Comparison of modified atmosphere packaging and vacuum packaging for long period storage of dry-cured ham: effects on colour, texture and microbiological quality.

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Abstract

Slices of dry-cured hams (*Biceps femoris* muscle) were stored during 8 weeks under vacuum and modified atmospheres (100% N_2 and a mixture of 20% CO_2 and 80% N_2) in order to study the modifications on colour, texture and microbial counts during that period. Luminosity was found to be more stable when samples were stored with 20% CO_2 and 80% N_2 without statistical differences between vacuum and 100% N_2. A slight whiteness was observed in the vacuum packed samples. Yellowness increased during time in vacuum packed samples, although no differences were found among the three conditions at the end of the study. Redness values were not affected by time nor by the packaging system. With regard to texture, values found for all samples were within the normal range for this type of products, although it was observed that modified atmosphere packaging preserved better samples from hardening than vacuum packaging. No safety problems were detected in relation to the microbial quality in any case. In general, no clear differences were found among the three packaging systems for colour, texture and microbial quality in the storage conditions studied.

Keywords

INTRODUCTION

Food industry has developed different packaging technologies trying to extend the shelf-life of perishable products such as meat and meat products. Among these technologies, vacuum packaging prevents product from contamination and evaporative losses, and modified atmosphere packaging also extends storage life (Stiles, 1990).

In general, modified atmosphere packaging (MAP) extends the shelf-life of fresh meat in a significant way, but a control of temperature and initial microbiological quality of raw matter is required. In relation to meat products, there is less potential to extend their shelf-life using MAP than with fresh meat since operations as drying, curing, smoking, fermentation, freezing, cooking and chilled storage already help to extend shelf-life (Church, 1993). Nevertheless, microbial spoilage and colour deterioration are considered the most common problems during the shelf-life of meat products (Church, 1993).

The application of MAP to processed meat has grown greatly in recent years, but optimisation of gas composition is critical to ensure both product quality and safety (Moller, Jensen, Olsen, Skibsted & Bertelsen, 2000). CO_2 is because of its antimicrobial activity, the most important component in the normally applied gas mixtures (Devlieghere, Debevere & Van Impe, 1998) and N_2 is used as a filler (Sorheim, Nissen & Nesbakken, 1999).

A lot of studies have been carried out in order to study the efectiveness of vacuum, different gas composition and packaging material on the preservation of fresh meat (García de Fernando, Nychas, Peck & Ordóñez, 1995; Houben, van Dijk, Eikelenboom & Hoving-Bolink, 2000; Sorheim et al., 1999; Buys, Nortjé, Jooste & Von Holy, 2000; Gill, 1996), cooked meat products (Houben & van Dijk, 2001; Moller, Jakobsen, Weber, Martinussen, Skibsted & Bertelsen, 2003; Mataragas, Drosinos &

Metaxopoulos, 2003) and dry fermented sausages (Yen, Brown, Dick & Acton, 1988; Fernández-Fernández, Vázquez-Odériz & Romero-Rodríguez, 2002). In relation to dry-cured ham not many research articles can be found in the bibliography. However, the use of MAP is becoming extensively used in industry and, above all, it is important for exportations (Ayuso, 2003).

Colour stability of cured meat packaged with modified atmospheres depends on a complex interaction between headspace oxygen level, product to headspace volume ratio and the level of illuminance. Moller et al. (2003) considering all these factors concluded that for a product/headspace volume ratio of 1:1 the colour is much better preserved than for larger headspace volumes, and in order to maintain a high a-value, it is necessary to keep the oxygen level low and also a low level of illuminance. García-Esteban, Ansorena, Sánchez and Astiasarán (2003b) found slight differences between vacuum and MAP storage of *Semimembranosus* muscle of dry-cured ham.

Studies carried out in fresh meat pointed out that vacuum packaging show lower drip loss than MAP (Sorheim, Kropf, Hunt, Karwoski & Warren, 1996). Dry cured products such as ham do not show the problem of drip loss, but some moisture losses can be produced during storage that could affect texture properties. Córdoba (1990) stated up that higher shear force values were observed in muscles of dry cured ham with significant lower moisture content.

