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Modified atmosphere packaging and vacuum packaging for long period chilled storage of dry-cured Iberian ham

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ABSTRACT

Dry-cured Iberian ham slices were stored under vacuum and under four different modified atmospheres (60/40 = 60%N_2 + 40%CO_2; 70/30 = 70%N_2 + 30%CO_2; 80/20 = 80%N_2 + 20%CO_2; argon = 70%argon + 30% CO_2) at 4 ± 1 °C during 120 days. Gas composition, moisture content, pH, colour, pigment content, and lipid stability were measured, as well as sensory and microbial analysis were carried out throughout storage. A loss of intensity of red colour (a^*-values) was observed during storage in ham slices ($P < 0.05$). Consistently, MbFe(II)NO content also decreased throughout storage ($P > 0.05$). Slices of ham packed in 40%CO_2 (60/40) and 30%CO_2 (70/30) showed lower a^*-values than the rest of the batches after 60 days ($P < 0.05$), though differences were not evident after 120 days ($P > 0.05$). TBARs values showed an upward trend during the storage of packaged slices ($P < 0.05$). Vacuum-packed slices showed the lowest TBARs values and those packed with 40%CO_2, the highest. Sensory attributes did not vary significantly ($P > 0.05$) throughout storage under refrigeration and packed either in vacuum or in modified atmospheres. No safety problems were detected in relation to the microbial quality in any case.

1. Introduction

Dry-cured Iberian ham is a typical meat product from the southwest of Spain, highly appreciated by consumers and with a considerable economical importance as a result of its unique and high sensory quality (Cava, Ventanas, Ruiz, Andrés, & Antequera, 2000). Over years, Iberian hams have been commercialized in a traditional way, which consists of presenting the whole leg with the hoof included. However, new ham retail presentations, such as packaged ham slices are being developed in recent years, as a result of new consumer demands and in order to increase competitiveness.

On the other hand, industry demands the use of preservation methods which increases the shelf life of manufactured foods ensuring food safety. In this sense, the food industry has developed different packaging technologies in order to extend the shelf life of products such as meat and meat products. Among these technologies, vacuum packaging and modified atmosphere packaging (MAP) prevent products from contamination and evaporative losses and also extend storage life (Stiles, 1990). In MAP, food products are packed in an atmosphere which has been modified so that its composition is something other than air (Hintlian & Hotchkiss, 1986). The optimisation of the gas mixture composition is critical to ensure both product quality and safety (Møller, Jensen, Olsen, Skibsted, & Bertelsen, 2000). The gases normally used for MAP include carbon dioxide, oxygen and nitrogen. The most important gas, from a microbiological standpoint is CO_2, which effectively inhibits the growth of many microorganisms, including spoilage bacteria (Hotchkiss, Werner, & Lee, 2006). Among the new gases, argon must be emphasized, this gas being very similar to nitrogen but being denser and more soluble in water than nitrogen and oxygen (Spencer & Humphreys, 2003), which could have relevant consequences on its effect on shelf life. Nevertheless, no scientific works have been carried out, as far as we are concerned, in order to evaluate the potential consequences of argon characteristics on its effect on shelf life of meat products.

Microbial growth, decolouration and lipid oxidation are important factors determining shelf life and consumer acceptance of packed dry-cured products. Colour and flavour characteristics are the main quality factors in dry-cured Iberian meat products (Cava et al., 2000), making necessary to achieve a better knowledge of the effect of different packaging conditions on parameters related to quality of these products during storage. Colour of dry-cured meat is mainly attributed to nitrosylmyoglobin content and discolouration is mainly ascribed to its oxidation (Møller et al., 2000). Recent studies by Adamsen, Moller, Parolari, Gabba, and Skibsted (2006) and Moller, Adamsen, Catharino, Skibsted, and Eberlin (2007)

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reveal that a Zn–protoporphyrin pigment constitutes a major chromophore in Iberian ham cured without nitrates and nitrites, though a complete absence of nitrosylmyoglobin was not demonstrated in this product. Salt was used in the curing process of the dry-cured hams for the present experiment, and though analysis for nitrate/nitrite content was not carried out, it is expected these chemical compounds are present as impurities (Andrés & Ruiz, 2001). On the other hand, as far as we are concerned, there is no published or accepted protocol for extracting specifically the Zn–protoporphyrin pigment. Colour stability of dry-cured meat packaged with modified atmospheres depends on a complex interaction between headspace oxygen level, product to headspace volume ratio and level of illuminance (Andrés, Adamsen, Møller, Ruiz, & Skibsted, 2005; García-Esteban, Ansorena, & Astiasarán, 2004). Lipid oxidation promotes rancidity problems which are considered unpleasant for consumers (Jeremiah, 2001). On the other hand, a close relationship between pigment and lipid oxidation has been pointed out by several authors (Faustman & Cassens, 1990; Skibsted, Mikkelsen, and Bertelsen, 1998).

A number of studies have been carried out in order to evaluate the effectiveness of vacuum, gas composition and packaging material on the preservation of fresh meat (Economou, Pournis, Ntzimani, & Savvaidis, 2009; Houben, van Dijk, Eikelenboom, & Hoving-Bolink, 2000), cooked meat products (Møller et al., 2003) dry fermented sausages (Fernández-Fernández, Vázquez-Odériz, & Romero-Rodriguez, 2002), cooked ham (Møller et al., 2000) and dry-cured ham (García-Esteban et al., 2004). Reports on Iberian dry-cured ham are much more scarce to the best of our knowledge (Andrés et al., 2005).

Thus, this work was focused on studying the evolution of instrumental colour, biochemical and sensory characteristics and microbiological quality in slices of dry-cured Iberian ham packed under vacuum and in four different modified atmospheres during chilled storage.

