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Effect of packaging during storage time on retail display microbial population of beef strip loins from two different production systems[1]

S. Luzardo,* D. R. Woerner,* I. Geornaras,* A. M. Hess,† and K. E. Belk*[2]

*Center for Meat Safety and Quality, Colorado State University, Fort Collins 80523-1171; and †Department of Statistics, Colorado State University, Fort Collins 80523-1877

ABSTRACT: Two studies were conducted 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 a high-concentrate diet (United States; US) on retail display life microbial growth. Four or 3 different packaging treatments were applied to UR and US strip loin roasts or steaks during 35 d of storage; treatments were applied 7 d following slaughter. After 35 d of storage, the samples were evaluated during simulated retail display for up to 6 d. In Exp. 1, the treatments were vacuum packaging (VP), low-oxygen modified atmosphere packaging (MAP) with N_2 and CO_2 (MAP/CO_2), low-oxygen MAP with N_2 plus CO_2 and CO, and VP plus an application of peroxyacetic acid (VP/PAA). In Exp. 2, block 1, the treatments were VP, MAP/CO, and VP with ethyl-*N*-lauroyl-L-arginate HCl incorporated into the film as an antimicrobial agent (VP/AM). In Exp. 2, block 2, the treatments were VP, MAP/CO_2, MAP/CO, and VP/AM. For retail display, VP treatments were sliced and repackaged in PVC overwrap, and MAP treatments were actually PVC overwrap trays that were removed from a master bag with the prescribed gas treatment. Regardless of production system and packaging treatment, mesophilic and psychrotrophic counts of 6.9 to 7.8 and 6.7 to 7.7 log10 CFU/cm^2, respectively, were obtained at the end of retail display, except for US samples in Exp. 2 (5.5 to 6.3 $\log_{10}$ CFU/cm^2). No differences ($P > 0.05$) were detected for *Pseudomonas* spp. counts among packaging treatments in US steaks at the end of the display time in Exp.1, whereas, for UR steaks, both MAP treatments had lower ($P < 0.05$) *Pseudomonas* spp. counts than VP treatments. *Pseudomonas* spp. counts were lower ($P < 0.05$) in the MAP/CO_2 treatment than in the other 3 treatments in US samples on d 6 of retail display for Exp. 2. At the end of display time and for Exp. 1, US steaks under MAP/CO had greater ($P < 0.05$) lactic acid bacteria (LAB) counts than samples in both VP treatments; no differences ($P > 0.05$) among packaging were detected for UR steaks. Both MAP and VP/AM treatments in the US samples for Exp. 2 had lower ($P < 0.05$) LAB counts on d 6 of display than the VP treatment, but no differences ($P > 0.05$) were found among packaging treatments for the UR samples. To maximize shelf life (storage and display life) of exported fresh beef, it is critical to minimize bacterial populations during processing and storage.

Key words: beef, microbial growth, packaging, production system, strip loin

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INTRODUCTION

The growing demand for foods around the world along with a globalized international market has led to an increased interest to extend the shelf life of food products. This is particularly important for fresh meat, which has been recognized as a highly perishable food product because of its biological composition (Lambert et al., 1991). Many factors, alone or in combination, such as atmospheric oxygen, moisture, endogenous enzymes, temperature, light, and, particularly, microorganisms, have a detrimental effect on meat quality (Lambert et al., 1991). There are 3 main mechanisms for meat spoilage after slaughtering and during processing and storage: 1) microbial spoilage, 2) lipid and pigment oxidation, and 3) autolytic enzymatic spoil-

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[2]Corresponding author: keith.belk@colostate.edu

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age. 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). The use of packaging systems represents a key tool to extend the shelf life of fresh meat.

Vacuum packaging (**VP**) preserves meat by maintaining an oxygen-depleted atmosphere that inhibits potent spoilage bacteria in normal pH meat under optimum VP conditions (Jeremiah, 2001). Modified atmosphere packaging (**MAP**) has been used to extend most of the shelf life properties of fresh meat, but anoxic forms of MAP without carbon monoxide do not provide bloomed red meat color, and MAP with O_2 may promote oxidation of lipids and pigments (McMillin, 2008). Active packaging with regard to antimicrobial films is also considered a promising technology to improve shelf life and food safety of fresh meat (Zhou et al., 2010).

