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Dissolved carbon dioxide and oxygen concentrations in meat purge and relationship to microbial populations and shelf life

Kristin Renee Adams

Iowa State University

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Dissolved carbon dioxide and oxygen concentrations in meat purge and relationship to microbial populations and shelf life

by

Kristin Renee Adams

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Meat Science

Program of Study Committee:
James S. Dickson, Major Professor
Steven Lonergan
Aubrey Mendonca

Iowa State University
Ames, Iowa
2014

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ABSTRACT

The shelf life of a fresh meat product can be measured by a total bacterial enumeration method, which is time-consuming, expensive, and destructive to conduct. Due to these issues, there is interest in developing a shelf life model from an instrument measurement, which has the capability to be less time-consuming, less expensive, and non-destructive. One instrument measurement that could be used to estimate shelf life is dissolved carbon dioxide (CO$_2$). There are few studies published that investigate dissolved CO$_2$ concentrations in the context of shelf life or its relationship to the microbial population. Therefore, the objectives of this study were to determine the dissolved CO$_2$ and O$_2$ concentrations in the purge of vacuum-packaged pork chops during storage, and to determine the relationship between dissolved CO$_2$ and O$_2$ concentrations to the microbial populations and shelf life. Scanning electron microscope (SEM) images were also taken of the packaging film in contact with the meat product to investigate how the biofilm develops on the packaging film throughout the shelf life.

These objectives were completed by taking sixty pork loin chops and placing them into twenty vacuum-packages. In each vacuum-package, a set of purge collection tubes were placed into each bag to collect the purge for the dissolved gas measurements. These packages were stored at 4°C for 60 days, and the packages were sampled randomly on days 0, 5, 15, 30, 45, and 60. On the sample days, the dissolved gases were measured and microbiological analysis conducted.

The results of the experiment demonstrated that the spoilage bacteria increased the dissolved CO$_2$ and decreased the dissolved O$_2$ concentrations during the 60 day
storage period by cellular respiration. The lactic acid bacteria (LAB) dominated the spoilage microflora, followed by Enterobacteriaceae and *Brochothrix thermosphacta*. Since the dissolved CO₂ concentrations increased due to the increase in microbial populations, this information was used to develop mathematical models. These mathematical models estimate microbial populations and shelf life based upon dissolved CO₂ concentrations in the purge of vacuum-packaged pork chops. The SEM images revealed a two-layer biofilm on the packaging film that was the result of a tri-phase growth environment.
Shelf life and meat spoilage will always be linked together because the time it takes for spoilage to occur determines the shelf life of a meat product. There are several ways in which to measure this time until spoilage, or to measure shelf life. Traditionally, they involve microbiological analysis, or total bacterial enumeration methods, to determine the populations of spoilage bacteria present. This method is effective and it will reveal the amount of spoilage bacteria present and how spoiled the product may be. However, this traditional method is time-consuming, destructive, and expensive to conduct (Bruckner, Albrecht, Petersen, & Kreyenschmidt, 2013; McDonald & Sun, 1999; McMeekin & Ross, 1996; Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). It is time-consuming because a total mesophilic aerobic bacterial enumeration requires three days to complete. In addition, once the data is collected, it is historical and provides information on the product 72 hours earlier. Total bacterial enumeration methods are also destructive because it requires taking samples from the product to enumerate the bacteria, so the product can no longer be sold. In addition, traditional shelf life estimates are expensive because they require a laboratory, laboratory personnel, supplies, and product, and all of these have costs associated with them. Because of these problems, there is interest in developing a method of estimating microbial populations based upon an instrument measurement (Bruckner et al., 2013; McDonald & Sun, 1999; McMeekin & Ross, 1996; Nychas et al., 2008).

This instrument measurement would be for dissolved carbon dioxide concentrations. Dissolved carbon dioxide is readily found in the purge of a meat product.
and it is produced by the spoilage bacteria and the meat product itself. The largest producer of the bacteria would be lactic acid bacteria (LAB) because they produce carbon dioxide as a metabolism by-product (Hammes & Hertel, 2006). Therefore, as the populations of the spoilage bacteria and LAB increase, so does the production and concentration of dissolved carbon dioxide. Potentially, a dissolved carbon dioxide concentration could estimate microbial populations and be used to estimate the shelf life of packaged meats. This instrument measurement could make estimating shelf life faster and easier to conduct, which would help the meat industry lower costs, improve distribution systems, and optimize storage management (Alderees & Hsieh, 2012; Kreyenschmidt et al., 2010).

Thesis Organization

This thesis is organized into four chapters and an appendix. The format and style of all chapters is in the style of the journal of *Meat Science*. Chapter one contains a general introduction into the thesis topic and Chapter 2 is a review of relevant literature pertaining to the thesis and the topic discussed. Chapter 3, titled “Dissolved carbon dioxide and oxygen concentrations in purge of vacuum-packaged pork chops and relationship to microbial population and shelf life” has been submitted to the journal of *Meat Science*. Chapter 4 contains a general conclusion for the entire thesis and the Appendix contains additional scanning electron microscope photographs of the biofilm on the packaging film.
References


CHAPTER 2. LITERATURE REVIEW

Sources of Meat Contamination

**Outer surfaces**

One of the main sources of contamination for fresh meat products are the outer surfaces of the live animals. These surfaces include the hide (fleece, skin) and hooves (Ayres, 1955; Bell, 1997; Gill, 1998; Grau, 1986; James & James, 2002; Phebus et al., 1997; Sheridan, 1998) and they are naturally contaminated with bacteria from the environment, soil, dust, and feces (Aberle, Forrest, Gerrard, & Mills, 2012; Ayres, 1955; Gill, 1986; C. O. Gill & K. G. Newton, 1977; Grau, 1986; Gregory, 2007; Lawrie & Ledward, 2006). Therefore, it is likely that the increased bacterial concentrations from the hide and hooves could be transferred to the muscle tissue by cross-contamination. For example of hide contamination, Samelis (2006) found an average aerobic plate count of between $10^4$ CFU/cm² and $10^7$ CFU/cm² on hides. Another study by Bacon et al. (2000) found initial bacterial numbers on the hide were log 8.2-12.5 for total plate count (TPC) and log 6.0-7.9 for *Escherichia coli* count (ECC). They also found that after hide removal, the bacterial levels were log 7.6 for TPC and log 4.1 for ECC on the carcass tissue, thus a large concentration of bacteria were transferred to the tissue underneath. However, these bacterial levels will not always contaminate the carcass to this extent and could be lower, to around log 2 CFU/cm² or fewer (Sofos et al., 1999).

Once the animals enter the slaughter facility, it is very easy for the contaminated hides to come in contact with the sterile meat tissue underneath the skin. Bacteria can be introduced to the underlying meat in a couple ways, either by knives or direct contact
When a worker makes a skin-opening cut through a contaminated hide, bacteria are transferred to the knife and then to the underlying muscle tissue (Gill, 2004, 2005). These skin-opening cuts occur during sticking and during hide removal, usually in the more heavily contaminated areas of the animal, such as the hindquarter, chest, belly, and neck area (Antic et al., 2010; Buncic, McKinstry, Reid, & Anil, 2002). Therefore, there is a greater chance of transferring bacteria from the hide to the underlying tissue by these skin-opening cuts by knives and workers (Collis et al., 2004). Contamination of the meat can also occur by direct contact with the hide. The hide is removed by hand or by a hide-puller by loosening the hide and then pulling down or up on the animal to fully remove the hide. As the hide is loosened from the animal, it can be moved around and come in direct contact with the tissue underneath, if workers are not careful to keep the outside of the hide away from the freshly skinned carcass (Gill, 2005; Scanga, 2005). Workers can also contaminate the carcass by touching the contaminated hide with their gloves and then touch the skinned carcass (Gill, 2005). Due to the many chances of contaminating the carcass, there are washing stations for the carcasses after hide removal to help limit some of the contamination from entering further into the slaughter facility and indirectly contaminating other operations in the facility, such as carcass-breaking and product production (Aslam, Greer, Nattress, Gill, & McMullen, 2004). In addition, many slaughter facilities wash their animals after sticking to help remove a majority of the fecal material embedded in the hides and hooves. This helps reduce the bacterial levels on the hides and hooves, so as to limit the number of bacteria transferred to the carcass.
Slaughter facilities also stress the importance of keeping the hide from contacting the carcass underneath and to keep equipment clean.

**Equipment**

Another source of contamination is the general equipment used in a slaughter operation. Equipment is a source of contamination because as animals and their carcasses come in contact with the equipment, they transfer bacteria (Gill, 1986; Youssef, Badoni, Yang, & Gill, 2013). Once the equipment is contaminated, it is not thoroughly sanitized until after the shift ends, so during operations, each carcass has been contaminated with bacteria that was on the equipment previously and contamination spreads throughout the processing line (Aslam et al., 2004; Duffy et al., 2001; Gill & Landers, 2004; Lawrie & Ledward, 2006; Stiles & Ng, 1981; Warriner, Aldsworth, Kaur, & Dodd, 2002).

Equipment means anything that can be contaminated with bacteria, such as chains, shackles, gambrels, conveyor belts, aprons, buckets, carts, knives, safety gear, saws, and much more. Conveyor belts are very hard to clean because the hinges that connect the plastic sections are very hard to access and clean thoroughly (Gill, 2005; Youssef et al., 2013). If these hinges are not cleaned well each day, they can be a source of contamination for fresh meat products because conveyor belts can have up to log 6 CFU/sample of aerobes and some coliforms (Aslam et al., 2004; Gill & Landers, 2004). Another study by Gill, McGinnis, and Bryant (2001) demonstrated that a carcass breaking conveyor belt had mean log 3.26 and log 3.08 CFU/1000 cm² for coliforms and *E. coli*, respectively, and a carcass cutting table had a mean log 1.56 CFU/1000 cm² for coliforms and log 1.36 CFU/1000 cm² for *E. coli*. The belt and table in the study were
depositing coliforms and *E. coli* onto the product cuts because the cuts had greater 
coliform (log 6 CFU/side) and *E. coli* (log 5.5 CFU/side) total counts than the sides being 
broken down (log 4 CFU/side coliforms and log 3.5 CFU/side for *E. coli*) (Gill et al., 
2001). In addition, a majority of the equipment used in a packing plant is made of 
stainless steel. Stainless steel may appear smooth, but microscopically it is rough and 
bacteria can attach themselves to the surface to form a biofilm (Hood & Zottola, 1997). 
Biofilms can contain upwards to $10^6$ cells/cm$^2$ or more, and if the biofilm is not 
adequately removed during cleaning, the biofilm can dislodge and contaminate the 
carcass and fresh meat products. These studies and examples show how the sanitizing and 
cleaning operation of the plant is a critical factor to reduce bacterial contamination so the 
equipment does not always add more bacteria to the carcasses.

Knives, as specific part of equipment, are another source of bacterial 
contamination to carcasses. Knives are contaminated from animal hides and from 
workers, which can then be spread onto the carcasses. Bell (1997) found that the 
contamination on a worker’s knife hands, after making cuts into the hide, had similar 
bacterial concentrations as found on the knife and on the hide. Furthermore, the knife 
blades themselves had about $1/10^{th}$ the contamination found on the worker’s hands. This 
means that knives can be contaminated from workers and hides then continue to spread 
the contamination throughout the carcass surface because they are not often 
decontaminated in-between cuts (Grau, 1986; Phebus et al., 1997). The stick knife can 
also be a contamination source because if the stick knife is not properly cleaned, it can 
transmit bacteria through the bloodstream in the animal during the sticking process 
(Aberle et al., 2012; Buncic et al., 2002; Gill, 2005; Jay et al., 2005). Knives can also be a
point of contamination during carcass breaking. Youssef et al. (2013) found that knives can have up to log 3.77 CFU/item of aerobes before work and the steel mesh gloves used for personal protective equipment had log 5.78 CFU/item aerobes before work and log 4.62 CFU/item aerobes after work in one beef packing plant. The log count decreased because bacteria will eventually come to an equilibrium between meat products and the equipment. These studies highlight the importance of clean, sanitized knives or steel mesh gloves during a slaughter or carcass breaking operation and to always sanitize them during operations to reduce bacterial contamination.

Workers, who use the equipment in a plant, are another source of contamination when it comes to fresh meat, whether it be from the workers themselves or by cross-contamination. Humans have been known to carry bacteria in their nose, mouth, and on their skin, so good hygiene is of upmost importance in a slaughter operation, for bad hygiene can contaminate carcasses (Jay et al., 2005). Workers who work in the stunning, dehiding, and evisceration areas of a slaughter line are more likely to be sources of contamination due to the increased bacterial levels found in these areas. Workers cause cross-contamination by their gloves, hands, and aprons from touching one carcass to the next without sanitizing in-between (Aberle et al., 2012; Sheridan, 1998). Youssef et al. (2013) found that workers who wore cotton gloves in a beef carcass breaking operation can indeed contaminate fresh meat products. Cotton gloves before work had log 5.8 CFU/item and log 2.74 CFU/item of aerobes and E. coli, respectively, and after work those numbers reduced to log 5.52 of aerobes and log 1.30 of E. coli (Youssef et al., 2013). The numbers reduced due to bacteria coming to an equilibrium between the fresh meat products and the cotton gloves of the workers. Despite the reduction in numbers,
this study showed that workers wearing cotton gloves can be a significant source of contamination for fresh meat products, so personal hygiene of the workers and sanitizing equipment thoroughly are of great importance to limit contamination. In addition, many times workers are in charge of cleaning their own protective equipment, so they are the ones who judge if their equipment is clean or not, and if it is not fully clean, the bacteria left on the equipment can contaminate other carcasses (Gill, 1998, 2009).

Sometimes, equipment not often thought to be contaminated, such as captive bolt guns, can have an impact on the carcass contamination level. The captive bolt gun is not cleaned or sanitized often during slaughter operations, so any bacteria on the captive bolt is introduced into the brain and bloodstream upon impact (Aberle et al., 2012; Gill, 2005). Buncic et al. (2002) found that most of the bacteria introduced by captive bolt were mostly in the lungs, liver, and spleen, but sometimes it made it to the surface of the carcass. This shows that a heavily contaminated captive bolt gun could influence the contamination levels of a carcass.

Between conveyor belts, chains, shackles, gambrels, buckets, carts, knives, safety gear, saws, aprons, steel mesh gloves, cotton gloves, workers, and captive bolt guns in a slaughter or carcass breaking facility, equipment can be a main source of contamination on meat carcasses. As carcasses come in contact with equipment, they transfer bacteria to the equipment, which then is transferred to the rest of the carcasses that use that equipment. This spreads contamination throughout the slaughter facility or carcass breaking operation, so sanitizing operations are crucial to keep contamination levels low.
Viscera

The viscera or intestinal tracts of animals are also a source of bacterial contamination, especially if the tracts are punctured during slaughter operations. If the viscera is punctured, or if fecal matter escapes the rectum during evisceration, it will contaminate the carcass and cause the potential for cross-contamination (Ayres, 1955; Grau, 1986). Cross-contamination will occur because once the viscera is punctured, it is on the worker’s knife and hands, and they may not notice the puncture until they spread bacteria all over the carcass. A rumen can have up to $10^{10}$ bacteria per gram, so one small puncture or leakage from the rumen, rectum, or esophagus can release a large amount of bacteria on the carcass (Jay et al., 2005). This is why tying off the esophagus and rectum are very important steps in a slaughter operation, along with not puncturing the viscera (Bell, 1997; Gill, 1998, 2005; Sheridan, 1998). Removing animals from feed overnight before slaughter reduces gastrointestinal contents, and for pigs, the viscera are reduced by 1 kg/pig (Murray, Robertson, Nattress, & Fortin, 2001). By reducing the amount of gastrointestinal contents, it helps to reduce nicking and spilling of visceral contents by workers, thereby reducing carcass contamination (Aberle et al., 2012; Gill, 2005; Murray et al., 2001; Nattress & Murray, 2000). In addition, feed withdrawal of 15 hours in pork can reduce the $E. \ coli$ and coliform counts in a pig’s stomach by 0.8 log CFU/g (Nattress & Murray, 2000). Miller, Carr, Bawcom, Ramsey, and Thompson (1997) found that out of 1,600 pork carcasses, 1.6%, 1.5%, 0.5%, and 0.7% had viscera punctured after a 0, 2, 4, and 6 hour feed withdrawal time before slaughter, respectively. This shows that a 2 hour or longer feed withdrawal time reduced puncturing the viscera by 50% during evisceration. Therefore, there is about a 50% less likely chance of contaminating the
carcass with ingesta if animals have feed withdrawn before slaughter because
evisceration is easier. Packers do not want to contaminate the carcass because that means
it has to be trimmed and potential product and profit will be lost in the trimming process.

**Cross-contamination**

Cross-contamination is another source of contamination for fresh meat products, and can occur from other carcasses, the slaughterhouse environment, and equipment. Cross-contamination encompasses a lot of items in one heading and cross-contamination means transferring bacteria or viruses from a product or surface, either indirectly or directly, to an uncontaminated product or surface (Carrasco, Morales-Rueda, & Garcia-Gimeno, 2012). Carcasses undergo a lot of handling from start to finish, whether live animal or not (Choi et al., 2013). They come in contact with workers, equipment, knives, conveyor belts, saws, surfaces, tables, aprons, gloves, and packaging materials. All of these areas where carcasses are handled or touched can have cross-contamination (Stiles & Ng, 1981). For example, Botteldoorn, Heyndrickx, Rijpens, Grijspeerdt, and Herman (2003) found that 27% of knife samples taken from four slaughter facilities were positive for *Salmonella*. The knives became contaminated from the pork carcasses. In addition, H.A.M van Hoek et al. (2012) found in a study at a Danish pork slaughter facility, over 35% of *Salmonella* found on the pork carcasses was due to cross-contamination. They also found that *Salmonella* Rissen, which was not present on any incoming carcasses, was found at significant levels (around log 2 CFU/g) at the end of the slaughter-line, so it came from cross-contamination and equipment in the slaughter process (H.A.M van Hoek et al., 2012). Other studies have shown that cross-contamination is responsible for
29-30% of positive *Salmonella* carcasses in pork packing plants (Botteldoorn et al., 2003; Carrasco et al., 2012). Air in the packing plant can also serve as a way to contaminate carcasses, for droplets of bacteria can be produced when pulling hides off the animals with APC counts of log 5 CFU/h and Enterobacteriaceae counts of log 3 CFU/h (Gill, 2005; Schmidt, Arthur, Bosilevac, Kalchayanand, & Wheeler, 2012). These studies demonstrate that cross-contamination in a slaughter or carcass-breaking operation is inevitable, but should be controlled by good sanitizing operations to reduce the chances of cross-contamination.