2. Material and methods

2.1. Samples

A total of 24 dry-cured hams obtained from Iberian pigs fed on acorn, weighing 11.7 ± 0.7 Kg were used in this study. Approximately 1.5 mm thick slices were obtained from hams. Homogeneous slices were selected in order to assign them to instrumental, sensorial or microbial analysis. Approximately 100 g of dry-cured Iberian ham slices were packed under vacuum or in the mixture of gases produced by a gas mixer (Witt-Gasetechnik GmbH and Co., Witton, Germany). The mixture of gases consisted in (i) 70%argon + 30%CO_2 = argon batch; (ii) =60%N_2 + 40% CO_2 = 60/40 batch; (iii) 70%N_2 + 30%CO_2 = 70/30 batch; (iv) 80%N_2 + 20%CO_2 = 80/20 batch. The laminated film used for packaging consisted of a mixture of PA (Polyamide) and PE (Polyethylene) (Viduca, S.L.), with an oxygen transmission rate (OTR) of 38 cm^3/m^2/24 h/atm. Packages had a headspace volume ratio of 1:1. All samples were stored in darkness at 4 ± 1 °C. The samples were opened for subsequent analysis after 1, 60 and 120 days of storage.

2.2. Gas composition

Gas composition of the headspace was analysed before opening packages in order to perform the determinations using a headspace analyser (Abiss, LS212, Germany). A septum was placed in the package and a 6 ml gas aliquot was withdrawn for analysis of relative oxygen (±1%) and carbon dioxide (±2%) content.

The oxygen level (%) was measured before opening the package and used to detect leaking packages (packages containing more than 10.3% and 20.6% oxygen after 60 and 120 days, respectively). These threshold values for leakage was chosen based on a theoretical calculation of the amount of oxygen permeating through the laminated packaging material during the days of storage using the upper limit for residual oxygen after packaging and upper limit for OTR ("worst case" conditions). The amount of oxygen inside the package was found from the following equation:

$$Q = P' \times A \times t \times \Delta P$$

where Q is the oxygen quantity, P' is oxygen transmission rate (corrected for decreased temperature, by reducing the OTR value twice for every 10 °C decrease on temperature). A is the total area of pouch, t is the time in days and ΔP is the pressure difference between the atmosphere and the headspace of the pouch.

Q = 10 cm^3/m^2/atm/24 h × 2 × 0.18 m × 0.35 m × 60 days × (0.21–0.005) = 15.5 ml O2, relative O_2 content after 60 days: (15.5 ml/150 ml) × 100 = 10.3%.

On the other hand, Q = 10 cm^3/m^2/atm/24 h × 2 x 0.18 m × 0.35 m × 120 days × (0.21–0.005) = 31.0 ml O2, relative O_2 content after 120 days: (31.0 ml/150 ml) x 100 = 20.6%.

Based on these assumptions 14 leaking packages were found.

CO_2 percent reduction was calculated considering the initial amount or concentration of the gas in the packaging (e.g. 40% CO_2 in the 60/40 batch: 60%N_2 + 40%CO_2,) and its concentration after 1 day.

2.3. Moisture content and pH measurement

Moisture content was determined following the ISO recommended method (ISO, 1973). The pH of dry-cured Iberian ham samples was measured using a micropHmeter model 2001 (Crison Instruments, Barcelona, Spain) after homogenizing 2 g of sample in 18 ml distilled water for 10 s at 1300 rpm with an Sorvall Omnimixer (Mod.17106).

2.4. Colour measurement

Colour measurements were taken in muscle BF (*Biceps femoris*) immediately after opening the package (to prevent colour degradation because of light and oxygen) in accordance with the recommendation on colour determination of the American Meat Science Association (AMSA, 1991).

The following colour coordinates were determined: lightness (L^*), redness (a^*, red ± green) and yellowness (b^*, yellow ± blue). The colour parameters were determined using a Minolta CR-300 colorimeter reflectance spectrophotometer (Minolta Camera Co., Osaka, Japan) (Illuminant D65/0° standard observer and 0.8 cm port/viewing area). a^* and b^* values were used to calculate spectral colour (hue = arctan $[b^*/a^*]$) and colour saturation (chroma = $[a^{*2} + b^{*2}]^{0.5}$). Before use, the colorimeter was standardized using a white tile (mod CR-A43). The measurements were repeated on five randomly selected locations on each slice BF and averaged for statistical analysis.

2.5. Determination of pigment content

Nitrosylmyoglobin (MbFe(II)NO) concentration was assessed following the method described by Hornsey (1956) for isolation of MbFe(II)NO in nitrite-cured meat products with slight modifications described by Andrés et al. (2005).

2.6. Lipid oxidation analysis

The extent of lipid oxidation was estimated as TBARs (thiobarbituric acid-reactive substances) by the extraction method

described in Sørensen and Jørgensen (1996). Absorbance at 532 nm (A532) was measured on three replicates of each sample on a Shimadzu UV-1201 spectrophotometer (UNICAM, Mod.Helios). Correction for sample turbidity was made by subtracting the absorbance at 600 nm (A600) from the absorbance at 532 nm (A532). TBARS were expressed as mg of malondialdehyde (MDA) per kg of muscle in samples using tetraethoxypropane (TEP) as a standard.

2.7. Sensory analysis

In order to evaluate the influence of gas composition and time of storage on sensory characteristics of ham slices, these were assessed by a trained panel of 15 members using a quantitative-descriptive analysis method (QDA) (Ruiz, Ventanas, Cava, Timón, & García, 1998) for different attributes. Panellists were trained and had participated in sensory evaluation of dry-cured hams for two years. Subjects had a total of 120 h of training in preparation for QDA of ham.

