THESIS

# AN EVALUATION OF THE EFFECTIVENESS OF FRESHCASE® TECHNOLOGY TO EXTEND THE SHELF LIFE OF BEEF AND PORK

Submitted by

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ABSTRACT

AN EVALUATION OF THE EFFECTIVENESS OF FRESHCASE® TECHNOLOGY TO EXTEND THE SHELF LIFE OF BEEF AND PORK

This research evaluated the effect of FreshCase®, a novel packaging technology that has been shown to extend the shelf life of whole muscle beef and ground beef, whole muscle pork and ground pork sausages by stabilizing fresh meat color. FreshCase® utilizes a high-barrier nitrite containing film in conjunction with vacuum packaging technology. Storage life was defined by the number of days required to reach an aerobic psychrotrophic plate count of $10^7$ log CFU/g, and all treatmes were stored and evaluated until storage life expired. The storage life for beef steaks stored in FreshCase® packages at 4°C was 36 days; and the shelf life for ground beef stored in FreshCase® packages at 4°C was 12 days. The shelf life for pork chops stored in FreshCase® packages at 1°C was 46 days; and the shelf life for ground pork sausages stored in FreshCase® packages at 1°C was 19 days. Values for CIE a* (redness) were greater ($P < 0.05$) for FreshCase®-packaged samples for both beef steaks and ground beef with the increase of storage time. Both pork chops and sausages stored in FreshCase® packages retained more acceptable redder color ($P < 0.05$) than those stored in Control packages throughout storage. By the point at which spoilage was detected, off-odors of putrid, acid, sour and rancidity for FreshCase®-packaged samples were detected, but were present at very low level. Likewise, by the point of spoilage, no significant differences ($P > 0.05$) were found between samples in control and FreshCase® packages in all off-odors detection for both pork chops and sausages and the intensities of these off-odors were very low. Also, beef

and pork samples resulted in very low (1.19 mg malonaldehyde/kg and 0.55 mg malonaldehyde/kg, respectively) TBA values throughout storage. Therefore, utilization of FreshCase® Technology in whole muscle beef and ground beef, whole muscle pork and ground pork sausages results in a more stable fresh red meat color with a low level of off-odors, and lipid oxidation. FreshCase® did not influence microbial growth in vacuum packaged samples.

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## OBJECTIVES OF THESIS

1) Evaluate the ability of FreshCase® technology to extend the shelf life of whole muscle beef and ground beef, whole muscle pork and ground pork sausages.

2) Identify the maximum time of refrigerated storage before bacteria grow to a level indicative of spoilage ($10^7$ CFU/g) in whole muscle beef and ground beef, whole muscle pork and ground pork sausage that is vacuum packaged with nitrite impregnated film.

# CHAPTER II

## Review of Literature

### Definitions of food shelf life

There are several definitions of food shelf life. According to the Institution of Food Technologits (IFT) in the United States, it was defined as "the period between the manufacture and the retail purchase of a food product, during which time the product is in a state of satisfactory quality in terms of nutritional value, taste, texture and appearance"(Anon., 1974). Accounting for consumers' perceptions of food quality, Labuza and Schmidl (1988) defined shelf life as "the duration of that period between the packing of a product and the end of consumer quality as determined by the percentage of consumers who are displeased by the product". Recently, shelf life was defined for the first time in EU legislation, in Commission Regulation (EC) No. 2073/2005 as: "either the period corresponding to the period preceding the 'used by' or the minimum durability date, which was defined as the 'date until which a foodstuff retains its specific properties when properly stored' in Articles 9 and 10 of Directive 2000/13/EC" (Roberson, 2010). The storage time for fresh meat until its spoilage, when adverse changes in color, flavor, aroma, texture and nutritive values occur, is considered as the shelf-life of meat and meat product (Borch et al., 1996; Gray, 1996).

### Factors affecting meat shelf life

There are both intrinsic and extrinsic factors that affect the shelf life of meat and meat products. The intrinsic factors include initial pH of meat, and the concentration

of nutrients that influence types and growth of bacteria, such as glycogen, glucose, glucose-6-phostate, L-lactate and fat (Blixt and Borch, 2002). The extrinsic factors include oxygen and light exposure, and storage temperature (Lambert et al., 1991). Additionally, packaging properties, such as modified gas mixtures, antimicrobial effect, etc. also play an important role in determining the shelf life of meat (Roberson, 2010).

Micro-organisms

Lambert et al. (1991) reported that the most significant factor causing spoilage of meat was microbial growth. The presence of bacteria influences all meat sensory properties, including appearance, texture, odor and flavor. Additionally, bacteria growth reduces product safety, which is of great concern to consumers of meat products (Prendergast, 1997). Generally speaking, during the refrigerated storage of meat, the maximum level that bacteria can grow to is $10^7$-$10^9$ CFU/cm$^2$ (Borch et al., 1996). Therefore, bacteria counts are considered as a primary indicator of spoilage point, combined with off-odor, off-flavor and discoloration that are associated with high plate counts (Ayres, 1955; Sutherland et al., 1976).

The initial bacterial level of meat fluctuates depending on species and other factors, but usually is around $10^2$-$10^3$ CFU/cm$^2$ or gram (Jackson et al., 1992). The initial bacterial load is extremely important to meat shelf life (Lambert et al., 1991). Holding other factors constant, it is known that lower initial bacteria counts are associated with the longer shelf life of meat. For instance, beef stored at 0°C with an initial microbial counts at 6 x $10^4$ CFU/cm$^{-2}$ had shelf life of 11days, while beef stored

at 0°C with an initial load of 65 CFU/$cm^{-2}$ displayed spoilage by day 21 (Ayre, 1960).

The predominant bacteria related to spoilage of pork and beef under refrigerated temperature, are *Brochotrix thermosphacta*, *Carnobacterium* spp., *Enterobacteriaceae*, *Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Shewanella putrefaciens* (Borch et al., 1996). Characteristics of major spoilage bacteria of fresh meat are shown in Table 2.1.

Presence of pathogenic bacteria is another growing issue resulting in meat safety problems today. This problem is that only a few cells of pathogens are required to cause a significant food safety risk and such cells may be present on meat long before plate counts become large enough to cause spoilage (Dainty, 1989).

pH

The level of free H+ ions and the concentration of undissociated acid affects survivability of bacteria, which when considered in combination with other environmental factors, can determine the types of bacteria on meat. Generally speaking, bacteria grow more rapidly on meat with high pH (>6.0) than those on meat with normal pH (5.5-5.7) (Rao, Nair and Sakhare, 1998). A study by Boers (1992) showed that vacuum-packaged pork had a shorter shelf life than vacuum-packaged beef. One reasonable explanation was that the depletion of glycogen and glucose in pork is faster than in beef, leading to a higher ultimate pH (Boers, 1992; Boers and Dijkmann, 1994).

Oxygen

Likewise oxygen concentrations can impact the specific types of bacteria on

meat during storage. Aerobic bacteria, such as *Pseudomonas*, require the presence of oxygen to survive. Anaerobic bacteria favor an environment without oxygen; an example is *Clostridium botulinum*, also a spore-forming, toxin producing pathogen. The facultative anaerobic bacteria can grow with or without oxygen; the examples include lactic acid bacteria and *Enterobacteriaceae* (Gill and Gill, 2010).

Oxygen molecules react with unsaturated lipids in meat and a lipid peroxidation cascade begins, which ultimately results in the rancid odor and flavor in meats (Campo et al., 2006). Additionally, lipid peroxidation also results in acceleration of the formation of metmyoglobin, which is responsible for brown color of meat (Lynch et al., 2001).

Removed of oxygen by 100% $CO_2$ in packages extends shelf life very effectively. For example, shelf life of chilled chicken is 12 days longer by using packaging with 100% $CO_2$ than using normal packages with only 6 days shelf life (Winger, 2000). Pork stored in pure $CO_2$ at 4°C, has shelf life of 40 days, compared with 10 days shelf life for pork stored in air (Bickstad et al., 1981).

Storage Temperature

Bacteria will grow and multiply unless meat and meat products are stored under -10℃, which is freezing temperature (Corry, 2007). There are four types of bacteria based on the temperature requirement for growth: 1) thermophiles, which prefer temperature as high as 55-65°C for growth; 2) mesophiles, which grow under room temperature ranging from 20-35°C; 3) psychrophiles, which grow at an optimal temperature of 25-30°C; and 4) psychrotrophs, which can grow attemperating less

than 0°C (ICMSF, 1980). In the United States, the psychrotrophic group is the most important bacteria responsible for spoilage of meat chilled at refrigerated temperature.

It was approved that storage life is inversely related to storage temperature (Lee, et al., 1983). Generally speaking, meat shelf life decreases proportionally to increased storage temperature over the optimum temperature. Gill and Shand (1993) concluded that "the storage life attainable is 100% at -1.5°C, 70% at 0°C, 50% at 2°C, and 15% at 10°C. Beef stored in vacuum packages at a temperature of -1.5°C has shelf life as long as 14 weeks when the bacterial counts reach about $10^7$ CFU/$cm^2$, but only three weeks when stored at 4°C (Blix & Borch, unpublished results). In addition, improper storage temperature promotes the growth of pathogenic microorganisms (Seideman & Durland, 1983). Furthermore, the solubility of oxygen in meat fluids increases when meat is stored at a low storage temperature, resulting in a deeper oxymyoglobin layer on meat surfaces (Hood, 1984). It was reported that low storage temperature has a negative effect on the rate of oxidation to metmyoglobin (Solberg, 1968) and a positive effect on the color stability of pork (Buys et al., 1993). Therefore, pork retains better color stability and displays better visual appearance when stored in atmosphere or packaging at subzero temperature of -1.5°C (Jeremiah and Gibson, 1997). Similarly, the solubility of $CO_2$, which has a microbial inhibitory effect, is increased when the storage temperature declines. This is the reason why the lag phase of microbial growth is longer at lower storage temperatures, achieving a longer shelf life of meat in modified atmosphere packages and vacuum packages (Dainty and Mackey, 1992).

**Meat Spoilage Signs**

Meat spoilage was defined by Gill (1986) as "any single symptom or group of symptoms of overt microbial activity, manifest by changes in meat odor, flavor or appearance". Generally speaking, there are two types of changes resulting in meat perishability, namely chemical changes and microbiological changes. The physical changes, such as bruising of fruits and vegetables, crushing of dried snack foods, freezer burn in frozen foods and so on, are less typical on meat during the storage time (Singh, 2000). The chemical changes include oxidative reactions, especially lipid oxidation that alters the flavor of lipid-containing meat, and non-enzymatic browning that causes the change of appearance.

The odor of meat changes gradually from a fresh "meaty" smell to a dairy/buttery/fatty/ cheesy non-fresh smell, and eventually to a sweet/fruity and finally to putrid odor with the growth of bacteria on meat (Dainty et al., 1985). Rancid odor develops soon after slaughtering and increases in intensity by the oxidation of lipids till the spoilage point (Campo et al., 2006). The sour/acid off-odor is typically related to lactic acid bacteria and their byproducts, lactic acid and acetic acid (Dainty & Mackey, 1992). Sulphur-containing compounds are produced by *Lactobacillus sake*, *pseudomonas* spp. and *Enterobacteriaceae*.

The color of fresh beef is bright cherry-red and the color of fresh pork is grayish-pink. During storage, meat color changes with the oxygen concentration and microbial activity. Under aerobic conditions, meat color changes to brown over time. Under anaerobic conditions, meat color turns to purple. Green color associated with

sulphmyoglobin sometimes can be observed, due to hydrogen sulphide produced from cysteine by *Lactobacillus sake* and when the glucose and oxygen concentration is low (Egan et al, 1989).

The four major components of meat are water, protein, lipid and carbohydrates, which are also the nutrients needed for microbial growth. The shelf life of meat can be determined by the growth rate and population of spoilage micro-organism. Different from pathogenic micro-organism, the spoilage micro-organisms, including bacteria, molds and yeast, do not usually cause illness. However, they are responsible for off-flavor, off-odor, as well as discoloration and gas production (Dainty et al., 1989). There are four phases of the natural growth curve of the spoilage bacteria, namely lag phase, exponential phase, stationary phase and death phase. Meat spoilage occurs when bacteria growth is in its stationary phase.

**Packaging Technology**

Since meats are also perishable commodities, the packaging technology has tremendous effect on meat quality during storage and distribution. In recent years, packaging technologies and the packaging industry have developed very rapidly, due to the increasing demand for extension of meat shelf-life and improvement of meat safety. Also, consumers desire more convenient, environmental friendly packages that require less product preparation time (Kerry, Grady & Hogan, 2006).

In order to inhibit or delay chemical and microbial changes, different types of packaging have been developed to avoid further microbial contamination, achieve slower lipid oxidation, reduce weight loss (i.e., shrink), retain better color and texture

appearance in meat at the retail level (Brody, 1996).

The primary packaging material is plastic in today's meat industry. There are several plastics commonly used. Polyolefins are the most widely used polymers as internal linings in flexible pouches for meat due to the heat sealing properties (Shorten, 1982). These polyolefins include polyethylene (PE) and Polypropylene (PP). Stöllman, Johansson and Leufven (2000) described Polyvinyl Chloride (PVC) as a hard, stiff, and clear, with excellent resistance to moisture, low gas permeability, and high impact strength film materials. It also provides good resistance to oil and fats. Due to its good gas barrier properties, ethylene vinyl alcohol (EVOH) is widely used in packaging. Sterilizable pouches and boil-in-bag often use polyethylene terephthalate (PET) as a packaging component (Stöllman, Johansson & Leufven, 2000). The development of novel packaging techniques stimulates the remarkable development of the packaging materials, which also meets the environmental considerations (Yokoyama, 1992).

Types of Packaging

For raw meat stored under refrigerated temperature, there are various packaging options, such as air-permeable packaging, vacuum packaging, high $O_2$ MAP, and low $O_2$ MAP with anoxic gases (Zhou et al., 2010). Specific spoilage bacteria can survive in meat stored in different packages (Table 2.2)

Air-Permeable Packaging

Red meats are mainly packaged in polystyrene trays overwrapped with a PVC film for retail display. A low density polyethylene (LDPE) or PVC coating may be

used for the inner surfaces of the trays, and the soaker pad is used to absorb the exudation from the meat. Consumers are familiar with overwrapped trays, which are also inexpensive. Additionally, air-permeable films allow off-odor volatiles to form from lipid oxidation and to escape from the package, which prevents lipid oxidation from becoming the major determinant of shelf life in overwrapped packages (Mart ńez et al., 2007). However, with the presence of $O_2$, aerobic bacteria, such as *Pseudomonas* spp., can grow to high concentrations, which will limit the shelf life of meat (Ledward, 1984). What's more, the permeability of the film is not high enough to maintain high oxygen level which is required to retain the bloomed bright red color (Siegel, 2010). Additionally, the oxygen depletion by bacterial respiration results in low oxygen partial pressure in the package, ultimately causing the unacceptable brown color due to the formation of metmyoglobin (Ledward, 1984). Therefore, fresh meat is preferably sold within two days of being prepared at a retail or central cutting facility when packaged in $O_2$-permeable film (Gill et al., 2002).

The development of master packs has extended the shelf life of meat in overwrapped trays. Multiple overwrapped tray packages can be enclosed in a large barrier pouch containing anoxic gas (McMillin et al., 1999). Once the overwrapped permeable film package is removed from the master pack for retail display, meat is exposed to air to become oxygenated and bloom to a bright red color associated with fresh meat pigments (Belcher, 2006).

Vacuum Packaging (VP)

Vacuum packaging is one of the most common methods used to change the

atmosphere of a meat package (Lambert, et al., 1991). A three layer co-extrusion of ethyl vinyl acetate/polyvinylidene chloride/ ethyl vinyl acetate, limiting the $O_2$ permeability less than 15.5 ml $m^{-2}$ /24h at 1 atmosphere because of the polyvinylidene chloride layer, which is usually used for primal cuts as the VP materials (Jenkins & Harrington, 1991). Less than 1% $O_2$ (v/v) and 10-20% $CO_2$ (v/v) are in the headspace of a package under good vacuum conditions (Lamber et al., 1991). According to results from an experiment conducted by Newton and Rigg (1979), it was known that shelf life of vacuum packaged meat declined with increased $O_2$ permeability of the film, as well as the increase of the growth rate and final counts of *Pseudomonas* species bacteria on the meat. Newton and Rigg (1979) also demonstrated that meat stored in packages using an $O_2$ scavenging system resulted in a shelf life of over 15 weeks.

