanging pork carcass sides ($n = 20$) during cooler storage at $0\pm 1^\circ\text{C}$ and 87.3% relative humidity for up to 21 days.



^{ab}Least squares means with different superscripts differ ($P \leq 0.05$).

Table 1. The number of yeast populations by sample location above the detection limit (DL)¹ of hanging pork carcass sides ($n = 20$) stored at $0\pm 1^\circ\text{C}$ and 87.3% relative humidity for up to 21 days.

	Sample Location			Total ²
	Flank	Shoulder	Jowl	
Below DL	50 ^a	44 ^a	29 ^b	123
Above DL	30 ^a	36 ^a	51 ^b	117
Total²	80	80	80	240

¹Flank and shoulder DL= $0.06 \log_{10} \text{CFU}/\text{cm}^2$; Jowl DL = $0.41 \log_{10} \text{CFU}/\text{cm}^2$.

²Number of samples pooled from all sampling days.

^{ab}Number within a row with different superscripts differ ($P \leq 0.05$).

Table 2. The number of yeast populations above the detection limit (DL)¹ of hanging pork carcass sides ($n = 20$) stored at $0\pm 1^\circ\text{C}$ and 87.3% relative humidity for up to 21 days.

	Days of Storage				Total ²
	1	7	14	21	
Below DL	24 ^a	27 ^a	28 ^a	44 ^b	123
Above DL	36 ^a	33 ^a	32 ^a	16 ^b	117
Total²	60	60	60	60	240

¹Flank and shoulder DL= $0.06 \log_{10} \text{CFU}/\text{cm}^2$; Jowl DL = $0.41 \log_{10} \text{CFU}/\text{cm}^2$.

²Number of samples pooled from flank, shoulder, and jowl.

^{ab}Number within a row with different superscripts differ ($P \leq 0.05$).

Pork Shoulder Blade Steaks

pH

There was a sampling day main effect ($P \leq 0.05$) for pH. Figure 6 and Appendix Table B.4 shows the pH of pork shoulder blade steaks. The pH was similar ($P \geq 0.05$) on d 0 and 7 at 5.73 and 5.78, respectively; however, on d 14 the pH increased ($P \leq 0.05$) to 5.89. On d 21, the pH declined ($P \leq 0.05$) to 5.7, and remained constant ($P \geq 0.05$) through d 28; however, the final pH on d 35 decreased ($P \leq 0.05$) to 5.69. Although the pork steaks pH varied throughout the storage time, pH on d 0 was similar to pH on d 35. These variations in pH may be due to numerous factors: The pH of muscle can vary among carcasses (Gill and Newton, 1978) as well as in different muscles within the same carcass (Topel, 1960). Additionally, the pH probe may have been inserted to contact fat surfaces which have higher pH than muscle tissue (Blickstad and Molin, 1983), resulting in pH differences when sampling. These pH readings did not agree with the results reported by Yang (2012) who found that vacuum packaged pork chop pH slightly increased during 50 d storage period at 1 °C. Similarly, Zhao et al. (2015), reported that pH on vacuum-packaged chilled pork increased ($P \leq 0.05$) from 5.72 on d 0 to 5.99 on d 21 in pork cuts stored at 0° C.

Microbiological

There was a day effect ($P \leq 0.05$) for APC on pork shoulder blade steaks (Figure 7 and Appendix Table B.5). The initial APC population was 1.61 log CFU/g. On d 7, APC populations declined to 1.18 log CFU/g, then increased to 2.44 log CFU/g ($P \leq 0.05$) on d 14. On d 14 and 21, aerobic plate counts were similar ($P \geq 0.05$); however, there was a 1.50 and 1.89 log CFU/g increase ($P \leq 0.05$) on APC population on d 28 and 35, respectively. On d 35, APC populations

reached 5.06 log CFU/g. As expected, APC populations increased as the cooler storage time was extended, which is in agreement with other studies (Mayr et al., 2003; Zhao et al., 2015). Holley et al. (2004) conducted a similar study in which boneless pork loins were stored at $-1.7 \pm 1^\circ\text{C}$, and APC populations reached 5.78 log CFU/cm² on d 56.

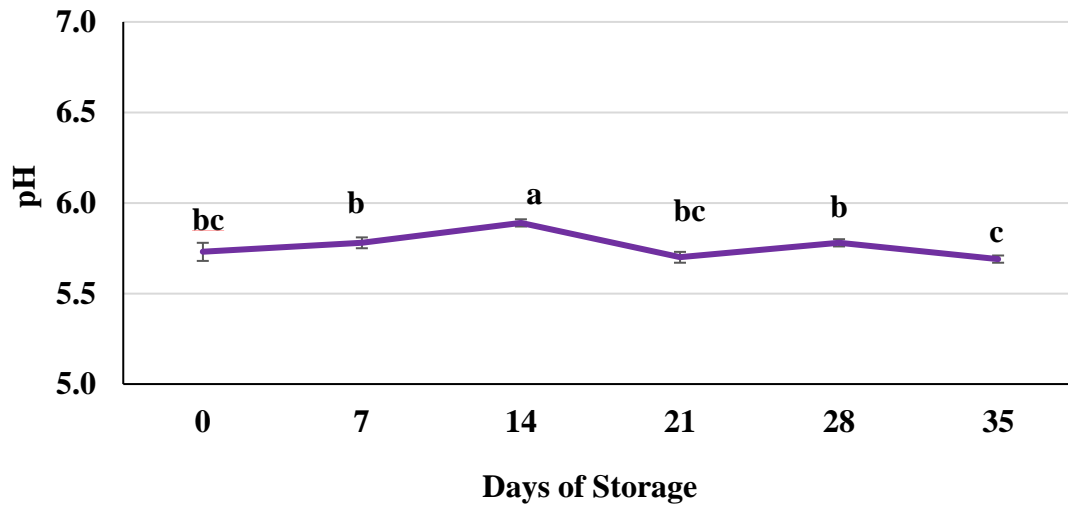
There was a day effect ($P \leq 0.05$) for EB and mold populations on pork shoulder steaks; but there was not a day effect ($P \geq 0.05$) for yeast populations. The DL for EB and yeast and mold populations on pork shoulder blade steak samples ($n = 102$) was 0.70 log CFU/g. It is noteworthy that the 3M method for EB enumeration only tests for presumptive positive samples. Results are reported in Table 3. On d 0 and 7, the percent of samples above the DL for EB populations was 0.0% ($P \geq 0.05$), which were similar ($P \geq 0.05$) to d 14 which proportion of EB populations above the DL was 23.5%. The percent of presumptive positive samples above the DL was 41.2% for EB populations on d 21, and was greater ($P \leq 0.05$) than d 0 and 7, but was not different ($P \geq 0.05$) than d 14. There was an increase ($P \leq 0.05$) for the EB populations on pork steaks at d 28. This sample day was the greatest (94.1%) for EB populations above the DL. On d 35, however, the EB populations above the DL declined ($P \leq 0.05$) to 41.2%. This EB population decrease at the last day of storage is in agreement with a recent study by Zhao et al. (2015) who evaluated the percentages of the primary microbiological flora on pork cuts stored at 0°C for up to 21 d. Zhao et al. (2015) reported that the EB population represented 4.70% of the total microbiological flora on d 7 and increased to 21.63% at day 14. On d 21, however, EB population decreased to 6.50%.

For all sampling days, none of the pork steak samples exceeded 4.40 log CFU/g for EB. Other studies have reported EB growth in pork under refrigeration temperatures. Mayr et al. (2003) reported that EB can grow in pork under vacuum package conditions when stored at 4°C .

