INTRODUCTION

Over the last decade, the poultry meat industry has developed a diverse range of products and presentations for consumers. In particular, the presentation of poultry meat has undergone modifications through the use of modified atmosphere packaging (MAP) with cold storage. This imposition by the industry on consumers has increased opportunities and opened markets by allowing poultry distribution to be managed differently, and by improving the shelf life and consumer handling of poultry meat.

The usual gas mixtures used for retail sliced poultry meat under MAP are 20% CO₂, 70% O₂, and 10% N₂, giving a shelf life of approximately 8 d. The elevated oxygen levels used in high-oxygen MAP saturate meat pigments with O₂ and slow the formation of metmyoglobin on the surface. Nevertheless, they accelerate lipid oxidation, off-flavor development, and premature browning during cooking (Cornforth and Hunt, 2008). Other anaerobic gas mixtures can extend the shelf life by 1 or 2 wk. These vary according to the quality, color, and temperature of the poultry meat (Fraqueza et al., 2008; Fraqueza and Barreto, 2009), although the aspect (color) of the meat is not so attractive to consumers. Processors continue to search for alternative technologies and new approaches to improve the acceptability, shelf life, and safety of fresh retail meat.

The most recent meat packaging technology, which uses low levels of CO, has been studied and adopted for red meat (Sorheim et al., 1997; Jeong and Claus, 2011). This packaging method presents several advantages over aerobic packaging (AP) or high-oxygen MAP.

Carbon monoxide, a colorless, odorless, and tasteless gas, is the result of incomplete combustion of materials containing carbon (Vreman et al., 1995). The main role of low levels of CO in MAP is to give a stable...
cherry-red color to the meat, a result of the strong linkage of CO to the porphyrin ring of myoglobin and the formation of carboxyhemoglobin. This molecule is more resistant to oxidation than is oxyhemoglobin because it is stable during storage (Sørheim et al., 1997).

The different mixtures of gases with CO for meat packaging can be balanced with CO2, N2, O2, air, or argon. Several gas mixture compositions have been tested on packaged beef or derived products, with color and shelf-life periods stabilized under refrigeration, which can be 30 to 35 d, compared with 3 to 4 d when packaged under aerobic conditions (Sørheim et al., 1997, 2004; Jeong and Claus, 2011). Among other advantages, the following have been mentioned: better flavor acceptability, no bone darkening, no premature browning during cooking, and increased tenderness (Cornforth and Hunt, 2008).

However, some disadvantages are related to CO MAP, such as the negative image consumers have of CO because it is a potentially hazardous gas. Carbon monoxide is considered toxic because it binds to hemoglobin in erythrocytes, leading to the formation of carboxyhemoglobin (Vreman et al., 1995, 2001). Exposure to CO that results in levels of carboxyhemoglobin higher than 1.5% must be avoided to protect susceptible members of the population (Sørheim et al., 1997, 2004). To avoid this hazard, gas distributors supply CO in mixtures of 0.4% CO, 60% CO2, and 39.6% N2. This is then mixed in the meat industries with CO2, or is mixed directly with complete mixtures of 0.4% CO, 60% CO2, and 39.6% N2.

Very little information is available on exposure to CO through the consumption of meat packaged with a CO gas mixture. However, even in the worst situation, treatment of the meat with CO or packaging meat with CO seems to make hardly any contribution to an increase in carboxyhemoglobin levels in consumers (Cornforth and Hunt, 2008). Carbon monoxide exposure from the packaging process or from the consumption of CO-packaged meat is well below US Environmental Protection Agency safety standards (US Environmental Protection Agency, 2010). A further concern relating to CO MAP is that products might look fresh even though bacterial levels might be high and the product spoiled.

At the end of 2001, the European Union Scientific Committee on Food issued a statement saying there were no problems or concerns with the use of CO mixtures in regard to consumers’ health, yet they did mention the fact that consumers could be misled by the color of meat as to its freshness (EC/SCF, 2001). In June 2003, the European Union decided not to accept the packaging of meat with low CO concentration (Sørheim, 2004). Another position was adopted in the United States. Since 2002, CO has been permitted as an MAP gas for use during distribution (Rulis, 2002). In 2004, CO at low levels was permitted as an MAP gas in retail packaging of fresh meat (Tarantino, 2004). The aim of this study was to evaluate the effect of an anaerobic gas mixture with CO on the growth of spoilage flora, color, and lipid oxidation stability of turkey meat under MAP stored at 0°C.