MAP has even been used to accelerate ripening of dry-cured boneless hams observing that it is feasible to ripen them without negative influence on quality (Wang, 2001). Ripening of dry-cured hams under MA could reduce cholesterol oxidation products and mite and fungus growth (Sánchez-Molinero, Arnau, García Regueiro & Rius, 2003). In Chinese-style sausages, a comparison of the lipid oxidation suffered by samples under vacuum and under MAP was carried out (Wang, Jiang & Lin, 1995). However, no

research works have been found studying the differences between vacuum and MAP conditions of storage of dry-cured ham.

The aim of this work was to compare the evolution of colour, texture and microbiological quality of dry-cured ham during chilled storage of slices packed under vacuum and under two MA conditions (100% N_2 and 80%N_2 + 20%CO_2).

MATERIALS AND METHODS

Samples

Samples were taken from twelve dry-cured hams ripened for approximately one year. These hams were sliced and packaged (with a packer Ramon Serie: VP Mod: 450) under one of the three conditions: vacuum, 100% N_2 (Extendapack 1, *Praxair*) and a gas mixture of 20% CO_2 and 80% N_2 (Extendapack 14, *Praxair*). Three slices of each ham were taken and the slices of four hams were stored under each condition. One slice of each ham was analysed just after packaging, another one three weeks later and the last one, eight weeks later. The film used for packaging consisted on seven layers: polyethylene (PE) of medium linear density, polyethylene (PE) of medium density, polyamide (PA) modified, ethylene-vinylidene alcohol (EVOH) alloy, polyamide (PA) modified, polyethilene (PE) of linear low density and 4.5% ethylene-vinylidene acetate (EVA) (Super7E2, thickness of 140µm, Vaessen-Schoemaker industrial, S.A.). This film has an oxygen transmission rate (OTR) of 8,3 $cc/m^2/atm/24hrs$ at 23ºC and 0% R.H. Packages were stored at chilling storage at 4ºC without light for the period mentioned. Packages had a headspace volume ratio of 1:1.

Instrumental colour measurement

Color measurements were taken in triplicate on the *Biceps femoris* muscle of 12 mm slices. Reflectance Spectra was determined with a UV/VS Perkin Elmer Lambda 5 Spectrophotometer from 400 to 700 nm, at 10 nm intervals, using an integrating sphere. Colour system employed was Hunter L a b with illuminant A and 10º observer angle (García-Esteban, Ansorena, Gimeno & Astiasarán, 2003a). The colour co-ordinates were calculated by means of the PECOL (Perkin Elmer) computer package.

Texture analysis

Texture parameters were determined by means of texture profile analysis (TPA) (Bourne, 1978; Henry, Katz, Pilgrim & May, 1971). Measurements were taken at room temperature, with a TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, UK) with a load cell of 5 kg. Measurements were taken on the *Biceps femoris* muscle of 12 mm slices, stored under the mentioned conditions. Compression was performed with a cylinder probe of one inch (P/1R, delrin). Two uniaxial compressions were carried out until 30% of deformation of the original height, applying a crosshead speed of 3 mm/sec. and a chart speed of 1 mm/sec.

Data collection and analysis were performed by means of the Texture Expert Stable Micro Systems computer program, version 1.16. Graphics allowed to calculate different texture parameters: hardness, springiness, cohesiveness, gumminess and chewiness. Hardness was the peak force during first compression cycle, springiness was the ratio of the time recorded between the start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reversal, cohesiveness was the ratio of the area under the second curve to the area under the first curve, gumminess was the product of hardness and cohesiveness and chewiness was the product of hardness, cohesiveness and springiness.

Microbial analysis

Microbial determinations were performed according to the following analytical methods: mesophile aerobic colonies (ISO 2293:1988), lactic acid bacteria (ISO 15214:1998), moulds and yeast counts (Berenguer, 1992), *Enterobacteriaceae* (ISO 5552:1997), coliforms (AOAC 982.36, 2002), *Escherichia coli* (AOAC 982.36), sulfite-reducing spored anaerobic bacteria (Pascual, 1992), *Staphylococcus aureus* (ISO 6888-

1:1999), *Salmonella* (Method ISO 3565:1975), *Listeria monocytogenes* (Automated Inmunoassay. Protocol Vidas. Validated by AFNOR: nº certificate BIO-12/03-03/96) and *Campylobacter jejuni* (Stern, Wojton & Kwiatek, 1992).

