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# Conservation of *Cervus elaphus* meat in modified atmospheres

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**Abstract**

This study examines the effect of three types of modified atmospheres, each with a different gas composition (A:40% $CO_2$ + 60% $N_2$; B:80% $CO_2$ + 20% $O_2$; C:80% $CO_2$ + 20% $N_2$), on the development of meat quality of *Cervus elaphus* in order to suggest a gas composition that best preserves this type of meat. Meat quality was assessed by examining pH, colour as $L^*$ $a^*$ $b^*$ values, drip loss (DL), cooking loss (CL) and shear force (SF). In samples of group A, pH values tended to be higher in all storage periods than those packed with 80% $CO_2$ and significant differences ($P < 0.001$) among the groups were found at 16 d of storage. Gas composition affected $a^*$ and $b^*$ parameters ($P < 0.001$), in samples packed with $O_2$, the $b^*$ values were higher than in other groups, while the opposite was true in $a^*$ values. Similar values of DL and CL were observed for all treatments and both parameters increased over time. SF values decreased with ageing, with similar values observed for all treatments.


*Keywords:* Meat quality; Deer; *Cervus elaphus*; Modified atmospheres; Ageing

## 1. Introduction

Deer is considered as the most recently domesticated species (Fletcher, 1998), and today deer (*Cervus elaphus*) farms provide a efficient use of land and also high-quality products, while offering a type of livestock that does not damage the environment (Milne, 1994). Although few farms raise deer in Spain, the introduction of this animal onto farms is quite feasible, specially because deer is a native animal well-adapted to the climate of the peninsula. According to data from the Federation of European Deer Farmers' Association (FEDFA) in Spain, there are ten farms, with an annual production that constitutes 1.5% of European production.

In addition, deer meat is very rich in protein and low in fat (21 and 1.2%, respectively) and in relation to phosfolipids half of the acids are polyunsaturated (Manley & Forss, 1979; Drew & Seman, 1987). Despite the fact that deer meat is considered a luxury product due to its organoleptic characteristics and high price, its consumption (presently 50 g per person and year) is increasing in Spain, this product now representing a viable alternative type in the consumption of meat. However, most deer meat is exported to other countries, thus requiring a method of preservation that permits it to arrive at its destination without a loss in quality.

Colour, tenderness and juiciness are the most important parameters that consumers value in meat. A great number of factors (intrinsic and extrinsic) affect meat quality and, therefore, the value of the product. One of the significant extrinsic factors is the method used to preserve meat (Sañudo, Sánchez, & Alfonso, 1998). Modified atmosphere packing has been used for increased distribution range and longer shelf-life. The effects and roles of the gases normally used in the modified atmospheres ($O_2$, $CO_2$ and $N_2$) have been extensively reported (Church, 1994; Gill, 1996; Jeremiah & Gibson 2001). Hood and Mead (1995) indicated that the effects which the mixture of gas produces in meat quality, such as colour and shelf life, are the principal factors that should be considered when choosing the gas mixture. In addition, Gill (1996) affirmed that the principal factors to be addressed in the preservation of chilled meat are the retention of an attractive, fresh

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appearance for the product displayed, and the retardation of bacterial spoilage.

In a previous paper (Vergara & Gallego, 2001) we analysed the development of the instrumental characteristics of lamb meat that most influence consumer satisfaction (colour, tenderness and juiciness) when meat is packed in modified atmospheres with different gas compositions (20–80% $CO_2$). However, the most suitable method (and therefore mixture of gas) will depend on many factors, including the type of meat packed, for example, beef, pork, lamb or deer meat (Hood and Mead, 1995).

Thus, using instrumental analysis, the objectives of this study were (1) to evaluate the characteristics that most influence consumer satisfaction (colour, tenderness and juiciness) in deer meat when packed in modified atmospheres with different gas compositions and (2) to determine a package atmosphere that best preserves the initial characteristics of deer meat.