Therefore, the objective of this study was to evaluate the influence of packaging during storage time of strip loins from steers fattened on grazing systems (Uruguay; **UR**) or a high-concentrate diet (United States; **US**) on retail display shelf life microbial growth.

MATERIALS AND METHODS

Slaughter and Carcass Sampling

Experiments were repeated 3 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 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*), and 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 on the basis of dentition (2 to 4 permanent incisors), and the HCW were between 250 and 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 "INACUR," 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 10th rib to the lumbar-sacral junction. After fabrication strip loins were vacuum packaged, properly boxed, and maintained under refrigerated conditions during air shipment to the US.

On the same day on which Uruguayan strip loins were fabricated, 20 strip loins (Institutional Meat Purchasing Specifications #180) from the left and right sides of each of 10 carcasses were collected in a federally inspected US meat packing plant and remained vacuum packaged under refrigerated conditions 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 loin 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 4 packaging treatments were evaluated within both production systems (UR and US) for each block. For 2 of the treatments (MAP treatments), strip loins were fabricated into 2.54-cm-thick steaks, and for the other 2 treatments (VP treatments) a 7.5-cm-thick roast from the strip loins was used.

Because of different strip loin fabrication procedures between countries, both strip loins from US carcasses were used, and just the right strip loins from UR carcasses were collected. The UR strip loins were longer than US strip loins, allowing for the application of the 4 packaging treatments in just 1 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, 3 different retail display times (0, 3, and 6 d) were randomly allotted.

In Exp. 1, the 4 packaging treatments were as follows: Treatment 1 was 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 for 24 h at 4.4°C and 0% relative humidity [**RH**] and moisture vapor transmission rate [**MVTR**] of 0.45 g/645.2 cm^2 for 24 h at 37.8°C and 100% RH; Cryovac Sealed Air Corp., Duncan, SC). Treatment 2 was low-O_2 MAP with 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.) overwrapped with polyvinyl chloride film (MAPAC DBL-TP film; OTR of 18,600 mL/m^2 for 24 h and MVTR of 28 g/645.2 cm^{2}for 24 h at 37.8°C and 90% RH;

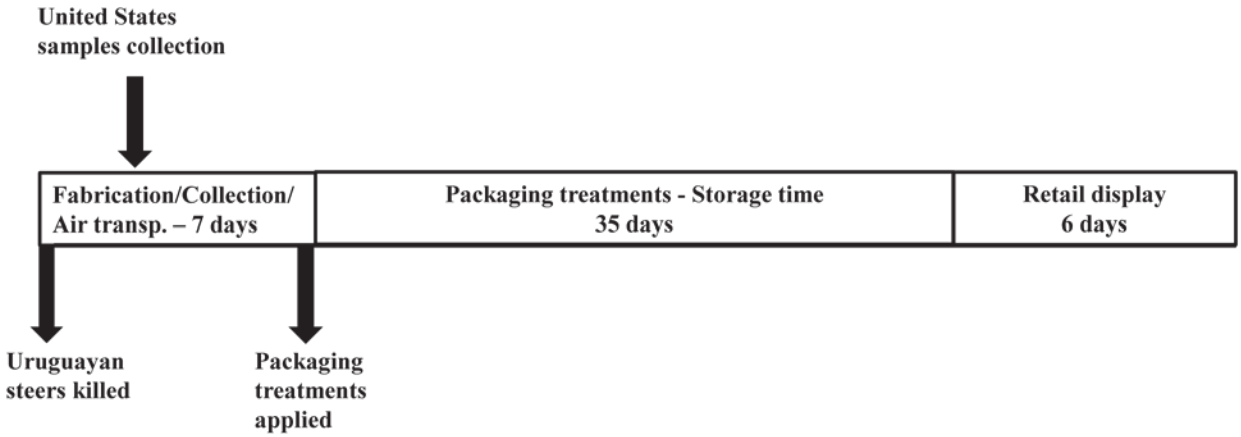

Figure 1. Chronological events from slaughter to the end of retail display.

