Influence of gas mixture on quality and shelf life of veal calf meat

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Abstract

The paper investigates the effect of two different gas mixtures on chemical, physical and microbiological quality of veal meat packed in modified atmosphere during chill storage. Experimental gas atmospheres tested were O₂46 (46% O₂, 31% N₂ and 23% CO₂) and O₂70 (70% O₂, 8% N₂ and 22% CO₂). Samples were stored at 4°C for 14 days and tested at 0, 2, 4, 6, 8, 10, 12 and 14 days after packaging. The different O₂ concentration influenced many parameters. Lower O₂ concentration showed a greater increase of a* values (P<0.01) from the 2nd to the 8th packaging day, and a lower increase in drip loss values, thiobarbituric acid reactive substances and protein oxidation (P<0.001). Total aerobic mesophilic and psychrophilic count showed a gradual increase in the pre-slaughtering management, but also by biochemical and microbiological modifications during post-mortem storage (Sierra et al., 2006). The gases commonly used for MAP are oxygen, carbon dioxide and nitrogen. Oxygen function was to maintain myoglobin in its oxygenated form and stabilise meat redness; carbon dioxide inhibited the growth of aerobic spoilage bacteria and nitrogen had a filler function (Jakobsen and Bertelsen, 2000). Several authors showed that high oxygen maintained oxyhemoglobin pigment and cherry red colour (O’Grady et al., 2000), but may induce other oxidative reactions resulting in undesirable flavours (Rhee and Ziprin, 1987; Estevez and Cava, 2004). Fatty acids oxidation caused rancidity and changed chromatic, physical and nutritional meat properties (Kanner, 1994). High oxygen during fresh meat chill storage resulted in a low enzyme activity, collagen solubility with worse tenderness (Rowe et al., 2004a) and caused the formation of protein complex and non-enzymatic browning products (Mercier et al., 2004).

The present study aims to investigate the relationship between two different gas mixtures in MAP and a number of indicators, such as colour stability, lipid and protein oxidation, tenderness and microbiological properties in veal calf meat. The choice of investigating veal meat was dictated by the poor literature about the effects of MAP on a bovine fresh meat fundamentally different from beef both from a physical and chemical perspective, such as low fat content, and a good smooth Browning products (Mercier et al., 2004), eating quality (Monteiro et al., 2013), fatty acids profile (Alldai et al., 2012), low myoglobin concentration (De Palo et al., 2013).

Introduction

Modified atmosphere packaging (MAP) in food industry is recognised to maintain the desired properties of meat for the desired period of storage and display (McMillin, 2008). Furthermore, fresh meat packaging is carried out to protect products and avoid cross-contamination, to facilitate food handling and identification, to present product in an attractive way to consumers and especially to reduce gas and water vapour deteriorative effects, delaying biological and physical food spoilage (Yam et al., 2005).

Different meat organoleptic properties are usually considered in meat evaluation by consumers, such as colour, flavour, tenderness and texture (Bredahl et al., 1998). These meat characteristics not only are affected by the pre-slaughtering management, but also by biochemical and microbiological modifications during post-mortem storage (Sierra et al., 2006). The gases commonly used for MAP are oxygen, carbon dioxide and nitrogen. Oxygen function was to maintain myoglobin in its oxygenated form and stabilise meat redness; carbon dioxide inhibited the growth of aerobic spoilage bacteria and nitrogen had a filler function (Jakobsen and Bertelsen, 2000).

Materials and methods

Animals

A total of 8 Italian Holstein Frisian bull calves were used in the present trial. They were reared in the same farm. Calves were housed individually in pens (1.2 m² per calf) during the first 8 wk and thereafter in groups of 4 calves (1.8 m² per calf) per pen as described by De Palo et al. (2013). After birth, animals received mother’s colostrum milked and supplied by artificial teats within 2 h of life. After the first colostrum supply, it was administered three times per day. Then, after 48 h and during the first 8 wk, they were fed commercial milk replacer (Alpuro B.V., Uddel, the Netherlands) 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, housing, and feeding techniques were adopted according the EU directive 2008/119. Animals were slaughtered at the age of 218 d with an average body weight of 237 kg, after transportation to a slaughterhouse 32 km far from farm, with a travel made almost exclusively on an state expressway.