**Methods that affect contamination**

Bacterial contamination in a slaughter facility is neither static or consistent, it can be affected by many factors, such as variations between individuals and herds, slaughter techniques for different species, line speeds, carcass interventions, and the season of the year (Ayres, 1955; Belk, 2001; C. O. Gill & K. G. Newton, 1977; Lawrie & Ledward, 2006). For example of season of the year, Aslam et al. (2004) found a mean log 3.94 CFU/100 cm² *E. coli* on beef hides in the summer and log 2.64 CFU/100 cm² *E. coli* in winter. This means the summer months tend to have increased bacterial contamination levels than the colder, winter months due to increased air temperatures in summer favoring more bacterial growth in the environment.

**Slaughter techniques**

Different slaughter techniques between species and plants can affect the amount of contamination on a carcass. For bovine and oovine carcasses, the hide is completely
removed, so that always leads to potential contamination by not preventing the hide from touching the carcass (Grau, 1986; Scanga, 2005). However, for a majority of swine slaughter facilities, they only remove the hair, not the hide. This means swine carcasses are exposed to polishers, dehairers, and scald tanks that can affect the levels of contamination by increasing (dehairers) or marginally reducing (scald tank) them (Gill & Bryant, 1992; Scanga, 2005). Contamination levels can increase with swine carcasses because many carcasses use the same dehairer and polisher. This results in a mixture of bacteria from multiple carcasses as well as transferring bacteria to many other carcasses (Gill, 2005; Warriner et al., 2002). Gill et al. (2000) found that in eight pork packing plants, aerobic counts sampled after polishing, after the final wash in the dressing process, and after cooling maintained bacterial levels of around log 3 CFU/cm². *E. coli* and coliform levels stayed around log 2 CFU/cm² at the same sampled sites, with coliforms only reducing a fraction to log 1.5 CFU/cm² after cooling (Gill et al., 2000). Another study by Warriner et al. (2002), showed that after scalding, scraping, and toe-nail removal of a pork carcass, Enterobacteriaceae counts decreased slightly from log 4.3 to log 3.7 CFU/100 cm² but increased after evisceration. As for *E. coli*, the counts were log 4.6 CFU/100 cm² pre-scald and after scalding, scraping, dry polishing, singeing, and wet polishing, *E. coli* was log 2.7 CFU/100 cm² (Warriner et al., 2002). Even with some removal and death of microorganisms with singeing and scalding, many times the carcasses were re-contaminated due to the polishers, scrapers, and other processing equipment (Gill, 2005). Both of these studies demonstrate that polishers, dehairers, singeing, and washing carcasses do not always lower bacterial levels, but stay the same due to cross-contamination. The scald tank can reduce bacterial numbers by the high
temperature in the tank (62 degrees Celsius), but it all depends upon maintaining that high temperature (Gill, 1998, 2004). All of these factors could be a potential reason why pork shelf life may be shorter than beef shelf life (Blixt & Borch, 2002). Pork could be exposed to more contamination due to the differences in slaughter technique, by leaving the hide on and using scald tanks, dehairers, and polishers compared to beef and lamb where the hide is removed and all possible contamination with the hide, which could cause a shorter shelf life.

Line speed

The line speed of slaughter operations can also have an effect on the amount of bacterial contamination per carcass. With slower line speeds, workers can take more time to carefully trim and remove the hide and any fecal contamination that they may not be able to do as well at faster line speeds. Bell (1997) supported this theory by testing bacterial contamination levels of carcasses with two different line speeds of 160 head/day and 440 head/day. He found that the lower line speed of 160 head/day had lower bacterial contamination levels than the faster line speed. Typical line speed in America is about 100 carcasses per hour for cattle, and around 200 carcasses per hour for swine (Gill, 2009). In addition, depending on the plant size there could be one worker preforming one task or many, or many workers performing only one task of the slaughter line (Gill, 2009). This can affect bacterial contamination because workers with many tasks will not take as great of care to complete each task as they would if they only had the one task to complete. Operator fatigue, knife skills, length of the working day, and levels of boredom of the worker(s) can also affect bacterial contamination with line speed (Sheridan, 1998).
If the workers are bored, fatigued, or have a long work day they may not be as focused on their task so they may miss any visible contamination on a carcass. On the other hand, if they are not fatigued, not bored, or have a short work day, they may be more focused on their task so they will remove any visible contamination. In addition, if a worker has great knife skills, they will easily remove and catch any visible contamination from a carcass without cutting away too much product, whereas poor knife skills may not remove all the visible contamination.

Carcass interventions

Another factor that can affect contamination are the types and processes of carcass interventions used. These carcass interventions can be knife trimming, hot water washing, organic acid washes, steam vacuums, or other antimicrobial compounds (Aberle et al., 2012; Bacon, 2005; Bell, 1997). These carcass interventions are employed to limit contamination by reducing bacterial numbers as well as comply with the USDA’s zero tolerance policy for any fecal or ingesta contaminants on a carcass (Aberle et al., 2012; Geornaras & Sofos, 2005; Jay et al., 2005). In order to reduce contamination on a carcass, these carcass interventions are generally applied pre-slaughter, after hide removal, and after evisceration. These points in the slaughter process are most prone to contamination from the hide or intestinal tracts (Dickson, 1995; Sheridan, 1998). Knife trimming is used mostly for small areas of contamination, but it can also be for large areas if need be. Its effectiveness depends largely upon the worker, such as are they consistent, are they tired, how long until shift end, their training, their skill level, sanitary status of the knives, and the assumption that the contamination is visible (Bacon, 2005; Geornaras & Sofos, 2005).
Gill and Landers (2004) found that knife trimming of visibly contaminated areas on a pork carcass did not reduce the number of aerobes, but it could reduce the number of E. coli and coliforms by 1.5 log units. However, trimming remained ineffective for reducing bacterial numbers (aerobes, E. coli, or coliforms) on other parts of the pork carcass, visibly contaminated or not. This is why other carcass interventions like hot water washing, steam vacuums, organic acid washes, and antimicrobial compounds are employed to give a “hurdle” approach to attack the contamination from many angles. Steam vacuums are used to augment knife trimming, but they are only meant for small areas of contamination. Furthermore, steam vacuums are only good as the worker and whether they see the contamination and take the time to remove it properly without embedding the bacteria deeper (Bacon, 2005; Geornaras & Sofos, 2005; Phebus et al., 1997). It is also a stationary, fixed system, so recontamination cannot be guarded against. Hot water washes are effective at removing bacteria, but they can also redistribute the bacteria on the carcass (Bacon, 2005; Bell, 1997; Gill, 2004). Many times hot water washes are used in cabinet systems to ensure a more even distribution of the hot water and that the surfaces of the carcasses reach the appropriate temperature. If the water is not at 80-85°C or greater, bacteria will only be redistributed to other areas of the carcass and not killed (Aberle et al., 2012; Sheridan, 1998). In addition, it is possible that not every part of the carcass is being sprayed with hot water, leading to increased contamination in areas that were missed or redistributed. Despite many of the issues with hot water washing, it is still a very common practice in the meat industry and is effective at killing bacteria, getting up to 2 log reductions (Bacon, 2005; Sheridan, 1998). To help combat the issues with hot water washing, many times organic acids, such as citric, acetic, or
lactic acid are incorporated into the washing cabinets, particularly after hide removal and evisceration (Aberle et al., 2012; Belk, 2001; Gill, 2009). These organic acids are used at a concentration of 1.5-2.5% and are sprayed at a temperature of 55°C to improve their effectiveness at killing bacteria, attaining log 2-3 reductions for aerobic organisms and 1-3 logs for E. coli (Acuff, 2005; Geornaras & Sofos, 2005; Gill, 2009). Another hurdle for bacteria is the use of antimicrobial chemicals such as lactoferrin, chlorine, acidified sodium chlorite, or trisodium phosphate (Aberle et al., 2012; Acuff, 2005). These antimicrobial chemicals work in different ways to either kill bacteria, inhibit their attachment to meat, or remove them from the carcass. They can be fairly effective if applied in the right conditions, as per manufacturer instructions, but they are generally more effective if used in conjunction with other carcass interventions. No matter what carcass intervention is used, they all reduce bacterial contamination in some way, which then affects the final contamination on fresh meat products (Bacon et al., 2000; Belk, 2001).

Meat Packaging Methods

Characteristics and environments

Aerobic

The most common type of packaging method for fresh meat is aerobic packaging or overwrap packaging. Many times the fresh meat product is placed on a Styrofoam or plastic tray with an absorbent pad underneath to collect any purge before its wrapped in a plastic film (Delmore, 2009).
The environment for an overwrap or aerobic package is very dynamic. It has an increased oxygen content due to the plastic film being very permeable to oxygen and moisture. Since this film is very permeable, oxygen, nitrogen, and carbon dioxide pass through the film at different rates (Gill, 1996). The spoilage organisms and the fresh meat product itself continue respiration throughout shelf life, so they are consuming oxygen and producing carbon dioxide (Gill, 1996). Nitrogen will also enter the package over time through the packaging film. Therefore, all of these effects combine to change the atmosphere throughout the shelf life, making it dynamic.

**Vacuum-packaging**

Vacuum-packaging is a very common practice in the fresh meat industry and has been used for over 35 years to extend the shelf life of fresh meat products. Vacuum-packaging requires a few elements for it to work effectively. The first element is the use of boneless fresh meat cuts because bones can puncture a vacuum package and render it useless (Gill, 1996). However, there are vacuum packages made to withstand bones and reduce punctures. The second element is a plastic pouch that has an oxygen gas permeability of 100 cm³/m²/atm/day or lower to ensure a longer shelf life (Lambert, Smith, & Dodds, 1991; Rao & Sachindra, 2002). If the film permeability for oxygen is 0 mL/m²/24h/atm at 25°C and 100% relative humidity, a shelf life of 15 weeks can be obtained but if it is increased to 818 mL/m²/24h/atm get only 4-6 weeks of shelf life (Newton & Rigg, 1979). This demonstrates the importance of selecting a low oxygen gas permeable film to obtain a longer shelf life. In addition, a low transmission rate of carbon dioxide is preferred, but many times the carbon dioxide transmission rate through the
plastic film is two to five times greater than oxygen (Jay et al., 2005). These low oxygen gas transmission rates, such as less than 15.5 mL/m²/24h at 1 atm, can be achieved by using a three-layered co-polymer plastic film of ethyl vinyl acetate, polyvinylidene chloride, and ethyl vinyl acetate again (Zhou, Xu, & Liu, 2010). There are also other types of plastic barriers made up of different compounds to give different properties, such as ethyl vinyl alcohol, polyethylene, or nylon (Aberle et al., 2012). Another element is that the air must be evacuated from the bag, usually by a vacuum in the range of 10-745 mm Hg (Gill, 1986; Jay et al., 2005). This vacuum can generally remove almost all of the air from the package creating a very small headspace, but a small amount of air remains for it is impossible to remove all the air from a package (Delmore, 2009; Lambert et al., 1991). In addition, it is important to select a package with as few of vacuities as possible to help minimize air re-entering the package and remaining there (Jeremiah, 2001). The package is then heat sealed to ensure that no oxygen can re-enter the bag in large amounts. Oxygen re-entering the vacuum package will cause a decrease in shelf life due to oxidation, increased aerobic bacterial growth, and color deterioration. A vacuum package can also be heat shrunk to create a tighter appearance of the package and the product within, by placing the package into a water bath of 80-90°C (Jay et al., 2005).

The environment inside a vacuum-package changes throughout the shelf life, with the greatest changes occurring within hours after sealing. At the time of packaging, the atmosphere is slightly aerobic, despite most of the air being evacuated by the vacuum applied. However, in a few hours after the package is sealed and throughout the end of its shelf life, the environment is anaerobic (Aberle et al., 2012; Gill, 1986). This anaerobic atmosphere is accomplished by the cellular respiration of the bacteria present and by the
fresh meat product itself (Enfors, Molin, & Ternstrom, 1979; Jay et al., 2005; Lambert et al., 1991), which converts the remaining oxygen in the package to carbon dioxide. This process results in a 10-20% carbon dioxide concentration inside the vacuum package within four hours, and then ultimately a 30% concentration or greater throughout the rest of the shelf life (Enfors et al., 1979; Jay et al., 2005; Jeremiah, 2001; Lee, Simard, Laley, & Holley, 1985; Rao & Sachindra, 2002). Despite the abundant carbon dioxide concentration, the vacuum package is not completely free of oxygen, around 1% or 100 ppm remains throughout the shelf life (Dainty & Mackey, 1992; Gill, 1996; Jeremiah, 2001; Nissen, Sorheim, & Dainty, 1996). This 1% oxygen level persists due to the gas transmission properties of the plastic film used. Oxygen is free to travel through the packaging film so it can penetrate inside the package. This gas transmission rate can be reduced by choosing a packaging film with a very low oxygen gas transmission rate, especially an oxygen impermeable one (Newton & Rigg, 1979). This oxygen transmission rate can also be increased by choosing a more oxygen permeable plastic film (Borch, Kant-Muermans, & Blixt, 1996). Therefore, the environment will change slightly over time due to the respiration of the bacteria and meat product, as well as the gas transmission rates of the package, but will remain anaerobic in nature and select for more anaerobic bacteria (Cayre, Garro, & Vignolo, 2005).

**Modified atmosphere packaging**

Modified atmosphere packaging (MAP) is another common method of packaging fresh meat products, particularly in the retail display case. A MAP has three defining characteristics: a packaging film with low gas transmission rates, a larger headspace than
vacuum-packaging, and an artificial atmosphere by the addition of gases. MAP needs to have a package with low gas transmission rates, between 10-100 cc O₂/m²/24h/atm, because the atmosphere put into the package at the beginning needs to remain the same to maintain a long shelf life (Gill, 2003; Rao & Sachindra, 2002). There will be some changes to the atmosphere over time in the package, but low gas transmission rate films will greatly reduce any drastic changes in the package atmosphere (Gill, 2003; Jay et al., 2005; Kraft, 1986). A larger headspace than vacuum-packaging is required because the atmosphere is artificially altered by the packager. Enough gas needs to be added to maintain a good product appearance and integrity, which Jeremiah (2001) found could be at a product ratio of 3:1 (three parts of gas mixture to 1 part product). Furthermore, enough gas needs to be added to take into account the changes in the atmosphere that will happen throughout the shelf life. Finally, the last defining characteristic of MAP is the artificial atmosphere added to the package. There are three gases commonly added to a MAP package: oxygen, carbon dioxide, and nitrogen. Oxygen is a crucial part of a MAP package because it determines what type of MAP package is created: high oxygen or low oxygen (Jay et al., 2005). A high oxygen MAP has 80% O₂ and 20% CO₂, and the 80% oxygen maintains the bright red color that consumers associate with fresh meat (Lambert et al., 1991; Sorheim, Nissen, & Nesbakken, 1999). Low oxygen MAP has <10% O₂, 20-30% CO₂, and 60-70% N₂, and it maintains the red color of the fresh meat product, but not as long as the high oxygen MAP would (Jay et al., 2005). Carbon dioxide is added to retard microbial growth and is usually added at a concentration of 20-30% (Delmore, 2009; Dixon & Kell, 1989; Sorheim et al., 1999). Lastly, nitrogen is added merely as a “filler” to maintain product integrity and shape, for nitrogen has no effect on
A modified atmosphere package is created in a similar fashion as a vacuum-package. A fresh meat product is placed into a plastic tray with plenty of headspace for that size of a product. A vacuum is then applied to remove the previous atmosphere and then a gas mixture of the packager’s choosing will be pumped into the package to fill up the headspace (Delmore, 2009; Stanbridge & Davies, 1998). After the gas mixture is added, the package film is heat-sealed on all the edges to seal the package.

The environment in a modified atmosphere package (MAP) is dependent upon the gas mixture applied at the start of packaging. However, the gas mixtures will change over the shelf life due to gas leakage through the plastic film, bacterial cellular respiration, and cellular respiration of the fresh meat product. The common gases added to a MAP package are oxygen, carbon dioxide, and nitrogen. Carbon monoxide can be added to a MAP package in 0.5% concentration to help maintain the bright red color, but is currently not allowed in the United States or European Union, and it does not inhibit microorganism growth in any way (Aberle et al., 2012; Sorheim et al., 1999). Since carbon monoxide cannot be used to maintain the bright red color consumers associate with fresh meat, oxygen is used. It is used in two amounts generally, high oxygen and low oxygen. High oxygen MAP has around 80% oxygen while low oxygen has less than 10% (Zhou et al., 2010). These levels will decrease due to cellular respiration of the bacteria and fresh meat product, and transmission of oxygen through the packaging film (Seideman, Carpenter, Smith, Dill, & Vanderzant, 1979). Sorheim et al. (1999) found that a MAP with 70% oxygen decreased to 60-65% after 21 days of storage. Seideman et al.
(1979) found that a MAP with 100% oxygen will decrease to 50% oxygen and carbon dioxide will increase to 44%, and a MAP with 80% oxygen and 20% carbon dioxide will change to 74% oxygen and 21% carbon dioxide after 35 days.

Carbon dioxide is added to help retard microbial growth and to extend shelf life (Gill, 1996; Rao & Sachindra, 2002). It can be added in any amount desired, but usually it is limited to a concentration of 30% to help reduce package collapse and avoid product discoloration (Jeremiah, 2001). In addition, this 30% carbon dioxide concentration added at the beginning of the shelf life will not remain at 30%. Carbon dioxide is very soluble in fresh meat at refrigeration temperatures, so the gas concentration will decrease slightly due to its solubility (Ercolini, Russo, Torrieri, Masi, & Villani, 2006). However, the carbon dioxide levels will also increase throughout the shelf life because of the cellular respiration by the bacteria and meat product (Jeremiah, 2001). Lastly, nitrogen is added as a “filler” to maintain the product appearance and package integrity (Rao & Sachindra, 2002). Nitrogen is an inert gas that will not react with the meat or the microorganisms present, so it maintains the same concentration throughout the shelf life (Zhou et al., 2010). Therefore, the amount and mixture of atmospheric gases added at the beginning of the shelf life of a fresh meat MAP will change over time due to cellular respiration and gas transmission through the packaging film.