The change of $O_2$ and $CO_2$ in vacuum packages affects the type of microbes and their population, which can determine the type of the spoilage of the meat. After using the limited quantity of $O_2$ for the respiration of microbes, the predominant microbes in the packages shift from aerobic *Pseudomonas* species to aerotolerant *Lactobacillus* species or facultative anaerobes, such as B. *thermosphacta* and *Enterobacteriaceae* (Gill and Tan 1979, 1980). During the later storage period, the condition of the package is anaerobic, and Lactobacillus grows more rapidly than either B.*thermosphata* or *Enterobacter* at refrigeration temperatures, becoming the predominant bacteria in vacuum-packaged meat (Raccach and Baker, 1978; Dubois et al. 1979; Ahn and Stiles, 1990).

With the absence of $O_2$ in vacuum packages, lipid oxidation can be minimized. Additionally, vacuum packages limit reductions in weight from dehydration, allow aging within the package, prevent additional contamination, and extend meat shelf life. For primal cuts of low or normal pH, the utilization of vacuum packaging can extend the shelf life by about five times over those using overwrapped packages (Johnson, 1974; Seman et al., 1988).

Meat displays brown color due to formation of metmyoglobin, which causes negative consumer perception. Discoloration due to residual $O_2$ in the package is a major disadvantage. At partial pressure of oxygen in the package of between 4-10 mmHg, metmyoglobin will be formed (Renerre, 2010). Even meat is perfectly vacuum-packaged, under anaerobic condition; the color of meat is purplish due to the deoxymyoglobin, which is still unacceptable to the consumers. Small cuts only have two-fold extension of shelf life due to this color deterioration, indicating that vacuum packaging is not suitable for display purpose. Furthermore, if the meat is exposed to an oxygen-containing atmosphere due to vacuities between a film and the meat surface by ineffective vacuum packaging, relatively early color deterioration of an undesirable brown color can be obtained due to the oxidation of myoglobin (O'Keefe and Hood, 1982; Grau, 1983). If bone is present, package may be punctured due to deformation of cuts, because package and meat are subjected to mechanical strain by using vacuum packaging technology (Seideman et al., 1979). Consequently, it is ineffective to apply vacuum packaging for whole carcasses or small cuts of any shape by impeding the application of the film to all surfaces closely (Gill, 1990).

Vacuum skin packaging (VSP), where a film of high $O_2$ permeability is first applied to the meat and subsequently applied a second film with low $O_2$ permeability over that, has been used for red meat. Therefore, meat can bloom to a bright red color after removal of the inner vacuum skin film and exposed to the air (Brody, 1996).

Modified Atmosphere Packaging (MAP)

MAP extends shelf life of fresh meat (Luño, et al., 2000). Young et al. (1988) defined MAP as the "enclosure of food products in high gas barrier materials, in which the gaseous environment has been changed or modified to slow respiration rates, reduce microbiological growth and retard enzymatic spoilage—with the intent of extending shelf life". The principle of MAP is to inhibit the growth and biochemical activities of aerobic *Pseudomonas* species, which are the predominant microbes limiting the shelf life of fresh meat (Lambert et al., 1991). The impermeability of packaging materials to both oxygen and carbon dioxide that can maintain the in the headspace determines the effectiveness of the modified atmosphere packages for meat. Furthermore, in order to minimize changes in moisture content in the package, film should have low water vapor transmission rates (WVTRs).

High $O_2$

The most common fresh meat MAP contains around 80% $O_2$: 20% $CO_2$, which improves meat shelf life for processors and retailers with Control distribution system (Eilert, 2005). The barrier tray used in high $O_2$ MAP is often polystyrene, polypropylene, or polyethylene (Belcher, 2006). High oxygen level is to maintain the

fresh meat, keep the oxygenated form of muscle pigment myoglobin. Additionally, the major function of $CO_2$ is to inhibit the growth of microbes. For instance, a concentration of $CO_2$ ranging from 10%-20% can inhibit *Pseudomonas* and A. *putrfaciens* (Gill and Tan, 1980). Mcmillin et al. (1999) figured out that certain concentration of $CO_2$ should be used to inhibit microbial growth. Higher than 40% $CO_2$ level would cause the collapse of package since meat can absorb $CO_2$, while the $CO_2$ level is less than 15% did not inhibit microbial growth effectively. Carbon dioxide has high inhibitory effect on gram-negative spoilage microorganisms, but has less effect on lactic acid bacteria (Silliker and Wolfe, 1980; Enfors and Molin, 1984). B. *thermosphacta* can grow in 50% $CO_2$ (Gardner, 1981), and Lactobacillus can even grow in concentrations of 100% $CO_2$ (Blickstad et al., 1981). Also, MAP with $CO_2$ for the major microbial inhibitory purpose, is not so effective at Controlling pathogenic microorganisms. Since the growth of spoilage bacteria is restricted by $CO_2$, pathogenic bacteria have relatively little competition to use the nutrients and grow (Winger, 2000).

Similar to air-permeable overwrapped trays, MAP with high oxygen levels accelerate lipid oxidation which results in the development of rancidity off-odors (O'Grady et al., 2000). Additionally, high levels of oxygen favor the rapid growth of aerobic bacteria, which limits the meat shelf life. What's more, there is economic loss due to the excessive space occupied by packaging rather than product (Holland, 1980; Taylor, 1985).

Low $O_2$

Anoxic atmosphere of $N_2$ and $CO_2$ are used in low $O_2$ MAP. The use of $N_2$ is to maintain integrity of the package without any interaction with meat pigments (Zhou et al., 2010). The use of low $O_2$ (without CO) MAP may be limited because of the purplish color of deoxymyoglobin, the oxidative form of myoglobin. In order to maintain desired red meat color, small amount of carbon monoxide (CO) has been used in low $O_2$ MAP. Carbon monoxide may be exposed to meat before packaging or be used to gas flush VSP packages before sealing (Belcher, 2006; Cornforth & Hunt, 2008; Eilert, 2005). Use of up to 1% CO in the MAP was approved to maintain meat oxymyoglobin during lag phase of spoilage bacteria (Clark et al., 1976). Carbon monoxide has strong myoglobin binding capacity, which can form carboxymyoglobin resulting in bright red color of meat. Additionally, the form carboxymyoglobin (MbCO) is more stable to oxidation than oxymyoglobin (El-Badawi, et al., 1964). After reviewing the toxicological aspects of CO used in MAP of meat (containing 0.3%-0.4% CO), Sorheim et al. (1999) concluded that gas mixtures with up to 0.5% CO concentration do not present toxic threat to consumers' health. In Norway, MAP containing low levels of CO is widely acceptable and used in beef packaging industry (Sorheim et al., 1999).

Active Packaging

There can be interactions among packaging materials, the internal atmosphere, and the food when using active packaging to maintain quality and safety of food (Rooney, 1995). Active packaging also can change the packaging environment at a specified time or condition via passive or active means without applying for the

Control atmosphere packaging (Yanyun, 1994). The development of active packaging systems is a fairly new conception. Specific additives or technologies are used to Control moisture, enhance flavor, generate oxygen, etc. (Table 2.3).

Packaging impregnated with antimicrobial substances to extend shelf life and improve food safety is an extremely challenging technology. There are several antimicrobial agents used in food packaging systems, for instance, organic acids, acid salts, acid anhydrides, pan-benzoic acids, alcohol, bacteriocins, fatty acids, fatty acid esters, chelating agents, enzymes, metals, antioxidants, antibiotics, fungicides, sterilizing gases, sanitizing agents, polysaccharides, phenolics, plant volatiles, plant and spice extracts and probiotics (Cutter, 2006). In 2006, the market volume for antimicrobial usage in polyoefins was 3300 tons, which would be expected to increase to 5480 tons in 2012 (McMillin, 2008).

As high levels of oxygen in packages may accelerate lipid oxidation, develop off-flavor, off-odor and discoloration, the Control of oxygen levels in packages is very important. Although low $O_2$ MAP and vacuum packaging technologies can be used for oxygen sensitive foods, the oxygen in the packages still cannot be completely removed. Packages using oxygen scavenging compounds can help absorbing the residual oxygen after packaging, which can minimize the quality change on oxygen sensitive foods (Vermeiren et al., 1999). However, it has not been used for fresh meat.

**Meat Color**

Fresh meat color is very important factor that affects meat shelf life. Consumers tend to judge meat quality via three sensory properties, namely appearance, texture

and flavor (Liu et al, 1995). To consumers, red color is one of the most important factors associated with good quality of meat (Luño et al., 2000; Veberg et al., 2006). The color stability of beef changed gradually with extension of chilled storage (Geer & Jones, 1991). Green & Zipser (1971) reported that since meat cuts lose their fresh color from bright red to brown red or discolored, about 30%-40% of consumers would reject purchasing. Consequently, the price of about 15% retail beef will be reduced, resulting in an annual revenue loss of $1 billion for the United States beef industry (Smith et al., 2000).

Both pre-harvest and post-harvest factors affect meat color. The pre-harvest factors include the breed of livestock, daily diet, housing, and pre-harvest handling. Post-harvest factors are chilling rate, antioxidant availability, antimicrobial compounds, package atmosphere, and cooking (Mancini & Hunt, 2005).

In the muscle tissue of animals, myoglobin is the major complex pigmented protein responsible for the color of meat, which comprises more than 90% of the total pigmentary compounds (Warris & Rhodes, 1977). Ledward (1984) considered that cytochromes, flavins and catalase have minor effect on pigmentation because they are only present in a small amounts. The biological function of myoglobin is to store and deliver oxygen (Siegel, 2010).

Renerre (2000) described myoglobin as “a monomeric, globular heme protein with a molecular weight of approximately 17000 and is formed by 140 to 160 amino-acid residues and a heme group in a crevice of the molecule”. The key residue that is responsible for myoglobin structure and function is histidine. Myoglobin also

has a prosthetic group within the protein's hydrophobic pocket. The centrally located iron atom of the heme ring can form six bonds. Pyrrole nitrogens binds to the four of these bonds, and the proximal histidine-93 coordinates with the 5th bond. The 6th bond can bind ligands reversibly (Mancini & Hunt, 2005).

Due to different oxygen concentrations, there are different chemical forms of myoglobin that are responsible for different meat color. When exposed to air, myoglobin binds to oxygen to form ferrous oxymyglobin ($MbO_2$), which is bright-red color indicating freshness of meat to consumers. Diatomic oxygen occupies the 6th coordination cite. However, iron's valence does not change during this oxygenation process. Furthermore, the structure of myoglobin is altered by the interaction of distal histidine and bound oxygen. Oxymyoglobin penetrates deeper into the interior of meat with the increased exposure to oxygen (Mancin and Hunt, 2005).

Since oxymyglobin keeps contacting with oxygen over time, metmyoglobin (MetMb) ultimately forms due to the oxidation of oxymyoglobin. MetMb is brown in color which indicates the discoloration resulting from the oxidation of ferrous myoglobin derivatives to ferric iron (Livingston & Brown, 1982).

With the absence of oxygen, no ligand is present at the $6^{th}$ coordination site and the heme iron is ferrous ($Fe^{2+}$), resulting in the formation deoxymyoglobin. Meat color changes to purplish-red or purplish-pink due to deoxymyoglobin, which occurs on vacuum packaging meat right after cutting (Mancini & Hunt, 2005). According to Brooks (1935), to maintain myoglobin in a deoxygenated state, the oxygen concentration should be below 1.4 mm Hg. When meat is exposed to air,

deoxymyoglobin is oxygenated to form oxymyoglobin, resulting in bright red color again.

Carbon monoxide can change the state of myoglobin to carboxymyoglobin. However, it is still unclear exactly which myoglobin derivatives can form the carboxymyoglobin. Carbon monoxide has strong ability to bind the vacant $6^{th}$ position of deoxymyoglobin and result in a very stable bright red color (Mancini & Hunt, 2005). Currently, use of low levels (1%-2%) of carbon monoxide in low $O_2$-modified atmosphere packages is permitted in some countries, such as USA and Norway (Luno et al., 2000; Hunt et al., 2004; Sorheim et al., 2001).

When the surface of meat is mostly converted to metmyoglobin, major discoloration occurs. The change of metmyoglobin to oxymyoglobin or the reduction of metmyglobin becomes important for meat industry to keep the meat in a fresh color appearance to consumers.

**The use of nitrite**

In 1899, an experiment was conducted to confirm that the red color of meat can be produced by the use of nitrite (Lehmann, 1899). Two years later, Haldane (1901) used the light to show the occurrence of red-ox reaction during the meat curing process. Furthermore, he discovered the NO-myglobin which was the key substance leading to the bright red color of cured meat. At the beginning of $20^{th}$ century, the nitrous acid (HNO2), instead of nitrite anion, is the key nitrite compound that reacts with myoglobin during the coloring process of cured meat (Hoagland, 1914).

The use of sodium nitrite ($NaNO_2$) combined with sodium chloride in processed

meat as a preservative to inhibit the growth of *Clostridium botulinum* for several years (Pierson and Smoot, 1987; Christiansen et al., 1974). From another point, nitrite can act as antioxidant in meat batters, retarding rancidity or a warmed over flavor (Honikel, 2008).

The most important effect of nitrite is to create red color of cured meat. The formation of NO-myglobin is responsible for the red color of meat. After cooking, meat color can turns grey or brown because oxymyoglobin is not heat stable. Nevertheless, the red $NO^-$ porphyrin ring system (usually called nitroso-myochromogen) can still exist even the protein moiety is denatured when heating the NO-myoglobin under 120°C. This heat stable red color is preferred by consumers as the color in fresh meat (Honikel, 2008).

In order to maintain bright red color of fresh meat in vacuum packages, a novel packaging technology called FreshCase® was recently developed by the Bemis Company. A limited amount of sodium nitrite is contained within the polymer matrix of the inner layer of the packaging film. Visible meat surface can absorb the sodium nitrite after oxygen has been removed by the vacuum packaging. Since the residual oxygen was consumed by meat metabolism and microbial activity, deoxymyoglobin is formed, displaying purple color. Nitrite catalyzes deoxymyoglobin to the metmyoglobin and is converted to Nitric Oxide. Metmyoglobin reduction activity occurs when the endogenous substrate donates the electron. Since metmyoglobin reduces to deoxymyoglobin, NO can be pulled into the heme pocket, and become a ligand, interacting with myoglobin, causing the bright red color for the meat contact

surface (Siegel, 2010). The vacuum packaged atmosphere minimizes the lipid oxidation and delays microbial growth. The use of nitrite contained in the film stabilizes the fresh color of meat. Meanwhile, the amount of nitrite is sufficient for the color change but not enough for the curing or preservation purpose.

**Conclusion**

The innovation and development of packaging technology result from the change of consumers' preferences, concerning both meat quality and meat safety. Since the researches on meat shelf life and packaging technology are done more and more, the novel packaging technology that can extend meat shelf life will become more applicable and more commercially viable.

**Table 2.1.**
Characteristics of major spoilage bacteria of fresh meat

| Species | Gram Reaction | Oxygen Requirement | Spoilage Products | Growth condition |
|---|---|---|---|---|
| B. *thermosphacta* | + | Facultative anaerobe | Diacetl, acetic, isovaleric and isobutyric acids | No anaerobic growth below pH 5.8 |
| *Enterobacteriaceae* | - | Facultative anaerobe | Amines, sulfides | No anaerobic growth below pH 5.8 |
| Lactic acid Bateria | + | Aerotolerant anaerobe | Lactic acid and ethanol | Ferment a restricted range of substrates |
| *Pseudomonas* | - | Aerobe | Amines, ethyl esters | Aerobic growth only |
| *Shewanella putrefaciens* | - | Facultative anaerobe | Sulfides | No anaerobic growth below pH 6.0 |

Source: Spoilage characteristics from Whitfield, F.B. 1998. Microbiology of food taints. International Journal of Food Science and Technology 33:31-51.

