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Effect of high pressure preservation on the quality of dry cured beef "Cecina de Leon"

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Abstract

Microbiological, physicochemical and sensory quality of "Cecina de León" vacuum packed was evaluated after high pressure treatment (500 MPa, 5 min) and further chilling storage at 6 °C for up to 210 days. The objective was to determine if high pressure processing is a valid preservation method to reduce the growth of spoliage microorganisms without modification of its quality properties along of the chilling storage time for this Spanish beef dried meat product. Since, this product is usually presented to the consumer in vacuum packed slices and cuts, these two retail sale systems were studied. High pressure processing at 500 MPa for 5 min avoided the growth of enterobacteria, enterococci and pseudomonads and delayed the growth of lactic acid bacteria, *Micrococcaceae* and yeasts and moulds. Besides, no change was found after pressure treatment and during refrigerated storage, in physicochemical and sensory parameters. It could be concluded, on the basis of the results, that the high pressure treatment was an efficient method for preserving the safety of "Cecina de León" without decreasing their sensory properties.

Keywords: Vacuum packaging; High pressure processing; Shelf-life; Dry cured beef; Cecina de León

Industrial relevance: High pressure processing is finding increasing use in the food industry because of its relative advantages versus other food processing methods in eliciting minimal changes in the flavour and nutritional qualities of the final product and in extending the shelf life. The study aimed the evaluation of microbiological, physicochemical and sensory characteristics at vacuum packed slices and cuts of "Cecina de León" during subsequent extended chilled storage. High pressure processing was a valid preservation method to reduce the growth of spoilage microorganisms without any changes on "Cecina de León" quality properties along wide chilled storage.

1. Introduction

"Cecina de León" is a salted, smoked and dried, beef meat product manufactured traditionally in the region of León (north-western Spain). It is an intermediate moisture meat product, and the preparation method is similar to that used in dry cured ham manufacture. The final product has a typical red colour, smoked flavour and a slight genuine salty taste. Two different retail sale systems are mostly used: vacuum packaged "cecina" slices or "cecina" cuts.

Normally, at the end of drying, internal flora of this product is present at low levels (10^3 cfu/g) in the overall "Cecina de León" (García, Zumalacárregui, & Díez, 1995). According to Rubio, Martínez, González-Fernández, García-Cachán, Rovira and Jaime (2006), cross-contamination during cutting or slicing and packaging leads to an increase of the concentration of total viable microorganisms. This fact reduces to 90 days the shelf life of vacuum packed "Cecina de León" slices. As heat treatment is not suitable for this product after packaging, an alternative process to minimise the microbiological counts is necessary.

In this sense, high hydrostatic pressure processing (HPP) is a very promising preservation technology of sliced meat cured products (Hugas, Garriga, & Monfort, 2002), which can be

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applied to the product after slicing and vacuum packaging. HPP at low or moderate temperature causes destruction of microbial vegetative cells without remarkable changes in odour, taste and nutrient content. Furthermore, the use of HPP will ensure the safety of meat cured products and extends the life of these products without decreasing organoleptic properties.

However, the effectiveness of the treatment or the resistance of the microorganisms is extremely variable and it depends on (1) the process parameters (achieved pressure, treatment temperature and exposure time); (2) the strain (gram-positive microorganisms are more resistant to HPP than gram-negative species as well as spores), cell morphology (bacilli are more sensitive to pressure than cocci) and the stage of growth of the microorganisms (bacteria from the early log phase of growth are more barosensitive than cells from stationary, dormant or death phase); and (3) the meat matrix to be treated (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989; Hugas et al., 2002; Saccani, Parolari, Tanzi, & Barbuti, 2004). In this way, it is important to experiment with real matrices because results obtained in buffers or synthetic media cannot be always extrapolated and applied to real situations (Garriga, Grébol, Aymerich, Monfort, & Hugas, 2004). Food composition can often have protective effect during pressurisation, and it is important to evaluate microbial resistance to pressure in foods. As for dry cured meat products, few studies have been carried out in order to study the effectiveness of the high pressure processing and to evaluate the microbial safety and the quality of these pressurised products throughout chilled storage (Andrés-Nieto, Møller, Adamsen, Ruiz, & Skibsted, 2004; Cava, González, Ladero, & Carrasco, 2005; Garriga et al., 2004; Saccani et al., 2004).