Meat sampling and packaging

The half carcasses were stored at 4±0.5°C for 24 h and then 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). Sixteen samples of longissimus dorsi muscle (LM) were collected from each of the 8 carcasses, for a total of 128 samples. Samples were placed in extruded polystyrene trays (AERPack PCM030; Coopbox Italia, Reggio Emilia, Italy) packed in MAP with the same barrier film (Cryovac LID 2050; Cryovac, Passirana di Rho, Italy) (Table 1) and two different gas mixtures: eight samples were packed with 46% O₂, 31% N₂, 23% CO₂ (O₂46) and eight with 70% O₂, 8% N₂, 22% CO₂.

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(O2:70). The MAP packed samples were processed using a heat sealer (CVS-PN 35; Mondini S.p.A., Cologne, Italy). Each sample (packed in each tray) consisted of 2 slices (thickness of 0.5 cm) and 3 pieces of meat. One slice was used to evaluate chromatic parameters and to perform microbiological analyses. The second slice was used to calculate water holding capacity (WHC), collagen and oxidative variables [protein oxidation, 2-thiobarbituric acid (TBA), i.e. thiobarbituric acid reactive substances (TBARS), and hydroperoxides]. One of the three pieces of meat (a parallelepiped with 3 by 6 by 6 cm sides) was used for the Warner Blazzer shear force (WBFS) evaluation on 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 a 1.5 cm per side) was used to measure cooking loss.

Samples were transported under controlled temperature (+4±0.5°C) to the laboratories 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 analysed 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**

**Colour evaluation**

Surface meat colour 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 colour of calf slices was determined according to the CIE L*, a*, b* (CIE, 1976) colour 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 lamp with a reading surface of 8 mm diameter. These measurements were performed in three different points of each sample. Moreover, on each point, three measurements were performed by rotating the detector system of 90° from the previous one, for a total of nine measurements for each sample. The 9 readings per sample were made at each time point and averaged for statistical analysis. The colorimeter was calibrated on the Hunterlab colour 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)½ and hue (°)=tan⁻¹(b/a) according to Little (1975) and Mancini and Hunt (2005).

**pH, water holding capacity, cooking loss and drip loss**

Intramuscular pH was recorded using a portable pH meter with glass electrode shaped to easily penetrate meat (Carlo Erba pH 710; Carlo Erba Reagenti, Milano, Italy). Before each measurement, the pH meter was automatically calibrated for muscle temperature and using solutions with 4 and 7 pH values (Crison, Lainate, 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 with an Allegra 64R centrifuge (Beckman Instruments Inc., Brea, CA, USA). After centrifugation, fluid was removed from the remaining samples 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 (IW)] 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 55C-TT-30-36; Omega Engineering Inc.). Cores (1.27 cm diameter) were cut from each cooked sample parallel to the muscle fiber direction. The cores were sheared perpendicular to the muscle fibres orientation using an Instron 1140 apparatus (Instron, High Wycombe, UK) provided with a computer, using a crosshead speed of 50 mm/min and a load cell of 50 N. 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 kg/cm². Total collagen and collagen solubility were calculated as described by De Palo et al. (2013).

**Thiobarbituric acid reactive substances, protein oxidation and hydroperoxides**

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 homogenised in 20 mL of 100 mM phosphate buffer (pH 7.0) for 2 min using a homogeniser. An aliquot of homogenate (1 mL) was transferred to a glass tube and added with 50 L of butyraldehyde (7.2% in ethanol) and with 1950 L of 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 in a bath. Subsequently, they reached room temperature (15 to 30°C) and were centrifuged at

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**Table 1. Properties of film package.**

| Property                                      | Value   |
|----------------------------------------------|---------|
| Thickness, μm                                | 25      |
| Density, g/cm²                               | 0.97    |
| O₂ permeability (at 23°C and 0% RH), cm³/m²/24 h | 15      |
| CO₂ permeability (at 23°C and 0% RH), cm³/m²/24 h | 54      |
| Moisture vapour transmission rate (at 35°C and 90% RH), g/m²/24 h | 16      |
2000×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 TBARS were calculated compared with a standard curve constructed with 1,1,3,3-tetramethoxypropane.