**Table 2.2.**
Expected shelf-life under chilled temperature, and growth ability of spoilage bacterial groups and specific bacteria and specific bacteria on fresh meat

| Product | Packaging types | Expected Shelf-life | Growth Ability[a] | | | |
|---|---|---|---|---|---|---|
| | | | *Pseudomonas* spp. | *Enterobacteriaceae* | LAB | B. *thermosphacta* |
| Meat, Normal pH | Air-wrapped | Days | * * * | * * | * * | * */* * * |
| | High O2-MAP | Days | * * * | * */* * * | * */* * * | * * * |
| | Vacuum | Weeks-months | * | */* * | * * * | * */* * * |
| | 100% CO2 | Months | * | */* * | * * * | * |
| Meat, High pH | Vacuum | Days | * | * */* * * | * * * | * */* * * |
| | 100%CO2 | Weeks-months | * | */* * | * * * | * |

[a] * * *, dominat bacteria of the mciroflora; * * intermediate bacteria of the microglora; *, minor bacteria of the microflora (Adapted from "Bacterial Spoilage of meat and cured meat products". Borch, E., Kant-Muermans, M.L., Blixt, Y. (1996). International Journal of Food Microbiology, 33, 103-120)

**Table 2.3.**

Examples of active packaging applications for use within the food industry

| | |
|---|---|
| Absorbing/scavenging properties | Oxygen, carbon dioxide, moisture, ethylene, flavors, taints, UV light |
| Releasing/emitting properties | Ethanol, carbon dioxide, antioxidants, preservative, sulphur dioxide, flavors, pesticides |
| Removing properties | Catalyzing food component removal: lactose , cholesterol |
| Temperature Control | Insulating materials, self-heating and self-cooling packaging,<br>microwave susceptors and modifiers,<br>temperature-sensitive packaging |
| Microbial and quality Control | UV and surface-treated packaging materials |

Source: Past, current and potential utilization of active and intelligent packaging systems for meat and muscle-based products: A review. From Kerry, J.P., O'Grady, M.N., Hogan, S.A. (2006). Meat Science, 74, 113-130

# Chapter III

## An Evaluation of the Effectiveness of FreshCase® Technology to Extend the Shelf Life of Whole Muscle Beef and Ground Beef


### Summary

This research evaluated the ability FreshCase®, a novel packaging technology which has been shown to extend the shelf life of whole muscle beef and ground beef by fixing beef color in a fresh-appearing state. The shelf life for beef steaks stored in FreshCase® packages at 4°C was 36 days; and the shelf life for ground beef stored in FreshCase® packages at 4°C was 12 days. Storage life was defined by the number of days required to reach an aerobic psychrotrophic plate count of $10^7$ log CFU/g. Higher ($P < 0.05$) a* (redness) values were detected in FreshCase®-packaged samples of both beef steaks and ground beef. When aerobic psychrotrophic plate counts exceeded $10^7$ log CFU/g, the point of spoilage, off-odors of putrid, acid, sour and rancid were detected at very low levels in all samples. Likewise, levels of oxidative rancidity in all packages were low with low TBA. Therefore, utilization of FreshCase® technology in whole muscle beef and ground beef is viable option to extend the storage life by improving the stability of beef color coupled with low levels of off-odors and lipid oxidation.



**Key words**: FreshCase®, vacuum packaging, shelf life; storage life, beef, beef color


### Introduction

The shelf life of fresh meat is defined as the storage time until it reaches spoilage (Borch et al., 1996). Spoilage can be defined by multiple characteristics of meat

including, but not limited to, bacterial load, appearance or color of lean and fat, oxidative rancidity, the presence of off-odors, and/or the presences of off flavors. There are both intrinsic and extrinsic factors that affect the shelf life of meat and meat products. Intrinsic factors include initial pH of meat, as well as the concentration of nutrients required for growth of bacteria, such as glycogen, glucose, glucose-6-phostate, L-lactate and fat (Blixt and Borch, 2001). Extrinsic factors include oxygen and light exposure, as well as temperature during storage (Lambert et al., 1991). Altering packaging technique is one of the most common methods for managing extrinsic factors influencing the shelf life of meat.

Consumers tend to judge meat quality via three sensory properties, namely appearance, texture, and flavor (Liu et al, 1995). A red color/appearance is most commonly associated with fresh meat and quality by consumers (Luño et al. 2000). Fresh meat color deteriorates gradually over time with extended periods of chilled storage (Greer & Jones, 1991). Consequently, the price of discolored beef is reduced by about 15%, resulting in an annual revenue loss of $1 billion for the United States' beef industry (Smith et al., 2000).

FreshCase® technology refers to vacuum packaging fresh meat with high barrier nitrite-containing film. The use of nitrite in the meat packaging material results in the development of a bright red surface color and reduces the rate of discoloration of meat under vacuum conditions (Siegel, 2010). On the other hand, when fresh meat is packaged using FreshCase® technology, the deterioration of fresh meat color is slowed and may give consumers a false indication of freshness.

The objective of this experiment was to identify the maximum time of refrigerated storage before aerobic psychrotrophic bacteria (APB) grew to a level indicative of spoilage ($10^7$ log CFU/g) or other indicators of spoilage were observed for whole muscle beef and ground beef packaged using FreshCase® technology.

## Materials and Methods

Sample Preparation

Whole Muscle Beef Steaks

Two cases of boneless, beef strip loins (NAMP 180) were collected from a commercial processing plant on day 5 postmortem and transported on ice (0 – 2 ℃) to the Colorado State University (CSU) Meat Laboratory. Each case was processed and stored independently from the other as case would serve as an experimental block. Immediately following arrival at CSU, strip loins were removed from their vacuum packages and cut into 2.54 cm thick steaks. Each steak was hand trimmed to a maximum remaining exterior fat thickness of 0.32 cm. Following trimming, steaks were randomly assigned to 1 of 2 packaging treatments. Every other steak was placed into a 15.24 cm x 30.48 cm x 2.54 cm FreshCase® pouch (FreshCase®). The FreshCase® pouches used were made using high barrier film with a 3 mil thickness and had sodium nitrite incorporated into the sealant layer. Sodium nitrite was present at a level providing less than 60 mg/$m^2$ to the beef contact surfaces. Every other steak was placed into an identical 15.24 x 30.48 cm x 2.54 cm bag that did not contain nitrite (Control). Each pouch was individually identified and vacuum packaged using

a dual chamber vacuum packaging machine (Multivac, Model C500) until 7 mbar of pressure was achieved. Then, pouches were boxed according to treatment and stored without light under refrigeration at 2 - 4 ℃.

Ground Beef

In order to achieve a lean (approximately 85 % lean) ground beef product, two cases of beef chuck rolls (NAMP 116A) were collected from a commercial processing plant on day 2 postmortem and transported on ice (0 – 2 ℃) to CSU. Each case was processed and stored independently from the other as case would serve as an experimental block. Using aseptic techniques, the chuck rolls were removed from their vacuum packages, cut into 5 cm x 5 cm pieces, and ground twice. Pieces were ground using a mixing-grinder (Hobart, Model 4346). The beef pieces were initially coarse ground using a 1.27 cm breaking plate and then ground a second time using a 0.48 cm plate. Immediately following grinding, 454 g of ground beef was tightly stuffed using a vacuum stuffer (Handtmann, Model VF 50) into 1 of 2 packaging treatments. Every other 454 g portion was stuffed into a FreshCase® pouch and a Control pouch. Ground beef pouches were individually labeled and packaged identically as described for strip loin steaks.

A total of 175 (7 pouches/day * 25 days) pouches of beef steaks and 175 pouches of ground beef were prepared for storage. Seven Control and 7 FreshCase® pouches were removed from storage for steaks and ground at time intervals of 1-5 days. Sampling intervals were variable and were determined in the interest of quantifying

APB on the day that the average count reached $10^7$ log CFU/g. The interval was be decreased as the bacterial growth reached the end of storage life in order to most accurately capture the total number of days before $10^7$ log CFU/g APB was observed. Refrigerated storage of untested samples continued until average APB counts reached $10^7$ log CFU/g for 3 consecutive days.

Instrumental Color Measurement

On each sampling day, objective color measurements were taken from every package removed from storage using a MiniScan® EZ spectrophotometer (Hunter Association Laboratory Inc., Reston, VA, USA). Packages were opened and exposed to the air for a maximum of 10 seconds. Within 10 seconds of opening, the surface lean color and external fat color of beef steaks was measured at three different locations. The three values for lean color and three values for fat color were averaged to obtain single lean color and fat color values for each sample. The surface color of ground beef was measured at three different locations on each sample and averaged to obtained single values for each sample. CIE L*(lightness), a*(redness) and b*(yellowness) values were recorded for each sample.

Microbiological Analysis

On each microbial sampling day, 7 pouches each of beef steaks and ground beef were randomly selected and analyzed for counts of total aerobic psychrotrophic bacteria (APB) and lactic acid bacteria (LAB). Individual steaks (approximately 100 g

of whole muscle) were aseptically cut into 1 cm cubes and placed into a Whirl-Pak filter bag (1.63 L; Nasco, Modesto, CA). For the ground beef, approximately 100 g samples were transferred from each pouch into individual Whirl-Pak filter bags. Diluent, comprised of 0.85% sodium chloride and 0.1% peptone, was added to each sample at a 1:1 ratio (sample weight to volume of diluent) followed by pummeling for 2 min (Masticator, IUL Instruments, Barcelona, Spain). Sample homogenates were serially diluted in 0.1% Buffered Peptone Water (Difco, Becton Dickinson, Sparks, MD) and spread-plated in duplicate onto Tryptic Soy Agar (TSA; Acumedia, Lansing, MI) for enumeration of total APB. For determination of LAB counts, sample dilutions (1 ml) were mixed with 10 ml of molten (45 ℃) Lactobacilli MRS agar (Difco); this was also done in duplicate. After setting, a 10 ml overlay of the molten Lactobacilli MRS agar was added to each plate. Colonies were counted after incubation of TSA plates at 7 ℃ for 10 days, and MRS plates at 25 ℃ for 5 days. Duplicate plate counts were averaged and a single count was reported for each sample.

pH Measurement

The pH of sample homogenates was measured after microbial analysis, using a Denver Instruments pH meter fitted with a glass electrode (UltraBasic-5, Arvada, CO).

Odor Panels

Before each odor panel, all steak and ground beef samples were cut into equal

parts; one-half of each sample was designated as raw and the other half was cooked for odor evaluation. The raw half of steak was cut into 1 cm x 1 cm x 1 cm pieces and put into 2 oz. lidded glass jars, and 50 g of raw ground beef was put in jars. Steaks and ground beef samples designated for cooking were cooked to an internal temperature of 71°C using double sided electric grills (Salton Clamshell Grill Model No. GR39A, Salton Inc., Lake Forest, IL), and put into 2 oz. lidded glass jars. All jars were labeled in a completely random order.

During the odor panel, at least 6 trained panelists who had been previously trained to become familiar with the sensory characteristics of meat were seated in individual booths in a light-controlled room. Each panelist received a set of 28 (7 raw and cooked beef steaks and 7 raw and cooked ground beef) samples to evaluate off-odors and general meat odors using a 15 cm unstructured line scale anchored on the extreme left indicating absence of the odor and the extreme right indicating a very strong presence. A single sensory value was obtained for each of the following odors: putrid, acid, sour, rancidity and meaty odor. Trained panelists marked the scale with a vertical line at the perceived intensity of the attributes. The results were expressed by the distance of the vertical line from the extreme left end of the 15 cm scale.

Thiobarbituric acid (TBA) value

The 2-thiobarbituric acid (TBARs) method described by Tarladgis et al. (1960) was used to measure the lipid oxidation for each sample designated for TBARS analysis. Four pouches of Control and FreshCase® beef steak samples and 4 ground

beef samples were selected for TBARS analysis on days indicating a significant increase in microbial growth. Thiobarbituric acid reacts with the oxidation products of fat to form malonaldehyde, which was measured on a spectrometer in solution. The TBA value was expressed by the mg malonaldehyde(MDA)/kg tissue.

Statistical Analysis

The experiment was a completely randomized block design. The effect of block was removed from the model as it was found to be insignificant in initial tests. A two-way analysis of variance (ANOVA) was conducted for each variable to investigate the fixed effects of packaging technology, storage time, and corresponding interactions. Rather than analyzing odor panel scores for individual samples by day, multiple sampling days were combined to represent 3 phases of microbial growth. Phase of microbial growth was utilized as a fixed effect in the model in the place of day for steak odor panel ratings. Phase 1 was the time period from day 0 to day 5; phase 2 was the time period from day 10 to day 20; time period of day 26 to day 47 was grouped in phase 3.

The General Linear Model procedure (PROC GLM) and mixed procedure (PROC MIXED) of SAS (Cary, NC Ver. 9.1; 2007) were used to analyze the data. Microbial counts were expressed as $\log_{10}$ CFU/g. The responsible variables, as CIE L*, a*, b* values, microbiological loads, pH, TBARS, and sensory panel scores were evaluated and significance of differences was defined as $\alpha = 0.05$. The mean separations were obtained using Fisher's Least Significant Difference test.

## Results and Discussion

### Beef Steaks

#### Objective Color

The main effects of packaging technology on objective color, microbial counts, and pH are presented in Table 3.1. Lean L* values tended to be slightly higher for Control steaks versus FreshCase® steaks ($P = 0.052$), while lean b* values were higher ($P < 0.05$) for FreshCase® steaks versus Control steaks. This indicates that while the lean of control steaks appeared to lighter colored, FreshCase® steaks had a brighter, more yellow appearance. The external fat of FreshCase® steaks had a redder/pinker appearance with higher ($P < 0.0001$) a* values. Higher ($P = 0.016$) b* values were recorded for the fat of Control steaks indicating a brighter, more yellow colored appearance. The main effect of storage time (day) was significant ($P < 0.05$) on steak lean L* and b* and fat L*, a*, and b* readings (Table 3.2). However, there were no discernible trends identified from these values. The interaction of packaging technology x storage time (day) existed for lean a* values (Figure 3.1). On day 0 of storage lean a* values for both control and FreshCase® steaks were similar, but as storage time increased, lean a* values for FreshCase® steaks remained higher ($P < 0.05$) translating to a much redder appearance.

#### Bacterial and pH Results

Averaging over all storage times, FreshCase® steaks had a lower number of APB ($P = 0.037$; Table 3.1), however the magnitude of difference provides little evidence

of a meaningful difference. Counts for LAB and pH were not different ($P > 0.05$) for packaging technologies (Table 3.1). However, steak pH values gradually decreased as storage time increased ($P < 0.0001$; data not shown).

Storage time (day) influenced levels of APB and LAB ($P < 0.05$; Table 3.3). Plate counts of APB and LAB increased over time. Initially, levels of APB and LAB were relatively low with counts of 2.52 log CFU/g and 1.75 log CFU/g, respectively. Both APB and LAB counts for beef steaks exceeded 2 logs of growth by 10 days of refrigerated storage. After 2 logs of growth for both classifications of bacteria was observed, sampling became more frequent and plate APB and LAB counts were steady and seemingly linear. Aerobic psychrotrophic bacteria (APB) exceeded 7 log CFU/g on day 41 of storage, while LAB exceeded 7 log CFU/g 15 days prior. These data indicated that LAB flourished more rapidly, as expected, in vacuum packaged beef steak samples. Consequently, the storage life of beef steaks, regardless of packaging technology in accordance with APB, expired after 36 days of storage.

Odor Panel Scores

Sensory odor scores for beef steaks are presented in Table 3.4. The main effect of packaging technology did not affect sensory odor scores for raw beef steak samples. Averaging over all storage periods, odor panel ratings of raw beef steaks were relatively low for all attributes indicating spoilage (Table 3.4). Similarly, putrid, sour, rancid, and meaty odors for cooked steaks were not affected by packaging technology ($P > 0.05$). The main effect of storage time (phase) significantly ($P < 0.05$; Table 3.5)

affected sensory panel scores of all attributes for raw beef steaks. As storage time increased, the intensity of putrid, acid, sour, and rancid increased while meaty odor intensity decreased. These results were as expected. Storage time did not affect putrid or meaty odors of cooked steak samples. The intensity of sour and rancid odors increased over time in cooked samples. Despite the notable increase in intensity of sour and rancid odors in cooked steak samples, the intensity remained low for both attributes. The intensity ratings for all attributes, except meaty odors, remained very low. Therefore, in reference to product odor, indications of spoilage remained low throughout storage. Most notably, APB and LAB counts grew beyond the recognized level of bacterial spoilage ($10^7$ log CFU/g) in Phase 3, and there was very little indication of spoilage via product odor. Additionally, throughout storage, the color of FreshCase® samples remained bright red and acceptable in appearance.

