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Effects of two different packaging materials on veal calf meat quality and shelf life[1]

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ABSTRACT: The aim of the present work was to evaluate the effects of 2 different packaging films on chemical, physical, and microbiological variables in veal calf meat. Slices from the right half carcass were sampled from each calf: 8 were packaged with Cryovac film (25 μm thick) and 8 were packaged with Weegal film (45 μm thick). In both packaging types, the same gas mixture was used. The samples were analyzed at these postpackaging times: 0, 2, 4, 6, 8, 10, 12, and 14 d. Chromatic patterns, water holding capacity (WHC), cooking loss, drip loss, protein oxidation, and hydroperoxide concentration were influenced by the packaging type ($P < 0.001$). Moreover, during the postpackaging time the WHC decreased but oxidative reactions increased ($P < 0.001$). The film that showed better characteristics in packaging of veal calf meat was the Weegal, characterized by decreased gas permeability due to its ability to reduce and delay chemical and physical alterative processes.

Key words: modified atmosphere, packaging material, shelf life, veal calf meat

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INTRODUCTION

Packaged food quality is strongly influenced by chemical and physical properties of the materials used in the packaging (Lee, 2010) because they can improve food shelf life, healthiness, and sensorial patterns (Coma, 2008). These advantages make packaging technology fundamental in food distribution and marketing. Traditionally, packaging was used to preserve food by environmental, chemical, physical, and microbiological agents that could affect and modify food quality during storage (Brody et al., 2008). The food packaging industry has rapidly developed due to increased demands for greater stringency in relation to hygiene and safety issues associated with fresh and processed meat products, retailer demand for cost-effective extensions to product shelf lives, and consumer expectations of convenience and quality (increased product range, easy use and minimum product preparation, more product information, and less packaging impact on the environment; Kerry et al., 2006).

In the fresh meat market, packaging is useful also for reducing microbiological contamination, for delaying deterioration, for allowing enzymatic activities that improve tenderness, for reducing drip loss, and for maintaining appearance appreciated by consumers (Brody, 1997). These characteristics are linked to the materials used in packaging production. In fact, plastic films used for vacuum and modified atmosphere packaging guarantee an exact water vapor and gas permeability (Sebranek and Houser, 2006). Therefore, food quality and shelf life are also due to the physical properties of films (passive packaging) and to bioactive agents that recently have been introduced in these materials (active and dynamic packaging; Lee, 2010).

Researchers have studied the effects of modified atmosphere packaging on beef (Brooks et al., 2008), pork (Lund et al., 2007), and poultry meat (Rokka et al., 2004), but no studies have been conducted on the effect of film thickness, particularly on veal calf meat.

The aim of the present work was to evaluate the effects of packaging materials with different physical characteristics on chemical, microbiological, and physical properties of veal calf meat.

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MATERIALS AND METHODS

The slaughtering procedures were performed in a European Economic Council (**EEC**) authorized slaughterhouse according to European laws on hygiene and animal welfare [Commission Regulation (**EU**) no.2004/853/CE, EU no. 2009/1099/CE].

Animals

A total of 8 Italian Holstein Friesian bull calves were used in the present trial. They were reared in the same farm. Calves were housed individually in pens (1.2 m^2 per calf) during the first 8 wk and thereafter in groups of 4 calves (1.8 m^2 per calf) per pen. During the first 8 wk, they were fed commercial milk replacer (Alpuro B.V., Uddel, the Nederlands) according to a commercial feeding schedule to produce veal meat. After 8 wk, besides milk replacer, a corn silage was supplied to calves. The structures, the housing, and the feeding techniques were adopted according the EU directive 2008/119. Animals were slaughtered at the age of 218 d with an average BW of 237 kg.

Meat Sampling and Packaging

The half carcasses were stored at 4 ± 0.5°C for 24 h and then were dissected in a temperature-controlled room (12 ± 1°C). Samples were obtained from the right side of carcasses and in particular from the LM (between the 7th and the 12th rib) muscle.

A total of 16 samples of LM muscle were collected. Each sample (packed in each tray) consisted of 2 slices and 3 pieces of meat. Slices (thickness of 0.5 cm) were used to evaluate chromatic parameters, water holding capacity (**WHC**), collagen, oxidative variables {protein oxidation, 2-thiobarbituric acid [thiobarbituric acid reactive substances (**TBARS**)], and hydroperoxides}, and microbiological analyses. One piece of meat (a parallelepiped with 3 by 6 by 6 cm sides) was used for the Warner Blatzer Shear Force (**WBSF**) evaluation on raw and cooked meat and pH measurement. Another piece of meat (a cube with 1.5 cm per side) was contained in a small polyvinyl chloride (**PVC**) container and used to calculate drip loss. The PVC container was a cube of 3 cm per side open on 1 surface. The last piece of meat (a cube with 1.5 cm per side) was used to measure cooking loss. Samples were placed in extruded polystyrene trays (AERpack PCM0330 produced by Coopbox Italia, Reggio Emilia, Italy) packed in modified atmosphere packaging (**MAP**) with the same combination of gases (46% O_2, 23% CO_2, and 31% N_2) and 2 different barrier films: 1) 8 with Cryovac LID 2050 (**CRY**): Samples were placed in extruded polystyrene trays (AERpack PCM0330 produced by Coopbox Italia) and heat sealed with a barrier polyolefin-based top film (Cryovac LID 2050) having specific technical properties (Table 1). The MAP packed samples were processed using a heat sealer (CVS-PN 35; Mondini S.p.A., Cologne (Bs), Italy); and 2) 8 with Weegal PEBAR film (**WEE**): Samples were placed in extruded polystyrene trays (STANDARD pack PCP1310 produced by Coopbox Italia) and heat sealed with a barrier polyolefin-based top film (Weegal PEBAR film produced by Fabbri Arti Grafiche S.p.A, Vignola (MO), Italy) having specific technical properties (Table 1). The MAP packed samples were processed using a heat sealer (ATMOPAK BDF; Fabbri Arti Grafiche S.p.A.).

