PRESENTING, PRODUCTS, AND FOOD SAFETY

The effect on turkey meat shelf life of modified-atmosphere packaging with an argon mixture

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ABSTRACT There is a lack of knowledge related to the action of Ar on microbial development and prevention of oxidation when applied to raw meat under modified-atmosphere package (MAP). The aim of this study was to evaluate the effect of an anaerobic gas mixture with Ar on spoilage flora growth, color, and lipid oxidation stability of turkey meat under MAP stored at 0°C. Breast muscles samples were collected on different working days from turkey carcasses (BUT9 and BIG6), fast-cooled in a tunnel (−2°C, 2 m·s⁻¹, 90% RH) for 2 h and selected to be deboned according current practices in industrial slaughterhouses. The breasts were cut into slices that were individually packaged under aerobiosis (P0) and in 4 different modified atmospheres containing different gas mixtures as (P1) 100% N₂, (P2) 50% Ar-50% N₂, (P3) 50% Ar-50% CO₂, and (P4) 50% N₂-50% CO₂. All samples were stored at 0 ± 1°C in the dark for between 12 and 25 d. Meat samples packaged in P0 were analyzed for their microbial and physicochemical characteristics on d 0, 5, and 12 of storage and then extended to 19 and 25 d when samples were under MAP. The microbial shelf life period extension of MAP sliced turkey meat compared with aerobic packaging (5-d shelf life) is 1 wk more for P1 and P2 mixtures, 2 wk for P4, and 3 wk for P3. The Ar-CO₂ mixture was more efficient in delaying flora development than CO₂-N₂ with 1 log difference on the 25th day of storage, for total psychrotrophic counts, total anaerobic counts, and Brochothrix thermosphacta. The presence of Ar on gas mixtures did not seem to have any additional protective effect on lipid turkey meat oxidation.

Key words: argon, modified-atmosphere packaging, poultry meat, shelf life, spoilage flora

INTRODUCTION

Meat market predictions for the future indicate an increase of poultry meat consumption in the worldwide market, with poultry being 40% of all the meat consumed in 2020 (Roeningk, 1998; Porter, 2002). This consumer preference has created a demand in the industry for the development of new poultry products and the application and development of new technologies, which consequently will increase poultry meat consumption even more. Meat presentation to the consumer has experienced over the few last years a modification through the use of modified-atmosphere packaging (MAP) with low storage temperature. These important contribute to microbial and lipid oxidation stability of meat products and increase their shelf life (Devlieghere et al., 2004). Also, the use of MAP gives a facility of storage, distribution, sale, and utilization (Farber, 1991; Church, 1994; Ohlsson, 1994; Smolander et al., 1997). The success of these technologies depends on the specificity of gas mixtures related to the product, type of meat, the nature and initial quality of meat, the temperature control, the barrier properties of the packaging film, and the efficacy of the equipment (Taylor, 1996).

The use of CO₂-enriched atmospheres extends the shelf life of raw poultry by inhibiting the psychrotrophic gram-negative bacteria and Pseudomonas spp. Although later meat spoilage and changes on organoleptic characteristics are observed because slower-growing microorganisms (Carnobacterium spp., Brochothrix thermosphacta, Lactobacillus spp.) proliferate (Blakistone, 1999).

Apart from the CO₂, O₂ and N₂ gas mixtures used on raw meat packaging, only CO has been adopted and studied on red meat (Sorheim et al., 1997), but its use is interdicted by law in Europe. However, other gases such as Ar, He, and N₂O are permitted in meat packaging in the European Union (Directive 95/2/CE; EU, 1995). Recently, there has been a great interest in the
potential benefits of Ar and other noble gases in MAP applications (Mostardini and Piergiovanni, 2002; Spencer, 2002).

According to Morgan (2007), Ar is increasingly used in MAP of foods. In fact, Ar is an inert gas, odorless and tasteless, and more dense and soluble than N2. The physical properties of this gas offer certain advantages over the N2, O2, or CO2 atmospheres, or other gas mixtures typically used in MAP to prolong the quality shelf life or freshness of packaged foods. Because it is chemically inert, Ar does not react with food components as O2 or CO2 might; it also inhibits the action of some oxidase enzymes that cause food spoilage and since it is denser and more soluble in both water and oil, it is more effective than N2 for displacing O2 from the oils and fats in foods.

