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Evaluation of the effectiveness of non-irradiated and chlorine-free packaging for fresh beef preservation

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ABSTRACT

This study evaluates the potential of using non-irradiated barrier-shrink bags containing ethylene-vinyl alcohol copolymer (EVOH), polyamide (PA) and ethylene ionomer in their structures to preserve vacuum-packaged fresh beef as an alternative to traditional gamma-ray cross-linked bags containing polyvinylidene chloride (PVDC). Boneless beef rib eye roll cuts were vacuum-packed in an industrial processing plant using EVOH 44% mol, EVOH 32% mol and a control PVDC barrier shrink bags. The cuts were evaluated during storage at 0.5 °C. The EVOH films presented similar performance compared to control PVDC barrier shrink bags related to bacteria growth and purge loss. Packages with EVOH 32% mol film presented better performance than control bag with respect to the meat sensorial attributes, including fewer bubbles and better adhesion. EVOH 44% mol bags presented the highest rate of colour loss. The EVOH 32% mol non-irradiated and chlorine-free film is as effective for the preservation of fresh beef as traditional PVDC-irradiated shrink bags.

1. Introduction

Loss in the quality of fresh beef during storage is related to discolouration, the presence of off-odours, surface dehydration and purge formation. Beef quality and extension of shelf life can be obtained by protecting the meat against environmental factors, such as oxygen, humidity, light and microbial contamination. Vacuum packaging, in which air is removed, is a traditional packaging technology used to preserve fresh meat. The low oxygen level inside a vacuum package coupled with production of carbon dioxide, an active antimicrobial gas, inhibits the growth of aerobic microorganisms that cause spoilage, discolouration and off-odours. These conditions also encourage the growth of anaerobes that produce lactic acid (Kropf, 2004).

Among the physical properties of packaging materials, gas permeability is very important for maintaining the quality of packed meat by controlling oxygen availability for microbial growth and pigment and fat oxidation. An oxygen barrier can be obtained by combining base polymeric materials with other gas barrier resins through coextrusion (Lee, 2010).

Heat-shrinkable bags for vacuum packaging of beef are considered to favour less drip loss than packaging in non-heat-contractile bags (Aspé, Roeckel, Marti, & Jimenez, 2008; Payne, Durham, Scott, & Devine, 1998). These effects might be attributed to the fact that the shrunken film provides less space for exudate or to the more flexible and softer nature of the packaging (Payne et al., 1998). For short period of storage the benefits of package shrinkage may not be verified (Yoon & Lee, 2001). However the drip loss is affected by so many other factors in addition to package, such as meat properties (breed of the animal, cut, size, temperature, etc.), vacuum operation conditions and storage temperature as previously reported by others (Gill, 1996; Payne et al., 1998, and McMillin, 2008).

Gamma ray cross-linked barrier-shrink films composed of several layers of ethylene-vinyl acetate copolymers (EVA), polyvinylidene chloride (PVDC) and polyethylene (PE) are the most common vacuum packaging offered to the market. Notwithstanding the films acknowledged capacity to preserve fresh beef, those films cannot be easily recycled into polymer streams because they are highly cross-linked, nor can they be disposed of in energy recycling plants that lack the necessary setups to treat the dioxin compounds formed during the combustion of PVDC.

As global environmental concerns grow, many companies seek to adopt packaging with reduced environmental impact and at the same time provide safety to industry workers and consumers.

The purpose of this study was to evaluate the potential of using barrier-shrink bags containing ethylene-vinyl alcohol copolymer (EVOH), polyamide (PA) and ethylene ionomer in their structures, non-irradiated and chlorine-free, to preserve the quality of fresh beef as an

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alternative to traditional gamma ray cross-linked barrier-shrink bags containing PVDC.

2. Materials and methods

2.1. Packages

Two barrier-shrink bags containing ethylene-vinyl alcohol copolymer (EVOH) with 44% mol or 32% mol of ethylene, semi-crystalline and amorphous polyamides (PA, amPA) blend, poly(ethylene terephthalate) (PET), ethylene ionomer and linear low-density polyethylene (LLDPE) in their coextruded structures were studied: EVOH44 (PET/tie/Ionomer/tie/PA + amPA/EVOH-44%mol/PA + amPA/tie/LLDPE, with respective thickness: 3.5/3.5/16.6/3.5/3.2/4.1/3.2/4.4/20.1 μm) and EVOH32 (PET/tie/Ionomer/tie/PA + amPA/EVOH-32%mol/PA + amPA/tie/LLDPE, with respective thickness: 3.8/3.8/19.2/3.5/5.0/4.6/5.0/3.8/19.2 μm). Traditional gamma ray cross-linked bags containing polyvinylidene chloride (PVDC) were used as a control: C (EVA/tie/PVDC/tie/LLDPE, with respective thickness: 14.8/3.8/4.6/4.6/31.4 μm). The oxygen permeance of the multilayer EVOH44, EVOH32, and control films were 14, 8 and 15 $cm^3/(m^2.day.bar)$ at 23 °C and 76–77% RH, respectively. The water vapour transmission rate of EVOH44, EVOH32, and control films were 11, 11 and 4.4 $g/(m^2.day)$ at 38 °C and 90% RH, respectively.