## 2. Materials and methods

### 2.1. Materials

One hundred and eight samples of 100 g each were used for this study. These samples were obtained from loins of 16-month-old female deer (*Cervus elaphus*) of very similar weight (approximately 91 kg) from the flock of the Experimental Farm (located in Albacete, Spain) of Castilla-La Mancha University. Animals were slaughtered using standard commercial procedures. After slaughter, the carcasses were chilled at 4 °C for 24 h and the loins were excised. None of the carcasses exhibited 24-h pH values higher than 5.70.

A thermoforming machine was used for packing (model TIROPAC 1000, CONTEL, Brescia, Italy). The samples were placed in clear semi-rigid trays (ECOFORM V 600 W, which have an oxygen permeability (OP) rate of 0.4 $cm^3/m^2$ at 1 atm and 20 °C, and a cover film with an OP of 30–40 $cm^3/m^2$ at 1 atm and 20 °C) with a volume two times that of the meat.

Samples were randomly assigned (36 loins per treatment) to one of the three types of packaging analysed (A, B and C treatment). The following gas mixtures used for packing were selected in order to evaluate the increase of $CO_2$,with or without $O_2$, in meat quality:

Treatment A: 40% $CO_2$ + 60% $N_2$
Treatment B: 80% $CO_2$ + 20% $O_2$
Treatment C: 80% $CO_2$ + 20% $N_2$

### 2.2. Analysis of samples

Meat samples (nine for each type of MA and period, i.e. 27 samples per period) were analysed at 4, 11, 18 and 25 days post-slaughter, [i.e. 2, 9, 16 and 23 days of storage in modified atmosphere (MA)]. Samples remained in chilled storage at 2 °C during this time.

The following variables were assessed:

- pH, measured with a penetrating electrode coupled to a Crison Instrument (Barcelona, Spain) model 507 portable pH meter.
- Colour as $L^*$, $a^*$ and $b^*$ values were determined 30 min after each package was opened, using a Minolta CR200 (Osaka, Japan) colorimeter calibrated against a standard white tile.
- Drip loss (DL), expressed as a percentage of the initial portion weight. DP was measured by the following procedure: (1) weight of pack before opening; (2) weight of dry sample (using a paper towel); (3) weight of pack after washing and drying. Water expelled (We) = (1)−(2 + 3); DL = (We × 100)/[We + (2)].
- Cooking loss (CL), expressed as the percentage of loss related to the initial weight, was determined in cuts of meat of approximately 50 g weight that were individually placed in polyethylene bags in a water bath at 80 °C for 60 min. Subsequently, the cuts were retrieved from the bags, dried with filter paper and weighed.
- After determining CL, shear force (SF) was determined using a TA.XT2 texture analyser (Surrey, England) equipped with a Warner–Bratzler device. The preparation of the samples and the measurement of shear force were performed as described by Vergara and Gallego (2000).

### 2.3. Data analysis

The data were analysed using analysis of variance to determine the effects of MA type (A, B, C) on the parameters of meat quality: pH, colour as $L^*$, $a^*$ and $b^*$ values, DL, CL and SF. When the differences among types of MA were significant ($P < 0.05$), Tukey's test was carried out to check the differences between pairs of groups. The effect of storage for each treatment packing on meat quality was analysed using Tukey's test at a significance level of $P < 0.05$. Data were analysed using the SAS (1988) statistical package.

## 3. Results and discussion

### 3.1. pH

Table 1 shows the pH variations of deer meat according to the type of MA and storage time. pH values from type A samples tended to be higher in all

Table 1
Values (means±S.E.) of pH of *Cervus elaphus* meat preserved in modified atmospheres with different gas composition

| Time post packing | MA | | | Analysis of variance |
|---|---|---|---|---|
| | Type A | Type B | Type C | |
| 2 days | 5.53±0.00a | 5.50±0.01a | 5.49±0.01a | NS |
| 9 days | 5.59±0.00b | 5.56±0.01a | 5.57±0.01b | NS |
| 16 days | 5.63±0.00c,x | 5.53±0.01a,y | 5.54±0.02ab,y | *** |
| 23 days | 5.80±0.01d | 5.78±0.03b | 5.77±0.02c | NS |