Resinite Packaging Film, AEP Industries Inc., Griffin, GA). Master bags (PM9120B, 2.0 mils; OTR of 5.3 mL/m^2 for 24 h at 23°C and 0% RH and MVTR of 9.5 g/m^2 for 24 h at 38°C and 90% RH; Cryovac Sealed Air Corp.) containing the trays were flushed with an 80% CO_2 and 20% N_2 gas mixture using a gas-flush packaging machine (Corr-Vac Mark III; M-Tek Inc., Elgin, IL). Treatment 3 was 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. Master bags containing the trays were flushed with an 80% N_2, 19.6% CO_2, and 0.4% CO gas mixture. Treatment 4 was VP plus peroxyacetic acid (**VP/PAA**) applied to a 7.5-cm-thick strip loin piece. Before VP (Multivac C500; Multivac Inc.), 28 to 30 mL of an 80 ul/l 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 1 O_2 scavenger (FreshPax CR20, Multisorb Tecnologies Inc., Buffalo, NY) was used for the MAP/CO treatment, according to the manufacturer's recommendations.

In Exp. 2, block 1, just 3 treatments were evaluated: VP, MAP/CO, and VP (B2620 bag; OTR of 3 to 6 mL/m^2 for 24 h at 4.4°C and 0% RH and MVTR of 0.5 to 0.6 g/645.2 cm^2 for 24 h at 37.8°C and 100% RH; Cryovac Sealed Air Corp.) with ethyl-*N*-lauroyl-L-arginate HCl (**LAE**) incorporated into the film as an antimicrobial agent (**VP/AM**). In Exp. 2, block 2, 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. 1). After storage, the master bags from the MAP/CO_2 and MAP/CO treatments were opened, and samples for d 0 of retail display were taken for corresponding measurements, and then the individual trays were placed in a multideck retail display case (Hussman, model M3X8GEP' Hussman, Bridgeton, MO) set at 2°C (±1°C) for up to 6 d. Additionally, the 7.5-cm-thick strip loin roasts from the VP, VP/PAA (Exp. 1), and VP/AM (Exp. 2) 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). The retail display case was equipped with light-emitting diode lighting that illuminated at an average light intensity of 900 lx (±184 lx). 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 bacteria, psychrotrophic bacteria, *Pseudomonas* spp., and lactic acid bacteria (**LAB**) were performed on the last steak from the anterior end of each strip loin before packaging treatments were applied (before storage). Microbiological analyses were also performed after 35 d of storage time (d 0 of retail display) and on d 3 and 6 of the retail display periods. At each sampling time, a 4 × 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 × 1 cm cubes, and the subcutaneous fat was removed. The cubes from each steak were placed into sterilized Whirl-Pak bags (207 mL; Nasco) and were frozen at −80°C for subsequent chemical analysis. The 4 × 4 cm squares for microbial analysis were homogenized in 72 mL of Dey/Engley 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 (Difco Laboratories). Appropriate dilutions were surface plated in duplicate onto 2 sets of tryptic soy agar (Acumedia, Neogen Corp, Lansing, MI) plates, 1 set for enumeration of mesophilic 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 bacteria and *Pseudomonas*) or 7°C for 10 d (psychrotrophic bacteria). Lactic acid bacteria counts were determined using the pour plate method (Lactobacilli MRS Agar; Difco Laboratories) 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.

Statistical Analysis and Design

Experiment 1 was analyzed separately from Exp. 2 because 1 of the packaging treatments evaluated was different. In Exp. 1, our collaborator provided us with 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 in Exp. 2. Data were analyzed at d 0, 3, and 6 of display time using the MIXED procedure of SAS (version 9.3; SAS Inst. Inc., Cary, NC), which included production system, packaging treatment, and time as fixed effects and the random effect of strip loin within production system and the packaging × strip loin within production system interaction. In the analysis of the data for blocks 1 and 2 together in Exp. 2, the block also was considered a random effect. Initial bacterial counts on the last steak from the anterior end of each strip loin before application of the packaging treatments were used as a covariate for the data analysis. Studentized residual plots were evaluated to test the homogeneity of variance and normality for all data. The experimental unit was the individual steak for the MAP treatments and the 7.5-cm-thick strip loin roast 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 ($P < 0.05$). Analyses of blocks 1 and 2 in Exp. 2 were conducted together in an incomplete design (no MAP/CO_2 in block 1), and the block effect was removed from the model when it was not significant.