2.2. Preparation of meat samples

Animals from the same lot were slaughtered on the same day according to standard procedures of a commercial slaughtering plant after having passed veterinary inspection. Boning and cutting were performed after the carcasses had been kept in a chilling room at 4 °C for 2 days. The whole 98 rib eye roll muscles (internal muscle block, the "eye" at the end of the ribs) (*longissimus thoracis*) were collected and were cut in halves, obtaining two chunks of approximately 18 cm width × 10 cm long × 8 cm height and weighing c.a. 1.0 kg, totalizing 196 samples that were randomly packaged in three treatments (65 samples/treatment). All cuts selected had a pH value less than or equal to 5.8 measured with a portable pH meter (DM-2, Digimed, Brazil) equipped with an insertion glass electrode, calibrated with 4.0 and 7.0 phosphate standard buffers.

All beef cuts were individually vacuum packaged in three groups of each tested shrink bag (EVOH44, EVOH32 and C) using a Sealed Air Cryovac® VS95 automatic belt vacuum packaging machine, operating at 6 mbar. A hot water shrink tunnel (Sealed Air Cryovac® model ST 98) operating at 86–88 °C was used to complete the vacuum bag packaging process (dwelling time around 2 s). The cuts were packaged in paper board boxes and shipped via refrigerated truck to the stocking area of a commercial cold storage room and maintained at 0.5 °C for up to 120 days. The first samples were analysed one day after packaging.

2.3. Analysis procedures

The analyses were performed in a single lot of beef cuts at 1, 30, 60, 75, 90, and 120 days, on triplicate samples randomly collected (except for the samples collected at 120 days) from each treatment.

2.3.1. Visual inspection

Visual inspection was conducted at intervals over a period of 120 days, noting and recording the presence of bubbles inside the vacuum packages. A significant amount of gas was present in all remaining samples on the 120th day, so these samples were categorized according to the level of bubbles (1 - very low; 2 - low; 3 - medium; 4 - high) inside the vacuum package. An exploratory study was performed to characterize this alteration and samples with different gas levels (total of 3 samples/treatment) were evaluated with respect to headspace gas composition within the package, microbiological counts, and pH values.

2.3.2. Microbiological evaluation

Total aerobic psychrotrophic bacteria were analysed at intervals up to 90 days of storage; lactic acid bacteria, enterobacteria, *Pseudomonas* spp., and sulphite-reducing clostridia were analysed on the 120th day. For bacterial analysis, meat samples (25 g) from each package were aseptically weighed and homogenized in peptone solution for 1 min in a Laboratory Blender (Stomacher Seward, 400, UK) at room temperature. Decimal dilutions were prepared, and 0.1 ml aliquots of the appropriate dilutions were either (1) spread-plated in duplicate on Plate Count Agar (PCA, Oxoid, UK) and incubated at 20 °C for 10 days for the enumeration of psychrotrophic bacteria; (2) pour-plated on de Man, Rogosa, Sharpe Agar (MRS, Oxoid) and incubated at 30 °C for 48 h for enumeration of lactic acid bacteria; (3) pour-plated on Violet Red Bile Glucose Agar (VRBGA, Oxoid) followed by incubation at 30 °C for 48 h for enterobacteria determination; (4) pour-plated on Pseudomonas Agar (Merck, Germany) with Pseudomonas CFC Selective Supplement (Merck) and incubated at 30 °C for 48 h for *Pseudomonas* spp. counts; or (5) spread-plated in Shahidi Ferguson Perfringens SFP (SFP Agar Base, Difco, USA) with 4% D-cycloserine (Inlab, Brazil) followed by incubation at 46 °C for 24 h for enumeration of sulphite-reducing clostridia. Except for *Pseudomonas* ssp., for which ISO 13720 (2010) methodology was applied, all microorganisms were analysed according to Downes and Ito (2001). The results are expressed as log cfu/g (colony-forming units).

2.3.3. pH measurement

pH measurements were performed on the external portion of each sample, using an insertion type electrode (Digimed pHmeter, Brazil). The electrode was calibrated with 4.0 and 7.0 phosphate standard buffers. Each measurement was performed in three repetitions on the same sample, taking the mean value as the assay result.

2.3.4. Purge loss

The methodology recommended by Aspé et al. (2008) was adapted for purge loss analysis. The weight of the entire package (meat sample and film) was taken; then, the sample and any purge were removed from the package, and the beef and the entire package surface were wiped clean with a paper towel. Finally, the bag and beef weight were separately recorded. The results are expressed as percentage of weight difference.

