DISSERTATION

EFFECT OF PACKAGING DURING STORAGE TIME ON RETAIL DISPLAY SHELF-LIFE OF BEEF STRIP LOINS FROM TWO DIFFERENT PRODUCTION SYSTEMS

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ABSTRACT

EFFECT OF PACKAGING DURING STORAGE TIME ON RETAIL DISPLAY SHELF-LIFE OF BEEF STRIP LOINS FROM TWO DIFFERENT PRODUCTION SYSTEMS

The objective of this study was to evaluate the influence of packaging during storage of strip loins (to simulate export shipment) from steers fattened on intensive grazing systems (Uruguay; UR) or on high concentrate diet (United States; US) on retail display life color, microbial growth, fatty acids profile, lipid peroxidation and vitamin E content. Four different packaging treatments were applied to UR and US striploins or steaks during 35 d storage; treatments were applied 7 d following slaughter. After 35 d storage, the samples were evaluated during simulated retail display for 6 d. In block 1, the treatments were: vacuum packaging (VP); low-oxygen modified atmosphere packaging (MAP) with nitrogen (N_2) and CO_2 (MAP/CO_2); low-oxygen MAP with N_2 plus CO_2 and carbon monoxide (CO); VP plus an application of peroxyacetic acid (VP/PAA). In block 2, the treatments were: VP, MAP/CO and VP with ethyl-N-lauroyl-L-arginate HCl (LAE) incorporated into the film as an antimicrobial agent (VP/AM). In block 3, the treatments were: VP, MAP/CO_2, MAP/CO and VP/AM. Regardless of production system and packaging treatment, mesophilic and psychrotrophic counts of 6.9 to 7.8 $\log_{10}$ CFU/cm^2, and 6.7 to 7.7 $\log_{10}$ CFU/cm^2, respectively, were obtained at the end of retail display, except for US samples in blocks 2 and 3 (5.5 to 6.3 $\log_{10}$ CFU/cm^2). The UR strip loins packaged with MAP/CO had greater ($P < 0.05$) a^* values than product packaged in VP/PA and MAP/CO_2 following 6 d of display. For US beef, the MAP/CO treatment resulted in the reddest lean color ($P < 0.05$) compared to the other three packaging treatments in block 1. In blocks 2 and 3, the UR strip loin steaks

packaged in MAP/CO also had the greatest a^* values compared to the other three treatments, but no differences ($P > 0.05$) were detected among the VP treatments and the MAP/CO in the US steaks at the end of retail display. Only system (in block 1, and blocks 2 and 3), and time (in block 1) affected ($P < 0.05$) lightness (L^*). In all blocks, US samples had greater L^* values than UR samples (32.6 vs. 28.5; $P = 0.0015$, for block 1; and 33.4 vs. 31.1; $P < 0.0001$ for blocks 2 and 3). Vitamin E content in UR steaks, regardless of packaging treatment, was greater ($P < 0.05$) than US steaks. No effect of packaging treatment ($P > 0.05$) was observed by country of origin at the different display times in block 1, but UR beef displayed for 0 d from the MAP/CO_2 treatment had greater ($P < 0.05$) vitamin E content than beef from the other three packaging treatments in blocks 2 and 3. Packaging x system, system x time and packaging x system x time interactions were not significant for any of the fatty acids analyzed on this study. Beef from UR had lower ($P < 0.05$) SFA and MUFA concentrations and greater ($P < 0.05$) PUFA, n-6 and n-3 concentrations than US beef when evaluated during retail display. Beef from UR developed more detectable ($P < 0.05$) oxidized odor than US samples while the latter exhibited a greater ($P < 0.05$) sour odor than UR grass-fed samples. Values from TBARS were influenced by significant packaging x system x time interaction in block 1 ($P = 0.0027$) and in blocks 2 and 3 ($P = 0.0104$). In block 1, UR beef had a greater ($P < 0.001$) TBARS values than US samples on d 0 of display, but TBARS values tended to decrease during retail display and differences almost disappear by the end of the display period. For blocks 2 and 3, TBARS value tended to increase between d 0 to d 6 of retail display in the UR and US samples. Complexity of fresh meat post-mortem chemistry warrants a more comprehensive and systemic approach to maximize shelf-life.

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CHAPTER I

INTRODUCTION

In 2011, the Food and Agriculture Organization (FAO) published a study reporting that approximately one-third of food produced for human consumption is lost or wasted globally, which represents approximately 1.3 billion tons per year (FAO, 2011). Food waste post-harvest is referred to as “food losses” and “spoilage” (Parfitt et al., 2010). Spoilage is characterized by any change in a food product that renders it unacceptable to the consumer from a sensory view point. This may be a result of physical damage, chemical changes (oxidation, color changes) or appearance of off-flavors and off-odors resulting from microbial growth and metabolism in the product (Gram et al., 2002). Food spoilage can be evident, but when spoilage results in changes in the texture or the development of off-odors due to (bio)chemical or microbial reactions, the underlying mechanisms may be difficult to identify (Huis in’t Veld, 1996). Thus, spoilage evaluation should always be, directly or indirectly, related to a sensory assessment (Huis in’t Veld, 1996).

Fresh meat is recognized as a highly perishable food product due to its biological composition (Lambert et al., 1991). There are three main mechanisms for meat spoilage after slaughtering and during processing and storage: (a) microbial spoilage, (b) lipid and pigment oxidation and, (c) autolytic enzymatic spoilage (Dave and Ghaly, 2011). Microbial growth and muscle metabolism depends upon the condition of the carcasses at the time of slaughter, the type of packaging and storage conditions. Microbial spoilage results in a sour taste, off-flavors, discoloration, gas production, pH change, slime formation, structural components degradation, off-odors and change in product appearance (Dave and Ghaly, 2011).

Meat preservation technologies mainly endeavor to inhibit microbial spoilage, although other methods of preservation have been explored to minimize other deteriorative changes such as color and oxidative changes. Packaging protects products against deteriorative effects, which may include discoloration, off-flavor and off-odor development, nutrient loss, texture changes, pathogenicity and other measurable factors (Zhou et al., 2010).

Modified atmosphere packaging (MAP) refers to the removal and/or replacement of the atmosphere surrounding the product before sealing in vapor-barrier materials. Modified atmosphere packaging includes vacuum packaging (VP), which removes most of the air before the product is enclosed in barrier materials, or forms of gas replacement, where air is removed by vacuum or flushing and replaced with another gas mixture before packaging and sealing in barrier materials (McMillin, 2008).

Vacuum packaging (VP) extends the storage life of chilled meats by maintaining an oxygen deficient environment within the pack (Bell et al., 1996). Vacuum packaging is considered an efficient packaging system to extend the shelf-life of fresh meat, preserving the sensory characteristics inherent to the product for a period sufficiently long. During refrigeration, the vacuum allows the shelf-life of the meat to be extended by reducing oxidation and the growth of aerobic microorganisms (Hernández-Macedo et al., 2011). Vacuum packaging is the most cost effective packaging (McMillin, 2008) and it has been the most widely used packaging system to merchandise fresh meat to export markets.

A bacteriostatic effect of carbon dioxide (CO_2) when used in MAP to extend shelf-life of chilled fresh meat has been well documented (Gill and Tan, 1980; Farber, 1991; Jakobsen and Bertelsen, 2002). Maximum antimicrobial effect of CO_2 is achieved when the storage

temperature of a MAP package is kept as low as possible, because the solubility of CO_2 decreases significantly with increasing temperature (Farber, 1991).

The bright red color of beef is used by consumers as an indicator of its freshness and wholesomeness (Hunt et al., 2004). Use of carbon monoxide (CO) in MAP can help to maintain cherry-red beef color. Carbon monoxide combines with myoglobin to form carboxymyoglobin, producing a bright cherry-red color in muscles that otherwise are more likely to discolor (Hunt et al., 2004).

Active packaging refers to the incorporation of additives into packaging systems (loose, attached or incorporated within the packaging materials) with the objective of maintaining or extending product quality and shelf-life (Kerry et al., 2006). Many techniques have potential for being incorporated into a packaging film surface to achieve shelf-life extension (Labuza and Breene, 1989). Particularly, antimicrobial packaging acts to reduce, inhibit or retard the growth of microorganisms that may be present in the packed food or packaging material itself (Appendini and Hotchkiss, 2002).

Furthermore, production systems (grazing vs. grain-fed) can have an effect on meat shelf-life due to its impact on color and lipid stability (Craig et al., 1959; Bidner et al., 1986; Zerby et al., 1999; Yang et al., 2002a,b; Realini et al., 2004; Descalzo et al., 2005; Gatellier et al., 2005).

Therefore, the objective of this study was to evaluate the influence of packaging during storage of strip loins (to simulate export shipment) from steers fattened on grazing systems (Uruguay) or on high concentrate diets (United States) on retail display shelf-life color, microbial growth, fatty acids profile, lipid peroxidation and vitamin E content.

CHAPTER II

REVIEW OF LITERATURE

Shelf-life definitions

Food shelf-life represents a broad concept where a large number of factors and mechanisms affect it. Because of that, it is not easy to find a comprehensive definition. Borch et al. (1996) defined shelf-life as the storage time until spoilage. They stated that a food may be spoiled when a certain maximum bacterial level is achieved, or an unacceptable off-odor/off-flavor is present, or the appearance of the food product changes making it undesirable. The Institute of Food Science and Technology (IFST) based in United Kingdom developed guidelines in 1993 where shelf-life was defined as "the time during which the food product will: (a) remain safe; (b) be certain to retain desired sensory, chemical, physical and microbiological characteristics; and (c) comply with any label declaration of nutritional data, when stored under the recommended conditions" (IFST, 1993). Iulietto et al. (2015) considered shelf-life "as the period of time in which the food keeps its qualitative characteristics".

Despite that shelf-life is based on the establishment of thresholds for many characteristics for which the food product becomes unacceptable, Hough et al. (2003) consider that sensory shelf-life depends on the interaction between the food and the consumer, because some consumers can accept a food product from the sensory view point that others reject.

Factors Affecting Microbial Spoilage of Meat

Proliferation of microorganisms in foods is determined by intrinsic and extrinsic factors, as well as the processing and preservation methods (Huis in't Veld, 1996). Intrinsic factors are the physical, chemical and structural properties of the meat. Among them, the most important

are: water activity, pH, available nutrients, natural antimicrobial substances (Huis in't Veld, 1996), composition, type, and extent of initial contamination (Koutsoumanis, et al., 2006). Extrinsic factors are those related to the environment in which the meat is stored (Huis in't Veld, 1996) and the most relevant are: temperature and packaging atmosphere (Koutsoumanis, et al., 2006).

Microorganisms

Bacteria found on meat may arise from the hide of the animal, from fecal material or from soil, water and air. The initial microbial load of fresh meat is directly related to good manufacturing practices during slaughter, particularly during the removal of the hide, evisceration and subsequent processing into primal cuts (Lambert et al., 1991). The latter author also indicated that the most important spoilage bacteria are aerobic gram-negative psychrotrophic strains of *Pseudomonas*, *Moraxella*, *Acinetobacter*, and *Aeromonas*; the facultative anaerobe *Shewanella putrefaciens*; and the gram-positive *Lactobacillus* and *Brochotrix thermosphacta* (Lambert et al., 1991).

On the other hand, Borch et al. (1996) reported that the predominant bacteria associated with meat spoilage under refrigerated conditions are *Brochothrix thermosphacta, Carnobacterium* spp., *Enterobacteriaceae, Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Shewanella putrefaciens.* They also stated that bacteria under refrigerated conditions causing defects such as off-flavors, discoloration, gas and slime production, and decreases in pH are *Brochothrix thermosphacta, Carnobacterium* spp., *Lactobacillus* spp., *Leuconostoc* spp., and *Weisella* spp.

The interaction among the different microorganisms determines synergistic effects and antagonistic processes. Synergistic effects refer to the production or availability of an essential

nutrient due to the growth of a specific microorganism that enables the growth of other microorganism which otherwise could not proliferate. Antagonistic processes are those related to competition for essential nutrients, change in pH values, or the production of antimicrobial substances that can have detrimental effects towards other organisms (Huis in't Veld, 1996). In this sense, lactic acid bacteria (LAB) that predominate in anaerobic packaging systems can produce bacteriocins that may inhibit closely related bacteria and also some food borne pathogens (Ahn and Stiles, 1990; O´Sullivan, et al, 2002). With regards to microbial spoilage, Nychas et al. (2008) referred to ephemeral spoilage organisms (ESO) which are the result of the factors that dynamically persist during processing, transportation and storage in the market. In other words, they are those which are able to adopt different ecological strategies.

Retail shelf-life of meat is estimated as the time required by the bacterial population to reach a level of 10^7 CFU/cm^2 (Borch et al., 1996).When bacteria consume glucose from the meat surface, no offensive by-products are produced; but, breaking down amino acids results in a variety of by-products which are detected organoleptically as putrid odors and flavors. Gill (1996) reported that when *Pseudomonas* spp. reach a number of 10^8 CFU/cm^2, the offensive byproducts accumulate rapidly and spoilage onset become an abrupt event.

Rapid methods to detect meat spoilage have been proposed that would measure volatile organic compounds utilizing proton transfer reaction mass spectrometry (Mayr et al., 2003) and multiplex PCR to characterize spoilage-related LAB populations (Yost and Nattress, 2000).

Meat pH

Growth of many important spoilage bacteria may be partially or totally inhibited when meat pH values are close to 5.5 (Gill and Newton, 1982). The undissociated acid and the low pH can affect the growth potential of certain spoilage bacteria. The importance of meat pH in the

development of anaerobic bacteria is well documented, since on high pH meat surfaces, species of high spoilage potential, such as *Brochothrix thermosphacta* and *Sehewanella putrefaciens,* can grow and cause early spoilage under VP conditiones. However, the effect of meat pH on aerobic spoilage bacteria is not very clear (Gill and Newton, 1982). Dark, firm, dry meat (high pH) and adipose tissue spoils faster than normal pH meat because amino acids are rapidly attacked (Borch et al., 1996).

Although most bacteria prefer a pH close to neutrality for growth, LAB tolerate lower pH values than the gram-negative bacteria commonly found on meats, especially under anaerobic storage conditions. In VP with a sufficiently low oxygen permeability film, LAB grow on the lean surface almost to the exclusion of all other types of bacteria in normal pH meat (5.4 to 5.9). If the pH of the meat is higher than 5.9, or the packaging film utilized has higher oxygen permeability, there is increased growth of gram-negative bacteria and *B. thermosphacta* (Egan, 1983).

Nutrient Availability

Gill (1983) reported that glucose is the initial substrate supporting growth of all the major types of bacteria found in red meats at a normal or high pH, stored under refrigerated conditions in air, VP or MAP. Depending on its initial concentration, glucose may be depleted and thereafter, other available substrates are metabolized. These substrates include lactate, amino acids and creatine under aerobic storage, and lactate and arginine during VP and MAP storage. Under aerobic conditions, spoilage is most frequently associated with amino acid utilization by *Pseudomonas* spp. after glucose depletion (Dainty, 1996).

Substrate preference by different types of aerobic bacteria has been studied by using a meat juice medium. *Pseudomonas* utilize in this order: glucose, amino acids and lactic acids.

Acinetobacter use: amino acids, lactic acid. *Enterobacter* use: glucose, glucose-6-phosphate, amino acids. Lastly, *Brochothrix thermosphacta* use: glucose, glutamate. All species grew at their maximum rate within the pH range 5.5–7.0 except *Acinetobacter* (Gill and Newton, 1977).

Temperature

Temperature is probably the most important single environmental factor influencing bacterial growth on meat (Lambert et al., 1991). The general effect of low temperatures is to diminish the growth rate of all spoilage bacteria, aside from the specific inhibitive effects on *Pseudomonas* spp. The optimum storage temperature for refrigerated meat is -1.5 ± 0.5°C (Gill, 1996). In MAP, antimicrobial effectiveness of CO_2 is greater at lower temperatures due to its higher solubility in the aqueous phase of the lean product (Lambert et al., 1991). However, solubility in beef fat increases with increasing temperatures reaching a maximum solubility at 22°C (Jakobsen and Bertelsen, 2002).

Oxygen Availability

When atmospheric pressures of O_2 are present, the shelf life of meat is limited by two important factors: the chemical effect of O_2 and the growth of aerobic spoilage micro-organisms (Lambert et al., 1991). The type of packaging is one of the factors that affects the composition of meat spoilage microflora (Cerveny et al., 2009).

Vacuum packaging represents a special case of oxygen-depleted atmosphere in which the volume of the pack atmosphere is close to zero (Gill, 1996). The concentration of O_2 decreases and the CO_2 levels increase during storage in VP due to tissue and microbial respiration (Lambert et al., 1991). During storage, aerobic microorganisms such as *Pseudomonas* spp. and molds are substituted by slower growing, facultative anaerobic organisms; e.g., LAB. The LAB utilize glucose to produce mixed organic acids resulting in a sour, cheesy odor and taste.

Proteolysis and lipolysis are unusual in VP products because of the limited ability of LAB to produce the enzymes required (Cerveny et al., 2009). Newton and Rigg (1979) found an inverse relationship between the shelf-life of VP meat and the oxygen transmission rate of the film package, mainly because more O_2 results in an increased growth rate and final counts of *Pseudomonas* spp. Egan (1983) reported that VP beef may have a storage life of 12 weeks at 0 to 1°C until off-flavor becomes unacceptable if compared to the frozen control samples. A major disadvantage limiting the use of VP is discoloration by metmyoglobin formation due to residual O_2 in the package (Lambert et al., 1991).

Normally, MAP packages contain differing concentrations of O_2, CO_2, and/or inert nitrogen N_2. In general, CO_2 percentages differ from 10% to 40% and O_2 from 90% to 60%, although a long shelf-life can be achieved with 100% CO_2 (García-López et al., 1998). High O_2 - MAP containing up to 80% O_2 and 20% CO_2 reduce color deterioration of retail cuts of meat, but the shelf-life is only slightly increased, compared to aerobic storage. In high O_2-MAP, a variety of bacteria are able to grow to high final counts, such as *Brochothrix thermosphacta*, *Pseudomonas* spp., *Leuconostoc* spp. and *Lactobacillus* spp. Most bacteria are more or less inhibited by CO_2 and, therefore, the growth rate is reduced and the shelf-life is increased (Borch et al., 1996). García-López et al. (1998) found that if O_2 is available, some genera of LAB, such as *Leuconostoc* may be favored but spoilage bacteria, such as *Pseudomonas* spp., *Enterobacteriaceae* and *Brochothrix* can still compete and higher counts are attained than under VP conditions. Jakobsen and Bertelsen (2002) reported that, when high CO_2 levels are flushed into the MAP, it dissolves in muscle and fat tissues until saturation or equilibrium is reached. The maximum preservative effect of CO_2 would be achieved if CO_2 is applied to the headspace above the levels required to saturate meat. In addition to the antimicrobial effect of CO_2, they

also stated that the CO_2 would affect the meat quality by lowering its pH as a consequence of carbonic acid dissociation to bicarbonate and hydrogen ions. Low ultimate meat pH promotes myoglobin oxidation (Faustman and Cassens, 1990). Low-O_2 MAP with CO are essentially anaerobic and include 0.4% of CO, 20 to 30% CO_2 and the remainder N_2, where LAB become the predominant bacteria (Cornforth and Hunt, 2008).

Lipid Oxidation

Lipid Oxidation – Reaction

It has been well documented that rates of lipid oxidation are an important deterioration factor which usually has a detrimental effect on meat quality attributes (Gray et al., 1996). Lipid peroxidation is a free radical chain reaction in which oxygen is the most important factor, and it consists of 3 primary steps: initiation, propagation, and termination (Min and Ahn, 2005). Lipid oxidation can take place by autoxidation, photoxidation, and enzymatic oxidation mechanisms. Autoxidation is the main oxidation process in meat and is initiated by reactive oxygen species (ROS) such as hydroxyl radicals ($^{\bullet}OH$) that remove hydrogen atoms from the fatty acyl group of polyunsaturated fatty acids (PUFA) and form lipid free radicals (Ahn et al., 2009). The free lipid radical reacts rapidly with O_2 to form a peroxyradical which removes another hydrogen from another hydrocarbon chain yielding a hydroperoxide and a new free radical which can perpetuate the chain reaction (Ladikos and Lougovois, 1990). Initiation, propagation and termination steps of lipid autoxidation are summarized below (equations 1 to 4), adapted from Frankel (1980).