A US patent was issued for the use of Ar in the preservation of cut and segmented fresh fruits (Powrie et al., 1990). Argon gas as a major component of the atmosphere in MAP has also been reported to reduce microbial growth and improve the quality retention of fresh produce such as broccoli and lettuce (Day, 1996, 1998; Jaime and Saltveit, 2002).

In other studies, Ar is reported to be biochemically active, probably due to its enhanced solubility in water when compared with N2 and it seems to interfere with enzymatic O2 receptor sites (Spencer, 2002). However, inconsistent results have been presented on the effect of Ar on inhibition and control of the growth of certain microorganisms, on the activity of quality-related enzymes, and on degradative chemical reactions in selected perishable food products, such as minimally processed fruit (Powrie et al., 1990; Spencer, 1995; Day, 1996, 1998; Kader and Watkins, 2000; Jaime and Saltveit, 2002; Spencer, 2002; Mostardini and Piergiovanni, 2002; Roculli et al., 2005). For chemical oxidation reaction, it has been found that noble gases strongly inhibit oxidation, generally in the order Xe > Kr > Ar > Ne > He, having a positive and unique ability to prevent oxidation even in the presence of O2, whereas N2 has no such capability except in the simple displacement of O2 (Spencer, 2002).

A study of sliced cooked ham packaged with Ar reports an improvement in oxidation control and microbial inhibition thus extending the product shelf life (Fachon, 2002). Although, there is a lack of knowledge related to the action of this gas on microbial development and prevention of oxidation when applied to raw meat under MAP. The aim of this study was to evaluate the effect of an anaerobic gas mixture with Ar in spoilage flora growth, color, and lipid oxidation stability of turkey meat under MAP stored at 0°C.

MATERIALS AND METHODS

Collection and Packaging of Samples

Breast muscle samples were collected on different working days from turkey carcasses (male turkeys, BUT9 and BIG6, 16 to 18 wk old), fast-cooled in a tunnel (−2°C, 2 m·s⁻¹, 90% RH) for 2 h and selected to be deboned according to current practices in industrial slaughterhouse. The carcasses were kept in a refrigeration chamber (0°C, 85% RH) until deboning (approximately 24 h postmortem). The breasts were then cut into slices by a meat deboning operator. The meat was placed in a polyethylene bag and transported in an isothermal box to the laboratory in less than 1 h.

Sliced meat samples were individually packaged under aerobicis (P0), using polypropylene trays (Tecknopack Plastics S/L, Barcelona, Spain) and polyvinyl chloride film, and in 4 different modified atmospheres containing different gas mixtures as (P1) 100% N2, (P2) 50% Ar-50% N2, (P3) 50% Ar-50% CO2, and (P4) 50% N2-50% CO2.

In MAP, polypropylene trays (Tecknopack Plastics S/L) and poly laminated plastic bags HBX-070 (R. Bayer, Veitsbronn, Germany) with 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 and water steam = 0.77 g/m²·d), due to a high barrier layer of ethylene vinyl alcohol, were used. The packages were sealed in an ETV-7-CD machine (Tecnoprip, Barcelona, Spain) after a vacuum of 97% and an introduction of a gas mixture of 60%. All samples were immediately stored in refrigeration (0 ± 1°C) in the dark for between 12 and 25 d. Meat samples packaged in aerobicis were analyzed for their microbial and physicochemical characteristics on d 0, 5, and 12 of storage. This evaluation was extended to 19 and 25 d when samples were under MAP. For each packaging condition, 7 replications (n = 7) were made on different storage days.