Table 1. Characteristics of films

Item	CRY[1]	WEE[2]
Thickness	25 µm	45 µm
Density	0.97 g/cm^2	43.33 g/m^2
O_2 permeability (at 23°C and 0% R.H. per day)	15 cm^3/m^2	6 cm^3/m^2/24 h
CO_2 permeability (at 23°C and 0% R.H.[3] per day)	64 cm^3/m^2	20 cm^3/m^2
Moisture vapor transmission rate (at 38°C and 90% R.H. per day)	16 g/m^2	11 g/m^2

[1]CRY = Cryovac LID 2050, Passirana di Rho, Milano, Italy.
[2]WEE = Weegal PEBAR film, Vignola, Modena, Italy.
[3]R.H. = relative humidity.

Samples were transported under controlled temperature (+4 ± 0.5°C) to the laboratories of the Animal Science Division of the Public Health and Animal Science Department and they were stored at +4 ± 0.5°C. All samples were stored in the same refrigerated cell, on a table, in dark conditions. Laboratory analyses were performed at 2, 4, 6, 8, 10, 12, and 14 d of storage. The samples identified as **T0** (24 h after slaughtering) were controls and they were analyzed on the same day of packaging within 2 h from collection time. Assignment of each sample to a treatment was made randomly.

Meat Quality and Microbiological Analysis

Surface meat color was measured at 1 min after opening the packaging during each experimental time: T0 (packaging day) and then 2, 4, 6, 8, 10, 12, 14 d of storage. The surface color of calf slices were determined according to the CIE L*, a*, b* (CIE, 1976) color system using Minolta CR-300 colorimeter (light source D65; Minolta Camera Co. Ltd., Osaka, Japan). Reflectance measurements were collected from a 0° viewing angle with an A pulsed xenon arc (**PXA**) lamp with a

reading surface of 8 mm diameter. Three measurements were performed on each sample by rotating the detector system of 90° from the previous on 3 different points. Then 9 readings per sample were made at each time point and averaged for statistical analysis. The colorimeter was calibrated on the Hunterlab color space system using a white title (L* = 99.2, a* = 1.0, b* = 1.9).

The a* and b* values were used to determine chroma = $(a^2 + b^2)^{1/2}$ and hue (°) = $\tan^{-1}$ (b/a) according to Little (1975) and Mancini and Hunt (2005).

Intramuscular pH was recorded using a portable pH meter with glass electrode shaped to easily penetrate meat (Forlab, Carlo Erba pH 710, Milano, Italy). Before each measurement, the pH meter was calibrated using solutions with 4 and 7 pH values (Crison, Lainate, Milano, Italy).

The WHC was measured using the centrifugation method according to Bouton et al. (1971). Samples weighing 3 g were collected from each slice and were then centrifuged at 60,000 × *g* for 1 h at 10°C. After centrifugation, the remaining samples were dried and weighed again, and the centrifugation loss was calculated as the difference in weight before and after centrifugation. Water holding capacity was measured twice on each sample.

For the cooking loss determination, cubic meat pieces with 1.5 cm per side were weighed [initial weight (**Wi**)] and then cooked in plastic bags in water bath at 80°C until they reached the internal temperature of 75°C, measured by a copper constantin fine-wire thermocouple fixed in the geometrical center of the sample (Model 5SC-TT-T-30-36; Omega Engineering Inc., Stamford, CT). Cooked samples were cooled, dried from fluids, and reweighed [final weight (**Wf**)]. The cooking loss was calculated as a percentage of weight loss: [(Wi – Wf)/Wi] × 100 (Bertram et al., 2003).

Drip loss was evaluated on the second cubic piece with 1.5 cm per side. They were placed in a PVC container with known weight (**Wc**) during packaging in MAP. When trays were opened, each container with meat and liquid was weighed (Wi). Then the meat sample was removed from the PVC container, dried, and weighed again (Wf). The drip loss was calculated according this formula: {[(Wi – Wc) – Wf]/(Wi – Wc)} × 100.

At each experimental time (0, 2, 4, 6, 8, 10, 12, and 14 d of storage), the WBSF was recorded on raw and cooked meat. First, the parallelepiped samples with 3 × 6 × 6 cm were subdivided in 2 equal parts. One was cooked in a plastic bag in water bath at 85°C to an internal temperature of 75°C, measured with a copper constantin fine-wire thermocouple fixed in the geometrical center of the sample (Model 5SC-TT-T-30-36; Omega Engineering Inc.). Cores (1.27 cm diameter) were cut from each raw and cooked sample parallel to the muscle fiber direction. Shear force was measured using an Instron 1140 apparatus (Instron, High Wycombe, UK) provided with a computer. Each core was sheared 3 times and these 3 values measured were used to obtain the mean value for each sample. Results were expressed in kilograms per cubic centimeter.

Samples were prepared according to Kristensen et al. (2002) modified. The meat was mixed with 20 mL of NaCl in a 50-mL glass centrifuge tube and heat treated for 2 h in circulating water at 80°C. The tube was cooled to 40°C and homogenized for 1 min, which afterward was flushed with 10.0 mL of water. The homogenate was centrifuged at 4,000 × *g* for 15 min at 4°C, and the supernatant was filtered through paper into a second glass centrifuge tube. The filter was added to the pellet, 30 mL of 6.0 *M* HCl was added to the supernatant, and 50 mL of 6.0 *M* HCl was added to the pellet. Both were then hydrolyzed overnight in a sand bath (100°C). The amount of soluble collagen was calculated from the hydroxyproline concentration in the supernatant, and the total collagen was calculated from the sum of the hydroxyproline concentration in the pellet and in the supernatant (Kolar, 1990). Total collagen content was determined using liquid chromatography to isolate the hydroxyproline as described by Hutson et al. (2003). Aliquots (900 μL) of homogenate supernatant or standard were transferred to 5-mL Reactivials (Supelco, St. Louis, MO) followed by addition of 200 μL of borate buffer (0.7 *M* boric acid, pH 9.5, with NaOH). Next, 100 μL of Phthaldialdehyde (**OPA**) solution were added (50 mg o-phthalaldehyde dissolved in 1 mL acetonitrile containing 26 μL of β-mercaptoethanol) followed 60 s later by 100 μL of iodoacetamide reagent (140 mg/mL of iodoacetamide in acetonitrile). One minute later, 300 μL of 5 m*M* fluorenylmethoxycarbonyl-chloride (**FMOC**) in acetone were added. The reaction vial was capped and vortexed between each addition of reagent. One minute after the addition of FMOC reagent, 2 mL of ethyl ether were added to each reaction vial. The vial was shaken vigorously for 30 s to wash the contents of the vial. The organic layers were discarded and the wash was repeated twice for a total of 3 washes. Then 50 μL of the remaining aqueous phase were aspirated by the HPLC. Samples aspiration was made every 40 min.