Two aliquots of homogenate (50 L each) previously prepared for the TBARS determination were added with 1 mL 10% TCA and then centrifuged at 1200×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 mM 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 sample s were vortexed for 30 s and centrifuged 3 times at 1200×g for 3 min at 4°C and the supernatant removed. The pellet was washed with 1 mL of ethanol:ethyl acetate (1:1), shaken, and centrifuged 3 times at 1200×g for 3 min at 4°C and the supernatant removed. The pellet was then dissolved in 1 mL 20 mM sodium phosphate 6 M guanidine hydrochloride buffer. Samples were then shaken and centrifuged at 1200×g for 3 min at 4°C. Carbonyl concentration was measured on the DNPH treated sample at 360 nm with a Beckman Coulter DU800 (Beckman Instruments Inc.) and expressed as micromoles carbonyl per milligrams protein. Protein concentration was measured 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 CH3COOH/CHCl 3 and 50 □L of KI (1.2 g/1 mL distilled water). Samples were then shaken and centrifuged at 4500×g for 10 min at 40°C. The homogenates were serially diluted 10-fold for microtubal count. Total aerobic mesophilic count and ECC were performed using TEMPO System (bioMérieux, Italy) for 60 s at room temperature. The homogenates were serially diluted 10-fold for microbial count.

**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 O2/CO2; PBI-Dansensor’s, Ringsted, Denmark). Each measurement was performed three times per sample, before opening the MAP pack, holing across the extruded polyolefins tray, on a sample never holed before, with a disposable needle inserted on the syringe of the instrument, inserted through an adhesive backed rubber septum (PBI Dansensor’s), placed on extruded polystyrene to prevent pack leakage between measurements. The mean value between the three recordings was used for the further statistical evaluations.

**Statistical analysis**

A total of 16 samples from each of 8 carcasses were collected. The samples were randomly assigned to 2 treatments: 8 with O246 and 8 with O270. The 8 samples for each gas mixture were tested at 8 different storage days. A 2×8 (gas mixture×storage days) treatment was performed. The data set was submitted to 2-way ANOVA using the general linear model (SAS Inst. Inc., Cary, NC, USA). The gas mixture, the storage time, and the binary interaction between these 2 variables were included as fixed effects.

Gas mixtures were analysed 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, 1999). All the data were expressed as least square mean and mean SE. Significance was set as P<0.05. Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g). Statistics were performed using Statview (SAS, 1999) with statistical significance settled at P<0.05.

**Results and discussion**

In both packagings, CO2 slowly increased during storage and O₂ decreased, but in different ways (Figure 1). In O246, oxygen decreased till 0% at the 14th storage day, with a greater drop between the 2nd and 4th day. In O270, oxygen dropped to 40.4% at the 14th storage day, with a greater drop between the 6th and 8th day.

Results showed an influence of gas mixture on the main quality parameters of fresh veal...
calf meat, such as colour, chemical, physical, oxidative and microbiological properties (Table 2). These data reflected the results obtained by Zakrys et al. (2008, 2009) on beef, by Lund et al. (2007a, 2007b) on pork, and by Berruga et al. (2005) on lamb.

The present trial showed that the gas mixture used for MAP influenced oxidation is responsible for meat colour and its main sensory characteristics. Gas exchange between inside and outside the trays was determined by several factors: gases partial pressure and differences in the partial pressure on both sides of the film, storage temperature and relative humidity, but also top film type, thickness and its gas permeability (Lee et al., 2008; De Palo et al., 2013).