Moisture determination

Moisture was determined according to the AOAC method (AOAC 950.46, 2002).

Oxygen analysis

Before opening packages (in order to perform determinations), oxygen in bags was measured with an Abiss Analyser (Modelo PAK01P nº 11 1631).

Data analysis

Experimental data were statistically processed using the SPSS version 9.0 software. Mean values, standard deviation and coefficient of variation are shown in the tables. For colour and texture parameters, an ANOVA test was carried out to determine statistical differences along the storage period or differences among the storage systems. Principal component analysis was carried out with colour, texture and moisture parameters.

RESULTS AND DISCUSSION

Color analysis

Results of colour measurement are shown in table 1. It includes the three primary (L, a and b) colour co-ordinates used in the Hunter system to determine colour. Luminosity (parameter *L)* at 0 days showed in all samples similar mean values to results of colour analysis performed in the same muscle in a previous study carried out by García-Esteban et al. (2003a) to optimise the instrumental colour analysis in dry cured ham. Luminosity increased during storage in vacuum packed samples showing significantly higher values by the third week of storage, which could be due to a whitening surface observed in these slices. No changes were observed for this parameter in samples under vacuum along the following weeks. Samples under both modified atmospheres kept L constant during the whole period of storage, showing values at the end of the storage period very similar to those at the packaging moment. The lowest L values at 3 and 8 weeks corresponded to samples under $CO_2 + N_2$ which did not show differences with samples under N_2 but were significantly different from those stored under vacuum storage.

Parameter b (yellowness) also increased by the third week of storage only in vacuum packed samples, but no significant differences were found among the three packaging conditions at the end of the eight weeks for yellowness. Differences in b along the storage period could be related to the intensity of the oxidation process that takes place during storage and might tend to increase yellowness of samples by rancidity. Wang et al. (1995) analysing the lipid oxidation in Chinesse-style sausage stored at two temperatures (4ºC and 15ºC) in vacuum and MA packaging found that TBA and peroxide values were lower in MA than in vacuum conditions at both temperatures. A slight but significant higher value was observed in this parameter at 0 days between

samples with N_2 and the two other conditions. This difference was probably due to the variability among the different hams. With regard to redness (a), which has been used as an indicator of colour stability in meat and meat products, no differences were detected nor during the storage periods nor during the three storage conditions. Yen et al. (1988) compared five packaging materials with OTR between 1 and 90 $cm^3/m^2/atm/24h$, in vacuum packed dry salami and found no significant decrease in a-value when OTR was below 30 $cm^3/m^2/atm/24h$. A decrease in a values was found in cooked cured ham packaged with films with OTRs of 10 (Moller et al., 2003), showing that colour stability decreased when residual oxygen increased. In the present work, packaging film used had a low OTR and the oxygen analysis performed showed very low levels of oxygen at the third week of storage (0.1%), and colour stability was kept in these samples. 0.1% or less residual oxygen protected sliced, pasteurised ham from discoloration when exposed to light during chilled retail display (Moller et al., 2000).

Consequently, only slight differences in luminosity were found between vacuum and MAP stored samples without differences in redness and yellowness among the three studied conditions.

Texture analysis

Changes in hardness during dry-cured ham ripening have been attributed to both water content and state of proteins (Monin, Marinova, Talmant, Martin, Cornet, Lanore & Grasso, 1997). These changes could continue also during the storage period, where modification of the water content could be observed. In vacuum and N_2 packed samples, there was a decrease in moisture at three weeks, decreasing significantly ($p<0.05$) from 55.56 to 53.85% and from 56.63% to 55.81%, respectively. No decrease was observed in samples packed with $CO_2 + N_2$. Bartkowiski, Dryden and Marchello, (1982) found that beef stored under controlled atmospheres contained more moisture than when it