Initiation: $RH + {}^{\bullet}OH \longrightarrow R^{\bullet} + H_2O$ (1)

Propagation: $R^{\bullet} + O_2 \longleftrightarrow ROO^{\bullet}$ (2)

$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$ (3)

Termination: $ROO^{\bullet} + ROO^{\bullet} \longrightarrow$ non-radical products (4)

There is still a controversy about the initiation mechanism of lipid peroxidation. Ground state oxygen does not have strong enough reactivity, but can be converted to ROS such as hydroxyl radical ($^{\bullet}OH$), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroperoxyl radical ($HO_2^{\bullet}$), lipid peroxyl radical ($LOO^{\bullet}$), alkoxyl radical ($LO^{\bullet}$), iron-oxygen complexes (ferryl- and perferryl radical) and singlet oxygen ($^1O_2$) (Min and Ahn, 2005). Hydroxyl radical ($^{\bullet}OH$) is the most reactive and is considered as the most damaging free species because it is capable of attacking any adjacent molecule. The Fenton reaction (equations 5 and 6) is the main path for $^{\bullet}OH$ formation, which depends on the availability of metal ions (Bekhit et al., 2013):

$$O_2^{\bullet -} + Fe^{3+} \longrightarrow O_2 + Fe^{2+} \quad (5)$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (6)$$

After slaughter, the mechanisms controlling metal ions, which operate in living animals, no longer are effective and, therefore, contribution of $^{\bullet}OH$ is high in post-mortem muscles. It is important to note that the reaction is not limited to iron, and other ions such as Cu^{2+}, Ti^{4+} and Co^{3+} can be involved (Bekhit et al., 2013). Buckley et al. (1995) reported that the rate and extent of lipid oxidation are affected by pre-slaughter and post-slaughter events such as stress, early postmortem pH, carcass temperature, cold shortening, and techniques such as electrical stimulation.

Lipid Oxidation - Antioxidant Defenses

A broad range of antioxidant mechanisms act to inhibit oxidative processes in meat (Decker et al., 2000), although their effectiveness decreases with increasing storage time (Monahan, 2000). These mechanisms include: inactivation of free radicals, control of oxidation catalysts, inactivation of oxidation intermediates, and interactions between antioxidants and secondary lipid oxidation products (Decker et al., 2000).

Free radical scavengers, or chain-breaking antioxidants, donate an electron to the free radical and the resulting scavenger forms a lower energy radical (Decker et al., 2000). Tocopherols (vitamin E) are the most powerful natural chain-breaking antioxidants present in muscle; they can scavenge two peroxyradical molecules that result in the formation of tocopherol radicals (Descalzo and Sancho, 2008). In his review, Buettner (1993) summarized that α-tocopherol radicals can be reduced (recycled) by ascorbate (vitamin C), which is regenerated by glutathione-dependent mechanisms that require NADPH. Vitamin C can also scavenge free radicals directly to form low-energy ascorbate radical. Carotenoids, ubiquinone, thiols, polyphenols, and nitrogenous compounds such as, uric acid, polyamines, amino-acids, and peptides also can inactivate free radicals (Decker et al., 2000).

Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play a role in inhibiting oxidation (Bekhit et al., 2013). The first two are coupled enzymes (equations 7 and 8), where the SOD catalyze the dismutation of superoxide anions, and CAT decompose hydrogen peroxide into water and oxygen (Descalzo and Sancho, 2008). Superoxide dismutase is present in the cytosol and the mitochondria. Copper and zinc are required for the cytosolic SOD, and manganese for the mitochondrial SOD (Decker et al., 2000).

$$O_2^{\bullet -} + 2\,H_2O \longrightarrow H_2O_2 \quad (7)$$

$$H_2O_2 \longrightarrow 2\,H_2O + O_2 \quad (8)$$

Glutathione peroxidase can decomposes hydrogen peroxide but also lipid peroxide (equations 9 and 10). This enzyme contains selenium and glutathione (GSH) acts as its cofactor allowing the reduction of hydrogen or lipid peroxide (Decker et al., 2000).

$$H_2O_2 + 2\,GSH \longrightarrow 2\,H_2O + GSSG \quad (9)$$

$$LOOH + 2\ GSH \longrightarrow LOH + H_2O + GSSG \quad (10)$$

Several studies were carried out to evaluate the effect of diet on antioxidant enzymes activity. Mercier et al. (2004) studied the influence of diet (pasture or mixed) in Charolias cows on antioxidant enzyme activity. Mixed diets mainly consisted of cereals, silage, and cattle-cake. They reported a greater SOD activity in pasture-fed cows, but lower GPx activity than in muscle from mixed diet fed cows. Diet did not affect catalase activity. These findings were in agreement with Gatellier et al. (2004), who also studied the influence of finishing diet (pasture or mixed) on SOD, catalase, and GPx activity in Charolais steers, heifers and cows. They observed the same pattern in SOD and GPx activity over the three groups of cattle. However, the catalase activity was greater in the mixed diet in muscle of heifers.

In his research, Descalzo et al. (2007) evaluated the overall antioxidant status in fresh beef from pasture or grain-fed crossbreed steers that were either provided or not provided vitamin E supplementation. In this research, they did not find any difference in CAT and GPx activities among the dietary treatments, but SOD activity was greater in beef from cattle in the pasture treatments than receiving concentrate diets. Petron et al. (2007) examined the effect of different types of pasture on the antioxidant status of meat from lambs. Different pastures did not affect SOD or CAT activity. However, GPx activity was higher in meat from lambs on the leguminous pasture compared to the intensive ryegrass and botanically diverse pasture.

Pradhan et al. (2000) studied the effects of refrigerated and frozen storage on CAT activity in skeletal muscles from different species. They found that CAT in ground meat was stable during refrigerated and frozen storage, and when the enzyme was inhibited, lipid oxidation increased. Thus, the authors concluded that CAT enzyme plays an important role in regulating lipid oxidation in raw meat (Pradhan et al., 2000). Renerre et al. (1996) demonstrated that lipid

oxidation and antioxidant enzyme activities were muscle-dependent. In his study, lipid oxidation decreased in the order of *Diaphragm* > *Psoas major* > *Longissimus lumborum* > *Tensor fasciae latae*, and increased with increased storage time under refrigerated conditions (2°C). Superoxide dismutase activity was higher postmortem in *Psoas major* and *Diaphragm* muscles than in *Longissimus lumborum* and *Tensor fasciae latae* muscles, while CAT and GPx activities were higher only in *Diaphragm* muscle.

The effect of dietary vitamin E retarding lipid and myoglobin oxidation has been well documented (Marusich et al., 1975; Arnold et al., 1993; Yin et al., 1993; Liu et al., 1995; Zerby et al., 1999; Phillips et al., 2001; Lanari et al., 2002; Descalzo et al., 2005), and it has been reported concentrations of vitamin E in meat at which pigment and lipid oxidation would be delayed and, therefore, the shelf-life of the product would be enhanced. Faustman et al. (1989) indicated that fresh ground sirloin containing approximately 3 μg of vitamin E/g of muscle exhibited the least pigment and lipid oxidation. Arnold et al. (1993) reported that a vitamin E concentration of 3.3 μg/g of muscle would provide protection against oxidation processes in the *Longissimus lumborum*. Similarly, Liu et al. (1995) recommended a vitamin E level of 3.5 μg/g of muscle to inhibit lipid oxidation and metmyoglobin formation.

Alpha-tocopherol, which is located in the phospholipid membranes, acts as a radical-quenching antioxidant to delay oxidative damage to membrane constituents (Faustman et al., 1998). Tocopherols react with lipid peroxyl radicals, resulting in a lipid hydroperoxide formation and a tocopheroxyl radical (Decker et al., 2000). This latter is relatively unreactive because the unpaired electron resonates across the phenolic ring system (Gregory, 2008). At slow oxidation rates, two tocopheroxyl radicals can interact and form a tocopheryl quinone and back transform tocopherol from the tocopheroxyl radical. If the lipid oxidation rates are high, and therefore there

are a high concentrations of lipid peroxyl radicals, a tocopherol-peroxyl can reacts with a second peroxyl radical resulting in the formation of tocopherol-peroxyl adducts. These adducts can be transformed to tocopherylquinone (Decker et al., 2000). Antioxidant efficiency of vitamin E is related to its regeneration from oxidized products. Redox cycles of α-tocopherol are considered to be important in the antioxidant function of the vitamin. It has been shown that tocopherol regeneration *in vitro* involves vitamin A, vitamin C and coenzyme Q. However, the importance of vitamins A and C in the back transformation to tocopherol from tocopheroxyl radical *in vivo* has been questioned (Wang and Quinn, 1999).

Lipid Oxidation – Sensory Attributes

The role of the lipid component on the chemistry of meat flavors produced during heating and storage is very complex. Literature indicates that multiple cascades of free radicals, hydrolytic and condensation reactions occur, each contributing to development of overall flavor, both positive and negative flavors (Kanner, 1994).

Despite that phospholipid concentrations in meat are very small compared to other lipid fractions, their susceptibility to oxidation makes them important in terms of meat quality. The lability of the phospholipids lies in their unsaturated fatty acid content. In beef phospholipids, 19% of the fatty acids have 4 or more double bonds, while 0.1% of the triglyceride fatty acids exhibit this degree of unsaturation (Love and Pearson, 1971).

Hydroperoxides are the primary products of lipid oxidation, which are colorless, tasteless and odorless (Gray and Monahan, 1992). Decomposition of these hydroperoxides results in a complex mixture of low molecular weight compounds with distinctive odor and flavor characteristics; including alkanes, alkenes, aldehydes, ketones, alcohols, esters and acids. These compounds cause rancid, fatty, pungent and other off-flavor characteristics in meat and the

contribution that a particular compound makes to the meat flavor or aroma depends on the concentration at which it is present and on its odor threshold (Gray and Monahan, 1992). Ladikos and Lougovois (1990) reported that lipid hydroperoxides also can form dimers and polymers which may, in turn, oxidize and break down into volatile products. Additional oxidation may occur in the original peroxides or in the unsaturated aldehydes, which then go through further degradation to form epoxides, cyclic peroxides and bicyclic endoperoxides. These secondary oxidation products can also decompose to form volatile compounds and dialdehydes, which contribute to flavor deterioration. The authors also indicated that thermal and rancid oxidation stem from many reactions involved in the formation of volatile aroma compounds from lipid that follow the same basic pathways, and similar volatile products are generated.

Mottram (1998) pointed out that phospholipids play an important role in the development of aroma during heating. Phospholipids have been associated with the off-flavor known as "warmed-over flavor", which develops in re-heated cooked meats. Nevertheless, the formation of lipid oxidation products from phospholipids may contribute to desirable aromas during the initial cooking of meat. Mottram (1998) also suggested that phospholipids or their degradation products inhibit reactions involved in the formation of heterocyclic aroma compounds from the Maillard reaction. Triglycerides from beef have had much less effect on the levels of Maillard volatiles than the phospholipid preparations.

Diet has a direct effect on meat fatty acid composition, which can be changed more easily in single-stomached pigs and poultry than in ruminants. Ruminal biohydrogenation can be mitigated by feeding PUFA which are protected either chemically, by processing, or naturally (Wood and Enser, 1997). Elmore et al. (2004) compared the volatile compound and fatty acid

composition of grilled beef steaks from Aberdeen Angus and Holstein-Friesian steers fed on cereal-based concentrates or grass silage. Concentration of linoleic acid was higher in the muscle from concentrate-fed animals, which in the cooked meat, resulted in increased levels of several compounds formed from linoleic acid decomposition. Meat from silage-fed steers had higher levels of α-linolenic acid, and thus some volatile compounds derived from this fatty acid. Steaks from the concentrate-fed steers had over 3 times higher 1-Octen-3-ol, hexanal, 2-pentylfuran, trimethylamine, cis- and trans-2-octene and 4,5-dimethyl-2-pentyl-3-oxazoline, while those from the silage-fed steers presented much greater levels of grass-derived 1-phytene. Regarding breed, the PUFA:SFA ratio was greater in the Holstein-Friesian cattle compared with the Aberdeen Angus cattle, but no other effects of breed were observed

Malonaldehyde is a secondary oxidation product of PUFA and is measured using thiobarbituric acid test. It is the most common technique to determine lipid oxidation (Ahn et al., 2009). Ang and Lyon (1990) evaluated development of warmed-over flavor during 5 days storage time of broiler breast, thigh and skin measured by thiobarbituric acid (TBA), headspace gas chromatography, and sensory methods. They reported that TBA values and levels of major headspace volatiles increased with advancing storage time. Additionally, intensities of cardboard, warmed-over, rancid/painty, and overall off-flavor characteristics increased over storage time.

Greene and Cumuze (1981) evaluated oxidized flavor in beef by inexperienced panelists and assessed its correlation with TBA values to determine the detection limit of the oxidized flavor. Results indicated that correlation coefficients for sensory scores versus TBA values were significant, but low, and variability among panelists appeared to account partly for the lower values. Furthermore, inconsistencies in TBA measurements may have been partially responsible

for low correlation values. Rhee (1978) recommended the addition of propyl gallate and ethylenediaminetetraacetic acid (EDTA) in the blending process of the distillation TBA test to minimize additional lipid oxidation occurring during the assay. However, Greene and Cumuze (1981) observed that of the 52 panelists, 28 of them were consistent in scoring. In this sub-population, the difference in intensity of oxidized flavor was detected in the range from 0.6 to 2.0 TBA numbers (mg TBARS/kg tissue).

Campo et al. (2006) also studied the relationship between human perception of lipid oxidation determined by trained panelists and chemical measurement of oxidation. They evaluated meat stored in MAP from animals fed diets differing in fatty acid composition. Sensory analysis and TBARS were used to assess beef steaks during display for 0, 4 or 9 days under simulated retail conditions. Correlations between analytical and sensory attributes were high (Spearman´s rho = 0.84) and TBARS were a good indicator of rancidity perception. Rancidity perception and beef flavor in relation to TBARS followed a sigmoidal curve as the best fit. Panelists identified less beef flavor and more rancidity when TBARS values were higher. Rancidity increased rapidly from the initial point of perception until it reached either a saturation point or an adaptation by panelists, in which greater oxidation - measured by TBARS - could not be perceived as such. A TBARS value of 2.28 was identified as the point at which rancidity perception overwhelmed beef flavor. The authors stated that this could be considered as the limiting threshold for acceptability of oxidation in beef, which is close to the maximum TBA values reported by Greene and Cumuze (1981). Despite these findings, the researchers concluded that it is difficult to determine by sensory evaluation the point at which beef would be rejected due to lipid oxidation. Perceptions depend on many factors and, among them, personal thresholds can vary due to experience.

Factors Affecting Lipid Oxidation - Diet

Lipid oxidation depends on several factors (Buckley et al., 1995), but the greater concentration of PUFA, the more the meat is susceptible to oxidation (Ahn et al., 2009). The latter authors also pointed out that the speed of oxidation relies on the degree of unsaturation of the fatty acids and the prooxidants to antioxidants balance.

Despite ruminal biohydrogenation, dietary PUFA can be incorporated into adipose tissue (Wood et al., 2008). Wood et al. (2003) reported that meat from grass-fed beef and lamb exhibits higher levels of linolenic acid (C18:3 n-3) and long chain n-3 PUFA. They also stated that, in ruminant muscle and adipose tissue, PUFA are present almost exclusively in the phospholipid fraction. Wood et al. (2008) stated that α-linolenic acid (C18:3 n-3) is an essential fatty acid representing the major fatty acid present in grass. Nevertheless, it does not compete well for insertion into phospholipid compared with linoleic acid, and its incorporation into adipose tissue and muscle is less efficient. Linolenic acid is subject of a more extensive biohydrogenation and a long rumen transit time for forage diets also limits the amount available for tissue uptake compared with C18:2 n-6 from concentrate diets. Ashes et al. (1992) studied the ruminal biohydrogenation and long-chain fatty acid utilization. They found that in ruminants the long-chain eicosapentaenoic (C20:5 n-3) and docosahexaenoic (C22:6 n-3) acids were not extensively biohydrogenated by ruminal microflora. Furthermore, they also reported that the long-chain fatty acids were incorporated into the muscle phospholipids fraction and not in the triacylglycerol adipose tissue.

Elmore et al. (1999) studied the aroma profiles of cooked steaks in relation to their fatty acid composition. Different fatty acid profiles in meat were obtained supplementing different fat sources that included palm oil, linseed, and fish oil. The researchers found, after cooking, higher

levels of lipid oxidation products (saturated and unsaturated aldehydes, alcohols and ketones) in the aroma extracts of all of the steaks with increased PUFA concentration. Aldehydes were quantitatively the most important and because they have low odor thresholds and are considered to be largely responsible for the changes in flavor. It is interesting the explanation for aldehyde production. The authors suggested that PUFA would induce an increase in thermal degradation of oleic and linoleic acid, since higher levels of aldehydes derived from these fatty acids were present in meat with greater PUFA content.

Factors Affecting Lipid Oxidation – Environmental Factors

Temperature and oxygen concentration are two of the most important environmental factors affecting lipid oxidation rates. In general, increasing temperature results in increase lipid oxidation rates, and decreasing oxygen availability decreases lipid oxidation (McClements and Decker, 2008). Compared to ambient temperatures, meat storage under refrigeration or freezing conditions decreases oxidative deterioration (Monahan, 2000). However, increasing temperature decreases oxygen solubility, and therefore, in some cases, high temperatures would slow the oxidation process. In terms of oxygen concentration, VP and low oxygen-MAP where the O_2 is replaced by N_2, are useful strategies to minimize lipid oxidation (McClements and Decker, 2008).

Jakobsen and Bertelsen (2000) evaluated the relationship between discoloration and lipid oxidation in beef and the combined effect of time, temperature and partial pressure of oxygen. The researchers concluded that temperature was the most important factor for maintaining the red oxymyoglobin color and retarding lipid oxidation. A low temperature (below approximately 4°C) delayed lipid oxidation, regardless of oxygen level, but, when the temperature is raised the oxygen level became more critical.

Protein oxidation

It is unclear to what extent proteolysis during meat aging is linked with oxidative processes (Martinaud et al., 1997). Stadtman (2006) pointed out that protein oxidation may include different changes in the protein, such as cleavage of the polypeptide chain, modification of amino acid side chains, and conversion of protein to derivatives that are highly sensitive to proteolytic degradation.

Peroxidized lipids interact with proteins and chemical changes occur resulting in protein-protein cross-links, protein scission, protein-lipid adducts, and amino acid damage (Gardner, 1979). Also secondary products from lipid oxidation can directly damage protein and amino acids by covalent bonds formation (Gardner, 1979). Xiong (2000) stated that formation of carbonyls (aldehyde and ketones) is one of the most noticeable changes due to protein oxidation, and that amino acids with reactive side chains, such as cysteine, methionine, lysine, arginine, histidine, and tryptophan, are particularly susceptible to oxidation. In their review, Bekhit et al. (2013) reported that carbonyl formation in oxidized protein can modify its tertiary structure resulting in unfolded protein. Hydrophobicity of the polypeptide and protein–protein interactions occur when proteins are unfolded. Thus, protein oxidation impairs its normal functions, such as enzymatic activity, and channel forming properties, and the proteins are more prone to proteolytic degradation.

On the other hand, Decker et al. (1993) supported the idea that protein oxidation can take place in absence of lipids through metal-catalyzed reactions via hydroxyl free radicals formed from hydrogen peroxide at specific iron-binding sites on protein. The researchers studied the iron and copper oxidation systems on turkey muscle myofibrillar proteins. They found that oxidized proteins had reduced solubility, gel strength, and gel water-holding capacity than controls, and

also formed protein polymers. Starke-Reed and Oliver (1989) stated that key metabolic enzymes are inactivated by metal-catalyzed oxidation reactions *in vitro* and the oxidative inactivation of these enzymes make them highly vulnerable to proteolysis.

Rowe et al. (2004) studied the impact of early postmortem protein oxidation on color and tenderness of beef steaks aged for 14 days. In order to get a range in protein oxidation levels, treatment levels evaluated included vitamin E supplementation (or not) and irradiation (or not) of the beef steaks from 24 to 26 h postmortem. Irradiation had a positive effect from the food safety standpoint but it also promoted oxidation that had a detrimental effect on meat quality. The authors found that increased early postmortem protein oxidation in both the sarcoplasmic and myofibrillar proteins was associated with increased shear force values at later times postmortem. They suggested that the aggregation and denaturation of myofibrillar proteins, and/or inactivation of some proteolytic enzymes, may have caused a negative impact on the tenderization mechanisms during beef aging. In terms of meat color, they observed that irradiated steaks had lower L* (lightness), a* (redness), and b* (yellowness) values than the non-irradiated meat samples. Thus, in general terms, irradiation had a negative effect on meat color. The researchers suggested that oxidation of myoglogin would be the main reason for the color change observed in irradiated steaks.