**Controlled atmosphere packaging**

Controlled atmosphere packaging (CAP) is similar to vacuum-packaging, but the main differences are the type of plastic films used and the no-change atmosphere. The process to make a controlled atmosphere package is the same as vacuum-packaging. The
fresh meat product is placed into a pouch, a vacuum is applied, and then the package is heat-sealed. However, controlled atmosphere packaging works on the principle that the atmosphere does not change throughout the shelf life, or changes very little (Gill, 2003; Rao & Sachindra, 2002). In order for that to happen, the plastic films used need to be gas impermeable. To make plastic films gas impermeable, they include foil laminates, thick layers of plastic, or some type of metallized packaging, such as aluminum (Gill, 2003; Jeremiah, 1997, 2001; Jeremiah, Gibson, & Argnosa, 1995). Since these films include foil laminates or aluminum, they are opaque films, which is not favored by consumers (Gill, 2003). Consumers like to see the product they are buying and the opaque films of CAP make that difficult. For this reason, CAP is not very common in retail outlets and is used mostly in distribution to stores and warehouses (Jeremiah, 2001).

Controlled atmosphere packaging has a more static environment than the other meat packaging types. Controlled atmosphere has a completely anaerobic environment, much like vacuum-packaging (Gill, 2003). It has an increased carbon dioxide content with less than 0.05% oxygen, and these gas concentrations will stay the same throughout shelf life (Jeremiah, Gibson, & Argnosa, 1995). They stay the same because controlled atmosphere uses completely gas impermeable packaging film with foil laminates. This film keeps gas transmission to almost nothing, so the environment does not change or changes very little over the shelf life.
Common Spoilage Bacteria

Influence of packaging type

Aerobic

Aerobic or overwrap packages have a dynamic, high-oxygen environment, so the common geneses found in this package are mostly aerobic microorganisms, with some facultative anaerobic microorganisms. The first common and dominant genus, making up 50-90% of the microflora in overwrap packages is *Pseudomonas*, with the dominant species being *P. fragi*, but *P. fluorescens, P. lundensis*, and *P. putida* can also be found (Dainty & Mackey, 1992; Gill, 1986; Gram et al., 2002; Jay et al., 2005; Kraft, 1986; Nowak, Rygala, Oltuszak-Walczak, & Walczak, 2012; Nychas et al., 2008; Samelis, 2006). The next common genus found is *Brochothrix*, particularly *Brochothrix thermosphacta* (Borch et al., 1996; Ercolini et al., 2011; Nychas et al., 2008; Pennacchia, Ercolini, & Villani, 2011). After *Brochothrix*, the Enterobacteriaceae family can be found, such as *E. coli, Hafnia alvei, Serratia liquefaciens, S. grimesii, S. marcescens, Enterobacter agglomerans, Rahnella, Shewanella putrefaciens*, and many more minor species (Borch et al., 1996; Doulgeraki, Ercolini, Villani, & Nychas, 2012; Ercolini et al., 2011; James & James, 2002; Nychas & Skandamis, 2005; Stiles & Ng, 1981). Lactic acid bacteria can also be found in aerobic packages, but generally at low levels because they are more anaerobic microorganisms. Other geneses such as *Aeromonas, Acinetobacter, Moraxella, Micrococcus*, and many others can also be found in overwrap packages, but they generally do not dominate the microflora. Furthermore, some geneses will be dominant in the beginning of the shelf life, but will fade by the end of shelf life because
the microflora is not static, it is always changing throughout the shelf life of a fresh meat product.

**Vacuum-packaging**

In a vacuum-package, the environment starts out aerobic before becoming anaerobic, so more anaerobic and facultative anaerobic geneses can be found in this package type. In the beginning of shelf life for a vacuum-packaged product, *Pseudomonas* can be found, up to 88.8% of the total microorganism population, but over 70 days and increasing carbon dioxide concentrations that decreases to 5.0% (Lee et al., 1985). As *Pseudomonas* decreases, lactic acid bacteria become the dominant genus and family and make up 88.5% of the microorganism population at the end of the shelf life (Enfors et al., 1979; Lambert et al., 1991; Lee et al., 1985). The most common species of lactic acid bacteria found in vacuum-packaging are *Lactobacillus carnis*, *L. divergens*, *L. sakei*, *L. algidus*, *Carnobacterium divergens*, *C. piscicola*, *Leuconostoc* spp., *L. gelidum*, and *L. mesenteroides* (Ahn & Stiles, 1990; Borch et al., 1996; Doulgeraki et al., 2012; Garcia-Lopez, Prieto, & Otero, 1998; Gill, 1996; Gram et al., 2002; Holley, M.D. Pierson, J. Lam, & K. B. Tam, 2004; Holzapfel, 1998; Jay et al., 2005; Knox, Laack, & Davidson, 2008; Newton & Gill, 1978; Nissen et al., 1996). After lactic acid bacteria, Enterobacteriaceae and *Brochothrix thermosphacta* can be found (James & James, 2002). For Enterobacteriaceae, some common species can be *E. coli*, *Serratia grimseii*, *S. liquefaciens*, and *Hafnia* spp. (Ercolini et al., 2011; Kraft, 1986; Labadie, 1999; Russo, Ercolini, Mauriello, & Villiani, 2006; Stiles & Ng, 1981). The microorganisms found in
vacuum-packages are not limited to these few geneses and families, for others can be found, but they are generally a minor part of the microflora and do not cause spoilage.

**Modified atmosphere packaging**

In a modified atmosphere package, the typical geneses found in the package varies according to the type of MAP used: high oxygen or low oxygen. In high oxygen MAP, the microorganisms are similar to an overwrap package, so the most common and dominant genus is *Pseudomonas* (Christopher, Vanderzant, Carpenter, & Smith, 1979; Gill, 1986; Stanbridge & Davies, 1998). *Pseudomonas* is then followed by lower concentrations of lactic acid bacteria, *B. thermosphacta*, and Enterobacteriaceae (Borch et al., 1996; Christopher et al., 1979; Garcia-Lopez et al., 1998). In low oxygen MAP, where the oxygen level is less than 10%, the microorganisms will be similar to a vacuum-package with more facultative anaerobic and anaerobic microorganisms. Therefore, the common geneses in low oxygen MAP are lactic acid bacteria (LAB), Enterobacteriaceae, and *B. thermosphacta* (Ercolini et al., 2006; Garcia-Lopez et al., 1998; Gill, 2003; Jay et al., 2005; Kraft, 1986; Kreyenschmidt et al., 2010; Nychas et al., 2008). The species of LAB can be *L. alimentarius, L. farcininis, L. sake, L. curvatus, L. plantarum, Carnobacterium divergens, C. maltaromaticum*, and a few other minor species (Doulgeraki et al., 2012; Labadie, 1999; McMullen & Stiles, 1993; Stanbridge & Davies, 1998). Enterobacteriaceae species can be *E. coli, Hafnia alvei, Serratia spp., S. liquefaciens, S. grimesii, S. proteamaculans, Enterobacter agglomerans, Rahnella, Weisella hellenica*, and many other minor species (Doulgeraki et al., 2012; Ercolini et al., 2006; Sade, Murros, & Bjorkroth, 2013; Stiles & Ng, 1981). The microorganisms present
can also be affected by the oxygen permeability of the packaging film, where if it is more permeable, more *Pseudomonas* and *B. thermosphacta* will be present due to the increased oxygen content. All these microorganisms change in dominance over the shelf life of the MAP package with some succeeding others or becoming a minor part as the environment changes over time.

**Characteristics of spoilage bacteria**

**Psychrotroph**

Psychrotrophs encompass are large group of microorganisms, typically those that can grow well at or below 7 degrees Celsius but have their optimum growth range between 20-30 degrees Celsius (Jay et al., 2005; Lawrie & Ledward, 2006). These microorganisms can be Gram-positive, Gram-negative, spore formers or not, motile or non-motile. Typical psychrotroph genera are *Corynebacterium*, *Microbacterium*, *Pseudomonas*, *Carnobacterium*, *Brochothrix*, Enterobacteriaceae, *Lactobacillus*, and lactic acid bacteria (Kraft, 1986).

**Pseudomonas**

The *Pseudomonas* genus contains Gram-negative, aerobic, and rod-shaped microorganisms. They grow rapidly at refrigeration temperatures (0-7°C), despite their optimum growth temperature at 20°C. If temperature is the only factor affecting the major population on a piece of fresh meat, *Pseudomonas* has a growth rate 30% faster than other microorganisms that could grow in the same atmosphere and temperature (McMeekin & Ross, 1996a). Due to this fast growth rate, they utilize a large amount of
glucose before switching to compounds like amino acids and lactate (Bruckner, Albrecht, Petersen, & Kreyenschmidt, 2012; Garcia-Lopez et al., 1998; Gill, 1976; C.O. Gill & K. G. Newton, 1977; Jay et al., 2005). Once they start consuming other metabolites besides glucose, they start to produce off-odors and compounds associated with spoiled fresh meat products, such as dimethysulfide, ammonia, cadaverine, and putrescine (C.O. Gill & K. G. Newton, 1977). However, *Pseudomonas* is affected greatly by the concentration of carbon dioxide, particularly if it is above 20%, and that inhibits their growth considerably (Gill, 1986, 2003; Jay et al., 2005; Sorheim et al., 1999). They cannot compete as well in an anaerobic environment, so they do not become the major component of the spoilage flora in anaerobic packaging conditions, but they can still be present. Therefore, *Pseudomonas* is more commonly found on aerobic packages, like overwrap packages or high oxygen MAP, particularly the species, *P. fragi*, followed by *P. ludensis* and *P. putida* (Garcia-Lopez et al., 1998; Labadie, 1999).

**Acinetobacter/Moraxella**

The *Acinetobacter/Moraxella* genus contains Gram-negative, aerobic microorganisms. They are widely found in soil, water, and on some foods, especially refrigerated fresh products (Jay et al., 2005). They do not use glucose, but they will use amino acids for their source of nutrients (Garcia-Lopez et al., 1998; Gill, 1986). However, they do not cause spoilage of food products because they do not make many malodorous compounds and so have a low spoilage potential.
Alteromonas (Shewanella) putrefaciens

*Alteromonas putrefaciens* is now known as *Shewanella putrefaciens* and is a Gram-negative rod that is facultative anaerobic. It cannot grow in fresh meat products below a pH of 6.0, so it is limited to higher pH meats, like dark, firm, and dry (DFD) meat (Gill, 1986). However, when it can grow, it uses amino acids like cysteine and cystine to produce hydrogen sulfide (H$_2$S) and other organic sulfides, which are common malodorous compounds associated with spoilage (Garcia-Lopez et al., 1998; Samelis, 2006). The H$_2$S produced can combine with myoglobin in meat to form sulfmyoglobin, the green pigment formed during spoilage that is responsible for “greening” of meat products (Garcia-Lopez et al., 1998; Lambert et al., 1991). Therefore, *S. putrefaciens* does have the capability to spoil fresh meat products, but it must have a pH greater than 6.0.

Brochothrix thermosphacta

*Brochothrix thermosphacta* is a Gram-positive, facultative anaerobic, non-spore forming rod microorganism (Gill, 1986; Jay et al., 2005). It can grow on fresh meat products quite readily because it can handle the more acidic pH, but it does tend to grow better with a pH greater than 5.8 (Knox et al., 2008). *B. thermosphacta* also grows on fresh meat products because it can grow at temperatures between 0-30 degrees Celsius, which encompasses the refrigeration temperatures (Jay et al., 2005; Nowak et al., 2012). This microorganism first utilizes glucose before switching to amino acids, and it produces acetic acid, acetoin, diacetyl, 3-methylbutanol, propionic acid, and isovaleric and isobutyric acids that help make up some of the off-odors associated with spoilage.
Despite utilizing glucose, *B. thermosphacta* has a slower growth rate than *Pseudomonas*, so in increased oxygen atmospheres (above 20%), it cannot compete well against *Pseudomonas* (Jay et al., 2005; McMullen & Stiles, 1993). *B. thermosphacta* is also influenced by oxygen film permeability, so if there is a increased gas transmission rate, *B. thermosphacta* will grow faster than under a low gas transmission rate (Cayre et al., 2005; Holley et al., 2004). In addition, *B. thermosphacta* is inhibited by carbon dioxide concentrations greater than 20%, so it can grow in anaerobic environments, just not as well (Lambert et al., 1991; Sorheim et al., 1999). *B. thermosphacta* is also inhibited by lactic acid, so lactic acid bacteria will typically outgrow *B. thermosphacta* (Grau, 1980; Jay et al., 2005; Lambert et al., 1991; Nowak et al., 2012). Despite these growth limitations, *B. thermosphacta* can be still found on many fresh meat products, and even though it is not the main spoiler microorganism in some conditions, it can still contribute by being able to grow on the product.

**Lactic acid bacteria**

Lactic acid bacteria (LAB) is a family of rod-shaped, Gram-positive microorganisms that are anaerobic and/or aerotolerant that grow at less than 20 degrees Celsius. Since they are aerotolerant and anaerobic, carbon dioxide does not inhibit their growth rate. They are also not influenced by oxygen film permeability, so they are commonly found in low oxygen MAP and vacuum-packages (Cayre et al., 2005; Kreyenschmidt et al., 2010; Lambert et al., 1991). LAB can be either homofermentative
or heterofermentative as they utilize glucose (Gill, 1976; McMullen & Stiles, 1993).
Homofermentative LAB produces greater than 85% lactic acid and heterofermentative
LAB produces lactic acid, carbon dioxide, ethanol, and acetic acid (Hammes & Hertel,
2006; Samelis, 2006). This lactic acid production makes LAB aciduric and it can inhibit
other microorganisms, preserve fresh meat products, and cause the typical “sour, acid”
spoilage typical of vacuum-packages (Borch & Agerham, 1992; Jay et al., 2005;
Koutsoumanis, Stamatiou, Skandamis, & Nychas, 2006; Kreyenschmidt et al., 2010;
Zhou et al., 2010). LAB can also produce bacteriocins, which are proteins and protein
complexes that inhibit closely related Gram-positive microorganisms (C. O. Gill & K. G.
Newton, 1977; Klaenhammer, 1988; Lambert et al., 1991). These can either be
bacteriostatic or bacteriocidal and the type produced depends on pH, temperature, and
presence of other microorganisms and metabolites such as lactic acid and hydrogen
peroxide (Ahn & Stiles, 1990). LAB have also been known to produce hydrogen sulfide
and hydrogen peroxide under reduced glucose concentrations, which can also inhibit
bacteria (Borch & Agerham, 1992).

**Enterobacteriaceae**

The family of Enterobacteriaceae consists of Gram-negative facultative anaerobic
spoilage microorganisms. They tend to grow better in vacuum-packaged fresh meat
products with a pH around 6.0 (Knox et al., 2008). Enterobacteriaceae utilize glucose
before they utilize amino acids, but once they do start utilizing amino acids, they release
compounds like ammonia, hydrogen sulfide (H\(_2\)S), amines, and other sulfides (Garcia-
Lopez et al., 1998; Gill, 1986; Samelis, 2006). These compounds are some of the typical
off-odors associated with spoiled fresh meat. Some of the typical genera and species within the family of Enterobacteriaceae found on fresh meat products are *Escherichia coli*, *Hafnia alvei*, *Enterobacter agglomerans*, *E. aerogenes*, *Rahnella*, *Serratia liquefaciens*, *S. marcescens*, *Proteus vulgarius*, and *P. mirabilis* (Ercolini et al., 2006; Garcia-Lopez et al., 1998; Russo et al., 2006; Sade et al., 2013; Samelis, 2006).

**Carnobacterium**

The *Carnobacterium* genus contains Gram-positive, facultative anaerobic microorganisms that can grow at 0 degrees Celsius and are common in vacuum-packaged and modified atmosphere packaged fresh meat products (Jay et al., 2005; McMullen & Stiles, 1993). They have a metabolism and behavior similar to heterofermentative lactic acid bacteria, by making carbon dioxide and lactic acid from glucose. Many times they are isolated with the lactic acid bacteria from fresh meat products, and the typical species are *C. divergens*, *C.pisicola*, *C. gallinarum*, *C. mobile*, and *C. inhibens* (Hammes & Hertel, 2006).

**Indicators of spoilage bacteria presence**

There are a few methods of indicating whether or not the bacteria mentioned above are present or not, and how many there are. These methods usually involve Enterobacteriaceae, coliform, fecal coliform, and *Escherichia coli* counts. These counts indicate whether or not a fresh meat product has been contaminated directly or indirectly, especially with visceral contents (Grau, 1986; Stiles & Ng, 1981). Some other methods
include the traditional aerobic plate count and total plate count to get a general idea of how many bacteria are present on a fresh meat product.

Meat Spoilage

Definition

Since fresh meat products can be easily contaminated with bacteria and are highly perishable, they are prone to spoilage. Spoilage can have several different meanings because spoilage is a subjective evaluation. It is subjective because it is based upon the first symptoms of spoilage, which can vary from person to person (James & James, 2002). Due to its subjective nature, fresh meat spoilage will be defined as any single symptom or group of symptoms of overt microbial activity that makes the meat product unfit for human consumption (Doulgeraki et al., 2012; Gill, 1986; Huis in't Veld, 1996; Nowak et al., 2012). Symptoms of fresh meat spoilage could be physical changes, chemical changes, or appearance of off-odors and off-flavors (Aberle et al., 2012; Ellis & Goodacre, 2001; Gram et al., 2002). Physical changes could be slime production, visible growth of microorganisms, or textural changes. Color changes or oxidation in the fresh meat product are possible chemical changes. Color changes occur because as fresh meat spoils, peroxides are formed by the spoilage bacteria which can cause the myoglobin in meat to change from bright red to metmyoglobin, a brown color, and sulfmyoglobin (when combined with hydrogen sulfide), a green color (Aberle et al., 2012; Lawrie & Ledward, 2006). However, the appearance of off-odors and off-flavors are the notable symptoms of spoilage. These off-odors and off-flavors are the accumulation of microbial growth by-products on the fresh meat products, and some common compounds are
aldehydes, ketones, esters, alcohols, organic acids, amines, and sulfur compounds (Ercolini et al., 2011). These compounds are made when the bacteria have finished utilizing glucose and have started to consume amino acids, and this will be discussed in detail later. In addition, these compounds can combine to form off-odors associated with spoilage, which are “sour/acid” or “buttery” or “cheesy” off-odors, and these off-odors are discussed later also (Ercolini et al., 2006). Despite what symptoms or off-odors develop due to overt microbial activity, the fresh meat product will be unfit for human consumption and be deemed “spoiled”.