2.3.5. Sensory evaluation

The evaluation panel consisted of a minimum of five trained panellists experienced in sensorial analyses of fresh beef and in the recognition of meat spoilage conditions, selected from a group of seven women and two men, aged 24 to 63 years. The panellists were chosen for their ability to recognize and discriminate colours according to the Farnsworth-Munsell 100 Hue Test (FM 100, Macbeth, New York) and for their ability to detect, discriminate and describe a range of odours. The training included the development of a descriptive sensory vocabulary and the discrimination of the sensory attributes of samples based on the difference from the control scale. Sensory analyses were performed on randomly collected triplicate samples of each treatment. On the first day of storage, the panellists performed a full sensory evaluation establishing a definition and description of attributes including adherence to packaging, presence of bubbles, amount and colour of exudate, lean colour and colour uniformity, odour and visual texture. As a reference, photographic standards used to illustrate characteristic and non-characteristic visual attributes were obtained with samples photographed in the first day of evaluation. All the images were set to an equal mean luminance, presented against a white background and printing designed in high resolution. The photograph standards were used by panellists at 30, 60, 75, 90 days to remember the initial day condition of the samples. Except for the odour attribute, panellists evaluated the samples using a degree of difference scale to compare the sensory condition of the sample on the evaluation day with its condition on the

initial day (first day of storage). The scale was a verbal structured 8-point scale with labelled interval ratings ranging from 0 to 8; the categories were defined as: 0 - no difference; 2 - slight difference; 4 - moderate difference; 6 - great difference and 8 - extreme difference. The panellists came to an agreement that grades differences from the initial samples higher than 4 or moderate were considered refused. To conduct the sensory appraisal, the three sample units of each treatment were coded with random three-digit codes. The panellists first evaluated closed package samples with respect to adhesion to the packaging inner surface, presence of bubbles, amount and colour of exudate, lean colour and colour uniformity. The same sample packages were then opened and immediately evaluated with respect to odour, discriminating and describing a range of odours. Lean colour and colour uniformity were appraised 15 min after the packages were opened and exposed to the room atmosphere to verify colour development after blooming, i.e., myoglobin pigment reaction with atmospheric oxygen. In the sensory evaluation the samples were assessed, on a majority score basis according to the modified method of Bell and Garout (1994). Each panellist evaluated the test samples independently. A panel moderator recorded the end evaluation and led discussions to resolve disagreements.

2.3.6. Package headspace gas composition

On the 120th day of storage, samples were randomly collected for determination of the gas composition of the headspace inside the packages in terms of oxygen (O_2), carbon dioxide (CO_2), nitrogen (N_2) and hydrogen (H_2). A 0.3 ml volume of headspace gas was collected from each sample using a gas-tight syringe through a silicon septum stuck on the external surface of each package. Identification and quantification of gases were carried out using a gas chromatograph (model 7890, Agilent, USA) operating with a thermal conductivity detector at 150 °C, molecular sieve columns (13X e Porapak N) at 40 °C and injector at 70 °C. The results were analysed using Chemstation/Agilent software version B 03.01 based on standard curves obtained using calibration gas mixtures and expressed in terms of gas volumetric percentage (% v/v). The composition was determined at 24 °C and 707 mmHg.

2.4. Statistical analysis

The results obtained for microbiology analysis, pH and purge loss were analysed using SAS System for Windows (Statistical Analysis System) version 9.1.3., Service Pack 3 (SAS Institute, 2003). The data were subjected to analysis of variance (ANOVA) and when the effects were significant, honestly significant differences were calculated for average comparisons using Tukey's test ($P < 0.05$). The model for microbial analysis and physicochemical traits included 3 film types, and 5 days of storage in combination, totalizing 15 treatments. Correlation coefficients of the variables among the types of films were generated using Pearson's correlation coefficient option (SAS Institute, 2003).

3. Results and discussion

3.1. Visual inspection: Gas production

On the 1st and the 30th days, samples that showed vacuum loss due to package sealing failures related to heat sealing area contamination with the product residues and wrinkles were discarded. On the 60th and the 90th storage days, very low gas production (small bubbles) was evident in <10% of the samples in all treatments. On the 120th day, all of the remaining samples presented some degree of gas production (Table 1). Samples presenting bubbles levels 3 and 4 on the 120th day of storage should be considered inadequate for commercialization. An exploratory study was performed aiming to characterize the gas production (subsection 3.4).

Table 1
Gas production in vacuum-packed rib eye roll during refrigerated storage at 0.5 °C expressed as percentage of remnant stored packages.

		% Treatment (N. of remnant packages)		
Time (days)	Gas production level[a]	C	EVOH44	EVOH32
1	1	0.0 (64)	0.0 (61)	0.0 (61)
30	1	0.0 (58)	0.0 (54)	0.0 (53)
60	1	9.6 (52)	6.3 (48)	8.5 (47)
90	1	7.3 (41)	2.6 (39)	0.0 (37)
120	2	19.2 (26)	7.7 (26)	24.0 (25)
	3	65.4 (26)	73.1 (26)	60.0 (25)
	4	15.4 (26)	19.2 (26)	16.0 (25)

[a] Level of gas bubbles: 1 - very low; 2 - low; 3 - medium; 4 - high.