In this study, HPP (500 MPa during 5 min) was assayed in dry cured beef "Cecina de León". The objective was to compare the microbiological, physicochemical and sensorial evolution between the HPP and untreated samples during a long chilled storage time (210 days) and thus to determine if HPP processing is a valid preservation method to reduce the growth of spoilage microorganisms without any changes on "Cecina de León" quality properties along wide chilled storage.

2. Materials and methods

2.1. Preparation of samples

The study was carried out on "Cecina de León" cuts and on "Cecina de León" slices.

2.1.1. "Cecina de León" cuts

The study was carried out on 4 pieces of "Cecina de Leon" manufactured according to the specifications of Protected Geographical Indication "Cecina de León" (Boletín Oficial de Castilla y León, 1994). The anatomical cut used was the knuckle, comprising mainly of *Quadriceps femoris*. Pieces of approximately 7 kg and 10–11 months of ripening were divided into portions (4–5 cm thick and about 500 g weight). A portion of each piece was used for initial analysis (day 0) and results corresponding to day 0 for all parameters were the average value of data from the 4 portions analysed.

2.1.2. "Cecina de León" slices

Two pieces of "Cecina de Leon" with the same characteristics above mentioned were sliced (1.5 mm thick) and 100 g of them were placed in polystyrene trays. Two trays from each piece were used for initial analysis and results corresponding to this sampling time for all parameters were the average value of four data.

2.1.3. Packaging

The cuts and trays of slice of "Cecina de León" were individually packaged in plastic bags (polyamide/polyethylene with an oxygen transmission rate of 30–40 cm^3/m^2/24 h/bar at 23 °C and 50% RH and a water vapour transmission rate of 2.5 g/m^2/24 h at 23 °C and 50% RH, supplied by WK Thomas España S.L., Rubí, Spain) which were subjected to vacuum and sealed using either a packer (EVT-7-TD Tecnotrip, Barcelona, Spain). After vacuum packaging, one group of samples of both cuts and slices remained untreated and the rest were high pressure treated. Then, all packs were stored at 6 °C for up to 210 days.

2.1.4. High pressure treatment

The pressurisation took place in an industrial hydrostatic pressurisation unit (Wave 6000/135. NC Hyperbaric, Burgos, Spain). The pressure level was 500 MPa, the treatment time of 5 min and the initial temperature, 18 °C. The time needed to achieve the treatment pressure was approximately 4 min and decompression was instantaneous.

2.1.5. Storage of the samples

After HPP, the pressurised samples (HP) were stored at 6 °C for up to 210 days together with the untreated control samples (CO). At selected times: after high pressure processing (1 day) and during chilled storage (15, 30, 60, 90, 150 and 210 days), microbiological, physicochemical and sensory analyses were carried out. Two packs of each treatment were opened for subsequent analysis after the determined days of storage.

2.2. Microbial analyses

Ten grams of each sample were taken aseptically and homogenised with 90 ml of tryptone water (Scharlau, Spain) for 2 min in a sterile plastic bag in a PK 400 Masticator (IUL, S.A., Barcelona, Spain). Serial decimal dilutions were made in sterile tryptone water and duplicate 1 ml or 0.1 ml samples of appropriate dilutions were poured or spread onto total count and selective agar plates.