Microbial Analysis

The preparation of meat samples for microbial analysis was performed in accordance with ISO 6887-1 (ISO, 1999). Microbial determinations were carried out to: total mesophilic aerobic counts (plate count agar, Sharlau, Barcelona, Spain) at 30°C for 2 d, in accordance with ISO 4833 (ISO, 2003); total psychrotrophic aerobic counts (plate count agar, Sharlau) at 7°C for 10 d (ISO/DIS 6730, ISO, 2005); anaerobic counts 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, ISO, 2004); Pseudomonas spp. counts (cephaloridene, fucidin, and cetrimide agar base; Oxoid, Cambridge, UK) after incubation at 30°C for 2 d (ISO 13720, ISO, 1995); lactic acid bacteria (LAB) counts in de Man, Rogosa, Sharpe agar (Oxoid) incubated at 30°C for 3 d (ISO 15214, ISO, 1998); and B. thermosphacta counts in streptomycin, actidione, thallous acetate agar (Oxoid) incubated for 2 d at 30°C (ISO 13722, ISO, 1996; Santé et al., 1994). Counts were expressed as log cfu·g⁻¹.
Physical-Chemical Analysis

pH Determination. The pH was determined with a portable pH meter (HI9023) equipped with a pH electrode (FC 230B, Hanna Instruments, Milan, Italy). Each value was the average of 3 tests on the meat slice.

Color. The color was measured on the surface of turkey meat slices, approximately 30 min. after opening the package, with a Minolta colorimeter CR-300 (Minolta, Osaka, Japan) using the L*, a*, b* coordinates (CIELAB color system). Each value resulted from the arithmetic mean of 3 measurements.

TBA Test. Lipid oxidation evaluation by TBA test was performed according to Pearson (1970) and Norma Portuguesa NP-3356 (1990) for meat and meat samples. Malondialdehyde (MDA) extraction was performed from 15 g of homogenized meat sample with trichloroacetic acid, propyl galate, and EDTA. The MDA reacted with TBA as a TBA reactive substance (TBARS) producing a red-colored complex, which was measured in a UV/Visible Ultrospec 2000 spectrophotometer (Pharmacia Biotech, Buckinghamshire, UK) at 538-nm wavelength. The results were expressed in milligrams of MDA per kilogram of meat sample comparing to a previous standard MDA curve prepared with a solution of 1,1,3,3-tetramethoxypropane at $10^{-8}$ mol·mL$^{-1}$.

Statistical Analysis

Statistical analysis was undertaken using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL). The comparison between different packaging conditions for analyzed parameters was performed by model adjustment of a 1-way ANOVA for each day. If F-test from ANOVA was significant, a least significant mean difference of a post hoc multiple comparisons test was performed. Regarding the effect of storage time, the comparison between storage days, considering each package, was made by t-test for dependent samples (Pestana and Gageiro, 2003).

RESULTS AND DISCUSSION

The evolution of microbial flora on sliced turkey meat packaged on different modified-atmosphere study conditions is reported on Figures 1, 2, and 3. The initial contamination of sliced turkey meat (d 0) for total aerobic mesophilic and psychrotrophic counts was approximately 4.7 log cfu·g$^{-1}$ (Figure 1A and B). The aerobic packaging (P0) of sliced turkey meat allowed aerobic mesophilic and psychrotrophic counts to reach 6.9 log cfu·g$^{-1}$ on the fifth day of storage and a higher level on the 12th day, about 9.8 and 9.7 log cfu·g$^{-1}$, respectively, outside of the limit of process hygiene criteria acceptability (6 log cfu·g$^{-1}$) recommended by the French government (Anonymous, 1998). The facultative anaerobic flora (Figure 1C) also reached high levels (8.1 log cfu·g$^{-1}$). From the analyzed microbiota, Pseudomonas spp. appears to dominate followed by B. thermosphacta and Enterobacteriaceae, as was stated also by Santé et al. (1994).

The anaerobic conditions created by packaging with all gas mixtures (P1 to P4) delayed significantly the development of dominant flora (aerobic mesophilic and psychrotrophic, Pseudomonas spp., and B. thermosphacta) when compared with the aerobic packaging on the fifth ($P < 0.01$) and 12th ($P < 0.001$) days of meat storage. The microbial development on meat MAP conditions P1 and P2 was similar (Figure 1, 2, and 3).