3.2. Microbiological evaluation, pH measurement, purge loss

As shown in Table 2, on the 1st day of storage the aerobic psychrotrophic bacteria counts for all treatments presented similar results, with values close to 2.0 log cfu/g. On the 30th day, the counts showed a significant increase, with counts higher than 7.0 log cfu/g for all treatments ($P < 0.05$). The counts remained at this level until the 90th day of storage, suggesting that the bacterial populations in the appraised samples achieved stationary growth phase. During the period of evaluation, no significant differences were found among the treatments ($P > 0.05$).

As mentioned by Small, Jenson, Kiermeier, and Sumner (2012), the high bacteriological counts found in this study on the 30th day of refrigerated storage are not uncommon. According to the microbiological profile of Australian vacuum-packed beef primals characterized since 1979, the stationary phase at 7.0 to 8.0 log cfu/cm^2 with a predominance of lactic acid bacteria was reached in 5 to 8 weeks at 0 °C, with sensorial signs of spoilage beginning at approximately 12 weeks. Under good processing and packaging conditions, the counts of lactic acid bacteria on the surface of the primals, which are considered a good index of microbiological quality, were approximately < 10^2 cfu/cm^2 and were expected to exceed 10^6 cfu/cm^2 after 2 to 3 weeks (Blixt & Borch, 2002).

Significant variation in pH value as a function of storage time was observed (Table 3). All pH values obtained were in the normal range for beef (5.18 to 5.75). The pH decreased during the first 30 days of storage in beef in C and EVOH32 films and at 60 days in beef in EVOH44 film. At 90 days of storage the pH increased slightly in beef in the EVOH44 and EVOH32 films, while in beef in C film the pH was stable until 90 days of storage. The pH values during the shelf life in beef in EVOH films are known (Adams & Moss, 2000). According to these authors after the death of the animals the supply of oxygen to the muscles and the respiration ends, the redox potential falls, but the glycolytic breakdown of glycogen continues leading to an accumulation of lactic acid and a decrease in muscle pH. In a mammalian muscle the pH will drop from around 7 to 5.4–5.5 with the accumulation of about 1% lactic

Table 2
Development of aerobic psychrotrophic bacteria (log cfu/g)[a] on vacuum-packed rib eye roll during refrigerated storage at 0.5 °C (mean ± standard error, n = 3).

	Treatment[1]		
Time (days)	C	EVOH44	EVOH32
1	$2.00^{c} \pm 0.00$	$2.43^{c} \pm 0.22$	$2.26^{c} \pm 0.14$
30	$7.69^{ab} \pm 0.12$	$7.92^{a} \pm 0.00$	$7.40^{ab} \pm 0.12$
60	$7.56^{ab} \pm 0.09$	$7.54^{ab} \pm 0.08$	$7.26^{b} \pm 0.07$
75	$7.52^{ab} \pm 0.07$	$7.44^{ab} \pm 0.07$	$7.44^{ab} \pm 0.04$
90	$7.45^{ab} \pm 0.09$	$7.76^{ab} \pm 0.20$	$7.27^{b} \pm 0.20$

Means with common superscript letters are not different ($P > 0.05$).
[1] Unit: log cfu/g (colony-forming unit).

Table 3
pH measurement of vacuum-packed rib eye roll during refrigerated storage at 0.5 °C (mean ± standard error, n = 9).

	Treatment		
Time (days)	C	EVOH44	EVOH32
1	$5.74^{a} \pm 0.06$	$5.75^{a} \pm 0.04$	$5.75^{a} \pm 0.03$
30	$5.19^{d} \pm 0.03$	$5.66^{a} \pm 0.09$	$5.24^{d} \pm 0.02$
60	$5.38^{bcd} \pm 0.08$	$5.38^{bcd} \pm 0.04$	$5.18^{d} \pm 0.03$
75	$5.30^{bcd} \pm 0.04$	$5.26^{cd} \pm 0.04$	$5.23^{d} \pm 0.04$
90	$5.35^{bcd} \pm 0.07$	$5.52^{ab} \pm 0.05$	$5.50^{abc} \pm 0.08$

Means with common superscript letters are not different ($P > 0.05$).

acid. When glucose becomes exhausted, the meat amino acids will be metabolized producing volatile sulfur compounds such as methane thiol, dimethyl sulfide and dimethyl disulfide. In the latter stages of shelf life an increase in the meat pH is verified as ammonia and amines are produced.