Meat Color

Pigments

Myoglobin, a globular single-chain protein present in the sarcoplasm, is the primary pigment responsible for meat color. A porphyrin or heme structure is located in the center of the myoglobin (Mancini, 2009). The iron atom present in the heme ring can form six bonds. Four of these coordination sites are in plane of the N atoms of a flat porphyrin ring and the other 2 are

perpendicular to this plane (Bekhit and Faustman, 2005). The 5th coordination site is connected to the proximal histidine-93 and the 6th site is available to reversibly bind ligands (Mancini and Hunt, 2005). The distal histidine-64 can interact with small molecules such as diatomic oxygen, carbon monoxide, nitric oxide, and aldehydes formed from lipid oxidation, which influence meat color stability. The ligand present on the iron and the redox state (Fe^{+2} or Fe^{+3}) determine the visible color changes in the meat surface (Mancini, 2009).

Beyond the ligand and the iron oxidation state in the myoglobin pigment, meat color is affected by several intrinsic (sex, breed, endogenous antioxidants, muscle type and metabolism, age of animal, ultimate pH, and the rate of postmortem pH decline) and extrinsic (temperature, packaging, light type exposed, type and growth of microorganism) factors (Bekhit and Faustman, 2005).

When the ligand donates electrons to the iron a, σ-bond is formed. The majority of the myoglobin ligands have this type of bonding (Livingston and Brown, 1981). On the other hand, π-bonding or "back bonding" occurs when the iron donates electrons back to the ligand. Back-bonding takes place when the metal has enough electron density for donation to its ligand, and this is what happens with the ferrous iron (Fe^{+2}). However, the ferric iron (Fe^{+3}) with its high nuclear charge does not form strong back-bonding (Livingston and Brown, 1981).

Four chemical forms of myoglobin are responsible for meat color: deoxymyoglobin, oxymyoglobin, metmyoglobin and carboxymyoglobin. Deoxymyoglobin is formed when no ligand is present in the 6th coordination site and the iron is in the ferrous state under very low oxygen tension (< 1.4 mm Hg) (Mancini and Hunt, 2005). The characteristic color of deoxymyoglobin is purplish-red or purplish-pink which is normally found in vacuum-packaged meat. Under oxygenated conditions, O_2 binds the 6th position and oxymyoglobin is formed while

the iron maintains its ferrous state, resulting in a bright cherry-red color (Mancini and Hunt, 2005). Oxidation of the ferrous states of myoglobin (deoxy- and oxy-) leads to the formation of metmyoglobin in which iron is in the ferric state and the meat color becomes brown in color. In this case, the 6th coordination site is H_2O that bonds to Fe^{+3} by σ donation. Carbon monoxide (CO) used in MAP, binds the 6th ligand and forms carboxymyoglobin resulting in a stable cherry-red meat color. Higher myoglobin affinity for CO than for O_2 provides stability to the ferrous oxidation state (Livingston and Brown, 1981). However, carboxymyoglobin stability in a high-oxygen MAP is not straightforward (Mancini, 2009). Use of CO in low-oxygen MAP represents an opportunity to maintain the cherry-red meat color preferred by consumers (Hunt et al., 2004).

Meat Discoloration

The appearance of meat is the most important sensory property influencing its purchase by consumers (Faustman and Cassens, 1990). Discolored meat is not associated with a fresh product and it is rejected by consumers. Thus, meat color stability becomes a relevant issue for the meat industry (Faustman and Cassens, 1990).

Autoxidation refers to the oxidation of myoglobin (deoxy or oxy) to metmyoglobin (MetMb) by free oxygen in a non-enzymatic process. This reaction implies that oxymyoglobin is converted into MetMb and free superoxide anion ($O_2^{\bullet -}$), the latter removing an electron from the iron (Giddings, 1977). The superoxide anion, in turn, will dismutate by a SOD catalyzed reaction into hydrogen peroxide (H_2O_2) and O_2 (Møller and Skibsted, 2006). It has been reported that O_2 must be reduced by 2 electrons, but only 1 electron is donated by ferrous myoglobin. Therefore, the second electron must be provided by another source (Livingston and Brown, 1981). Castro (1971), in his theory of hemeprotein reactivity, stated that Fe^{2+} donates an electron to O_2 when

secondary metal ions or protons are present. Snyder and Skrdlant (1966) demonstrated that copper greatly accelerated oxymyoglobin autoxidation, while iron and zinc had much less effect catalyzing this reaction. Additionally, Castro (1977) stated that Fe^{+2} porphyrin can be oxidized very rapidly by quinones involved in the electron transport chain in the mitochondria. Andersen et al. (1988) reported that autoxidation is enhanced by acidic conditions. Therefore, during the conversion of muscle to meat, postmortem anaerobic glycolysis forms lactic acid that helps to prevent microbial spoilage, but also increases autoxidation leading to a decrease in color stability (Møller and Skibsted, 2006).

Sevanian and McLeod (1997) reported that the formation of H_2O_2 takes place by myoglobin oxidation in the presence of electron donors such as hydroquinones, nitrates, and aminophenols. This involves a concerted two-electron process where donation of an electron from the heme iron to oxygen forms a superoxoferrimyoglobin intermediate according to the following reaction (equations 11 and 12):

$$Mb–Fe^{+2} – O_2 + H^+ \rightarrow Mb–Fe^{+3} – O_2^{\bullet -} \quad (11)$$

Donation of the second electron to $O_2^{\bullet -}$ yields metmyoglobin and H_2O_2:

$$Mb–Fe^{+3} – O_2^{\bullet -} + RH \rightarrow Mb–Fe^{+3} + H_2O_2 + R \quad (12)$$

Meat Discoloration and Bacteria Contamination

Increased MetMb formation has been related to the logarithmic growth phase of aerobic bacteria such as *Pseudomonas*, *Achromobacter* and *Flavobacterium*, but facultative anaerobic bacteria seems to not be associated with meat discoloration (Renerre, 1990). Oxygen partial pressure would be reduced by bacteria on the surface of meat to the critical levels at which MetMb formation is favored (Faustman and Cassens, 1990). By- products generated by some bacteria oxidize the iron molecule. Walters (1975) reported that hydrogen sulphide (H_2S) and

hydrogen peroxide (H_2O_2) react with myoglobin to produce sulphmyoglobin and choleglobin or cause degradation beyond porphyrins to bile pigments.

Pigment and Lipid Oxidation

An interrelationship between lipid and myoglobin oxidation is reasonable, although this link has not always been shown (Faustman et al., 2010). Faustman et al. (1999) demonstrated that α-β unsaturated aldehydes formed as a secondary lipid oxidation products, and particularly the 4-hydroxynonenal (HNE) accelerate oxymyoglobin oxidation through covalent attachment. This would change the tertiary structure of the protein making it more susceptible to oxidation. However, a prooxidant effect of HNE was noted at pH 7.4, but was not apparent at pH 5.6 even though oxymyoglobin oxidation was faster at pH 5.6 than at 7.4. This would happen because of the rapid oxymyoglobin autooxidation at pH 5.6.

On the other hand, myglobin may play a role as a facilitator of lipid oxidation (Faustamn et al, 2010). Rhee and Ziprin (1987a) reported that total pigment and myoglobin concentrations best explained differences in a modified thiobarbituric acid (TBA) determination in raw muscles of beef, pork and chicken. Kanner and Harel (1985) demonstrated that activated MetMb by H_2O_2 initiates lipid peroxidation. Autoxidation of oxyhemoglobin and oxymyoglobin lead to the formation of methemeproteins and the superoxide radicals ($O_2^{\bullet -}$), which dismutate to H_2O_2. Rhee et al. (1987b) also reported that the heme pigment system (MetMb-H_2O_2) plays an important role in the catalysis of lipid oxidation in raw and cooked meat. Oxidation of oxymyoglobin to MetMb is a common phenomenon during display of red meat; thus, enough H_2O_2 could be produced from the pigment oxidation for the MetMb-H_2O_2 catalysis of lipid oxidation.

Chan et al. (1997) studied the relative role of MetMb and oxymyoglobin in lipid oxidation, and the potential involvement of H_2O_2 and superoxide anion in oxymyoglobin-catalyzed lipid oxidation. Results showed that oxymyoglobin increases rates of lipid oxidation to a greater degree than MetMb at equimolar concentrations in phosphatidylcholine liposome. Decreased oxymyoglobin and lipid oxidation was found to occur when catalase enzyme was added to the myoglobin-liposome system, suggesting a role for H_2O_2 in the interaction between oxymyoglobin and lipid.

Sevanian and McLeod (1997) reported that, besides of being a source of H_2O_2 and further oxyradicals, MetMb can be oxidized by H_2O_2 to the ferryl state ($Mb–Fe^{+4} = O$), leading to a hyper-accelerated rate of oxidation, in essence, the second electron resides on the protein as a transient radical. This higher oxidation state has been shown to initiate lipid peroxidation according to the following reaction (equation 13):

$$HX–Fe^{+4} = O + RH \rightarrow HX–Fe^{+3} + HO^{-} + R^{\bullet} \quad (13)$$

Aside from formation of ferryl myoglobin from the myoglobin oxidation process, the dissociated heme from myoglobin and the iron from heme may also play a role in the mechanism by which myoglobin promotes lipid oxidation (Faustman et al., 2010).

Despite many studies showing the relationship between myoglobin and lipid oxidation, others have found no linkage (Faustman et al., 2010). George and Stratmann (1952) showed a relationship between oxygen partial pressure and first order rate constants for the autoxidation of myoglobin to MetMb. They found the maximum rate constant at approximately 1 mm Hg of oxygen partial pressure. Ledward (1970) reported that MetMb formation was maximal at oxygen partial pressure of 6 ± 3 mm Hg at 0°C in the semitendinosus muscle. Therefore, MetMb formation is favored under low partial pressure of oxygen. Oxymyoglobin redox state is

enhanced under high-oxygen atmospheres in which lipid oxidation also would occur readily. Hence, the conditions at very low or very high O_2 partial pressure environments do not support a close interrelationship between lipid and myoglobin oxidation processes (Faustman et al., 2010).

Metmyoglobin Reducing Activity

Bekhit and Faustman (2005) reported that NADH-cytochrome b_5 MetMb reductase is the best known enzyme responsible for hemeprotein reduction. This system involves the enzyme NADH-cytochrome b_5 MetMb reductase, cytochrome b_5 as an electron transfer mediator, and NADH. Cytochrome b_5 reductase acts as an electron donor from NADH to cytochrome b_5, which in turn reduces Fe^{+3} myoglobin to Fe^{+2} (Livingston et al., 1985).

Hagler et al. (1979) demonstrated *in vitro* that a reducing MetMb enzyme was present in beef heart muscle. Enzymatic activity was dependent on NADH, an acceptable myoglobin susbtrate, and ferrocyanide. They also found that an equimolar amount of cytochrome b_5 was more effective than ferrocyanide in the enzymatic reduction of MetMb. Optimum pH and temperature for the enzymatic reduction was 6.5 and 37°C, respectively, in harmony with what would be expected in exercising muscle. The enzyme also was unaffected by absence of O_2. Reddy and Carpenter (1991) proposed a novel procedure to isolate muscle extract for MetMb reductase activity assay. They reported significant differences in enzyme activity among beef muscles from the same animal. The order of enzyme activity in the muscles expressed on the basis of muscle myoglobin content was: tensor fasciae latae > longissimus dorsi > gluteus medius > diaphragm medialis > semimembranosus = psoas major. In agreement with Hagler et al. (1979), they found also that the enzyme activity was highest at pH 6.4 as compared to 5.8 or 7.0 and at 30°C compared to 4°C.

Arihara et al. (1995) studied localization of the MetMb-reducing enzyme system components (NAD-cytochrome b_5, reductase cytochrome b_5, and outer mitochondrial membrane cytochrome b) in bovine skeletal muscle. NADH-cytochrome b_5 reductase was identified mostly in the mitochondrial fraction and in the microsomal fraction to a lesser extent. Outer mitochondrial membrane (OM) cytochrome b was detected mainly in the mitochondrial fraction, while cytochrome b_5 was found only in the microsomal fraction. The researchers suggested that NADH-cytochrome b_5 reductase reduces metmyoglobin by using OM cytochrome *b* at the mitochondrial surface and, in part, by using cytochrome b_5 at the sarcoplasmic reticulum.
In their review, Bekhit and Faustman (2005) summarized that NADH-cytochrome b_5 reductase is capable of reducing electron acceptors such as methylene blue, ferricyanide, 2-6-dichlorophenol-indophenol, and the physiological acceptors OM cytochrome *b* and cytochrome b_5.

Madhavi and Carpenter (1993) determined the effects of postmortem aging, processing method and retail display time on MetMb reductase activity, oxygen consumption rate, and color stability in *Psoas major* and *Longissimus dorsi* muscles. They reported that surface MetMb formation, MetMb reductase activity, and oxygen consumption rate were affected by muscle type, postmortem aging, and fabrication method. They found that *Psoas major* steaks had greater MetMb accumulation, lower MetMb reductase activity, and greater oxygen consumption rate than *Longissimus dorsi* steaks, but after grinding, the color stability was similar between both muscles. Meat color was more stable in steaks fabricated at 4 or 7 days after slaughter. The order of color stability in terms of processing method was knife-cut steaks > saw-cut steaks > ground muscle.

Zhu and Brewer (1998) studied relationships between color stability, MetMb reductase activity and oxygen consumption rate in pale, soft and exudative (PSE), dark, firm and dry

(DFD), and normal pork. The highest MetMb reductase activity and oxygen consumption rate was found in the DFD pork, while the lowest enzyme activity was measured in PSE pork. No differences were registered in oxygen consumption rates between PSE and normal samples. MetMb reductase activity dropped slowly during meat storage and oxygen consumption rate rapidly decreased during the first day of storage.

Mikkelsen et al. (1999) demonstrated the presence of a MetMb reducing enzyme system in pork *Longissimus dorsi* muscle. Presence of NADH was critical for the reduction, but the electron transfer mediator was less important. The latter observation suggested that MetMb in pork would be more closely associated to the NADH-cytochrome b_5 reductase enzyme compared to the bovine MetMb. In addition, porcine MetMb was more easily reduced by NADH in a non-enzymatic process compared to bovine and equine MetMb.

Echevarne et al. (1990) examined the localization of the MetMb reducing system, the effects of pH and temperature on *in vitro* MetMb reductase activity, and the influence of O_2 in enzymatic activity. Homogenates from four different color-stable beef muscles were used: *Longissimus dorsi, Tensor fasciae latae, Psoas major,* and *Diaphragma medialis*. They observed that the greatest reducing activity was in the fraction comprised of microsomes and mostly intact mitochondria. The most color unstable muscles also had the highest reducing activity, and no differences were registered between aerobic and anaerobic activities. From their findings, the researchers concluded that MetMb reductase activity is not associated to color stability regulation in during meat shelf-life.

Lanari and Cassens (1991) analyzed differences in mitochondrial activity of color-stable *Longissimus dorsi* and color-labile *Gluteus medius* muscles from Holstein and crossbreed steers. Oxygen consumption rate decreased, but MetMb reductase activity was unaffected by the storage

time. *Gluteus medius* muscle and Holstein steers presented the highest oxygen consumption rate and MetMb reductase activity. Results lead to a question about the role of MetMb reductase activity in beef discoloration while mitochondrial oxygen consumption rate would be a contributing factor in the effects of muscle and breed on the rate of discoloration (Lanari and Cassens, 1991).

O´Keeffe and Hood (1982) measured different biochemical parameters in muscles with different color stability characteristics. They concluded that pigment reduction takes place aerobically and anaerobically. They also stated that the rate of MetMb formation (or discoloration rate) in different muscles would be more related to the enzymatic activity and oxygen consumption rate than by MetMb reducing activity.

King et al. (2011) reported that differences in color stability across muscles have been mostly attributed to greater oxygen consumption in muscles with less stable color, due to oxygen-scavenging enzymes compete with myoglobin for oxygen resulting in greater deoxymyoglobin concentration, which is more susceptible to oxidation than oxymyoglobin. These differences in oxygen consumption would be associated to differences in muscle fiber type across muscles (King et al., 2011).

Bekhit and Faustman (2005) stated that, even when MetMb reducing activity has been demonstrated in postmortem muscle, the extent to which this system contributes to maintenance of fresh meat color stability still remains unclear. One of the main constraints to support the role of MetMb reducing activity is the rapid NADH oxidation under postmortem conditions, particularly at normal meat pH (approximately 5.6). Moreover, extrapolation of *in vitro* results to more complex biological systems as in fresh meat leave questions unanswered.

Furthermore, a non-enzymatic reduction of MetMb has been shown to occur when NADH or NADPH were present, as well as EDTA was present (Brown and Snyder, 1969), in presence of ascorbate but absence of NADH (Hagler et al., 1979), or by direct reduction of cytochrome b_5 by α-tocopherol (Lynch et al., 1998).

CHAPTER III

EFFECT OF PACKAGING DURING STORAGE TIME ON RETAIL DISPLAY SHELF-LIFE OF BEEF STRIP LOINS FROM TWO DIFFERENT PRODUCTION SYSTEMS

INTRODUCTION

Growing demand for foods around the world, along with a globalized international market, has led to increased interest in extending shelf-life of food products. This is particularly important for fresh meat that is considered one of the most perishable of foods, because its composition is ideal for the growth of a wide range of spoilage bacteria (Mayr et al., 2003). Many factors, alone or in combination, such as atmospheric oxygen (O_2), moisture, endogenous enzymes, temperature, light, and bacteria, have a detrimental effect on meat quality (Lambert et al., 1991).

Meat quality deterioration does not refer just to microbial contamination. Other mechanisms, such as lipid and myoglobin oxidation, play an important role in this deterioration process. Lipid oxidation is related to the development of off-flavors, while pigment oxidation is responsible for meat discoloration, and both processes are interrelated (Faustman et al., 2010). Oxidation of fat and myoglobin depends on, besides environmental conditions, the balance between endogenous and/or exogenous pro-oxidant and antioxidant compounds present in meat (Martínez et al., 2014). Cattle feeding systems (grass vs. grain) affect the fatty acid profile and the antioxidant capacity of meat (Daley et al., 2010).

Development of new packaging systems represents, along with other preservation methods such as chilling, freezing, etc., a suitable strategy to extend meat shelf-life that includes storage and retail display life.

Therefore, the objective of this study was to evaluate the influence of packaging during storage of strip loins (to simulate export shipment) from steers fattened on grazing systems (Uruguay) or on high concentrate diets (United States) on retail display shelf-life color, microbial growth, fatty acids profile, lipid peroxidation and vitamin E content.

MATERIALS AND METHODS

Slaughter and Carcass Sampling

Experiments were repeated three times and each repetition was considered to be a different block of the overall study. For each block, 10 strip loins were collected from the right side of carcasses in a commercial meat packing plant in Uruguay (**UR**) from steers fattened on an intensive grazing system with improved pastures. Pastures consisted mainly of tall fescue (*Lolium arundinaceum*), italian ryegrass (*Lolium multiflorum*), red clover (*Trifolium pratense*), black oat (*Avena strigosa*). Carcasses were graded after slaughter using the Uruguayan grading system as specified by the National Meat Institute (INAC, 1997), and carcass data were recorded (conformation, age, degree of finishing, dentition). Carcasses were classified as young steers based on dentition (2 to 4 permanent incisors) and the HCW were between 250 to 296 kg. Different muscling grades, according to the Uruguayan grading system (INAC, 2004), were based on visual assessment of muscle mass development and were identified by the letters belonging to the word I - N - A - C - U - R, from very muscular development to thinly-muscled, and carcasses were graded as N or A. Strip loins were fabricated after 48 h of slaughter from a "pistola" cut by cutting from the 10^{th} rib to the lumbar-sacral junction. After fabrication strip loins were vacuum packaged, properly boxed and maintained under refrigerated conditions during its air shipment to United States (**US**).