In this study, the initial bacterial counts of EB was 3.56 log CFU/g, and increased ($P \leq 0.05$) to 4.02 log CFU/g after 11 d of cold storage. Another study conducted by Ariyapitipun et al. (1999) showed a similar trend, in which the EB population increased gradually from 1 log CFU/cm² at day 0 to 4.8 log CFU/cm² at day 56 in vacuum-packaged beef stored at 4°C. *Enterobacteriaceae* species that can grow in vacuum-packed meat at temperatures between 0 and 10°C, are *Serratia liquefaciens* and *Hafnia* spp. (Labadie, 1999). Research conducted by Brightwell et al. (2007) in New Zealand studied the effect of *Hafnia*, *Enterobacter*, *Serratia*, *Rahnella*, and *Ewingella*, which belong to the EB family, after being inoculated on fresh lamb. They found these bacteria were responsible for the blown pack spoilage of vacuum-packaged lamb stored at 4°C for 21 d.

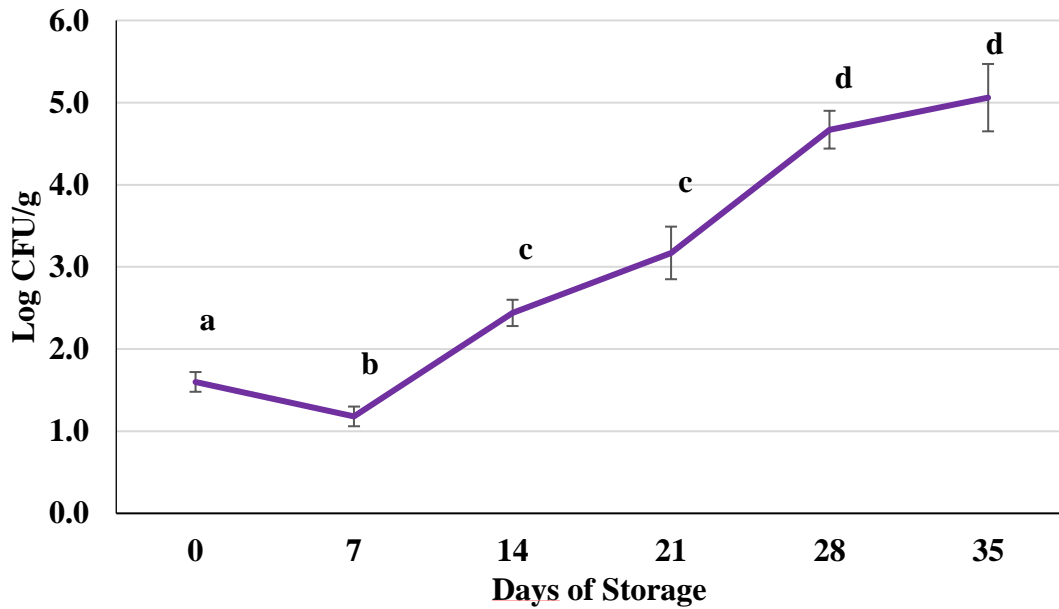
Mold populations on pork shoulder steaks (Table 4 and Appendix Table B.6), were not different ($P \geq 0.05$) on d 0, 7, and 14 with 100.0% of pork steaks below the DL. However, on d 21, 28, and 35, 18, 24, and 12%, respectively, were above the DL for mold populations. None of the steak samples exceeded 2.68 log CFU/g for mold populations. Even though a day effect was reported using a pairwise comparison for mold populations, SAS did not detect any statistical differences ($P = 0.1026$) between days due to the low sample number. For yeast populations, 56.8% of pork steaks samples pooled over 35 days of storage were above the DL, but none were greater than 3.69 log CFU/g (Appendix Table B.7).

Figure 4. Pork shoulder blade steak ($n = 12$) pH during cooler storage at $0\pm 1^\circ\text{C}$ for up to 35 days.



^{abc}Least squares means with different superscripts differ ($P \leq 0.05$).

Figure 5. Aerobic plate count populations of pork shoulder blade steaks ($n = 17$) during cooler storage at $0\pm 1^\circ\text{C}$ for up to 35 days.



^{abcd}Least squares means with different superscripts differ ($P \leq 0.05$).

Table 3. The number of presumptive positive *Enterobacteriaceae* (EB) populations above the detection limit (DL)¹ of pork shoulder blade steaks ($n = 17$) stored at $0\pm 1^\circ\text{C}$ for up to 35 days.

	Sampling Days						Total ²
	0	7	14	21	28	35	
Below DL	17 ^a	17 ^a	13 ^{ab}	10 ^b	1 ^c	7 ^b	65
Above DL	0 ^a	0 ^a	4 ^{ab}	7 ^b	16 ^c	10 ^b	37
Total²	17	17	17	17	17	17	102

¹DL = $0.70 \log_{10}$ CFU/g.

²Number of samples measured.

^{abc}Number within a row with different superscripts differ ($P \leq 0.05$).

Table 4. The number of mold populations above the detection limit (DL)¹ of pork shoulder blade steaks ($n = 17$) stored at $0\pm 1^\circ\text{C}$ for up to 35 days.

	Sampling Days						Total ²
	0	7	14	21	28	35	
Below DL	17	17	17	14	13	15	93
Above DL	0	0	0	3	4	2	9
Total²	17	17	17	17	17	17	102

¹DL = $0.70 \log_{10}$ CFU/g.

²Number of samples measured.

^{abc}Number within a row with different superscripts differ ($P \leq 0.05$).

Harvest Day

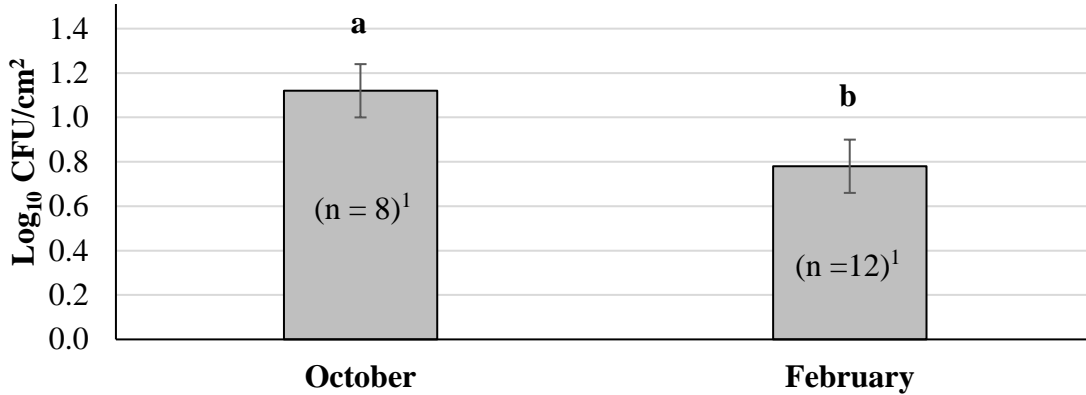
The comparison between harvest days of pork carcasses is shown in Figure 8 and Appendix Table B.8. There was a harvest day main effect ($P = 0.0297$) for APC. Overall, pork carcasses samples collected from the October harvest day had a higher ($P \leq 0.05$) APC population than pork carcasses harvested in February with 1.12 and 0.78 log CFU/cm², respectively, resulting in a 0.34 log CFU/cm² difference.

There was a harvest day by sampling day interaction ($P \leq 0.019$) for pork shoulder blade steaks (Figure 9 and Appendix Table B.9). On d 0 and 7 of sampling, the October and February harvest days had similar ($P \geq 0.05$) APC populations; however, from 7 d onward, all sampling days for the October and February harvest days differed ($P \leq 0.05$). On d 14, the carcass APC population was 0.71 log CFU/cm² higher ($P \leq 0.05$) for the October harvest than when harvested in February. Similarly, carcasses harvested in October had higher ($P \leq 0.05$) APC populations on d 21 and 28 of hanging than those harvested in February by more than 1.50 log CFU/cm². The largest log difference between October and February occurred on d 35 with the APC population being 2.89 log CFU/cm² higher in October.