MA: modified atmosphere [Type A (40% $CO_2$+60% $N_2$); Type B (80% $CO_2$+20% $O_2$); Type C (80% $CO_2$+20% $N_2$)]. NS: not significant. Values in the same row with different letters (x–z) are significantly different ($P<0.05$). Values in the same column with different letters (a–c) are significantly different ($P<0.05$).
*** Indicate significance level at 0.001.

storage periods than those packed with 80% $CO_2$ (type B and C) and significant differences ($P<0.001$) among the groups were found at 16 days of storage. The lower values of pH in groups B and C (both with 80% $CO_2$) may be due to the absorption of $CO_2$ ($H_2CO_3$) in the muscle (Jeremiah & Gibson, 2001).

The storage time caused differences in meat pH and in agreement with other studies (Doherty, Sheridan, Allen, McDowell, & Blair, 1996; Moore & Gill, 1987; both in lamb meat) we also found an increase in the pH of meat. Moore and Gill (1987) suggested that tissue breakdown may be responsible for this increase. Also, in other works analysing the length of chilling of venison loins packed with 100% $CO_2$, (Seman, Drew, & Littlejohn, 1989), pH values increased with time but only to the 12th week.

According a previous reports (Gill, 1990) that meat pH declines when it is packed in atmospheres rich in $CO_2$, the increase in pH with storage time in all groups of present study was unexpected. On the other hand, the development of the pH values in this study is opposite that of a previous work where the effect of MA in preservation of lamb meat was analysed (Vergara and Gallego, 2001). The present results show different behaviour for meat of different species with the same gas composition in pack.

### 3.2. Colour

Table 2 shows the differences in meat colour among the three treatments. Until 23 days post packing, significant differences among groups in $L^*$ values (lightness) were not found, but at this moment this parameter was higher ($P<0.01$) in samples with low $CO_2$ content (group A). In this same group, $L^*$ increased ($P<0.05$) after 16 days post packing but this development was not so obvious in group B and C (80% $CO_2$). Significant differences ($P<0.001$) between groups were observed from 9 days post packing in $a^*$ values (redness), and this parameter was higher in group A. The development in $a^*$ was different between all groups. From 9 days post packing $a^*$ increased in group A (less $CO_2$), was opposite in group B, while in group C was first a decrease and after an increase. The reasons for this different evolution of redness are complex. As Ledward (1992) indicated, the stability of colour depends on the $O_2$ consumption rate in the early post mortem period, but after ageing, the metmyoglobin-reductase activity dominates once the oxygen consumption rate is reduced. However, in samples from the A group, this parameter increased with time until 23 days post-packing. The gas composition of the packs affected $b^*$ (yellowness) significantly, as this value was in general higher in the group with $O_2$ (group B) than in packs without $O_2$ (groups A and C). In all groups this parameter increased from 2 to 9 days in storage and remained constant afterwards. The more rapid changes in this parameter of meat group with $O_2$ (B) suggest that this gas is responsible for the colour. Moore and Gill (1987) also found increases in $b^*$ values with time, in agreement with our results. The increment in $b^*$ may be associated with transformation of the meat pigment and the formation of metmyoglobin, which is faster at an relatively low oxygen concentration (Brody, 1970).

### 3.3. Drip loss and cooking loss

A high drip loss has been recognized as a disadvantage associated with using $CO_2$ modified atmosphere packaging methods (Gill, 1986; Seman et al., 1989). However, in this experiment (Table 3) there is an absence of differences among the various treatments. In addition, in the three groups (A, B and C), DL increased significantly in the first 9 days after packing but then remained constant in all groups (DL values oscillated between 1.37%, at 2 days post-packing, and 4.57% at 23 days post-packaging). This is in agreement with Zarate and Zaritzky (1985), who indicated that most of the exudates are lost from primal cuts within the first two weeks of their preparation. Also, CL (Table 4) increased with time in all groups ($P<0.05$ in groups A and B) and in general there were not differences between groups. On the other hand, other reports (Seman et al., 1989) found that DL did not vary with storage period (although these authors determined DL at 12 and 18 weeks of storage).