On the same day on which Uruguayan strip loins were fabricated, 20 strip loins (IMPS #180) from the left and right sides of each of 10 carcasses, were collected in a federally inspected US meat packing plant and maintained vacuum packaged under refrigerated conditions (2°C) in the Meat Laboratory at Colorado State University until the packaging treatments were applied. Carcasses were representative of US conventional feedlot production systems and all were graded as USDA Choice with A maturity and an average HCW of 387 kg. One week after Uruguayan steers were slaughtered, strip loins samples arrived at the Meat Laboratory of Colorado State University and packaging treatments were applied to the samples from both countries.

Packaging Treatments

All sample processing took place in the Meat Laboratory at Colorado State University. Before packaging treatments were applied, samples were trimmed to 0.6 cm of external fat thickness. Up to four packaging treatments were evaluated within both production systems (UR and US) for each block. For two of the treatments, strip loins were fabricated into 2.54-cm-thick steaks, and for the other two treatments a 7.5-cm-thick piece from the strip loins was used.

Because of different strip loin fabrication procedure between countries, both strip loins from US carcasses were used and just the right strip loin from UR carcasses were collected. The UR strip loins were longer than US strip loins allowing to apply the four packaging treatments in just one strip loin. Packaging treatments were assigned randomly within each strip loin for UR samples and within each pair of strip loins (right and left) for US samples. Within each packaging treatment and country of origin, three different retail display times (0, 3 and 6 d) were randomly allotted.

In block 1, the four packaging treatments were: 1) vacuum packaging (**VP**; Multivac C500; Multivac Inc., Kansas City, MO) of a 7.5-cm-thick strip loin piece with a barrier bag (B6620 bag;

oxygen transmission rate [**OTR**] of 4.5 mL/m^2/24 h at 4.4°C and 0% RH and moisture vapor transmission rate [**MVTR**] of 0.45 g/645.2 cm^2/24 h at 37.8°C and 100% RH; Cryovac Sealed Air Corp., Duncan, SC). 2) low-O_2 MAP with nitrogen (N_2) as a filling gas and CO_2 (**MAP/CO_2**) of the individual 2.54-cm-thick steaks on #2 polystyrene trays (Genpak LLC, Glens Falls, NY) containing absorbent pads (Dri-Loc AC-50, Cryovac Sealed Air Corp., Duncan, SC) and overwrapped with polyvinyl chloride film (MAPAC DBL-TP film; OTR of 18,600 mL/m^2/24 h and MVTR of 28 g/645.2 cm^2/24 h at 37.8°C and 90% RH; Resinite Packaging Film, AEP Industries Inc., Griffin, GA). Trays were flushed with a 80% CO_2 and 20% nitrogen gas mixture (Airgas Inc., Fort Collins, CO) in a master bag (PM9120B, 2.0 mils; OTR of 5.3 mL/m^2/24 h at 23°C and 0% RH and MVTR of 9.5 g/m^2/24 h cm^2 at 38°C and 90% RH; Cryovac Sealed Air Corp., Duncan, SC) using a gas-flush packaging machine (Corr-Vac Mark III; M-Tek Inc., Elgin, IL). 3) low-O_2 MAP with N_2 plus CO_2 and CO (**MAP/CO**) of the individual 2.54-cm-thick steaks using the same equipment, trays and films used for the MAP/CO_2 treatment. Trays were flushed with a 80% N_2, 19.6% CO_2, and 0.4% CO gas mixture (Airgas Inc., Salt Lake City, UT). 4) VP plus peroxyacetic acid (**VP/PAA**) applied to a 7.5-cm-thick strip loin piece. Before VP (Multivac C500; Multivac Inc., Kansas City, MO), 28 to 30 ml of a 80 ppm PAA solution (16% PAA; DiverContact P16, Diversey Sealed Air Corp., Sturtevant, WI) was sprayed onto each strip loin piece. Two ready-to-use O_2 scavengers (FreshPax CR14, Multisorb Tecnologies Inc., Buffalo, NY) were placed in the headspace of the master bags corresponding to the MAP/CO_2 treatment and one O_2 scavenger (FreshPax CR20, Multisorb Tecnologies Inc., Buffalo, NY) was used for the MAP/CO treatment, according to the manufacturer´s recommendations.

In block 2, three treatments were evaluated and included: VP, MAP/CO and VP (B2620 bag; OTR of 3-6 mL/m^2/24 h at 4.4°C and 0% RH and MVTR of 0.5-0.6 g/645.2 cm^2/24 h at

37.8°C and 100% RH; Cryovac Sealed Air Corp., Duncan, SC) with ethyl-N-lauroyl-L-arginate HCl (**LAE**) incorporated into the film as an antimicrobial agent (**VP/AM**). In block 3, the treatments were: VP, MAP/CO_2, MAP/CO and VP/AM.

Retail Display

After the packaging treatments were applied, samples were stored in a cooler set at 2°C under dark conditions for 35 d to simulate export shipment (Fig. 3.1). After storage, the mother bags from the MAP/CO_2 and MAP/CO treatments were opened, samples for d 0 of retail display were taken for corresponding measurements, and then the individual trays were placed in a multi-deck retail display case (Hussman, Model No. M3X8GEP) set at 2°C (±1°C) for up to 6 days. Additionally, the 7.5-cm-thick strip loin piece from the VP, VP/PAA (block 1) and VP/AM (blocks 2 and 3) treatments were fabricated into 2.54-cm-thick steaks and overwrapped on individuals trays with the same materials used for the MAP treatments, and samples for d 0 of retail display were taken for the corresponding determinations. Therefore, all the samples displayed in the retail case were steaks on individual trays overwrapped with polyvinyl chloride film (MAPAC DBL-TP film). Retail display case was equipped with light emitting diodes (LED) lighting that illuminated at an average light intensity of 900 Lux (±184 Lux). Samples were exposed to light during the entire evaluation period. Every 8 h, samples were rotated to account for any variation in light intensity or temperature. Retail case temperature was monitored during display using temperature data loggers (iLog Console Pro, Cryopak, Monticello, AR).

Microbiological Analyses

Initial bacterial counts for mesophilic, psychrotrophic, *Pseudomonas* spp. and lactic acid bacteria (**LAB**) were performed on the vein steak of each strip loin before packaging treatments were applied (before storage). Microbiological analyses were also carried out after 35 d of storage

time (d 0 of retail display) and on d 3 and d 6 of the retail display periods. At each sampling time, a 4 x 4 cm square was aseptically excised from the center of 10 steaks per treatment using disposable scalpels (Feather Sterile Scalpels 2975#21; Graham-Field Inc., Atlanta, GA) and placed into individual sterile Whirl-Pak bags (710 mL; Nasco, Fort Atkinson, WI). The remaining part of each steak was cut into 1 x 1 cm cubes and the subcutaneous fat was removed. The cubes from each steak were placed into a sterilized Whirl-Pak bags (207 mL; Nasco, Fort Atkinson, WI) and were frozen at -80°C for subsequent chemical analysis. The 4 x 4 cm squares for microbial analysis were homogenized in 72 ml of Dey/Engley (D/E) neutralizing broth (Difco Laboratories, Sparks, MD), using a masticator paddle blender (IUL Industries, Barcelona, Spain) for 2 min. Tenfold serial dilutions were prepared in test tubes with 9 ml of 0.1% buffered peptone water (BPW; Difco Laboratories, Sparks, MD). Appropriate dilutions were surface-plated in duplicate onto two sets of tryptic soy agar (TSA; Acumedia, Neogen Corp, Lansing, MI) plates; one set for enumeration of mesophilic microbial populations and the second set for enumeration of psychrophilic microorganisms. Appropriate dilutions were also surface-plated on *Pseudomonas* selective agar (Pseudomonas Agar CFC Selective Agar; Oxoid Ltd, Basingstoke, UK) to obtain total *Pseudomonas* spp. counts. Colonies were enumerated after incubation of plates at 25°C for 72 h (mesophilic and *Pseudomonas*) or 7°C for 10 d (psychrotrophic). Lactic acid bacteria counts were determined using the pour plate method (Lactobacilli MRS Agar; Difco Laboratories, Sparks, MD) in a double layer technique using 10 mL for each layer to maintain anaerobic conditions. Plates were counted after 72 h at 25°C incubation.

Instrumental Color

Instrumental color measurements were recorded every 8 hours on the steaks displayed in the retail case for the total exhibition period (6 d). In blocks 1 and 2, measurements were obtained

using a handheld reflectance spectrophotometer (Hunter MiniScan XE, Model 45/O-S; Hunter Associates Laboratory Inc., Reston, VA) equipped with a 6 mm measurement port, calibrated at an illuminant D_{65} and 10° standard observer angle. In block 3, the measurements were collected using a Hunter MiniScan EZ (Model 4500 S; Hunter Associates Laboratory Inc., Reston, VA) provided with a 6 mm port size and a D_{65} light source. Hunter CIE L^*(lightness), a^*(redness), and b^* (yellowness) values were recorded in triplicate on the lean and subcutaneous fat of each steak through the overwrap film and the averages were used for statistical analysis. Spectrophotometers were calibrated with the black glass and white tile before each use.

Visual Color

Trained color panelists (n = 6 to 8) evaluated lean lightness, lean redness, percent lean discoloration and fat color every 8 h on steaks exhibited during the total display period (6 d). Panelists used a 15 cm unstructured line scales anchored at both ends with descriptive terms. For lean lightness, 0 cm denoted flat or dull, and 15 cm represented very bright/vivid. For redness of the predominant lean color, 0 cm denoted dark red or brown, and 15 cm indicated bright cherry-red. For fat color, 0 cm represented yellow/tan or brown/green, and 15 cm denoted very white color. For percent color discoloration, 0 cm represented 0% lean surface discoloration and 15 cm indicated 100% lean surface discoloration. After each scoring session, individual panelist ratings were averaged to obtain a single panel rating for each visual attribute of each sample.

Odor Panel

Odor panels were carried out at the end of the retail display period (6 d) for each block. Between 14 and 15 trained panelists who had previously been trained to assess and rate off-odors evaluated 16 samples (2 samples/packaging treatment x system combination). The samples were evaluated on trays, carefully removing the PVC film to avoid any microbial contamination, using

a 15-cm unstructured line scale anchored on the extreme left indicating absence of odor and the extreme right indicating a very strong presence. A single sensory value was obtained for each of the following odors: oxidized, putrid, and sour. Panelists marked the scale with a vertical line at the perceived intensity of the attributes. Results were expressed as the distance of the line measured from the extreme left end of the 15-cm scale.

Chemical Analysis

Fatty Acids Analysis. Composite samples (n = 5) selected randomly from each packaging x country of origin combination and for each block were used for the fatty acid analysis. Before compositing samples were homogenized using a Robot Coupe BLIXER 6V (Robot Coupe USA Inc., Ridgeland, MS). Fatty acid (**FA**) analysis was conducted for samples from d 0, 3 and 6 of the simulated retail display period. Total lipid content was determined from 1 g of homogenized sample using the chloroform:methanol lipid extraction method described by Folch et al. (1957). Fatty acids were analyzed by gas chromatography using a Hewlett Packard (Model 6890 series II; Avondale, PA) gas chromatograph fixed with a series 7683 injector and flame ionization detector. The analytical method was the same as that described by Phillips et al. (2010). Fatty acids were identified by comparing the relative retention times of sample fatty acid methyl ester peaks with those of standards. The methyl ester peaks were calculated as normalized area percentages of fatty acids.

Vitamin E. Composites samples were used for the vitamin E content determination on d 0, 3 and 6 of the retail display period. The analytical procedure used was as described by Njeru et al. (1995).

Thiobarbituric Acid Reactive Substances (TBARS). Lipid peroxidation was determined by quantifying the malondialdehyde (MDA) concentrations in each sample per the manufacturer's

instructions. Briefly, MDA concentrations were determined using a colorimetric endpoint MDA quantitation kit (OxiSelect TBARS Assay Kit; Cell Biolabs Inc., San Diego, CA) on all samples following 0, 3 and 6 d retail display. Malondialdehyde-thiobarbituric acid adducts were created per the manufacturer's directions and read at 532 nm in a microplate reader (Synergy HT Multi-detection reader; BioTek Instruments Inc., Winooski, VT), computer-controlled (Gen5 Data Analysis Software; BioTek Instruments Inc., Winooski, VT) and quantified using a MDA equivalent standard provided by the manufacturer. Assays were completed no more than 1 month after the samples from each block were frozen at -80°C.

Statistical Analysis and Design

Block 1 was analyzed separately from blocks 2 and 3 due to one of the packaging treatments evaluated being different. For block 1, our collaborator provided us the PAA solution to be sprayed on strip loin pieces before VP to evaluate its antimicrobial effectiveness. A new technology became available and we decide with our collaborator to substitute the previous treatment (VP/PAA) with a VP film with LAE incorporated into the film as an antimicrobial agent for blocks 2 and 3. Data were analyzed as a split-plot, repeated measures design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, version 9.3) with country of origin or production system (n = 2) as fixed main effect, packaging treatment (n = 4) as a subplot, time as a repeated measure and strip loin as a random effect. Studentized residuals plots were evaluated to test homogeneity of variance and normality for all data. Kenward-Roger approximation was used to calculate denominator degrees of freedom for different covariance structures for adjustment of the F-statistic. The experimental unit was the individual steak for the MAP treatments and the 7.5-cm-thick strip loin portion for the VP treatments, which was fabricated into 3 steaks (for d 0, 3 and 6) before retail display. After ANOVA, least squares means were calculated for treatment

comparisons with a significance level of $\alpha = 0.05$, using the PDIFF option of LSMEANS, when F-tests were significant ($P < 0.05$). Analysis of blocks 2 and 3 were conducted together in an incomplete design (no MAP/CO_2 in block 2) and the block effect was removed from the model when it was not significant.

Microbiological data were analyzed at d 0, 3 and 6 of display time using a mixed model which included packaging treatment, system and time as fixed effects and the random effect of strip loin within system and packaging x strip loin within system interaction. In the analysis of the data for blocks 2 and 3 together, the block also was considered as a random effect. Initial bacterial counts from the vein steak evaluated before application of the packaging treatments was used as a covariate for the data analysis. Fatty acid composition, TBARS values, and vitamin E content were analyzed with the same model as the microbiological data, except that no covariates were used.

For odor panel, putrid odor was analyzed using the LOGISTIC procedure for binary response variables. After normalization of the data using square root transformation, oxidized and sour odors were analyzed using the GLIMMIX procedure of SAS.

For instrumental and visual color variables, the best covariance structure was determined based on the Akaike Information Criterion that selects a model from a set of models. Autoregressive (AR [1]), heterogeneous autoregressive (ARH [1]), compound symmetry (CS), and heterogeneous compound symmetry (CSH) covariance structures were used for the repeated measures analysis. Packaging and system were considered as fixed effects and the random effect of strip loin within system and packaging x strip loin within system interaction were included in the REPEATED statement. Time was considered as a continuous variable for the data analysis. Principal component analyses (PCA) were conducted considering L^*, a^*, and b^* parameters for muscle, redness and percent discoloration.

RESULTS AND DISCUSSION

Microbiological Contamination

Different antimicrobial intervention systems (for food safety control) were used in the meat packing plants in UR versus the US. Furthermore, postmortem conditions might be different considering UR samples transportation. For these reasons, microbiological data analyses were performed within each country of origin because results were thus confounded among a number of factors that were not controlled. Initial bacterial counts before application of packaging treatments for US strip loins in block 1 were: 1.2 ± 0.7, 1.0 ± 0.7, 0.9 ± 0.8, and 0.6 ± 0.5 $\log_{10}$ CFU/cm^2, for mesophilic, psychrotrophic, *Pseudomonas* spp., and LAB, respectively; bacteria load on UR strip loins were: 2.1 ± 0.5, 0.9 ± 0.5, 0.4 ± 0.2, and 1.0 ± 0.4 $\log_{10}$ CFU/cm^2, for mesophilic, psychrotrophic, *Pseudomonas* spp., and LAB, respectively. For blocks 2 and 3, the initial microbial contamination levels in US samples were: 2.1 ± 0.6, 1.8 ± 0.6, 2.0 ± 0.8, and 1.8 ± 0.4 $\log_{10}$ CFU/cm^2, for mesophilic, psychrotrophic, *Pseudomonas* spp., and LAB, respectively; in UR strip loins the counts were: 3.8 ± 1.0, 3.1 ± 0.9, 1.7 ± 0.9, and 3.5 ± 1.0 $\log_{10}$ CFU/cm^2, for mesophilic, psychrotrophic, *Pseudomonas* spp., and LAB, respectively.

Because samples were stored under refrigerated conditions, it was expected that mesophilic bacteria were mainly psychrotrophic. One of the most important environmental factors that determines bacterial growth on meat is the temperature (Lambert et al., 1991). Growth of psychrotrophic bacteria is favored under refrigerated conditions and they are generally responsible for meat spoilage (Ercolini, 2009). At the end of storage time (d 0 of retail display) and for block 1, mesophilic and psychrotrophic bacteria counts were lower ($P < 0.05$) in VP and VP/PAA treatments in US samples and VP/PAA has lower ($P < 0.05$) bacteria load than MAP treatments in UR samples (Tables 3.1, 3.2, 3.5, and 3.6).

No significant differences ($P > 0.05$) in mesophilic and psychrotrophic bacteria were detected among treatments on d 3 and d 6 of display for the US samples in block 1 (Tables 3.1 and 3.5). For the UR steaks in block 1, no significant differences ($P > 0.05$) in mesophilic bacteria population were detected among treatments on d 3 and d 6 of retail display (Table 3.2), and on d 6 for psychrotrophic bacteria (Table 3.6). However, psychrotrophic bacteria count was lower ($P < 0.05$) in the VP/PAA than both MAP treatments on d 3 of display (Table. 3.6). The results found in block 1 for both production systems are not in agreement with the well documented bacteriostatic effect of CO_2 in MAP (Farber, 1991; Gill, 1996; Jakobsen and Bertelsen, 2002). Under anaerobic conditions as those imposed by the four packaging treatments, LAB growth is favored when the initial counts of spoilage bacteria are low (Gill, 1996), and become the predominant microorganisms of meats (Egan, 1983). One characteristic of LAB is that they are resistant to inhibition by CO_2 (Egan, 1983), that could explain the non-bacteriostatic effect observed in both MAP treatments for block 1 (Tables 3.1, 3.2, 3.5, 3.6, 3.13, and 3.14). However, in blocks 2 and 3 for the US samples on d 6 of display, there was an inhibitory effect ($P < 0.05$) of CO_2 on the mesophilic bacteria counts in the MAP treatments compared to the VP (Table 3.3), and on psychrotrophic bacteria load ($P < 0.05$) compared to the VP treatment (Table 3.7). Packaging treatment had no effect ($P > 0.05$) on mesophilic and psychrotrophic bacteria population at the end of the retail display time (d 6) in the UR samples from blocks 2 and 3 (Tables 3.4 and 3.8). It is important to note that, in block 1 for both production systems and in blocks 2 & 3 for the UR samples, mesophilic and psychrotrophic counts at the end of the retail display period in any packaging treatment were close to or even exceeds 7 $\log_{10}$ CFU/cm^2 whose level is considered as retail shelf-life (Borch et al., 1996; Tables 3.1, 3.2, 3.4, 3.5, 3.6, and 3.8). No effect of packaging treatments at high contamination levels may be associated with the stationary phase of growth

curve reached by bacteria population. In the present study, the total period from slaughter to retail display was 42 d (7 d from slaughter to the application of packaging treatments plus 35 d storage), hence explaining, in part, the high bacteria counts.

In regard to LAB, at the end of display and for block 1, US samples under MAP/CO had greater ($P < 0.05$) counts than samples treated with both VP (Table 3.13); no differences ($P > 0.05$) among packaging were detected for UR samples (Table 3.14). Both MAP and VP/AM treatments in the US samples for blocks 2 and 3 had lower ($P < 0.05$) LAB counts on d 6 than the VP treatment (Table 3.15), but no differences ($P > 0.05$) were found among packaging treatments for the UR samples (Table 3.16).