**Numerical value**

Despite having a general definition, the spoilage threshold also has a numerical value. This numerical value is generally considered at log 7 CFU/g or cm² because by the time the spoilage bacteria have reached this concentration, they are actively producing detectable off-odors and off-flavors (Garcia-Lopez et al., 1998; Jay et al., 2005; Knox et al., 2008). Therefore, spoilage defects can be seen. At greater values, such as log 8 CFU/g, slime formation starts to begin (Ayres, 1960; Jay et al., 2005; Kraft, 1986; Lawrie & Ledward, 2006), and at log 9 CFU/g, spoilage defects become rapidly pronounced and the fresh meat is considered putrid (Ingram & Dainty, 1971). Hence, the numerical value of log 7 CFU/g or cm² is the agreed upon value by many researchers for the spoilage threshold because it is before the clearest symptoms of spoilage are evident, such as slime formation or off-odors.
**Surface phenomenon**

Meat spoilage is generally considered a surface phenomenon because the spoilage bacteria do not penetrate very deeply into the meat surface (Gill, 1976; Gill, 1986; Ingram & Dainty, 1971; Kraft, 1986). This is because most of the nutrients and oxygen availability are better at the surface than the deeper tissues of meat (Aberle et al., 2012). In addition, fresh intact muscle is generally considered sterile so unless bacteria are introduced manually into the deeper tissues, they remain on the surface.

**Spoilage composition**

The type of spoilage that develops for fresh meat products can have varying compositions. Not all fresh meat products spoil the same, especially when it comes to the type of spoilage flora that develops. Intrinsic or extrinsic factors all have an effect on the spoilage composition that develops. Intrinsic factors of the fresh meat products, such as species, pH, glucose concentrations, and water activity, can cause different types of spoilage flora to develop based upon changes in these areas (Blixt & Borch, 2002; Jay et al., 2005; Koutsoumanis et al., 2006; Labadie, 1999; McDonald & Sun, 1999; Zhou et al., 2010). Extrinsic factors could be packaging conditions, oxygen concentrations, carbon dioxide concentrations, the original number of bacteria present, storage temperature, light intensity, and many other factors can all have an effect on the type of spoilage that develops (Gill, 1996; Ingram & Dainty, 1971; Jeremiah, 1997; Knox et al., 2008; Kraft, 1986; Nychas et al., 2008; Samelis, 2006). The following sections will discuss some of the factors that can influence the spoilage composition, such as the conversion process of...
Conversion of muscle to meat

After animals are slaughtered, their muscles begin the process of converting into meat, and this process can have effects on the spoilage composition. After animals die, there is no more blood circulation and ATP is minimally replenished, and lactic acid is no longer removed from the muscles via the bloodstream (Jay et al., 2005; Lawrie & Ledward, 2006), so this results in a different metabolism than normal, live muscles. Despite the death of the animal, the muscles continue to perform glycolysis, fueled by the glycogen stores in the muscles (Aberle et al., 2012; Gill & Newton, 1982). However, since the animal is no longer alive and breathing, oxygen is not supplied to the tissues, so the pyruvate made at the end of glycolysis cannot be burned to make ATP in the following TCA/Krebs cycle. Without the production of many moles of ATP, the muscle can no longer relax all the bonds formed between actin and myosin, so they remain bonded, forming a long-lasting adenosine cross-bridge known as rigor mortis (Lawrie & Ledward, 2006). As the TCA cycle remains non-functional, pyruvate begins to increase in concentration and the muscles are depleting their supply of NADH to continue glycolysis. This molecule, NADH, is in limited quantities and when quantities are low, NADH is oxidized back to NAD+ by dropping off the extra electron and hydrogen proton onto another molecule, reducing that molecule. The molecule that is abundant to be reduced is pyruvate, forming lactate. The muscles continue to reduce pyruvate to form lactate in order to keep the amount of free NAD+ available for glycolysis to continue to
make ATP (Aberle et al., 2012). As lactate concentrations increase, they start to build-up in the muscles and under normal, live muscles, this lactate would be removed by the bloodstream to the liver and heart to be used for other energy production processes (Aberle et al., 2012). However, the bloodstream has stopped circulating, so lactate continues to build up in the muscles. As lactate concentrations increase, so does the concentration of free hydrogen ions because the buffering capacity of the muscles will be reduced due to the increase in lactate and decrease in ATP production. Therefore, lactic acid concentrations increase (lactate plus a hydrogen ion) and they begin to lower the resulting internal pH of the muscles from the original value of around 7.0 (Lawrie & Ledward, 2006). This pH decline is fueled by the glycogen concentrations in the muscles, so as long as glucose or glycogen can be utilized for glycolysis, lactic acid will be produced. Glycolysis will continue to function in this manner until the pH of the muscles reaches 5.5-5.8. At this pH, the supply of NAD+ and ATP will be exhausted and the pH is low enough to denature the glycolytic enzymes. After the enzymes stop due to lack of glycogen and the acidic pH, the internal muscle pH stabilizes at 5.5-5.8 and the muscle is fully converted into meat around 24 hours post-mortem (Aberle et al., 2012; James & James, 2002). This reduction in pH helps limit some bacterial growth, so not all bacteria can grow on the meat, which determines a spoilage composition of bacteria that can grow in the 5.5-5.8 pH range (C. O. Gill & K. G. Newton, 1977; James & James, 2002).

The lactic acid pH decline outlined above is for normal muscles, but the pH decline can be influenced by stress in the live animal. If the animal was stressed for a long time, such as throughout its lifespan before being slaughtered, it will have lower glycogen concentrations stored in the muscles than normal, unstressed animals (Gregory,
This means the pH decline post-mortem will not reduce the internal pH of the muscles to the same extent as a normal pH decline. This happens because there is less glycogen available to use for glycolysis so less lactic acid is produced. This lower lactic acid production results in a pH decline to a value of 5.8-6.0, which produces meat called “dark, firm, and dry” or DFD (Aberle et al., 2012; C. O. Gill & K. G. Newton, 1977). This pH value of 5.8-6.0 is high for meat and as a result, different bacteria will be able to grow on DFD meat, resulting in a different spoilage composition than normal meat. This spoilage composition will have a faster spoilage rate than normal because those spoilage bacteria present on DFD meat are prolific producers of off-odors (James & James, 2002), so those off-odors will arise sooner than a normal spoilage rate. The effects of DFD meat and how it relates to spoilage is discussed in more detail in the “Meat quality” section under “Methods to Control Meat Spoilage”.

On the other hand, if the animal was stressed immediately before slaughter, within a few hours, the glycogen concentrations in the muscles are burned unnecessarily due to the stress response. This causes the pH decline to begin, even before the animal’s death, so the pH decline is rapid post-mortem. This rapid pH decline drops the internal pH of the meat to 5.3-5.6 in under 6 hours, and that pH value is low for normal muscles (Aberle et al., 2012; C. O. Gill & K. G. Newton, 1977), so the type of meat developed is called “pale, soft, and exudative” or PSE (Gregory, 2007). The low muscle pH denatures the proteins so the muscle cannot hold water (exudative), has a pale color, and a soft texture. This low pH range can limit the types of bacteria that can grow on the fresh meat to aciduric or acidophilic types, resulting in a different spoilage composition than DFD.
meat. Due to these acidic conditions, these bacteria could cause a slower spoilage rate than normal meat due to a possibly slower microbial growth rate.

As it has been explained, the conversion of muscle to meat can have an effect on the spoilage composition due to the pH declines created. These pH declines would affect the types of bacteria that can grow on these meat types (DFD or PSE) that would be different from normal meat, resulting in different spoilage compositions and spoilage symptoms. For more detailed information on the different spoilage bacteria present, see the section of “Meat Quality” under “Methods to Control Meat Spoilage”.

Storage temperature

The storage temperature of fresh meat products can also influence the spoilage composition. A majority of the fresh meat products made today are stored at refrigeration temperatures, so that selects for bacteria that can grow at those temperatures (Gill, 1986), such as psychrotrophs (Kraft, 1986). Psychrotrophs grow slower than mesophiles, so psychrotrophs will have a slower rate of spoilage and will spoil the fresh meat differently than mesophiles. Psychrotrophs and mesophiles spoil fresh meat products differently because they have different spoilage bacteria present. Psychrotrophic spoilage is dominated by Pseudomonas, Enterobacteriaceae, lactic acid bacteria, Carnobacterium, and Brochothrix thermosphacta while mesophilic spoilage is mostly Acinetobacter, Buttiauxella, Serratia, and Carnobacterium (Doulgeraki et al., 2012; Ercolini, Russo, Nasi, Ferranti, & Villiani, 2009; Pennacchia et al., 2011). Carnobacterium can be found in mesophilic and psychrotrophic spoilage because of its diverse temperature growth range. These examples demonstrate that storage temperature is a factor influencing
spoilage composition (Doulgeraki et al., 2012; Nychas et al., 2008) by determining whether the spoilage bacteria are psychrotrophic or mesophilic.

Metabolites

Meat is a highly nutritious medium for bacteria, with its composition of 71-76% water, 20-22% protein, 3-8% lipid, 1.2% lactic acid, 0.9% glycogen, 0.3% glucose, and 2.3% other non-protein organic compounds (Garcia-Lopez et al., 1998; Nychas, Drosinos, & Board, 1998; Samelis, 2006). This composition is highly nutritious due to the abundance of low molecular weight compounds, such as glucose, amino acids, and nucleotides that bacteria utilize (Faucitano et al., 2010; Gill, 1986; C. O. Gill & K. G. Newton, 1977; Nychas et al., 2008). The first low molecular weight compound utilized is glucose, which can support up to 10⁸ CFU/g of bacteria despite its low concentration of 1 mg/g wet weight of muscle (Ercolini et al., 2011; Garcia-Lopez et al., 1998; C. O. Gill & K. G. Newton, 1977; Jay et al., 2005; Kraft, 1986; Newton & Rigg, 1979). After glucose, different low molecular weight compounds are consumed in different orders depending on the type of bacteria. *Pseudomonas* will consume these compounds in this order: glucose, glucose-6-phosphate, lactic acid, pyruvate, gluconate, gluconate-6-phosphate, ethanol, acetate, to amino acids while *Enterobacter* will consume glucose, glucose-6-phosphate, amino acids such as lysine, arginine, and threonine before lactic acid (Ellis & Goodacre, 2001; Nychas et al., 1998; Nychas & Skandamis, 2005). *S. putrefaciens* will consume glucose, lactic acid, pyruvate, gluconate, propionate, ethanol, acetate, and then amino acids while *B. thermosphacta* will consume glucose then amino acids such as valine, glucine, leucine, and isoleucine before ribose and glycerol (Ellis & Goodacre,
Therefore, if there are different levels of low molecular weight compounds in the fresh meat products, different spoilage compositions can be created because different bacteria will dominate as a result of adjusting to the concentrations of metabolites available (Gill, 1976). For example, if there is more glucose, *Pseudomonas* will dominate while if there is more lactic acid, *S. putrefaciens* will dominate. After glucose and the glycolytic intermediates have been utilized, the spoilage bacteria will begin to utilize amino acids and nucleotides (Lawrie & Ledward, 2006). As the metabolite concentrations of the fresh meat products vary, it will affect the spoilage composition by determining which bacteria dominate the spoilage flora.

**Off-odors**

There are many off-odors produced by the spoilage bacteria as they utilize amino acids and nucleotides after utilizing glucose and the glycolytic intermediates. This off-odor production is a common symptom of spoilage (Ingram & Dainty, 1971; Knox et al., 2008) that can make fresh meat products unfit for human consumption. Some common off-odors created are hydrogen sulfide (H\textsubscript{2}S), ethanol, acetoin, ethyl esters, short-chain fatty acids, amines, diamines, cadaverine, putrescine, indole, ammonia, methanediol, dimethyl disulfide, and many more (Ercolini et al., 2011; Garcia-Lopez et al., 1998; Gill, 1986; Huis in't Veld, 1996; Jay et al., 2005; Kraft, 1986; Lawrie & Ledward, 2006; Nychas & Skandamis, 2005). These off-odors are produced at different rates, depending upon package type and oxygen content. For example, if there is an increased oxygen atmosphere, such as an aerobic package, off-odors would develop in 2-7 days while it
could take as long as 6 weeks in a vacuum-package (Jeremiah, Gibson, & Argnosa, 1995; Nissen et al., 1996; Sorheim et al., 1999). The faster off-odor production in aerobic packages is due to the increase in oxygen film permeability and the increased growth rates of the aerobic bacteria (Newton & Rigg, 1979). Different types of microorganisms working together can create different kinds of spoilage off-odors. For example, Enterobacteriaceae, *B. thermosphacta*, and lactic acid bacteria can produce hydrogen sulfide, acetoin, diacetyl, acetic acid, isovaleric and isobutyric acids, and 3-methylbutanol for a “cheesy, sour, acid” off-odor while *Pseudomonas* and other aerobic microorganisms will produce a “sweet, fruity, putrid” off-odor (Borch et al., 1996; Dainty, Edwards, Hibbard, & Marnewick, 1989; Dainty & Mackey, 1992; Nychas et al., 1998; Nychas et al., 2008; Samelis, 2006). Many of these off-odor compounds incorporate the atoms of the amino acid utilized into the off-odor, such as sulfur off-odors come from sulfur incorporating amino acids (Aberle et al., 2012). By knowing the different off-odors produced during spoilage, the different spoilage compositions can also be determined.

**Water activity**

Fresh meat has a very high water activity, around 0.99, with pork and poultry having water activities around 0.985 and 0.986, respectively (Aberle et al., 2012; James & James, 2002; Lawrie & Ledward, 2006). This high water activity does not impose any kind of barrier to microorganism growth, so it will not influence spoilage composition on fresh meat products (Gill, 1986). Water activity would influence spoilage composition if the water activity was below 0.985, such as in processed meat products, because only certain types of microorganisms can grow with that low of a water activity.
Molds and yeasts

Molds and yeasts can spoil fresh meat products and be a part of the spoilage composition, but only under specific conditions. Molds and yeasts are very hardy microorganisms compared to spoilage bacteria because molds and yeasts can grow in lower water activities, lower pH, increased salinity, or in the presence of preservatives (Huis in't Veld, 1996). However, they do need oxygen to grow, so they are not common in vacuum-packaging and some types of modified atmosphere packaging (Samelis, 2006). In addition, they have slower growth rates compared to spoilage bacteria, so they cannot compete as well to be a major part of the spoilage composition (Dillon, 1998). If they do become a major part of the spoilage composition, it is because the bacteria are inhibited (Dillon, 1998; Gill, 1986; James & James, 2002). If the bacteria are inhibited, molds and yeasts will dominate the spoilage composition and spoil the fresh meat product, producing slime, off-odors, and pigmented spots where they colonize on the surface (Huis in't Veld, 1996; Jay et al., 2005). The typical genuses of molds and yeasts that will colonize and spoil fresh meat are Candida, Cryptococcus, Debaroyomyces, Rhodotorula, Thamnidium, Mucor, Penicillum, Rhizopus, Cladosporium herbarum, and Sporotorichum carnis (Ayres, 1960; Dillon, 1998; James & James, 2002; Jay et al., 2005; Lawrie & Ledward, 2006; Samelis, 2006). C. herbarum and S. carnis are commonly known as “black spot” and “white spot” mold, respectively.
Methods to Control Meat Spoilage

There are several methods in which to control the rate of meat spoilage and thus extend the shelf life of a fresh meat product. Some of the major methods to control meat spoilage are ultimate pH and quality of the fresh meat, storage temperature, carbon dioxide, number of microorganisms, and package choice. However, the rate of spoilage cannot be influenced by the fat content, addition of glucose, and surface drying. All of these methods work by influencing the growth rate or type of microorganisms present on the fresh meat product by introducing some type of inhibitor. As more inhibitors are applied, the spoilage microorganisms will change from non-fermentative, Gram-negative psychrotrophs to fermentative, Gram-negative psychrotrophs, to lactic acid bacteria, to yeasts and molds (Gram et al., 2002; Jeremiah, 2001). Each inhibitor addition results in hardier and tougher microorganisms that can withstand those inhibitors, which will affect the rate of meat spoilage. The methods discussed below are possible inhibitors that could be applied to influence the meat spoilage rate.

**Meat quality and ultimate pH**

The quality of fresh meat products, and their corresponding ultimate pHs, should be held in consideration when trying to control meat spoilage. The ultimate pH is the post-mortem stabilized pH of the meat, and most red meat, beef and pork, have an ultimate pH around 5.6-5.8 (Aberle et al., 2012; Borch et al., 1996; Knox et al., 2008; Lawrie & Ledward, 2006). Any changes either above or below this pH range can affect the quality of fresh meat and the rate of fresh meat spoilage, such as dark, firm, and dry (DFD) meat and pale, soft, and exudative (PSE) meat. DFD meat has a shorter shelf life.
than normal meat because it has a higher pH (around 6.0) than the normal 5.8 value (Aberle et al., 2012). This increased pH is more favorable for microorganism growth because it is closer to a neutral pH. For example, DFD pork can reach log 5 CFU/cm² psychrotrophs while normal pork has log 4 CFU/cm² at 5°C for 9 days aerobically (Rey, Kraft, Toppel, Jr., & Hotchkiss, 1976). There is also a lower glycogen concentration in DFD meat because the animal was stressed before slaughter and used those reserves in the stress response. This lower glucose concentration causes the production of off-odors and off-flavors to occur sooner because the spoilage bacteria utilize amino acids earlier due to the lack of glucose to metabolize first (Faucitano et al., 2010; Gill, 1986; James & James, 2002; Jeremiah, Gibson, & Argonosa, 1995). Therefore, the combination of a higher pH and no glucose present causes a shorter shelf life than normal pH meat. Rao and Sachindra (2002) summarized that DFD meat has a shelf life of 6 weeks while normal pH meat has a shelf life of 8 weeks. On the other hand, Knox et al. (2008) found that vacuum-packaged pork loins in the normal pH range have a shelf life of 6 weeks at 0°C while DFD pork loins have a shorter 3-4 week shelf life. Either way, these examples demonstrate that DFD meat has a shorter shelf life than normal meat. In addition, the higher pH of DFD meat selects for different spoilage bacteria, such as *Shewanella putrefaciens* and *S. liquefaciens*, which are not common on normal pH meat (Samelis, 2006; Stanbridge & Davies, 1998). These spoilage bacteria are prolific producers of off-odors that characterize meat spoilage, which also help to shorten the fresh meat product’s shelf life.