There are several factors that may have led to APC populations being higher in October than in February. Pork carcasses from both harvest days were utilized for several activities while hanging in the cooler. It was difficult to keep track of when the carcasses were pulled from the cooler to a classroom. Additionally, the hot water wash used during harvest ranged from 77°C to 94°C, and this variation can lead to a change in the microflora of a pork carcasses surface (Gill et al., 1995). According to the National Weather Service (2017), the average temperature in October 2016 and February 2017 when pork harvest occurred, was 16.5°C and 6°C, respectively. Hald and Andersen (2001) indicated microbial populations on pork carcasses may increase if the

outdoor ambient temperature becomes warmer, leading to a higher risk of contamination of pork carcass during processing.

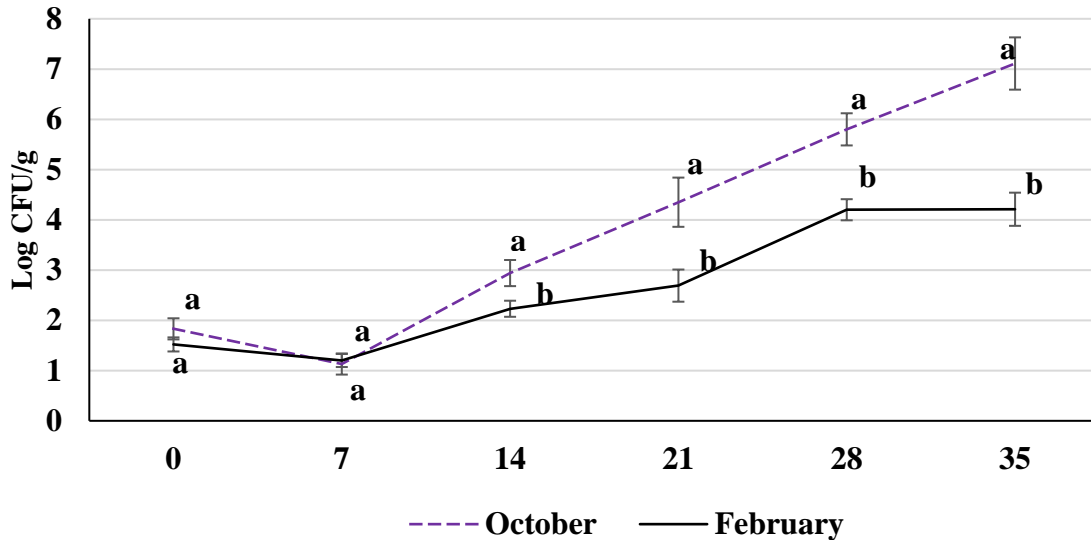
Figure 6. Aerobic plate count populations pooled from the flank, shoulder, and jowl of pork carcasses harvested on two separate production days and hung up to 21 days at $0\pm 1^\circ\text{C}$ and 87.3% relative humidity.



¹Number of hogs harvested.

^{ab}Least squares means with different superscripts differ ($P \leq 0.05$).

Figure 7. Aerobic plate count populations of pork shoulder blade steaks fabricated from hogs harvested on two separate production days [October ($n = 5$)¹ and February ($n = 12$)¹] and stored up to 35 days at $0\pm 1^\circ\text{C}$.



¹Number of pork shoulders fabricated.

^aLeast squares means with different superscripts within the same day differ ($P \leq 0.05$).

Conclusions

Post-harvest extended hanging time did not have a negative effect on the microbial quality of pork carcasses hung for up to 21 d. However, APC populations were affected by the location within a pork carcass. The jowl had the highest APC population and highest number of yeast populations that were above the DL. Additionally, the proportion of yeast populations above the DL on pork carcasses decreased during 21 days of cold storage. The higher prevalence of APC and yeast populations on the jowl compared to the flank and shoulder may be due to the inherent dripping of water after the hot water wash, resulting in water accumulation on that location. Additionally, this location is commonly used to handle pork carcasses when moved, increasing the likelihood of bacterial contamination. Overall, the microbiological results indicate that pork carcasses have acceptable quality for 21 d $0\pm 1^{\circ}\text{C}$ and 87.3% relative humidity.

Pork carcasses harvested in February had higher microbial quality than those harvested in October. It is perhaps noteworthy that even though pork carcasses harvested at separate times (February and October) may be statistically different for APC populations, these populations remained very low for both harvest times, around 1 log CFU/cm² throughout the storage time. This microbiological difference may be due to hot water wash temperature, cross-contamination, and different ambient temperatures when harvest occurs.

The microbial quality for the pork blade steaks fabricated from the pork carcasses previously hung for 21 d prior to fabrication was acceptable and below 10^7 log CFU/g, which is when bacteria present on meat begin to produce slime, leading to spoilage. However, pork blade steaks of carcasses harvested in February had higher microbial quality than pork blade steaks fabricated from carcasses harvested in October. This may be the result due to the reduction in

number of carcasses used in October ($n = 5$) compared to February ($n = 12$), which potentially led to the APC population difference between the two harvest times.

References

- Aberle, E. D., Forrest, J. C., Gerrard, D.E., and Mills, E. W. 2001. Principles of meat science. 4th ed. Kendall Hunt Publ. Company, Dubuque, IA.
- Adam, J. R., and Huffman, D. L. 1972. Effect of controlled gas atmospheres and temperatures on quality of packaged pork. *J. Food Sci.* 37(6):869-872. doi:10.1111/j.1365-2621.1972.tb03690.x.
- Agunbiade, S. O., Akintobi, O. A., and Ighodaro, O. M. 2010. Some biochemical and organoleptic changes due to microbial growth in minced beef packaged in alluminium polyethylene trays and stored under chilled condition. *Life Sci. J.* 7(2):47-51.
- Ahnström, M. L., Seyfert, M., Hunt, M. C., and Johnson, D. E. 2006. Dry aging of beef in a bag highly permeable to water vapour. *Meat Sci.* 73(4):674-679. doi:10.1016/j.meatsci.2006.03.006.
- AOAC. 2008. Official methods of analysis. Method 2008.06. 17th Ed. AOAC, Gaithersburg, MD.
- Ariyapitipun, T., Mustapha, A., and Clarke, A. D. (1999). Microbial shelf life determination of vacuum-packaged fresh beef treated with polylactic acid, lactic acid, and nisin solutions. *J. Food Prot.* 62(8) 913-920. doi:10.4315/0362-028X-62.8.913.
- Bailey, C. 1986. Current issues affecting meat chilling and distribution. In: *Recent advances and developments in the refrigeration of meat by chilling*. Int. Inst. Refrigeration. Paris, France. p.18-20.
- Berk, Z. 2013. Refrigeration: Chilling and freezing. In: *Food process engineering and technology*. Academic Press 2nd ed. San Diego, CA. p.439-460. <http://www.sciencedirect.com/science/article/pii/B9780124159235000198>. Accessed 18 August 2017.
- Blagojevic, B., Antic, D., Ducic, M., and Buncic, S. 2011. Ratio between carcass-and skin microflora as an abattoir process hygiene indicator. *Food Control.* 22(2):186-190. doi:10.1016/j.foodcont.2010.06.017.
- Blaise, C. R., and Armstrong, J. B. 1973. Lipolytic bacteria in the Ottawa River. *Appl. Microbiol.* 26(5):733-740.
- Blickstad, E., and Molin, G. 1983. Carbon dioxide as a controller of the spoilage flora pork, with special reference to temperature and sodium chloride. *J. Food. Protect.* 46(9):756-63. doi:10.4315/0362-028X-46.9.756.