Lipid oxidation was assessed by the TBARS method (Buege and Aust, 1978) and expressed as milligrams of malondialdehyde (**MDA**) per kilogram meat. Meat samples (2 g) were homogenized in 20 mL of 100 m*M* phosphate buffer (pH 7.0) for 2 min using a homogenizer. An aliquot of homogenate (1 mL) was transferred to a glass tube and added with 50 μL of butylated hydroxytoluene (**BHT**; 7.2% in ethanol) and with 1,950 mL of thiobarbituric acid(**TBA**)/trichloracetic acid (**TCA**)/HCl solution (0.375% TBA, 15% TCA, and 0.25 *N* HCl). Samples

were shaken and incubated for 15 min at 90°C. Subsequently, they reached room temperature (15 to 30°C) and were centrifuged at 2,000 × *g* for 15 min at 4°C. Supernatant absorbance at 531 nm was measured against the blank containing 2 mL of TBA/TCA/HCl solution in 1 mL of distilled water. The thiobarbituric acid reactive substances were calculated compared with a standard curve constructed with 1,1,3,3-tetramethoxypropane.

Two aliquots of homogenate (50 µL each) previously prepared for the TBARS determination were added with 1 mL 10% TCA and then centrifuged at 1,200 × *g* for 3 min at 4°C to measure protein oxidation. The first aliquot was used as a standard and added with 1 mL of 2 *M* HCl solution. The second aliquot was added with 1 mL of 2 *M* HCl containing 10 m*M* 2,4-dinitrophenyl hydrazine (**DNPH**). Samples were incubated for 1 h at room temperature (15 to 30°C) and shaken every 20 min, and then 1 mL of 10% TCA was added. The samples were vortexed for 30 s and centrifuged 3 times at 1,200 × *g* for 3 min at 4°C and the supernatant removed. Care was taken not to disrupt the pellet. The pellet was washed with 1 mL of ethanol:ethyl acetate (1:1), shaken, and centrifuged 3 times at 1,200 × *g* for 3 min at 4°C and the supernatant removed. The pellet was then dissolved in 1 mL 20 m*M* sodium phosphate 6 *M* guanidine hydrochloride buffer. Samples were then shaken and centrifuged at 1,200 × *g* for 3 min at 4°C. Carbonyl concentration was calculated on the DNPH treated sample at 360 nm with a Beckman Coulter DU800 (Beckman Instruments Inc., Brea, CA) and expressed as nanomoles carbonyl per milligrams protein. Protein concentration was calculated according to Biuret assay (Tokur and Korkmaz, 2007).

To determine hydroperoxides, 2 mL of homogenate (previously prepared for the TBARS determination) were added with 4 mL of CH_3OH and 2 mL of $CHCl_3$. The samples were vortexed for 30 s and were added with 2 mL of $CHCl_3$ and 1.6 mL of 0.9% NaCl. The samples were shaken for 1 min and then centrifuged at 3,500 × *g* for 10 min at 4°C. Two milliliters of lipid extract were sampled from the lower chloroform phase and processed with 1 mL of $CH_3COOH/CHCl_3$ and 50 µL of KI (1.2 g/1 mL distilled water).

Samples were stored for 5 min in a dark room and added with 3 mL of 0.5% of CH_3COOCd and then vortexed and centrifuged at 4,500 × *g* for 10 min at 40°C. Absorbance at 353 nm was measured against a blank title in which meat homogenate was replaced by 2 mL of distilled water. Results were expressed in micromoles per gram according to Buege and Aust (1978).

For each sample, these tests were performed: total aerobic mesophilic count (**TAMC**), total aerobic psychrophilic count (**TAPC**), and *Escherichia coli* count (**ECC**). Each test was performed on d 0, 2, 8, 10, 14, and 16 of storage at 4°C.

For each packing, 10 g of meat was removed aseptically, transferred to stomacher bags (bioMèrieux, Bagno a Ripoli, Firenze, Italy) containing 90 mL of 0.85% sterile tryptone salt solution, and homogenized using a stomacher (International Pbi S.p.a., Milan, Italy) for 60 s at room temperature. The homogenates were serially diluted 10-fold for microbial count. Total aerobic mesophilic count and ECC were performed using TEMPO System (bioMèrieux). Samples used for TAMC measurement were incubated for 40 h at 30°C and samples used for ECC measurement were incubated for 24 h at 37°C.

Total aerobic psychrophilic count was performed with pour-plate method (Harrigan, 1998) using Plate Count Agar (Oxoid Basingstoke, Hampshire, England) incubated aerobically for 7 d at 4°C.

Evaluation of Gas Mixture Changes during Storage

Headspace gas concentrations were measured at each time point using a gas detector provided with a syringe (PBI-Dansensor CheckPoint O_2/CO_2, Ringsted, Denmark). Each measurement was performed 3 times per sample, before opening the MAP pack, by penetrating the extruded polystyrene tray with a disposable needle inserted on the syringe of the instrument through an adhesive backed rubber septum (PBI Dansensor A/S, Ringsted, Denmark) placed on extruded polystyrene to prevent pack leakage between measurements. The mean value between the 3 recordings was used for the further statistical evaluations. Variation in gas mixtures were reported in Figure 1.

Statistical Analysis

A total of 16 samples from each animal were collected. The samples were randomly assigned to 2 treatments: 8 with CRY and 8 with WEE. The 8 samples for each packaging film were tested at 8 different storage days. A 2 × 8 (packaging films × storage days) treatment was performed. The data set was submitted to 2-way ANOVA using the general linear model (SAS Inst. Inc., Cary, NC). The packaging film, the storage time, and the binary interaction between these 2 variables were included as fixed effects.