The samples were analysed for: *mesophilic aerobic bacteria* determined on 3 M Petrifilm Aerobic Count Plate (Bioser, Spain), incubated at 30 °C for 48 h; *psychrotrophic bacteria* on Plate Count Agar (Scharlau, Spain), incubated at 7 °C for 10 days; *anaerobic bacteria* on Schaedler Agar (Scharlau, Spain), overlayed with 5 ml of the same medium and incubated at 37 °C for 48 h; *enterobacteria* on 3 M Petrifilm Enterobacteriaceae Count Plate (Bioser, Spain), incubated at 37 °C for 24 h; *enterococci* on Slanetz Bartley Agar (Scharlau, Spain), incubated at 37 °C for 48 h; *pseudomonads* on Pseudomonads

Agar (Oxoid, Spain) supplemented with Cetrimide, Fucidine and Cephaloridine (CFC) (Oxoid, Spain), incubated at 30 °C for 48 h; *lactic acid bacteria* (LAB) on MRS Agar (Scharlau, Spain), incubated anaerobically in 6% CO_2, at 30 °C for 72 h; *Micrococcaceae* on MSA (Scharlau, Spain), incubated at 37 °C for 48 h; *yeasts and moulds* on 3 M Petrifilm Yeast and Mold Count Plate (Bioser, Spain), incubated at 25 °C for 5 days.

For experimental purposes, the lowest detection limit of the above techniques was 10 cfu/g except for enterococci, pseudomonads, LAB and *Micrococcaceae* whose limit was 10^2 cfu/g. Microbiological counts were expressed as log cfu/g. When microorganism counts were above 10^7 cfu/g the product was considered unsuitable for consumption (ICMSF (International Commission on Microbiological Specifications for Foods), 1983).

Besides, *Listeria monocytogenes* was investigated in 25 g by preenrichment in Half-Fraser broth (Biomerieux, Spain) at 30 °C for 24–26 h, enrichment in Fraser broth (Biomerieux, Spain) at 30 °C for 24–26 h, followed by immune-detection test VIDAS LM02 (Biomerieux, Spain). The results were expressed as absence or presence in 25 g.

2.3. pH and a_w analyses

The pH values were determined in "Cecina de León" cuts by puncture with a pH meter model 507 (Crison Instruments, Barcelona, Spain). For "Cecina de León" slices, the pH was measured by blending 10 g of product with 10 ml of distilled water for 2 min. Water activity (a_w) was measured with a Decagon CX-2 AQUA LAB equipment (Decagon Devices, Inc., Pullman, WA, USA).

2.4. Instrumental colour measurement

Objective measurement of colour was performed at the surface of "Cecina de León" cuts and on "Cecina de León" sliced, using a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan). The illuminant used was D65 (colour temperature of 6504 K) and the standard observer position was 10°. Colour coordinates were determined in the CIE-LAB system and the results were expressed as lightness (L^*), redness (a^*) and yellowness (b^*). Each value was determined on 4 different surface locations per cut or slice.

2.5. Instrumental texture measurement

The texture instrumental measure was only determined on "Cecina de León" cuts, due to the slices thickness (1.5 mm). Instrumental Texture Profile Analysis (TPA) (Breene, 1975) was performed with a texture analyzer TA-XT2 (Stable Micro Systems, Haslemere, UK). The Texture Expert, version 1.20 (Spanish), computer program by Stable Micro Systems was used for data collection and calculations. Six cubes of "Cecina de León" (1×1×1 cm), obtained of the cut centre, were compressed twice with a cylindrical probe of 1 cm diameter, at 1 mm/s speed and the level of compression was 60% of the thickness of the sample. The test was always accomplished at room temperature and the parameters determined from the force–time curves were hardness, springiness, cohesiveness and chewiness. Hardness was defined by peak force during first compression cycle and expressed in g. Springiness was defined as a ratio of 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 calculated as the ratio of the area under the second curve to the area under the first curve. Chewiness was obtained by multiplying hardness, springiness and cohesiveness and expressed in g.

2.6. Sensory evaluation

Sensory evaluation was carried out on "Cecina de León" slices after each storage time by an experienced 8-member sensory panel. Eight training sessions were held to familiarise the judges with the attributes to evaluate and the scale to use. Evaluation was always done regarding initial characteristics of the fresh product prior to packing. Colour, odour, taste, hardness, juiciness and overall acceptability were scored on a 5-point hedonic scale as follow: 5=excellent, 4=good, 3=acceptable, 2=fair and 1=unacceptable (Kotzekidou & Bloukas, 1996). Moreover, when the overall acceptability score was less than 3 the product was considered expired and when scores lower than 3 were given, the reason had to be stated.