Pseudomonas spp. represent one of the most important spoilage bacteria on refrigerated meat, mainly under aerobic conditions (Lambert, 1991; García de Fernando et al., 1995; Gill, 1996; Pennacchia et al., 2011) due to its greater ability to use glucose and amino acids than other bacteria at refrigerated temperatures (Ercolini et al., 2006). *Pseudomonas* spp. produce gluconic acid and 2-oxogluconate in the Entner-Doudoroff pathway from glucose under aerobic conditions, which accumulate outside the cells and are further utilized; whereas, competing bacteria are unable to do so. After *Pseudomonas* organisms reach an 8 $\log_{10}$ CFU/cm^2 concentration on meat surfaces, the glucose supply is not enough to meet their growth requirements and then amino acids are degraded generating sulfur-containing compounds (Zhang et al., 2011) that are related to putrid odors (Gill, 1996). Proteolytic activity of *Pseudomonas* spp. lead to their penetration into the meat, representing an ecological advantage because they have access to a new niche with newly available nutrients not accessible to non- or less proteolytic bacteria (Nychas et al., 2008). Additionally, it has been documented that *Pseudomonas fluorescens* plays a main role in meat discoloration due to the increased MetMb formation via increased oxygen consumption (Chan et al., 1998).

For block 1, no differences ($P > 0.05$) were detected among packaging treatments in US samples at the end of the display time (d 6); whereas, for UR samples, both MAP treatments had a lower ($P < 0.05$) *Pseudomonas* spp. counts than VP treatments (Tables 3.9 and 3.10). *Pseudomonas* spp. counts were lower ($P < 0.05$) in the MAP/CO_2 than the other three treatments in US samples on d 6 of retail display for blocks 2 and 3 (Table 3.11). This could be explained by less residual oxygen in packaging during storage because anaerobic conditions inhibit all growth of the *Pseudomonas* spp. (Gill, 1996). It is important to keep in mind that, in retail display, steaks from all treatments were equal in condition, overwrapped with an oxygen permeable film and the packaging treatments were applied previously during the 35 d storage time. Exposure to air entails a fast *Pseudomonas* spp. growth (Borch et al., 1996). In UR samples and for blocks 2 and 3, VP/AM treatment resulted in a lower ($P < 0.05$) *Pseudomonas* spp. counts than the MAP/CO and VP treatment (Table 3.12).

The VP/PAA (block 1) and VP/AM (blocks 2 and 3) treatments were not effective in inhibiting bacterial growth at the end of retail display compared to the other three treatments (Tables 3.1 to 3.16). Use of PAA solution at 80 ppm may be explains the lack of inhibitory effect on bacteria population observed on the VP/PAA treatment. Food and Safety Inspection Service of USDA approved to use PAA up to a concentration of 220 ppm (FSIS, 2015). Peroxyacetic acid is a disinfectant that oxidizes and denatures proteins and lipids of microorganisms, causing a disorganization of the membrane (Maris, 1995). Gill and Badoni, (2004) reported inconsistencies in PAA efficacy as an antimicrobial agent with aerobic counts reductions between <0.5 log and 1 log unit. King et al. (2005) observed that use of PAA as an antimicrobial intervention to control *Escherichia coli* O157:H7 and *Salmonella* Typhimurium was not effective when applied to chilled inoculated carcass piece surfaces. Ransom et al. (2001) evaluated the efficacy of different

intervention technologies to decontaminate beef carcasses and lean pieces surfaces on *Escherichia coli* O157:H7. They reported that 0.02% PAA reduced pathogen populations in 1 log CFU/g when applied on lean tissue pieces. Pohlman et al. (2009) reported about 1.6 log CFU/g reduction in aerobic plate counts on d 7 of simulated retail display compared to the untreated control when 0.02% PAA was applied on beef trimmings before grinding. Geornaras et al. (2012) reported reductions of pathogen counts of 0.6 to 1.0 log CFU/cm^2 when PAA was used at 200 ppm as an immersion treatment for decontamination of beef trimmings inoculated (3.4 to 3.9 log CFU/cm^2) with *Escherichia coli* O157:H7 or non-O157 Shiga toxin–producing E. coli.

On the other hand, VP/AM with LAE did not reduce microbial activity in this study. The LAE is a cationic preservative derived from lauric acid and arginine, which causes disturbance in membrane potential and structural changes and loss of cell viability, although no disruption of cells has been detected (Rodríguez et al., 2004). It has been reported that a 1.78 to 5.81 $\log_{10}$ reduction on chicken breast fillets was obtained when LAE was incorporated into a chitosan film (Higueras et al., 2013). Pezo et al. (2012) indicated that the critical point in an antimicrobial active packaging is the kinetics of release of the antimicrobial agent from the packaging, although the migration kinetics of LAE have shown its progressive release to the food for at least 24 days. Joerger (2007) conducted a review on the antimicrobial films used in foods and concluded that they still face limitations; but even when they fail to completely remove higher numbers of target bacteria, they can be used as an additional post-processing safety measure. Thus, antimicrobial packaging represents promising form of active packaging to control microbial contamination by reducing the growth rate and/or extending the lag-phase of the target bacteria, or by inactivating bacteria by contact (Quintavalla and Vicini, 2002).

Color Measurements

Fresh meat quality attributes vary around the world depending on consumer preferences in different regions (Joo et al., 2013). However, the color of fresh meat is considered to be the single most important characteristic influencing a consumer´s purchase decision (Faustman and Cassens, 1990; Zerby et al, 1999). Meat color depends on the concentration and chemical state of the pigments and the physical characteristics of the meat (Renerre, 1990). Meat discoloration can be interpreted as a deviation from ideal color preferred by consumers to something less desirable (Faustman and Cassens, 1990). Prolonged meat storage promotes the oxidation of oxymyoglobin to metmyoglobin, resulting in an unattractive brown meat color (O´Grady et al, 1998).

Instrumental parameters and subjective attributes were evaluated to assess meat color in the present study, and in order to reduce multidimensional data and identify patterns in the color dataset, principal component analyses were performed. The first 2 PC explained 73% of the orthogonal variation in the color data (Table 3.17). An eigenvalue greater than 1 points out that PCs account for more variance than accounted by one of the original variables. Loadings represent the weights in the linear transformation when computing principal components scores (Suhr, 2005). For the PC 1 the loadings were: -0.198, 0.585, 0.515, 0.436, and -0.404 for L*, a*, b* measured on steak surface, redness, and percent discoloration, respectively. For the PC 2 the loadings were: 0.659, -0.190, -0.292, 0.541, and -0.390 for L*, a*, b* measured on steak surface, redness, and percent discoloration, respectively. The a* value was positively associated explaining the variation in the PC 1 while percent discoloration had a negative association. These results seem logical, considering that increase in a* values and percent discoloration measured on the steak surfaces are positively and negatively associated, respectively, to meat color preferred by consumers. Renerre (2000) indicated that the a^* value represents an important meat color

parameter related to meat discoloration (conversion of oxymyoglobin to metmyoglobin), that it is characterized by a decrease in its values. Zerby et al. (1999) determined an a^* value of 7.7 measured on the surface of a strip loin steak below which it would be discounted in a retail display case. These findings agreed with Carpenter et al. (2001) who reported that consumers prefer to purchase bright red beef rather than purple or brown beef even though packaging systems did not affect eating satisfaction experience.

The UR strip loins under MAP/CO had a greater ($P < 0.05$) a^* values than the VP/PA and MAP/CO_2 treatments on d 6 of display period. For the US samples, the MAP/CO treatment generated the most red-colored lean ($P < 0.05$) compared to the other three packaging for block 1 (Fig. 3.2). In blocks 2 and 3, the UR strip loin steaks in MAP/CO also had the greatest a^* values compared to the other three treatments, but no differences ($P > 0.05$) were detected among the VP treatments and the MAP/CO in the US steaks at the end of the retail display time (Fig. 3.3). In general, the results confirmed previous findings that meat exposed to CO extend the cherry-red color in fresh meat (Gee and Brown, 1978; Sørheim et al., 1999; Luño et al. 2000; Carpenter et al., 2001; Jayasingh et al., 2001; Hunt et al., 2004). The ability of the iron located in the center of the myoglobin´s porphyrin ring to bind ligands, and also its valence plays, a major role in meat color (Mancini, 2009). Myoglobin´s heme ring has a strong affinity for CO forming ferrous carboxymyoglobin that has a visible spectrum similar to that of oxymyoglobin (Livingston and Brown, 1981). These authors also reported that the CO complex is stable even when denaturation of the proteins takes place and CO dissociates from ferrous myoglobin 1000 times more slowly than oxygen. However, they also pointed out that carboxymyoglobin is extremely labile to photooxidation, which could be a problem in retail case conditions. Values for a* of lean after d 4 of retail display for the UR samples under MAP/CO_2 treatment were similar to those reported by

Isdell et al. (1999) for steaks from the *Longissimus dorsi* muscle stored in MAP (50% CO_2/50% N) with O_2 scavengers for 6 wk and displayed for 4 d. In the present study, a* values measured on steak surfaces were generally lower than those reported by Yang et al. (2002b) on vacuum packaged *Longissimus dorsi* stored during 47 days and thereafter overwrapped in oxygen permeable film for 7 days in darkness. Gatellier et al. (2005) also reported greater a* values on *Longissimus dorsi* steaks aged in VP for 14 d and subsequently stored in an oxygen permeable film during 6 d in dark conditions.

Deterioration based on a^* values (Fig. 3.2 and 3.3) during retail display, independent of the packaging treatment, was less pronounced in UR steaks than in US samples. This difference was likely due to the greater ($P < 0.05$) vitamin E content in the UR beef muscle than in the US samples (Fig. 3.4 and 3.5) which would relate to a greater intrinsic antioxidant activity against pigment and lipid oxidation (Descalzo and Sancho, 2008). Also, it was possible that US samples had an ultimate pH lower than those for UR beef, promoting MetMb formation and leading to a low color intensity (Renerre, 1990). A potential higher MetMb reducing activity in the UR samples could also explain, although it was not measured in this study, its greater color stability (Bekhit and Faustman, 2005). O´Sullivan et al. (2003) reported greater a^* values for steaks from pasture-fed steers than for steaks from steers fed a concentrate diet when meat samples were under MAP (80% O_2 and 20% CO_2). Lanari et al. (2002) reported that, after 30 d aging, beef samples from pasture-fed steers had similar color and color stability to grain-fed steers supplemented with vitamin E, and better than those beef samples from steers fed with grain but not supplemented. As pigmentation is positively correlated with a^* values (Vestergaard et al., 2000), its level could be greater in UR steaks than in US samples. Some works have shown that pasture-diet finishing animals present higher myoglobin concentration than concentrate-fed cattle (Bidner et al., 1986; Vestergaard et al., 2000). However,

Gatellier et al. (2005) did not report differences in myoglobin content between grass- and grain-fed steers.

In the analysis of lightness (L^*) values on the surface of the steak, country of origin (in block 1, and blocks 2 and 3), and time (in block 1) were the only significant ($P < 0.05$) effects. Therefore, packaging treatment and its interactions did not affect ($P > 0.05$) L^* values of muscle. In block 1, US samples had greater (32.6 vs. 28.5; $P = 0.0015$) L^* values than UR samples; which also was true in block 2 and 3 (33.4 vs. 31.1; $P < 0.0001$). These results were in agreement with the findings reported by Vestergaard et al. (2000) and Gatellier et al. (2005) in which *Longissimus dorsi* samples from grain-finished steers were lighter-colored (higher L^* values) than those from pasture-finished steers due to a higher ultimate pH normally achieved on grazing systems (i.e., since pH is inversely related to lightness of meat). Vestergaard et al. (2000) also demonstrated that pasture-finished steers have skeletal muscle fiber types characteristic of slow contraction and more oxidative metabolism, which would explain darker-colored meat (lower L^* values) with higher myoglobin concentrations compared to grain-finished steers. Darker lean color in pasture-fed compared to grain-fed steers also was documented by Bidner et al. (1986).

In terms of yellowness (b*) measured on the steak surface, packaging treatment had a significant effect in block 1 ($P < 0.0001$) and blocks 2 and 3 ($P < 0.0011$). Country of origin and its interaction with packaging treatments had no effect ($P = 0.6517$ and $P = 0.9320$, respectively) on yellowness (b^* parameter) in block 1, but did in blocks 2 and 3 ($P = 0.0010$ and $P = 0.0265$, respectively). Additionally, in blocks 2 and 3 the production system x packaging x time interaction was not significant ($P = 0.4480$). In block 1, both VP treatments had greater ($P < 0.05$) b* values than the MAP treatments at day 0 of retail display, but not differences ($P > 0.05$) were detected among packaging at the end of retail display time (Table 3.18). Furthermore, there was no effect

($P > 0.05$) of packaging treatments on UR and US samples at the end of the display time for blocks 2 and 3 (Tables 3.19 and 3.20).

Chemical Determinations

Effect of grazing system on vitamin E content in beef muscle has been well documented, resulting in greater levels than grain-fed animals (Yang et al., 2002a; Yang et al., 2002b; Mercier et al., 2004; Realini et al., 2004; Descalzo et al., 2005; Descalzo and Sancho, 2008; Luciano et al., 2011). Vitamin E content in UR samples regardless of the packaging treatment were greater ($P < 0.05$) than in US samples (Fig. 3.4 and 3.5). No packaging treatment effect ($P > 0.05$) was observed within production system over simulated retail display times in block 1 (Fig. 3.4) and, just for d 0 of display time in UR samples, the MAP/CO_2 treatment had a greater ($P < 0.05$) vitamin E content than the other three packaging treatments in blocks 2 and 3 (Fig. 3.5). It is interesting to note that the concentration of vitamin E in all UR samples was between 3.22 and 3.98 μg/g of muscle (Fig 3.4 and 3.5), which attained the threshold level proposed by Faustman et al. (1989), Arnold et al. (1993) and Liu et al. (1995) to delay pigment and lipid oxidation. Although antioxidant enzyme activity was not measured in this study, some studies have shown (Mercier et al., 2004; Gatellier et al., 2004; Descalzo and Sancho, 2008) greater superoxide dismutase (SOD) activity in beef of pasture compared to grain-fed animals, but no diet effect was documented in catalase activity. Strip loins from pasture-fed steers (UR) may have an increased SOD activity compared to grain-fed steers (US).

Packaging x production system, production system x time and packaging x production system x time interactions were not significant ($P > 0.05$) for any of the fatty acids categories analyzed in this study. For this reason, fatty acid data are presented by packaging treatment across production system (Tables 3.21 and 3.22) and by system over packaging type (Tables 3.23 and

3.24). The VP treatment had the lowest ($P < 0.05$) SFA and greatest ($P < 0.05$) UFA and MUFA concentrations compared to the other three packaging treatments on d 6 of retail display in block 1. The VP/PAA had greater concentrations of omega 3 (**n-3**) fatty acids than the VP and MAP/CO_2 treatments at the end of the display time in block 1 (Table 3.21). For blocks 2 and 3, beef from the MAP/CO_2 treatment had a lower concentration of UFA ($P < 0.05$) and greater concentration of SFA ($P < 0.005$) than beef packaged with MAP/CO and VP/AM at the end of retail display. Omega 6 concentrations were greater ($P < 0.05$) in both VP treatments than in the MAP/CO, but no differences ($P > 0.05$) were detected among packaging treatments in n-3 percentage after d 6 of display time (Table 3.22).

Regarding to fatty acid composition by country of production, UR samples had lower ($P < 0.05$) SFA and MUFA concentrations, and greater ($P < 0.05$) PUFA, n-6 and n-3 concentrations than the US samples over retail display (Tables 3.23 and 3.24). These results agreed with previous research showing that PUFA and, particularly the n-3 fatty acids concentration, are greater in muscles from grass fed- than high grain-fed animals (Duckett et al., 1993; French et al., 2000; Yang et al., 2002b; Realini et al., 2004; Descalzo et al., 2005; Gatellier et al., 2005; Purchas et al., 2005) even when a high proportion of PUFA are biohydrogenated in the rumen by the microorganisms (Wood et al., 2008). Additionally, greater ($P < 0.05$) PUFA/SFA and lower ($P < 0.05$) n-6/n-3 ratios were observed in pasture-fed steers (UR) than in concentrate-fed steers (US) across display time (Tables 3.23 and 3.24).

Results from the odor panels performed at the end of each display time are presented in Table 3.25. In general terms, there were a very low off-odor levels detected by the panelists. All packaging treatments evaluated were anaerobic and the meat samples were exposed to oxygen (overwrapped with polyvinyl chloride film) only after 35 d storage. During the storage time, LAB

became predominant (Tables 3.13 to 3.16), resulting in a long odor-free beef shelf-life normally developed under anaerobic conditions (Renerre and Labadie, 1993). However, UR beef samples developed a greater ($P < 0.05$) oxidized odor than US samples while the latter exhibited a greater ($P < 0.05$) sour odor than beef from grass-fed cattle. The more prevalent oxidized odor in UR beef may have resulted from its greater ($P < 0.05$) PUFA concentration (Tables 3.23 and 3.24) that are more prone to oxidation.

Lipid peroxidation determined by TBARS presented a significant packaging x system x time interaction in block 1 ($P = 0.0027$) and blocks 2 and 3 ($P = 0.0104$). In general terms and for block 1, UR beef had greater levels of TBARS ($P < 0.001$) than US samples on d 0 of display; but TBARS values tended to decrease during retail display and these differences almost disappeared by the end of the display period (Fig. 3.6). Greater TBARS values in the UR samples on d 0 of retail display may have been associated with greater PUFA concentrations (Table 3.23) which are more prone to oxidation. Decreased TBARS values in beef from UR on d 6 of display may have related to more antioxidant agents (i.e., α-tocopherol) present in pasture-fed steers. For blocks 2 and 3, TBARS values followed a different pattern, increasing in general terms from d 0 to d 6 of retail display in UR and US samples (Fig. 3.7). There was no evident explanation for this dissimilar behavior in TBARS values during retail display between block 1, and blocks 2 and 3. Lipid oxidation promotes meat discoloration (Faustman et al., 2010). Thus, oxymyoglobin oxidation is accelerated in presence of oxidizing lipids, but significant increases in lipid oxidation can take place before significant oxymyoglobin oxidation is observed (O´Grady et al., 2001). The relationship between lipid oxidation and meat discoloration seems not be tightly linked when very high or very low oxygen concentrations are present in the environment (Faustman et al., 2010). Additionally, the oxidative stability of meat stems from the balance between endogenous (α-

tocopherol, peptides, uric acid, polyamines, ascorbate; antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase; and minerals such as selenium and zinc) and exogenous (nitrites, phenols and ascorbate) antioxidant and prooxidant substances (Martínez et al., 2014). Furthermore, dipeptides carnosine and anserine play a fundamental role in meat as an endogenous antioxidants (Antonini et al., 2002).

CONCLUSIONS

It is crucial to have microbial contamination levels as low as possible before to store meat under refrigerated conditions in order to extend the retail display shelf-life. At spoilage levels any packaging treatment seems to have any effect on the microbial population. Low-O_2 MAP treatments had lower mesophilic and psychrotrophic bacteria counts at the end of the retail display time when spoilage levels were still not reached.

From the color standpoint, the a* value measured on the steak surface was the most relevant attribute that explained the variability of the data in the PC 1. The a* values decreased across retail display time but in lesser extent in the UR samples, probably due to its greater vitamin E concentration and total antioxidant capacity. Low-O_2 MAP/CO treatment improved the redness (greater a* values) of the meat in both production systems, but in more extent in the UR samples. The L* parameter (lightness) was not affected by packaging treatments and its interactions, but US samples were lighter than UR samples. Packaging treatments had no effect on yellowness (b* parameter) at the end of retail display time.

At the end of the storage time (d 0 of retail display), VP and MAP/CO_2 treatments had greater proportions of PUFA, PUFA/SFA ratio, n-6, and lower n-6/n-3 ratio than the other 2 treatments in block 1. No packaging treatment effect on FA profiles were observed in blocks 2

and 3 on d 0 of display time. The UR samples had greater proportions of PUFA, PUFA/SFA ratio, n-6, n-3, and lower n-6/n-3 ratio than US samples.

In terms of lipid oxidation, and for block 1, greater TBARS values were observed, in general, for UR samples than US samples on day 0 of retail display. Minor differences were detected among packaging x production system interaction at the end of display time. For blocks 2 and 3, no significant differences were found among packaging treatments within each country of origin at the end of retail display.

Odor was only affected by the production system in block 1. Panelists detected more oxidized odor in UR samples than in US beef, but sourer odor in US samples than in UR samples.

IMPLICATIONS

To maximize shelf-life (storage and display life) of exported fresh beef, it is critical to minimize bacterial populations during processing and storage. At the same time, enhancing total antioxidant capacity of beef also is key to delaying lipid and myoglobin oxidation, which lead to formation of off-flavors and lean discoloration. Low O_2 - MAP/CO represents a packaging system that can extend storage life of fresh beef during export, particularly with regard to maintaining desirable beef color. Sensory evaluation undoubtedly would have contributed to a more comprehensive understanding of beef shelf life characteristics of importance for consumers, and should be considered in further studies. Complexity of fresh meat post-mortem chemistry warrants a more comprehensive and systemic approach to maximize its shelf-life.