MATERIALS AND METHODS

Collection of Samples and Packaging Procedure

On different working days, samples of breast muscle were collected after turkey carcasses were deboned and cut into slices. The meat was placed in a polyethylene bag and transported in an isothermal box to the laboratory, where it arrived in less than 1 h.

The sliced meat samples were individually packaged under aerobiosis (AP), using polypropylene trays (Tecknopen Plastics S/L, Barcelona, Spain) and polyvinyl chloride film, and in 4 different modified atmospheres containing the following different gas mixtures: MAP 1, 50% N2 and 50% CO2; MAP 2, 0.5% CO, 50% CO2, and 49.5% N2; MAP 3, 0.5% CO, 80% CO2, and 19.5% N2; and MAP 4, 100% N2.

For the MAP, polypropylene trays (Tecknopen Plastics S/L) were used, and poly laminated plastic bags (HBX-070, R. Bayer, Veitsbronn, Germany) with a high impermeability to O2 and CO2 (permeability: O2 = 7.5 cm3/m2∙d∙bar, 75% RH, 23°C; CO2 = 32 cm3/m2∙d∙bar, 75% RH, 23°C; N2 = 3 cm3/m2∙d∙bar, 75% RH, 23°C; steam = 0.77 g/m2∙d) because of a high barrier layer of ethylene vinyl alcohol. Packages were sealed with an EVT-7-CD instrument (Tecnorip, Barcelona, Spain) after a vacuum of 97% and the introduction of a 60% gas mixture. All meat samples were stored under refrigeration (0 ± 1°C) in the dark for 12 to 25 d.

Meat samples in AP were analyzed for their microbial and physicochemical characteristics on d 0, 5, and 12 of storage. This evaluation was extended to d 19 and 25 when samples were under MAP.

After the storage period, the meat samples were removed from the MAP and cooked at 85°C for 15 min under vacuum (with an internal end-point temperature of 82.2 ± 0.9°C). After being cooled to 0°C, the meat sample color was evaluated. For each packaging condition, 5 replications (n = 5) were carried out on different storage days.

Microbial Analysis

The preparation of meat samples for microbial analysis was performed in accordance with ISO standard 6887-1:1999 (ISO, 1999). Microbial determinations were carried out on total mesophilic aerobic counts (plate count agar, Scharlau, Barcelona, Spain) at 30°C for 2 d in accordance with ISO standard 4833:2003 (ISO, 2003); total psychrotrophic aerobic counts (plate count agar, Scharlau) at 7°C for 10 d (ISO/DIS 6730:2005; ISO, 2005); anaerobic count at 7°C for 10 d (Brewer anaerobic agar, Merck, Darmstadt, Germany); Enterobacteriaceae counts in violet red bile agar (Merck) at 37°C for 2 d (ISO 21528-2:2004; ISO, 2004); Pseudo-
monas spp. counts (cephaloridene, fucidin, and cetrimide agar base, Oxoid, Basingstoke, Hampshire, UK) after incubation at 30°C for 2 d (ISO 13720:1995; ISO, 1995), lactic acid bacteria counts in de Man, Rogosa, Sharpe agar (Oxoid) incubated at 30°C for 3 d (ISO 15214:1998; ISO, 1998), and Brochothrix thermosphacta counts in streptomycin, actidione, and thallous acetate agar (Oxoid) incubated for 2 d at 30°C [ISO 13722:1996 (ISO, 1996); Santé et al., 1994]. Counts were expressed as log colony-forming units per gram. 

Physicochemical Analysis

Color. Approximately 30 min after opening a package of turkey meat slices, the color was measured on the surface of the meat with a Minolta colorimeter CR-300 (Minolta, Osaka, Japan) by using the L* (lightness), a* (redness), b* (yellowness) coordinates (CIELAB Color System, Commission Internationale de l’Éclairage, Vienna, Austria). Cooked meat samples were measured after being cooled to 0°C. Each value resulted from the arithmetic mean of 3 measurements.

TBA Test. Lipid oxidation evaluation by the TBA test was performed according to standard NP-3356 (Instituto Português de Qualidade, 1990) and the method of Pearson (1970) for meat and meat samples. Malondialdehyde (MDA) extraction was performed from 15 g of homogenized meat sample with trichloroacetic acid, propyl gallate, and EDTA. The MDA reacted with TBA as a TBA-reactive substance, producing a red-colored complex that was measured with a UV/visible Ultrospec 2000 spectrophotometer (Pharmacia Biotech, Buckinghamshire, UK) at a wavelength of 538 nm. The results were expressed in milligrams of MDA per kilogram of meat sample and were compared with a previous standard MDA curve prepared with a solution of 1,1,3,3-tetramethoxypropane at 10⁻⁸ mol/mL.