was stored with vacuum. Ruiz, Ventanas, Cava, Timón & García (1998) studying the influence of slice location on the sensory characteristics of Iberian ham observed significant changes in the appearance and texture. Texture parameters were evaluated by TPA analysis carried out in *Biceps femoris* (Table 2). When studying their evolution throughout the storage period, it could be observed that samples packed with N_2 or N_2+CO_2 showed no statistical differences in any parameter during the period studied with regard to those values observed at the packaging moment. However, vacuum packaging increased hardness, cohesiveness, gumminess and chewiness already by the third week of storage and these values were maintained at eight weeks. Guerrero, Gou and Arnau (1999) evaluating texture of *Biceps femoris* of dry-cured hams found values for hardness compressed between 2600g in hams elaborated with high pH meat and 4700g in those elaborated with normal pH meat. Values around 2320g were obtained for hardness in the same muscle by Monin et al. (1997) after 251 days of ripening. Virgili, Parolari, Schivazappa, Soresi Bordini and Borri (1995) evaluating the texture of 120 hams at the end of maturation detected a hardness range between 730-4370 g also in the *Biceps femoris* muscle. At the end of the storage period, vacuum packed samples showed, among the three packaging systems, the highest hardness value (2669g), significantly higher than the one found for samples with N_2 but without significant differences with samples with CO_2+N_2.

It could be concluded that modified atmosphere packaging preserved samples from hardening and deterioration of textural properties more efficiently than vacuum packaging. Nevertheless, taking into account the normal variability found for this parameter, all values were considered normal for this type of product.

Microbiologycal quality

Table 3 shows results of the microbiological analysis of hams at the beginning and at the end of the study. Wang (2001) applying MAP for reducing ripening time of dry cured boneless hams stated up that the stability of microbiological quality is attributed to low water activity in dry-cured ham and bacteriostatic effect of modified atmospheres. *Enterobacteriaceae,* considered to be hygienic indicators, were found to be less than 10 cfu/g at the end of the curing process. In all the storage conditions, the final product showed no differences in the parameters related to hygienic-sanitary quality compared to the initial slices. A special care has to be taken of the growth of survival psychrotrophic pathogens as *Listeria monocytogenes* under modified atmospheres. García de Fernando et al. (1995) stated up that both vacuum and modified atmosphere packaging under 100% N_2 readily support the growth of some pathogens such as *L. monocytogenes*. In our work *L. monocytogenes* was not detected in any sample. *Lactic acid bacteria* (*LAB*) counts were around 10^2 during the whole period of storage. These *LAB* naturally dominate the microflora of many meat products chill-stored under vacuum or in an environment enriched with CO_2 (Holzapfel, Geisen & Schillinger, 1995; Stiles, 1996). Mataragas et al. (2003) proved that some *LAB* strains may be used as protective cultures to inhibit the growth of *L. monocytogenes* or its bacteriocins in vacuum or MA ($80\% CO_2 + 20\% N_2$) packaged sliced cooked cured pork at 4ºC.

Staphylococcus aureus has also been controlled because it is one of the risk factors in food poisoning. Portocarrero, Newman and Mikel (2002) pointed out that higher salt content and lower water activity values on country-cured hams play an important role in controlling the growth and toxin production of *Staphylococcus aureus.* In this work no increases were found in *S. aureus* counts along the 8 weeks of storage in none of the

studied conditions and values of water activity were always lower than 0.8. There was absence of *Salmonella* and *Campylobacter jejuni*.

Final aerobic mesophile counts were, in all cases, lower than 10^4 cfu/g, which were the values found at the end of both fast and slow curing of dry-cured ham (Marín, Carrascosa & Cornejo, 1996). Mesophile aerobic colonies at eight weeks slightly increased with regard to initial counts in vacuum packed samples whereas a decrease was found in samples under 100% N_2 and 20% CO_2+ 80% N_2.

As it can be seen in figure 1, despite the differences found in the ANOVA test for luminosity, moisture and hardness between vacuum and MAP conditions at the end of the study, the results of the principal component analysis showed that no clear advantage of the modified atmospheres was observed with regard to vacuum packaging of dry-cured ham. The plot of samples in function of the two principal components resulted in an overlapped diagram, without clear differences among the different storage conditions.

In summary, it could be concluded that, with regard to colour, texture, moisture and microbiological stability, dry-cured ham stored in vacuum and modified atmospheres (100% N_2 and 20% CO_2 + 80% N_2) did not show clear differences.

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TABLE 1. Colour evolution in *Biceps femoris* muscle during the storage period for all the packaging conditions.