The meat purge loss was not significantly ($P < 0.05$) different between conventional PVDC film versus EVOH structures (Table 4). Only at 75 days it was verified less purge loss in beef packaged in both EVOH films. At 90 days there was no more difference among the packages. Payne et al. (1998) reported 2.7% beef purge loss for shrink vacuum package and 3% for non-shrink bag after 6 weeks at −1.5 °C. Aspé et al. (2008) verified 1.6% beef purge loss for shrink package and 2.6% for non-shrinkable beef vacuum package after 8 weeks at 0 ± 2 °C. Smulders, Hiesberger, Hofbauer, Dögl, and Dransfield (2006) reported considerable purge loss (3.52 ± 0.99%) in beef loin packed in non-shrinkable polyamide-polyethylene vacuum film over a shorter period of storage (23 days) at 2 ± 2 °C compared to the film used in this work. Some authors (Yoon & Lee, 2001) did not verify significant differences in exudate loss when compared the shelf life of vacuum-packaged beef loin cuts in PA/PE film and vacuum and shrink-packaged with PVDC/EVA copolymer (VSP) during storage at 2 °C for 4 weeks.

The water-holding capacity of meat without added salt displays a distinct minimum at pH 5.0, which is also the average isoelectric pH of the meat structural proteins; the average, because proteins have different isoelectric points, and even different parts of large proteins have different isoelectric points. At pH values higher than the isoelectric pH, there is a steep increase in water-holding with a maximum at approximately pH 10. On the acid side, the maximum water-holding capacity occurs at pH 3–4 (no salt) (Puolanne & Peltonen, 2013). In general, the present results are consistent with this observation; as the pH value decreased during storage, the amount of purge loss increased at a specific rate.

Drip is composed primarily of sarcoplasmic proteins (Savage, Warriss, & Jolley, 1990). The exudate originates from the spaces between the fibre bundles and the perimysial network and from the spaces between fibres and the endomysial network (Offer & Cousins, 1992). Drip increases nonlinearly with storage time (McMillin, 2008) and may represent from 2 to 10% of the lean weight in steaks and chops (Offer & Knight, 1988).

A disadvantage of vacuum-packaging technology is that it requires subjecting the meat to a mechanical effort that can liberate intracellular liquids, which provide an excellent growth medium for microorganisms and also contribute to weight loss, reduced meat juiciness, increasing toughness and consequently lower acceptance by consumers. An excess of exudate is also harmful to the product's appearance, an attribute highly valued by consumers. Thus, exudation is not desirable from an economical, commercial or preservation point of view.

Variations in water-holding capacity at a given meat pH and storage temperature have been proposed to be partially due to variations in proteolysis and subsequent shrinkage and movement of water into extracellular spaces (Huff-Lonergan & Lonergan, 2005). Water holding capacity is a measure of the ability of meat to hold water, and the amount of bound water affects the appearance of products and their economic value (Offer & Cousins, 1992). Packages of meat containing a pool of fluid surrounding the meat are not desirable to consumers (McMillin, 2008). Consumers also do not like handling meat packages that leak fluids. Packaging in polyethylene or in non-shrinking ethylene-Saran composites did not affect drip, but shrinking films reduced drip by 51–68% compared with non-shrinking films (Zarate & Zaritzky, 1985).

The correlation coefficients among the film types, microbial counts, pH value and purge loss are presented in Table 5. The correlations between microbial counts and all films were highly, significantly and positively correlated. The purge loss values were significantly and positively correlated to the film types. The pH values were significantly and positively correlated to the films C and EVOH32, but were not consistent among films C and EVOH44, and among films EVOH44 and EVOH32. The EVOH packages presented similar performance in relation to aerobic psychrotrophic counts and purge loss compared to control packages.

3.3. Sensory evaluation

The acceptance of vacuum-packed beef at the moment of purchase is considered according to the attributes of appearance perceived by consumers, whereas after the opening of the package other attributes such as odour, firmness and colour after blooming are evaluated.

With the exception of colour uniformity of treatment C, beef packaged with all of the tested treatments presented some degree of change in all sensorial characteristics with storage time (Table 6).

The adhesion of the packaging film to the beef is desirable as a means of reducing purge loss. On the 60th day of storage, treatments C and EVOH44 showed adhesion scores greater than or equal to 4 (a moderate difference from the initial evaluation). Treatment C film presented the lowest adhesion, and EVOH32 packaging presented the highest adhesion during the evaluation period. The good shrinkage of ionomer in the PVDC-free films improves the adhesion between the film and the meat. The sensorial panellists considered that the EVOH44 packaging was the least flexible and the most difficult to pinch, suggesting that these factors influenced the perception of the film's adhesion on the beef. In addition, EVOH32 packaging was less flexible than treatment C film. This perception was associated with the highest stiffness of ionomer incorporated in the EVOH32 and EVOH44 films.

The panellists verified higher purge loss from samples of treatment C at 75 and 90 days, whereas samples of treatments EVOH44 and EVOH32 were rejected only at the 90th day. According to the sensorial panellists,

Table 4
Percentage of purge loss in rib eye roll after vacuum packaging and storage under refrigeration at 0.5 °C (mean ± standard error, n = 6).