Packaging types were analyzed separately, considering as fixed effect the storage time, and applying the post hoc Tukey's test for repeated measures to evaluate the differences between the experimental times (SAS). All the data were expressed as least square mean and mean SE. Significance was set as $P < 0.01$.

All data were submitted to Pearson's correlation indices analysis. The WHC, protein oxidation, hydroperoxides, and TBARS showed high correlation indices and they were analyzed with simple linear regression analysis, considering interaction between variables 2 at a time (SAS).

Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g). Statistics were performed using Statview (SAS) with statistical significance settled at $P < 0.05$.

RESULTS

Color (L*, a*, b*, and chroma), pH, WHC, drip loss, protein oxidation, and hydroperoxides were influenced by packaging materials ($P < 0.001$; Table 2). Storage time affected surface color (a*, b*, chroma, and hue), pH, WHC, drip loss, cooking loss, protein oxidation, TBARS, and hydroperoxide measurements ($P < 0.001$). The binary interaction between these 2 variables had effects on cooking loss, protein oxidation, hydroperoxides ($P < 0.001$), a*, chroma ($P < 0.01$), and pH ($P < 0.05$).

In the samples packaged using CRY top film, the instrumental a* values (redness) increased from the second to the eighth storage day ($P < 0.05$; Fig. 2) and it increased at 12 and 14 d after packaging ($P < 0.01$). Greater a* values were measured in CRY samples than in WEE samples at 2 ($P < 0.001$), 4 ($P < 0.01$), 6, and 8 ($P < 0.05$) d of storage.

In samples packaged with CRY, yellowness measured from the 2nd to the 14th storage day was greater than that measured on the day of packaging ($P < 0.01$; Fig. 2). Similarly, in samples packaged with WEE, yellowness measured from the second to the 14th storage day was greater than that measured on the day of packaging ($P < 0.05$). In the samples packaged with CRY, chroma values increased until the second day after packaging ($P < 0.01$; Fig. 2), remained constant up to the 10th day, and then decreased significantly at the 12th day ($P < 0.05$). In addition, the chroma values in the CRY samples were greater than in WEE samples, particularly at 2, 4 ($P < 0.001$), 6, 8 ($P < 0.01$), and 10 ($P < 0.05$) d of storage.

Table 2. Analyses of variance

Variables[1]	Packaging	Time	Packaging × time
L*	<0.0001	0.2230	0.9487
a*	<0.0001	<0.0001	0.0047
b*	0.0007	0.0006	0.3649
Chroma	<0.0001	<0.0001	0.0028
Hue	0.5709	0.0005	0.1563
pH	<0.0001	0.0004	0.0104
WHC	<0.0001	<0.0001	0.3595
Cooking loss	0.0004	<0.0001	<0.0001
Drip loss	<0.0001	<0.0001	0.2201
WBSF (cooked meat)	0.3190	0.0343	0.0036
TBARS	0.3184	<0.0001	0.1368
Protein oxidation	<0.0001	<0.0001	<0.0001
Hydroperoxides	0.0001	<0.0001	<0.0001

[1]L* = lightness; a* = redness; b* = yellowness; WHC = water holding capacity; WBSF = Warner Blatzer Shear Force; TBARS = thiobarbituric acid reactive substances.

In the samples wrapped with WEE top film, the hue values increased until the second ($P < 0.01$) and the fourth ($P < 0.05$) day after packaging (Fig. 2) whereas in CRY ones, the hue rose later (at 12 and 14 storage d; $P < 0.01$). The hue showed greater values in CRY packed samples only during the last storage day ($P < 0.05$).

In the samples packed with CRY top film, the pH values measured on d 2, 8, 12, and 14 of storage were greater than the packaging day ($P < 0.01$). In the samples packed with WEE, the pH values increased similarly to CRY samples but at 12th and 14th storage day decreased ($P < 0.05$; Fig. 3).

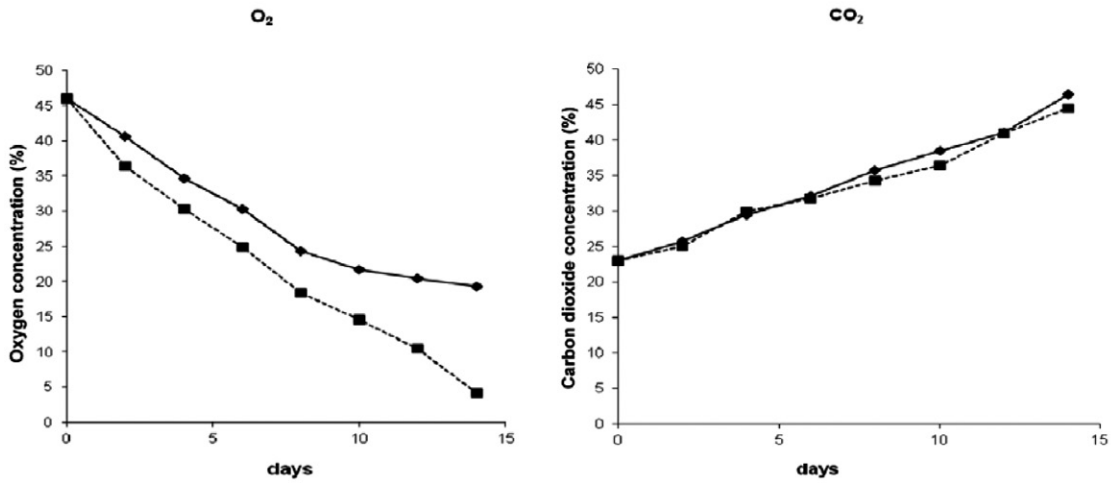

Figure 1. Gas packaging variation during storage. CRY = Cryovac LID 2050 (Passirana di Rho, Milano, Italy); WEE = Weegal PEBAR film (Vignola, Modena, Italy).