Four hams with different gas mixtures within the package were successively evaluated in each session. The sample order was randomised within the sessions. The panel was held at 11 am, 3 h after breakfast. Two thin slices (1 mm thick), containing *Biceps femoris* muscle, were served on plates to panellists. A glass of about 100 ml of water at room temperature was provided for each assessor between samples. All sessions were held in a 6 booth sensory panel room at 22 °C equipped with white fluorescent lighting (220–230 V 35 W). Descriptor selection was carried out on the basis of previous publications on Iberian ham (Andrés, Cava, Ventanas, Thovar, & Ruiz, 2004; Cava et al., 2000) and the acquired experience of our research group. Twenty two sensory traits of Iberian hams, grouped in appearance of the lean (redness, brightness and marbling), odour (intensity, rancidity, cured), texture of the lean (firmness, dryness, fibrousnesses, juiciness and pastiness), taste (saltiness, sweetness and bitterness), and aroma (intensity, cured, rancid, after-taste, toasted, off-flavours) were assessed. The panellists answered using an unstructured 10 cm line, ranging from the lowest intensity of each trait (left side) to the highest (right side), following the sensory descriptive test previously developed by Andrés et al. (2004). Definitions of sensory traits and extremes are explained elsewhere (Cava et al., 2000; Ruiz et al., 1998).

2.8. Microbial analysis

After opening the packages, 10 g of meat were taken aseptically from the slices of ham, and diluted in 90 ml of 1% peptone water (Pronadisa, Alcobendas, Madrid, Spain). Each sample was homogenised in a Stomacher Lab-Blender 400 (A.J. Seward Lab., London, England) for 1 min. Additional decimal dilutions were prepared, and the following analysis were carried out: (i) total aerobic bacterial counts on Plate Count Agar (Scharlau, Barcelona, Spain) incubated for 72 h at 30 °C (Martín et al., 2008); (ii) *Enterobacteriaceae* counts on Violet Red Bile Glucose Agar (bioMerieux, Madrid, Spain) incubated for 24 h at 37 °C (Martín et al., 2008); (iii) *Escherichia coli* counts on eosin methylene blue agar (Levine) incubated for 24–48 h at 44.5 °C (García-Esteban et al., 2004); (iv) *moulds and yeast* counts on Malt Extract Agar (Oxoid, Spain) incubated for four days at 25 °C (Martín et al., 2008). Counts were expressed as the $\log_{10}$ of colony forming units (CFU/g).

2.9. Statistical analysis

The effect of different gas mixtures during storage on samples of sliced hams was analysed by a two-way analysis of variance together with their interaction (5 treatments or gas composition × 3 sampling days of storage), using the GLM procedure of SPSS (SPSS 13.0). The Tukey test was used at the 5% level to make comparisons between sample means when pertinent. Pearson's correlation coefficient between variables was also calculated using the GLM procedure of SPSS (SPSS 13.0).

3. Results and discussion

3.1. Gas composition

Oxygen and carbon dioxide levels in packages containing dry-cured Iberian ham slices with different gas mixtures are shown in Table 1.

Oxygen contents were maintained within the acceptable levels according to the calculations explained above, and no significant variations for this parameter were detected during storage ($P > 0.05$). CO_2 content in the packages declined during storage ($P < 0.05$). This decline was presumably due to carbon dioxide absorption by the sample. CO_2 is a highly soluble gas in water and fat and it can be absorbed by the muscle and fat tissue, and thus, part of the gas injected into the package is absorbed by the sample (Jakobsen, 2003). A sharp reduction in carbon dioxide content in the packages after one day of storage is also remarkable (39.5% in argon, 24.6% in 60/40, 25.6% in 70/30 and 26.4% in 80/20). CO_2 percent reduction was similar in all the batches except for argon batch whose percentage of reduction was higher (39.5%). These results could be related to argon characteristics. In this sense, several authors have observed that argon is biochemically active, probably due to its higher solubility in water than nitrogen (Mostardini & Piergiovanni, 2002; Spencer & Humphreys, 2003). On the other hand, the absorption rate of CO_2 was approx-

Table 1
Gas evolution (O_2 and CO_2 percentages) in packages containing dry-cured ham slices stored at 4 °C during 120 days.

Days of storage	Treatment[A]	O_2 (%)	CO_2 (%)
1 day[B]	Vacuum	–	–
	Argon	0.54	20.51c[1]
	60/40	0.73	28.22a[1]
	70/30	0.36	22.80b[1]
	80/20	0.84	14.91d[1]
	SEM	0.13	0.73
	P	NS	**
60 days	Vacuum	–	–
	Argon	0.55	17.13c[2]
	60/40	0.17	23.30a[2]
	70/30	0.89	19.11b[2]
	80/20	0.31	13.14d[2]
	SEM	0.16	0.62
	P	NS	**
120 days	Vacuum	–	–
	Argon	0.39	16.63b[2]
	60/40	0.30	21.71a[2]
	70/30	0.11	16.83b[2]
	80/20	0.33	11.80c[2]
	SEM	0.10	0.60
	P	NS	***

[1,2], different superscripts in the same column within the same treatment, mean significant differences between days of storage ($P < 0.05$).
a–c, different letters in the same column within the same day of storage, mean significant differences between treatments ($P < 0.05$).
SEM = standard error of the mean.
NS = not significant ($p > 0.05$).
** ($p < 0.01$).
*** ($p < 0.001$).
[A] Argon = 70%argon + 30%CO_2; 60/40 = 60%N_2 + 40%CO_2; 70/30 = 70%N_2 + 30%CO_2; 80/20 = 80%N_2 + 20%CO_2.
[B] After 1 day of storage under refrigeration.

imately 30% in every batch during the rest of the storage, which is consistent with results obtained by Cilla, Martínez, Beltrán, and Roncalés (2006) for dry-cured ham.