### 3.4. Shear force

SF values are shown in Table 5. The initial values of SF are intermediate when compared to those found by Stevenson, Seman, and Littlejohn (1992) in the same species (9 kg/cm$^2$ in framed red deer with a carcass weight of 127.9 and 95.6 kg), and with those found by Duranti, Casoli, Coli, Cardinali, and Donnini (1994) in

Table 2
Values (means±S.E.) of colour ($L^*$, $a^*$, $b^*$) parameters of *Cervus elaphus* meat preserved in modified atmospheres with different gas composition

| Coordinate | Time post packing | Type A | Type B | Type C | Analysis of variance |
|---|---|---|---|---|---|
| $L^*$ | 2 days | 32.86±0.27a | 32.85±0.32a | 32.69±0.43ab | NS |
| | 9 days | 32.35±1.16a | 34.38±0.34b | 34.70±0.31b | NS |
| | 16 days | 34.25±0.28ab | 33.42±0.39ab | 33.77±0.42ab | NS |
| | 23 days | 35.46±0.44b,x | 33.12±0.44 ab,xy | 31.36±1.22a,y | ** |
| $a^*$ | 2 days | 17.67±0.76a | 16.24±0.69a | 17.58±0.69a | NS |
| | 9 days | 18.72±1.19ab,x | 7.56±0.30b,y | 10.11±0.71b,y | *** |
| | 16 days | 21.38±0.39bc,x | 7.68±0.26b,z | 13.31±1.71b,y | *** |
| | 23 days | 22.04±0.66c,x | 7.06±0.20b,z | 17.83±0.87a,y | *** |
| $b^*$ | 2 days | 6.20±1.12a,x | 7.53±0.35a,y | 5.89±0.34a,x | *** |
| | 9 days | 7.73±0.28b,x | 9.73±0.23c,y | 8.55±0.22b,x | *** |
| | 16 days | 8.95±0.21c,xy | 9.33±0.23bc,y | 8.53±0.22b,x | * |
| | 23 days | 9.66±0.32c,x | 8.56±0.30ab,xy | 8.30±0.35b,y | * |

MA: modified atmosphere [Type A (40% $CO_2$ + 60% $N_2$); Type B (80% $CO_2$ + 20% $O_2$); Type C (80% $CO_2$ + 20% $N_2$)]. NS: not significant. Values in the same column with different letters (a–c) are significantly different ($P<0.05$). Values in the same row with different letters (x–z) are significantly different ($P<0.05$).

* Significance level 0.05.
** Significance level 0.01.
*** Significance level 0.001.

Table 3
Values (means±S.E.) of drip loss (% DL) of *Cervus elaphus* meat preserved in modified atmospheres with different gas composition

| Time post packing | MA | | | Analysis of variance |
|---|---|---|---|---|
| | Type A | Type B | Type C | |
| 2 days | 1.35±0.14a | 1.42±0.21a | 1.35±0.13a | NS |
| 9 days | 3.35±0.47b | 3.22±0.38b | 3.08±0.49b | NS |
| 16 days | 3.95±0.24b | 4.17±0.29b | 3.86±0.14b | NS |
| 23 days | 4.28±0.29b | 4.43±0.44b | 4.99±0.30b | NS |

MA: modified atmosphere [Type A (40% $CO_2$ + 60% $N_2$);Type B (80% $CO_2$ + 20% $O_2$); Type C (80% $CO_2$ + 20% $N_2$)]. NS: not significant. Values in the same column with different letters (a, b) are significantly different ($P<0.05$).

Table 4
Values (means±e.s.) of cooking loss (%CL) of *Cervus elaphus* meat preserved in modified atmospheres with different gas composition

| Time post packing | MA | | | Analysis of variance |
|---|---|---|---|---|
| | Type A | Type B | Type C | |
| 2 days | 33.86±0.50a | 35.18±0.23a | 35.24±0.31 | NS |
| 9 days | 36.01±0.37b | 36.61±0.43ab | 35.68±0.39 | NS |
| 16 days | 36.54±0.36bc,xy | 36.97±0.26b,y | 35.75±0.28x | * |
| 23 days | 37.77±0.24c | 37.35±0.56b | 35.96±0.37 | NS |

MA: modified atmosphere [Type A (40% $CO_2$ + 60% $N_2$); Type B (80% $CO_2$ + 20% $O_2$); Type C (80% $CO_2$ + 20% $N_2$)]. NS: not significant. Values in the same column with different letters (a, b) are significantly different ($P<0.05$). Values in the same row with different letters (x, y) are significantly different ($P<0.05$).