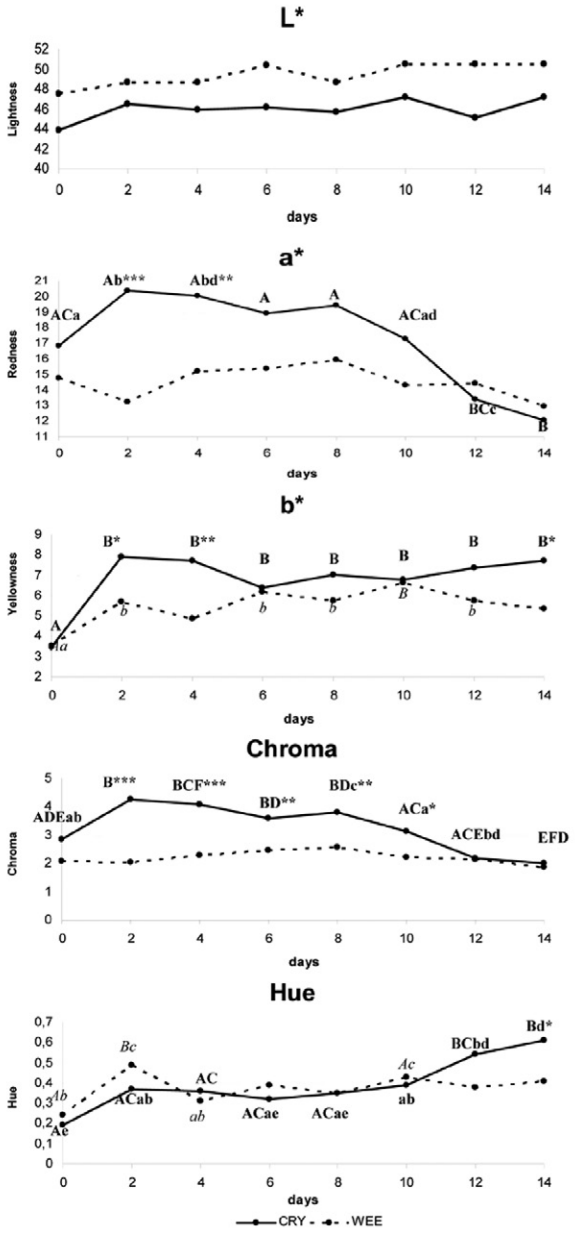

Figure 2. Effect of packaging on colorimetric parameters during storage. Different letters in the same packaging line show statistical differences (A–F: $P < 0.01$; a–e: $P < 0.05$). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ show statistical differences between packagings in the same day. CRY = Cryovac LID 2050 (Passirana di Rho, Milano, Italy); WEE = Weegal PEBAR film (Vignola, Modena, Italy); L* = lightness, a* = redness, b* = yellowness.

In both packaging types, WHC values decreased during storage ($P < 0.01$). Moreover, these values were greater in meat wrapped with WEE film than in CRY after the fourth day of storage (Fig. 4).

The drip losses increased with increasing storage time in both packaging and they were greater in samples wrapped with CRY top film from the second storage day to the trial end ($P < 0.001$; Fig. 3).

The WBSF values recorded on raw and cooked meat (Fig. 3) did not differ between the 2 packagings used. In the raw meat wrapped with the WEE top film, the WBSF values showed no difference during the storage period. In the raw meat packed with CRY top film, the WBSF values at the 12th day of storage were greater than the fourth, the sixth ($P < 0.01$), the second, and the packaging day ($P < 0.05$). Cooked meat packed with CRY top film showed lower WBSF values at the 4th and the 10th days of storage than the 12th ($P < 0.01$) and the packaging day ($P < 0.05$). Cooked meat packed with WEE showed at the sixth and eighth day of storage lower WBSF values than the packaging day and the second ($P < 0.01$) and the fourth days of storage ($P < 0.05$). Moreover, at the 10th and 12th days of storage these values were lower than the second day of storage ($P < 0.01$).

The TBARS increased with increasing storage time both in CRY and in WEE samples ($P < 0.01$) and without statistical differences between the 2 packaging films (Fig. 4).

The protein oxidation rose during storage time in both packaging systems ($P < 0.01$; Fig. 4). The meat preserved with CRY top film showed a more rapid and greater protein oxidation on T0 ($P < 0.05$) and then during storage time ($P < 0.001$).

The hydroperoxides increased during storage time in both packaging systems ($P < 0.01$; Fig. 4). The samples wrapped with CRY top film had hydroperoxide values lower than WEE at the first and the second storage day ($P < 0.01$) and greater from the 8th to the 14th day ($P < 0.001$).

Both in CRY and in WEE packed samples, there was a direct proportionality between oxidative parameters (Table 3). This direct proportionality was observed between hydroperoxides and protein oxidation. In samples packed with CRY top film, with the increase of 1 nmol DNPH/mg there was an increase of 0.4196 μmol/g ($R^2 = 0.9736$, $P < 0.01$) and in samples packed with WEE top film with the increase of 1 nmol DNPH/mg there was an increase of 0.3902 μmol/g ($R^2 = 0.9409$, $P < 0.01$). Moreover, between TBARS and hydroperoxides, an increase of 1 mg MDA/kg corresponds to a 2.7 mmol/g hydroperoxide gain in CRY packed meat ($R^2 = 0.9174$, $P < 0.01$) and to a 2.2 mmol/g hydroperoxide gain in WEE samples ($R^2 = 0.9094$, $P < 0.001$). The same direct proportionality was observed between TBARS and protein oxidation. In fact, an increase of 1 mg MDA/kg corresponds to a 6.9 mmol DNPH/mg protein oxidation gain in CRY packed meat ($R^2 = 0.9631$, $P < 0.01$) and to 5.6 mmol DNPH/mg protein oxidation gain in WEE samples ($R^2 = 0.9225$, $P < 0.01$). Moreover, angular coefficients measured between hydroperoxides and protein oxidation ($P < 0.01$), TBARS and hydroperoxides ($P < 0.001$), and TBARS and protein oxidation ($P < 0.001$) were affected by packaging.