3.2. pH and moisture

pH and moisture content in dry-cured Iberian ham slices packed in different gas mixtures during chilled storage are shown in Table 2. Moisture content in sliced dry-cured ham remained constant throughout storage ($P > 0.05$) and was not affected by gas composition introduced into the packs ($P < 0.05$). pH values ranged from 5.4 to 5.7 which were values considered as normal for this product (Ruiz et al., 1998) but slightly lower in comparison to hams from other commercial pig breeds (Cilla et al., 2006). pH of samples was not significantly affected by the type of gas mixture used in this study ($P > 0.05$). On the contrary, several authors observed that packaging with carbon dioxide produced a lower pH in ham (Cilla et al., 2006) and other meat products (Juncher et al., 2003; Martínez, Djenane, Cilla, Beltrán, & Roncalés, 2005). This effect has been related to the absorption of CO_2 by meat, which results in the formation of carbonic acid (Bruce, Wolfe, Jones, & Price, 1996; Dixon & Kell, 1989). A low pH has been reported to increase lipid oxidation in a variety of meat products (Martínez et al., 2005).

3.3. Colour analysis

Results for CIE (Commission Internationale de ÍEclairage, 1976) colour parameters measured on the surface of Iberian ham slices either vacuum-packaged (VP) or modified-atmosphere-packaged (MAP) throughout refrigerated storage, are shown in Table 2.

Lightness (L^*) was not significantly affected by storage time, this parameter remaining constant all throughout the experiment ($P > 0.05$). This result agrees with that found by García-Esteban, Ansorena, Gimeno, and Astiasarán (2003) in dry-cured ham stored during two months. Water content is one of the most important factors determining lightness in dry-cured ham (Sánchez-Rodríguez et al., 2001). As mentioned above, moisture content did not significantly change during storage of ham slices, which is consistent with the evolution of lightness in this study.a^*-Values in surface slices decreased significantly during storage ($P > 0.05$), indicating a loss of intensity of red colour in ham slices. Colour fading of dry-cured ham is normally attributed to the oxidation of nitrosylmyoglobin (MbFe(II)NO) resulting in the formation of metmyoglobin, which is primarily responsible for meat browning (Lindahl, Lundström, & Tornberg, 2001). MbFe(II)NO is highly unstable in the presence of oxygen (Andersen & Skibsted, 1992). Therefore, maintaining the lowest levels in oxygen in packs of dry-cured ham would keep colour for a longer period as has been demonstrated by Andrés et al. (2005). Residual oxygen levels in the current study reached 0.20–0.89%, this fact explaining the decrease in a^*-values. This relationship is also evident in this study, as indicated by Pearson's correlation coefficient between a^*-values and percentage of oxygen within the packages, which was significant and positive ($r = 0.413$; $P = 0.003$). Consistently to what has been detailed for a^*-values, MbFe(II)NO content followed a decreasing trend throughout storage in this study, from $3.0–3.7 \times 10^{-6}$ M to $2.5–2.9 \times 10^{-6}$ M (Table 2), though statistical significance was not reached ($P > 0.05$) for this parameter.

Packaging atmospheres presented a limited effect on instrumental colour parameters. L^*-values were not significantly affected by gas composition ($P > 0.05$). Significant differences for a^* ($P < 0.05$) and chroma values ($P < 0.05$) were observed after 60 days of storage. Slices of ham packed in 40% CO_2 and nitrogen (60/40) and 30% CO_2 and nitrogen (70/30) showed a less intense red colour than the rest of the samples at this stage. However, differences in colour surface were not evident after 120 days ($P > 0.05$). The presence of higher levels of CO_2 in 60/40 and 70/30 batches, may have determined the colour differences observed after 60 days. Some studies have identified negative effects of CO_2 on the colour of pig, lamb or venison meat (Anjaneyulu & Smidt, 1986; Seideman,

Table 2
Effect of packaging and time on the instrumental colour (L^*, a^*, b^*, C, h), nitrosylmyoglobin content (M), pH and moisture content (mean values) on dry-cured ham slices stored at 4 °C during 120 days.

Days of storage	Treatment[A]	pH	% Moisture	L^*	a^*	b^*	C	h	MbFe(II)NO (M)
1 day[B]	Vacuum	5.4	39.9	36.4	27.0[1]	16.1	31.6	30.2	3.2×10^6
	Argon	5.6	40.1	39.1	26.5[1]	17.0[1]	31.6[1]	32.1	3.0×10^6
	60/40	5.4	41.6	35.9	25.4	14.6	29.4	29.5	3.7×10^6
	70/30	5.5	40.5	37.9	26.3[1]	15.3	30.6	29.5	3.2×10^6
	80/20	5.5[2]	39.7	36.5	28.2[1]	14.8	31.9[1]	27.0	3.2×10^6
	SEM	0.0	0.4	0.7	0.6	0.6	0.8	0.7	2.4×10^7
	P	NS	NS	NS	NS	NS	NS	NS	NS
60 days	Vacuum	5.5	38.6	37.3	25.3ab[12]	14.2	29.1ab	29.2	3.6×10^6
	Argon	5.5	38.0	39.5	26.8a[1]	17.7[1]	32.2a[1]	33.0	3.1×10^6
	60/40	5.4	39.3	35.6	23.6ab	14.1	27.7ab	30.4	3.3×10^6
	70/30	5.5	38.3	35.5	22.0b[12]	13.8	26.2b	31.1	2.3×10^6
	80/20	5.6[12]	37.7	36.9	26.5a[1]	15.0	30.5ab[1]	29.2	3.6×10^6
	SEM	0.0	0.3	0.6	0.5	0.5	0.7	0.6	1.4×10^7
	P	NS	NS	NS	*	NS	*	NS	NS
120 days	Vacuum	5.6	39.7	36.1	21.8[2]	14.8	26.5	32.6	2.5×10^6
	Argon	5.7	40.9	35.5	20.2[2]	12.3[2]	23.6[2]	31.7	2.7×10^6
	60/40	5.6	41.4	33.9	20.5	12.6	24.3	31.0	3.2×10^6
	70/30	5.6	40.9	35.8	19.6[2]	12.9	23.9	31.8	2.8×10^6
	80/20	5.7[1]	39.9	35.9	20.3[2]	12.8	24.3[2]	32.1	2.9×10^6
	SEM	0.0	1.2	0.6	0.7	0.6	0.9	0.8	1.4×10^7
	P	NS	NS	NS	NS	NS	NS	NS	NS