* Indicate significance levels at 0.05.

fallow deer meat (4.68 kg/cm$^2$, with a carcass weight of 41 kg). The differences found with other reports might be associated with fat content, although in our study we did not measure this parameter.

Table 5
Values (means±S.E.) of SF (kg/cm$^2$) of *Cervus elaphus* meat preserved in modified atmospheres with different gas composition

| Time post packing | MA | | | Analysis of variance |
|---|---|---|---|---|
| | Type A | Type B | Type C | |
| 2 days | 7.02±0.78a | 7.05±0.64a | 7.57±0.87a | NS |
| 9 days | 5.93±0.74ab | 6.30±0.63ab | 5.07±0.46b | NS |
| 16 days | 5.04±0.46ab | 5.37±0.50ab | 4.10±0.50b | NS |
| 23 days | 3.83±0.34b | 4.23±0.37b | 4.85±0.51b | NS |

MA: modified atmosphere [Type A (40% $CO_2$ + 60% $N_2$); Type B (80% $CO_2$ + 20% $O_2$); Type C (80% $CO_2$ + 20% $N_2$)]. NS: not significant. Values in the same column with different letters (a, b) are significantly different ($P<0.05$).

There were no significant differences between different groups of MA for any of the ageing times. SF values tended to decrease ($P<0.05$) with increasing storage periods in all treatments. SF values varied from 2 to 23 days post packing and ranged from 7.22 to 4.3 kg/cm$^2$.

Other studies have found that tenderness increases with ageing in deer meat (Wiklund, Stevenson-Barry, Duncan, & Littlejohn, 2001), and also in other species such as lamb (Jeremiah, Tong & Gibson, 1997; Pinkas, Voinova, & Popoviska, 1978). The absence of differences in SF between groups of different gas composition is also found in other species, for example, lamb (Vergara & Gallego, 2001) or beef (Bell, Penney, & Moorhead, 1996).

## 4. Conclusions

With this study we have observed the evolution of the main parameters that affect deer meat quality, pH, colour

as $L^*$ $a^*$ $b^*$ values, drip loss, cooking loss and shear force when preserved in modified atmospheres with different mixtures of gas. The greatest changes in the instrumental values of the parameters occur principally during the first 9 days post-packing. In all treatments, pH and water loss increased, thus making it impossible to maintain initial meat quality in any of the groups tested. Also, the samples in all groups turn yellow with time (increase in $b^*$) and this change is more rapid in group packed in MA with mixtures containing oxygen. In addition, the colour values (redness and yellowness) of meat packed in MA with 40% $CO_2$ + 60% $N_2$ (type A) suggest that this mixture is the most appropriate for the preservation of deer meat.

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## References

Bell, R. G., Penney, N., & Moorhead, S. M. (1996). The retail display life of steaks prepared from chill stored vacuum and carbon dioxide packed sub-primal beef cuts. *Meat Science*, *42*(2), 165–178.

Brody, A. L. (1970). Shelf life of fresh meat. *Modern Packaging*, *1*, 81–86.

Church, N. (1994). Development in modified-atmosphere packaging and related technologies. *Trends in Food Science & Technology*, *5*, 345–352.

Doherty, A. M., Sheridan, J. J., Allen, P., McDowell, D. A., & Blair, I. S. (1996). Physical characteristics of lamb primal packaged under vacuum or modified atmospheres. *Meat Science*, *42*, 315–324.

Duranti, E., Casoli, C., Coli, R., Cardinali, A., & Donnini, D. (1994). Carne di diano: aspectti productivi, qualitativi e nutrizionaly. *Annali Facoltà di Agraria Univ Perugia*, *48*, 75–98.

Drew, K. R., & Seman, D. L. (1987). The nutrient content venison. *Proceedings of the Nutrition Society of New Zealand*, *12*, 49–55.