A packaging technology x storage time interaction existed for acid odor intensity in cooked beef steaks ($P = 0.018$; Table 3.6). Even though the intensity of acid odors increased over time for both treatments, and FreshCase® samples had higher ($P < 0.05$) intensities of acid odor in the last phase of storage, the most extended storage phase resulted in a very low presence of detectable acid odors.

TBA

The TBA values of beef steaks are shown in Table 3.7. Statistically, there was a storage time x packaging technology interaction ($P < 0.05$) for beef steaks. The level of oxidative rancidity, as indicated by TBA values, remained very low for all samples

over all time periods and treatments. The maximum tested TBA value for Control beef steaks was 0.44mg/kg, and the maximum TBA value for FreshCase® samples was 0.32 mg MDA/kg. Green and Cumuze (1981) concluded that the development of strong off-flavor occurred when TBA values of at least 2.0 mg MDA/kg were reached in meat samples. Therefore, despite the fact that after extended periods of storage samples reached APB and LAB exceeding $10^7$ log CFU/g, oxidative rancidity was not reached in the sampling periods included in this study. These findings also support the low intensity levels of putrid and rancid odors in the sensory portion of this study.

## Ground Beef

### Objective Color

The main effect of packaging technology on objective color, microbial counts, and pH are shown in Table 3.8. No significant difference ($P = 0.937$) was found on L* values for ground beef. The main effect of storage time (day) was significant ($P < 0.0001$) on L* values of ground beef (Table 3.9). However, there was no discernible trend identified from these values. The interaction of packaging technology x storage time (day) existed for a* and b* values (Figure 3.2; Figure 3.3). On day 0 of storage, a* and b* values for FreshCase® ground beef were lower, but as storage time increased, a* and b* values for FreshCase® ground beef remained higher ($P < 0.05$) indicating a much redder, and brighter, more yellow colored appearance.

Bacterial and pH Results

According to Table 3.8, counts for APB and LAB were not different ($P > 0.05$) for ground beef stored in different packages. Averaging over storage time, pH for Control ground beef was 5.61, which was statistically higher ($P = 0.008$) than the pH of FreshCase® ground beef, which was 5.58. However, the magnitude of difference provides little evidence of a meaningful difference. Ground beef pH gradually declined from 5.63 to 5.53 as storage time increased ($P < 0.0001$; data not shown).

Plate counts of APB and LAB increased ($P < 0.05$) with the increase of storage time (Table 3.10). Initially, levels of APB and LAB were relatively high with counts of 4.12 log CFU/g and 3.06 log CFU/g, respectively. Both APB and LAB grew very rapidly and exceed 6 log CFU/g by 10 days of refrigerated storage. Both classifications of bacteria exceeded 7 log CFU on day 12 of storage. Consequently, the storage life of ground beef, regardless of packaging technology in accordance with APB, expired after 10 days of storage.

Odor Panel Scores

Odor scores for ground beef are demonstrated in Table 3.11. The main effect of packaging technology did not ($P > 0.05$) influence odor scores for raw ground beef samples. Likewise, putrid and meaty odors for cooked ground beef samples were not affected by packaging technology ($P > 0.05$). Cooked FreshCase® had lower ($P < 0.05$) intensity of sour and rancid odors. Averaging over all storage period, odor panel ratings of raw and cooked ground beef were relatively low for all attributes indicating

spoilage (Table 3.11). The main effect of storage time (phase) significantly ($P < 0.05$; Table 3.12) affected odor panel scores of all attributes for all raw ground beef. As storage time increased, the intensity of putrid, sour, and rancid increased while meaty odor intensity decreased. Similarly, the intensity of putrid and rancid increased over time in cooked samples. The intensity of sour odor was stable throughout storage except a higher ($P < 0.05$) odor score on day 13 of storage. Similarly, meaty odor scores were stable throughout storage except a lower ($P < 0.05$) score on day 10 of storage. The intensity ratings for all attributes, except meaty odors, remained very low. Therefore, in reference to product odor, indications of spoilage remained low throughout storage. Most notably, APB and LAB counts grew beyond the recognized level of bacterial spoilage ($10^7$ log CFU/g) in Phase 3, and there was very little indication of spoilage via product odor. Additionally, throughout storage, the color of FreshCase® samples remained bright red and acceptable in appearance.

A packaging technology x storage time interaction was found for acid odor intensity in cooked ground beef ($P = 0.002$; Table 3.13). However, there was no discernible trend identified from these values. The intensity of acid odor remained low for both Control and FreshCase® samples throughout storage.

TBA

The TBA values of ground beef are showed in Table 3.14. Statistically, there was a packaging technology x storage time interaction ($P < 0.0001$) for ground beef. TBA values for Control samples increased by the storage time from 0.29 mg MDA/kg to

1.19 mg MDA/kg. TBA values for FreshCase® samples increased from 0.25 mg MDA/kg to 0.82 mg MDA/kg through day 0 to day 13, but ending with 0.41 mg MDA/kg on day 14. This result agreed with the study which found that the TBA values increase to a certain point during the storage period, followed by a decline in these values (Gokalp et al., 1983; Babji et al., 1998). The TBA values for ground beef remained lower than the minimum value of TBA for strong off-odor development established at a concentration of 2 mg MDA/kg (Green and Cumuze, 1981). Therefore, despite the fact that after extended periods of storage samples reached APB and LAB exceeding $10^7$ log CFU/g, oxidative rancidity was not reached in the sampling periods included in this study. These findings also support the low intensity levels of putrid and rancid odors for ground beef in the sensory portion of this study.

The results that both beef steaks and ground beef displayed redder color (higher a* values) during the storage from this study was strong supported by other studies that the use of nitrite can improve and stabilize the red color of meat (McClure, et al., 2011; Skibsted, 2011; ).

In our research, the a* values of beef steaks and ground beef were changed by the storage time without any trend, which was different with the results from some papers that the a* values for meat in vacuum packages tend to decrease with the increase of storage time (Luno et al., 1999; Jeremiah & Gibson, 2001;). However, the studies by Filgueras et al. (2010) and Suman et al. (2010) found that the redness of the muscle color was highly stable in the vacuum packages during the storage. Furthermore, a study by Grobbel et al. (2008) concluded that vacuum packages or

modified-atmosphere package (64.6% $N_2$, 35% $CO_2$, 0.4% CO) allowed longer period for the stabilization of myglobin in red color form and can delay the onset of metmyoglobin , which was brown in color, due to the oxidation of myoglobin.

From other aspect, nitrite can be used as an important antioxidant in cured meat (Skibsted, 2011). Oxygen molecule reacts with unsaturated fat of meat, the process lipid oxidation begins, which results in the development of rancid odor and flavor in meat (Campo et al., 2006). Nitric oxide from nitrite can scavenge the lipid derived radicals and form non-radical products. Additionally, nitric oxide can also deactivate peroxide without forming hypervalent heme pigment which is responsible for the initiation of lipid and protein oxidation (Kanner et al., 1991; Carlsen, 2005). This may explain why the ultimate TBA value of ground beef in FreshCase® packages was lower than that in Control samples, although the level of nitrite used in FreshCase® packages was relatively low. Moreover, lipid oxidation also accelerates the oxidation of myoglobin, resulting in the formation of metmyoglobin, which is the responsible for brown color of meat (Frustman et al., 2010; Lynch, et al., 2000). For meat stored in high O2 modified atmosphere packages, bright red color can be stabilized due to high concentration of oxygen, but over time, metmyoglobin will be formed as well as a much higher than 2 mg MDA/kg TBA value indicating high level of oxidative rancidity (Bingol and Ergun, 2011). Therefore, the use of FreshCase® Packaging Technology will not only Control the oxygen level, minimizing the lipid oxidation, but also minimize the oxidation of pigment, improving the red color by applying nitrite to the package films.

The shelf life of ground beef in this research was extended to 12 days compared to ground beef stored in over-wrapped packages, which is usually 3 days (Robert, 2009). However, the shelf life of ground beef could have a longer shelf life with the small change of the storage condition. The initial bacterial load is extremely important to meat shelf life (Lambert et al., 1991). Keeping other factors consistent, it is known that the lower initial micro-organism counts, the longer shelf life of meat would last. For instance, beef stored at 0°C with an initial microbial counts at 6 x 104 cm-2 had shelf life of 11days, while beef stored at 0°C with an initial load of 65 cm-2 displayed spoilage on day 21 (Ayre, 1960). What's more, it was proved that storage life is inversely related to storage temperature (Lee, et al. 1983). Generally speaking, a proportional shorten in meat shelf life results from an increase in storage temperature over the optimum temperature. Gill and Shand (1993) concluded that "the storage life attainable is 100% at -1.5°C, 70% at 0°C, 50% at 2°C, and 15% at 10°C. Beef stored in vacuum package at temperature of -1.5°C has shelf life as long as 14 weeks when the bacterial counts reach about $10^7$ CFU/$cm^2$, but only three weeks that stored at 4°C (Blix & Borch, unpublished results). The ground beef with an initial microbial load of 4.12 log CFU/g, stored at 4°C appeared a relatively shorter shelf life of 12 days, compared to those using the same packaging technology with lower initial microbial level of 3.84 log CFU/g and stored in lower temperature at 1°C, which had shelf life as long as 35 days (Yang, unpublished data).

**Conclusion**

The results of this research identified that the shelf life of whole muscle beef and

ground beef stored at 4°C is 36 days and 12 days, respectively. It also confirmed that shelf life of whole muscle beef and ground beef can be extended by using FreshCase® Packaging Technology, displaying redder color than beef stored in the regular vacuum packages, without any other adverse change of the meat quality. Further research may be carried out to evaluate the effect of FreshCase® Packaging Technology on the display color for beef and pork, which allows the red color to be stable during the display time after consumers open the packages. Additionally, visual color by panelists should also be tested.

**Table 3.1**. Means for traits of beef steaks stored at 4 ℃.

| | Control | FreshCase® | SEM | *P*-value |
|---|---|---|---|---|
| Lean L*[1] | 34.64 | 34.03 | 0.22 | 0.052 |
| Lean b*[1] | 8.94[b] | 9.35[a] | 0.10 | 0.005 |
| Fat L*[1] | 51.04 | 50.61 | 0.38 | 0.428 |
| Fat a*[1] | 3.52[b] | 5.76[a] | 0.19 | <0.0001 |
| Fat b*[1] | 14.4[a] | 13.9[b] | 0.14 | 0.016 |
| TSA[2] | 5.48[a] | 5.32[b] | 0.05 | 0.037 |
| MRS[2] | 6.01 | 5.95 | 0.04 | 0.309 |
| pH | 5.47 | 5.48 | 0.00 | 0.697 |

[a,b] Means with different superscriptal letters within columns differ ($P<0.05$)

[1] L* reflects the lightness of meat color; a* reflects the redness; b* reflects the yellowness

[2] APB: Aerobic psychrotrophic bacteria
LAB: Lactic acid bacteria

**Table 3.2.** Objective color (CIE L*, a*, b*)[1] by storage time (day) of beef steaks for lean and fat meat stored at 4°C.

| Day | Lean | | Fat | | |
|---|---|---|---|---|---|
| | L* | b* | L* | a* | b* |
| 0 | 33.56$^{c}$ | 8.04$^{g}$ | 51.14$^{bcde}$ | -0.01$^{g}$ | 10.51$^{h}$ |
| 5 | 33.91$^{bc}$ | 9.00$^{ef}$ | 52.23$^{bc}$ | 2.44$^{f}$ | 13.06$^{g}$ |
| 10 | 37.40$^{a}$ | 10.60$^{ab}$ | 57.28$^{a}$ | 5.54$^{abc}$ | 16.43$^{a}$ |
| 13 | 34.18$^{bc}$ | 8.72$^{fg}$ | 51.89$^{bc}$ | 3.81$^{def}$ | 13.54$^{efg}$ |
| 14 | 34.77$^{bc}$ | 10.67$^{a}$ | 57.18$^{a}$ | 4.55$^{cde}$ | 15.68$^{ab}$ |
| 15 | 34.49$^{bc}$ | 8.59$^{fg}$ | 49.34$^{cdef}$ | 5.80$^{abc}$ | 15.21$^{bc}$ |
| 16 | 33.11$^{c}$ | 9.62$^{cde}$ | 48.14$^{ef}$ | 5.50$^{abc}$ | 13.89$^{defg}$ |
| 17 | 33.46$^{c}$ | 9.83$^{bcd}$ | 47.82$^{f}$ | 6.63$^{a}$ | 14.77$^{bcd}$ |
| 18 | 34.49$^{bc}$ | 9.98$^{abc}$ | 47.94$^{f}$ | 6.31$^{ab}$ | 15.02$^{bcd}$ |
| 19 | 33.35$^{c}$ | 9.12$^{def}$ | 48.44$^{def}$ | 5.28$^{abcd}$ | 14.67$^{bcde}$ |
| 20 | 34.65$^{bc}$ | 8.33$^{fg}$ | 52.49$^{b}$ | 3.89$^{def}$ | 14.07$^{defg}$ |
| 26 | 34.07$^{bc}$ | 8.93$^{ef}$ | 51.10$^{bcde}$ | 4.85$^{bcde}$ | 14.31$^{cdef}$ |
| 31 | 34.40$^{bc}$ | 8.49$^{fg}$ | 51.21$^{bcd}$ | 3.64$^{ef}$ | 13.25$^{fg}$ |
| 36 | 35.53$^{b}$ | 8.88$^{ef}$ | 50.54$^{bcdef}$ | 5.28$^{abcd}$ | 14.28$^{cdef}$ |
| 41 | 34.49$^{bc}$ | 9.02$^{ef}$ | 48.39$^{def}$ | 4.75$^{cde}$ | 13.52$^{fg}$ |
| 47 | 33.52$^{c}$ | 8.47$^{fg}$ | 48.14$^{ef}$ | 6.01$^{abc}$ | 14.20$^{cdef}$ |
| SEM | 0.62 | 0.29 | 1.08 | 0.55 | 0.41 |

[a-g] Means with different superscript letters within columns differ ($P < 0.05$)

[1] CIE L* (lightness), a* (redness), b* (yellowness)

**Table 3.3.**
Microbial counts for beef steaks for beef steaks by day effect stored at 4°C

| | APB* | LAB* |
|---|---|---|
| Day | Log CFU/g | Log CFU/g |
| 0 | $2.52^{h}$ | $1.75^{j}$ |
| 5 | $2.69^{h}$ | $3.06^{i}$ |
| 10 | $4.34^{g}$ | $5.37^{h}$ |
| 13 | $4.65^{fg}$ | $5.64^{gh}$ |
| 14 | $4.94^{ef}$ | $5.75^{g}$ |
| 15 | $5.27^{de}$ | $6.17^{f}$ |
| 16 | $5.26^{de}$ | $5.93^{fg}$ |
| 17 | $5.77^{c}$ | $6.57^{de}$ |
| 18 | $5.64^{cd}$ | $6.27^{ef}$ |
| 19 | $5.68^{c}$ | $6.23^{ef}$ |
| 20 | $5.79^{c}$ | $6.69^{cd}$ |
| 26 | $6.43^{b}$ | $7.09^{b}$ |
| 31 | $6.75^{ab}$ | $7.01^{bc}$ |
| 36 | $6.74^{ab}$ | $7.22^{ab}$ |
| 41 | $7.08^{a}$ | $7.48^{a}$ |
| 47 | $6.93^{a}$ | $7.47^{a}$ |
| SEM | 0.14 | 0.12 |

$^{a-i}$ Means with different superscriptal letters within columns differ ($P < 0.05$)

* APB: Aerobic psychrotrophic bacteria
LAB: Lactic acid bacteria

**Table 3.4**. Odor panel scores by packaging effect for beef steaks stored at 4 ℃.

| | Control | FreshCase® | SEM | *P*-value |
|---|---|---|---|---|
| Raw | | | | |
| Putrid | 0.84 | 0.57 | 0.20 | 0.177 |
| Acid | 0.55 | 0.44 | 0.10 | 0.276 |
| Sour | 1.38 | 1.32 | 0.19 | 0.739 |
| Rancid | 2.04 | 1.95 | 0.25 | 0.723 |
| Meaty | 2.81 | 2.80 | 0.16 | 0.932 |
| Cooked | | | | |
| Putrid | 0.19 | 0.13 | 0.08 | 0.496 |
| Sour | 0.35 | 0.44 | 0.10 | 0.387 |
| Rancid | 1.82 | 1.68 | 0.27 | 0.604 |
| Meaty | 8.86 | 8.91 | 0.27 | 0.91 |

[a,b] Means without superscript letters within columns do not differ ($P < 0.05$)

**Table 3.5**. Odor panel scores by storage time (Phase) effect for raw and cooked beef steaks stored at 4 ℃

| Phase* | Raw | | | | | Cooked | |
|---|---|---|---|---|---|---|---|
| | Putrid | Acid | Sour | Rancid | Meaty | Sour | Rancid |
| Phase 1* | $0.01^{c}$(0.22) | $0.05^{b}$(0.12) | $0.22^{c}$(0.22) | $0.16^{c}$(0.3) | $3.42^{a}$(9.19) | $0.05^{c}$(0.12) | $0.12^{b}$(0.32) |
| Phase 2* | $0.72^{b}$(0.09) | $0.11^{b}$(0.05) | $1.19^{b}$(0.09) | $2.36^{b}$(0.13) | $2.78^{b}$(0.08) | $0.33^{b}$(0.05) | $2.29^{a}$(0.14) |
| Phase 3* | $1.38^{a}$(0.18) | $1.32^{a}$(0.1) | $2.64^{a}$(0.18) | $3.47^{a}$(0.24) | $2.23^{c}$(0.15) | $0.81^{a}$(0.1) | $2.86^{a}$(0.26) |

a,b,c Means with different superscriptal letter within columns differ ($P < 0.05$)

* Phases are grouped by microbial counts from TSA plate cut-off (log CFU/g).