The anaerobic conditions given by P1 and P2 inhibited the aerobic mesophilic and psychrotrophic flora (Figure 1) so that the meat shelf life could be longer than the usual 5 d of storage in aerobic conditions. However, on the 12th storage day, the flora counts on meat under P1 and P2 was over the limit of acceptability. This fact is related to the growth of Pseudomonas spp. (Figure 2) and B. thermosphacta (Figure 3) at a slower rate compared with their development in aerobic conditions, as a subsequent metabolic adaptation to the anaerobic conditions established in these MAP.

Concerning the anaerobic psychrotrophic flora (Figure 1C), it was observed that meat under P1 and P2 presented similar values to those when meat was under aerobic conditions (fifth day of storage) but significantly higher than those of the other meat packaging conditions. The anaerobic psychrotrophic flora initially was not inhibited with gas mixtures P1 and P2. In this study, it appears that the main anaerobic flora on meat under P1 and P2 was Enterobacteriaceae because they are facultative anaerobes, the storage temperatures at 0°C being the key factor of their inhibition.

The introduction of Ar replacing N$_2$, in mixture P2, does not add a microbial inhibitory effect. Despite the delay of flora development, with P1 and P2 gas mixtures that had been relevant in particular to Pseudomonas spp. and B. thermosphacta (Figure 2A and 3B, respectively), it was the presence of CO$_2$ in mixtures P3 and P4 that added a significant ($P < 0.05$) bacteriostatic effect on flora development (Figure 1).

After 12 d of storage, all meat under MAP with CO$_2$ mixtures (P3 and P4) had aerobic mesophilic counts of approximately 4.5 and 5.5 log cfu·g$^{-1}$ (Figure 1A) under the limit of the criteria of acceptability recommended by the French government (Anonymous, 1998). The inhibitory effect of CO$_2$ affects mainly Pseudomonas spp. (Figure 2A), increasing lag phase and generation time (Saucier et al., 2000), but also Enterobacteriaceae counts (Figure 2B) with differences of 1 to 2 log cfu·g$^{-1}$ in relationship with the values observed in gas mixtures without this gas (P1 and P2).

The Ar/CO$_2$ mixture, P3, was more efficient in delaying flora development than P4, without Ar. In these last study conditions (P3 and P4), there was more than 1 log difference on the 25th day of storage for total mesophilic and psychrotrophic counts ($P < 0.05$), to-
tial anaerobic counts ($P < 0.05$), and *B. thermosphacta* ($P < 0.05$). This synergetic effect of Ar-CO$_2$ appeared to be connected to the development delay of *B. thermosphacta* and consequent relationship of anaerobic and aerobic psychrotrophic flora counts, which was lower than microbial criteria limit.

According to Sneath and Jones (1986), the *B. thermosphacta* is a facultative anaerobic bacteria but it grows better in the presence of air. The reduction of O$_2$ in gas mixtures or its complete absence can justify the growth inhibition more than the presence of CO$_2$ at certain concentrations. Holley (2000) stated that the introduction of CO$_2$ itself will not be inhibitory for *Brochothrix* until 50% concentration with presence of O$_2$. In fact, Ordoñez et al. (1991) reported that its metabolism continued to be mainly aerobic in atmospheres of CO$_2$ enriched with O$_2$. Low concentrations of O$_2$ do not have any effect in the development of this microbial growth until decreasing below 0.2%. Santé et al. (1994) studied turkey meat under MAP and observed an inhibition of *B. thermosphacta* only when meat was under 100% CO$_2$. With a mixture of 25% CO$_2$, 9% N$_2$, and 66% O$_2$, the numbers of these microorganisms were similar to mixtures with 100% N$_2$. However, the introduction of

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**Figure 1.** Evolution of total aerobic mesophilic (A), psychrotrophic (B), and anaerobic (C) flora in sliced turkey meat packaged under aerobic atmosphere (P0) and in 4 different modified-atmosphere package gas mixtures: P1 = 100% N$_2$; P2 = 50% Ar, 50% N$_2$; P3 = 50% Ar, 50% CO$_2$; and P4 = 50% N$_2$, 50% CO$_2$. *Values on the same day of storage not sharing a common letter were significantly different ($P < 0.05$); n = 7.*