Figure 3.1. Chronological events from slaughter to the end of retail display.

Table 3.1. Mesophilic bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from US samples for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	5.4^{bx}	0.19	6.4^{ax}	0.19	6.6^{ax}	0.19	5.3^{bx}	0.19	<0.0001
d 3	6.3^{y}	0.19	6.6^{x}	0.19	6.9^{x}	0.19	6.3^{y}	0.19	0.0511
d 6	6.9^{z}	0.19	7.3^{y}	0.19	7.4^{y}	0.19	7.1^{z}	0.19	0.1162
P-value	<0.0001		<0.0001		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).
$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.2. Mesophilic bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from UR samples for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	5.9^{abx}	0.19	6.5^{ax}	0.19	6.4^{ax}	0.19	5.5^{bx}	0.19	0.0001
d 3	6.9^{y}	0.19	6.9^{xy}	0.19	7.0^{y}	0.19	6.4^{y}	0.19	0.0544
d 6	7.7^{z}	0.19	7.2^{y}	0.19	7.5^{z}	0.19	7.2^{z}	0.19	0.1100
P-value	<0.0001		<0.0001		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.

$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.3. Mesophilic bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from US samples for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	4.6^{x}	0.15	4.8^{x}	0.20	4.7^{x}	0.15	4.6^{x}	0.15	0.8438
d 3	5.5^{ay}	0.15	5.0^{abxy}	0.20	4.7^{bx}	0.15	4.9^{bx}	0.15	0.0001
d 6	6.3^{az}	0.15	5.5^{by}	0.20	5.6^{by}	0.15	6.0^{aby}	0.15	0.0002
P-value	<0.0001		<0.0001		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

[a, b]: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

[x, y, z]: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.4. Mesophilic bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from UR samples for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	6.8^{ax}	0.15	7.7^{b}	0.20	7.8^{b}	0.15	6.7^{ax}	0.15	<0.0001
d 3	7.3^{by}	0.15	7.7^{ab}	0.20	7.9^{a}	0.15	7.2^{by}	0.15	0.0003
d 6	7.7^{z}	0.15	7.7	0.20	7.8	0.15	7.7^{z}	0.15	0.7946
P-value	<0.0001		0.9337		0.9668		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.5. Psychrotrophic bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from US samples for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	5.4^{bx}	0.19	6.4^{ax}	0.19	6.5^{ax}	0.19	5.7^{bx}	0.19	<0.0001
d 3	6.3^{y}	0.19	6.5^{x}	0.19	6.9^{xy}	0.19	6.3^{y}	0.19	0.0629
d 6	6.7^{y}	0.19	7.1^{y}	0.19	7.3^{y}	0.19	6.9^{z}	0.19	0.1373
P-value	<0.0001		<0.0001		0.0007		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).
$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.6. Psychrotrophic bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from UR samples for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	6.1^{abx}	0.19	6.5^{ax}	0.19	6.5^{ax}	0.19	5.7^{bx}	0.19	0.0015
d 3	6.7^{aby}	0.19	7.0^{ay}	0.19	7.1^{ay}	0.19	6.4^{by}	0.19	0.0213
d 6	7.5^{z}	0.19	7.3^{y}	0.19	7.5^{z}	0.19	7.2^{z}	0.19	0.3548
P-value	<0.0001		0.0016		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).
$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.7. Psychrotrophic bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from US samples for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	4.8^{x}	0.14	4.9^{x}	0.19	4.8^{x}	0.14	4.7^{x}	0.14	0.8341
d 3	5.6^{ay}	0.14	5.1^{abx}	0.19	4.9^{bx}	0.14	5.0^{by}	0.14	0.0009
d 6	6.3^{az}	0.14	5.7^{bcy}	0.19	5.5^{cy}	0.14	6.1^{abz}	0.14	<0.0001
P-value	<0.0001		0.0020		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

[a, b]: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

[x, y, z]: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.8. Psychrotrophic bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from UR samples for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	6.7^{bx}	0.14	7.5^{a}	0.19	7.6^{a}	0.14	6.6^{bx}	0.14	<0.0001
d 3	7.1^{by}	0.14	7.6^{a}	0.19	7.7^{a}	0.14	7.1^{by}	0.14	0.0006
d 6	7.6^{z}	0.14	7.5	0.19	7.7	0.14	7.6^{z}	0.14	0.7890
P-value	<0.0001		0.9109		0.8491		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.9. *Pseudomonas* spp. bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from US samples for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	3.8^{ax}	0.36	4.2^{ax}	0.36	3.5^{ax}	0.36	2.5^{bx}	0.36	0.0032
d 3	4.3^{x}	0.36	4.0^{x}	0.36	4.0^{x}	0.36	3.5^{y}	0.36	0.3168
d 6	5.5^{y}	0.36	5.5^{y}	0.36	4.9^{y}	0.36	5.2^{z}	0.36	0.5557
P-value	<0.0001		<0.0001		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).
$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.10. *Pseudomonas* spp. bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from UR samples for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	3.0^{ax}	0.36	1.3^{bcx}	0.36	0.9^{cx}	0.37	2.1^{bx}	0.36	<0.0001
d 3	4.0^{ay}	0.36	1.2^{cx}	0.36	1.3^{cx}	0.36	2.7^{bx}	0.36	<0.0001
d 6	6.3^{az}	0.36	2.7^{by}	0.36	3.2^{by}	0.36	5.6^{ay}	0.36	<0.0001
P-value	<0.0001		<0.0001		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).
$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.11. *Pseudomonas* spp. bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from US samples for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	3.1^{ax}	0.72	$<1.3^{bx}$	0.76	$<1.5^{bx}$	0.72	$<1.9^{bx}$	0.72	<0.0001
d 3	3.7^{ay}	0.72	$<1.4^{bx}$	0.76	$<1.9^{by}$	0.72	$<2.0^{bx}$	0.72	<0.0001
d 6	4.8^{az}	0.72	2.5^{cy}	0.76	$<3.3^{bz}$	0.72	3.3^{by}	0.72	<0.0001
P-value	<0.0001		<0.0001		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Least squares means with a less than symbol (<) indicate one or more of the samples within the treatment had plate counts below the analysis detection limit (0.4 log CFU/cm^2)

Table 3.12. *Pseudomonas* spp. bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from UR samples for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	2.8^{bx}	0.72	$<3.3^{bc}$	0.76	3.9^{a}	0.72	2.0^{cx}	0.72	<0.0001
d 3	3.2^{by}	0.72	3.6^{bc}	0.76	$<3.8^{a}$	0.72	2.4^{cy}	0.72	0.0001
d 6	4.5^{az}	0.72	3.4^{bc}	0.76	4.1^{ab}	0.72	3.1^{cz}	0.72	<0.0001
P-value	<0.0001		0.4256		0.2222		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Least squares means with a less than symbol (<) indicate one or more of the samples within the treatment had plate counts below the analysis detection limit (0.4 log CFU/cm^2)

Table 3.13. Lactic acid bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from US samples for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	5.6^{bx}	0.17	6.4^{ax}	0.18	6.6^{ax}	0.17	5.4^{bx}	0.17	<0.0001
d 3	6.3^{y}	0.17	6.5^{x}	0.17	6.7^{x}	0.17	6.2^{y}	0.17	0.0847
d 6	6.7^{bz}	0.17	7.0^{aby}	0.17	7.4^{ay}	0.17	7.0^{bz}	0.17	0.0153
P-value	<0.0001		0.0003		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).
$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.14. Lactic acid bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from UR samples for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	6.0^{bcx}	0.17	6.4^{abx}	0.17	6.5^{ax}	0.17	5.6^{cx}	0.17	0.0002
d 3	6.7^{ay}	0.17	6.9^{ay}	0.17	6.9^{ay}	0.17	6.3^{by}	0.17	0.0136
d 6	7.4^{z}	0.17	7.2^{z}	0.17	7.6^{z}	0.17	7.1^{z}	0.17	0.1730
P-value	<0.0001		<0.0001		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).
$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.15. Lactic acid bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from US samples for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	4.8^{x}	0.25	4.6^{x}	0.29	4.4^{x}	0.25	4.5^{x}	0.25	0.2688
d 3	5.1^{y}	0.25	5.2^{y}	0.29	5.0^{y}	0.25	5.0^{y}	0.25	0.6835
d 6	6.3^{az}	0.25	5.6^{by}	0.29	5.6^{bz}	0.25	5.9^{bz}	0.25	<0.0001
P-value	<0.0001		<0.0001		<0.0001		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.16. Lactic acid bacteria count (log_{10} CFU/cm^2) by packaging treatment and retail display time from UR samples for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	6.8^{bx}	0.24	7.7^{a}	0.27	7.7^{a}	0.24	6.8^{bx}	0.24	<0.0001
d 3	7.3^{by}	0.24	7.6^{ab}	0.27	7.9^{a}	0.24	7.3^{by}	0.24	0.0038
d 6	7.8^{z}	0.24	7.9	0.27	8.0	0.24	7.7^{z}	0.24	0.5182
P-value	<0.0001		0.4123		0.1408		<0.0001		

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

$^{a, b}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

$^{x, y, z}$: Least squares means (LSMean) within a column without a common superscript differ ($P < 0.05$).

Table 3.17. Eigenvalues of the correlation matrix.

Variable	Eigenvalue	Proportion	Cumulative
PC 1	2.47	0.49	0.49
PC 2	1.20	0.24	0.73

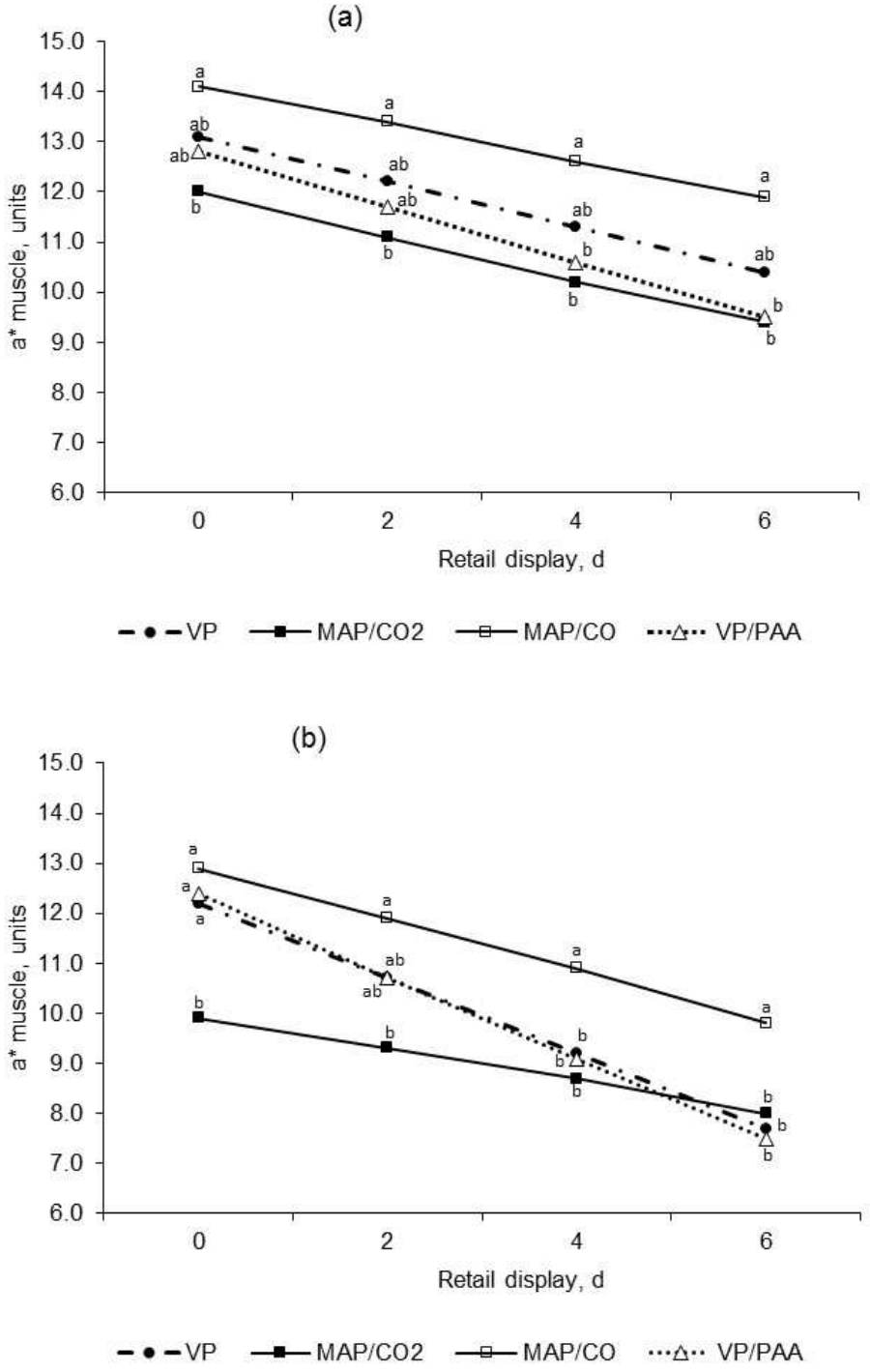

Figure 3.2. Redness (a* values) during 6 d of simulated retail display for UR (a) and for US (b) strip loins steaks by packaging treatment for block 1. VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid. At each time point least squares means without a common superscript differ ($P < 0.05$).

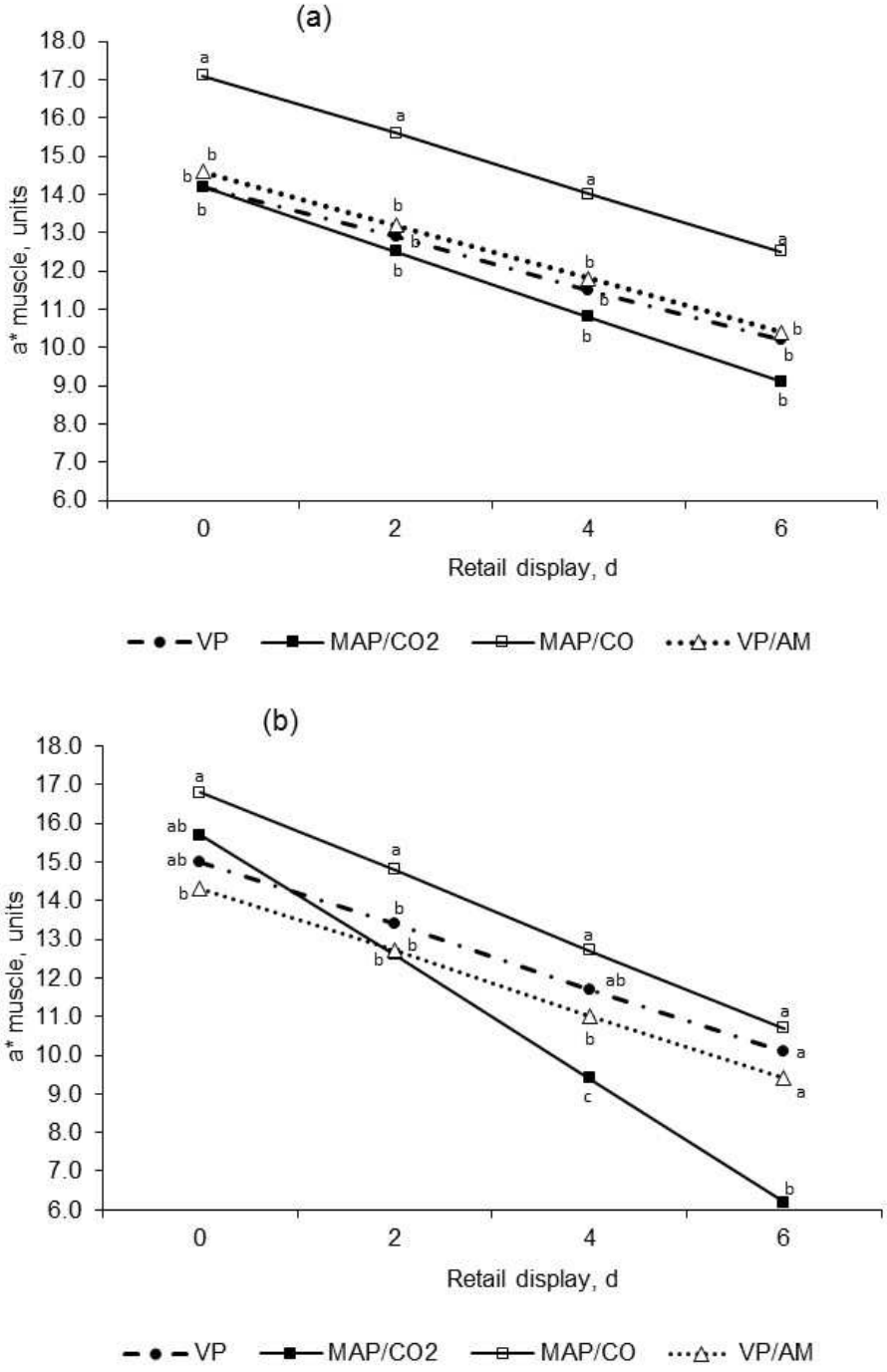

Figure 3.3. Redness (a* values) during 6 d of simulated retail display for UR (a) and US (b) strip loin steaks by packaging treatment for blocks 2 and 3. VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film. At each time point least squares means without a common superscript differ ($P < 0.05$).

Table 3.18. Effect of packaging treatments averaged over production system on yellowness (b^*) measured on the steak surface during retail display for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	13.7[a]	0.3	12.2[b]	0.3	12.6[b]	0.3	14.2[a]	0.3	<0.0001
d 2	13.9[a]	0.3	12.8[c]	0.3	13.2[b]	0.3	13.6[ab]	0.3	<0.0001
d 4	13.2[a]	0.3	12.5[b]	0.3	13.1[a]	0.3	12.8[ab]	0.3	0.0064
d 6	11.7	0.3	11.4	0.3	12.1	0.3	11.7	0.3	0.3719

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
SEM: standard error the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table 3.19. Effect of packaging treatments in UR steaks on yellowness (b^*) measured on the steak surface during retail display for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	14.1^{a}	0.5	13.2^{ab}	0.6	13.2^{b}	0.5	14.0^{ab}	0.5	0.0095
d 2	13.5^{ab}	0.5	12.8^{b}	0.5	14.0^{a}	0.5	13.4^{b}	0.5	0.0002
d 4	12.9^{b}	0.5	12.2^{c}	0.5	13.7^{a}	0.5	12.9^{b}	0.5	<0.0001
d 6	12.4	0.5	11.4	0.6	12.4	0.5	12.4	0.5	0.0525

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

SEM: standard error the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table 3.20. Effect of packaging treatments in US steaks on yellowness (b^*) measured on the steak surface during retail display for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	14.6^{ab}	0.5	15.5^{a}	0.6	13.9^{b}	0.5	14.7^{ab}	0.5	0.0037
d 2	14.1^{ab}	0.5	14.2^{ab}	0.5	14.4^{a}	0.5	13.5^{b}	0.5	0.0007
d 4	13.3^{b}	0.5	12.9^{bc}	0.5	13.9^{a}	0.5	12.6^{c}	0.5	<0.0001
d 6	12.2	0.5	11.6	0.6	12.4	0.5	12.0	0.5	0.2742

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

SEM: standard error the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

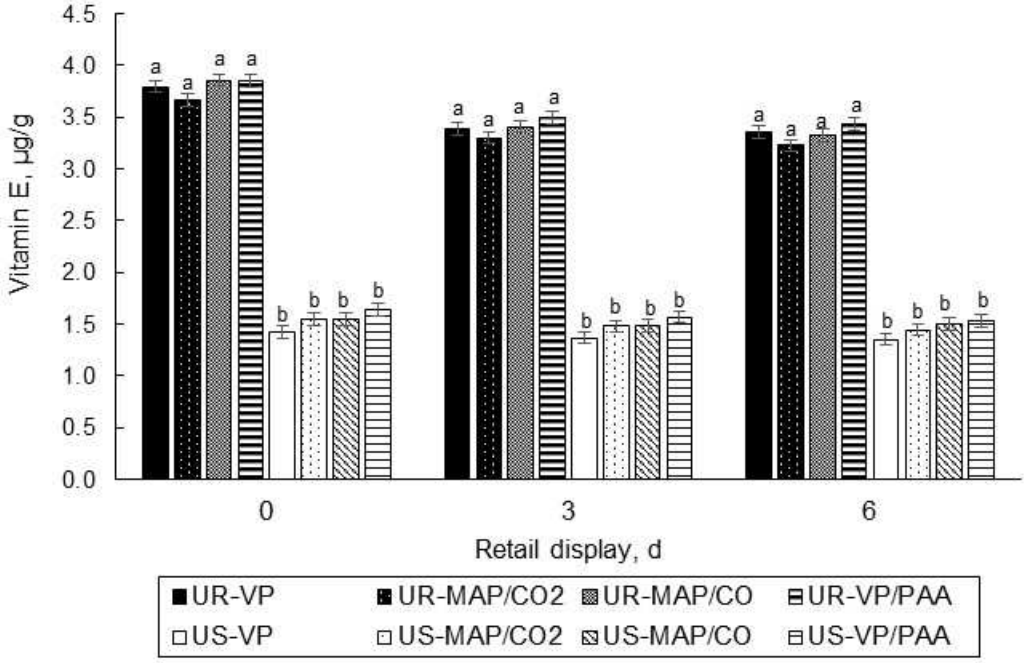

Figure 3.4. Vitamin E content (µg/g of muscle) during simulated retail display for UR and US strip loin steaks by packaging treatment for block 1. VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid. At each time point least squares means without a common superscript differ ($P < 0.05$).