Pale, soft, and exudative pork (PSE) is another quality to keep in mind when it comes to controlling meat spoilage. PSE has a low pH, around 5.3-5.6, compared to the
normal pH range of 5.6-5.8 (Aberle et al., 2012; Fox, Wolfram, Kemp, & Langlois, 1980). Due to this low pH, there can be fewer microorganisms present compared to normal or DFD pork (Rey et al., 1976). For example, total microorganism load for 8 days at 37°C is log 8 CFU/g for normal pork but only log 4 CFU/g for PSE pork (Fox et al., 1980). Jeremiah, Gibson, and Argonosa (1995) found that PSE pork loins could reach log 5 CFU/cm² and DFD pork loins could reach log 6 CFU/cm² for psychrotrophs when stored for 15 weeks under carbon dioxide at -1.5°C. In addition, Rey et al. (1976) found that PSE pork loins stored aerobically at 5°C for 9 days reached log 3 CFU/cm² psychrotrophs compared to log 5 CFU/cm² for normal and DFD pork. These studies highlight there are fewer microorganisms present on PSE pork than DFD pork, so the shelf life of PSE pork may be longer than DFD pork due to the differences in pH and microbial numbers. When PSE pork is compared to normal pork, PSE may or may not have a longer shelf life because the spoilage rate can be similar between the two and have the same amount of microorganisms present. However, the lack of quality and poor appearance of PSE pork keep most consumers from buying the fresh meat product, despite the possible increase in shelf life.

**Storage temperature**

Storage temperature is one of the main factors in controlling fresh meat spoilage. By reducing the storage temperature of fresh meat products to 0-10°C, there is a selective action for a greater concentration of psychrotrophs in the spoilage bacteria than mesophiles (Kraft, 1986; Zhou et al., 2010). A greater concentration of psychrotrophs can result in a slower rate of meat spoilage because psychrotrophs may have slower
generation times than mesophiles. However, different bacteria within the encompassing psychrotroph classification will have different growth rates depending on their species, ability to utilize metabolites at different rates, ability to compete against other bacteria, or ability to handle environmental stress (Gill, 1986). This potential variation in the meat spoilage rate can be controlled by reducing the storage temperature of the fresh meat products. By reducing the storage temperature it causes an increase in the lag phase duration and generation time of the spoilage bacteria, resulting in slower rate of meat spoilage (Kraft, 1986; McDonald & Sun, 1999). For example, vacuum-packaged pork chops held at -4°C had log 4 CFU/g fewer psychrotrophs than vacuum-packaged pork chops held at 3°C at the end of a 49 day storage period (Lee et al., 1985). Another example of storage temperature slowing bacterial growth is that for a generic pseudomonad, its generation time would be 1 hour at 20°C, 2.5 hours at 10°C, and 11 hours at 0°C (James & James, 2002). Slower bacterial growth means a longer shelf life due to a slower meat spoilage rate, and for every degree reduction in storage temperature, the shelf life will increase around 10% (Jeremiah, 1997). Shelf life can be maximized when fresh meat products are stored at the optimum temperature of -1.0°C for unwrapped product and -1.5°C for packaged product (Borch et al., 1996; Gill, 1996, 2003; James & James, 2002; Jeremiah, 1997) because those storage temperatures cause the greatest increase in the lag phase and generation time of the spoilage bacteria. Meat does not freeze until -2.2°C, so fresh meat products can be stored at those temperatures and still be considered “fresh.” On the other hand, if the fresh meat products are stored at storage temperatures greater than -1.5°C, the spoilage rate will also increase due to increases in the bacterial growth rates (Kreyenschmidt et al., 2010), so shelf life will be decreased. If
fresh meat products are stored at 2°C or 5°C, only 50% or 30%, respectively, of the shelf life can be attained than would be if it was stored at -1.5°C (Gill, 1996). The meat spoilage rate can also be increased if the storage temperature fluctuates by ±5-15°C during storage. During increased temperature periods (+5-15°C), the spoilage bacteria can grow faster than at the normal storage temperature, so the resulting meat spoilage rate will also increase in those periods. Therefore, it is crucial to keep fresh meat products stored at the optimum storage temperature of -1.0°C to -1.5°C to obtain the slowest rate of fresh meat spoilage, and as a result, a longer shelf life.

**Carbon dioxide**

Carbon dioxide is another factor that controls meat spoilage and extends the shelf life of fresh meat products. Carbon dioxide (CO₂) inhibits bacteria when added at a concentration of 20-30% inside the package. Carbon dioxide inhibits bacteria by altering the intracellular pH, inhibiting decarboxylating enzymes, or disrupting the cell membrane (Dixon & Kell, 1989; Gill, 1986; Guan & Hoover, 2005). Carbon dioxide disrupts the intracellular pH because CO₂ is soluble in water and can pass freely through biological membranes. Once CO₂ dissolves, it forms carbonic acid which can acidify the cytoplasm (Guan & Hoover, 2005; Jay et al., 2005) causing enzymes and proteins to denature due to the decrease in intracellular pH. Carbon dioxide can also limit bacterial growth by inhibiting decarboxylating enzymes. Decarboxylating enzymes are inhibited, because in principle, decarboxylating enzymes function by removing a CO₂ molecule from a metabolite. If there is a greater CO₂ concentration present in the cell, the decarboxylating enzymes will not function properly because they are naturally inhibited by increased CO₂
concentrations. Without the ability to remove CO₂ molecules, bacterial cells cannot utilize some metabolites because they cannot break the bonds inside the metabolite to release the energy for their biological processes. Lastly, CO₂ can inhibit bacterial growth by disrupting the biological cell membrane. Carbon dioxide dissolves into the fat phase of a food or cell, so it can dissolve in the non-polar region of the cell membrane (Devlieghere, Debevere, & Impe, 1998; Dixon & Kell, 1989). Once it is dissolved, the cell membrane’s selective permeability will be compromised, and the cell will not be able to maintain homeostasis of the molecules and ions inside the cell. By denaturing proteins and enzymes, reducing the energy released from metabolites by inhibiting decarboxylating enzymes, and compromising the cell membrane, the bacteria cell cannot maintain its biological functions or homeostasis so the cell will be impaired or will die. Fewer bacterial cells cause a slower rate of meat spoilage because fewer bacterial cells require more time to reach the log 7 CFU/g spoilage threshold than many cells.

Carbon dioxide can also inhibit bacterial growth by acidifying the environment of the spoilage bacteria, such as the fresh meat product itself. Carbon dioxide acidifies the fresh meat product because at low temperatures, such as refrigeration temperatures, CO₂ is soluble (Guan & Hoover, 2005; Jay et al., 2005). Once CO₂ is soluble, it dissolves into the fresh meat tissue and forms carbonic acid which dissociates inside the fresh meat tissue to release acidic protons (Dixon & Kell, 1989; Gill, 1988). Carbon dioxide is soluble at a rate of 960 mL of CO₂ at 1 atm, 0°C, and pH 5.5 per kilogram of fresh muscle tissue, and this rate decreases by 19 mL/kg for every one degree increase in the storage temperature (Gill, 1988; Jeremiah, 2001). These rates are the same for beef, pork, or lamb muscle. Therefore, it is critical to keep fresh meat products stored at refrigeration
temperatures or colder to achieve an increased rate of CO$_2$ solubility (Jeremiah, 2001; Nissen et al., 1996; Samelis, 2006; Sorheim et al., 1999). An increased CO$_2$ solubility rate means a greater production of carbonic acid to acidify the fresh meat product and disrupt bacterial cell function as described previously. Due to carbon dioxide’s rate of solubility, it dissolves quickly into a fresh meat product. For example, within an hour, up to 78-87% of the original CO$_2$ amount in the package could be dissolved (Devlieghere & Debevere, 2000). However, cold temperatures must be maintained to achieve and sustain this increased solubility rate.

Despite these inhibitory effects of carbon dioxide, its effectiveness depends on the type of bacteria inside the fresh meat package. Not all bacterial geneses and species are inhibited at the same rate, for some geneses and species are more resistant to CO$_2$ than others. Gram-negative bacteria tend to be more sensitive to CO$_2$ (Gill & Tan, 1980) due to their double cell membrane structure that is affected by CO$_2$, as explained previously. The most resistant genus and/or specie of bacteria is lactic acid bacteria, followed by $B$. thermosphacta, Enterobacteriaceae, and then $P$. pseudomonas (Enfors et al., 1979; Gill & Tan, 1980; Lawrie & Ledward, 2006; Nowak et al., 2012). Therefore, CO$_2$ can inhibit some of the faster growing spoilage bacteria, such as $P$. pseudomonas, but CO$_2$ cannot completely stop spoilage bacteria growth by itself. The fresh meat product will eventually spoil, no matter how much carbon dioxide is added or produced during the storage period, but the spoilage rate will be decreased by inhibiting the spoilage bacteria to slower growing types, such as lactic acid bacteria.
Number of microorganisms

Another factor that can be used to control the rate of meat spoilage is the number of microorganisms present at the beginning of packaging. If fewer microorganisms are present at initial packaging, it will take more time for the spoilage bacteria to reach the log 7 CFU/g spoilage threshold (Gill, 1996; Nissen et al., 1996; Nychas & Skandamis, 2005), so the meat spoilage rate overall is slower. It takes longer to reach the threshold because the microorganisms need to multiply to greater numbers to cause spoilage, which takes time (James & James, 2002). For example, Holley et al. (2004) found that if there is a bacterial concentration of less than log 2 CFU/cm² initially present at packaging, a shelf life of 49 days can be achieved for pork at -1.5°C. Another study by Ayres (1960) found that meat with an initial microbial concentration of 59,000 microorganisms per cm² had an 11 day shelf life, while meat with an initial microbial population of 69 microorganisms per cm² had a 21 day shelf life at 0°C. Furthermore, Ayres (1960) found that an initial bacterial load of log 2 CFU/cm² had a 12 day shelf life while an initial load of log 5.2 CFU/cm² had a 6 day shelf life at 5°C in an overwrap package. These examples demonstrate that a reduced initial bacterial concentration at packaging will have a longer shelf life than a greater initial bacterial concentration. In addition, increased initial levels of spoilage bacteria on a fresh meat product at packaging will reduce the time prior to the onset of spoilage, irrespective of meat quality, carbon dioxide concentrations, storage temperature, and package choice (Borch et al., 1996; C. O. Gill & K. G. Newton, 1977; Jeremiah, 1997). By reducing the number of microorganisms present at packaging of a fresh meat product, the rate of fresh meat spoilage can be reduced and the shelf life extended.
Package choice

The package choice for a fresh meat product can also control the meat spoilage rate. The package protects the fresh meat from the environment, discoloration, off-odor and off-flavor development, nutrient loss, texture changes, and other deteriorative effects (Zhou et al., 2010). It also determines the rate of fresh meat spoilage and the length of shelf life by the type of gas atmosphere used (Sade et al., 2013). If the atmosphere contains a greater oxygen concentration than any other gas, the spoilage rate will increase because aerobic microorganisms, particularly *Pseudomonas*, have a fast growth rate, (Ingram & Dainty, 1971). On the other hand, if the gas atmosphere has a greater carbon dioxide concentration, the package will be anaerobic, such as vacuum-packaging. The microorganisms inside an anaerobic package will grow slower, as most anaerobic microorganisms do, but there are always exceptions to this generalization. An exception would be *Clostridium perfringens*, a purely anaerobic microorganism, that has a generation time of less than 10 minutes anaerobically (Jay et al., 2005), which is a faster generation time than some aerobic microorganisms. Despite this exception, anaerobic microorganisms have slower growth rates, such as lactic acid bacteria, than aerobic microorganisms, so the spoilage rate for an anaerobic package would be slower. This slower spoilage rate results in a longer shelf life than the faster spoilage rate of an aerobic package with a shorter shelf life. Therefore, the package choice for a fresh meat product can influence its meat spoilage rate by selecting for different bacteria with different growth rates.
**Fat content**

The fat content of the fresh meat product plays a minimal role in controlling the meat spoilage rate. Fat and lean have varying growth properties where fat tends to have more microorganism growth than lean muscle, but only if the fat is not dried out. If the fat is dried out, it would result in a lower water activity than the lean in which most microorganisms could not grow (Holley et al., 2004). However, if the fat is covered with a layer of purge, the fat and lean will have the same growth properties as the rest of the muscle in the package (Gill, 1986). In addition, most consumers want leaner fresh meat products so the fat content will be lower than they have been in the past. Therefore, between the lower fat content in fresh meat currently and similar growth rate between fat and purge, fat content has little effect on controlling the meat spoilage rate.

**Addition of glucose**

The addition of glucose to fresh meat products does not inhibit the meat spoilage rate, but rather increases it. Spoilage microorganisms utilize glucose as their primary food source in fresh meat, and once the glucose is depleted, they start to utilize amino acids that result in the typical off-odors associated with spoilage. The amount of glucose present in muscle post-mortem is affected by the condition of the animal before slaughter, such as age, nutritional state, stress, and exercise levels (Bruckner et al., 2012). The amount of glucose declines after slaughter because it is used during glycolysis to create lactic acid to lower the ultimate pH of the meat. Bruckner et al. (2012) found that pork has a D-glucose value of 0.101 grams of glucose per 100 grams of meat that decreases constantly throughout the shelf life while poultry has 0.014 grams of glucose per 100
grams of meat. This demonstrates there is little glucose available after the post-mortem pH decline for microorganisms to utilize. Therefore, researchers wondered if adding glucose to fresh meat would slow the rate of fresh meat spoilage by delaying the microorganisms’ utilization of amino acids, and thus the onset of spoilage. However, it is completely deleterious if glucose is added to fresh meat because it stimulates microorganism growth since more glucose is available for their use than normal (Gill, 1986). More microorganism growth will result in a faster spoilage rate than normal, so adding glucose to fresh meat does not inhibit the rate of fresh meat spoilage, but rather increases it.

**Surface drying**

In terms of surface drying to control meat spoilage, it is not very effective. It can work to a limited degree but it causes a detrimental effect on appearance (Gill, 1986) in order to achieve its limited effectiveness. It also causes a problem with molds and yeasts because surface drying can inhibit bacterial growth, so molds and yeasts will dominate the spoilage process. However, the meat industry is concerned more about spoilage bacteria because they will spoil the fresh meat product faster than yeasts and molds would. Therefore, the meat industry wants methods that inhibit spoilage bacteria, but surface drying is not one of those methods because it is ineffective and has detrimental effects on meat quality.
Shelf Life

**Average estimated shelf lives**

Different types of fresh meat packages have different estimated, average shelf life times. Shelf life can have different meanings, but it will mean the length of time a fresh meat product can be stored until spoilage is evident and the product is unfit for human consumption. Beef and pork fresh meat products tend to have similar shelf life times, despite being of different meat species (Blixt & Borch, 2002). On average, vacuum-packaged normal pH pork has a shelf life of 8-9 weeks while beef has 8-12 weeks, and the end of shelf life is marked by the “cheesy, sour, acid” smell typical of spoiled vacuum-packages (Aberle et al., 2012; Blixt & Borch, 2002; Delmore, 2009; Small, Jenson, Kiermeier, & Sumner, 2012). Vacuum-packaging is also how fresh meat products are shipped overseas to export markets because it is effective at preserving the products during inter-continental transport while minimizing water losses and space requirements (Pennacchia et al., 2011). In overseas transport, the packages are stored at -1.5°C to provide a longer shelf life, so fresh pork products have a shelf life of 6 weeks and when it arrives at its export market, it has a 2-5 day retail display shelf life (Jeremiah, 1997). As for the other meat packaging types, overwrap packages can last 5-9 days, low oxygen MAP 25-35 days, and high oxygen MAP 10-21 days (Aberle et al., 2012; Borch et al., 1996; Delmore, 2009; Ercolini et al., 2011; Jeremiah, 1997, 2001; Stanbridge & Davies, 1998). These shelf life times were obtained at a storage temperature of about 0°C and represent average industry shelf lives. There are no absolute shelf life times for fresh meat products because spoilage happens gradually over time, not suddenly and all at once (Man, 2002). Therefore, shelf life times will vary depending upon the type of fresh meat.
product, amount of oxygen and carbon dioxide inside the package, any additives present, permeability of the packaging film, storage temperature, and number of microorganisms present, as discussed previously. Ground fresh meat products have shorter shelf lives than fresh intact muscles because the bacteria were mixed more throughout the ground products than it would have been if left intact (Jeremiah, 1997). Many times companies make the shelf life estimates shorter than the actual shelf life (Man, 2002), based upon microbiological data or sensory analysis, to provide a safety margin to protect the consumer and the quality of their products.