Statistical Analysis

Statistical analysis was undertaken using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL). The comparisons between different packaging conditions for the analyzed variables were performed by model adjustment of a one-way ANOVA for each day. If the F-test from ANOVA was significant, the least significant mean difference was determined using a post hoc multiple-comparisons test. The comparisons between days for each package were made using the t-test for dependent samples (Pestana and Gageiro, 2003).

RESULTS AND DISCUSSION

Microbial Development on Packaged Turkey Meat

Figures 1, 2, and 3 illustrate the development of microbes on turkey meat packaged under study conditions using different gas mixtures. Total aerobic mesophilic and psychrotrophic counts on sliced turkey meat under AP after 12 d of storage reached 9.8 and 9.7 log cfu/g, respectively (Figure 1A and 1B). The facultative anaerobic flora also attained a high count (8.1 log cfu/g; Figure 1C) after this storage period. Pseudomonas spp. was the dominant flora on meat under AP conditions, followed by B. thermosphacta and Enterobacteriaceae (Figures 2 and 3).

Figure 1. Total aerobic mesophilic (A), psychrotrophic (B), and anaerobic (C) flora counts present in sliced turkey meat packaged under an aerobic atmosphere and in 4 different gas mixtures: modified atmosphere packaging (MAP) 1, 50% N₂ and 50% CO₂; MAP 2, 0.5% CO, 50% CO₂, and 49.5% N₂; MAP 3, 0.5% CO, 80% CO₂, and 19.5% N₂; MAP 4, 100% N₂. Values on the same day of storage not sharing a common letter (a–c) were significantly different (P < 0.05).
All the anaerobic gas mixtures tested significantly delayed the development of dominant flora (aerobic mesophilic and psychrotrophic microbes, *Pseudomonas* spp., and *B. thermosphacta*) when compared with the AP on the 5th (*P* < 0.01) and 12th (*P* < 0.001) days of meat storage.

Turkey meat under MAP with CO2 after 12 d of storage had aerobic counts of approximately 5 log cfu/g, which was below the limit of acceptability recognized by the French Government law “Arrêté” of 5 March, 1985 (Direction de l’Information Légale et Administrative, 1998).

The aerobic psychrotrophic flora presented a behavior similar to the mesophilic flora, but not to the anaerobic psychrotrophic flora on meat packaged with CO2 mixtures (Figure 1). These microbial groups presented a lower count when the concentration of CO2 increased to 80% with CO. However, the increase in CO2 concentration to more than 50% did not make a significant difference in anaerobic counts, as was observed in meat after a storage period of 25 d.

The CO2 inhibition affected the *Pseudomonas* spp. in particular, increasing the lag phase and generation time (Saucier et al., 2000), and in *Enterobacteriaceae* (Figure 2A and 2B), with count differences of 2 to 3 log cfu/g compared with the values registered in MAP packages without this gas (MAP 4).

In meat packages with CO2 gas mixtures, a decrease in meat pH occurs that is as high as the CO2 concentration increase (Chin et al., 1976; Jones and Greenfield, 1982; Tan and Gill, 1982; Gill, 1988; Dixon and Kell, 1989; Blakistone, 1999). This contributed to the inhibi-
tion of microbial flora; however, independently of the presence of CO, microbial inhibition did not increase linearly with an increase in CO2 concentration to 80%.

The introduction of a 0.5% concentration of CO in the gas mixtures did not inhibit aerobic mesophilic, psychrophilic, and anaerobic psychrotrophic microorganisms. No significant differences were observed between the above-mentioned microbial group counts obtained in meat under MAP 1 and MAP 2. Furthermore, Luño et al. (1998) concluded that the presence of 1% CO in gas mixtures of packaged sliced or minced beef did not have a significant inhibitory effect on aerobic psychrotrophic flora.

The presence of CO, in addition to the fact that residual O2 in packages was less than 0.5%, had an inhibitory effect on B. thermosphacta (Figure 3A). After 19 and 25 d of storage at 0°C, it was possible to observe this significant inhibitory effect (P < 0.001) on the development of B. thermosphacta in packaged turkey meat. Gee and Brown (1981) concluded that gas mixtures with CO had a selective effect on the type of microorganisms that grew in a mixture culture. Luño et al. (2000) studied the influence of CO on beef under MAP with concentrations between 0.1 and 1% and obtained counts of 1 log cfu/cm2 for B. thermosphacta, whereas for CO concentrations of 0.1 and 0.25%, it was 2 log cfu/cm2.