Phase 1: Day 0-Day 5 (< 3 log CFU/g); Phase 2: Day 10-Day 20 ( 4 log CFU/g – 6 log CFU/g);

Phase 3: Day 26-Day 47 (> 6 log CFU/g).

**Table 3.6**. Acid evaluation scores by package x storage time (phase) with SE for cooked beef steaks stored at 4°C.

| Phase* | Cooked | |
|---|---|---|
| | Control | FreshCase® |
| Phase 1 | $0.01^{c}$(0.08) | $0.06^{c}$(0.08) |
| Phase 2 | $0.08^{c}$(0.04) | $0.08^{c}$(0.04) |
| Phase 3 | $0.52^{b}$(0.07) | $0.8^{a}$(0.06) |

$^{a,b,c}$ Means with different superscriptal letter differ ($P < 0.05$)

* Phases are grouped based on aerobic psychrotrophic bacteria counts cut-off
Phase 1: Day 0-Day 5 (< 3 log CFU/g);
Phase 2:Day10-Day 20 ( log CFU/g – 6 log CFU/g);
Phase 3: Day 26-Day 47 (> 6 log CFU/g).

**Table 3.7.**

Thiobarbituric acid (TBA) values by package x storage time (day) effect for beef steaks stored at 4°C

| | Day | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 26 | 31 | 36 | 41 |
| Control | $0.44^{a}$ | $0.26^{bcde}$ | $0.28^{bcd}$ | $0.07^{g}$ | $0.11^{fg}$ | $0.33^{ab}$ | $0.34^{ab}$ | $0.23^{bcde}$ | $0.31^{bc}$ |
| FreshCase® | $0.18^{defg}$ | $0.29^{bcd}$ | $0.20^{cdef}$ | $0.15^{efg}$ | $0.15^{efg}$ | $0.20^{cdef}$ | $0.29^{bcd}$ | $0.32^{ab}$ | $0.18^{defg}$ |

SEM is 0.04.

$^{a-g}$ Means with different superscript letters differ ($P < 0.05$)

**Table 3.8.** Means of traits by package effect for ground beef stored at 4°C.

| | Control | FreshCase® | SEM | P-value |
|---|---|---|---|---|
| L*[1] | 39.93 | 39.97 | 0.31 | 0.937 |
| APB[2] | 6.59 | 6.50 | 0.06 | 0.354 |
| LAB[2] | 5.97 | 5.93 | 0.05 | 0.808 |
| pH | 5.61[a] | 5.58[b] | 0.01 | 0.008 |

[1] L* reflects the instrumental color of lightness
[2] APB: Aerobic psychrotrophic bacteria
LAB: Lactic acid bacteria

**Table 3.9.**
Objective color of L* (lightness) by storage time (day) for ground beef stored 4 ℃

| Day | 0 | 5 | 10 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|
| L* | $37.09^{c}$ | $38.25^{bc}$ | $43.80^{a}$ | $38.58^{bc}$ | $39.15^{b}$ | $42.84^{a}$ |

SEM=0.54

$^{a,b,c}$ Means with different superscript letters within column differ ($P < 0.05$)

**Table 3.10.** Microbial counts by storage time (day) effect for ground beef stored at 4°C

| | APB* | LAB * |
|---|---|---|
| Day | Log CFU/g | Log CFU/g |
| 0 | 4.12[e] | 3.06[e] |
| 5 | 5.21[d] | 4.87[d] |
| 10 | 6.76[c] | 6.42[c] |
| 12 | 7.37[b] | 7.07[ab] |
| 13 | 7.88[a] | 7.31[a] |
| 14 | 7.94[a] | 6.98[b] |
| SEM | 0.1 | 0.1 |

[a-e] Means without same superscript within column differ ($P < 0.05$)

* APB: Aerobic psychrotrophic bacteria
LAB: Lactic acid bacteria

**Table 3.11**. Odor panel scores by package effect for ground beef stored at 4°

| | Control | FreshCase® | SEM | *P*-value |
|---|---|---|---|---|
| Raw | | | | |
| Putrid | 0.38 | 0.39 | 0.12 | 0.97 |
| Acid | 0.06 | 0.09 | 0.04 | 0.56 |
| Sour | 0.54 | 0.42 | 0.10 | 0.21 |
| Rancid | 1.08 | 0.98 | 0.15 | 0.51 |
| Meaty | 3.13 | 3.12 | 0.11 | 0.91 |
| Cooked | | | | |
| Putrid | 0.21 | 0.19 | 0.06 | 0.71 |
| Sour | 0.73[a] | 0.32[b] | 0.13 | 0.004 |
| Rancid | 1.32[a] | 0.8[b] | 0.14 | 0.001 |
| Meaty | 8.23 | 8.17 | 0.15 | 0.71 |

**Table 3.12.** Odor panel scores by storage time (day) for raw and cooked ground beef of stored at 4°C.

| | Raw | | | | | Cooked | | | |
|---|---|---|---|---|---|---|---|---|---|
| Day | Putrid | Acid | Sour | Rancid | Meaty | Putrid | Sour | Rancid | Meaty |
| 0 | $0.33^{ab}$ | $0.05^{b}$ | $0.02^{d}$ | $0.23^{d}$ | $2.50^{c}$ | $0.03^{d}$ | $0.11^{b}$ | $0.21^{c}$ | $8.46^{a}$ |
| 5 | $0.10^{b}$ | $0.02^{b}$ | $0.12^{d}$ | $0.16^{d}$ | $3.33^{ab}$ | $0.31^{ab}$ | $0.10^{b}$ | $0.58^{bc}$ | $8.22^{a}$ |
| 10 | $0.05^{b}$ | $0.10^{ab}$ | $0.33^{cd}$ | $1.03^{bc}$ | $3.21^{ab}$ | $0.13^{bcd}$ | $0.07^{b}$ | $0.80^{b}$ | $6.94^{b}$ |
| 12 | $0.67^{a}$ | $0.24^{a}$ | $0.75^{ab}$ | $0.8^{c}$ | $3.03^{b}$ | $0.1^{cd}$ | $0.15^{b}$ | $0.68^{bc}$ | $8.52^{a}$ |
| 13 | $0.41^{ab}$ | $0.01^{b}$ | $0.64^{bc}$ | $1.34^{b}$ | $3.49^{a}$ | $0.28^{abc}$ | $0.19^{a}$ | $1.90^{a}$ | $8.66^{a}$ |
| 14 | $0.75^{a}$ | $0.03^{b}$ | $1.04^{a}$ | $2.65^{a}$ | $3.22^{ab}$ | $0.39^{a}$ | $0.06^{b}$ | $2.34^{a}$ | $8.39^{a}$ |
| SEM | 0.15 | 0.05 | 0.12 | 0.19 | 0.13 | 0.07 | 0.16 | 0.17 | 0.18 |

$^{a,b,c}$ Means without same superscript within same column differ ($P < 0.05$)

**Table 3.13.** Acid scores by package x time effect of cooked ground beef stored at 4°C

| Day | Acid | |
|---|---|---|
| | Control | FreshCase® |
| 0 | $0.00^{c}$ | $0.25^{ab}$ |
| 5 | $0.1^{bc}$ | $0.11^{bc}$ |
| 10 | $0.17^{abc}$ | $0^{c}$ |
| 12 | $0.12^{bc}$ | $0.19^{abc}$ |
| 13 | $0.34^{a}$ | $0^{c}$ |
| 14 | $0.11^{bc}$ | $0.01^{bc}$ |

$^{a,b,c}$ Means without same superscript letters differ at $P < 0.05$

**Table 3.14**. Thiobarbituric acid (TBA) values for raw ground beef stored in different packages by day effect at 4°C

| | Day | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 12 | 13 | 14 |
| Control | $0.29^{d}$ | $0.75^{bc}$ | $0.95^{b}$ | $0.78^{bc}$ | $0.95^{b}$ | $1.19^{a}$ |
| FreshCase® | $0.25^{d}$ | $0.75^{bc}$ | $0.74^{c}$ | $0.76^{bc}$ | $0.82^{bc}$ | $0.41^{d}$ |

SEM = 0.07

$^{a-d}$ Means without the same superscript differ at $P < 0.05$

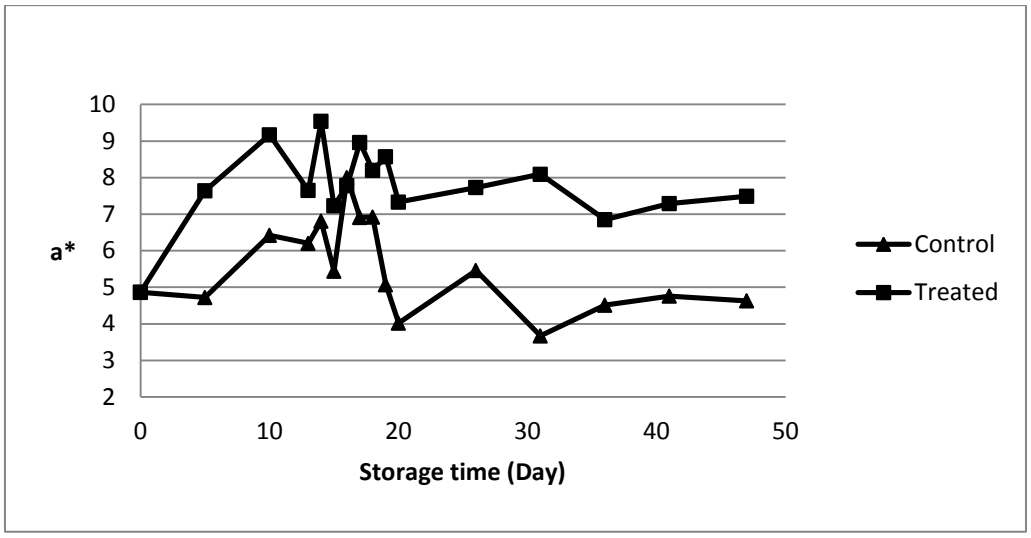


**Figure 3.1.** Least squares means for a* (redness) value of lean beef steaks stored at 4°C

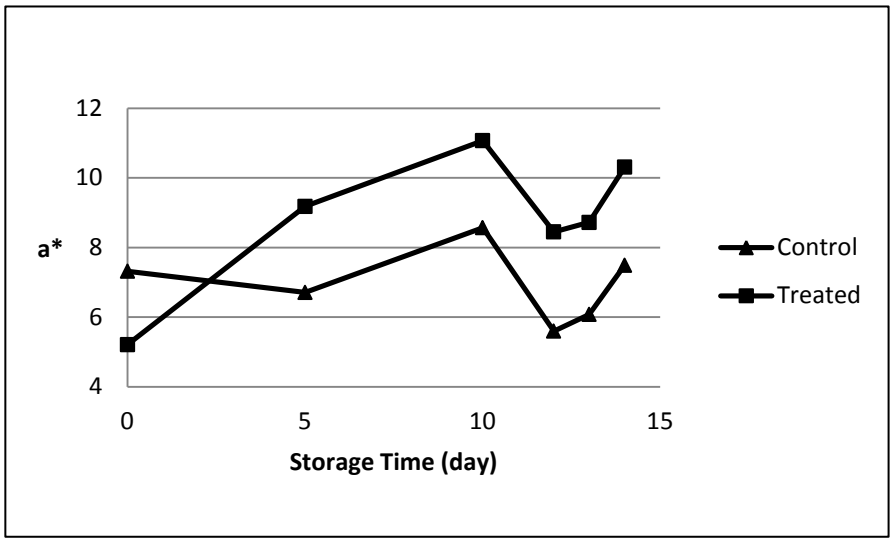


**Figure 3.2.** Least square means for the a* (redness) values of fresh ground beef during storage at 4°C.

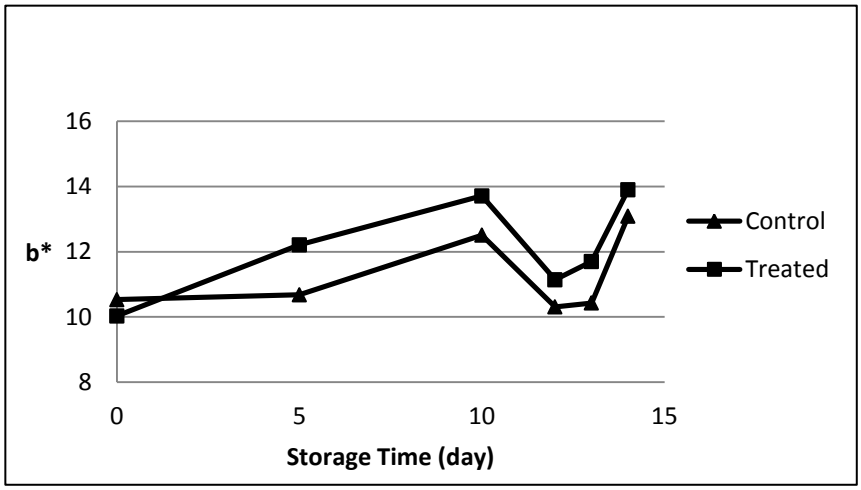


**Figure 3.3.** Least square means for the b* (yellowness) values of ground beef during storage at 4°C.

# Chapter IV

## An Evaluation of the Effectiveness of FreshCase® Technology to Extend the Shelf Life of Whole Muscle Pork and Ground Pork Sausage


### Summary

The effect of FreshCase®, a novel packaging technology that has been shown to extend the shelf life of whole muscle pork and pork sausages, was evaluated in this research. FreshCase® packaging refers to vacuum packaging fresh meat with high barrier nitrite containing film. Pork chops and pork sausages packaged using conventional vacuum packaging or FreshCase® technology were compared with respect to microbial counts, pH, instrumental color measurements, and sensory properties. Storage life was defined by the number of days required to reach an aerobic psychrotrophic plate count of $10^7$ log CFU/g. The storage life for pork chops stored in FreshCase® packages at 1°C was 48 days; and the storage life for ground pork sausages stored in FreshCase® packages at 1°C was 19 days. Additionally, the results indicated that both pork chops and sausages stored in FreshCase® packages retained redder color ($P < 0.05$) than those stored in Control packages throughout the storage time. No significant differences ($P > 0.05$) were found between Control and FreshCase®-Packaged samples of all off-odor detection for both pork chops and sausages. Likewise, levels of oxidative rancidity in all packages were low with TBA values.