- Bolton, D. J. 2003. The EC decision of the 8th June 2001 (EC/471/ 2001): excision versus swabbing. *Food C.* 14(3):207–209. doi:10.1016/S0956-7135(02)00093-2.
- Bolton, D. J., Pearce, R. A., Sheridan, J. J., Blair, I. S., McDowell, D. A., and Harrington, D. 2002. Washing and chilling as critical control points in pork slaughter hazard analysis and critical control point (HACCP) systems. *J. Appl. Microbiol.* 92(5):893-902. doi:10.1046/j.1365-2672.2002.01599.x.
- Brightwell, G., Clemens, R., Ulrich, S., and Boerema, J. (2007). Possible involvement of psychrotolerant *Enterobacteriaceae* in blown pack spoilage of vacuum-packaged raw meat. *Int. J. Food Microbiol.* 119(3):334–339. doi:10.1016/j.ijfoodmicro.2007.08.024.
- Briley, G. C. 2004. 100 years of refrigeration. *The Amer. Soc. Heating, Refrigerating, and Air-Conditioning Eng.* p. S31-S34. <https://www.ashrae.org/.../ASHRAE-D-22923-20050105.pdf>. Accessed 10 May 2017.
- Breidenstein, B. C., Kinsman, D. M., and Kotula, A. W. 2013. Muscle foods. *Meat Poultry Seafood Tech.* <https://books.google.com/books?id=Qc3eBwAAQBAJ&printsec=frontcover&hl=fr#v=onepage&q&f=false>. Accessed 28 January 2017.
- Buchanan, R. L., and Palumbo, S. A. 1985. *Aeromonas hydrophila* and *aeromonas sobria* as potential food poisoning species: A review. *J. Food Saf.* 7(1):15-29. doi:10.1111/j.1745-4565.1985.tb00490.x..
- Buncic, S., Nychas, G. J., Lee, M. R., Koutsoumanis, K., Hébraud, M., Desvaux, M., Chorianoopoulos, N., Bolton, D., Blagojevic, B., Dragan., Antic, D. 2014. Microbial pathogen control in the beef chain: recent research advances. *Meat Sci.* 97(3):288-297. doi: 10.1016/j.meatsci.2013.04.040.
- Buzby, J., Roberts, T., Lin, C. T. J., and MacDonald, J. M. 1996. Bacterial foodborne disease: Medical costs and productivity losses. U.S. Department of Agriculture, Economic Research Service. p. 1-93. https://www.ers.usda.gov/webdocs/publications/40722/28371_aer741fm_1_.pdf?v=41063. Accessed 28 January 2017.
- Campbell, R. E., Hunt, M. C., Levis, P., and Chambers, E. 2001. Dry-aging effects on palatability of beef longissimus muscle. *J. Food Sci.*, 66(2):196-199. doi:10.1111/j.1365-2621.2001.tb11315.x.
- Cano-Muñoz, G. 1991. Storage conditions for chilled animal products. Manual on meat cold store operation and management. Food and Agricultural Organization. Vol. 92. Rome, Italy. <http://www.fao.org/docrep/004/T0098E/T0098E00.HTM>. Accessed 10 May 2017.

- Cenci-Goga, B. T., Karama, M., Sechi, P., Lulietto, M. F., Novelli, S., and Mattei, S. 2014. Evolution under different storage conditions of anomalous blue coloration of mozzarella cheese intentionally contaminated with a pigment-producing strain of *Pseudomonas fluorescens*. *J. Dairy Sci.* 97(11):6708-6718. doi:10.3168/jds.2014-8611.
- Chung, K. T., Dickson, J. S., and Grouse, J. D. 1989. Attachment and proliferation of bacteria on meat. *J. Food Prot.*, 52(3):173-177. doi:10.4315/0362-028X-52.3.173.
- Dainty, R. H., and Mackey, B. M. 1992. The relationship between the phenotypic properties of bacteria from chill-stored meats and spoilage processes. *J. Appl. Bacteriol.* 73(s21):103S-114S. doi:10.1111/j.1365-2672.1992.tb03630.x
- Dainty, R. H., Shaw, B. G., de Boer, K. A., Scheps, E. S. J. 1975. Protein changes caused by bacterial growth on beef. *J. Appl. Bacteriol.* 39(1):73-81. doi:10.1111/j.1365-2672.1975.tb00547.x
- Dave, D., and Ghaly, A. E. 2011. Meat spoilage mechanisms and preservation techniques: a critical review. *Am. J. Agric. Biol. Sci.* 6(4):486-510.
- Davies, A., and Board, R. G. *Microbiology of meat and poultry.* Blackie Academic and Professional, London, UK.
- DeGeer, S. L., Hunt, M. C., Bratcher, C. L., Crozier-Dodson, B. A., Johnson, D. E., and Stika, J. F. 2009. Effects of dry aging of bone-in and boneless strip loins using two aging processes for two aging times. *Meat Sci.* 83(4):768-774. doi:10.1016/j.meatsci.2009.08.017.
- Dorsa, W. J., Siragusa, G. R., Cutter, C. N., Berry, E. D., and Koohmaraie, M. 1997. Efficacy of using a sponge sampling method to recover low levels of *E. coli* O157:H7, *Salmonella typhimurium* and aerobic bacteria from beef carcass surface tissue. *Food Microbiol.* 14(1):63-69. doi:10.1006/fmic.1996.0060.
- Doulgeraki, A. I., Ercolini, D., Villani, F., and Nychas, G. J. E. 2012. Spoilage microbiota associated to the storage of raw meat in different conditions. *Intl. J. Food Microbiol.* 157(2):130-41. doi:10.1016/j.ijfoodmicro.2012.05.020.
- Eddy, B. P. 1960. The use and meaning of the term psychrophilic. *J. Appl. Microbiol.* 23(2):189-190. doi: 10.1111/j.1365-2672.1960.tb00195.x.
- Egan, A. F., and Roberts, T. A. 1987. *Microbiology of meat and meat products.* In: Norris, J.R., Pettipher, G. L, editors, *Essays in agricultural and food microbiology.* Wiley New York, United States of America. p. 167-197.
- Ellis, D. I., and Goodacre, R. 2001. Rapid and quantitative detection of the microbial spoilage of muscle foods: Current status and future trends. *Trends in Food Science and Technology*, 12(11):414-424. doi:/10.1016/S0924-2244(02)00019-5.