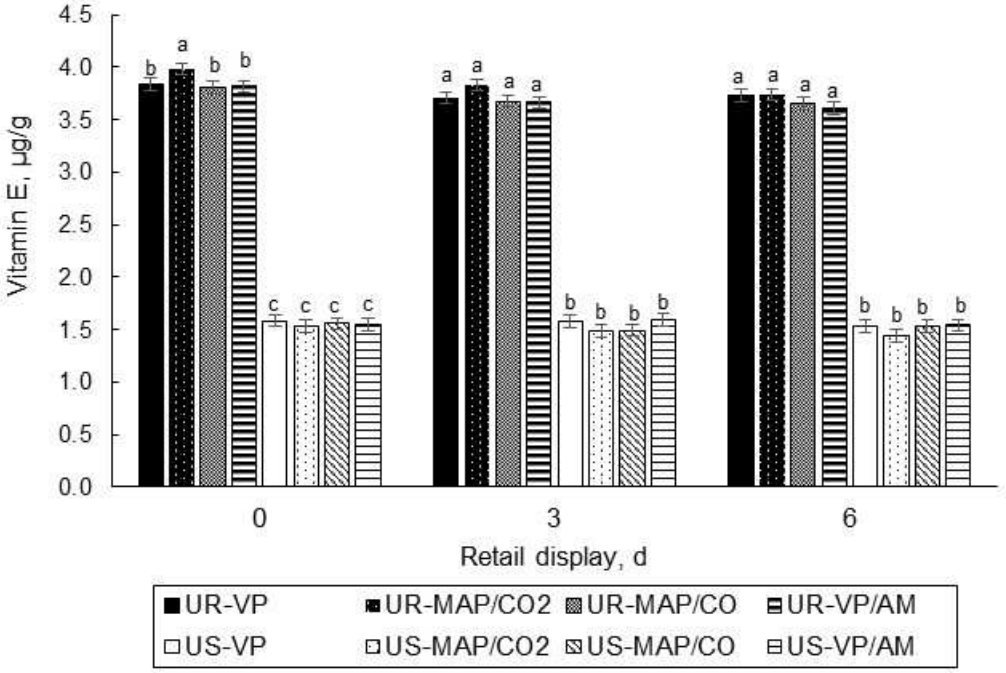

Figure 3.5. Vitamin E content (µg/g of muscle) during simulated retail display for UR and US strip loin steaks by packaging treatment for blocks 2 and 3. VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film. At each time point least squares means without a common superscript differ ($P < 0.05$).

Table 3.21. Fatty acid composition (%) by packaging treatment and retail display time averaged over production system for block 1.

d 0	VP		MAP/CO_2		MAP/CO		VP/PAA		
Fatty acid	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
SFA	41.7[a]	0.42	41.4[ab]	0.42	42.3[a]	0.42	40.5[b]	0.42	0.0301
UFA	53.0[b]	0.44	52.8[b]	0.44	52.1[b]	0.44	54.4[a]	0.44	0.0038
MUFA	45.1[b]	0.43	45.4[b]	0.43	45.8[b]	0.43	48.5[a]	0.43	<0.0001
PUFA	7.85[a]	0.27	7.41[a]	0.27	6.28[b]	0.27	5.90[b]	0.27	<0.0001
PUFA/SFA[1]	0.175[a]	0.007	0.164[a]	0.007	0.139[b]	0.007	0.123[b]	0.007	<0.0001
n-6	5.67[a]	0.25	5.49[a]	0.25	4.20[b]	0.25	3.94[b]	0.25	<0.0001
n-3	1.86[a]	0.06	1.61[b]	0.06	1.83[a]	0.06	1.64[b]	0.06	0.0015
n-6/n-3[1]	4.93[a]	0.28	5.60[a]	0.28	3.62[b]	0.28	3.79[b]	0.28	<0.0001
Unknown	5.36	0.35	5.81	0.35	5.68	0.35	5.18	0.35	0.5652
d 3									
SFA	41.2	0.42	42.0	0.42	42.1	0.42	41.9	0.42	0.3811
UFA	53.5[a]	0.44	52.1[b]	0.44	52.5[ab]	0.44	51.8[b]	0.44	0.0408
MUFA	47.9[a]	0.43	46.0[bc]	0.43	46.5[b]	0.43	45.3[c]	0.43	0.0006
PUFA	5.62	0.27	6.04	0.27	5.94	0.27	6.54	0.27	0.1329
PUFA/SFA[1]	0.118[b]	0.007	0.132[ab]	0.007	0.129[ab]	0.007	0.146[a]	0.007	0.0325
n-6	3.69[b]	0.25	4.09[ab]	0.25	4.09[ab]	0.25	4.69[a]	0.25	0.0471
n-3	1.58	0.06	1.60	0.06	1.48	0.06	1.54	0.06	0.4564
n-6/n-3[1]	3.53[b]	0.28	3.86[ab]	0.28	4.21[ab]	0.28	4.61[a]	0.28	0.0454
Unknown	5.34	0.35	5.96	0.35	5.39	0.35	6.30	0.35	0.1561
d 6									
SFA	38.1[c]	0.42	40.4[b]	0.42	41.3[ab]	0.42	42.3[a]	0.42	<0.0001
UFA	56.2[a]	0.44	54.0[b]	0.44	53.5[b]	0.44	52.3[c]	0.44	<0.0001
MUFA	50.7[a]	0.43	46.8[b]	0.43	45.6[c]	0.43	45.8[c]	0.43	<0.0001
PUFA	5.49[c]	0.27	7.17[b]	0.27	7.94[a]	0.27	6.42[bc]	0.27	<0.0001
PUFA/SFA[1]	0.109[c]	0.007	0.154[b]	0.007	0.176[a]	0.007	0.142[b]	0.007	<0.0001
n-6	3.74[c]	0.25	5.19[b]	0.25	5.91[a]	0.25	4.35[c]	0.25	<0.0001
n-3	1.39[c]	0.06	1.63[b]	0.06	1.69[ab]	0.06	1.82[a]	0.06	<0.0001
n-6/n-3[1]	4.07[b]	0.28	4.89[a]	0.28	5.31[a]	0.28	3.68[b]	0.28	0.0002
Unknown	5.75	0.35	5.64	0.35	5.20	0.35	5.43	0.35	0.6911

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
SFA: Saturated Fatty Acids; UFA: Unsaturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; PUFA/SFA: PUFA to SFA ratio; n-6: omega 6 fatty acids; n-3: omega 3 fatty acids. n-6/n-3: omega 6 to omega 3 ratio.
SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).
[1]: ratios were calculated as the average of the ratios for each observation.

Table 3.22. Fatty acid composition (%) by packaging treatment and retail display time averaged over production system for blocks 2 and 3.

d 0	VP		MAP/CO_2		MAP/CO		VP/AM		
Fatty acid	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
SFA	41.1	0.41	42.4	0.64	41.7	0.41	41.2	0.41	0.3596
UFA	53.7	0.43	52.5	0.61	53.2	0.43	53.9	0.43	0.3021
MUFA	47.8	0.47	46.7	0.67	47.2	0.47	47.7	0.47	0.5206
PUFA	5.91	0.18	5.81	0.25	6.05	0.18	6.14	0.18	0.6779
PUFA/SFA[1]	0.125	0.005	0.125	0.007	0.129	0.005	0.131	0.005	0.7914
n-6	3.93	0.15	3.98	0.21	4.13	0.15	4.21	0.15	0.5679
n-3	1.65	0.05	1.46	0.07	1.59	0.05	1.57	0.05	0.1242
n-6/n-3[1]	3.66	0.15	4.18	0.22	4.03	0.15	4.02	0.15	0.1616
Unknown	5.19	0.22	5.13	0.31	5.12	0.22	4.97	0.22	0.9071
d 3									
SFA	40.7	0.41	40.9	0.64	41.4	0.40	40.0	0.42	0.1193
UFA	53.9	0.43	53.5	0.61	53.2	0.42	54.5	0.44	0.2046
MUFA	47.7	0.47	46.9	0.67	47.1	0.46	48.8	0.49	0.0546
PUFA	6.20	0.18	6.52	0.25	6.15	0.17	5.75	0.18	0.0881
PUFA/SFA[1]	0.131	0.005	0.140	0.007	0.132	0.005	0.119	0.005	0.0558
n-6	4.34[a]	0.15	4.63[a]	0.21	4.29[ab]	0.15	3.89[b]	0.16	0.0325
n-3	1.51	0.05	1.53	0.07	1.55	0.05	1.52	0.05	0.9556
n-6/n-3[1]	4.36[a]	0.15	4.60[a]	0.21	4.21[ab]	0.15	3.88[b]	0.16	0.0338
Unknown	5.41	0.22	5.70	0.31	5.41	0.21	5.56	0.23	0.8466
d 6									
SFA	41.3[ab]	0.41	42.4[a]	0.64	40.6[bc]	0.41	39.8[c]	0.41	0.0029
UFA	53.4[bc]	0.43	52.2[c]	0.61	54.3[ab]	0.43	54.9[a]	0.43	0.0015
MUFA	46.9[b]	0.47	46.1[b]	0.67	48.5[a]	0.47	48.5[a]	0.47	0.0022
PUFA	6.54[a]	0.18	6.10[ab]	0.25	5.78[b]	0.18	6.42[a]	0.18	0.0149
PUFA/SFA[1]	0.140[a]	0.005	0.134[ab]	0.007	0.121[b]	0.005	0.134[ab]	0.005	0.0286
n-6	4.62[a]	0.15	4.37[ab]	0.21	3.87[b]	0.15	4.50[a]	0.15	0.0037
n-3	1.55	0.05	1.45	0.07	1.58	0.05	1.58	0.05	0.4033
n-6/n-3[1]	4.54[a]	0.15	4.57[a]	0.22	3.79[b]	0.15	4.34[a]	0.15	0.0021
Unknown	5.31	0.22	5.43	0.31	5.12	0.22	5.31	0.22	0.8520

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film. SFA: Saturated Fatty Acids; UFA: Unsaturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; PUFA/SFA: PUFA to SFA ratio; n-6: omega 6 fatty acids; n-3: omega 3 fatty acids; n-6/n-3: omega 6 to omega 3 ratio.

SEM: standard error of the mean.

[a-c]: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

[1]: ratios were calculated as the average of the ratios that were computed for each observation.

Table 3.23. Fatty acid composition (%) by country of production and retail display time averaged over packaging for block 1.

d 0	UR		US		
Fatty acid	LSMean	SEM	LSMean	SEM	*P*-value
SFA	40.7[b]	0.30	42.2[a]	0.30	0.0008
UFA	53.3	0.31	52.8	0.31	0.2735
MUFA	44.9[b]	0.31	47.5[a]	0.31	<0.0001
PUFA	8.37[a]	0.19	5.35[b]	0.19	<0.0001
PUFA/SFA[1]	0.187[a]	0.005	0.114[b]	0.005	<0.0001
n-6	5.11[a]	0.18	4.53[b]	0.18	0.0224
n-3	2.83[a]	0.04	0.63[b]	0.04	<0.0001
n-6/n-3[1]	1.83[b]	0.20	7.15[a]	0.20	<0.0001
Unknown	6.00[a]	0.24	5.02[b]	0.24	0.0053
d 3					
SFA	41.2[b]	0.30	42.4[a]	0.30	0.0037
UFA	52.8	0.31	52.2	0.31	0.1570
MUFA	45.3[b]	0.31	47.5[a]	0.31	<0.0001
PUFA	7.46[a]	0.19	4.60[b]	0.19	<0.0001
PUFA/SFA[1]	0.165[a]	0.005	0.097[b]	0.005	<0.0001
n-6	4.48[a]	0.18	3.81[b]	0.18	0.0084
n-3	2.49[a]	0.04	0.61[b]	0.04	<0.0001
n-6/n-3[1]	1.80[b]	0.20	6.30[a]	0.20	<0.0001
Unknown	6.07	0.24	5.43	0.24	0.0679
d 6					
SFA	39.8[b]	0.30	41.2[a]	0.30	0.0009
UFA	54.4	0.31	53.6	0.31	0.0521
MUFA	46.0[b]	0.31	48.5[a]	0.31	<0.0001
PUFA	8.45[a]	0.19	5.06[b]	0.19	<0.0001
PUFA/SFA[1]	0.185[a]	0.005	0.105[b]	0.005	<0.0001
n-6	5.33[a]	0.18	4.27[b]	0.18	<0.0001
n-3	2.65[a]	0.04	0.61[b]	0.04	<0.0001
n-6/n-3[1]	2.02[b]	0.20	6.96[a]	0.20	<0.0001
Unknown	5.80	0.24	5.21	0.24	0.0934

UR: Uruguayan samples; US: United States samples; SFA: Saturated Fatty Acids; UFA: Unsaturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; PUFA/SFA: PUFA to SFA ratio; n-6: omega 6 fatty acids; n-3: omega 3 fatty acids; n-6/n-3: omega 6 to omega 3 ratio.
SEM: standard error of the mean.
[a,b]: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).
[1]: ratios were calculated as the average of the ratios that were computed for each observation.

Table 3.24. Fatty acid composition (%) by country of production and retail display time averaged over packaging for blocks 2 and 3.

d 0	UR		US		
Fatty acid	LSMean	SEM	LSMean	SEM	*P*-value
SFA	41.0[b]	0.33	42.2[a]	0.33	0.0073
UFA	53.8	0.34	52.9	0.34	0.0575
MUFA	46.3[b]	0.37	48.4[a]	0.37	<0.0001
PUFA	7.52[a]	0.14	4.43[b]	0.14	<0.0001
PUFA/SFA[1]	0.163[a]	0.004	0.092[b]	0.004	<0.0001
n-6	4.48[a]	0.12	3.64[b]	0.12	<0.0001
n-3	2.54[a]	0.04	0.60[b]	0.04	<0.0001
n-6/n-3[1]	1.78[b]	0.12	6.16[a]	0.12	<0.0001
Unknown	5.26	0.17	4.94	0.17	0.1885
d 3					
SFA	40.1[b]	0.33	41.3[a]	0.33	0.0114
UFA	54.2	0.34	53.3	0.34	0.0541
MUFA	46.6[b]	0.37	48.6[a]	0.37	0.0002
PUFA	7.62[a]	0.14	4.69[b]	0.14	<0.0001
PUFA/SFA[1]	0.164[a]	0.004	0.097[b]	0.004	<0.0001
n-6	4.67[a]	0.12	3.91[b]	0.12	<0.0001
n-3	2.46[a]	0.04	0.59[b]	0.04	<0.0001
n-6/n-3[1]	1.90[b]	0.12	6.63[a]	0.12	<0.0001
Unknown	5.64	0.17	5.40	0.17	0.3319
d 6					
SFA	40.4[b]	0.33	41.6[a]	0.33	0.0134
UFA	54.2	0.34	53.2	0.34	0.0586
MUFA	46.4[b]	0.37	48.6[a]	0.37	<0.0001
PUFA	7.74[a]	0.14	4.68[b]	0.14	<0.0001
PUFA/SFA[1]	0.168[a]	0.004	0.097[b]	0.004	<0.0001
n-6	4.78[a]	0.12	3.91[b]	0.12	<0.0001
n-3	2.50[a]	0.04	0.59[b]	0.04	<0.0001
n-6/n-3[1]	1.92[b]	0.12	6.69[a]	0.12	<0.0001
Unknown	5.41	0.17	5.18	0.17	0.3503

UR: Uruguayan samples; US: United States samples; SFA: Saturated Fatty Acids; UFA: Unsaturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; PUFA/SFA: PUFA to SFA ratio; n-6: omega 6 fatty acids; n-3: omega 3 fatty acids; n-6/n-3: omega 6 to omega 3 ratio.
SEM: standard error of the mean.
[a,b]: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).
[1]: ratios were calculated as the average of the ratios that were computed for each observation.

Table 3.25. Effect of packaging, production system and its interaction on odor attributes (15 cm. line).

Item		Oxidized	Sour	Putrid
P-values				
Block 1				
Packaging		0.3133	0.4680	0.4205
Production system (PS)		0.0139	0.0013	0.8859
	UR	$1.64^{a} \pm 0.01$	$0.47^{b} \pm 0.38$	-
	US	$0.73^{b} \pm 0.01$	$3.10^{a} \pm 0.38$	-
Packaging x PS		0.8281	0.6676	0.5826
Blocks 2 & 3				
Packaging		0.7762	0.5396	0.5987
Production system		0.1437	0.3310	0.3087
Packaging x PS		0.0888	0.1067	0.9149

UR: Uruguayan samples; US: United States samples.
^{a,b}: Least squares means within a column without a common superscript differ ($P < 0.05$).

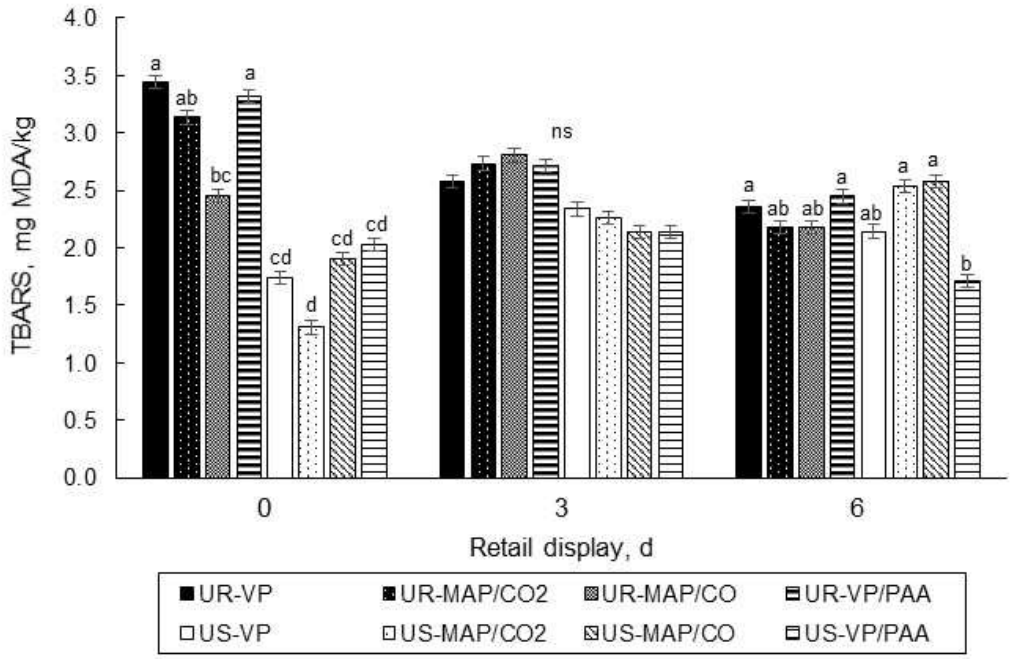

Figure 3.6. Thiobarbituric acid reactive substances (TBARS) values during 6 d of simulated retail display for UR and US strip loin steaks by packaging treatment for block 1. VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid. At each time point least squares means without a common superscript differ ($P < 0.05$). Ns: not significant.