**Key words:** FreshCase®, vacuum packaging, pork color, shelf life, storage life

## Introduction

Meat and meat products are highly perishable commodities. Shelf life of meat is the period of time between packaging of the product and the occurrence of the adverse changes of texture, flavor, odor, color and nutritional values until an unacceptable level, if one or more of these properties is attained (Gray, 1996). It has been shown that off-odor and slime were detected strongly on meat when bacterial populations grow to $10^7$ $cm^{-2}$ and $10^8$ $cm^{-2}$, respectively (Ayres, 1960).

Due to a high level of oxygen, meat shelf life may be reduced by the microbial growth, the development of off-flavor and off-odor, the change of color and nutritional losses (Kerry et al., 2006). Vacuum packaging is one of the most common methods used in wholesale meat marketing to Control the level of oxygen (Lambert, et al., 1991). For primal cuts on low or normal pH, the utilization of vacuum packaging can extend the shelf life by about five times over those using overwrapped packages. However, the major disadvantage of vacuum packaging is the purple color resulting from the formation of deoxymyoglobin. Consumers tend to consider meat color as the indicator of freshness and anticipated palatability (Brewer, 2002; Jeremiah, 1982). Green & Zipser (1971) reported that since meat cuts lose their fresh color from bright red to brown red or discolored, 30%-40% of consumers would reject purchasing.

FreshCase® technology refers to vacuum packaging fresh meat with high barrier nitrite containing film. The reaction between nitrite and myoglobin consistently provide a bright red color to the surface of the meat. However, no microbiological data

has been provided to the USDA/FSIS on FreshCase® packaged pork, which indicates the spoilage of meat.

The aims of this research were to identify the microbiological shelf life, which is defined by the level that aerobic psychrotrophic bacteria (APB) grew to indicating spoilage ($10^7$ log CFU/g), of whole muscle pork and pork sausages, and to evaluate the effects of FreshCase® Technology on the instrumental color and sensory scores of the same products.

## Materials and Methods

Sample Preparation

Pork Chops

Two cases of boneless, pork loins (NAMP 413B) were collected from a USDA-inspected commercial processing plant on day 3 postmortem and transported on ice (0 – 2℃) to the Colorado State University (CSU) Meat Laboratory. Immediately following arrival at CSU, pork loins were removed from their vacuum packages and sliced into 2.54cm thick pork chops. Each chop remained maximum exterior fat thickness of 0.64 cm. Following trimming, pork chops were randomly assigned to 1 of 2 packaging treatments. Every other pork chop was placed into a 15.24 cm x 30.48 cm x 2.54 cm FreshCase® pouch (FreshCase®).The FreshCase® pouches used were made using high barrier film with a 3 mil thickness and had sodium nitrite incorporated into the sealant layer. Sodium nitrite was present at a level providing less than 60 mg/$m^2$ to the pork contact surfaces. Every other pork chop was

placed into an identical 15.24 x 30.48 cm x 2.54 cm bag that did not contain nitrite (Control). Each pouch was individually identified and vacuum packaged using a dual chamber vacuum packaging machine (Multivac, Model C500) until 7 mbar of pressure was achieved. Then, pouches were boxed according to treatment and stored without light under refrigeration at 0 - 2 ℃.

Pork Sausages

In order to achieve a lean specification of approximately 60 %, two cases of pork shoulder, Boston Butts (NAMP 406) were chosen as a typical cut and collected from a USDA-inspected commercial processing plant on day 3 postmortem and transported on ice (0 – 2 ℃) to CSU. Each case was processed and stored independently from the other as case would serve as an experimental block. Upon arrival at the meat laboratory, using sanitary techniques, the butts were removed from vacuum packages, cut into 5 cm x 5 cm pieces, and ground twice. Using a mixing-grinder (Hobart, Model 4346), the pork pieces were coarse ground using a 1.27 cm breaking plate. The coarse ground pork was mixed with a commercially available irradiated pork sausage seasoning blend that contained salt and spices including sage and pepper (Legg's Old Plantation Seasonings, Blend WS-00-027-001, A.C. Legg, Inc. Calera, AL) according to the label (11.75 oz. of seasoning per 25 lbs. of meat). The seasoning blend did not contain nitrites. The mixed pork was then ground the second time using a 0.48 cm plate. Immediately following grinding, 454 g of ground pork sausage was tightly stuffed using a vacuum stuffer (Handtmann, Model VF 50) into 1 of 2 packaging treatments. Every other 454 g portion was stuffed

into a FreshCase® pouch and a Control pouch. Ground pork sausage pouches were individually labeled and packaged identically as described for pork loins.

A total of 150 (6 pouches/day * 25 days) pouches of pork chops and 150 pouches of ground pork sausages were prepared for storage. Six Control and 6 FreshCase® pouches were removed from storage for pork chops and sausage at time intervals of 1-5 days. Sampling intervals were variable and were determined in the interest of quantifying APB on the day that the average count reached $10^7$ log CFU/g. The interval was be decreased as the bacterial growth reached the end of storage life in order to most accurately capture the total number of days before $10^7$ log CFU/g APB was observed. Refrigerated storage of untested samples continued until average APB counts reached $10^7$ log CFU/g for 3 consecutive days.

Instrumental Color Measurement

On each sampling day, objective color measurements were taken from every package removed from storage using a MiniScan® EZ spectrophotometer (Hunter Association Laboratory Inc., Reston, VA, USA). Packages were opened and exposed to the air for a maximum of 10 seconds. Within 10 seconds of opening, the surface lean color and external fat color of pork chops was measured independently at three different locations. The three values for lean color and three values for fat color were averaged to obtain single lean color and fat color values for each sample. The surface color of ground pork sausage was measured at three different locations on each sample and averaged to obtained single values for each sample. The color was

presented in terms of CIE L*(lightness), a*(redness) and b*(yellowness) values.

Microbiological Analysis

On each microbial sampling day, 6 pouches each of pork chop and ground pork sausage were randomly selected and analyzed for counts of total aerobic psychrotrophic bacteria (APB) and lactic acid bacteria (LAB). Individual pork chops (approximately 100 g of whole muscle) were aseptically cut into 1 cm cubes and placed into a Whirl-Pak filter bag (1.63 L; Nasco, Modesto, CA). For the ground pork sausage, approximately 100 g samples were transferred from each pouch into individual Whirl-Pak filter bags. Diluent, comprised of 0.85% sodium chloride and 0.1% peptone, was added to each sample at a 1:1 ratio (sample weight to volume of diluent) followed by pummeling for 2 min (Masticator, IUL Instruments, Barcelona, Spain). Sample homogenates were serially diluted in 0.1% Buffered Peptone Water (Difco, Becton Dickinson, Sparks, MD) and spread-plated in duplicate onto Tryptic Soy Agar (TSA; Acumedia, Lansing, MI) for enumeration of total APB. For determination of LAB counts, sample dilutions (1 ml) were mixed with 10 ml of molten (45 ℃) Lactobacilli MRS agar (Difco); this was also done in duplicate. After setting, a 10 ml overlay of the molten Lactobacilli MRS agar was added to each plate. Colonies were counted after incubation of TSA plates at 7 ℃ for 10 days, and MRS plates at 25 ℃ for 5 days. Duplicate plate counts were averaged and a single count was reported for each sample.

pH Measurement

The pH of sample homogenates was measured after microbial analysis, using a Denver Instruments pH meter fitted with a glass electrode (UltraBasic-5, Arvada, CO). The pH meter was calibrated with standard buffers of pH 4.0 and 7.0 and pH was measured at room temperature (25 ℃).

Odor Panels

Before each odor panel, 4 pork chop and ground pork sausage samples were cut into equal parts; one-half of each sample was designated as raw and the other half was cooked for odor evaluation. The raw half of pork chop was cut into 1 cm x 1 cm x 1 cm pieces and put into 2 oz. lidded glass jars, and 50 g of raw ground pork sausage was put in jars. Pork chop and ground pork sausage samples designated for cooking were cooked to an internal temperature of 71°C using double sided electric grills (Salton Clamshell Grill Model No. GR39A, Salton Inc., Lake Forest, IL), and put into 2 oz. lidded glass jars. All jars were labeled in a completely random order.

During the odor panel, at least 6 trained panelists who had been previously trained to become familiar with the sensory characteristics of meat were seated in individual booths in a light-controlled room. Each panelist received a set of 8 (2 raw and cooked pork chops and 2 raw and cooked ground pork sausage) samples to evaluate off-odors and general meat odors using a 15 cm unstructured line scale anchored on the extreme left indicating absence of the odor and the extreme right indicating a very strong presence. A single sensory value was obtained for each of the

following odors: putrid, acid, sour, rancidity and meaty odor. Trained panelists marked the scale with a vertical line at the perceived intensity of the attributes. The results were expressed by the distance of the vertical line from the extreme left end of the 15 cm scale.

Thiobarbituric acid (TBA) value

The 2-thiobarbituric acid (TBARs) method described by Tarladgis et al. (1960) was used to measure the lipid oxidation for each sample designated for TBARS analysis. Four pouches of Control and FreshCase® pork chops samples and 4 ground pork sausage samples were selected for TBARS analysis on days indicating a significant increase in microbial growth. Thiobarbituric acid reacts with the oxidation products of fat to form malonaldehyde, which was measured on a spectrometer in solution. The TBA value was expressed by the mg malonaldehyde (MDA) /kg tissue.

Statistical Analysis

The experiment was a completely randomized block design. The effect of block was removed from the model as it was found to be insignificant in initial tests. A two-way analysis of variance (ANOVA) was conducted for each variable to investigate the fixed effects of packaging treatment, storage time, and corresponding interactions. Rather than analyzing odor panel scores for individual samples by day, multiple sampling days were combined to represent 3 phases of microbial growth. Phase of microbial growth was utilized as a fixed effect in the model in the place of

day for pork chops and ground pork sausage odor panel ratings. For pork chops, Phase 1 was the time period from day 0 to day 5; phase 2 was the time period from day 10 to day 20; the time period of day 26 to day 47 was grouped in phase 3. For ground pork sausages, phase 1 was the time period from day 0 to day 5; phase 2 was the time period from day 10 to day 19; phase 3 was the time period from day 20 to day 23.

The General Linear Model procedure (PROC GLM) and mixed procedure (PROC MIXED) of SAS (Cary, NC Ver. 9.1; 2007) were used to analyze the data. Microbial counts were expressed as $\log_{10}$ CFU/g. The responsible variables, as CIE L*, a*, b* values, microbiological loads, pH, TBARS, and sensory panel scores were evaluated and significance of differences was defined as $\alpha = 0.05$. The mean separations were obtained using Fisher's Least Significant Difference test.

## Results and Discussion

### Pork Chops

#### Objective Color

The main effects of packaging treatment on objective color, microbial counts, pH and TBA values are presented in Table 4.1. Lean L* and b* values were lower ($P < 0.05$) for FreshCase® pork chops versus Control pork chops. Likewise, the external fat of FreshCase® pork chops also had lower ($P < 0.05$) L* and b* values. This indicates that control pork chops appeared to lighter and more yellow colored. Both lean and the external fat of FreshCase® pork chops displayed a redder/pinker appearance with higher ($P < 0.0001$) a* values. The main effect of storage time (day) was significant

($P < 0.05$) on pork chops L*, a*, and b* readings (data not shown). However, there were no discernible trends identified from these values.

Bacteria and pH Results

Counts for APB and LAB, as well as pH were not influenced ($P > 0.05$) by packaging types. The storage time had a positive effect ($P < 0.05$) on pH. The slight increase of pH by day has agreed to the result concluded by Moore and Gill (1987). The average pH values of pork chopped stored in two types of packages ranged from 5.56 to 5.92 (data not shown).

Plate counts of APB and LAB increased ($P < 0.05$) throughout storage time (day; Table 4.2). Initially, levels of APB and LAB were relatively low with counts of 1.53 log CFU/g and 1.56 log CFU/g, respectively. Both APB and LAB counts for pork chops exceeded 3 logs of growth by 25 days of refrigerated storage. After 3 logs of growth for both classifications of bacteria was observed, sampling became more frequent and plate APB and LAB counts were steady and seemingly linear. Aerobic psychrotrophic bacteria (APB) exceeded 7 log CFU/g on day 49 of storage, while LAB exceeded 7 log CFU/g 48 days prior. Consequently, the storage life of beef steaks, regardless of packaging technology in accordance with APB, expired after 47 days of storage.

Odor Panel Scores

Odor panel scores were presented in Table 4.3. The main effect of packaging treatment did not influence ($P > 0.05$) all sensory odor scores for both raw and cooked

pork chop samples, indicating that the use of nitrite didn't accelerate the developments of these off-odors. The main effect of storage time (phase) significantly ($P < 0.05$; Table 4.4) affected odor panel scores of all attributes for raw pork chops. As storage time increased, the intensity of putrid, acid, sour, and rancid increased while meaty odor intensity declined. These results were expected. Storage time did not ($P = 0.2294$) affect acid odor of cooked pork chops. Despite the notable increase in intensity of putrid, sour and rancid odors in cooked pork chop samples, the intensity remained low for all these attributes. Therefore, in reference to product odor, indications of spoilage remained low throughout storage. Most notably, APB and LAB counts grew beyond the recognized level of bacterial spoilage ($10^7$ log CFU/g) in Phase 3, and there was very little indication of spoilage via product odor. Additionally, throughout storage, the color of FreshCase® samples remained bright red and acceptable in appearance.

TBA Results

The TBA value for pork chops stored in the FreshCase® packages was slightly higher ($P = 0.0002$) than Control ones (Table 4.1). However, averaging the storage time, the difference was only 0.08 mg MDA/kg. Although pork chops using FreshCase® packaging technology did have a higher TBA value, the overall TBA value was still lower than 1 mg MDA/kg, which was accepted by human threshold for detection of rancidity (Tarladgis et al., 1964). The main effect of storage time did not ($P = 0.204$) influence the TBA value for pork chops.

## Ground Pork Sausage

### Objective Color

The main effects of packaging treatment on objective color, microbial counts, and pH are presented in Table 4.5. There was no ($P > 0.05$) main effect of package treatment for fresh pork sausages on lightness (L*) and yellowness (b*), while FreshCase® ground pork sausage had higher ($P < 0.0001$) a* values translating to a much redder appearance. The main effect of storage time (day) was significant ($P < 0.0001$) on ground pork sausage L*, a*, and b* readings (data not shown). However, there were no discernible trends identified from these values.

### Bacterial and pH Results

As shown in Table 4.5, the main effect of packaging treatment did not ($P > 0.05$) affect the counts for APB and LAB. The average pH throughout storage time for FreshCase® ground pork sausages was slightly higher ($P = 0.005$; Table 4.5) versus Control ground pork sausage. However, this 0.03 difference was of little practical importance. The pH of ground pork sausages ranged from 6.19 to 6.35, which was significantly ($P < 0.0001$) influenced by the storage time effect, but no discernible trends identified from these value (data not shown).

Storage time (day) influenced levels of APB and LAB ($P < 0.05$; Table 4.6). Initially, levels of APB and LAB were relatively high compared to that of whole muscle pork with counts of 3.42 log CFU/g and 2.48 log CFU/g, respectively. Both APB and LAB counts for ground pork sausage exceeded 2 logs of growth by 13 days

of refrigerated storage. After 2 logs of growth for both classifications of bacteria was observed, sampling became more frequent and plate APB and LAB counts were steady and seemingly linear. Aerobic psychrotrophic bacteria (APB) exceeded 7 log CFU/g on day 20 of storage, while LAB exceeded 7 log CFU/g 19 days prior. Consequently, the storage life of ground pork sausage, regardless of packaging type in accordance with APB, expired after 19 days of storage.

Odor Panel Scores

As shown in Table 4.7, the main effect of packaging treatment did not affect ($P > 0.05$) sensory odor scores for both raw and cooked ground pork sausage samples. The main effect of storage time (phase) significantly ($P < 0.05$; Table 4.8) influenced odor panel scores of all attributes for raw ground pork sausages. As storage increased, the intensity of putrid, acid, sour, and rancid increased while meaty odor intensity decreased. These results were as expected. The rancid odor and meaty odor increased in cooked samples. Storage time (phase) did not affect putrid, acid, or sour odors of cooked ground pork sausage samples. The use of sausage mix blending in ground pork sausages might affect panelist to detect the weak intensity of these off odors for cooked pork sausages, since the spices had very offensive odor after cooking the sausages. The intensity ratings for all attributes, except meaty odors, remained very low. Therefore, in reference to product odor, indications of spoilage remained low throughout storage. Most notably, APB and LAB counts grew beyond the recognized level of bacterial spoilage ($10^7$ log CFU/g) in Phase 3, and there was very little

indication of spoilage via product odor. Additionally, throughout storage, the color of FreshCase® samples remained bright red and acceptable in appearance.