RESULTS AND DISCUSSION

Different antimicrobial intervention systems (for food safety control) were used in the meat packing plants in UR vs. 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 Exp. 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 bacteria, psychrotrophic bacteria, *Pseudomonas* spp., and LAB, respectively; bacteria loads 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 bacteria, psychrotrophic bacteria, *Pseudomonas* spp., and LAB, respectively. In Exp. 2, 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 bacteria, psychrotrophic bacteria, *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 et al., 2009). In Exp. 1 at the end of storage time (d 0 of retail display), mesophilic and psychrotrophic bacteria counts were lower ($P < 0.05$) in VP and VP/PAA treatments in US samples, and VP/PAA had a lower ($P < 0.05$) bacteria load than MAP treatments in UR samples (Tables 1 and 2).

Mesophilic and psychrotrophic bacteria counts were lower ($P < 0.05$) in the VP treatments than in both MAP treatments on d 0 of display (end storage time), but no significant differences ($P > 0.05$) were detected among packaging treatments on d 3 and 6 of

Table 1. Mesophilic bacteria counts (log_{10} CFU/cm^2) by country of origin, packaging treatment, and retail display time in Exp.1

	Treatment[2]								
	VP		MAP/CO_2		MAP/CO		VP/PAA		
Country[1]	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
US									
d 0	$5.4^{b,x}$	0.19	$6.4^{a,x}$	0.19	$6.6^{a,x}$	0.19	$5.3^{b,x}$	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		
UR									
d 0	$5.9^{a,b,x}$	0.19	$6.5^{a,x}$	0.19	$6.4^{a,x}$	0.19	$5.5^{b,x}$	0.19	0.0001
d 3	6.9^{y}	0.19	$6.9^{x,y}$	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		

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

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

[1]US: United States; UR: Uruguay.

[2]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.

retail display for the US samples in Exp. 1 (Tables 1 and 2). For the UR steaks in Exp. 1, no significant differences ($P > 0.05$) in mesophilic bacteria population were detected among treatments on d 3 and 6 of retail display (Table 1) and on d 6 for psychrotrophic bacteria (Table 2). However, psychrotrophic bacteria counts for the UR samples in Exp. 1 were lower ($P < 0.05$) in the VP/PAA treatment than in both MAP treatments on d 0 and 3 of display (Table 2). The results found in Exp. 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 such as those imposed by the 4 (or 3) packaging treatments, LAB growth is favored when the initial counts of spoilage bacteria are low (Gill, 1996), and LAB become the predominant microorganisms of meats (Egan, 1983). One characteristic of LAB is that they are resistant to inhibition by CO_2 (Egan, 1983), which could explain the nonbacteriostatic effect observed in both MAP treatments for Exp. 1 (Tables 1 and 2). At the end of storage time (d 0 of display) in Exp. 2, no differences ($P > 0.05$) among packaging were observed on mesophilic and psychrotrophic counts in the US samples, but both

Table 2. Psychrotrophic bacteria counts (log_{10} CFU/cm^2) by country of origin, packaging treatment, and retail display time in Exp. 1

	Treatment[2]								
	VP		MAP/CO_2		MAP/CO		VP/PAA		
Country[1]	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
US									
d 0	$5.4^{b,x}$	0.19	$6.4^{a,x}$	0.19	$6.5^{a,x}$	0.19	$5.7^{b,x}$	0.19	<0.0001
d 3	6.3^{y}	0.19	6.5^{x}	0.19	$6.9^{x,y}$	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		
UR									
d 0	$6.1^{a,b,x}$	0.19	$6.5^{a,x}$	0.19	$6.5^{a,x}$	0.19	$5.7^{b,x}$	0.19	0.0015
d 3	$6.7^{a,b,y}$	0.19	$7.0^{a,y}$	0.19	$7.1^{a,y}$	0.19	$6.4^{b,y}$	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		

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

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

[1]US: United States; UR: Uruguay.

[2]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.