The low growth of lactic acid bacteria during the storage period was not different among the different gas mixtures studied in turkey meat packages (Figure 3B). These flora were not inhibited by anaerobic conditions or by the introduction of CO2 or CO. This anaerobic facultative group without sensitivity to CO2 (Farber, 1991; Tewari et al., 1999) was present after 25 d of storage in turkey meat under MAP, with values of 5 to 6 log cfu/g, contributing as spoilage flora in MAP with CO2.

The appropriate concentrations of CO2 to achieve the maximum microbial inhibition have been discussed by several authors. Seideman and Durland (1984) and Brody (1996) concluded that 20 to 30% CO2 is the adequate concentration. However, Luño et al. (1998) stated that 20% CO2 (when gas mixtures with CO2, N2, O2, and CO were used) was not sufficient to achieve the maximum microbial inhibition. Gill and Tan (1979) verified that the inhibitory effect increased linearly with concentrations up to 50 to 60%. Huffman et al. (1975) and Santé et al. (1994) showed that a package with 100% CO2 was the most efficient for increasing the shelf life of fresh meat.

For meat packaged with MAP 3, the total mesophilic and psychrotrophic counts were significantly lower (P < 0.001) than those observed in MAP 1. The introduction of CO added to a greater concentration of CO2 inhibited the microbial flora in general, with particular action on B. thermosphacta. Regarding microbial quality, the shelf life of poultry meat under the MAP in this study condition was longer than that for meat in AP (5 d): 12 d for mixture MAP 4, 19 d for MAP 1 and MAP 2, and 25 d for MAP 3.

**Color and Lipid Oxidation of Packaged Turkey Meat**

Figure 4 presents the evolution of color parameters using the L*a*b* system on sliced turkey meat packaged under an aerobic atmosphere and different MAP conditions. The characterization of meat color after opening the MAP package registered a slight increase in L* and b* during storage time, but there was no decrease in a* as was recorded for meat under AP, and there was a significant increase in b* (P < 0.05) after 12 d of storage (Figure 4C). This discoloration of turkey meat, characterized by a pale yellowish-brown color instead of pink, is related to oxidative processes. Furthermore, the growth of microorganisms affects meat color. The presence of H2O2 and acids from the metabolism of lactic acid bacteria or fat oxidation induces myoglobin oxidation with coelmyoglobin production, which is responsible for the pale green meat color (Lawrie, 1998; Ranken, 2000).

Santé et al. (1994, 1996) referred to discoloration with increased L* and b* values during storage time in turkey meat under AP. They reported a decrease in a* in turkey meat samples packaged under aerobic conditions and under gas mixtures with O2 in contrast to those packaged with 100% CO2 or under vacuum, where increases in a* and b* during storage time were observed. Myoglobin oxidation is the cause of color modification, initiating turkey meat oxidation and the process of becoming rancid, which is dependent on the high number of polyunsaturated fatty acids present in poultry meat (Cantor et al., 2000; Rhee, 2000). Sorheim et al. (1997) studied the effect of MAP under 100% CO2 on the color and microbial flora of pork meat and concluded that the level of O2 in the package had a great effect on meat color. They observed a greater change of color, with higher L* and b* and lower a* values when the residual O2 was 0.5 to 1%. Under these O2 concentrations, the bacterial counts did not change and increased only with values of O2 greater than 2%.

In the present study, a significant increase in a* values (P < 0.001) for turkey meat under MAP with CO (MAP 2 and MAP 3) was observed, compared with a* values under the other MAP storage conditions (Figure 4B). However, no significant increase in a* values was noticed during storage time. In fact, the formation of carboxymyoglobin induced by the presence of CO stabilized the meat color pigment during the storage time, as also demonstrated by Luño et al. (2000), Krause et al. (2003), Hunt et al. (2004), Pettersen et al. (2004),
According to Fraqueza et al. (2005), when turkey meat was under MAP with a gas composition of CO₂, N₂, and CO, consumers preferred the bright pink meat color to the brown or gray meat color induced by the anoxic gas composition used in other packaging.

When compared with raw meat, the color of cooked, sliced turkey meat samples under the study conditions (Figure 5) registered an increase in L* and b* parameters, whereas a* values decreased. The cooked turkey meat is yellow-white. The L*a*b* values of cooked, sliced turkey meat from MAP with CO (MAP 2 and MAP 3) did not register any significant differences compared with those under MAP 1 and MAP 4.