**Measuring shelf life**

Over the last couple of decades, research has developed ways to estimate or measure remaining shelf life faster than normal methods. Normal, conventional methods usually rely upon microbiological methods or sensory analysis, which takes time to complete (Nychas & Skandamis, 2005). These conventional methods could be challenge tests or accelerated shelf life determination (ASLD), and each method has limitations. Challenge tests are laboratory investigations of the behavior of a meat product subjected to a controlled set of conditions (Man, 2002). These challenge tests require microbial analysis to determine when spoilage occurs, which requires a two to three day downtime, resulting in “historical” data. This “historical” data is why companies would like a rapid instrument measurement of shelf life. In addition, the challenge test results are limited to the conditions tested (McMeekin & Ross, 1996a), so any change to the tested conditions will require repetition of the of the test (Koutsoumanis et al., 2006). Shelf life can also be determined by accelerated shelf life determination (ASLD), where the product is held at
elevated storage temperatures to bring about any adverse spoilage effects sooner than the normal storage temperature (Man, 2002). Unfortunately, ASLD is product-specific, can have unexpected, false results, and cannot be used for fresh meat products. ASLD cannot be used for fresh meat products because spoilage at elevated storage temperatures causes a completely different method of spoilage than at refrigeration temperatures. This would result in an irrelevant shelf life estimate for that product. In addition, microbiological sampling for shelf-life is expensive (Bruckner, Albrecht, Petersen, & Kreyenschmidt, 2013; McDonald & Sun, 1999). The company has to sacrifice potential product, ship it to the laboratory, pay the laboratory for their services and labor, and pay to have separate tests conducted for each product they produce. Sensory analysis is even more costly and time-consuming than microbiological sampling because trained panelists are required, and it takes time and money to train the panelists and to use their services (Man, 2002). Due to the expensive nature of determining shelf life, not all companies have access to the resources needed to conduct these challenge tests or sensory analyses, so a quicker, inexpensive way of measuring shelf life or estimate the amount of time remaining until the fresh meat product is spoiled is desired (Alderees & Hsieh, 2012; McDonald & Sun, 1999). This would include an instrument reading that could be used by anyone without extensive training, is economical to use, and ideally, not destructive to the product (McMeekin & Ross, 1996a; Nychas et al., 2008). The above three criteria are the ideal way for a company to estimate shelf life because anyone could use the instrument and the product could still be sold. Furthermore, the instrument reading would result in a “real-time” measurement of remaining shelf life by approximating a microbial population, not “historical”, three-day old data with microbial analysis. In addition, if a better method of
measuring shelf life was developed, there could be a reduction in the 25% of all foods produced globally that is lost to microbial spoilage (Gram et al., 2002; McMeekin & Ross, 1996a). This would make more fresh meat products available to everyone because not as much would be lost to microbial spoilage, and as a result the profitability of fresh meat products would also increase since more product could be sold.

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CHAPTER 3. DISSOLVED CARBON DIOXIDE AND OXYGEN
CONCENTRATIONS IN PURGE OF VACUUM-PACKAGED PORK
CHOPS AND RELATIONSHIP TO MICROBIAL POPULATION AND
SHELF LIFE

A paper that has been submitted to the journal *Meat Science*.

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Abstract

The objectives of this study were to determine the dissolved CO\(_2\) and O\(_2\)
concentrations in the purge of vacuum-packaged pork chops over a 60 day storage period,
and to elucidate the relationship of dissolved CO\(_2\) and O\(_2\) to the microbial populations
and shelf life. As the populations of spoilage bacteria increased, the dissolved CO\(_2\)
increased and the dissolved O\(_2\) decreased in the purge. Lactic acid bacteria dominated the
spoilage microflora, followed by Enterobacteriaceae and *B. thermosphacta*. The surface
pH decreased to 5.4 due to carbonic acid and lactic acid production before rising to 5.7
due to ammonia production. A mathematical model was developed which estimated

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\(^3\) Principal Investigator who provided insight and feedback into the design, overall progress of the project, and while writing the manuscript and thesis
microbial populations based on dissolved CO\textsubscript{2} concentrations. Scanning electron microscope images were also taken of the packaging film to observe the biofilm development. The SEM images revealed a two-layer biofilm on the packaging film that was the result of the tri-phase growth environment.

**Keywords:** pork; dissolved carbon dioxide; dissolved oxygen; shelf life; spoilage

**Introduction**

Spoilage is an inevitable problem for fresh meat products because fresh meat is contaminated during slaughter and processing with bacteria from the feces, hide, and hooves of the animal (Ayres, 1955). Meat also has a favorable 5.4-6.4 pH, high water activity (0.99), and abundance of low molecular weight compounds, such as glucose and amino acids, for bacteria to utilize (Samelis, 2006). However, spoilage can be managed and extended by storage temperature, initial bacterial populations, packaging conditions, and carbon dioxide (CO\textsubscript{2}) concentrations (Gill, 1986). Storage temperature can slow bacterial growth by increasing the lag phase duration and generation time of the bacteria. The greatest increases in lag phase duration and generation time is a storage temperature of -1.5°C (Gill, 1986; Jeremiah, 1997), but lag phase duration and generation times can increase at refrigeration temperatures (less than 4°C). Initial bacterial populations present at packaging can also influence the length of shelf life for a fresh meat product. For example, fewer than 2 colony forming units (CFU)/g present at packaging resulted in a 7 week shelf life of fresh pork (Holley et al., 2004).
Packaging also affects the shelf life of fresh meat, especially if it involves altering the atmosphere by vacuum or modified atmosphere packaging. Overwrap packages have a high oxygen (O_2) content in their package so the shelf life is, on average, 5-7 days (Delmore, 2009; Jeremiah, 1997). The aerobic atmosphere selects for *Pseudomonas* spp., *Brochothrix thermosphacta*, and Enterobacteriaceae spp. (Doulgeraki et al., 2012; Gill, 1986). Modified-atmosphere packages (MAP) may be either high or low oxygen. High oxygen MAP typically have 80% O_2 and 20% CO_2 present and the meat has a similar spoilage microflora as aerobic overwrap packages, with *Pseudomonas* spp., *B. thermosphacta*, and Enterobacteriaceae spp. (Borch et al., 1996). A typical low oxygen MAP will have 20% CO_2, 70% nitrogen, and less than 10% O_2, on average, and lactic acid bacteria (LAB), Enterobacteriaceae spp., *B. thermosphacta*, and *Pseudomonas* spp. dominate the spoilage microflora (Doulgeraki et al., 2012). Because of the differences in atmosphere and spoilage composition, meat packaged in high oxygen MAP has an average shelf life of 10-21 days, while low oxygen MAP packaged meat is 25-35 days (Delmore, 2009; Jeremiah, 1997). Vacuum-packaging is similar to low oxygen MAP, but it has an anaerobic environment that selects for LAB, Enterobacteriaceae spp., *B. thermosphacta*, and a few *Pseudomonas* spp. in the beginning of the shelf life (Borch et al., 1996; Doulgeraki et al., 2012). Vacuum-packaging, due to its anaerobic environment, can have a shelf life of 45-90 days (Delmore, 2009; Jeremiah, 1997).

The shelf life of vacuum-packaged meats is also affected by the CO_2 concentration present in the package, which is a metabolic by-product of microbial growth. Carbon dioxide is known to inhibit bacteria by affecting the cell membrane permeability, decarboxylating enzymes, and acidifying the intracellular pH (Dixon &
Carbon dioxide can inhibit bacteria because it is soluble in water at refrigeration temperatures, forming dissolved CO$_2$, and carbonic acid (Dixon & Kell, 1989; Gill, 1986). Carbon dioxide is also soluble in the fresh meat tissue at a rate of 960 mL of CO$_2$/kg of fresh meat at 1 atm, 0°C, and pH 5.5 (Gill, 1988). This rate is similar for pork, beef, and lamb and it can be affected by pH and storage temperature (Gill, 1988). This means CO$_2$ will dissolve into the fresh meat product, forming carbonic acid and inhibiting the spoilage bacteria by acidifying the intracellular pH and affecting cell membrane permeability.

CO$_2$ inhibits different types of bacteria at different rates. LAB have the greatest resistance to CO$_2$ because they produce CO$_2$ as a by-product of respiration, whereas Pseudomonas spp. have the least resistance (Dixon & Kell, 1989). Intermediate resistance to CO$_2$ concentrations are B. thermosphacta and Enterobacteriaceae spp. (Dixon & Kell, 1989; Nowak et al., 2012).

The standard method of determining microbial populations in packaged meat involves microbiological analysis to determine the populations of spoilage bacteria present. However, this method is time-consuming, destructive, and expensive to conduct (Bruckner et al., 2013; McDonald & Sun, 1999; McMeekin & Ross, 1996b). A total mesophilic aerobic bacterial enumeration requires three days to complete, and delivers historical data on the population in the product 72 h earlier. Because of this, there is interest in developing a method of estimating microbial populations based upon an instrument measurement (Bruckner et al., 2013; McDonald & Sun, 1999; McMeekin & Ross, 1996b). This instrument measurement would focus on dissolved CO$_2$ concentrations because LAB produce it as a metabolism by-product (Hammes & Hertel,
so as the LAB populations increase, the dissolved CO$_2$ concentration also increases. Potentially, a dissolved CO$_2$ concentration could estimate microbial populations and be used to estimate shelf life of packaged meats. There are very few studies conducted which determine the interaction of dissolved CO$_2$ and microbial populations using a meat system and in the context of shelf life. Previous work by Devlieghere and Debevere (2000) and Devlieghere et al. (1998) used Brain-Heart-Infusion media, or a similar broth system, to determine how dissolved CO$_2$ affected certain types of spoilage bacteria.

The objectives of this study were to determine the dissolved CO$_2$ and O$_2$ concentrations in the purge of vacuum-packaged pork chops during storage, and to determine the relationship between dissolved CO$_2$ and O$_2$ concentrations to the microbial populations and shelf life. Scanning electron microscope (SEM) images were also taken of the packaging film in contact with the meat product to investigate how the biofilm develops on the packaging film throughout the shelf life. The hypothesis was that dissolved CO$_2$ concentrations will increase and dissolved O$_2$ concentrations will decrease inside the vacuum-package, and that the concentrations of the dissolved gases could be used to estimate microbial populations.

**Materials and Methods**

**Meat sample preparation**

Sixty bone-in thick cut pork *Longissimus thoracis et lumborum* (LTL) chops were purchased from a retail store the day of initial packaging, with a post-mortem age of 3-14 days. The bones were removed at the laboratory with a flame-sterilized knife before
being placed into a vacuum-package (B470T, Cryovac Sealed Air Corporation, Duncan, SC; oxygen transmission rate of 3-6 cc/m², 24 h, 1 atm, at 4.4°C and 0% relative humidity, and a water vapor transmission rate of 0.5-0.6 g at 37.7°C (100% relative humidity, 100 in.², 24 h). Three pork chops were placed into each vacuum-package. Before sealing, a pair of autoclaved 3x22 mm flat-bottom glass test tubes, with a 3x6 mm flea magnetic stir bar in each tube, were placed into an autoclaved 0.95 x 2.54 x 10.16 cm Teflon stand (Fig. 1). These glass tube and Teflon stands were placed aseptically into the vacuum-package. The tubes were placed upright in the package approximately 7.6 cm away from the pork chops. The vacuum-packages were then vacuum-sealed (975-980 mm Hg vacuum) with a Multivac C350 (Kansas City, MO). After sealing, the vacuum-packages were stored in a 4°C cooler for 60 days.

**Dissolved oxygen and carbon dioxide concentration measurements**

Two vacuum-packages were randomly selected on days 0, 5, 15, 30, 45, and 60 for dissolved gas concentration measurements. The vacuum-packages were placed into a biosafety cabinet and allowed to temper to 20-22°C for 10 min. After 10 min., the
packages were placed onto a stir plate (Dataplate Digital Hotplate/Stirrer Series 730, Thermolyne Corporation, Dubuque, IA), set to an stirring rate of 300 rpm and allowed to mix for 1 min. The outside of the package near the glass tubes was wiped with ethanol and allowed to evaporate before sterilized scissors were used to cut a cross aseptically into the top of one glass tube. After opening the package, the dissolved oxygen probe (Hach LDO101 optical probe; Hach HQ30d meter, Ames, IA) was slipped inside one of the glass tubes until halfway submerged. The dissolved oxygen concentration was measured four times before the measurements were averaged for an average dissolved oxygen concentration. The dissolved oxygen probe was calibrated before use according to the manufacturer’s directions for a 100% oxygen concentration.

For dissolved carbon dioxide, a Thermo Scientific Orion Carbon Dioxide Electrode 9502BNWP (Beverly, MA) with a Thermo Scientific Orion Star A214 pH/ISE benchtop meter (Beverly, MA) were used to measure the concentration. The electrode was calibrated to 100 and 1,000 mg/L using a 0.1M NaHCO$_3$ (Thermo Scientific Orion Application Standard 950206, Beverly, MA) calibration standard with Carbon dioxide buffer added (Thermo Scientific Orion Application Solution 950210, Beverly, MA), according to the manufacturer’s directions. After finishing the dissolved O$_2$ measurement, the other glass tube was used for dissolved CO$_2$ measurements. Another cross was cut aseptically into the package on top of the glass tube and the Carbon Dioxide Buffer solution was added in a 10% concentration to the amount of purge inside the glass tube. The buffer was needed to convert all bicarbonate and carbonate into CO$_2$ inside the purge. After allowing the buffer to mix for 1 min., the electrode was submerged halfway down into the purge. The dissolved CO$_2$ concentrations were measured after the millivolt
readings stabilized, and then five measurements were recorded. The measurements were averaged together to obtain an average measurement for dissolved CO$_2$ per package.

**Microbiological analysis**

After the dissolved gas measurements were obtained, the pork chops and purge were used for microbiological analysis. 1 mL of purge was removed from the glass tube used for the dissolved O$_2$ measurement and placed into a tube with 9 mL of 0.1% buffered peptone water (Difco, Benton Dickson, Sparks, MD). Three cores were removed from each pork chop, getting at least one from each side, with an autoclaved 1.5 cm diameter corer. The outer 2 mm surfaces were aseptically removed from each core (total surface area of 21.2 cm$^2$), and each sample was placed into a WhirlPak™ filter bag (VWR International, Radnor, PA) along with 90 mL of 0.1% buffered peptone water. The core samples were homogenized (Neutec Group, Inc., Masticator Homogenizer, Farmingdale, NY) on a normal setting for 30 s. Following homogenization, appropriate ten-fold serial dilutions were made using 0.1% buffered peptone water for both core and purge samples. 0.1 mL of the appropriate dilutions were surface-plated in duplicate on Tryptic Soy Agar (TSA; Difco, Benton Dickson, Sparks, MD) incubated at 22°C aerobically for 72 h for Aerobic Plate Counts (APC), Violet Red Bile Glucose Agar (VRBG; Difco, Benton Dickson, Sparks, MD) incubated at 35°C for 48 h aerobically for Enterobacteriaceae, Man, de Rogosa, Sharpe agar (MRS; Difco, Benton Dickson, Sparks, MD) incubated at 32°C for 48 h anaerobically for LAB, and Streptomycin-thallous acetate-actidione agar supplemented with STAA Selective Supplement (STAA; Oxoid Ltd., Basingstroke, Hampshire, UK) incubated at 22°C for 48 h aerobically for $B$. 
thermosphaeta. All plates with growth between 25-250 colonies were enumerated. If a plate had less than 25 colonies, the plate was enumerated and an estimated population was recorded.

Surface pH determination

The surface pH of the pork was measured both before the pork chops were placed inside the vacuum-package (initial) and before microbiological analysis (Thermo Scientific Orion 8135BN ROSS Flat surface pH probe, Beverly, MA; Jenco 6230N Meter, San Diego, CA). The pH meter was calibrated using 4.0 and 7.0 phosphate buffers at 4°C. The surface pH of each pork chop was measured three times, obtaining at least one measurement from each side of the pork chop. All measurements for pork chops of the same vacuum-package were averaged to obtain a pre-package pH, and for a post-package pH, all pH measurements of the same vacuum-package taken after the package was opened were averaged.

Surface electron microscopy

After removing the core samples, the pork chops were removed. Sterilized scissors were used to remove 1 cm² samples of the packaging film which was in contact with the lean portion of the pork chops. Duplicate samples were obtained, and the samples were fixed with 2% paraformaldehyde and 2% glutaraldehyde in 0.1M cacodylate buffer at 4°C for 48 h. Samples were rinsed in deionized water and post-fixed in 2% aqueous osmium tetroxide for 1 h at 22°C followed by dehydration in a graded ethanol series up to 100% ultra-pure ethanol. The samples were washed three times in
100% ultra-pure ethanol before they were dried using a Denton DCP-2 critical point dryer (Denton Vacuum, LLC, Moorestown, NJ). The samples were placed onto adhesive coated aluminum stubs, sputter coated (Denton Desk II sputter coater, Denton Vacuum, LLC, Moorestown, NJ) with palladium/gold alloy, and imaged using a JEOL 5800LV SEM (Japan Electron Optics Laboratory, Peabody, MA) at 10kV with a SIS ADDA II for digital image capture (Olympus Soft Imaging Systems Inc., Lakewood, CO).

**Statistical analysis**

Three independent replications of the experiment were conducted. Statistical analysis was conducted using the Statistical Analysis System (SAS 9.3, SAS, Institute Inc., Cary, NC, 2011). The microbial populations were transformed to log₁₀ CFU, and the PROC mixed function was used to analyze any effects on the log populations (the dependent variable). The fixed effects of the PROC mixed function were block, sample type (core or purge), microbial population type (APC, LAB, Enterobacteriaceae, *B. thermosphacta*), day, dissolved CO₂ and O₂ concentrations, and pre-package and post-package surface pH using the Kenward-Roger adjustment for degrees of freedom. Due to a significant interaction between day and microbial population type, the least square means for microbial population type were pairwise compared using PROC mixed. For pre-package surface pH, post-package surface pH, dissolved O₂, and dissolved CO₂ concentrations, the PROC reg function was used to determine differences amongst means for each day. For any significant differences by day for pre-package surface pH, post-package surface pH, dissolved O₂, or dissolved CO₂ concentrations, the PROC mixed
statement was used to pairwise compare the means and determine the differences.
Statistical significance decisions were made at p < 0.05.

Model development

After statistical analysis, there was no significant difference (p > 0.05) between the MRS and APC counts, so the counts were pooled for core and purge populations separately. A simple quadratic model was fitted to the plots of dissolved CO₂ concentrations and the bacterial populations using the global curve fit function in SigmaPlot (Systat Software, San Jose, CA). The result was a mathematical model for estimating microbial populations in vacuum-packaged pork based upon the concentration of dissolved CO₂ in the purge.