[1,2], different superscripts in the same column within the same treatment, mean significant differences between days of storage ($P < 0.05$).
a–c, different letters in the same column within the same day of storage, mean significant differences between treatments ($P < 0.05$).
SEM = standard error of the mean.
NS = not significant ($p > 0.05$).
* ($p < 0.05$).
[A] Argon = 70%argon + 30%CO_2; 60/40 = 60%N_2 + 40%CO_2; 70/30 = 70%N_2 + 30%CO_2; 80/20 = 80%N_2 + 20%CO_2.
[B] After 1 day of storage under refrigeration.

Vanderzant, Smith, Dill, & Carpenter, 1980). In the same sense, Martínez et al. (2005) observed that carbon dioxide up to 60% promoted the oxidation of myoglobin in fresh sausages. Nevertheless, CO_2 levels were substantially higher in the mentioned reports than in the present study. On the other hand, hams packed with 30% CO_2 and argon in this experiment show higher a^*-values than the rest of the batches after 60 days storage ($P < 0.05$). In this sense, some studies suggest that argon interfere with the enzyme substrates receptors by oxygen (Spencer, 1995; Spencer & Humphreys, 2003) and consequently is more efficient in displacing oxygen than nitrogen. Nevertheless, a^*-values differences for argon batch are not significant after 120 days.

3.4. Oxidative stability

Results for TBARs values (expressed as mg malondialdehyde (MDA)/kg sample) from dry-cured Iberian ham slices in different packaging conditions are shown in Fig. 1.

TBARs values ranged from 1.10–1.27 mg MDA/kg of sample to 1.21–1.82 mg MDA/kg of sample at the end of the storage. TBA values showed an upward trend during the storage of packaged sliced ham, reaching statistical differences in some batches ($P < 0.05$). These results evidence that lipid oxidation occur despite of the low percentage of residual oxygen in this experiment (0.20–0.89%). Lipid oxidation promotes rancidity problems which are considered unpleasant for consumers (Jeremiah, 2001) and is related to the discolouration of meat (Faustman & Cassens, 1990; Skibsted et al., 1998).

The composition of the modified atmospheres significantly affected TBARs values after 60 and 120 days of refrigeration storage in this report ($P < 0.05$). Slices of ham packed in vacuum showed the lowest TBARs values and 60/40 batch the highest. In accordance with these findings, Cilla et al. (2006) observed a higher oxidation stability of slices of ham packed in vacuum in comparison to those packaged in modified atmospheres.

On the other hand, the results above mentioned points to a slight relationship between discolouration and lipid oxidation in this report. In fact, the calculated Pearson's correlation coefficient between a^* and the TBARs values was significant ($r = -0.197$; $P = <0.001$) as well as between chroma and TBARs values ($r = -0.161$; $P < 0.05$).

3.5. Sensory analysis

Sensory characteristics did not significantly vary ($P > 0.05$) throughout storage under refrigeration and packed in vacuum or in modified atmospheres. In relation to the appearance of the dry-cured slices, lightness and yellowness of subcutaneous fact included in the slices showed statistically differences due to the gas

Table 3
Sensory characteristics related with appearance in dry-cured ham slices and MAPs dry-cured ham slices stored at 4 °C during 120 days.

		Subcutaneous fat		Lean	
Time	Packaging[A]	Brightness	Yellowness	Redness	Brightness
1 days[B]	Vacuum	4.5	3.0	5.8	4.3ab
	Argon	5.5	3.7	6.3	4.4ab
	60/40	5.2	3.5	6.6	4.9a
	70/30	5.0	2.8	5.7	3.7b
	80/20	5.1	3.1	6.1	4.6ab
	SEM	0.2	0.2	0.1	0.1
	P	NS	NS	NS	*
60 days	Vacuum	5.2	3.3	6.1	4.8
	Argon	6.0	3.4	6.4	4.7
	60/40	5.6	3.4	6.5	4.2
	70/30	5.5	3.7	5.9	3.7
	80/20	5.8	2.9	5.5	4.3
	SEM	0.2	0.2	0.1	0.2
	P	NS	NS	NS	NS
120 days	Vacuum	3.9b	3b	5.8	3.6
	Argon	5.7a	4.3ab	6.5	4.3
	60/40	5.3ab	3.6ab	6.1	4.6
	70/30	4.9ab	5.1a	6.2	3.7
	80/20	5.3a	3.6ab	6.0	3.4
	SEM	0.2	0.2	0.1	0.2
	P	*	**	NS	NS

Least-squares means and standard mean error are shown.
a–c, different letters in the same column within the same day of storage, mean significant differences between treatments ($P < 0.05$).
SEM = standard error mean.
NS = not significant ($p > 0.05$).
* ($p < 0.05$).
** ($p < 0.01$).
[A] Argon = 70%argon + 30%CO_2; 60/40 = 60%N_2 + 40%CO_2; 70/30 = 70%N_2 + 30% CO_2; 80/20 = 80%N_2 + 20%CO_2.
[B] After 1 day of storage under refrigeration.