TBA

The TBA values of beef steaks are shown in Table 4.9. Statistically, there was a packaging treatment x storage time (day) interaction ($P$ = 0.0018) for ground pork sausages. The level of oxidative rancidity, as indicated by TBA values, remained very low for all samples over all time periods and treatments. The maximum tested TBA value for Control beef steaks was 0.35 mg MDA/kg, and the maximum TBA value for FreshCase® samples was 0.55 mg MDA/kg. Tarladgis et al. (1964) reported that human can accept the intensity of rancidity when the TBA value is low than 1 mg MDA/kg. Therefore, despite the fact that after extended periods of storage samples reached APB and LAB exceeding $10^7$ log CFU/g, oxidative rancidity was not reached in the sampling periods included in this study. These findings also support the low intensity levels of putrid and rancid odors in the sensory portion of this study.

Sodium nitrite ($NaNO_2$) combined with sodium chloride has been used in processed meat as a preservative to inhibit the growth of *Clostridium botulinum* for several years (Pierson and Smoot, 1987; Christiansen et al., 1974). However, the amount of nitrite in FreshCase® packages is relative low, which is not enough for any preservative antimicrobial purpose. Additionally, a certain level of nitrite can inhibit the growth of some spoilage bacteria, like *Enterobacteriaceae* and B. *thermosphacta*, but not lactic acid bacteria (Nielsen, 1983). Furthermore, it is well known that the

major bacteria growing on the vacuum-packaged meat at chill temperature is lactic acid bacteria (Blickstad and Molin, 1983; Shaw and Harding, 1989; von Holy et al., 1991). This is why we did not expect any package effect on microbial growth in this study, and the results are all support our assumption.

As expected, both pork chops and fresh pork sausages displayed redder color (higher a* values) by using FreshCase® packages which containing nitrite in the film. This result confirms the results reported in other studies that nitrite is the common curing agent used in meat to maintain fresh red color of meat (Pourazrang et al., 2002; Zarringhalami et al., 2005). The formation of NO-myoglobin produces a red meat color. (Siegel, 2010).

Additionally, florescent green color was observed on pork chops in Control packages at day 30 of storage. Hydrogen sylpide produced from cysteine by Lactobacillus sake, when the glucose and oxygen availability is limited, converting the myoglobin to sulphmyoglobin, is responsible for the green color of meat (Egan et al,. 1989). Meat with high pH may have high incidence of greening, however, normal pH meat also has green pigment (Borch, et al., 1996).

It was also observed that the internal color of the cooked sausages, which were stored in the FreshCase® packages, was pink as well. Generally speaking, after cooking, meat color can turns grey or brown because oxymyoglobin is not heat stable. However, the red $NO^-$ porphyrin ring system (usually called nitroso-myochromogen) can still exist even when heating the NO-myoglobin under 120°C and the protein is denatured (Honikel, 2008). It is not good for cooked sausage to maintain a pink color

after cooking, which may mislead the doneness of the sausages to the consumers, resulting overcooking and negative effect on palability of the product.

The small TBA values of pork chops and pork sausages stored in the vacuum packages confirmed the result that vacuum packages can minimized the oxidation of lipid effectively from another study (Cayuela, et al, 2004; John et al., 2004; John et al., 2005). In Krause et al. (2003) study, it was noticed that pork chops stored in vacuum packaged and modified-atmosphere package (MAP) of 0.5% CO, 70% $CO_2$ and 29.5% N2 has the lowest TBA values, compared to those stored in over-wrapped package or MAP of 20% $CO_2$ and 80% $N_2$. The most common commercial use of MAP is containing 20%-80% oxygen (Eilert, 2005). Therefore, the application of MAP with low level of CO is not used widely in the market because CO is known as a potentially hazardous gas to consumers (Cornforth, 2008).

**Conclusion**

The results of this study approved that the use of FreshCase® Packaging Technology can extend shelf life of pork and pork products by stabilizing a bright red color, with low levels of lipid oxidation and off-odors. Compared to MAP technology, the FreshCase® Packaging Technology does not require any additional equipment to modify the atmosphere of the packaging. It can run with existing vacuum packaging equipment, but still provide the same bright red color as using the MAP with low CO concentration.

**Table 4.1.** Means of traits by packaging effect for pork chops stored at 1°C.

| Traits | Control | FreshCase® | SEM | *P*-value |
|---|---|---|---|---|
| Lean L*[1] | 47.58 | 46.75 | 0.25 | 0.017 |
| Lean a*[1] | -1.47 | 0.71 | 0.08 | <0.0001 |
| Lean b*[1] | 6.23 | 5.83 | 0.10 | 0.007 |
| Fat L*[1] | 68.48 | 67.77 | 0.18 | 0.007 |
| Fat a*[1] | 0.44 | 1.18 | 0.08 | <0.0001 |
| Fat b*[1] | 10.85 | 9.93 | 0.14 | <0.0001 |
| APB[2] | 5.41 | 5.26 | 0.06 | 0.088 |
| LAB[2] | 5.49 | 5.41 | 0.06 | 0.325 |
| pH | 5.74 | 5.75 | 0.01 | 0.501 |
| TBA | 0.27 | 0.33 | 0.01 | 0.0002 |

[1] L* reflects the lightness of meat color; a* reflects the redness; b* reflects the yellowness

[2] APB: Aerobic psychrotrophic bacteria
LAB: Lactic acid bacteria

**Table 4.2.** Microbial counts for pork chops stored at 1°C

| | APB* | LAB* |
|---|---|---|
| Day | Log CFU/g | Log CFU/g |
| 0 | $1.53^{n}$ | $1.56^{l}$ |
| 5 | $1.33^{n}$ | $1.25^{l}$ |
| 10 | $1.56^{n}$ | $1.22^{l}$ |
| 15 | $2.41^{n}$ | $2.41^{k}$ |
| 20 | $3.33^{m}$ | $3.6^{j}$ |
| 25 | $4.79^{k}$ | $4.97^{i}$ |
| 28 | $4.71^{k}$ | $4.94^{i}$ |
| 30 | $5.01^{jk}$ | $5.27^{hi}$ |
| 32 | $5.44^{hij}$ | $5.45^{hi}$ |
| 34 | $5.24^{ijk}$ | $5.31^{hi}$ |
| 36 | $5.97^{efgh}$ | $6.32^{ef}$ |
| 37 | $5.77^{ghi}$ | $5.7^{gh}$ |
| 38 | $6.05^{efg}$ | $6.13^{fg}$ |
| 39 | $6.05^{efg}$ | $6.49^{def}$ |
| 40 | $6.52^{cde}$ | $6.6^{cdef}$ |
| 41 | $6.42^{cdef}$ | $6.71^{bcde}$ |
| 42 | $6.38^{cdefg}$ | $6.78^{bcde}$ |
| 43 | $6.26^{def}$ | $6.44^{def}$ |
| 44 | $6.55^{cde}$ | $6.62^{cdef}$ |
| 45 | $6.52^{cde}$ | $6.58^{def}$ |
| 46 | $6.9^{bc}$ | $6.97^{bcd}$ |
| 47 | $6.97^{abc}$ | $7.14^{abc}$ |
| 48 | $6.83^{bcd}$ | $6.95^{bcd}$ |
| 49 | $7.23^{ab}$ | $7.27^{ab}$ |
| 50 | $7.5^{a}$ | $7.58^{a}$ |
| SEM | 0.23 | 0.21 |

$^{a,b,c}$ Means with different superscript within column differ ($P > 0.05$)

* APB: Aerobic psychrotrophic bacteria
LAB: Lactic acid bacteria

**Table 4.3.** Odor panel scores by packaging effect for pork chops stored at 1°C.

| | Control | FreshCase® | SEM | *P*-value |
|---|---|---|---|---|
| Raw | | | | |
| Putrid | 1.17 | 1.11 | 0.16 | 0.807 |
| Acid | 0.47 | 0.45 | 0.09 | 0.882 |
| Sour | 1.21 | 1.27 | 0.14 | 0.749 |
| Rancid | 1.05 | 1.06 | 0.16 | 0.967 |
| Meaty | 4.35 | 4.40 | 0.13 | 0.778 |
| Cooked | | | | |
| Putrid | 0.49 | 0.68 | 0.20 | 0.339 |
| Acid | 0.37 | 0.45 | 0.15 | 0.623 |
| Sour | 0.37 | 0.37 | 0.12 | 0.943 |
| Rancid | 1.36 | 1.35 | 0.29 | 0.965 |
| Meaty | 8.19 | 7.99 | 0.26 | 0.46 |

**Table 4.4.** Odor panel scores by storage time (phase) effect for raw and cooked pork chops stored at 1 ℃

| Phase* | Raw | | | | Cooked | | | |
|---|---|---|---|---|---|---|---|---|
| | Putrid | Acid | Sour | Rancid | Putrid | Sour | Rancid | Meaty |
| Phase 1* | $0.1^{b}$(0.28) | $0^{c}$(0.15) | $0.06^{c}$(0.22) | $0.08^{c}$(0.26) | $0.07^{b}$(0.23) | $0.02^{c}$(0.15) | $0.07^{c}$(0.33) | $8.93^{a}$(0.32) |
| Phase 2* | $1.62^{a}$(0.16) | $0.44^{b}$(0.09) | $1.55^{b}$(0.13) | $1.15^{b}$(0.15) | $0.53^{b}$(0.13) | $0.39^{b}$(0.08) | $1.29^{b}$(0.19) | $7.26^{c}$(0.19) |
| Phase 3* | $1.68^{a}$(0.13) | $0.81^{a}$(0.07) | $2.03^{a}$(0.11) | $1.83^{a}$(0.12) | $1.05^{a}$(0.11) | $0.66^{a}$(0.07) | $2.49^{a}$(0.16) | $7.95^{b}$(0.15) |

a,b,c Means with different superscript within columns are different at $P < 0.05$

* Phases are grouped based on aerobic psychrotrophic bacteria plate counts cut-off (log CFU/g).
Phase 1: Day 0-Day10 (< log 2 CFU/g); Phase 2:Day 15-Day 37 (log 2 CFU/g – log 6 CFU/g);
Phase 3: Day 38-Day 50 (> log 6 CFU/g).

**Table 4.5**. Means of traits by packaging effect for pork sausages stored at 1°C.

| | Control | FreshCase® | SEM | P-value |
|---|---|---|---|---|
| L*[1] | 47.79 | 47.50 | 0.27 | 0.453 |
| a*[1] | 6.05 | 6.69 | 0.08 | <0.0001 |
| b*[1] | 14.59 | 14.65 | 0.12 | 0.727 |
| APB[2] | 6.03 | 5.98 | 0.03 | 0.202 |
| LAB[2] | 5.98 | 5.94 | 0.03 | 0.252 |
| pH | 6.25 | 6.28 | 0.01 | 0.005 |

[1] L* reflects the instrumental color of lightness;
a* reflects the instrumental color of redness;
b* reflects the instrumental color of yellowness

[2]APB: aerobic psychrotrophic bacteria
LAB: lactic acid bacteria

**Table 4.6**. Microbial counts by storage time for pork sausages stored at 1 ℃

| | APB* | LAB* |
|---|---|---|
| Day | logCFU/g | logCFU/g |
| 0 | 3.42$^{h}$ | 2.48$^{i}$ |
| 5 | 3.36$^{i}$ | 3.16$^{h}$ |
| 10 | 4.63$^{h}$ | 4.65$^{g}$ |
| 13 | 5.39$^{g}$ | 5.40$^{f}$ |
| 15 | 6.01$^{f}$ | 6.08$^{e}$ |
| 17 | 6.57$^{e}$ | 6.63$^{d}$ |
| 19 | 6.84$^{d}$ | 7.04$^{c}$ |
| 20 | 7.14$^{c}$ | 7.19$^{c}$ |
| 21 | 7.37$^{b}$ | 7.49$^{b}$ |
| 22 | 7.62$^{a}$ | 7.64$^{ab}$ |
| 23 | 7.69$^{a}$ | 7.80$^{a}$ |
| SEM | 0.06 | 0.06 |

$^{a-i}$ Means with different superscript within column are different at $P<0.05$

* APB: aerobic psychrotrophic bacteria
LAB: lactic acid bacteria

**Table 4.7.** Odor panel scores by packaging effect for pork sausages stored at 1°C.

| | Control | FreshCase® | SEM | *P*-value |
|---|---|---|---|---|
| Raw | | | | |
| Putrid | 0.16 | 0.21 | 0.07 | 0.58 |
| Acid | 0.68 | 0.63 | 0.09 | 0.73 |
| Sour | 0.71 | 0.64 | 0.07 | 0.8 |
| Rancid | 0.48 | 0.57 | 0.06 | 0.51 |
| Meaty | 6.58 | 6.38 | 0.07 | 0.42 |
| Cooked | | | | |
| Putrid | 0.08 | 0.14 | 0.05 | 0.56 |
| Acid | 0.11 | 0.18 | 0.05 | 0.62 |
| Sour | 0.21 | 0.20 | 0.08 | 0.88 |
| Rancid | 0.53 | 0.48 | 0.06 | 0.73 |
| Meaty | 8.92 | 8.54 | 0.17 | 0.26 |

**Table 4.8.** Odor panel scores with standard error by storage time (phase) effect for raw and cooked pork sausages stored at 1 ℃

| Phase* | Raw | | | | | Cooked | |
|---|---|---|---|---|---|---|---|
| | Putrid | Acid | Sour | Rancidity | Meaty | Rancidity | Meaty |
| Phase 1* | $0^{b}(0.1)$ | $0.08^{c}(0.16)$ | $0^{b}(0.18)$ | $0.09^{b}(0.12)$ | $6.61^{a}0.39)$ | $0^{b}(0.16)$ | $7.84^{b}(0.39)$ |
| Phase 2* | $0.09^{b}(0.06)$ | $0.52^{b}(0.1)$ | $0.44^{b}(0.11)$ | $0.54^{a}(0.07)$ | $7.1^{a}(0.25)$ | $0.54^{a}(0.10)$ | $9.13^{a}(0.25)$ |
| Phase 3* | $0.39^{a}(0.07)$ | $1.12^{a}(0.11)$ | $1.31^{a}(0.13)$ | $0.73^{a}(0.08)$ | $5.63^{b}(0.28)$ | $0.71^{a}(0.11)$ | $8.65^{ab}(0.28)$ |

$^{a,b,c}$ Means with different superscript within columns are different at $P < 0.05$

* Phase 1: Day 0-Day 5 (< 3 log CFU/g); Phase 2: Day 10-Day 19 (2 log CFU/g – 7 log CFU/g); Phase 3: Day 20-Day 23 (> 7 log CFU/g).

**Table 4.9.** Thiobarbituric acid (TBA) values for pork sausages by packaging x storage time (day) effect stored at 1 ℃.

| | Day | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 13 | 15 | 17 | 19 | 20 |
| Control | $0.34^{bcd}$ | $0.21^{efg}$ | $0.33^{bcde}$ | $0.20^{efg}$ | $0.23^{defg}$ | $0.18^{fg}$ | $0.35^{bc}$ | $0.32^{bcde}$ |
| FreshCase® | $0.27^{cdefg}$ | $0.24^{cdefg}$ | $0.25^{cdefg}$ | $0.29^{bcdefg}$ | $0.30^{bcdef}$ | $0.17^{g}$ | $0.36^{bc}$ | $0.55^{a}$ |

SEM is 0.04.