	Treatment		
Time (days)	C	EVOH44	EVOH32
1	$0.3^{e} \pm 0.1$	$0.2^{e} \pm 0.0$	$0.3^{e} \pm 0.0$
30	$2.0^{cd} \pm 0.1$	$1.3^{de} \pm 0.1$	$1.6^{cd} \pm 0.3$
60	$2.3^{cd} \pm 0.2$	$2.3^{cd} \pm 0.5$	$2.2^{cd} \pm 0.3$
75	$4.4^{a} \pm 0.1$	$2.5^{c} \pm 0.1$	$2.4^{c} \pm 0.2$
90	$3.7^{ab} \pm 0.3$	$2.6^{bc} \pm 0.2$	$2.7^{bc} \pm 0.3$

Means with common superscript letters are not different ($P > 0.05$).

Table 5
Pearson's correlation coefficients and probabilities of film type (C, EVOH44, EVOH32), microbial counts, purge loss and pH value.

	Aerobic psychrotrophic bacteria	Purge loss	pH
C - EVOH44	0.99034	0.74754	0.17262
	<0.0001	<0.0001	0.2568
C - EVOH32	0.99396	0.76146	0.53840
	<0.0001	<0.0001	0.0001
EVOH44 - EVOH32	0.98331	0.63168	0.43677
	<0.0001	0.0002	0.0027

Table 6
Sensory attributes of rib eye roll after vacuum packaging and storage at refrigerated temperatures (0.5 °C).

	Time (days)											
	30			60			75			90		
Attribute	C	EVOH44	EVOH32	C	EVOH44	EVOH32	C	EVOH44	EVOH32	C	EVOH44	EVOH32
Closed package												
Adhesion	3	2	3	5	4	3	6	4	3	6	5	3
Purge loss	4	1	2	4	4	3	6	4	3	7	5	5
Bubbles	2	4	3	4	5	3	6	4	3	6	6	4
Colour of purge	0	0	0	0	0	0	0	2	1	1	1	1
Lean colour	0	1	0	1	5	1	0	4	2	1	5	2
Lean colour uniformity	0	0	0	0	0	0	0	3	2	0	2	2
Opened package												
Firmness	1	0	1	2	3	2	2	1	1	3	2	3
Lean colour	0	0	0	1	4	1	1	5	3	2	5	3
Lean colour uniformity	0	0	0	0	0	3	1	4	4	2	5	4

An eight-point graduated scale based on comparison with the corresponding attribute of the sample at the beginning of the storage period (Day 0) was used: 0 - no difference; 2 - slight difference; 4 - moderate difference; 6 - great difference; 8 - extreme difference.
Values higher than 4 were considered "refused".

one reason that could explain the adherence of the product to the film was the amount of liquid inside the pack.

Gas bubble volume scores higher than 4 were recorded at 60 and 75 days for the EVOH44 and C treatments, respectively. The panellists did not refuse meat that had received EVOH32 treatment after 90 days of storage. These results may be associated with the lower oxygen permeability of EVOH32, which reduces the oxygen intake compared to that of other packaging. The gas bubbles appear as a slight separation of the plastic film from the product, mainly over the fatty area of the meat. In general, they appeared in the first days just after packaging. These bubbles consist mainly of nitrogen because its solubility in fat decreases as the fat crystallizes on cooling. During storage, the bubbles increase in size, in part because CO_2 is produced by microbial activity.

Consumers rely heavily on the colour of fresh meat as an indicator of its wholesomeness at the point of sale. Deviations from the bright cherry-red colour of fresh meat lead to product rejection and revenue loss (Suman & Joseph, 2013). Of the several quality attributes of fresh meat, colour has the most important influence on purchasing decisions (Mancini & Hunt, 2005). At the point of sale, consumers, in general, cannot evaluate the odour or feel the texture of meat without opening the packages. Generally, EVOH44 treatment did not present good acceptance regarding meat colour, either for closed or opened packs for blooming, compared to treatments EVOH32 and C. Treatment EVOH44 presented refusing scores from the 60th day of storage onward concerning beef colour (closed pack), with a brownish-red colour of the cut surface. Regarding the colour uniformity (stain presence) evaluated after blooming, meat packaged using EVOH44 treatment films was refused from the 90th day of storage.

The samples from treatments EVOH32 and C were accepted by the panellists with respect to colour and uniformity during the entire 90-day storage period. From the 60th day of storage onward, the colour of the meat in C and EVOH32 packaging appeared pale, either in the closed pack or after blooming. In the case of EVOH32, the pale red layer that appeared after blooming represented the portion of the meat that had been in contact with the sealed area of the bottom of the pack. Samples of meat in EVOH32 and C packaging presented higher colour uniformity and better colour after blooming at all evaluated time points. On the other hand, samples in EVOH44 packaging presented a brownish-red colour on the surface of the cuts after blooming. On the 75th day, two of the three samples showed no uniformity and presented greenish stains in samples both in and out of the packaging. The colour of the samples packaged in EVOH44 appeared paler in both closed and opened packs from the 75th day of storage onward. On the 90th day of storage, one of the three EVOH44 samples showed a brownish-red colour on the whole cut surface. On the 75th day, two of the three opened samples from treatment EVOH32 showed brownish and greenish stains. On the 90th day, one EVOH32 sample showed a greenish stain on the beef surface.