Table 3. Mesophilic bacteria counts (log_{10} CFU/cm^2) by country of origin, packaging treatment, and retail display time in Exp. 2

	Treatment[2]								
	VP		MAP/CO_2		MAP/CO		VP/AM		
Country[1]	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
US									
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^{a,y}	0.15	5.0^{a,b,x,y}	0.20	4.7^{b,x}	0.15	4.9^{b,x}	0.15	0.0001
d 6	6.3^{a,z}	0.15	5.5^{b,y}	0.20	5.6^{b,y}	0.15	6.0^{a,b,y}	0.15	0.0002
P-value	<0.0001		<0.0001		<0.0001		<0.0001		
UR									
d 0	6.8^{b,x}	0.15	7.7^{a}	0.20	7.8^{a}	0.15	6.7^{b,x}	0.15	<0.0001
d 3	7.3^{b,y}	0.15	7.7^{a,b}	0.20	7.9^{a}	0.15	7.2^{b,y}	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		

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

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

[1]US: United States; UR: Uruguay.

[2]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.

VP treatments had lower ($P < 0.05$) counts than MAP treatments in UR steaks (Tables 3 and 4). In Exp. 2 and for the US samples, there was an inhibitory effect ($P < 0.05$) of CO_2 on the mesophilic and psychrotrophic bacteria counts in both MAP treatments compared to the VP treatment on d 6 of retail display, whereas packaging treatment had no effect ($P > 0.05$) in the UR steaks at the end of display (Tables 3 and 4). It is important to note that in Exp.1 for both production systems and in Exp. 2 for the UR samples, mesophilic and psychrotrophic counts at the end of the retail display period in all packaging treatments were close to or even exceeded 7 log10 CFU/cm^2, a level considered the maximum bacterial load for retail shelf life (Borch et al., 1996; Tables 1 to 4). No effect of packaging treatments at high contamination levels may be associated with the stationary phase of the growth curve reached by the 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), explaining, in part, the high bacteria counts.

In regard to LAB, US steaks in Exp. 1 under both VP treatments had lower counts ($P < 0.05$) than MAP/CO at the end of storage time (d 0), but no differences

Table 4. Psychrotrophic bacteria counts (log_{10} CFU/cm^2) by country of origin, packaging treatment, and retail display time in Exp. 2

	Treatment[2]								
	VP		MAP/CO_2		MAP/CO		VP/AM		
Country[1]	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
US									
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^{a,y}	0.14	5.1^{a,b,x}	0.19	4.9^{b,x}	0.14	5.0^{b,y}	0.14	0.0009
d 6	6.3^{a,z}	0.14	5.7^{b,c,y}	0.19	5.5^{c,y}	0.14	6.1^{a,b,z}	0.14	<0.0001
P-value	<0.0001		0.0020		<0.0001		<0.0001		
UR									
d 0	6.7^{b,x}	0.14	7.5^{a}	0.19	7.6^{a}	0.14	6.6^{b,x}	0.14	<0.0001
d 3	7.1^{b,y}	0.14	7.6^{a}	0.19	7.7^{a}	0.14	7.1^{b,y}	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		

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

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

[1]US: United States; UR: Uruguay.

[2]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.

Table 5. Lactic acid bacteria counts (log_{10} CFU/cm^2) by country of origin, packaging treatment, and retail display time in Exp. 1

	Treatment[2]								
	VP		MAP/CO_2		MAP/CO		VP/PAA		
Country[1]	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
US									
d 0	5.6^{b,x}	0.17	6.4^{a,x}	0.18	6.6^{a,x}	0.17	5.4^{b,x}	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^{b,z}	0.17	7.0^{a,b,y}	0.17	7.4^{a,y}	0.17	7.0^{b,z}	0.17	0.0153
P-value	<0.0001		0.0003		<0.0001		<0.0001		
UR									
d 0	6.0^{b,c,x}	0.17	6.4^{a,b,x}	0.17	6.5^{a,x}	0.17	5.6^{c,x}	0.17	0.0002
d 3	6.7^{a,y}	0.17	6.9^{a,y}	0.17	6.9^{a,y}	0.17	6.3^{b,y}	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		

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

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

[1]US: United States; UR: Uruguay.