$^{a-g}$ Means with the different superscript are different at $P < 0.05$

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APPENDIX

**Appendix 1.1.** Instrumental color (CIE L*, a*, b*) of beef steaks for lean and fat meat stored at 4°C.

| Day | Lean | | Fat | | |
|---|---|---|---|---|---|
| | L* | b* | L* | a* | b* |
| 0 | 33.56$^{c}$ | 8.04$^{g}$ | 51.14$^{bcde}$ | -0.01$^{g}$ | 10.51$^{h}$ |
| 5 | 33.91$^{bc}$ | 9.00$^{ef}$ | 52.23$^{bc}$ | 2.44$^{f}$ | 13.06$^{g}$ |
| 10 | 37.40$^{a}$ | 10.60$^{ab}$ | 57.28$^{a}$ | 5.54$^{abc}$ | 16.43$^{a}$ |
| 13 | 34.18$^{bc}$ | 8.72$^{fg}$ | 51.89$^{bc}$ | 3.81$^{def}$ | 13.54$^{efg}$ |
| 14 | 34.77$^{bc}$ | 10.67$^{a}$ | 57.18$^{a}$ | 4.55$^{cde}$ | 15.68$^{ab}$ |
| 15 | 34.49$^{bc}$ | 8.59$^{fg}$ | 49.34$^{cdef}$ | 5.80$^{abc}$ | 15.21$^{bc}$ |
| 16 | 33.11$^{c}$ | 9.62$^{cde}$ | 48.14$^{ef}$ | 5.50$^{abc}$ | 13.89$^{defg}$ |
| 17 | 33.46$^{c}$ | 9.83$^{bcd}$ | 47.82$^{f}$ | 6.63$^{a}$ | 14.77$^{bcd}$ |
| 18 | 34.49$^{bc}$ | 9.98$^{abc}$ | 47.94$^{f}$ | 6.31$^{ab}$ | 15.02$^{bcd}$ |
| 19 | 33.35$^{c}$ | 9.12$^{def}$ | 48.44$^{def}$ | 5.28$^{abcd}$ | 14.67$^{bcde}$ |
| 20 | 34.65$^{bc}$ | 8.33$^{fg}$ | 52.49$^{b}$ | 3.89$^{def}$ | 14.07$^{defg}$ |
| 26 | 34.07$^{bc}$ | 8.93$^{ef}$ | 51.10$^{bcde}$ | 4.85$^{bcde}$ | 14.31$^{cdef}$ |
| 31 | 34.40$^{bc}$ | 8.49$^{fg}$ | 51.21$^{bcd}$ | 3.64$^{ef}$ | 13.25$^{fg}$ |
| 36 | 35.53$^{b}$ | 8.88$^{ef}$ | 50.54$^{bcdef}$ | 5.28$^{abcd}$ | 14.28$^{cdef}$ |
| 41 | 34.49$^{bc}$ | 9.02$^{ef}$ | 48.39$^{def}$ | 4.75$^{cde}$ | 13.52$^{fg}$ |
| 47 | 33.52$^{c}$ | 8.47$^{fg}$ | 48.14$^{ef}$ | 6.01$^{abc}$ | 14.20$^{cdef}$ |
| SEM | 0.62 | 0.29 | 1.08 | 0.55 | 0.41 |

$^{a-g}$ Means with different superscriptal letters within columns differ ($P < 0.05$)

**Appendix 1.2.**Change of Beef Lean Color a* values by Day x Package Effect

| Day | Package Type | |
|---|---|---|
| | Control | FreshCase® |
| 0 | $4.86^{jkl}$ | $4.87^{jkl}$ |
| 5 | $4.72^{kl}$ | $7.64^{cdef}$ |
| 10 | $6.42^{fghi}$ | $9.17^{ab}$ |
| 13 | $6.20^{ghij}$ | $7.65^{cdef}$ |
| 14 | $6.81^{efgh}$ | $9.54^{a}$ |
| 15 | $5.44^{hijkl}$ | $7.23^{defg}$ |
| 16 | $8.00^{bcdef}$ | $7.78^{bcdef}$ |
| 17 | $6.91^{efg}$ | $8.96^{abc}$ |
| 18 | $6.92^{efg}$ | $8.20^{abcde}$ |
| 19 | $5.07^{ijkl}$ | $8.57^{abcd}$ |
| 20 | $4.02^{kl}$ | $7.33^{defg}$ |
| 26 | $5.46^{hijk}$ | $7.73^{bcdef}$ |
| 31 | $3.67^{l}$ | $8.09^{bcde}$ |
| 36 | $4.51^{kl}$ | $6.85^{efgh}$ |
| 41 | $4.76^{jkl}$ | $7.29^{defg}$ |
| 47 | $4.63^{kl}$ | $7.49^{defg}$ |

SEM=0.73

$^{a-l}$ Means with different superscriptal letters differ ($P < 0.05$)

**Appendix 1.3.** Sensory evaluation (off-odor) scores for raw and cooked beef steaks by day of storage at 4°C

| | Raw | | | Cooked | |
|---|---|---|---|---|---|
| Day | Putrid | Sour | Rancidity | Acid | Sour |
| 0 | $0.00^{f}$ | $0.08^{h}$ | $0.07^{e}$ | $0.04^{d}$ | $0.02^{e}$ |
| 5 | $0.02^{f}$ | $0.37^{fgh}$ | $0.25^{e}$ | $0.03^{d}$ | $0.08^{e}$ |
| 10 | $0.04^{f}$ | $0.19^{gh}$ | $0.62^{e}$ | $0.11^{d}$ | $0.07^{e}$ |
| 13 | $0.38^{ef}$ | $0.46^{fgh}$ | $0.49^{e}$ | $0.15^{d}$ | $0.53^{bcd}$ |
| 14 | $0.18^{ef}$ | $0.74^{fg}$ | $2.09^{d}$ | $0.09^{d}$ | $0.57^{bcd}$ |
| 15 | $0.44^{def}$ | $1.62^{cd}$ | $2.32^{d}$ | $0.07^{d}$ | $0.85^{ab}$ |
| 16 | 0.42 | $0.94^{ef}$ | $2.06^{d}$ | $0.12^{d}$ | $0.59^{bcd}$ |
| 17 | $1.18^{bcd}$ | $2.51^{ab}$ | $3.56^{b}$ | $0.00^{d}$ | $0.05^{e}$ |
| 18 | $0.50^{def}$ | $1.68^{cd}$ | $2.71^{cd}$ | $0.09^{d}$ | $0.36^{cde}$ |
| 19 | $1.45^{abc}$ | $0.65^{fgh}$ | $3.23^{bc}$ | $0.00^{d}$ | $0.18^{de}$ |
| 20 | $1.76^{ab}$ | $1.37^{de}$ | $4.28^{a}$ | $0.07^{d}$ | $0.03^{e}$ |
| 26 | $0.85^{cde}$ | $1.74^{cd}$ | $2.28^{d}$ | $0.01^{d}$ | $0.11^{e}$ |
| 31 | $0.50^{def}$ | $2.06^{bc}$ | $3.33^{bc}$ | $0.22^{cd}$ | $0.64^{bc}$ |
| 36 | $1.40^{abc}$ | $2.59^{ab}$ | $3.45^{b}$ | $0.67^{b}$ | $0.62^{bc}$ |
| 41 | $2.05^{a}$ | $2.78^{a}$ | $3.58^{b}$ | $0.41^{c}$ | $0.88^{ab}$ |
| 47 | $1.59^{abc}$ | $3.13^{a}$ | $3.51^{b}$ | $0.92^{a}$ | $1.09^{a}$ |
| SEM | 0.27 | 0.22 | 0.25 | 0.08 | 0.15 |

$^{a-h}$ Means with different superscriptal letter within columns differ ($P < 0.05$)

**Appendix 2.1.** Instrumental color (CIE L*, a*, b*) of pork chops stored at 1°C.

| | Lean | | | Fat | | |
|---|---|---|---|---|---|---|
| Day | L* | a* | b* | L* | a* | b* |
| 0 | 45.96$^{defg}$ | -0.72$^{defgh}$ | 5.11$^{fg}$ | 67.70$^{cdefgh}$ | -0.29$^{h}$ | 8.20$^{h}$ |
| 5 | 46.01$^{defg}$ | -0.06$^{bcde}$ | 5.62$^{defg}$ | 69.45$^{c}$ | 0.52$^{defg}$ | 9.90$^{cdefg}$ |
| 10 | 45.71$^{efg}$ | -0.26$^{bcdef}$ | 6.15$^{abcde}$ | 67.88$^{cdef}$ | 0.76$^{bcdefg}$ | 10.35$^{cdef}$ |
| 15 | 58.90$^{a}$ | -1.50$^{i}$ | 5.69$^{defg}$ | 85.62$^{a}$ | 0.27$^{fgh}$ | 12.78$^{a}$ |
| 20 | 48.30$^{cd}$ | -1.21$^{hi}$ | 5.01$^{g}$ | 68.65$^{cd}$ | 0.83$^{bcdefg}$ | 10.55$^{cde}$ |
| 25 | 46.23$^{defg}$ | -0.75$^{efghi}$ | 5.79$^{cdefg}$ | 68.45$^{cde}$ | 0.66$^{cdefg}$ | 10.37$^{cdef}$ |
| 28 | 47.69$^{cde}$ | -0.65$^{defgh}$ | 5.47$^{defg}$ | 68.80$^{cd}$ | 0.36$^{efgh}$ | 9.36$^{efgh}$ |
| 30 | 45.71$^{efg}$ | -0.94$^{fghi}$ | 5.28$^{efg}$ | 68.70$^{cd}$ | 0.53$^{defg}$ | 8.86$^{gh}$ |
| 32 | 47.19$^{cdef}$ | -0.65$^{defgh}$ | 5.55$^{defg}$ | 68.72$^{cd}$ | 0.53$^{defg}$ | 8.77$^{gh}$ |
| 34 | 46.38$^{defg}$ | -0.53$^{cdefgh}$ | 5.91$^{cdefg}$ | 67.39$^{defghi}$ | 0.69$^{cdefg}$ | 9.90$^{cdefg}$ |
| 36 | 46.81$^{defg}$ | -0.14$^{bcde}$ | 6.20$^{abcde}$ | 67.70$^{cdefgh}$ | 0.51$^{defg}$ | 9.82$^{cdefg}$ |
| 37 | 54.09$^{b}$ | -1.06$^{ghi}$ | 6.80$^{abc}$ | 76.85$^{b}$ | 1.02$^{bcdef}$ | 11.20$^{bc}$ |
| 38 | 49.52$^{c}$ | -0.52$^{cdefgh}$ | 6.77$^{abc}$ | 66.72$^{efghijk}$ | 1.14$^{bcde}$ | 11.15$^{bc}$ |
| 39 | 42.78$^{gh}$ | 0.05$^{bcd}$ | 6.12$^{bcdef}$ | 65.23$^{kl}$ | 1.10$^{bcde}$ | 9.11$^{fgh}$ |
| 40 | 44.62$^{gh}$ | -0.39$^{cdefg}$ | 5.91$^{cdefg}$ | 65.17$^{kl}$ | 0.80$^{bcdefg}$ | 10.28$^{cdef}$ |
| 41 | 44.83$^{fgh}$ | -0.28$^{bcdef}$ | 5.68$^{defg}$ | 65.91$^{hijkl}$ | 1.28$^{bcd}$ | 10.75$^{bcd}$ |
| 42 | 46.53$^{defg}$ | -0.37$^{cdefg}$ | 6.34$^{abcd}$ | 66.28$^{fghijkl}$ | 1.12$^{bcde}$ | 10.71$^{bcde}$ |
| 43 | 47.00$^{defg}$ | -0.16$^{bcde}$ | 6.21$^{abcde}$ | 66.04$^{ghijkl}$ | 1.25$^{bcd}$ | 10.13$^{cdefg}$ |
| 44 | 46.89$^{defg}$ | -0.43$^{cdefg}$ | 6.04$^{cdef}$ | 67.04$^{defghij}$ | 0.40$^{efgh}$ | 9.86$^{cdefg}$ |
| 45 | 46.18$^{defg}$ | -0.56$^{cdefgh}$ | 5.66$^{defg}$ | 67.76$^{cdefg}$ | 0.16$^{gh}$ | 9.50$^{defgh}$ |
| 46 | 46.81$^{defg}$ | -0.46$^{cdefgh}$ | 6.29$^{abcde}$ | 65.17$^{kl}$ | 0.76$^{bcdefg}$ | 10.76$^{bcd}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 47 | 45.74$^{efg}$ | 0.41$^{ab}$ | 6.30$^{abcde}$ | 66.11$^{fghijkl}$ | 1.45$^{bc}$ | 12.09$^{ab}$ |
| 48 | 46.93$^{defg}$ | 0.19$^{bc}$ | 6.71$^{abc}$ | 65.88$^{ijkl}$ | 0.46$^{efgh}$ | 10.73$^{bcde}$ |
| 49 | 46.34$^{defg}$ | 0.98$^{a}$ | 7.07$^{ab}$ | 65.38$^{jkl}$ | 1.51$^{b}$ | 11.96$^{ab}$ |
| 50 | 45.96$^{defg}$ | 0.49$^{ab}$ | 7.16$^{a}$ | 64.56$^{l}$ | 2.42$^{a}$ | 12.69$^{a}$ |
| SEM | 0.87 | 0.28 | 0.37 | 0.65 | 0.28 | 0.5 |

$^{a,b,c}$ Means with different superscript within column are different at $P < 0.05$

**Appendix 2.2** Sensory evaluation (0ff-odor) scores by day effect for raw and cooked pork sausage stored at 1°C

| | Raw | | | | | Cook | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Day | Putrid | Acid | Sour | Rancidity | Meaty | Putrid | Acid | Sour | Rancidity | Meaty |
| 0 | 0.00 | $0.03^{cd}$ | $0.00^{d}$ | $0.10^{c}$ | $4.69^{f}$ | 0.00 | $0.00^{c}$ | 0.00 | $0.00^{d}$ | $8.64^{bc}$ |
| 5 | 0.00 | $0.12^{cd}$ | $0.00^{d}$ | $0.08^{c}$ | $8.52^{a}$ | 0.00 | $0.00^{c}$ | 0.06 | $0.00^{d}$ | $7.05^{e}$ |
| 10 | 0.02 | $0.00^{d}$ | $0.04^{d}$ | $0.07^{c}$ | $7.48^{b}$ | 0.00 | $0.00^{c}$ | 0.00 | $0.00^{d}$ | $10.71^{a}$ |
| 13 | 0.14 | $0.16^{cd}$ | $0.17^{d}$ | $0.60^{ab}$ | $6.50^{cd}$ | 0.17 | $0.04^{c}$ | 0.53 | $0.76^{abc}$ | $8.59^{bc}$ |
| 15 | 0.11 | $1.02^{ab}$ | $0.08^{d}$ | $0.73^{ab}$ | $8.25^{a}$ | 0.12 | $0.51^{ab}$ | 0.27 | $0.11^{d}$ | $8.27^{cd}$ |
| 17 | 0.17 | $0.75^{b}$ | $0.78^{c}$ | $0.41^{bc}$ | $6.46^{cd}$ | 0.19 | $0.13^{c}$ | 0.14 | $1.16^{a}$ | $8.55^{bcd}$ |
| 19 | 0.00 | $0.61^{bc}$ | $1.12^{bc}$ | $0.89^{a}$ | $6.84^{c}$ | 0.09 | $0.07^{c}$ | 0.48 | $0.68^{c}$ | $9.58^{ab}$ |
| 20 | 0.64 | $1.43^{a}$ | $0.82^{bc}$ | $0.94^{a}$ | $4.65^{f}$ | 0.00 | $0.21^{bc}$ | 0.07 | $0.66^{c}$ | $7.41^{ed}$ |
| 21 | 0.47 | $1.20^{ab}$ | $1.90^{a}$ | $0.37^{bc}$ | $5.95^{e}$ | 0.00 | $0.03^{c}$ | 0.54 | $0.72^{bc}$ | $9.49^{b}$ |
| 22 | 0.29 | $0.91^{ab}$ | $1.27^{b}$ | $0.68^{ab}$ | $5.72^{e}$ | 0.19 | $0.64^{a}$ | 0.03 | $0.38^{cd}$ | $9.03^{bc}$ |
| 23 | 0.16 | $0.96^{ab}$ | $1.26^{b}$ | $0.94^{a}$ | $6.20^{de}$ | 0.44 | $0.00^{c}$ | 0.15 | $1.09^{ab}$ | $8.70^{bc}$ |
| SEM | 0.16 | 0.20 | 0.16 | 0.13 | 0.17 | 0.12 | 0.18 | 0.12 | 0.14 | 0.39 |

$^{a-f}$ Means with different superscript within same column are different at $P < 0.05$.