The texture of the beef cuts changed only slightly and was not refused during storage.

A variety of odours (Table 7) were mentioned during the sensory evaluation, including "aged" meat, "egg" and "putrid" odours. The majority of samples presented an aged odour varying among acid, fruity, and sweet aged during the storage period. Raw aged beef has a characteristic aged aroma (aroma is a more pleasant and descriptive term for meat than odour) (Romans, Costello, Carlson, Greaser, & Jones, 2001).

An "egg" odour was detected in samples from treatments EVOH32 and EVOH44 on the 30th day. On the 60th day, at least one sample of each treatment presented egg odour; surprisingly, on the 75th day, no samples presented egg or putrid odour. On the 90th day, one sample from treatment EVOH44 presented a slightly putrid egg odour.

It is important to note that the utilization of meat as a substrate for bacterial growth includes some well-defined stages. According to Ellis and Goodacre (2001), once surface levels of glucose have been depleted, bacteria present on the meat metabolize secondary substrates such as free amino acids obtained by the activity of protease and lactate. The

Table 7
Odour evaluation after the opening of films of vacuum-packed rib eye roll stored at refrigerated temperatures (0.5 °C).

Time (days)	Odour rating	Treatment[a]		
		C	EVOH44	EVOH32
1	Fresh beef	3	3	3
30	Aged	2	–	–
	Aged, less intense	–	2	–
	Aged, sweet	1	–	1
	Aged, acid	–	–	1
	Aged, slight egg	–	1	–
	Slight egg, sulphur	–	–	1
60	Aged	1	2	1
	Aged, fruity	–	–	1
	Slight egg, prevailing aged	1	–	–
	Slight egg	–	–	1
	Egg, slightly putrid	1	1	–
75	Aged	–	–	2
	Aged, acid	2	2	–
	Aged, sweet	–	1	–
	Aged, intense	1	–	1
90	Aged	3	1	2
	Aged, fruity	–	–	1
	Aged, intense	–	1	–
	Egg, slightly putrid	–	1	–

[a] Number of samples displaying the indicated odours (of a total of 3 samples per treatment).

utilization of free amino acids by bacteria leads to an increase in ammonia levels, and it has been observed that the switch from a saccharolytic to an amino acid-degrading metabolism occurs while considerable levels of glucose are still present deep within the muscle tissue (Seymour, Cole, & Coote, 1994). In addition to ammonia, the by-products of amino acid utilization include sulphides, indoles, scatoles and amines such as the diamines putrescine and cadaverine (Adams & Moss, 2000; Dainty, Edwards, Hibbard, & Ramantanis, 1986; Dainty, Edwards, Hibbard, & Marnewick, 1989; Doyle, 2007). The production of these compounds and others leads to the characteristic changes associated with spoiled meat, such as off-odours and an increase in pH. Large amounts of purge loss might also contribute to the higher detection of off-odours in vacuum skin pack beef (Lagerstedt, Ahnström, & Lundström, 2011).

3.4. Evaluation of samples after 120 days of storage

The gases (O_2 and N_2) detected inside the packages originated from (1) the presence of residual air after the vacuum-packing operation; (2) desorption of gases from air solved in the meat; and (3) permeation of O_2 and N_2 present in the environment through the packaging material. CO_2 was produced by microorganisms during their growth in the product. The H_2 detected in samples that received EVOH32 treatment probably originated from microbiological growth (Table 8). It was verified that the most intensive gas production (level 4) occurred in samples with higher CO_2 concentrations; this could have resulted from microbiological activity. The increased CO_2 level is partially associated with O_2 consumption and could indicate the growth of aerobic and facultative anaerobic microorganisms.

To establish a relationship between the gas production detected in the beef samples and the presence of specific bacterial populations, a more specific evaluation of the biodeterioration was performed. On the 120th day of storage, the microbiological counts of enterobacteria, lactic acid bacteria and *Pseudomonas* spp. were high as a result of the spoilage process of the samples, which includes gas production. Samples from treatment C and EVOH44 (gas level 2) presented higher counts of enterobacteria and *Pseudomonas* spp. compared to samples with gas levels of 3 or 4. It is possible that the bacteria present in the samples with higher levels of gases could already be largely dead on the day of analysis, resulting in a reduction in bacterial counts. High levels of O_2 were detected in samples of gas level 2, indicating that some factors, including film permeability, favoured the growth of aerobic and facultative anaerobic microorganisms. Conversely, the anaerobic bacteria counts, represented by the sulphite-reducing clostridia, were below the limit of detection of the analytical method used. The relatively large amount of CO_2 in samples with gas level 4 (all treatments) could be related to the reduction in *Pseudomonas* spp. counts compared to samples with gas levels of 2 and 3. The growth of this group of bacteria is apparently inhibited at CO_2 concentrations of 20% or higher. Under these conditions, lactic acid bacteria are the dominant group; nevertheless, strict aerobic bacteria such as *Pseudomonas* spp. are not completely eliminated and may occasionally be detectable. In addition to CO_2, the inhibition of microbiological growth may be attributed to the products of secondary metabolism.