Results and Discussion

Microbial populations

Fig. 2 and Fig. 3 illustrate the changes in the spoilage bacteria populations on the pork chops over the 60 day storage period. There was a significant difference between the core and purge samples (p < 0.0047), with the core samples having slightly greater populations than the purge samples, by an average of log₁₀ 0.5 CFU/cm². Although statistically significant, this suggests that the microbial populations in the purge samples followed the same trend as the core samples, because the microbial populations for each bacterial type are similar. The greater populations on the core samples may be attributable to more available substrates, or simply dilution of the microbes in the purge. As expected there was a significant difference between microbial population type (p <
0.0001) and a significant interaction between microbial population type and day (p < 0.0188). This is consistent with previously reported data (Blixt & Borch, 2002; Borch et al., 1996; Holley et al., 2004; Knox et al., 2008).

As Fig. 2 and Fig. 3 illustrate, APC and LAB had similar bacterial populations throughout the 60 day storage period, for both core and purge samples. There was no significance difference (p > 0.05) between the APC and LAB counts on any sample day within sample type. LAB dominates the spoilage flora of vacuum-packaged pork chops, because of their ability to grow in the specific environment created by vacuum-packaging (Borch et al., 1996; Newton & Gill, 1978). LAB are capable of rapid growth under these conditions, with reported generations times of 6.5 h at 5°C, whereas the generation times for Enterobacteriaceae was 23.2 h and *B. thermosphacta* was 20.1 h under the same conditions (Gill, 1986). On day 0, the LAB populations averaged log_{10} 3.66 CFU/cm² for core samples and increased...
to $\log_{10} 6.51 \text{ CFU/cm}^2$ by day 5 (Fig. 2). The LAB populations reached $\log_{10} 7.37 \text{ CFU/cm}^2$ on day 15 and increased to approximately $\log_{10} 8 \text{ CFU/cm}^2$ until the end of the storage period. For purge samples (Fig. 3), the LAB populations were $\log_{10} 3.58 \text{ CFU/mL}$ on day 0 and increased to $\log_{10} 5.80 \text{ CFU/mL}$ by day 5. After day 5, the LAB populations increased to $\log_{10} 6.50 \text{ CFU/mL}$ on day 15 before reaching $\log_{10} 7.71 \text{ CFU/mL}$ by day 30, and then approximately $\log_{10} 8 \text{ CFU/mL}$ by the end of the storage period. These LAB populations for both core and purge are consistent with a study conducted by Blixt and Borch (2002), who found a $\log_{10} 8 \text{ CFU/g}$ concentration of LAB on pork loins after 56 days at 4°C. It is also consistent with a study done by Knox et al. (2008), who found a $\log_{10} 7.5 \text{ CFU/g}$ of LAB on pork loins in a similar pH to those used in this study (5.7-5.8) after 35 days at 4°C.

Enterobacteriaceae was a major contributor to spoilage because they had the second greatest bacterial population, after LAB and APC, either for core or purge samples. For core samples (Fig. 2), the Enterobacteriaceae populations were $\log_{10} 3.46 \text{ CFU/cm}^2$ on day 0 and increased to $\log_{10} 4.36 \text{ CFU/cm}^2$ and $\log_{10} 5.72 \text{ CFU/cm}^2$ on days 5 and 15, respectively. After day 15, the Enterobacteriaceae populations increased to approximately $\log_{10} 6.6 \text{ CFU/cm}^2$ and remained in that range throughout the rest of the storage period. The Enterobacteriaceae populations in purge samples were approximately $\log_{10} 3.0 \text{ CFU/mL}$ on day 0 and increased to $\log_{10} 4.90 \text{ CFU/mL}$ by day 5 (Fig. 3). On day 15 and 30, the Enterobacteriaceae populations were approximately $\log_{10} 5.7 \text{ CFU/mL}$ and increased to approximately $\log_{10} 6 \text{ CFU/mL}$ for the remainder of the storage period. These Enterobacteriaceae populations are also consistent with the study done by Blixt and Borch (2002) who reported similar populations of $\log_{10} 5-7 \text{ CFU/g}$ on pork loins after
56 days at 4°C. However, we had a lower population of Enterobacteriaceae than Knox et al. (2008), who found log$_{10}$ 7-8 CFU/g Enterobacteriaceae after 35 days at 4°C. This difference could be attributable to a greater initial population than in our study, or because of slight differences in muscle pH between the two studies (Knox et al., 2008).

*B. thermosphacta* contributed to spoilage but had a reduced microbial population compared to the other types of bacteria enumerated in the core and purge samples. *B. thermosphacta* populations on core samples for day 0 were log$_{10}$ 2.9 CFU/cm$^2$ and increased to log$_{10}$ 3.47 CFU/cm$^2$ by day 5, and remained at approximately log$_{10}$ 4.5 CFU/cm$^2$ for the remainder of the storage period (Fig. 2). Similar populations for *B. thermosphacta* were found in the purge samples, with log$_{10}$ 2 CFU/mL on day 0 and increasing to log$_{10}$ 3.7 CFU/mL by day 30 (Fig. 3). On day 45, the *B. thermosphacta* populations reached log$_{10}$ 4.1 CFU/mL before decreasing to log$_{10}$ 2.91 CFU/mL by day 60. The *B. thermosphacta* populations in this study were lower than those found by Blixt and Borch (2002), who reported *B. thermosphacta* populations of approximately log$_{10}$ 3-4 CFU/g on pork loins after 56 days at 4°C. However, this may be attributable to greater concentrations of lactic acid in the purge of this study, as *B. thermosphacta* is inhibited by lactic acid at 5°C (Grau, 1980). *B. thermosphacta* also has a faster anaerobic growth rate when the pH is greater than 5.8 (Knox et al., 2008). The surface pH in the study presented here was 5.7, which may have resulted in a slower growth rate for *B. thermosphacta*. The initial populations of *B. thermosphacta*, in the present study, log$_{10}$ 2.9 CFU/cm$^2$, were similar to those reported by Nowak et al. (2012), who reported *B. thermosphacta* populations on pork loins between log$_{10}$ 3-6 CFU/g.
There was a significant interaction between dissolved CO$_2$ and microbial population type (p < 0.0484). This was expected because different microbial populations have different sensitivities to dissolved CO$_2$ concentrations, with LAB having the greatest resistance, and *B. thermosphacta* and Enterobacteriaceae having intermediate resistance (Dixon & Kell, 1989). There was also a significant interaction between the dissolved O$_2$ concentration and microbial population type (p < 0.0066). The dissolved O$_2$ concentration effect had the most influence during the first 5 days within the package because that is when the greatest decrease in O$_2$ occurred (section “Dissolved carbon dioxide and oxygen concentrations”). This decrease in O$_2$ concentration resulted in an anaerobic environment in the vacuum-package. The APC bacterial population increased by log$_{10} 0.87$ CFU/g, while the *B. thermosphacta* and Enterobacteriaceae populations increased by log$_{10} 2.48$ CFU/g and by log$_{10} 2.96$ CFU/g, respectively. In contrast, the LAB population decreased by log$_{10} 1.83$ CFU/g. This observed decrease in population could be attributable to the increased oxygen content in the initial vacuum-package resulted in a temporary population reduction, as LAB have a primarily anaerobic metabolism (Hammes & Hertel, 2006). As the oxygen concentrations decreased after day 5, LAB were no longer influenced by the dissolved O$_2$ concentration and grew rapidly.

The initial pre-package surface pH had no effect (p > 0.2856) on the microbial populations because the surface pH was still in a favorable range (see section “Surface pH”). However, the post-package surface pH did influence the microbial populations (p < 0.0051), with a lower surface pH associated with a lower microbial population, or the metabolism by-products of the microorganisms could have affected the surface pH. A
surface pH on the meat products in the range of pH 5.4-5.7 can be unfavorable to spoilage bacteria (Gill, 1986), thus resulting in slower growth rates.

**Dissolved carbon dioxide and oxygen concentrations**

Dissolved CO$_2$ concentrations increased significantly over time ($p < 0.0184$) in the purge of vacuum-packaged pork chops (Fig. 4). The dissolved CO$_2$ concentrations increased over the 60 day storage period by cellular respiration of the spoilage bacteria, especially LAB, and the metabolic activity of pork chops themselves (Dainty & Mackey, 1992). The dissolved CO$_2$ concentrations were 0 mg/L on day 0, increasing to 783 mg/L on day 5, 1,500 mg/L on day 15, and 2,167 mg/L on day 30. On day 45 and 60, the dissolved CO$_2$ concentrations stabilized at 3,000 mg/L. The dissolved CO$_2$ concentrations may have stabilized because the LAB entered stationary phase at $\log_{10} 8$ CFU/g, or because 3,000 mg/L is the maximum concentration that can be maintained in purge. This also demonstrates that LAB are not affected by dissolved CO$_2$ concentrations (Dixon & Kell, 1989). The greatest increase in dissolved CO$_2$ concentrations per day, at 156.6 mg/L, were between day 0 and day 5 because the LAB populations increased rapidly by $\log_{10} 2.2$ CFU/mL for purge and $\log_{10} 2.8$ CFU/cm$^2$ for core samples during this time period. As the LAB concentrations increased, the
production of dissolved CO\textsubscript{2} also increased proportionally since it is a metabolism by-product (Hammes & Hertel, 2006). Throughout the rest of the storage period, there was a slower but proportional increase in dissolved CO\textsubscript{2} concentrations per day because the LAB populations increased by a slower rate after the initial rapid increase. The observed dissolved CO\textsubscript{2} concentrations were also consistent with the previous work conducted by Devlieghere and Debevere (2000), who reported 2,000 mg/L of dissolved CO\textsubscript{2} after 26 hours in BHI media. We would expect a slower rate for a fresh meat product because of the different food matrix between a broth system and a meat system, but we found approximately the same overall final concentration. In addition, Gill (1988) achieved greater than 1,000 mg/L dissolved CO\textsubscript{2} in pork with a pH 5.8 at 4\degree C. These studies and the present study support a final dissolved CO\textsubscript{2} concentrations of 3,000 mg/L after 60 days.

The dissolved O\textsubscript{2} concentrations decreased significantly over time (p < 0.0001) in the purge of the vacuum-packaged pork chops (Fig 4). On day 0, the dissolved O\textsubscript{2} concentrations was 8.31 mg/L before decreasing to 0.326 mg/L on day 5. This decrease in dissolved O\textsubscript{2} concentrations occurred because there was an approximate log\textsubscript{10} 3 CFU/g increase in bacterial populations between day 0 and day 5. As the bacterial populations increased, their cellular respiration rate would have also increased with more bacteria present, so the O\textsubscript{2} would have been converted to CO\textsubscript{2} (Dainty & Mackey, 1992). Between day 15 and day 45, the dissolved O\textsubscript{2} concentrations remained around 0.11 mg/L but on day 60, they increased slightly to 0.15 mg/L. The dissolved O\textsubscript{2} concentrations increased slightly at the end of the storage period because there was a loss in vacuum and gas leakage through the packaging film. Gas leakage through the packaging film occurred
because the film used in this study was not gas impermeable, it had an O2 transmission rate of 3-6 cc/m², 24 h, 1 atm, at 4.4°C and 0% relative humidity. Since the dissolved O2 concentrations in the present study remain below 0.20 mg/L, or 2%, throughout the storage period after day 5, it is consistent with the literature that O2 concentrations in vacuum-packages remains around 1% after sealing (Dainty & Mackey, 1992). These results also demonstrate that it is impossible to remove all of the air from a vacuum-package upon sealing, since less than 2% O2 is present inside the packages throughout the 60 day storage period.

Surface pH

The surface pH changed throughout the storage period (Table 1). The pre-package surface pH was consistently around pH 5.8, which is within the normal ultimate pH range of pork (Samelis, 2006). On day 0, the pre-package surface pH decreased by 0.1 pH units, but between day 5 to day 15, the post-package surface pH was 5.48, 0.3 pH units lower than the pre-package surface pH. This pH decrease occurred because of carbonic acid and lactic acid production by the spoilage bacteria and the meat product (Huis in't Veld, 1996). Carbonic acid is formed when CO2 produced by cellular respiration dissolves into the meat tissue, which acidifies the meat product. Our 0.3 unit pH decrease corresponded

<table>
<thead>
<tr>
<th>Sample Daya</th>
<th>Pre-package pHb</th>
<th>Post-package pHc</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.81 (0.11)</td>
<td>5.78 (0.08)</td>
</tr>
<tr>
<td>5</td>
<td>5.81 (0.15)</td>
<td>5.49 (0.19)</td>
</tr>
<tr>
<td>15</td>
<td>5.84 (0.20)</td>
<td>5.48 (0.11)</td>
</tr>
<tr>
<td>30</td>
<td>5.87 (0.16)</td>
<td>5.68 (0.13)</td>
</tr>
<tr>
<td>45</td>
<td>5.93 (0.16)</td>
<td>5.72 (0.17)</td>
</tr>
<tr>
<td>60</td>
<td>5.90 (0.16)</td>
<td>5.75 (0.14)</td>
</tr>
</tbody>
</table>

aEach sample day has a sample size of n=6.
bPre-package pH was measured before the pork chops were placed into the vacuum-package.
cPost-package pH was measured before microbiological analysis.
to 1,500 mg/L dissolved CO$_2$ concentration on day 15 and this decrease was similar to the 0.17 unit pH decrease seen by Devlieghere and Debevere (2000) with 2,000 mg/L of dissolved CO$_2$. LAB can also produce lactic acid as a metabolism by-product (Hammes & Hertel, 2006), so as their populations increase, so does the amount of lactic acid. On day 30, the post-package pH increased to 5.67, and increased further to pH 5.7 for day 45 to day 60. The post-package surface pH increased due to ammonia production by the spoilage bacteria as they utilized amino acids (Gill, 1976). Spoilage bacteria consume amino acids after the glucose has been completely utilized (Gill, 1976). Studies by Borch and Agerham (1992) and Gill (1976) demonstrated that the glucose in the fresh meat was completely utilized when bacterial concentrations reached $10^7$ g or log$_{10}$ 7 CFU/g. This bacterial concentration corresponds to day 15 in our results, so by day 15 all the glucose was consumed in our pork chops. After day 15, the spoilage bacteria consumed amino acids and ammonia production began causing an increase the post-package surface pH on day 30 to day 60.

Models

We found that a shelf life model could be developed from the pooled APC and LAB populations and the dissolved CO$_2$ concentrations in the purge of vacuum-packaged pork chops. Since LAB produce CO$_2$ as a by-product of their metabolism (Hammes & Hertel, 2006) and are not affected by CO$_2$ levels (Dixon & Kell, 1989), dissolved CO$_2$ concentrations could be used to estimate the LAB populations. In addition, since the APC and LAB microbial populations were shown to be not statistically different (p > 0.05), the data were pooled. However, the pooled LAB and APC populations were separated by
sample type (core and purge), as the two sample types were significantly different from each other (p < 0.0047). We developed two different models, one for core samples and another for purge samples. These models estimate microbial populations, so if the microbial population exceeds an agreed upon level for spoilage, such as log_{10} 7 CFU/g (Holley et al., 2004; Knox et al., 2008), the meat product could be considered spoiled.

The model for the core samples was determined to be a simple quadratic model:

\[ 1 \] \[ \log_{10} \text{CFU} = 4.165 + [(3.535 \times 10^{-3}) \times (\text{dissolved CO}_2)] + [(-7.171 \times 10^{-7}) \times (\text{dissolved CO}_2)^2] \]

with an adjusted R^2 of 0.89. For purge samples, the model is:

\[ 2 \] \[ \log_{10} \text{CFU} = 3.988 + [(2.898 \times 10^{-3}) \times (\text{dissolved CO}_2)] + [(-5.294 \times 10^{-7}) \times (\text{dissolved CO}_2)^2] \]

with an adjusted R^2 value of 0.87. A measured dissolved CO_2 concentration from the purge of the fresh meat product is determined, and then an estimate of the corresponding microbial population can be determined, and thus an indication of the approximate time of remaining shelf life.

These models have acceptable adjusted R^2 values for being a simple quadratic models using limited (three replications) data (Fig. 5). If additional data were available, the adjusted R^2 values would improve with a resulting improvement in the accuracy of the models. The fit would also improve if there was less variation in the initial bacterial populations. The initial microbial populations in this study differ because the pork chops were of different post-mortem ages when we received them from the retail store, varying from 3, 7, and 14 days post-mortem. If the meat was obtained from the production source during fabrication, there would likely be less variation in the initial populations. Despite
this variation, the models still had a good fit and adjusted $R^2$ values. However, these models are limited to vacuum-packaged pork chops stored at 4°C because those were the conditions tested. These models also only estimate microbial populations, and not sensory shelf life, which could be different from the microbial spoilage threshold. Despite these limitations, these models show proof of concept and prove that shelf life models can be developed from an instrument measurement, such as dissolved CO$_2$ concentrations. It also promisingly suggests that non-destructive, rapid, and relatively easy to use instruments, such as those that measure dissolved CO$_2$, can be used to estimate remaining shelf life by an approximate microbial population. These models also provide an indication of a “real-time” measurement of shelf life, which is an improvement upon the three-day, “historical” data delivered currently by traditional plate count methods to determine shelf life.