**Figure 2.** Evolution of *Pseudomonas* spp. (A) and Enterobacteriaceae (B) in sliced turkey meat packaged under aerobic atmosphere (P0) and in 4 different modified-atmosphere package gas mixtures: P1 = 100% N$_2$; P2 = 50% Ar, 50% N$_2$; P3 = 50% Ar, 50% CO$_2$; and P4 = 50% N$_2$, 50% CO$_2$. *Values on the same day of storage not sharing a common letter were significantly different ($P < 0.05$); n = 7.*
Ar in our study had a higher inhibitory effect in the *B. thermosphacta* because the counts were significantly lower (*P* < 0.05) on meat under MAP P3.

The LAB (Figure 3A), a slower growing microorganism group, was an exception because none of the gas mixture packaging had any inhibitory effect, as was also stated by other authors (Blakistone, 1999; Saucier et al., 2000). The LAB was not inhibited by anaerobic conditions or by CO2 introduction in gas mixture. Being a facultative anaerobic bacteria group not sensitive to CO2 (Farber, 1991; Tewari et al., 1999), LAB counts in meat package under the various MAP study conditions after 25 storage days were 5 to 6 log cfu·g−1, contributing as major spoilage flora particularly in atmospheres with CO2. The microbial shelf life period extension of MAP sliced turkey meat compared with aerobic packaging (5-d shelf life) is 1 wk more for P1 and P2 mixtures, 2 wk for P4, and 3 wk for P3.

The initial pH of turkey meat packaged under aerobic condition (P0) increased significantly (*P* < 0.05) from 5 to 12 d of storage (Figure 4). This significant increase during storage period was generated by spoilage flora growth, aerobic and anaerobic facultative psychrotrophic microorganisms, principally *Pseudomonas* spp. and Enterobacteriaceae, with the use of amino acid and the release of NH3 as stated by Gram et al. (2002).

In meat under MAP with CO2 (P3 and P4), there is a slight meat pH decline associated with the dissolution of CO2 in meat constituents and the production of HCO3− (Gill, 1988; Zhao et al., 1995). However, the meat buffer effect (Dixon and Kell, 1989) and the microbial growth during the storage period of meat under MAP contraries a major decline of pH. Devlieghere (2000) found changes of 0.3 pH units in cooked meat products when a concentration of 80% CO2 and a ratio of gas volume:products of 4:1 was applied.

The microbial flora present in meat under MAP with anaerobic mixtures with or without CO2 was not the cause of an accentuated pH increase as was observed in meat packaged under aerobic condition. The modification of microbiota nature due to the inhibition created by gas mixtures in packages, associated with the use of alternative anaerobic metabolic ways by microorganisms, could explain why there was not a significant pH increase. It has been found that pH is not suitable as an early indicator of spoilage produced by microorganisms, although it increases in meat under aerobic package conditions. In fact, there was a significant increase of flora counts at 5 d of storage, but a significant pH increase was not observed. This increase was observed only when meat in aerobiosis had putrefactive signs, with abnormal smell and slime (data not shown) after the 12th day of storage.

Figure 5 represents the evolution of color evaluated by the L*, a*, b* system on turkey meat packaged under different study conditions. The turkey meat color under aerobicosis (P0) changed, becoming significantly (*P* < 0.05) lighter during the first 5 d of storage (increase of L*); however, after 5 d of storage, the meat turned dark, less reddish, and more yellow as it is described by the significant (*P* < 0.05) decrease of L* and a* and the increase of b*. This could be related initially to the oxidation of myoglobin and after to the high bacterial counts found in turkey meat under aerobicosis after 5 d of storage.
The turkey meat discoloration, characterized by a paler color instead of pink, is attributed to an oxidative process. Santé et al. (1994, 1996) observed a discoloration in meat under aerobic conditions characterized by increase of L* and b* during storage time. The bacterial growth responds to the change of myoglobin to metmyoglobin, which gives an undesirable brownish red color (Renerre et al., 1999; Ranken, 2000; Toldrá, 2006).