- El-Kest, S. E., and Marth, E. H. 1992. Freezing of *listeria monocytogenes* and other microorganisms: a review. *J. Food Prot.*55(8):639-648. doi: 10.4315/0362-028X-55.8.639.
- Fenlon, D. R., Wilson, J., and Donachie, W. 1996. The incidence and level of *listeria monocytogenes* contamination of food sources at primary production and initial processing. *J. Appl. Microbiol.* 60(6):641-650. doi: 10.1111/j.1365-2672.1996.tb03559.x.
- Garcia-Lopez, M. L., Prieto, M., and Otero, A. 1998. Physiological attributes of gram-negative bacteria associated with spoilage of meat and meat products. In: Davies, A., and Board, R., editors, *Microbiology of meat and poultry*. Blackie Academic and Professional, London, UK. p.1-34.
- Gentry, J. G., McGlone, J. J., Miller, M. F., and Blanton, J. R. 2004. Environmental effects on pig performance, meat quality, and muscle characteristics. *J. Anim. Sci.* 82(1):209-217. doi:10.2527/2004.821209x.
- Gill, C. O. 1976. Substrate limitation of bacterial growth at meat surfaces. *J. Appl. Microbiol.* 41(3):401-410. doi: 10.1111/j.1365-2672.1976.tb00652.x.
- Gill, C. O., and Newton K. G. 1978. The ecology of bacterial spoilage of fresh meat at chill temperatures. *Meat Sci.* 2(3):207-217. doi:10.1016/0309-1740(78)90006-2.
- Gill, C. O. 1982. Microbial interaction with meats. In: Brown, M.H. editor, *Meat microbiology*.
- Gill, C. O. 1997. Assessment of the hygienic performances of an air-cooling process for lamb carcasses and a spray-cooling process for pig carcasses. *Int. J. Food Microbiol.* 38(2):85-93. doi:10.1016/S0168-1605(97)00087-1.
- Gill, C. O., McGinnis, D. S., Bryant, J., and Chabot, B. 1995. Decontamination of commercial, polished pig carcasses with hot water. *Food Microbiol.* 12(1):143-149. doi: 10.1016/S0740-0020(95)80090-5.
- Gill, C. O., Deslandes, B., Rahn, K. Houde, A., and Bryant, J. 1998. Evaluation of the hygienic performances of the processes for beef carcass dressing at 10 packing plants. *J. Appl. Microbiol.* 84(6):1050–1058. doi:10.1046/j.1365-2672.1998.00441.x.
- Gill, C. O., and Mollin, G. 1991. Modified atmospheres and vacuum packaging. In: Russel, NJ and Gould, G W, editors, *Food Preservatives*, Glasgow, Blackie Academic and Professional, p. 172-199.
- Gill, C. O., Dussault, F. H. R. A., Holley, R. A., Houde, A., Jones, T., Rheault, N., Rosales, A., and Quessy, S. 2000. Evaluation of the hygienic performances of the processes for cleaning, dressing and cooling pig carcasses at eight packing plants. *Int. J. Food Microbiol.* 58(1):65-72. doi:10.1016/S0168-1605(00)00294-4.

- Gill, C. O., and Newton, K. G. 1980. Development of bacterial spoilage at adipose tissue surfaces of fresh meat. *Appl. Environ. Microbiol.* 39(5):1076-1077. doi:0099-2240/80/05-1076/02\$02.00/0.
- Gram, L., Ravn, Rasch, M., Bruhn, J. B., Christensen, A. B., and Givskov, M. 2002. Food spoilage- interactions between food spoilage bacteria. *Int. J. Microbiol.* 78(1-2):79-97. doi:10.1016/S0168-1605(02)00233-7.
- Grau, F. H. 1981. Role of pH, lactate, and anaerobiosis in controlling the growth of some fermentative gram-negative bacteria on beef. *Appl. Environ. Microbiol.* 42(6):1043-1050. doi:0099-2240/81/121043-08\$02.00/0.
- Grau, F. H. 1979. Fresh meats: Bacterial association. *Archiv fur Lebensmittel hygiene.* 30(3):87-92.
- Hald, T., and Andersen, J. S. 2001. Trends and seasonal variations in the occurrence of Salmonella in pigs, pork and humans in Denmark, 1995-2000. 4th International symposium on the epidemiology and control of salmonella and other food borne pathogens in pork. Iowa State University.
- Heinz, G., and Hautzinger, P. 2007. Meat processing technology for small to medium scale producers. FAO. Rome, Italy.
- Holley, R. A., Peirson, M. D., Lam, J., and Tan, K. B. 2004. Microbial profiles of commercial, vacuum-packaged, fresh pork of normal or short storage life. *Int. J. Food Microbiol.* 97(1):53-62.
- Huff-Lonergan, E., Page J. 2010. The role of carcass chilling in the development of pork quality. Extension. <http://articles.extension.org/pages/27298/the-role-of-carcass-chilling-in-the-development-of-pork-quality>. Accessed 9 March 2018.
- Hurley, D. 1981. Images of historical Cincinnati. G. E. Peterson. Cincinnati Museum Center. <http://library.cincymuseum.org/topics/a/files/artists/ima-233.pdf>. Accessed 10 May 2017.
- Ingram, M., and Dainty, R. H. 1971. Changes caused by microbes in spoilage of meats. *J. Appl. Microbiol.* 34(1):21-39. doi:10.1111/j.1365-2672.1971.tb02266.x.
- International Institute of Refrigeration. 1967 and 1971. Recommended conditions for cold storage of perishable products. Paris, France.
- International Organization for Standardization. 2015. Carcass sampling for microbiological analysis ISO 17604:2015. <https://www.iso.org/standard/62769.html>. Accessed 10 May 2017.
- James, S. J. James, C. 2002. Microbiology of meat refrigerated. In: Taylor and Francis, editors, Meat Refrigeration. Woodhead Publishing Limited. p. 6-7. United States of America.

- <http://app.knovel.com/hotlink/toc/id:kpMR000005/meat-refrigeration/meat-refrigeration>. Accessed 10 May 2017.
- Jensen, H. H., and Unnevehr, L. J. 1999. HACCP in pork processing: costs and benefits. http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1251&context=card_workingpapers. Accessed 18 August 2017.
- Jeremiah, L. E., and Gibson L. L. 1997. The influence of storage and display conditions on the color stability of display-Ready pork loin roasts. *Meat Sci.* 47(1):1-16. doi:10.1016/S0309-1740(97)00036-3.
- Korkeala, H., and Alanka, T., 1988. The prediction of food-product shelf-life. In Proc. 34th Intl. Congr. Meat Sci. Tech. Part B, 537-538. Brisbane, Australia.
- Kotula B. W. 1987. Control of extrinsic and intrinsic contamination of pork. In: Linton, A., Hinton, M., Smulders, F, editors, Elimination of pathogenic organisms from meat and poultry.p. 187-201.
- Kraft, A. A. 1992. Psychotropic bacteria in foods disease and spoilage. In: Background, definition, and description of psychotropic bacteria. CRC Press. p. 4-10.
- Labadie, J. (1999). Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Sci.* 52(3):299-305. doi:10.1016/S0309-1740(99)00006-6.
- Lee, B. H., Simard, R. E., Laleye, L. C., and Rolley, R. A. 1985. Effects of temperature and storage duration on the microflora, physicochemical and sensory changes of vacuum- or nitrogen-packed pork. *Meat Sci.* 13:99-112. doi:10.1016/0309-1740(85)90039-7.
- Lee, C. W., Lee, J. R., Kim, M. K., Jo, C., Lee, K. H., You, I., and Jung, S. 2016. Quality improvement of pork loin by dry aging. *Korean J. for Food Sci. of Anim. Res.* 36(3):369–376. doi:10.5851/kosfa.2016.36.3.369.
- Leet, N. G., and Locker, R. H. 1973. A prolonged pre-rigor condition in aerobic surfaces of ox muscle. *J. Sci. Food Agric.* 24(10):1181-1191. doi:10.1002/jsfa.2740241007.
- Lovatt, S. J. 2014. Refrigeration and freezing technology. In: *Ency. Meat Sci.* 2nd ed. Elsevier, p.1-57. <http://app.knovel.com/hotlink/toc/id:kpEMSE0003/encyclopedia-meat-sciences/encyclopedia-meat-sciences>. Accessed 28 January 2017.
- Lulietto, M. F., Sechi, P., Borgogni, E., and Cenci-Goga, B. T. 2015. Meat spoilage: a critical review of a neglected alteration due to ropy slime producing bacteria. *Ital. J. of Anim. Sci.* 14(3):4011. doi:10.4081/ijas.2015.4011.
- Mackey, B. M. and Roberts, T. A. 1993. Improving slaughter hygiene using HACCP and monitoring. *Fleischwirtsch. Intl.* 73(1):58-61.