Some authors (Adams & Moss, 2000; Varnam & Sutherland, 1995) have reported that vacuum packing changes the microbial population of the meat and consequently the time course and characteristics of spoilage. Theoretically, low O_2 concentrations, ca. 1%, would support growth of a relatively large population of *Pseudomonas* spp. As the respiration of meat tissues continues, rapid depletion of O_2 and an increase in the CO_2 concentration to ca. 20% occurs. Under these conditions, the growth of *Pseudomonas* spp. is usually restricted, although high bacterial counts (10^6 cfu/cm^2) may be present in primal joints; more typically, high CO_2 gives rise to a microflora dominated by Gram-positive bacteria (particularly lactic acid bacteria) and Gram-negative bacteria of the *Enterobacteriaceae* family. Microbiological spoilage of vacuum- packed meat is characterized by the development of sour acid odours that are far less objectionable than the odour associated with aerobically stored meat. Even when the microorganisms reach their maximum population density of approximately 10^7 cfu/cm^2, souring develops only slowly thereafter. When microbial numbers reach levels of approximately 10^8 cfu/cm^2, further indication of spoilage becomes apparent in the form of a visible surface slime on the meat (Adams & Moss, 2000).

Lactic acid bacteria are able to grow rapidly at low temperatures and low O_2 tensions, and their growth is strongly favoured by their tolerance for CO_2. At temperatures below 5 °C, the growth of *Enterobacteriaceae* in vacuum-packed beef is inhibited by CO_2, low pH and the presence of lactic acid. At higher temperatures and pH values, CO_2 is markedly less inhibitory and growth is possible.

On the 120th day of storage, all of the EVOH32 and EVOH44 samples showing level 1 gas production displayed high pH values (following an initial pH of <5.8). The microbiological activity in these samples presented intense growth of enterobacteria, lactic acid bacteria and *Pseudomonas* spp. Formation of by-products from proteolysis during microbial metabolism could be the cause of the high pH value observed in these samples.

After very long periods of storage, the meat quality was not maintained by the package preservation properties.

Table 8
Headspace gas composition, microbiological counts, and pH of vacuum-packed rib eye roll after 120 days of refrigerated storage at 0.5 °C.

Parameter		Gas production level[a]	Treatment		
			C	EVOH44	EVOH32
Headspace gas composition (% v/v)	H_2 – hydrogen	2	<QL[b]	<QL	1.3
		3	<QL	1.0	3.7
		4	<QL	<QL	1.0
	O_2 – oxygen	2	14.8	14.5	11.7
		3	11.9	13.5	1.6
		4	2.0	<QL	<QL
	N_2 – nitrogen	2	66.5	62.6	62.0
		3	56.4	62.7	31.5
		4	26.8	22.4	15.5
	CO_2 – carbon dioxide	2	16.5	19.3	22.8
		3	27.0	19.8	60.0
		4	66.4	75.1	81.3
Microbiological counts (log cfu/g)[c]	*Enterobacteriaceae*	2	6.81	7.52	6.43
		3	4.48	5.88	6.40
		4	3.40	2.58	6.59
	Lactic acid bacteria	2	6.80	8.00	7.49
		3	6.48	7.61	8.15
		4	6.54	6.72	8.11
	Sulphite-reducing clostridia	2	<DL[d]	<DL	<DL
		3	<DL	<DL	<DL
		4	<DL	<DL	<DL
	Pseudomonas ssp.	2	6.34	7.20	6.36
		3	4.20	6.52	6.15
		4	3.67	4.04	5.74
pH		2	5.56	6.64	6.21
		3	5.35	5.75	5.90
		4	5.44	5.41	6.77

[a] Level 2: low; level 3: medium; level 4: high.
[b] QL: quantitation limit (H_2: 0.1% and O_2: 0.04%).
[c] Unit: log colony-forming units/g.
[d] DL = detection limit (for sulphite-reducing clostridia < 1.00 log cfu/g).

4. Conclusions

The multilayer EVOH films presented the same performance compared to control PVDC barrier shrink bags related to psychrotrophic aerobic growth and purge loss. Package with EVOH 32% mol presented better performance than control bag with respect to the meat sensorial attributes, including fewer bubbles and better adhesion to the meat. EVOH 44% mol bag presented a higher rate of meat colour loss than

the others. The multilayer film with EVOH 32% mol, non-irradiated and chlorine-free is as effective for the preservation of fresh beef during cold storage as traditional PVDC-irradiated shrink bags.

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