Different gas mixtures influenced colour parameters recorded (Figure 2). The instrumental a* values displayed a negative correlation with days, indicating a decrease in red colour of samples packed in O246 and O270 over time, as described by Zakrys et al. (2009) about beef steaks. The samples packed in O270 showed a* values constant until the 12th day and then significantly lower on the 14th (P<0.01), so the decrease in the redness was more delayed in the presence of a higher concentration of O2, as already widely described in literature. Previous finding by Jayasingh et al. (2002) reported that ground beef packed in high oxygen MAP maintained a bright red colour until the 10th day of storage. Sarhein et al. (1999) also reported a gradual discoulouration in beef loin steaks packed under high oxygen between days 3 and 10 of display. According to John et al. (2005) beef steaks packaged under high oxygen had a desirable red colour until the seventh day of storage, and then browned by day 14. In the current study it appears that the O270 atmosphere also provided lower values of redness and chroma compared to O246, especially at 48 h (P<0.05) and 96 h (P<0.01) after the packaging. Oxygen maintained the fresh meat red colour through the formation of oxymyoglobin (Mancini and Hunt, 2005) but, in this case, it probably had a reduced effect on the oxidative stability on veal calf meat, maybe due to a lower myoglobin content of this meat because of young slaughtering age and animal feeding (Gariépy et al., 1998). Important were also top film properties, particularly their permeability to water vapour and gas, and the residual concentration of O2, which was progressively reduced in the package over storage time.

The instrumental L* and b* values showed an opposite trend compared to a*, indicating an increase in lightness over time and a rise of yellowness up to 2 storage days. The oxidation does not involve only the myoglobin, but also lipids with a consequent increasing in meat yellowness (O'Grady et al., 2000; Tang et al., 2006). The lipid oxidation involved the formation of free radicals, which influenced myoglobin oxidative stability (Faustman et al., 1989): therefore lipid oxidation promoted myoglobin oxidation (Lin and Hultin, 1977).

Although high oxygen levels promoted oxidation (Estevez and Cava, 2004), instrumental b* values showed no significant differences between the two gas mixtures. These values were very low, and appeared acceptable relative to oxidation of lipids. Moreover, the O270 samples maintained higher L* values, indicating that they became lighter than those packed with less oxygen during storage. The increased lightness over time was also linked to the cell membranes denaturation in muscle myofibrils, moving intracellular water towards the extracellular space (Yu et al., 2005; Mortensen et al., 2006). So, meat lightness was linked to WHC. In fact, the WHC was defined as the meat ability to retain water (Offer and Trinick, 1983), which is normally present into muscle cells and bound to protein in the intercellular spaces with a reduced mobility (Huff-Lonergan and Lonergan, 2005). The proteolysis occurring physiologically with cell death reduced WHC (Huff-Lonergan and Lonergan, 2005). In the current trial the WHC (Figure 3) progressively reduced over time showing no significant differences between the two atmospheres. Meat with low WHC had higher drip loss (den Hertog-Meischke et al., 1997). Both in O246 and O270 samples, drip loss increased over storage time, but slices packed under higher oxygen showed significantly greater drip loss, probably due to reduced proteolysis. A negative correlation between degradation of desmin by proteolysis and drip loss has been demonstrated by Melody et al. (2004) and Morrison et al. (1998). These proteins degrade from 45 min to 6 h post-mortem. Degradation of these proteins produces the expulsion of water from the intramyofibrillar spaces and the retention of water in the intracellular space. So, reduced degradation of proteins such as desmin causes an increased shrinking of the muscle cell and, then, of drip loss. Lund et al. (2007a) also described an increase in drip loss over chill storage time in pork slices packed under high O2 concentrations. Moreover, desmin degradation, played an important role in meat WHC as well as contributed to meat aging and tenderising during post-mortem storage (Huff-Lonergan et al., 1996; Zhang et al., 2006). In beef meat the calpain system (µ-calpain and m-calpain) is believed to play a central role during post-mortem tenderisation (Huff-Lonergan et al., 1996). Because calpains contain histidine, protein oxidation influenced their activities (Lametsch et al., 2007), degrading histidine and inactivating or inhibiting their protease activity (Harris et al., 2001; Rowe et al., 2004a, 2004b). In fact, in the current study, instrumental WBSF of cooked meat, although there were some differences between the two gas mixtures, declined early during storage and then remained constant over time (Figure 4). According to Lund et al.
(2007a) high oxygen atmosphere storage promoted a significant decrease in tenderness for beef and porcine meat, due to protein cross-linking from oxidative processes. These results coincide with consumer assessment on beef steaks packed in MAP with oxygen levels higher than 40%, imparting more toughness and less juiciness potentially due to protein oxidation (Zakrys et al., 2009). So, protein oxidation is considered to play a role in meat tenderness, because of its demonstrated action in controlling proteases (Starke-Reed and Oliver, 1989).