- Mallikarjunan, P., and Mittal, G. S. 1995. Optimum conditions for beef carcass chilling. *Meat Sci.* 39(2):215-223. doi: 10.1016/0309-1740(94)P1822-D.
- Mayr, D., Margesin, R., Klingsbichel, E., Hartungen, E., Jenewein, D., Schinner, F., and Märk, T. D. 2003. Rapid detection of meat spoilage by measuring volatile organic compounds by using proton transfer reaction mass spectrometry. *Appl. Environ. Microbiol.* 69(8):4697-4705. doi: 10.1128/AEM.69.8.4697-4705.2003.
- Mazur, P. 1966. Physical and chemical basis of injury in single-celled microorganisms subjected to freezing and thawing. In: Meryman, H.T. editor, *Cryobiology*, San Diego, CA, Academic Press, 213.
- Milligan, S. D., Ramsey, C. B., Miller, M. F., Kaster, C. S., and Thompson, L. D. 1998. Resting of pigs and hot-fat trimming and accelerated chilling of carcasses. *J. Anim. Sci.* 76(1):74-86. doi: 10.2527/1998.76174x.
- Murray, A. C. 1995. The evaluation of muscle quality. In: Morgan Jones, S.D. editor, *Quality and grading of carcasses of meat animals*, CRC Press, Boca Raton, FL. 83-107. <https://books.google.com/books?hl=fr&lr=&id=3FFz4bOMXiUC&oi=fnd&pg=PA83&q=The+evaluation+of+muscle+quality,&ots=cJBzPTfKWF&sig=gVuuFwKffmv9k2FwZ3Ht0woIa4s#v=onepage&q=The%20evaluation%20of%20muscle%20quality%2C&f=false>. Accessed 12 June 2017.
- Newton, K. G., Harrison, J. C. L., and Wauters, A. M. 1978. Sources of psychrotrophic bacteria on meat at the abattoir. *J. Appl. Microbiol.* 45(1):75-82. doi: 10.1111/j.1365-2672.1978.tb04200.x.
- Nortje, G. L., Nel, L., Jordaan, E., Badenhorst, K., Goedhart, E., and Holzapfel, W. H. 1990. The aerobic psychrotrophic populations on meat and meat contact surfaces in a meat production system and on meat stored at chill temperatures. *J. Appl. Microbiol.* 68(4):335-344. doi: 10.1111/j.1365-2672.1990.tb02883.x.
- Nottingham, P. M. 1982. Microbiology of carcass meats. In: Brown, M. H. editor, *Meat Microbiol.* London Appl. Sci. Pub, London, UK. p. 13-65.
- Nychas, G. J., Dillon, V. M., and Board, R. G. 1988. Glucose, the key substrate in the microbiological changes occurring in meat and certain meat products. *Biotechnol. Appl. Biochem.* 10(3):203-231. doi: 10.1111/j.1470-8744.1988.tb00014.x.
- Nychas, G. J. E., Drosinos, E. H., and Board R. G. 1998. Chemical changes in stored meat. In: Davies, A., and Board, R.G., editors, *Microbiology of meat and poultry*. Blackie Academic and Professional, London, UK. p. 288-327.
- Nychas, G. J. E., Skandamis, P. N., Tassou, C. C., and Koutsoumanis, K. P. 2008. Meat spoilage during distribution. *Meat Sci.* 78(1-2):77-89. doi: 10.1016/j.meatsci.2007.06.020.

- Pennacchia, C., Ercolini, D., and Villani, F. 2011. Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. *Food Microbiol.* 28(1):84-93. doi:10.1016/j.fm.2010.08.010.
- Pepperell, R., Reid, C. A., Nicolau, S., Hutchison, M. L., Walters, L. D., Johnston, A. M., and Buncic, S. 2005. Experimental comparison of excision and swabbing microbiological sampling methods for carcasses. *J. Food Prot.* 68(10):2163–2168. doi: 10.4315/0362-028X-68.10.2163.
- PIC. 2016. Meat Quality: Understanding Industry Measurements and Guidelines. http://na.pic.com/Uploads/Files/News/Meat%20Quality_Understanding%20Industry%20Measurements201610%2031.pdf. Accessed 10 March 2018.
- Ray, B., and Bhunia, A. 2013. *Fundamental food microbiology*, 5th ed. CRC Press, Boca Raton, FL, USA.
- Remenant, B., Jaffres, E., Dousset, X., Pilet, M. F., and Zagorec, M. 2015. Bacterial spoilers of food: behavior, fitness, and functional properties. *Food Microbiol.* 45(Part A):45-53. doi: 10.1016/j.fm.2014.03.009.
- Rey, C. R., Kraft, A. A., Topel, D. G., Parrish, F. C., and Hotchkiss, D. K. 1976. Microbiology of pale, dark and normal pork. *J. Food Sci.* 41(1):111-116. doi: 10.1111/j.1365-2621.1976.tb01114.x.
- Rossaint, S., Klausmann, S., and Kreyenschmidt, J. 2015. Effect of high-oxygen and oxygen-free modified atmosphere packaging on the spoilage process of poultry breast fillets. *Poultry Sci.* 94(1):93-103. doi:10.3382/ps/peu001.
- Rossel, R., Jouffe, L., and Belceil, P. A. 2009. Analysis of factors associated with *Salmonella* isolation on pork carcass using bayesian networks. *Journées Recherche Porcine*, 41:43-8.
- Savell, J. W., Mueller, S. L., and Baird, B. E. 2005. The chilling of carcasses. *Meat Sci.* 70(3):449-459. doi: 10.1016/j.meatsci.2004.06.027
- Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M., Roy, S. L. and Griffin, P. M. 2011. Foodborne illness acquired in the united states-major pathogens emerging infectious diseases. *Emerg. Infect. Dis.* 17(1):7-15. doi: 10.3201/eid1701.P11101.
- Scott, W. J. 1936. *Aust. Coun. Sci. Ind. Res. J.* 9:117.
- Sebranek, J. G. 2008. *Meats frozen*, World Food Logistics Organization-Commodity storage manual. <http://www.gcca.org/wp-content/uploads/2012/09/MeatsFrozen.pdf>. Accessed 10 May 2017.

- Shamberger, R. L., Andreone, T. L., and Willis, C. E. 1974. Antioxidants and cancer. IV. Malonaldehyde has initiating activity as carcinogen. *J. Natl. Cancer Inst.* 53(6):1771-1773. doi: 10.1093/jnci/53.6.1771.
- Sofos, J. N. 1994. Quality attributes and their measurement in: Meat, poultry and fish products. Springer, Boston, MA. p. 359-403.
https://books.google.com/books?hl=fr&lr=&id=YlzmBwAAQBAJ&oi=fnd&pg=PA1&q=Quality+Attributes+and+Their+Measurement+In:+Meat,+Poultry+and+Fish+Products&ots=kypWKlG_IM&sig=jszHKlzWyOUgPItp93fKhQwk60#v=onepage&q=Quality%20Attributes%20and%20Their%20Measurement%20In%3A%20Meat%2C%20Poultry%20and%20Fish%20Products&f=false.
- USDA-ERS. 2016. Long-term Projections-Report OCE-2016-1.
https://www.ers.usda.gov/webdocs/publications/37809/56729_oce-2016-1.pdf?v=42508. Accessed 20 May 2017.
- USDA-ERS. 2017. Livestock and meat domestic data.
[https://www.ers.usda.gov/data-products/livestock-meat-domestic-data/livestock-meat-domestic-data/#Red meat and poultry production](https://www.ers.usda.gov/data-products/livestock-meat-domestic-data/livestock-meat-domestic-data/#Red%20meat%20and%20poultry%20production). Accessed 20 May 2017.
- USDA-FSIS. 2013. Food safety information. Fresh pork from farm to table.
https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/meat-preparation/fresh-pork-from-farm-to-table/CT_Index. Accessed 20 May 2017.
- USDA-FSIS. 2010. Food safety information. Refrigeration and food safety.
https://www.fsis.usda.gov/shared/PDF/Refrigeration_and_Food_Safety.pdf. Accessed 20 May 2017.
- USDA-FSIS. 1996. The pathogen reduction/HACCP final rule- Federal Register, 61(144):38806-38810.
<https://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance/haccp/pr-and-haccp-guidance-documents>. Accessed 20 May 2017.
- USDA-FSIS. 1995. Pathogen reduction: hazard analysis and critical control point (HACCP) systems. Proposed rule. Federal Register, 60(23):3.
<https://www.gpo.gov/fdsys/pkg/FR-1995-02-03/pdf/95-2366.pdf>. Accessed 20 May 2017.
- USDA-FAS. 2015. FAO, World meat and poultry consumption. Pork check off.
<http://www.pork.org/pork-quick-facts/home/stats/u-s-pork-exports/world-per-capita-pork-consumption-2/> Accessed 20 May 2017.
- USDA-NASS-ASB. 2016. Overview of the United States slaughter industry.
<http://usda.mannlib.cornell.edu/usda/current/SlauOverview/SlauOverview-10-27-2016.pdf>. Accessed 12 June 2017.