Fletcher T. J. (1998). The first new domesticant for 5000 years? In: *Proceedings of the 2nd World Deer Farming Congress, Limerik, Ireland*, (pp. 5–14).

Gill, C. O. (1986). Prospects for extending the shelf life of chilled meats. The MIRINZ-UEB process. *Proceeding 24th Meat Ind. Res. Conf.,* (Vol. 24, pp. 236–240).

Gill, C. O. (1990). Controlled atmosphere packaging of chilled meat. *Food Control*, *April*, 74–78.

Gill, C. O. (1996). Extending the storage life of raw chilled meats. *Meat Science*, *43*(S), S99–S109.

Hood, D. E., & Mead, G. C. (1995). Almacenamiento en atmósfera modificada de carnes frescas In: *Envasado de los Alimentos en Atmósfera Modificada Ed A. Madrid Vicente* (pp. 300–331).

Jeremiah, L. E., & Gibson, L. L. (2001). The influence of storage temperature and storage time on color stability, retail properties and case-life of retail-ready beef. *Food Research International*, *34*, 815–826.

Jeremiah, L. E., Tong, A. K. W., & Gibson, L. L. (1997). Hot-boning, elevated temperature conditioning, and vacuum packaged ageing influences on lamb cooking losses and palatability. *Journal of Food Science*, *62*, 1026–1027.

Ledward, D. A. (1992). Colour of raw and cooked meat. In D. E. Johnson, M. K. Knight, & D. A. Ledward (Eds.), *The chemistry of muscle-based foods*. (pp. 129–144). The Royal Society of Chemistry.

Manley, T. R., & Forss, D. A. (1979). Fatty acids of meat lipids from young red deer (*Cervus elaphus*). *Journal of the Science of Food and Agriculture*, *30*, 931–947.

Milne, J. (1994). Lighting up their lives. *The Deer Farmer*, *111*, 27–29.

Moore, V. J., & Gill, C. O. (1987). The pH and display life of chilled lamb after prolonged storage under vacuum or under $CO_2$. *New Zealand Journal of Agriculture Research*, *30*, 449–452.

Pinkas, A., Voinova, R., & Popoviska, T. (1978). Changes in lamb meat tenderness as affected by postmortem biochemical modifications in myofibrillar protein. *Zhivotrovundi Nauki*, *15*, 47–53.

Sañudo, C., Sánchez, A., & Alfonso, M. (1998). Small ruminant production systems and factors affecting lamb meat quality. 44th International Congress of Meat Science and Technology, Barcelona, Spain. *Meat Science*, *49*(Suppl. 1), S29–S64.

SAS/STAT. (1988). *User's guide, release 6.03 edn*. Cary, NC: SAS Institute Inc.

Seman, D. L., Drew, K. R., & Littlejohn, R. P. (1989). Packaging venison for extended chilled storage: comparison of vacuum and modified atmosphere packaging containing 100% carbon dioxide. *Journal of Food Protection*, *52*(12), 886–893.

Stevenson, J. M., Seman, D. L., & Littlejohn, R. P. (1992). Seasonal variation in venison quality of mature, farmed red deer stag. *New Zealand Journal of Animal Science*, *70*, 1389–1396.

Vergara, H., & Gallego, L. (2000). Effect of electrical stunning on meat quality of lamb. *Meat Science*, *56*, 345–349.

Vergara, H., & Gallego, L. (2001). Effect of gas composition in modified atmosphere packaging on the meat quality of Spanish Manchega lamb. *Journal of Science of Food and Agriculture*, *81*, 1353–1357.

Wiklund, E., Stevenson-Barry, J. M., Duncan, S. L., & Littlejohn, R. P. (2001). Electrical stimulation of red deer (*Cervus Elaphus*) carcasses-effects on rate of pH-decline, meat tenderness, colour stability and water-holding capacity. *Meat Science*, *59*, 211–220.

Zarate, J. R., & Zaritzky, N. E. (1985). Production of weep in packaged refrigerated beef. *Journal of Food Science*, *50*, 155–159.