[2]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.

($P > 0.05$) among packaging treatments were observed on d 3 of retail display (Table 5). For UR samples in Exp. 1, the VP/PAA treatment had lower ($P < 0.05$) LAB counts than both MAP treatments on d 0 of display and also lower ($P < 0.05$) counts than the other 3 treatments on d 3 of retail display (Table 5). At the end of display and in Exp.1, US samples under MAP/CO had greater ($P < 0.05$) LAB counts than samples under both VP treatments, but no differences ($P > 0.05$) among packaging were detected for UR samples (Table 5). In Exp. 2, no differences ($P > 0.05$) among packaging treatments were observed on LAB counts on d 0 and 3 in the US steaks. However, UR samples under both VP treatments had lower ($P < 0.05$) LAB counts than MAP treatments on d 0 of display, whereas no differences ($P > 0.05$) were found among both VP treatments and MAP/CO_2 on d 3 (Table 6). Both MAP treatments and VP/AM in the US steaks in Exp. 2 had lower ($P < 0.05$) LAB counts on d 6 of retail display than the VP treatment, but no differences ($P > 0.05$) were found among packaging treatments for the UR samples (Table 6).

Pseudomonas spp. represent 1 of the most important spoilage bacteria on refrigerated meat, mainly under aerobic conditions (Lambert et al., 1991; García de Fernando et al., 1995; Gill, 1996; Pennacchia et al., 2011) because of its greater ability to use glucose and amino acids compared with 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 log10 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. leads 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 nonproteolytic or less proteolytic bacteria (Nychas et al., 2008). Additionally, it has been documented that *Pseudomonas fluorescens* plays the main role in meat discoloration because of the increased metmyoglobin formation via increased oxygen consumption (Chan et al., 1998).

In Exp. 1, the lowest ($P < 0.05$) *Pseudomonas* spp. counts in US steaks were observed in the VP/PAA treatment on d 0 of display, but no differences ($P > 0.05$) among packaging were detected on d 3 (Table 7). For UR samples, *Pseudomonas* spp. load was lower ($P < 0.05$) in MAP/CO than in both VP treatments at the end of storage time (d 0), whereas both MAP treatments had lower ($P < 0.05$) counts than VP treatments on d 3 of display (Table 7). No differences ($P > 0.05$) were detected on *Pseudomonas* spp. counts among packaging treatments in US samples at the end of display time (d 6) in Exp. 1, whereas for UR samples, both MAP treatments had a lower ($P < 0.05$) *Pseudomonas* spp. load than VP treatments (Table 7).

In Exp. 2 and for US steaks, both MAP treatments and VP/AM had lower ($P < 0.05$) *Pseudomonas* spp. numbers than VP treatment on d 0 and 3 of retail display. *Pseudomonas* spp. counts were lower ($P < 0.05$) in the MAP/CO_2 treatment than the other 3 packag-

Table 6. Lactic acid bacteria counts (log_{10} CFU/cm^2) by country of origin, packaging treatment, and retail display time in Exp. 2

	Treatment[2]								
	VP		MAP/CO_2		MAP/CO		VP/AM		
Country[1]	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
US									
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^{a,z}	0.25	5.6^{b,y}	0.29	5.6^{bz}	0.25	5.9^{b,z}	0.25	<0.0001
P-value	<0.0001		<0.0001		<0.0001		<0.0001		
UR									
d 0	6.8^{b,x}	0.24	7.7^{a}	0.27	7.7^{a}	0.24	6.8^{b,x}	0.24	<0.0001
d 3	7.3^{b,y}	0.24	7.6^{a,b}	0.27	7.9^{a}	0.24	7.3^{b,y}	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		

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

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

[1]US: United States; UR: Uruguay.

[2]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.

ing treatments in US samples at the end of retail display in Exp. 2 (Table 8). 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 fast *Pseudomonas* spp. growth (Borch et al., 1996). For UR samples in Exp. 2, *Pseudomonas* spp. populations were lower ($P < 0.05$) in VP/AM than in the other 3 packaging treatments on d 0 and 3 of display in the UR samples. Also, steaks from UR treated with VP/AM resulted in lower ($P < 0.05$) *Pseudomonas* spp. counts than MAP/CO and VP on d 6 of retail display in Exp. 2 (Table 8).