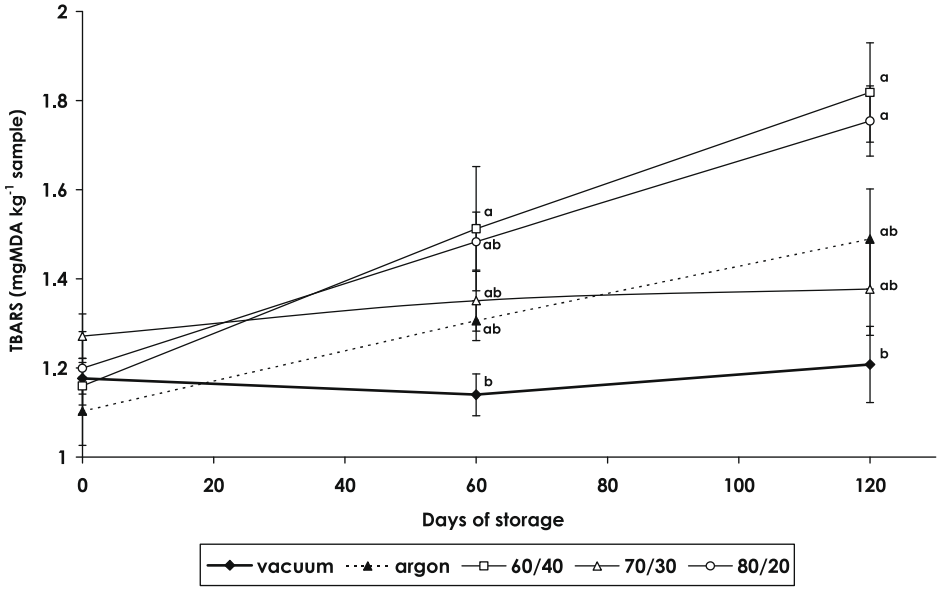

Fig. 1. Thiobarbituric acid-reactive substances (TBARs) in dry-cured ham slices stored in different atmospheres at 4 °C during 120 days.

composition ($P < 0.05$ and $P < 0.01$, respectively) after 120 days (Table 3). Vacuum-packed ham slices presented a less intense yellowness at the end of storage in comparison to the slices packed in gases. Yellowness of the fat is due to lipid oxidation and is considered a defect in fresh meat and some meat products (Arvanitoyannis, Bloukas, Pappa, & Psomiadou, 2000). Consistently, as mentioned above, vacuum-packed slices showed lower oxidation indexes than the rest of batches. Different atmospheres of packaging did not exert a significant effect on the red colour of lean ($P > 0.05$) during storage.

Sensory attributes associated with the odour and flavour from dry-cured Iberian ham slices with different packaging conditions, are shown in Table 4. Several studies reveal the importance of flavour in the overall quality of dry-cured ham (Parolari, Virgili, & Schivazappa, 1994; Ruiz, García, Muriel, Andrés, & Ventanas, 2002). Odour and flavour intensity remained unchanged after 120 days of storage whereas in other studies it was observed that odour and flavour intensity were reduced in time (Cilla et al., 2006). On the other hand, rancid odour and flavour intensity slightly increased during storage in the present study. It must be pointed out that rancid odour and aroma in vacuum-packed ham and stored for 120 days, reached lower scores than in the slices packed in modified atmospheres, though not to a significant extent. Professionals and consumers consider that very rancid hams are defective. In fact, Ruiz et al. (2002) reported a certain negative influence of rancidity on the acceptability of Iberian ham. The lower rancidity of vacuum slices is in good agreement with results explained previously for lipid oxidation, since these samples were also the least oxidised ($P < 0.05$). However, when Pearson's correlation analysis was carried out, no significant coefficients were obtained between rancidity and TBARs. In agreement with results found in this report, Cilla et al. (2006) found that the flavour is better preserved in vacuum rather than in packages with 80% N_2 and 20% CO_2. Fernández-Fernández et al. (2002) reported similar results for a dry sausage subjected to vacuum and modified atmosphere packaging.

The presence of off-flavours was also evaluated in this study. It is noteworthy that slices packed in 70% argon and 30% CO_2 showed significantly higher scores for this attribute than vacuum and the rest of gas mixtures ($P < 0.01$) after 120 days of storage. We have no ready explanation for this result. Related experiments are being carried out in this sense in our laboratory.

Attributes related to taste are summarized in Table 6. No significant differences were observed during storage or among the different treatments of packaging. Slices packed in 70% argon and 30% CO_2 showed a slight higher intensity of acid and bitter taste in comparison to the rest of the batches after 4 months of storage ($P > 0.05$). As it was mentioned above, the slices packaged with argon showed a higher intensity of off-flavours than the remaining samples. The aroma is the sensation caused by the introduction of a food into the mouth, and includes sensations related to the odour and taste. Therefore, the more intense acidity and bitterness of argon batch may be related to the off-flavours. This relationship is also found when calculating the correlation coefficients between off-flavours and acidity and between off-flavours and bitterness, which were positive and statistically significant ($r = 0.176$; $P = 0.000$ and $r = 0.438$; $P = 0.000$, respectively).

None of the attributes associated with the texture showed significant changes throughout storage time (Table 5), which agrees with the results by Cilla et al. (2006). In accordance with the findings by these authors, the attribute adhesiveness was the most affected by packaging conditions in this report, ham slices in vacuum packaging presenting a higher adhesiveness in comparison with slices in MAP. In fact, one of the major problems of the vacuum packaging is the difficulty of separation between ham slices. Therefore, according to the results of this experiment, packing ham slices in gases, minimizes the problems of adherence between slices.

Table 4
Sensory characteristics related to odour and flavour of dry-cured ham slices stored at 4 °C during 120 days.