PACKAGING	HUNTER A10	0 days	3 weeks	8 weeks
Vacuum	**L**	41.69aA (1.22; 2.93)	46.64bB (2.71; 5.28)	46.65bB (3.30; 7.07)
	a	21.27aA (1.29; 6.06)	19.36aA (2.50; 12.91)	20.09aA (2.37; 11.80)
	b	22.01aA (0.70; 3.18)	23.59bA (1.37; 5.81)	23.79bA (0.96; 4.03)
N₂	**L**	42.43aA (1.28; 3.02)	43.85aAB (1.40; 3.19)	44.26aAB (1.95; 4.41)
	a	21.57aA (1.60; 7.42)	22.53aA (1.69; 7.50)	21.74aA (0.96; 4.41)
	b	23.78aB (1.37; 5.76)	24.48aA (1.18; 4.82)	24.04aA (1.48; 6.16)
CO₂ + N₂	**L**	41.94aA (0.67; 1.60)	43.33aA (2.11; 4.87)	41.47aA (2.75; 6.63)
	a	20.92aA (2.67; 12.76)	20.66aA (2.63; 12.73)	20.56aA (3.40; 16.54)
	b	22.31aA (0.37; 1.66)	23.02aA (1.24; 5.39)	22.07aA (1.60; 7.25)

Results are the mean of the three measurements made in four dry-cured hams.
Values in brackets are (standard deviation; coefficient of variation).
Statistics in small letters compare the three periods in each storage condition and parameter.
Statistics in capital letters compare the three storage conditions for each parameter at certain moment.

TABLE 2. Texture parameters measured on *Biceps femoris* muscle of dry-cured ham slices packaged under different conditions for the period of time studied.

PACKAGING	PARAMETERS	0 days	3 weeks	8 weeks
Vacío	**Hardness (g)**	2030aA (147; 7)	2544bA (443; 17)	2669bB (334; 12)
	Springiness (mm)	0.813aA (0.03; 4)	0.839aA (0.07; 8)	0.850aB (0.07; 8)
	Cohesiveness	0.556aA (0.03; 5)	0.610bB (0.01; 2)	0.613bB (0.03; 5)
	Gumminess (g)	1130aA (113; 10)	1551bB (260; 17)	1636bB (227; 14)
	Chewiness (g x mm)	922aA (126; 13)	1303bB (235; 18)	1391bB (227; 16)
N$_2$	**Hardness (g)**	1552aA (542; 35)	1922aA (573; 30)	1679aA (528; 31)
	Springiness (mm)	0.705aA (0.07; 10)	0.777aA (0.129; 16)	0.690aA (0.09; 13)
	Cohesiveness	0.508aA (0.02; 5)	0.498aA (0.06; 11)	0.505aA (0.03; 5)
	Gumminess (g)	781aA (252; 32)	974aA (353; 36)	855aA (293;34)
	Chewiness (g x mm)	554aA (195; 35)	787aA (369; 47)	609aA (270; 44)
CO$_2$ + N$_2$	**Hardness (g)**	1954aA (804; 41)	2160aA (902; 42)	2178aAB (815; 37)
	Springiness (mm)	0.787aA (0.12; 16)	0.757aA (0.07; 9)	0.802aB (0.09; 12)
	Cohesiveness	0.548aA (0.06; 11)	0.548aA (0.06; 11)	0.569aB (0.07; 11)
	Gumminess (g)	1100aA (541; 49)	1230aAB (615; 50)	1291aAB (616; 47)
	Chewiness (g x mm)	914aA (552; 60)	954aAB (519; 54)	1085aB (613; 56)

Values in brackets are (standard deviation; coefficient of variation).
Results are the mean of the four measurements made in four dry-cured hams.
Statistics in small letters compare the three periods in each storage condition and parameter.
Statistics in capital letters compare the three storage conditions for each parameter at certain moment.

TABLE 3. Microbiological stability of dry-cured ham slices packed under different conditions during the period of time studied.