The VP/PAA (Exp. 1) and VP/AM (Exp. 2) treatments were not effective in inhibiting bacterial growth at the end of retail display compared to the other 3 treat-

Table 7. *Pseudomonas* spp. bacteria counts (log_{10} CFU/cm^2) by country of origin, packaging treatment, and retail display time in Exp. 1

	Treatment[2]								
	VP		MAP/CO_2		MAP/CO		VP/PAA		
Country[1]	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
US									
d 0	3.8^{a,x}	0.36	4.2^{a,x}	0.36	3.5^{a,x}	0.36	2.5^{b,x}	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		
UR									
d 0	3.0^{a,x}	0.36	1.3^{b,c,x}	0.36	0.9^{c,x}	0.37	2.1^{b,x}	0.36	<0.0001
d 3	4.0^{a,y}	0.36	1.2^{c,x}	0.36	1.3^{c,x}	0.36	2.7^{b,x}	0.36	<0.0001
d 6	6.3^{a,z}	0.36	2.7^{b,y}	0.36	3.2^{b,y}	0.36	5.6^{a,y}	0.36	<0.0001
P-value	<0.0001		<0.0001		<0.0001		<0.0001		

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

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

[1]US: United States; UR: Uruguay.

[2]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.

Table 8. *Pseudomonas* spp. bacteria counts (log_{10} CFU/cm^2) by country of origin, packaging treatment, and retail display time in Exp. 2

	Treatment[2]								
	VP		MAP/CO_2		MAP/CO		VP/AM		
Country[1]	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM	*P*-value
US									
d 0	3.1^{a,x}	0.72	<1.3^{b,x}	0.76	<1.5^{b,x}	0.72	<1.9^{b,x}	0.72	<0.0001
d 3	3.7^{a,y}	0.72	<1.4^{b,x}	0.76	<1.9^{b,y}	0.72	<2.0^{b,x}	0.72	<0.0001
d 6	4.8^{a,z}	0.72	2.5^{c,y}	0.76	<3.3^{b,z}	0.72	3.3^{b,y}	0.72	<0.0001
P-value	<0.0001		<0.0001		<0.0001		<0.0001		
UR									
d 0	2.8^{b,x}	0.72	<3.3^{a,b}	0.76	3.9^{a}	0.72	2.0^{c,x}	0.72	<0.0001
d 3	3.2^{b,y}	0.72	3.6^{a,b}	0.76	<3.8^{a}	0.72	2.4^{c,y}	0.72	0.0001
d 6	4.5^{a,z}	0.72	3.4^{b,c}	0.76	4.1^{a,b}	0.72	3.1^{c,z}	0.72	<0.0001
P-value	<0.0001		0.4256		0.2222		<0.0001		

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

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

[1]US: United States; UR: Uruguay.

[2]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. Least squares means with a less than symbol (<) indicate 1 or more of the samples within the treatment had plate counts below the analysis detection limit (0.4 $log1_0$ CFU/cm^2).

ments (Tables 1 to 8). Use of a PAA solution at 80 ul/l may explain the lack of inhibitory effect on bacteria population observed in the VP/PAA treatment. The Food and Safety Inspection Service (2015) of the USDA approved the use of PAA up to a concentration of 220 ul/l. 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 count reductions between <0.5 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 piece 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 ul/l 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 d. Joerger (2007) conducted a review of 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 postprocessing safety measure. Thus, antimicrobial packaging represents a 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).

Conclusions

Even when all the treatments were the low-O_2 (or depleted O_2) packaging type, mesophilic and psychrotrophic bacteria achieved spoilage levels at the end of display time or before, particularly in the case of UR samples. At spoilage levels any packaging treatment seems to have an effect on the microbial populations. For the US steaks, low-O_2 MAP treatments had lower mesophilic and psychrotrophic bacteria counts at the end of the retail display

time in Exp. 2, when spoilage levels had still not been reached. To maximize shelf life (storage and display life) of exported fresh beef, it is critical to minimize bacterial populations during processing and storage.

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