Figure 5. Regression lines for the models for vacuum-packaged pork chops at 4°C for 60 days for core (A) and purge (B) samples. LAB populations for each replication are shown to demonstrate the varying initial microbial populations.
**Packaging film biofilm**

SEM images were taken of the packaging film touching the lean portion of the pork chops on each sample day to document the biofilm formation on the packaging film throughout the storage period. The first noticeable development of the biofilm was on day 5, as illustrated by Fig. 6A. This SEM image (Fig. 6A) shows a protein layer deposited on the packaging film that comes from the purge and from touching the lean portion of the pork chops. There is also a smooth globular sphere, which is a fat globule, so fat was deposited as well. On day 15, some spoilage bacteria were evident, embedded into the biofilm matrix. The biofilm also showed a distinct two layer appearance (Fig. 6B), and the black line in the image shows the approximate division of these two layers. This two

![Figure 6. SEM images of the packaging film in contact with the lean portion of the vacuum-packaged pork chops. A = day 5; B = day 15; C = day 30; D = day 60.](image)
layer biofilm consists of the protein layer seen on day 5 covered by the biofilm matrix formed by the spoilage bacteria (black arrow). This matrix formed by the bacteria is more structured than the protein layer and has a “honeycomb” appearance. Also in Fig. 6B, the black background is the plastic of the packaging film, which the biofilm has pulled away from during SEM sample preparation. On day 30, more spoilage bacteria were evident, embedded in the biofilm, as shown by the arrows pointing to the smooth rod-shaped objects (Fig. 6C). This is consistent with more spoilage bacteria present overall in the package, so more would be embedded in the packaging film. On day 45 and 60, the biofilm appeared the same, with more bacteria embedded in the biofilm and the biofilm filling up with fat deposits and loose protein, as shown by Fig. 6D. More spoilage bacteria were embedded in the biofilm because of the larger concentration of spoilage bacteria present, upwards to log_{10} 8 CFU/cm^{2}. Although relatively few bacteria are evident in the biofilm, most may not be visible because they are embedded beneath the surface. The biofilm on day 45 and 60 had more fat deposits filling in the honeycomb structure of the biofilm as well as another protein layer being deposited on top of the biofilm matrix (Fig. 6D). This second protein layer was starting to occur because the pork chops inside the package were decomposing due to the extensive microbial spoilage occurring. This microbial spoilage would have freed up some protein from the pork chops and allowing it to settle on top of the biofilm layer. The protein could also come from the purge because as the storage period approached 60 days, the purge proteins started to precipitate. This protein precipitation could have also contributed to the second protein layer.
These SEM images demonstrate that a biofilm does form on the packaging film of vacuum-packages. It first begins with a protein layer and then the matrix formed by the bacteria forms on top of this protein layer. As the spoilage bacteria concentration increases, the amount of bacteria embedded into the biofilm also increases. Not all the bacteria can be seen because they could be embedded beneath the surface. Towards the end of shelf life, the biofilm matrix fills in with fatty deposits and loose protein from the purge and the pork chops due to the extensive microbial spoilage occurring. The significant element these SEM images demonstrate is that there is a tri-phase system occurring inside the vacuum-package, which consists of the purge, the fresh meat product, and the biofilm on the packaging film.

Conclusion

The results of these experiments demonstrated that the spoilage bacteria in vacuum-packaged pork chops increase the dissolved CO$_2$ and decrease the dissolved O$_2$ concentrations. The dissolved CO$_2$ concentrations increased because of increasing LAB concentrations that produce CO$_2$ as a metabolism by-product (Hammes & Hertel, 2006). The increasing spoilage bacteria concentrations also decrease the dissolved O$_2$ concentrations because of the increased rates of cellular respiration occurring (Dainty & Mackey, 1992). These results also showed that LAB dominated the spoilage flora of vacuum-packaged pork chops because they maintained a log$_{10}$ 7 CFU/cm$^2$ or greater population throughout the majority of the storage period. After LAB, the Enterobacteriaceae had a population of around log$_{10}$ 5 CFU/cm$^2$, so they contributed to spoilage but did not dominate the spoilage flora. In addition, *B. thermosphacta*
contributes to spoilage, but they also do not dominate because they had a reduced population \( \log_{10} 4 \text{ CFU/cm}^2 \) in comparison to the other bacterial types measured.

The results also show that as the LAB population increases, so do the concentrations of dissolved CO\(_2\), so dissolved CO\(_2\) concentrations could be used to predict the population of LAB present. This data was used to develop models which estimated microbial populations based on dissolved CO\(_2\) concentrations for the core and purge samples. These models can be used to estimate shelf life by approximating a microbial population from a dissolved CO\(_2\) concentration from the purge of vacuum-packaged pork chops. The models had a good fit despite variation in initial microbial populations and few replications, but the fit would improve with more replications. The models could also have a practical application by being able to compensate for variation in initial microbial populations, since the post-mortem age and transportation conditions of fresh meat products will vary. However, these models are limited to vacuum-packaged pork chops that predict microbial shelf life and not sensory shelf life, which can be different from microbial shelf life. Despite these limitations, our results demonstrate proof of concept that it is possible to develop a shelf life model from an instrument measurement, such as dissolved CO\(_2\) concentrations. It also shows that a “real-time” measurement of shelf life is possible, which is favored over the “historical” data delivered by microbiological methods (Bruckner et al., 2013; McDonald & Sun, 1999; McMeekin & Ross, 1996b). These models are also relatively inexpensive, less-time consuming, and non-destructive, because the purge is being used, not the product to predict the remaining shelf life. The models we developed could direct more
development into using instrument measurements to base more shelf life models for other food products and different temperature conditions.

The SEM images of the packaging film clearly show that a biofilm develops on the packaging film throughout the shelf life of a fresh meat product. A protein layer developed first, followed by the matrix of bacteria on top of that layer by day 15 in the storage period. This causes a distinct two layer appearance to the biofilm and the spoilage bacteria are found embedded within this biofilm. By day 45 and 60 of the storage period, another protein layer begins to deposit on top of the matrix because of the loose protein precipitating from the purge and being released from the extensive spoilage of the pork chops. However, the SEM images demonstrate there is a tri-phasic system involved inside the vacuum-package to influence spoilage. It is the purge, the meat product, and the biofilm and they interact together to influence the spoilage composition and process.

Acknowledgements

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References


CHAPTER 4. GENERAL CONCLUSIONS

Since the shelf life of meat products will always have to be determined, the meat industry will want to make shelf life determination faster, easier to conduct, and less expensive. Using instrument measurements, particularly for dissolved carbon dioxide, for shelf life determination could help solve this issue. This study showed proof of concept that it is possible to develop mathematical models based upon dissolved carbon dioxide concentrations to estimate microbial populations. It is possible because the spoilage bacteria inside the vacuum-packaged pork increased the dissolved carbon dioxide and decreased the dissolved oxygen concentrations through cellular respiration. As the populations of the spoilage bacteria increased, especially the LAB who produce carbon dioxide as a metabolism by-product, so did the concentration of dissolved carbon dioxide. This information about increasing microbial and LAB populations was used to develop mathematical models.

The two mathematical models developed were for core and purge samples because they were significantly different from each other (p < 0.0047). The model for core samples is:

\[ [1] \log_{10} \text{CFU} = 4.165 + [(3.535 \times 10^{-3}) \times (\text{dissolved CO}_2)] + [(-7.171 \times 10^{-7}) \times (\text{dissolved CO}_2)^2] \]

with an adjusted \( R^2 \) of 0.89. For purge samples, the model is:

\[ [2] \log_{10} \text{CFU} = 3.988 + [(2.898 \times 10^{-3}) \times (\text{dissolved CO}_2)] + [(-5.294 \times 10^{-7}) \times (\text{dissolved CO}_2)^2] \]

with an adjusted \( R^2 \) of 0.87.

These models estimate shelf life by approximating a microbial population from a dissolved carbon dioxide concentration inside the purge of the vacuum-packaged pork.
chops. For example, if the microbial population is greater than $\log_{10} 7$ CFU/g, the product could be spoiled because $\log_{10} 7$ CFU/g is the spoilage threshold.

Currently, these models are limited to vacuum-packaged pork chops stored at 4°C and only predict microbial shelf life, not sensory shelf. However, despite these limitations, this study demonstrates it is possible to use instrument measurements to estimate microbial populations and the shelf life of packaged meats. This study also highlights that instrument measurements have a capability of being faster, less expensive, and non-destructive for estimating microbial populations than traditional bacterial enumeration methods. An instrument measurement is faster because it takes about 10 minutes to complete, resulting in an almost “real-time” measurement. Whereas, bacterial enumeration requires three days and delivers “historical” data. An instrument measurement could also be less expensive because it may not need laboratory personnel or a laboratory to be conducted. It only requires the equipment to conduct the measurement and a calculator or computer to run the mathematical model. Furthermore, an instrument measurement could be non-destructive because it only uses the purge of the meat product, which is undesirable by the consumer anyway. A sample of purge could be removed from the product and the rest of the product could be sold. All of these benefits of instrument measurements can make them more favorable than traditional shelf life estimates, so they would receive more consideration and research in the near future.

Possible continued research for instrument measurements for estimating shelf life are more validation of the current models provided in this study, temperature abuse conditions, using different meat cuts and their different mitochondria levels to see how they affect dissolved carbon dioxide concentrations, and also a retail package model. The
retail package model would look at retail (overwrap) packages that originate from vacuum-packages and see how the dissolved carbon dioxide measured in the vacuum-package corresponds to retail display shelf life. There could also be more research with other purge-producing products or other products in different industries that have a measurable aqueous portion to determine dissolved carbon dioxide concentrations.
Figure 7. Original Packaging Film. This is an image of the inner surface of a vacuum-package film. The water bubbles formed during the fabrication of the package is evident, along with dust particles.

Figure 8. Inner Packaging Surface. Another image of the inner surface of a vacuum-package.
Figure 9. Plain Package Detail. This is a close up image of the dust particles and water bubbles seen in Fig. 7 and 8.

Figure 10. Close Detail of Dust and Water Bubble Edge. This image shows a closer detail of the dust particles and water bubble edge seen in Fig. 7, 8, and 9.
Figure 11. Water Bubble Edge. Another image of the water bubble edge formed during the fabrication process.

Day 5 SEMs

Figure 12. Day 5 Biofilm. Broad view of the biofilm starting to develop on the packaging film. There is a smooth layer from either fat or purge, whereas the broken up sections consist of a protein layer.
Figure 13. Day 5 Biofilm Protein Layer. This is a closer up of the “broken-up” sections seen in Fig. 12. This is the protein layer that develops first before the biofilm forms on top.

Figure 14. Day 5 Protein Layer. This SEM image demonstrates the protein layer that is deposited on the packaging film.
Figure 15. Day 5 Protein Fat Connection. This image reveals a closer look at the protein layer merging with the smooth layer seen on top of the protein, which could be a layer of fat and purge.

Figure 16. Biofilm Initiator. This image reveals the very first layer that develops on the packaging film. It is fragile, but it forms the base of the protein layer seen in Figures 13, 14, 15.
Figure 17. Protein Layer Detail. This image shows a close view of the protein layer seen developing on day 5 of the storage period. It shows the protein fragments attaching to the very thin film seen in Fig. 16.

Figure 18. Purge Layer. This image shows the purge layer that covers the protein layer underneath (as seen by the holes in the purge layer).
Figure 19. Protein Purge Layers. More detail of the protein layer on day 5 of the storage period. The purge layer is thin and stretches over the protein layer.

Figure 20. Protein, Purge, and Initiator Film. This image reveals the initiator (large object stretched across the image) that forms on the packaging film. It also shows a section of the purge still covering the developing protein layer.
Figure 21. Fleck Detail. This is a close-up of the fleck seen on the purge glob in Fig. 20.

Figure 22. Initial Stages. This image shows the initial stages of the biofilm, before it is clearly formed into its layers. It shows the protein layer, fat deposits, and some purge sections.
Figure 23. Initial Stage of the Protein Layer. This reveals the initial protein layer (long spindle-like fibers) being formed on the thin film initiator seen in Fig. 16 before the larger, deeper protein layer appears.

Figure 24. Initial Protein Layer Detail. This image shows a detailed view of the initial protein layer as the larger protein layer forms over top.
Figure 25. Protein Layer Development. Another image displaying the development of the protein layer.

Figure 26. Overview of Packaging Film. This SEM image reveals the thin layer formed on the packaging film, then the various stages of the other components of the biofilm, the purge layer and the protein layer. It also shows the biofilm is uneven and still in its developmental stages.
Figure 27. Detail of the Purge Layer. This shows detail of the smooth yet uneven purge layer and some of the chunks of the protein layer forming on top.

Figure 28. Another Overview of the Packaging Film. More views of the purge layer and protein layers that develop unevenly.
Figure 29. **Protein Islands.** There are spots where the protein layer develops in clumps, showing that biofilm development is uneven.

Figure 30. **Possible Bacterial Clump.** This is a close up of a clump seen on the packaging film, and by the small spheres, it could be a bacterial clump.
**Figure 31. Irregular, Uneven Biofilm.** This again shows there are clumps of bacteria or protein that form before the full biofilm has matured.

**Figure 32. Protein or Bacteria Clump.** This shows a bacteria or protein clump resting on the purge layer (rippled surface).
Day 15 SEMs

**Figure 33. Day 15 Biofilm.** This image reveals the detail of the developing biofilm. There is a bacterium embedded in the film in the upper left hand corner, and there are smooth globules present, which are fat deposits.

**Figure 34. Embedded Spoilage Bacteria.** This image shows how the spoilage bacteria are embedded into the biofilm (center of image).
Figure 35. Bacteria Layer Development. This SEM image shows the beginnings of the “honeycomb” matrix that is formed by the bacteria that is evident throughout the rest of the storage period (upper left). There is also bacteria present (lower right hand corner).

Figure 36. Bacteria, Fat, Protein. This image shows more spoilage bacteria embedded in the biofilm, in the protein layer before the “honeycomb” matrix forms (upper left, center bottom, upper right). There are also many fat globules present.
Figure 37. Matrix Detail. This image shows the detail of the “honeycomb” matrix. More bacteria are embedded as well (upper left corner).

Figure 38. Two Layer Biofilm. This SEM reveals the two layer nature of the biofilm, with the protein layer underneath the “honeycomb” matrix layer.
**Figure 39. Two Layer Edge.** This shows the edge of the “honeycomb” matrix formed by the spoilage bacteria over the protein layer seen on day 5. There are also bacteria embedded in the protein layer as well (center).

**Figure 40. Bacteria!** This SEM shows a close-up of the bacteria found within the biofilm.
Figure 41. **Detail View of Fat Globules.** This is a detailed view of the fat globules seen on some undeveloped sections of the biofilm, even at day 15.

**Day 30 SEMs**

Figure 42. **Day 30 Biofilm.** This SEM image shows the structure of the biofilm and how it begins to fill in overtime with fat deposits. There is also some bacteria present (upper right corner).
Figure 43. Bacteria and Biofilm. There is bacteria present on the surface of the biofilm, as well as being embedded in the biofilm (upper right, lower right, left middle).

Figure 44. Honeycomb Matrix. This image shows the “honeycomb” matrix present in the biofilm formed by the bacteria.
**Figure 45. Lone Bacterium.** This image shows that in the “honeycomb” matrix seen in Fig. 44, there are bacteria present, as shown here in the center of this image.

**Figure 46. Loose Strings and Protein.** This image show that even though the biofilm may be developed on the packaging film, there are still unique areas present. This one has more protein present followed by many “strings”. There is also a spiral object present near the center of the image.
Figure 47. **Spiral.** This is a close-up of the spiral object seen in Fig. 46. This could be a bacterium because of its smooth appearance.

Figure 48. **More Spoilage Bacteria.** This image clearly shows the abundance of spoilage bacteria embedded in the biofilm. More bacteria could be beneath the surface.
Figure 49. Five Bacteria. This SEM image shows a close-up of the spoilage bacteria embedded or on the surface of the biofilm on day 30 of storage.

Day 45 SEMs

Figure 50. Unique Biofilm Sections. This SEM image shows that at day 45 of the storage period there will be different sections to the biofilm. This section shows possible bacteria or smaller fat deposits as well as other types of bacteria. It even shows how deep the biofilm can be.
Figure 51. Honeycomb Edge. This image reveals that at the edge of the honeycomb matrix, there is a smooth section of undeveloped biofilm. It also shows that the matrix forms by reaching out in tendrils and building upon those layers.

Figure 52. Many Biofilm Layers. Even though the honeycomb matrix is the dominant part of the biofilm, there are many layers present. This one has some protein and bacteria clumps forming on top of the honeycomb layer.
Figure 53. Honeycomb Mess. This image shows that as the storage period increases, the honeycomb matrix becomes less neat and becomes more filled in with fatty deposits, giving it a “messier” look.

Figure 54. Frozen Bacteria. This image caught a bacterium in the middle of cell reproduction. This image also shows how the honeycomb is getting “messier” and more filled in the later stages of the storage period, as seen in Fig. 53.
**Figure 55. Undeveloped Biofilm.** This image reveals some of the undeveloped biofilm sections. It has the thin purge layer that starts the process, along with the protein layer being deposited on top, but it lacks the honeycomb.

**Figure 56. More Protein Layer.** This image shows that as the storage period increases, another protein layer, like that seen in day 5, begins to develop on top of the honeycomb matrix. This protein comes from the purge proteins and from the pork chops.
Figure 57. Embedded Bacteria Day 45. This is a close-up of the center of Fig. 56, which shows how well the bacteria are embedded into the biofilm.

Day 60 SEMs

Figure 58. Late Stage Protein Layer Detail. This SEM image on day 60 of the storage period shows some detail of the second protein layer that develops on top of the honeycomb matrix.
**Figure 59. Filled-In Honeycomb.** This image shows how much the honeycomb matrix has filled in since its appearance at day 15. The honeycomb part is harder to distinguish with the protein and fat being deposited.

**Figure 60. Overview Day 60 Biofilm.** This image shows how complex the biofilm has become throughout the storage period. It also shows that the biofilm is not a level, even surface, it protrudes and has uneven sections.
Figure 61. Elongated Bacterium? This image shows a possible elongated, spiral like bacterium present. This is a close-up of the elongated bacterium seen in Fig. 60.

Figure 62. Honeycomb and Two Bacteria. In this image, the honeycomb matrix again shows how it is being filled in protein and fat deposits. However, there are still bacteria present (middle, middle bottom left).
**Figure 63. More Protein.** This image shows how the protein layer seems to fit with the honeycomb layer as it develops. There are also always bacteria present at this stage (middle top right).

**Figure 64. Hidden Bacteria.** This SEM shows a detailed view of the bacteria seen in Fig. 63.
Figure 65. Always Bacteria. This SEM shows that there will always be bacteria present in the biofilm, especially at day 60 when microbial counts are around log_{10} 8 CFU/g. It also shows the “messy” texture of the honeycomb as it fills in with fat and protein, and of the protein layer.

Figure 66. Messy Detail. This SEM shows a detailed view of the bacteria seen in Fig. 65. It also shows more detail of the “messy” texture of the honeycomb as the biofilm advances in age.