		Odour			Flavour				
Days of storage	Treatment[A]	Intensity	Rancid	Cured	Intensity	Cured	Rancid	Persistence	Off-flavours
1 day[B]	Vacuum	6.2	3.0	6.0	6.2	5.7	2.3	6.0	0.8
	Argon	5.8	3.3	5.2	6.0	5.3	3.5	6.2	0.9
	60/40	6.0	2.9	5.4	6.0	5.4	2.9	6.1	1.1
	70/30	6.2	2.7	5.5	5.9	5.1	2.5	5.7	0.6
	80/20	6.1	2.5	5.4	6.3	5.6	2.3	6.0	0.6
	SEM	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.1
	P	NS	NS	NS	NS	NS	NS	NS	NS
60 days	Vacuum	6.8	3.6	6.0	6.2	5.7	2.9	6.0	0.7
	Argon	6.1	3.5	5.3	5.8	4.6	3.1	5.6	1.2
	60/40	6.0	3.5	5.4	6.1	5.7	3.1	5.7	0.6
	70/30	6.0	3.1	4.9	6.7	5.3	3.1	6.0	0.9
	80/20	6.3	2.9	5.6	6.5	5.6	2.9	6.1	0.4
	SEM	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.1
	P	NS	NS	NS	NS	NS	NS	NS	NS
120 days	Vacuum	6.6a	2.6	5.6	6.2	5.0	2.7	5.1	0.9b
	Argon	5.5b	4.1	4.6	6.1	4.5	4.1	5.7	1.8a
	60/40	6.0ab	4.2	5.6	6.5	5.3	3.8	6.1	0.8ab
	70/30	5.8ab	4.3	4.7	6.4	5.0	4.2	5.5	1.1ab
	80/20	6.5a	3.3	5.6	6.2	5.4	2.8	5.2	0.5b
	SEM	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.1
	P	*	NS	NS	NS	NS	NS	NS	**

a–c, different letters in the same column within the same day of storage, mean significant differences between treatments ($P < 0.05$).
SEM = standard error of the mean.
NS = not significant ($p > 0.05$).
* ($p < 0.05$).
** ($p < 0.01$).
[A] Argon = 70%argon + 30%CO_2; 60/40 = 60%N_2 + 40%CO_2; 70/30 = 70%N_2 + 30%CO_2; 80/20 = 80%N_2 + 20%CO_2.
[B] After 1 day of storage under refrigeration.

Table 5
Sensory characteristics related to texture of dry-cured ham slices stored at 4 °C during 120 days.

Days of storage	Treatment[A]	Adhesiveness	Hardness	Dryness	Fibrousness	Juiciness	Pastiness
1 day[B]	Vacuum	3.0	3.1b	2.4	3.3	5.1	3.4
	Argon	2.4	4.9ab	2.9	3.6	4.6	3.0
	60/40	2.9	4ab	2.7	3.4	4.9	3.0
	70/30	2.6	4.1ab	3.5	3.6	4.3	3.2
	80/20	2.8	3.3b	2.8	3.6	4.8	3.0
	SEM	0.2	0.1	0.1	0.2	0.2	0.2
	P	NS	***	NS	NS	NS	NS
60 days	Vacuum	3.7a	4ab	2.6	3.5	5.0	3.0
	Argon	3.0b	3.5b	2.5	3.7	4.9	3.8
	60/40	3.1b	3.4ab	2.4	3.0	4.9	2.8
	70/30	3.0b	4.4a	2.9	4.1	4.3	3.1
	80/20	2.9b	3.5b	3.0	3.7	4.7	3.0
	SEM	0.2	0.1	0.1	0.2	0.2	0.2
	P	*	*	NS	NS	NS	NS
120 days	Vacuum	3.6	3.1bc	2.5	3.6	5.0	3.4
	Argon	3.0	3.2bc	2.4	3.3	5.0	3.4
	60/40	3.0	3.5c	2.6	3.2	4.7	2.9
	70/30	2.5	4.7a	3.4	3.5	4.2	3.0
	80/20	3.2	4.3ab	3.1	3.8	4.8	2.9
	SEM	0.2	0.2	0.2	0.2	0.2	0.2
	P	NS	***	NS	NS	NS	NS

a–c, different letters in the same column within the same day of storage, mean significant differences between treatments ($P < 0.05$).
SEM = standard error of t he mean.
NS = not significant ($p > 0.05$).
* ($p < 0.05$).
*** ($p < 0.001$).
[A] Argon = 70%argon + 30%CO_2; 60/40 = 60%N_2 + 40%CO_2; 70/30 = 70%N_2 + 30%CO_2; 80/20 = 80%N_2 + 20%CO_2.
[B] After 1 day of storage under refrigeration.

Table 6
Sensory characteristics related to taste of dry-cured ham slices stored at 4 °C during 120 days.

Days of storage	Treatment[a]	Saltiness	Sweet	Bitter	Acid
1 day[b]	Vacuum	4.9	2.2	1.1	1.3
	Argon	5.0	2.4	1.4	1.4
	60/40	4.8	2.8	1.1	1.4
	70/30	5.5	2.1	1.2	1.3
	80/20	5.8	2.1	1.2	1.4
	SEM	0.2	0.2	0.1	0.1
	P	NS	NS	NS	NS
60 days	Vacuum	5.0	2.6	1.4	1.9
	Argon	5.0	3.0	1.3	1.9
	60/40	4.9	3.0	1.4	1.8
	70/30	6.3	1.9	1.8	1.9
	80/20	5.7	2.4	1.4	1.9
	SEM	0.2	0.2	0.1	0.2
	P	NS	NS	NS	NS
120 days	Vacuum	4.6	2.7	1.3	1.6
	Argon	5.3	2.5	1.8	2.0
	60/40	4.6	2.6	1.1	1.5
	70/30	5.6	2.3	1.5	1.5
	80/20	6.2	1.8	1.6	1.7
	SEM	0.2	0.2	0.1	0.1
	P	NS	NS	NS	NS

SEM = standard error of the mean.
NS = not significant ($p > 0.05$).
[a] Argon = 70%argon + 30%CO_2; 60/40 = 60%N_2 + 40%CO_2; 70/30 = 70%N_2 + 30%CO_2; 80/20 = 80%N_2 + 20%CO_2.
[b] After 1 day of storage under refrigeration.