Hydroperoxides, TBARS, and protein oxidation had an indirect linear proportionality with the WHC and showed high values of R^2 ($P < 0.001$). In particular, a

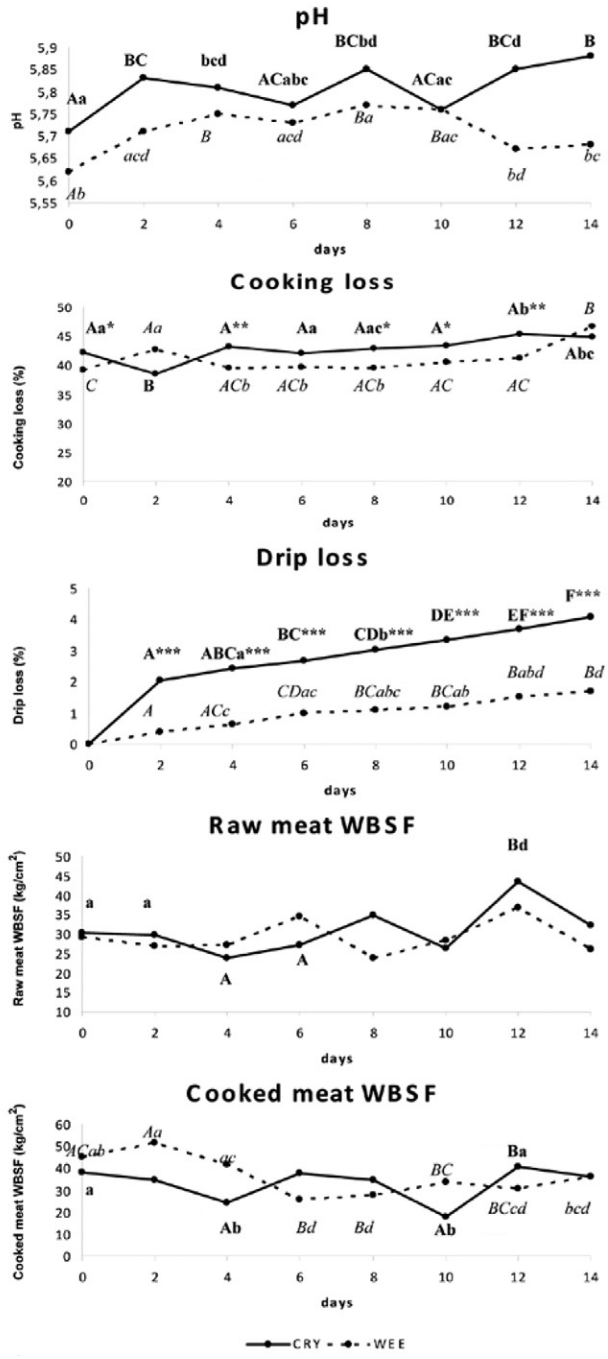

Figure 3. Effect of packaging on pH, cooking loss, drip loss, Warner Blatzer Shear Force (WBSF) on raw and cooked meat during storage. Different letters in the same packaging line show statistical differences (A–F: $P < 0.01$; a–d: $P < 0.05$). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ show statistical differences between packagings in the same day. CRY = Cryovac LID 2050 (Passirana di Rho, Milano, Italy); WEE = Weegal PEBAR film (Vignola, Modena, Italy).

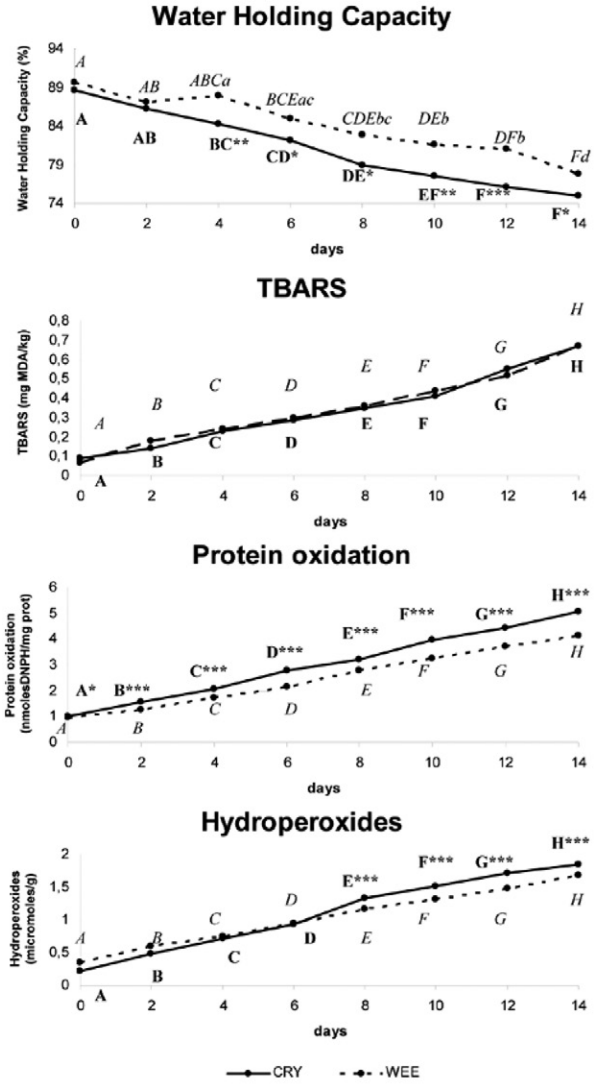

Figure 4. Effect of packaging on water holding capacity, thiobarbituric acid reactive substances (TBARS), protein oxidation, and hydroperoxides during storage. Different letters in the same packaging line show statistical differences (A–H: $P < 0.01$; a–d: $P < 0.05$). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ show statistical differences between packagings in the same day. MDA = malondialdehyde; DNPH = 2,4-dinitrophenyl hydrazine; prot = protein; CRY = Cryovac LID 2050 (Passirana di Rho, Milano, Italy); WEE = Weegal PEBAR film (Vignola, Modena, Italy).

WHC decrease of 1% corresponded to an increase in hydroperoxides equal to 0.1 µmol/g in CRY samples and to 0.08 µmol/g in WEE packed. Both in CRY and in WEE samples a WHC decrease of 1% corresponded to a protein oxidation increase equal to 0.23 mmol DNPH/mg and to a TBARS increase equal to 0.03 mg MDA/kg.