Controlling the initiation of the pink color defect in cooked meat is extremely difficult because it can be associated with several factors (Ahn and Maurer, 1990a,b; Trout, 1991; Sørheim et al., 2001; Holownia et al., 2003). The formation of heme pigment cannot be attributed only to the presence of CO because the prior formation of carboxymyoglobin does not cause the heme pigment responsible for the pink color in meat in quantities detectable by sensory analysis (data not shown). It is possible to state that the previous contact

Figure 4. Color parameters L* (lightness; A), a* (redness; B), and b* (yellowness; C) in sliced turkey meat packaged under an aerobic atmosphere and in 4 different gas mixtures: modified atmosphere packaging (MAP) 1, 50% N₂ and 50% CO₂; MAP 2, 0.5% CO, 50% CO₂, and 49.5% N₂; MAP 3, 0.5% CO, 80% CO₂, and 19.5% N₂; MAP 4, 100% N₂. Values on the same day of storage not sharing a common letter (a–c) were significantly different (P < 0.05).

Figure 5. Color parameters L* (lightness; A), a* (redness; B), and b* (yellowness; C) in cooked, sliced turkey meat packaged under an aerobic atmosphere and in 4 different gas mixtures: modified atmosphere packaging (MAP) 1, 50% N₂ and 50% CO₂; MAP 2, 0.5% CO, 50% CO₂, and 49.5% N₂; MAP 3, 0.5% CO, 80% CO₂, and 19.5% N₂; MAP 4, 100% N₂. Values on the same day of storage not sharing a common letter (a, b) were significantly different (P < 0.05).
of turkey meat under MAP with CO for a long period and the formation of carboxymyoglobin in turkey meat did not induce the defect related to the appearance of undercooked meat.

Lipid oxidation was influenced by time and storage conditions, as shown in Figure 6. In turkey meat under AP, a significant increase was observed in TBA (P < 0.001) during storage time, presenting values of 0.66 to 0.78 mg of MDA/kg after 12 d. The protective antioxidant effect of anaerobic MAP was not observed in turkey meat under our study conditions after 12 d of storage. Meat from turkeys raised on feed enriched with vitamin E could have added a protective antioxidant effect (Renerre et al., 1999; Batifoulier et al., 2002; Mielnik et al., 2003; Wood et al., 2004).

The stabilized meat color (Figures 4 and 5) masked the lipid oxidation (Figure 6) that occurred in MAP 1, MAP 2, and MAP 3, and did so to a greater degree than that in anaerobic MAP 4 (d 19 and 25). Contrary to previous statements by various authors (Besser and Kramer, 1972; Luño et al., 2000; Krause et al., 2003; Sørheim et al., 2004; John et al., 2005) regarding the fact that CO prevents lipid oxidation of pork meat under MAP, the presence of 0.5% CO in the MAP gas mixtures did not prevent the oxidation of turkey meat. The antioxidant effect was observed for beef under MAP with O2, CO2, and CO only with CO concentrations greater than 0.75 and 1% (Luño et al., 2000). Krause et al. (2003) studied the effect on pork of MAP with an anaerobic gas mixture with 0.5% CO, and they recorded less lipid oxidation under that condition than in pork under AP. The authors did not observe significant differences in TBA between pork under vacuum packaging, MAP with 20% CO2 and 80% N2, and MAP with 70% CO2, 29.5% N2, and 0.5% CO, but a higher TBA value was present under this last condition.

According to Ryter and Otterbein (2004), the role of CO on biological systems has not been fully explained. Its endogenous formation results from lipid oxidation, among other pathways (Vreman et al., 1998; Archakov et al., 2002), whereas the development of lipid oxidation in the cerebral tissue of mice after they were subjected to higher concentrations of CO has been reported (Thom, 1990; Kudo et al., 2001).

Only MAP 4, without CO2 or CO, prevented the lipid oxidation of meat. The influence of bacterial flora on turkey meat discoloration and lipid oxidation under MAP conditions is not clear. Under MAP 4, turkey meat had higher bacterial counts but lower TBA values and no differences regarding L*a*b* color values.

Meat pigments and lipid oxidation are related, and oxidation is induced by several factors: apart from packaging conditions, the presence or absence of O2, and storage temperature, lipid oxidation comes with meat aging, a potential reduction in enzyme activity, such as that of metmyoglobin reductase, or the activation of other enzymatic systems during the storage period (Hagler et al., 1979; Livingston and Brown, 1981; Trout, 1991; Renerre et al., 1999; Alasnier et al., 2000; Jakobsen and Bertelsen, 2000).

The introduction of CO into anoxic gas mixtures with CO2 used for turkey meat under MAP is useful because it gives the bright pink color preferred by consumers without inducing an undercooked meat appearance, it inhibits B. thermosphacta, and it increases shelf life.

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