3.6. Microbial analysis

Table 7 shows the results of the microbial analysis of dry-cured Iberian ham slices with different packaging conditions in refrigerated storage initially (0 day) and after 60 and 120 days.

Wang (2001) observed that the microbiological quality of ham depends on water activity (a_w), which in the cured ham is low (about 0.85) as well as the bacteriostatic effect of modified atmospheres. In this sense, Devlieguere and Debevere (2000) demonstrated that carbon dioxide concentration in the aqueous-phase of meat determines the level of inhibition of microorganisms growth in the atmosphere used.

Mesophilic aerobic bacteria counts were in the range of 2.1–4.1 log cfu/g during 120 days of storage in all samples. The type of gas mixture did not produce different counts after 0, 60 or 120 days ($P > 0.05$). On the contrary, García-Esteban et al. (2003) found that vacuum packaging reduced the level of counts in cured ham from white pigs during two months of storage. However, Rubio et al. (2006) did not found differences between vacuum packaging and atmospheres containing 20% CO_2 and 80% N_2 and 80% N_2/20% CO_2 in a dry-cured product, "Cecina de Leon".

Microorganisms belonging to the family *Enterobacteriaceae* are considered indicators of hygiene and initially were present in all samples. A downward trend for counts of *Enterobacteriaceae* is observed in every batch. Counts of *Enterobacteriaceae* were not significantly inhibited by vacuum or modified atmosphere packaging ($P > 0.05$). These results are in agreement with those found by García-Esteban et al. (2003), who did not found significant differences for counts of *Enterobacteriaceae* between vacuum packaging and an atmosphere containing 80% of nitrogen and 20% of dioxide carbon in ham. On the contrary, the results obtained in this study contrast with those observed by Rubio et al. (2006), who found a significant inhibitory effect of 20%/80% CO_2/N_2 and 80%/20% CO_2/N_2 in comparison with vacuum packaging. Regarding the counting of *E. coli,* there were not differences between packaging systems ($P > 0.05$). Similar results were found in the studies carried out in ham and "cecina" (García-Esteban et al., 2003; Rubio et al., 2006). With respect to molds and yeasts, the counts were reduced during storage to the same extent in all the packaging although it was not significant ($P > 0.05$). García-Esteban et al. (2003) did not find significant differences either but, these authors also observed a reduction in counts of molds and yeasts on vacuum and MAP ham.

Table 7
Effect of packaging and time on microbial counts (log cfu/g) (mean values) of dry-cured ham slices stored at 4 °C during 120 days.

Time	Packaging[a]	Mesophilic aerobic bacteria	*Enterobacteriaceae*	*Escherichia coli*	Moulds and yeast
1 day[b]	Vacuum	3.7	1.3	0.3	2.7
	Argon	2.9	1.1	ND	2.6
	60/40	3.3[2]	1.2	1.0	3.0
	70/30	2.8	1.2	0.6	2.7
	80/20	3.6[1]	0.3	ND	2.8
	SEM	0.3	0.2	0.1	0.4
	P	NS	NS	NS	NS
60 days	Vacuum	3.1	0.9	0.7	2.6
	Argon	2.3	0.3	0.2	2.5
	60/40	3.8[1]	1.1	0.3	3.1
	70/30	2.3	0.4	ND	2.9
	80/20	2.1[2]	1.1	0.4	2.9
	SEM	0.3	0.1	0.1	0.3
	P	NS	NS	NS	NS
120 days	Vacuum	3.3	0.9	0.4	2.6
	Argon	3.5	0.9	0.5	2.5
	60/40	4.1[1]	0.7	0.5	3.0
	70/30	3.6	0.6	0.3	3.2
	80/20	3.4[12]	0.9	0.3	3.2
	SEM	0.1	0.1	0.1	0.1
	P	NS	NS	NS	NS

[1,2], different superscripts in the same column within the same treatment, mean significant differences between days of storage ($P < 0.05$).
SEM = standard error of the mean.
NS = not significant ($p > 0.05$).
ND: not detected.
[a] Argon = 70%argon + 30%CO_2; 60/40 = 60%N_2 + 40%CO_2; 70/30 = 70%N_2 + 30%CO_2; 80/20 = 80%N_2 + 20%CO_2.
[b] After 1 day of storage under refrigeration.

In this study other microorganisms such as *E. coli* O157:H7, *Campylobacter* sp., *Salmonella* sp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus* and *Vibrio* sp. were also analysed. There was a total absence of these microorganisms for all packaging atmospheres used for our experiment (data not shown).

In summary, it could be concluded that the studied gas mixtures did not promote clear differences in physico-chemical characteristics of dry-cured Iberian ham slices such as pH, moisture content or colour surface. However, preservation of lipid oxidative stability was best achieved using vacuum packaging rather than modified atmosphere packaging, the latter samples being perceived, to some extent, as more rancid.

Packaging conditions used in this experiment did not lead to differences in microbiological counts on dry-cured Iberian ham slices. Moreover, regarding microbiological values after 4 months, all the samples were suitable for consumption.

Finally, the mixture of CO_2 with argon instead of nitrogen, in the conditions of the present experiment does not lead to significant differences in dry-cured ham slices quality.

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