The color of turkey meat under anaerobic MAP conditions at fifth day of storage was significantly different \((P < 0.05)\) from that observed on meat under aerobic packaging regarding the a* and b* parameters. On the 12th day of storage, the difference of meat color was noticeable when protected by anaerobic packaging conditions; the L* and a* parameters were significantly higher \((P < 0.05)\) and the b* significantly lower \((P < 0.05)\) from those presented by meat under aerobic packaging. On the 25th day of storage, there was no differences of L*, a*, and b* parameters in meat under MAP study conditions (P1 to P4). The meat color seems to be more preserved when anaerobic MAP is used because the rate of myoglobin oxidation or pigment modifications by other causes was less important than that observed in meat under aerobiosis.

The turkey meat under anaerobic MAP conditions turned slightly lighter (increase of L* to 47.37 to 48.79) after storage when compared with 0 d \((P < 0.05)\), independently of the anaerobic gases mixtures used. The b* value increased significantly \((P < 0.05)\) during 12 d of storage time independently of MAP mixtures used in this study, with a decrease after 19 d of storage. It seems that there is always a discoloration of meat during storage time, which could be related to the slow growth of spoilage flora (Ranken, 2000).

The myoglobin oxidation is the cause of color change, inducing also the development of turkey meat oxidation and rancidity when it is under aerobic conditions, and is also conditioned by the elevated content of polyunsaturated fatty acid (Cantor et al., 2000; Rhee, 2000).

The results of lipid oxidation evaluation expressed by TBA determination are presented in Figure 6. The initial TBA values in turkey meat were low (0.3 mg of MDA/kg of meat) as stated also by Jo and Ahn (1998). The MDA concentrations in turkey meat were lower than those that were found in broiler meat storage at 4°C, during 48 h (Tichivangana and Morrissey, 1985). Rhee (2000) reported that poultry meat presented lower TBA values than beef or pork meat, increasing little during 2 to 6 d of storage at 4°C.

It was observed that for aerobic packaging (P0) meat, a significant increase \((P < 0.05)\) of TBA value occurred after 5 d of storage (0.3 to 0.5 mg of MDA/kg). The TBA value of aerobic package meat storage on the 12th day had increased (0.7 mg/kg) but was not significantly different from the fifth day. This last value does not

Figure 5. Evolution of color parameters L* (A), a* (B), and b* (C) in sliced turkey meat packaged under aerobic atmosphere (P0) and in 4 different modified-atmosphere package gas mixtures: P1 = 100% N₂; P2 = 50% Ar, 50% N₂; P3 = 50% Ar, 50% CO₂; and P4 = 50% N₂, 50% CO₂. **Values on the same day of storage not sharing a common letter were significantly different \((P < 0.05)\); n = 7.
Figure 6. Development of lipid oxidation measured by TBA content (mg of malondialdehyde [MDA]/kg) in sliced turkey meat packaged under aerobic atmosphere (P0) and in 4 different modified-atmosphere package gas mixtures: P1 = 100% N2; P2 = 50% Ar, 50% N2; P3 = 50% Ar, 50% CO2; and P4 = 50% N2, 50% CO2. Values on the same day of storage not sharing a common letter were significantly different (P < 0.05); n = 7.

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Argon is not able to participate in chemical reactions and chemical compounds with it are not known; because it is chemically inert, Ar does not react with food components as O2 or CO2 might (Brody, 1996). The biochemical benefits from the effect of the replacement of Ar by N2 in gas mixtures seems to be minimal. Contrary to what was expected, the presence of Ar in gas mixtures did not appear to have any additional protective effect on lipid turkey meat oxidation.

In mixtures P1 and P2 or P3 and P4, there were no significant differences in TBA observed in turkey meat under MAP with replacement of 50% N2 by Ar. Because Ar had no protective effect on the lipids of meat packaged, there is indifference whether Ar or N2 should be used as filling gases.

In conclusion, the Ar-CO2 mixture was more efficient in delaying flora development than CO2-N2 with 1 log difference on the 25th day storage, for total psychrotrophic counts, total anaerobic counts, and B. thermosphaacta. However, the presence of Ar on gas mixtures did not seem to have any additional protective effect on lipid turkey meat oxidation.

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