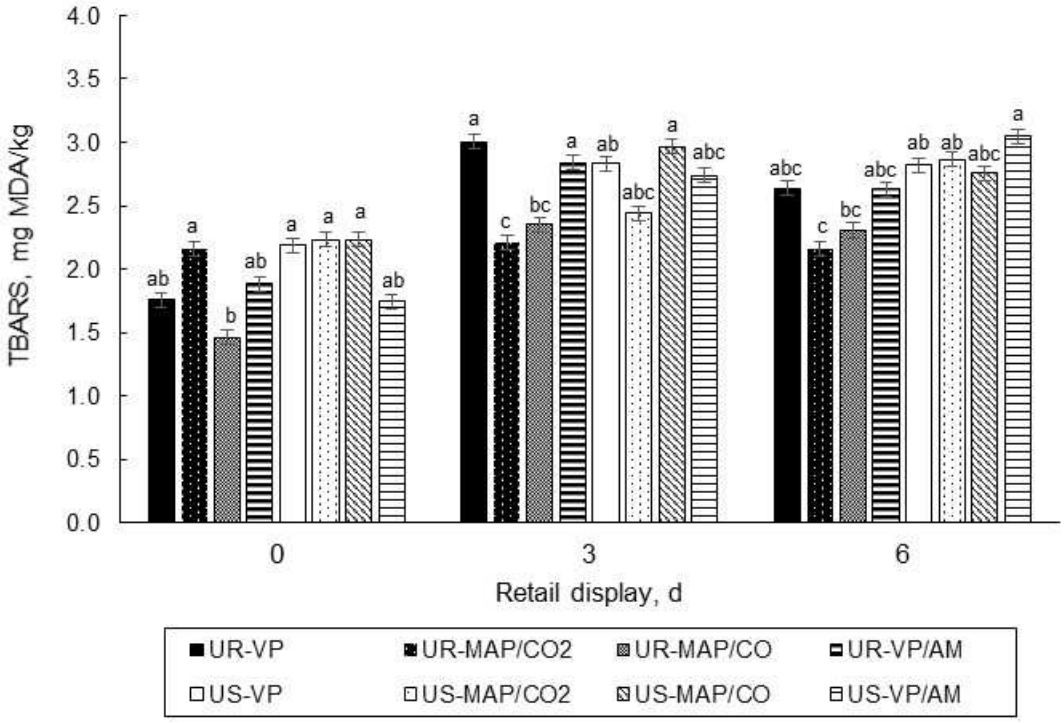

Figure 3.7. Thiobarbituric acid reactive substances (TBARS) values during 6 d of simulated retail display for UR and US strip loin steaks by packaging treatment for blocks 2 and 3. VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film. At each time point least squares means without a common superscript differ ($P < 0.05$).

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APPENDIX A

Panelist: _____ **Panel Date & Time :** _____

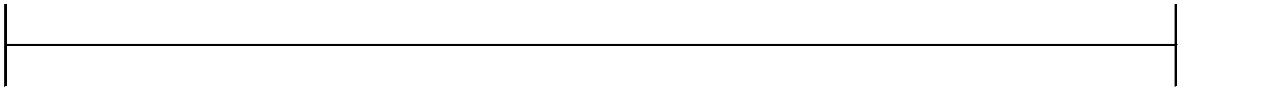
Sample ID:

APPENDIX B

Panelist: _____ **Panel Date & Time :** _____

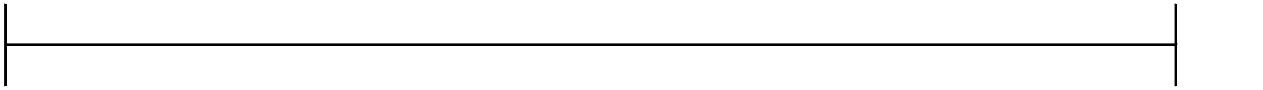
Sample ID: _____

Oxidized



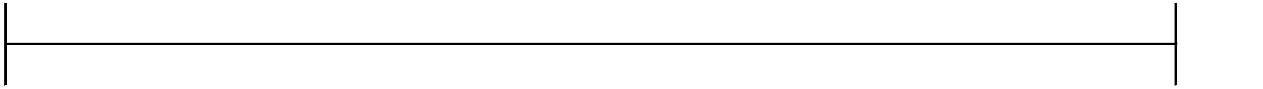
no presence very strong presence

Putrid



no presence very strong presence

Sour



no presence very strong presence

APPENDIX C

Table C.1. Effect of packaging treatments in UR samples on muscle lightness (L^*) during retail display for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	28.8	1.0	28.8	1.0	27.9	1.0	29.7	1.0	0.4491
d 2	28.6	0.9	28.6	0.9	27.9	0.9	29.4	0.9	0.5825
d 4	28.4	0.9	28.4	0.9	28.0	0.9	29.0	0.9	0.7877
d 6	28.2	1.0	28.2	1.0	28.0	1.0	28.7	1.0	0.9439

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
SE: standard error the mean.
LSMean: Least squares means.

Table C.2. Effect of packaging treatments in US samples on muscle lightness (L^*) during retail display for block 1.

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	32.3	1.0	33.3	1.0	33.8	1.0	32.6	1.0	0.5225
d 2	32.2	0.9	33.0	0.9	33.2	0.9	32.5	0.9	0.6884
d 4	32.0	0.9	32.7	0.9	32.6	0.9	32.4	0.9	0.8689
d 6	31.8	1.0	32.4	1.0	32.1	1.0	32.2	1.0	0.9419

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
SE: standard error the mean.
LSMean: Least squares means.

Table C.3. Effect of packaging treatments in UR samples on muscle lightness (L^*) during retail display for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	30.7	0.8	30.4	1.2	32.0	0.8	31.4	0.8	0.5962
d 2	30.5	0.6	30.8	0.8	31.9	0.6	31.2	0.6	0.2914
d 4	30.2	0.6	31.3	0.8	31.8	0.6	31.1	0.6	0.2221
d 6	30.0	0.8	31.7	1.2	31.6	0.8	30.9	0.8	0.4443

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.
SE: standard error the mean.
LSMean: Least squares means.

Table C.4. Effect of packaging treatments in US samples on muscle lightness (L^*) during retail display for blocks 2 and 3.

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	32.8	0.8	34.0	1.2	34.6	0.8	33.9	0.9	0.3923
d 2	32.6	0.6	34.0	0.8	34.1	0.6	33.5	0.6	0.1702
d 4	32.4	0.6	33.9	0.8	33.5	0.6	33.0	0.6	0.2900
d 6	32.3	0.8	33.9	1.2	32.9	0.8	32.6	0.9	0.6766

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.
SEM: standard error the mean.
LSMean: Least squares means.

APPENDIX D

Table D.1. Effect of packaging treatments and country of production on subcutaneous fat lightness (L^*) during retail display for block 1.

	URUGUAY								UNITED STATES								
	VP		MAP/CO_2		MAP/CO		VP/PAA		VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	*P*-value
d 0	51.9	1.7	54.1	1.7	54.8	1.7	53.0	1.7	55.9	1.7	58.7	1.7	56.4	1.8	56.6	1.7	0.1657
d 2	54.1	1.5	56.0	1.5	56.5	1.5	53.7	1.5	56.4	1.5	58.4	1.5	58.2	1.5	58.4	1.5	0.1738
d 4	56.3	1.5	58.0	1.5	58.2	1.5	54.4	1.5	56.9	1.5	58.2	1.5	60.1	1.5	60.1	1.5	0.1416
d 6	58.5	1.7	59.9	1.7	59.9	1.7	55.0	1.7	57.4	1.7	57.9	1.7	61.9	1.7	61.9	1.7	0.1016

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
SE: standard error the mean.
LSMean: Least squares means.

Table D.2. Effect of packaging treatments and country of production on subcutaneous fat lightness (L^*) during retail display for blocks 2 and 3.

	URUGUAY								UNITED STATES								
	VP		MAP/CO_2		MAP/CO		VP/AM		VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	*P*-value
d 0	62.8	1.1	63.8	1.5	63.1	1.1	61.4	1.1	64.5	1.0	61.3	1.5	64.5	1.1	64.6	1.1	0.1825
d 2	63.0	0.9	64.1	1.3	63.4	0.9	61.9	0.9	65.1	0.9	61.8	1.3	64.4	0.9	64.7	0.9	0.0909
d 4	63.3	0.9	64.3	1.3	63.7	0.9	62.4	0.9	65.6	0.9	62.3	1.3	64.3	0.9	64.8	0.9	0.1208
d 6	63.5	1.1	64.5	1.5	64.0	1.1	62.9	1.1	66.2	1.0	62.8	1.5	64.3	1.1	64.9	1.1	0.3268

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.
SE: standard error the mean.
LSMean: Least squares means.

Table D.3. Effect of packaging treatments and country of production on subcutaneous fat redness (a^*) during retail display for block 1.

	URUGUAY								UNITED STATES								
	VP		MAP/CO_2		MAP/CO		VP/PAA		VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	*P*-value
d 0	5.5^{bc}	0.4	4.9^{cd}	0.4	6.0^{b}	0.4	7.5^{a}	0.4	5.1^{bc}	0.4	3.9^{d}	0.4	5.7^{bc}	0.4	5.0^{bc}	0.4	<0.0001
d 2	4.4^{bc}	0.3	3.7^{de}	0.3	4.6^{b}	0.3	5.9^{a}	0.3	4.0^{bcd}	0.3	3.2^{e}	0.3	4.6^{b}	0.3	3.9^{cd}	0.3	<0.0001
d 4	3.3^{bc}	0.3	2.5^{d}	0.3	3.1^{bc}	0.3	4.4^{a}	0.3	2.9^{cd}	0.3	2.5^{cd}	0.3	3.5^{ab}	0.3	2.8^{cd}	0.3	<0.0001
d 6	2.2	0.4	1.3	0.4	1.7	0.4	2.8	0.4	1.8	0.4	1.7	0.4	2.4	0.4	1.8	0.4	0.0800

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
SE: standard error the mean.
$^{a-e}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table D.4. Effect of packaging treatments and country of production on subcutaneous fat redness (a^*) during retail display for blocks 2 and 3.

	URUGUAY								UNITED STATES								
	VP		MAP/CO_2		MAP/CO		VP/AM		VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	*P*-value
d 0	5.7^{bcd}	1.0	5.1^{d}	1.1	8.6^{a}	1.0	6.4^{bc}	1.0	4.8^{d}	1.0	7.4^{ab}	1.1	6.9^{b}	1.0	5.6^{cd}	1.0	<0.0001
d 2	5.4^{bc}	1.0	5.1^{bcd}	1.1	6.6^{a}	1.0	5.6^{ab}	1.0	4.2^{d}	1.0	4.1^{d}	1.1	4.8^{bcd}	1.0	4.3^{cd}	1.0	0.0054
d 4	4.3^{ab}	1.0	4.4^{ab}	1.1	4.8^{a}	1.0	4.2^{ab}	1.0	3.4^{b}	1.0	1.8^{c}	1.1	3.0^{bc}	1.0	3.1^{bc}	1.0	0.0052
d 6	2.3	1.0	3.0	1.1	3.1	1.0	2.5	1.0	2.3	1.0	0.3	1.1	1.5	1.0	2.0	1.0	0.0612

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

SE: standard error the mean.

$^{a-d}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table D.5. Effect of packaging treatments and country of production on subcutaneous fat yellowness (b^*) during retail display for block 1.

	URUGUAY								UNITED STATES								
	VP		MAP/CO_2		MAP/CO		VP/PAA		VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	*P*-value
d 0	13.1^{b}	0.5	12.6^{bc}	0.5	13.4^{b}	0.5	15.9^{a}	0.5	12.3^{bc}	0.5	11.1^{c}	0.5	12.8^{b}	0.5	13.1^{b}	0.5	<0.0001
d 2	12.7^{bc}	0.4	12.0^{cde}	0.4	12.8^{b}	0.4	14.6^{a}	0.4	11.5^{de}	0.4	11.1^{e}	0.4	12.5^{bc}	0.4	12.2^{bcd}	0.4	<0.0001
d 4	12.2^{bcd}	0.4	11.4^{cd}	0.4	12.3^{b}	0.4	13.3^{a}	0.4	10.8^{e}	0.4	11.2^{de}	0.4	12.2^{bc}	0.4	11.3^{de}	0.4	<0.0001
d 6	11.8^{ab}	0.5	10.9^{abc}	0.5	11.7^{ab}	0.5	12.0^{a}	0.5	10.1^{c}	0.5	11.3^{abc}	0.5	11.9^{a}	0.5	10.4^{bc}	0.5	0.0412

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.

SE: standard error the mean.

$^{a-e}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table D.6. Effect of packaging treatments and country of production on subcutaneous fat yellowness (b^*) during retail display for blocks 2 and 3.

	URUGUAY								UNITED STATES								
	VP		MAP/CO_2		MAP/CO		VP/AM		VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	LSMean	SE	*P*-value
d 0	15.5^{bc}	0.7	15.1^{bcd}	1.0	17.3^{a}	0.7	15.9^{ab}	0.7	13.0^{d}	0.7	16.5^{ab}	1.0	14.6^{bcd}	0.7	13.9^{cd}	0.7	0.0003
d 2	14.8^{ab}	0.7	14.1^{bc}	0.9	16.1^{a}	0.7	15.0^{ab}	0.7	12.5^{c}	0.6	14.4^{ebc}	0.9	13.7^{bc}	0.7	12.7^{c}	0.7	0.0018
d 4	14.1^{ab}	0.7	13.1^{abc}	0.9	14.9^{a}	0.7	14.1^{ab}	0.7	12.0^{c}	0.6	12.4^{bc}	0.9	12.8^{bc}	0.7	11.5^{c}	0.7	0.0040
d 6	13.5^{ab}	0.7	12.2^{abc}	1.0	13.7^{a}	0.7	13.2^{ab}	0.7	11.5^{c}	0.7	10.4^{c}	1.0	11.9^{bc}	0.7	10.3^{c}	0.7	0.0027

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

SE: standard error the mean.

$^{a-d}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

APPENDIX E

Table E.1. Effect of packaging treatments in UR samples on redness during retail display for block 1 (15 cm. line).

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	10.7^{b}	0.3	10.4^{b}	0.3	11.7^{a}	0.3	10.9^{ab}	0.3	0.0027
d 2	9.5^{b}	0.2	9.4^{b}	0.2	10.7^{a}	0.2	9.6^{b}	0.2	<0.0001
d 4	8.4^{b}	0.3	8.5^{b}	0.3	9.7^{a}	0.3	8.3^{b}	0.3	<0.0001
d 6	7.2^{b}	0.3	7.6^{ab}	0.3	8.6^{a}	0.3	7.0^{b}	0.3	0.0003

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid. SEM: standard error the mean.

^{a,b}: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table E.2. Effect of packaging treatments in US samples on redness during retail display for block 1 (15 cm. line).

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	11.5^{a}	0.3	9.9^{b}	0.3	11.8^{a}	0.3	11.3^{a}	0.3	<0.0001
d 2	10.1^{ab}	0.2	8.9^{c}	0.2	10.6^{a}	0.2	9.9^{b}	0.2	<0.0001
d 4	8.7^{b}	0.3	7.9^{c}	0.3	9.4^{a}	0.3	8.5^{bc}	0.3	<0.0001
d 6	7.3^{ab}	0.3	6.9^{b}	0.3	8.2^{a}	0.3	7.2^{ab}	0.3	0.0113

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid. SEM: standard error the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table E.3. Effect of packaging treatments in UR samples on redness during retail display for blocks 2 and 3 (15 cm. line).

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	10.1^{b}	0.1	10.9^{a}	0.2	11.3^{a}	0.1	10.3^{b}	0.1	<0.0001
d 2	8.9^{b}	0.2	10.3^{a}	0.2	10.5^{a}	0.2	9.1^{b}	0.2	<0.0001
d 4	7.7^{b}	0.2	9.7^{a}	0.3	9.8^{a}	0.2	8.0^{b}	0.2	<0.0001
d 6	6.4^{b}	0.3	9.2^{a}	0.4	9.0^{a}	0.3	6.9^{b}	0.3	<0.0001

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

SEM: standard error the mean.

^{a,b}: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table E.4. Effect of packaging treatments in US samples on redness during retail display for blocks 2 and 3 (15 cm. line).

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	11.1^{a}	0.1	10.2^{b}	0.2	11.5^{a}	0.1	11.1^{b}	0.1	<0.0001
d 2	10.2^{a}	0.2	8.2^{b}	0.2	10.3^{a}	0.2	10.1^{a}	0.2	<0.0001
d 4	9.2^{a}	0.2	6.2^{b}	0.3	9.0^{a}	0.2	9.1^{a}	0.2	<0.0001
d 6	8.3^{a}	0.3	4.2^{b}	0.4	7.7^{a}	0.3	8.1^{a}	0.3	<0.0001

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

SEM: standard error the mean.

^{a,b}: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

APPENDIX F

Table F.1. Effect of packaging treatments in UR samples on brightness during retail display for block 1 (15 cm. line).

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	10.0^{b}	0.3	9.7^{b}	0.3	11.2^{a}	0.3	9.9^{b}	0.3	<0.0001
d 2	9.3^{b}	0.2	9.2^{b}	0.2	10.5^{a}	0.2	9.2^{b}	0.2	<0.0001
d 4	8.6^{b}	0.2	8.7^{b}	0.2	9.8^{a}	0.2	8.4^{b}	0.2	<0.0001
d 6	8.0^{b}	0.3	8.1^{b}	0.3	9.1^{a}	0.3	7.7^{b}	0.3	0.0015

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
SEM: standard error the mean.
^{a,b}: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table F.2. Effect of packaging treatments in US samples on brightness during retail display for block 1 (15 cm. line).

	VP		MAP/CO_2		MAP/CO		VP/PAA		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	10.4^{a}	0.3	8.7^{b}	0.3	10.9^{a}	0.3	10.2^{a}	0.3	<0.0001
d 2	9.3^{b}	0.2	8.1^{c}	0.2	10.0^{a}	0.2	9.1^{b}	0.2	<0.0001
d 4	8.3^{b}	0.2	7.6^{c}	0.2	9.1^{a}	0.2	7.9^{bc}	0.2	<0.0001
d 6	7.2^{b}	0.3	7.0^{b}	0.3	8.3^{a}	0.3	6.8^{b}	0.3	0.0007

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/PAA: vacuum packaging plus an application of peroxyacetic acid.
SEM: standard error the mean.
$^{a-c}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table F.3. Effect of packaging treatments in UR samples on brightness during retail display for blocks 2 and 3 (15 cm. line).

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	10.3^{c}	0.1	10.8^{b}	0.2	11.3^{a}	0.1	10.5^{bc}	0.1	<0.0001
d 2	9.2^{b}	0.1	10.2^{a}	0.2	10.5^{a}	0.1	9.4^{b}	0.1	<0.0001
d 4	8.0^{b}	0.2	9.6^{a}	0.3	9.7^{a}	0.2	8.3^{b}	0.2	<0.0001
d 6	6.9^{b}	0.2	9.0^{a}	0.3	8.8^{a}	0.2	7.2^{b}	0.2	<0.0001

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

SEM: standard error the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).

Table F.4. Effect of packaging treatments in US samples on brightness during retail display for blocks 2 and 3 (15 cm. line).

	VP		MAP/CO_2		MAP/CO		VP/AM		
Time	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
d 0	11.1^{b}	0.1	10.4^{c}	0.2	11.6^{a}	0.1	11.1^{b}	0.1	<0.0001
d 2	10.1^{a}	0.1	8.6^{b}	0.2	10.3^{a}	0.1	10.1^{a}	0.1	<0.0001
d 4	9.1^{a}	0.2	6.9^{b}	0.3	9.0^{a}	0.2	9.0^{a}	0.2	<0.0001
d 6	8.1^{a}	0.2	5.0^{b}	0.3	7.7^{a}	0.2	8.0^{a}	0.2	<0.0001

VP: vacuum packaging; MAP/CO_2: low oxygen modified atmosphere packaging with carbon dioxide (80% N_2 and 20% CO_2); MAP/CO: low oxygen modified atmosphere packaging with carbon dioxide and carbon monoxide (80% N_2, 19.6% CO_2, and 0.4% CO); VP/AM: vacuum packaging with an antimicrobial agent incorporated into the film.

SEM: standard error the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript differ ($P < 0.05$).