- USDA-NASS. 2017. Livestock Slaughter Summary, 2016.
<http://usda.mannlib.cornell.edu/usda/current/LiveSlauSu/LiveSlauSu-04-19-2017.pdf>.
Accessed 12 June 2017.
- U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Weather Service.
<http://forecast.weather.gov/MapClick.php?textField1=39.14&textField2=-96.67#.WhfOLVXibiU>. Accessed 18 November 2017.
- Van Netten, P., Mossel, D. A. A., and In't Veld, J. H. 1995. Lactic acid decontamination of fresh pork carcasses: A pilot plant study. *Int. J. Food Microbiol.* 25(1):1-9. doi:10.1016/0168-1605(94)00039-9.
- Vanderzant, C., Hanna, M. O., Ehlers, J.G., Savell, J. W., Griffin, D. B., Johnson, D. D., Smith, G. C., and Stiffler, D. M. 1985. Methods of chilling and packaging of beef, pork and lamb variety meats for transoceanic shipment: microbiological characteristics. *J. Food. Prot.* 48(9):765-769. doi: 10.4315/0362-028X-48.9.765.
- Voyle, C. A. 1974. Structural and histological changes associated with the freezing of meat. In *Meat Res. Inst. Symposium* 3(6).
- Wilkins, W., Rajić, A., Parker, S., Waddell, L., Sanchez, J., Sargeant, J., and Waldner, C. 2010. Examining heterogeneity in the diagnostic accuracy of culture and PCR for *Salmonella* spp. in swine: A systematic review/meta regression approach. *Zoonoses and Public Health.* 57(s1):121-134. doi: 10.1111/j.1863-2378.2010.01366.x.
- Wong, A. C. L. 1998. Biofilms in food processing environments. *J. Dairy Sci.* 81(10):2765-2770. doi: 10.3168/jds.S0022-0302(98)75834-5.
- Wright, L. I., Scanger, J. A., Belk, K. E., Engle, T. E., Tatum, J. D., Person, R. C., McKenna, D. R., Griffin, D. B., McKeith, F. K., Savell, J. W., and Smith, G. C. 2005. Benchmarking value in the pork supply chain: characterization of U.S. pork in the retail marketplace. *Meat Sci.* 71(3):451-463. doi: 10.1016/j.meatsci.2005.04.024.
- Yang, X. 2012. An evaluation of the effectiveness of Freshcase® technology to extend the shelf life of beef and pork. M.S. thesis, Colorado State Univ., Fort Collins.
(https://dspace.library.colostate.edu/bitstream/handle/10217/67324/Yang_colostate_0053N_11095.pdf?sequence=1&isAllowed=y).
- Zhao, F., Zhou, G., Ye, K., Wang, S., Xu, X., and Li, C. 2015. Microbial changes in vacuum-packed chilled pork during storage. *Meat Sci.* 100:145-149. doi: 10.1016/j.meatsci.2014.10.004g.

Appendix A - Statistical Codes

Pork Carcass

pH

```
title 'pH Analysis';
proc mixed data=carc covtest plots=none;
class carcass day;
model ph = day/ddfm=KR;
random carcass;
lsmeans day/pdiff;
run;
```

Moisture Content

```
title 'Moisture Analysis by Phase';
proc mixed data=carc covtest plots=none;
class carcass phase day;
model moisture = phase|day/ddfm=KR;
repeated day/subject= carcass type=un;
lsmeans day/pdiff;
lsmeans phase phase*day;
run;
```

```
title 'Moisture Analysis';
proc mixed data=carc covtest plots=none;
class carcass day;
model moisture = day/ddfm=KR;
repeated day/subject= carcass type=un;
lsmeans day/pdiff;
run;
```

Microbiological

APC

```
title 'APC Analysis with Day as a Class Variable';
proc mixed data=carc covtest plots=none;
class carcass location day;
model apc = location day location*day/ddfm=KR;
random carcass;
repeated day / subject=carcass*location type=ar(1);
lsmeans location|day/pdiff;
```



```

contrast 'linear day' day -3 -1 1 3;
contrast 'quadratic day' day 1 -1 -1 1;
contrast 'cubic day' day -1 3 -3 1;
run;

```

EB, Yeast and Mold

```

data carc;
  set carc;
  ebpos = ((Location<=3)*(EB>=0.062683)) + ((Location=3)*(EB>=0.414765));
  ypos = ((Location<=3)*(y>=0.062683)) + ((Location=3)*(y>=0.414765));
  mpos = ((Location<=3)*(m>=0.062683)) + ((Location=3)*(m>=0.414765));
run;

```

```

title "Marginal Analyses of EB, Yeast & Mold";
proc freq data=carc;
  table ebpos*(Location Day)/exact norow;
  table ypos*(Location Day)/exact norow;
  table mpos*(Location Day)/exact norow;
run;

```

EB

```

title "Conditional Analyses of EB";
proc freq data=carc;
  table Day*ebpos*location/exact norow;
  table location*ebpos*Day/exact norow;
run;

```

Yeast

```

title "Conditional Analyses of Yeast";
proc freq data=carc;
  table Day*ypos*location/exact norow;
  table location*ypos*Day/exact norow;
run;
proc freq data=carc;
  where location ne 3;
  table ypos*Location/exact norow;
run;

```

```

proc freq data=carc;
  where location ne 2;
  table ypos*Location/exact norow;
run;

```

```
proc freq data=carc;
where location ne 1;
table ypos*Location/exact norow;
run;
```

```
proc freq data=carc;
where Day in (1 7);
table ypos*Day/exact norow;
run;
```

```
proc freq data=carc;
where Day in (1 14);
table ypos*Day/exact norow;
run;
```

```
proc freq data=carc;
where Day in (7 14);
table ypos*Day/exact norow;
run;
```

```
proc freq data=carc;
where Day in (1 21);
table ypos*Day/exact norow;
run;
```

```
proc freq data=carc;
where Day in (7 21);
table ypos*Day/exact norow;
run;
```

```
proc freq data=carc;
where Day in (14 21);
table ypos*Day/exact norow;
run;
ods rtf close;
```

Mold

```
title "Conditional Analyses of Mold";
proc freq data=carc;
  table Day*mpos*location/exact norow;
  table location*mpos*Day/exact norow;
run;
ods rtf close;
```