In both types of packaging, the results of TAMC and TAPC showed a gradual increase in bacterial load over time. At T0, values of TAMC were 5.0 log cfu/g in CRY samples and 5.3 log cfu/g in WEE samples (Fig. 5). At the 14th day, the end of time of storage at refrigeration temperature, the bacteria present values of 9.2 log cfu/g in CRY samples and 9.6 log cfu/g in WEE samples (Fig. 5), with a mean values in the 2 samples of 9.4 log cfu/g.

On the day of packaging, TAPC values were 4.4 log cfu/g and 4.3 log cfu/g in CRY and WEE samples, respectively (Fig. 5). At 14th day, the mean value of psychrophilic microbes in the 2 samples had reached 8.4 log cfu/g. The EEC has maintained very low loads over time, with average values in the 2 samples at 14th day of 1 log cfu/g.

Table 3. Linear regression analysis

Parameters	CRY[1]	WEE[2]	*P*-value
Hydroperoxides × protein oxidation			
R^2	0.9736	0.9409	–
P-value	$P < 0.01$	$P < 0.01$	–
Intercept	0.1655	0.0586	NS[3]
Angular coefficient	0.4196	0.3902	**
Hydroperoxides × TBARS[4]			
R^2	0.9174	0.9094	–
P-value	$P < 0.01$	$P < 0.001$	–
Intercept	0.1171	0.2601	NS
Angular coefficient	2.6789	2.2368	***
Protein oxidation × TBARS			
R^2	0.9631	0.9225	–
P-value	$P < 0.01$	$P < 0.01$	–
Intercept	0.6478	0.5523	NS
Angular coefficient	6.9357	5.6270	***
Hydroperoxides × WHC[5]			
R^2	0.8507	0.7581	–
P-value	$P < 0.001$	$P < 0.001$	–
Intercept	9.5115	8.1217	NS
Angular coefficient	–0.1036	–0.0846	*
Protein oxidation × WHC			
R^2	0.8443	0.7434	–
P-value	$P < 0.001$	$P < 0.001$	–
Intercept	22.7180	20.3680	NS
Angular coefficient	–0.2426	–0.2129	NS
TBARS × WHC			
R^2	0.7877	0.7145	–
P-value	$P < 0.001$	$P < 0.001$	–
Intercept	3.0340	2.9914	NS
Angular coefficient	–0.0332	–0.0317	NS

[1]CRY = Cryovac LID 2050 (Passirana di Rho, Milano, Italy).

[2]WEE = Weegal PEBAR film (Vignola, Modena, Italy).

[3]NS = not significant.

[4]TBARS = thiobarbituric acid reactive substances.

[5]WHC = water holding capacity.

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

In addition, at 14th day all meat samples were characterized by unpleasant odors and surface slime. No statistically significant differences were observed in microbial growth in the 2 types of samples over time ($P > 0.05$).

DISCUSSION

Food quality is greatly influenced by the packaging characteristics, its plastic properties, and its gas and vapor transmission rates (Lee, 2010). This trial found that, for the same gas mixture used in MAP packaging, differences in the top film gas permeability significantly influenced chemical and physical properties of veal calf meat.

Color variables were influenced by different packaging properties. The redness (a*) and chroma showed changes only on meat packaged using the CRY top film, characterized by a permeability to gas. These values initially increased and then, after the 10th day, tended to decrease. Gas mixture were the same at T0 but changed differently during the storage period. Gas transition through the top films depends on various factors: gas partial pressure, storage temperature, relative humidity (constant parameters in both packaging), and film characteristics (thickness and gas transmission rates; Lee et al., 2008). Probably, the greater oxygen permeability resulted in a greater introduction of this gas from the atmosphere with consequent increased oxygen availability in the tray. These conditions could have promoted the initial oxygenation of myoglobin to oxymyoglobin (O'Grady et al., 2000), which gives meat a cherry red color, and then the oxidation of oxymyoglobin to metmyoglobin (Mancini et al., 2005), responsible for meat browning. Zakrys et al. (2008a) found similar results in beef meat, which revealed a negative correlation between the redness and the storage time and between the oxygen concentration and the metmyoglobin formation during storage period. The yellowness increased in the first 24 h and more rapidly in meat wrapped with the more permeable film. Because yellowness is affected by lipid chemical composition (Mancini et al., 2005), the greater oxygen concentration, due to the greater gas permeability of this film, promoted the lipid oxidation (Okayama et al., 1995; Estevez and Cava, 2004) particularly of PUFA (Kanner, 1994), which could cause a greater variation of yellowness (Faustman and Cassens, 1990; O'Grady et al., 1998; Tang et al., 2006). Kim et al. (2010) reported that a greater oxygen concentration increased the lipid oxidation, which reduced the color stability. Moreover, free radical formation resulting from lipid oxidation is closely associated with the myoglobin oxidation (Faustman et al., 1989), so the lipid oxidation promoted myoglobin oxidation (Lin and Hultin, 1977). Oxidation of unsaturated fatty acids produced hydroperoxides and TBARS. These tend to increase during the

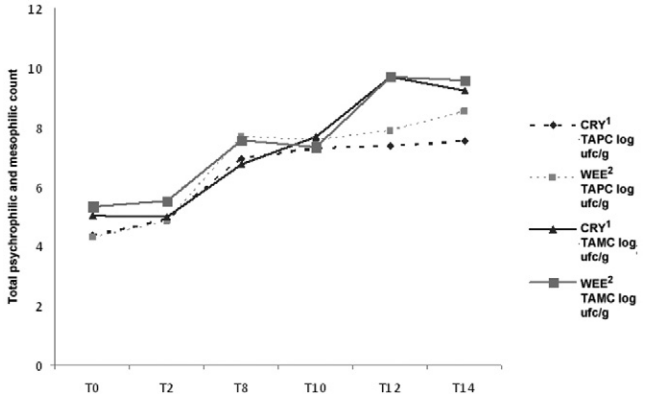

Figure 5. Total aerobic mesophilic count (TAMC) and total aerobic psychrophilic count (TAPC) variation during storage. [1]CRY = Cryovac LID 2050 (Passirana di Rho, Milano, Italy). [2]WEE = Weegal PEBAR film (Vignola, Modena, Italy).

storage time confirming a positive correlation between storage time and lipid oxidation (Gray, 1978; Lund et al., 2007; McMillin, 2008). Therefore, although the veal calf meat has a reduced myoglobin concentration, it shows the same color variations of beef meat. The oxidation of myofibrils myosin occurs naturally in meat during aging (Huff Lonergan et al., 2010). Even in the present study a positive correlation between protein oxidation and storage time was found. Protein oxidation processes were greater in samples packed using the film with greater oxygen transmission rate. The protein oxidation increased during storage and showed a positive correlation with the oxygen (Zakrys et al., 2008a,b). The greater protein oxidation values, resulting from the packaging with the greater gas permeability, may be due to a greater oxygen access and availability.