MICROBIOLOGICAL PARAMETERS	VACUUM		100% N_2		20% CO_2 + 80% N_2	
	0 days	8 weeks	0 days	8 weeks	0 days	8 weeks
Mesophile aerobic colonies (cfu/g)	$7,5 \times 10^2$	$9,45 \times 10^3$	$7,4 \times 10^3$	$1,95 \times 10^3$	$1,65 \times 10^4$	$4,35 \times 10^3$
Lactic acid bacteria (cfu/g)	$< 1,0 \times 10^2$	$< 1,0 \times 10^2$	$< 1,0 \times 10^2$	$< 1,0 \times 10^2$	$< 1,0 \times 10^2$	$< 1,0 \times 10^2$
Moulds and yeasts count (cfu/g)	$5,0 \times 10^2$	$4,0 \times 10^2$	$8,0 \times 10^2$	$3,5 \times 10^2$	$3,15 \times 10^2$	$3,5 \times 10^2$
Enterobacteriaceae (cfu/g)	$< 1,0 \times 10^1$	$< 1,0 \times 10^1$	$< 1,0 \times 10^1$	$< 1,0 \times 10^1$	$< 1,0 \times 10^1$	$< 1,0 \times 10^1$
Coliforms (cfu/g)	< 3	< 3	< 3	< 3	< 3	< 3
E.coli /g	< 3	< 3	< 3	< 3	< 3	< 3
Sulfite-reducing spored anaerobic bacteria (cfu/g)	$< 1,0 \times 10^1$	$< 1,0 \times 10^1$	$< 1,0 \times 10^1$	$< 1,0 \times 10^1$	$< 1,0 \times 10^1$	$< 1,0 \times 10^1$
Salmonella (25g)	Absence	Absence	Absence	Absence	Absence	Absence
Staphylococcus aureus (cfu/g)	$< 10^2$	$< 1,0 \times 10^2$	$< 1,0 \times 10^2$	$< 1,0 \times 10^2$	$< 1,0 \times 10^2$	$< 1,0 \times 10^2$
Listeria monocytogenes (25g)	Absence	Absence	Absence	Absence	Absence	Absence
Campylobacter jejuni (25g)	Absence	Absence	Absence	Absence	Absence	Absence

FIGURE 1. Plot of the results obtained for the principal component analysis test carried out with colour parameters, texture parameters and moisture .
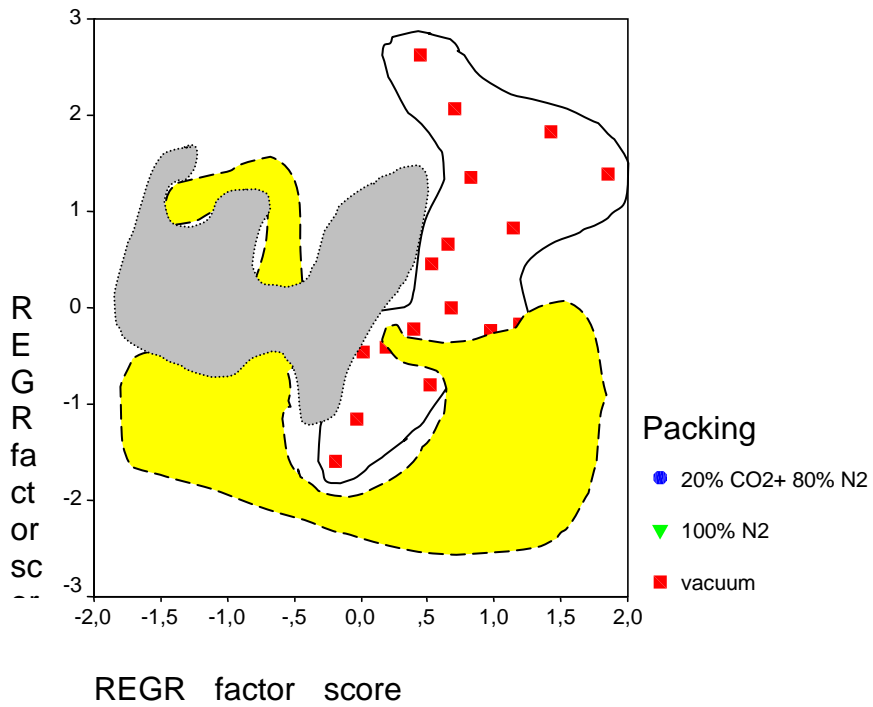
Component 1 explained 55,24% of the variance. Component 2 explained 18,16% of the variance.