Pork Shoulder Blade Steaks

pH

```
title "Analysis of Ph by Day";
proc mixed data=final plots=none;
  class chop day;
  model ph=day/ddfm=kr;
  repeated day/subject=chop type=arh(1);
  lsmeans day/pdiff ;
  contrast 'Linear' day -5 -3 -1 1 3 5;
  contrast 'Quad' day 5 -1 -4 -4 -1 5;
  contrast 'Cubic' day -5 7 4 -4 -7 5;
  contrast 'Quart' day 1 -3 2 2 -3 1;
  contrast 'Quint' day -1 5 -10 10 -5 1;
run;
```

APC

```
title "Analysis of APC with All Half Carasses";
proc mixed data=chops covtest;
  class Chop Day;
  model APC = Day/ddfm=kr;
  repeated Day/subject=chop type=csh;
  lsmeans day/pdiff adjust=none
  contrast 'linear' day -5 -3 -1 1 3 5;
  contrast 'quad' day 5 -1 -4 -4 -1 5;
  contrast 'cubic' day -5 7 4 -4 -7 5;
  contrast 'quartic' day 1 -3 2 2 -3 1;
  contrast 'quintic' day -1 5 -10 10 -5 1;
run;
```

EB, Yeast and Mold

```
title "Marginal Analyses of EB, Yeast & Mold";
proc freq data=chops6;
  table ebpos*Day/exact norow;
  table ypos*Day/exact norow;
  table mpos*Day/exact norow;
run;
```

Harvest Day

Pork Carcass APC

title 'APC Analysis with Day as a Class Variable by Phase (Carcasses 1-8 vs 9-20): Equal Variances for Phase';

```
proc mixed data=carc covtest plots=none;
  class carcass phase location day;
  model apc = location|phase|day/ddfm=KR;
  random carcass;
  repeated day / subject=carcass*location type=ar(1);
  lsmeans location|day/pdiff;
  contrast 'linear day' day -3 -1 1 3;
  contrast 'quadratic day' day 1 -1 -1 1;
  contrast 'cubic day' day -1 3 -3 1;
run;
```

Pork Blade Steaks APC

title "Analysis of APC with Chops in Two Phases";

```
proc mixed data=chops4;
  class chop day phase;
  model APC=phase|day /ddfm=kr;
  repeated Day/subject=chop(phase) type=csh;
  lsmeans phase|day/pdiff;
run;
```

Appendix B - Microbiological and pH tables

Table B.1. Least squares means and standard error¹ for pH and moisture content of right pork carcass sides ($n = 20$) measured on four dates and stored at $0\pm 1^\circ\text{C}$ and 87.3% relative humidity for up to 21 days.

	pH	Moisture, %
Day 1	5.88 ± 0.04^a	65.10 ± 0.79^a
Day 7	5.88 ± 0.04^a	60.90 ± 1.13^b
Day 14	5.84 ± 0.04^a	58.87 ± 0.98^c
Day 21	5.72 ± 0.04^b	50.52 ± 1.39^d
<i>P</i> -value	0.02	≤ 0.01

^{abcd}Least squares means in the same column lacking a common superscript differ ($P \leq 0.05$).

¹Pooled SE of the least squares means.

Table B.2. Aerobic plate count (APC)¹ populations of right pork carcass sides ($n = 20$) stored at $0\pm 1^\circ\text{C}$ and 87.3% relative humidity for up to 21 days.

	Sample Location			<i>P</i> -value
	Flank	Shoulder	Jowl	
APC¹	0.77 ± 0.12^a	0.76 ± 0.12^a	1.21 ± 0.12^b	0.01

^{ab}Least squares means \pm standard error with different superscripts differ ($P \leq 0.05$).

¹APC \log_{10} CFU/cm².

Table B.3. Aerobic plate count (APC)¹ populations of right pork carcass sides ($n = 20$) stored at $0\pm 1^\circ\text{C}$ and 87.3% relative humidity for up to 21 days.

	Days of Storage				<i>P</i> -value
	1	7	14	21	
APC ¹	0.98±0.11 ^a	0.99±0.11 ^a	0.86±0.11 ^a	0.84±0.11 ^a	0.55

^aLeast squares means±standard error with different superscripts differ ($P \leq 0.05$)

¹APC log₁₀ CFU/cm².

Table B.4. Least squares means and standard error¹ for pH of pork shoulder blade steaks ($n = 17$) stored at $0\pm 1^\circ\text{C}$ for up to 35 days.

	pH
Day 0	5.73±0.05 ^{bc}
Day 7	5.78±0.03 ^b
Day 14	5.89±0.02 ^a
Day 21	5.70±0.03 ^{bc}
Day 28	5.78±0.02 ^b
Day 35	5.69±0.02 ^c
<i>P</i> -value	≤ 0.05

^{abc}Least squares means in the same column lacking a common superscript differ ($P \leq 0.05$).

¹Pooled SE of the least squares means.

Table B.5. Aerobic plate count (APC)¹ populations of pork shoulder blade steaks ($n = 17$) stored at $0\pm 1^\circ\text{C}$ for up to 35 days.

	Days of Storage						<i>P</i> -value
	0	7	14	21	28	35	
APC ¹	1.61±0.12 ^a	1.18±0.12 ^b	2.44±0.15 ^c	3.17±0.31 ^c	4.67±0.23 ^d	5.06±0.41 ^d	0.01

^{abcd}Least squares means±standard error with different superscripts differ ($P \leq 0.05$).

¹ APC log₁₀ CFU/g.

Table B.6. Mold populations of pork shoulder blade steaks ($n = 17$) stored at $0\pm 1^\circ\text{C}$ for up to 35 days.

	Days of Storage						Total ²
	0	7	14	21	28	35	
Below DL ¹	17	17	17	14	13	15	93
Above DL ¹	0	0	0	3	4	2	102
Total²	17	17	17	17	17	17	102

¹Number of observations below or above the detection limit (DL).

²Number of samples measured.

DL = 0.70 log₁₀ CFU/g.

Table B.7. Yeast populations of pork shoulder blade steaks ($n = 17$) stored at $0\pm 1^\circ\text{C}$ for up to 35 days.

	Days of Storage						Total ²
	0	7	14	21	28	35	
Below DL ¹	8	6	6	6	10	8	44
Above DL ¹	9	11	11	11	7	9	58
Total²	17	17	17	17	17	17	102

¹Number of observations below or above the detection limit (DL).

²Number of samples measured.

DL = 0.70 log₁₀ CFU/g.

Table B.8. Aerobic plate count (APC)¹ populations pooled from the flank, shoulder, and jowl of pork carcasses ($n = 20$) harvested on two separate production days and hung up to 21 days at $0\pm 1^\circ\text{C}$ and 87.3% relative humidity.

	Harvest Month		
	October	February	<i>P</i> -value
APC ¹	1.12±0.12 ^a	0.78±0.09 ^b	0.03

^{ab}Least squares means±standard error with different superscripts differ ($P \leq 0.05$).

¹APC log₁₀ CFU/cm².

Table B.9. Aerobic plate count (APC)¹ populations of pork shoulder blade steaks fabricated from hogs harvested on two separate production days October ($n = 5$)¹ and February ($n = 12$)¹ and stored up to 35 days at $0\pm 1^\circ\text{C}$.

Harvest Month	Days of Storage					
	0	7	14	21	28	35
October ¹	1.83 ^a	1.13 ^a	2.94 ^a	4.35 ^a	5.80 ^a	7.10 ^a
February ¹	1.52 ^a	1.20 ^a	2.23 ^b	2.69 ^b	4.20 ^b	4.21 ^b
SEM ²	0.25	0.24	0.30	0.58	0.38	0.61
<i>P</i> -value	0.24	0.77	0.03	≤0.01	≤0.01	≤0.01

^{ab}Least squares means with different superscripts within the same column differ ($P \leq 0.05$).

¹APC log₁₀ CFU/cm².

²Pooled SE of the least squares means.