Protein oxidation increased during storage time both in O$_2$46 than in O$_2$70 samples (Figure 5), showing values significantly greater in slices packed with higher oxygen compared to others since from the second day.

Figure 2. Effect of gas mixture on colorimetric parameters during storage. Different letters in the same gas mixture line show statistical differences (A-D, P<0.01). *P<0.05 and **P<0.01 show statistical differences between gas mixtures in the same day.

Figure 3. Effect of gas mixture on pH, water holding capacity, cooking and drip loss during storage. Different letters in the same gas mixture line show statistical differences (A-E, P<0.01). *P<0.05, **P<0.01 and ***P<0.001 show statistical differences between gas mixtures in the same day.
Previous studies by Lund et al. (2007a), Zakrys et al. (2008, 2009), showed increase in protein oxidation with the growth of O2 concentration, resulting in lower tenderness and juiciness, flavour deterioration and discoloration (Xiong, 2000). Physical and chemical changes in oxidised meat protein include hydroperoxides formation.

Lipid oxidation, measured by TBARS, also increased during shelf-life and in relation to oxygen concentration. In fact, O₂70 slices had TBARS values significant greater than O₂46 since 6th day of storage. These results are supported by previous studies (O’Grady et al., 2000; Zakrys et al. 2008, 2009), which reported that oxidative stability decreased with storage time and treatments aimed to modify TBARS concentration may be useful only in the presence of low O₂.

The positive correlation between storage time and lipid oxidation are confirmed by the increased levels of hydroperoxides formed by unsaturated fatty acids oxidation (McMillin, 2008), and TBARS, used as lipid oxidation measurement (Lund et al., 2007a). Despite veal calf meat usually presents low levels of myoglobin (Gariépy et al., 1998), it showed the same discoloration of beef meat.

The two different atmosphere packaging did not affect total aerobic mesophilic and psychrophilic counts. In addition the results related to the TAMC and the TAPC (Figure 6) showed a significant increase in bacterial load over time with an increase of about 4 log cfu/g and 3 log cfu/g respectively in association with the appearance of unpleasant odors and slime formation.

Figure 4. Effect of gas mixture on total collagen, collagen solubility and Warner Blatzer shear force on cooked meat during storage.

Figure 5. Effect of gas mixture on thiobarbituric acid reactive substances, protein oxidation, and hydroperoxides during storage. Different letters in the same gas mixture line show statistical differences (A-H, P<0.01). **P<0.05, ***P<0.01 and ****P<0.001 show statistical differences between gas mixtures in the same day.
surface. Moreover, at 8th day, the TAMC and the TAPC have reached values of about 7 log cfu/g, which is considered as the upper limit for acceptable microbiological quality meat as defined by ICMFS (1986).

Conclusions

The results clearly showed that O₂70 atmosphere was responsible for an earlier decline in meat quality, evidenced by increased drip and cooking loss and higher protein and lipid oxidation. Particularly, protein oxidation, higher in O₂70 MAP, has proved critical in determining meat quality (colour, WHC, drip and cooking loss) and shelf-life. The mixture with the highest concentration of oxygen did not benefit colour, because of the particular chemical and colourimetric properties of veal calf meat. So, it seems more advantageous to use MAP gas mixtures with O₂ concentration of 46 than 70%, in order to improve shelf-life and avoid an increase in oxidative reactions and physical changes related to them.

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Figure 6. Total aerobic mesophilic and psychrophilic count.
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