Water holding capacity and drip loss are closely interconnected through an indirect proportionality (Zhang et al., 2006). During storage time, results obtained showed a decreasing trend of the WHC and an increasing trend of drip and cooking loss. These variables are influenced by animal genetics (Hamilton et al., 2000), storage time (Kristensen and Purslow, 2001), temperature (Maribo et al., 1998), and protein oxidation (Xiong, 2000; Rowe et al., 2004a,b). These factors did not change in the 2 theses considered, except for the protein oxidation. In fact, the meat with greater protein oxidation also showed lower WHC values and consequently greater drip losses. Melody et al. (2004) reported that protein oxidation affects the meat WHC, causing the conversion of some AA, including histidine, into carbonyl derivatives (Levine et al., 1994; Martinaud et al., 1997) resulting in the formation of intra- and interprotein disulfide bonds (Stadtman, 1990; Martinaud et al., 1997).

A simple linear regression about oxidative variables was made about the interrelation between oxidative variables (TBARS, hydroperoxides, and protein oxidation) and WHC. High R^2 values were found between WHC and oxidative variables, particularly with the protein oxidation. Several authors observed in beef and pork meat a positive correlation between storage time and protein oxidation and a negative relationship between protein oxidation and the WHC (Morrison et al., 1998; Melody et al., 2004; Zhang et al., 2006; Huff-Lonergan et al., 2010). Many researchers have observed and demonstrated a positive correlation between lipid oxidation and storage time (Jayasingh et al., 2002; Zakrys et al., 2008a). Different authors correlated the fatty acid oxidation and the amount of meat hydroperoxides (Gray, 1978; McMillin, 2008) and TBARS (Lund et al., 2007) used for measuring lipid oxidation. In fact, these 2 variables showed a mutual $R^2 > 0.90$. Moreover, between protein oxidation and TBARS there was a $R^2 > 0.95$. As noted by several authors, oxygen promotes lipid and protein oxidation (Zakrys et al., 2008a). According to these results it is possible, through the calculation of 1 meat variable (for example protein oxidation), to predict the performance of the others, obtaining economic and practical advantages.

The TAMC and TAPC counts at T0 were very low, showing an acceptable microbiological quality of meat as indicated by the EU no. 2073/2005. The oxygen value probably contributed to the selection of aerobic spoilage flora. In fact both the TAMC and TAPC at eighth day had reached high load with values of 7.56 and 6.74 log cfu/g for the TAMC and 6.95 and 7.69 log cfu/g for TAPC, in CRY and WEE samples, respectively.

At d 8 in both samples, there was a change in the composition of the mixture of gases; the percentage of O_2 was decreased by 20 to 25% with values showing a gradual increase in CO_2 due to microbial metabolism and to the different permeability of the protective film used.

The results we obtained show that the progressive decrease of O_2 led to the selection and then the gradual increase of aerobic and microaerophilic flora such as *Lactobacillus* spp. (Borch et al., 1996); the latter together with the residual aerobic flora has determined, at the end of shelf life, the onset of unpleasant odors and slime. According to some authors, these signs of alteration of meat occur when TAMC and TAPC have reached values between 10^7 and 10^8 cfu/g. Instead, other authors observed these signs when the bacteria load exceed the value of 3.2×10^9 cfu/cm^2.

In fact, during storage, the alteration of chilled meat is due to the modification of available substrate with the increase in time of low molecular weight compounds and to the selection of a particular microbial association, the so-called specific spoilage organisms (**SSO**; Jay et al., 2005; Nychas et al., 2008; Limbo et al., 2010). Furthermore, Nychaset et al. (2008) argue that the deterioration of the meat depends primarily on a small fraction of SSO, called ephemeral spoilage organisms (**ESO**). Moreover, environmental factors (e.g., storage temperature, gas mixture, pH) are particularly important because they could influence the microbial replication and the subsequent meat alteration (Borch et al., 1996; James and James, 2002).

The metabolic activity of bacteria also involves changes of meat color for the reduction of surface tension of O_2 and the production of oxidizing agents (Insausti et al., 2001; McMillin, 2008). In this trial, the packaging film did not affect the microbial parameters tested (TAMC, TAPC, and ECC).

The packaging industry investigates the effects of gas mixture and packaging materials on fresh meat. Particularly, quality and shelf life of meat is influenced not only by gas mixture but also by the top film characteristics. For a given gas mixture, it was observed that the

fresh meat shelf life is extended by using films with a low oxygen transmission rate (Gill and Molin, 1991). Even in this trial it is clear that films with greater oxygen permeability, as can maintain color most appreciated by the consumer, encourage lipid and protein oxidation, changing meat chemical, physical, and organoleptic properties.

Conclusion

The results showed that texture of LM slices from veal calf was affected by packaging materials and properties, particularly by gas transmission rates. Top film with reduced gas permeability slowed down and reduced the meat deterioration from a chemical, physical, and colorimetric point of view. These variables undergo changes that reduce fresh meat quality during storage, so they are important for assessing veal calf meat quality in addition to microbiological features. Additionally, the regression equations between meat oxidative variables and water holding capacity suggest an estimate of some of these variables by calculating just 1 of them. Moreover, the present study showed no differences in bacterial count in the 2 investigated packaging materials.

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