The panel sessions were held at mid-morning, in a sensory panel room at 22 °C. For "Cecina de León" cuts, slices (1.5 mm thick) were obtained with a slicing machine and served on plates to panellist. The two first slices were always removed. Four "Cecina de León" slices from different cuts or slices of the two different treatment methods were successively evaluated in each session. The sample order was randomised within sessions and water at room temperature and unsalted bread were provided between successive samples.

2.7. Statistical analysis

Data were statistically analysed using one-way analysis of variance (ANOVA), and means were separated by Tukey-honest significant difference test at 5% level. Data analyses were conducted using the statistical package STATISTICA 7.0.

3. Results and discussion

3.1. Development of microflora

Tables 1 and 2 show microbiological results of "Cecina de León" cuts and "Cecina de León" slices during the storage period. In general, the pressurisation treatment exerts an inhibitory effect on microflora.

In treated "Cecina de León" cuts (Table 1), after pressurisation and up to 210 days, mesophilic aerobe, anaerobe and psychrotroph counts were very low and 2–3 log below the counts obtained in untreated samples (CO). Enterobacteria counts were always below the detection limit. High pressure treatment did not show significant effect on enterococci counts ($p>0.05$) which

Table 1
Microbial evolution (log cfu/g) (mean values) of the "Cecina de León" cuts control (CO) and treated with high pressures (HP) along of storage time

Microorganisms	Initial value	1 days	15 days	30 days	60 days	90 days	150 days	210 days
Mesophilic aerobic bacteria								
CO	$_{a}5.31^{A}$	$_{a}5.03^{B}$	$_{a}4.66^{A}$	$_{a}5.12^{B}$	$_{a}4.05^{B}$	$_{a}4.07^{B}$	$_{a}4.44^{B}$	$_{a}4.04^{B}$
HP	$_{c}5.31^{A}$	$_{b}3.37^{A}$	$_{bc}3.73^{A}$	$_{ab}3.27^{A}$	$_{a}1.50^{A}$	$_{a}1.48^{A}$	$_{ab}2.22^{A}$	$_{ab}2.10^{A}$
Psychrotrophic bacteria								
CO	$_{bc}4.47^{A}$	$_{c}4.53^{B}$	$_{abc}3.50^{B}$	$_{abc}2.70^{B}$	$_{a}1.87^{A}$	$_{abc}2.31^{B}$	$_{ab}2.14^{A}$	$_{abc}3.78^{B}$
HP	$_{b}4.47^{A}$	$_{a}1.73^{A}$	$_{a}1.08^{A}$	$_{a}1.37^{A}$	$_{a}1.45^{A}$	$_{a}1.08^{A}$	$_{ab}2.67^{A}$	$_{a}2.25^{A}$
Anaerobic bacteria								
CO	$_{a}5.34^{A}$	$_{a}5.02^{B}$	$_{a}4.69^{B}$	$_{a}4.63^{B}$	$_{a}3.55^{B}$	$_{a}4.01^{A}$	$_{a}4.34^{B}$	$_{a}3.68^{B}$
HP	$_{c}5.34^{A}$	$_{ab}3.05^{A}$	$_{ab}3.17^{A}$	$_{ab}2.76^{A}$	$_{a}1.19^{A}$	$_{ab}2.91^{A}$	$_{ab}2.23^{A}$	$_{a}1.88^{A}$
Enterobacteria								
CO	ND	ND	ND	ND	ND	ND	ND	ND
HP	ND	ND	ND	ND	ND	ND	ND	ND
Enterococci								
CO	$_{a}2.38^{A}$	$_{a}2.38^{A}$	$_{ab}2.74$	$_{b}3.80$	ND	ND	ND	ND
HP	$_{a}2.38^{A}$	$_{a}2.08^{A}$	ND	ND	ND	ND	ND	ND
Pseudomonads								
CO	2.29^{A}	ND	ND	ND	ND	2.43	ND	ND
HP	2.29^{A}	ND	ND	ND	ND	ND	ND	ND
Lactic acid bacteria								
CO	$_{a}5.15^{A}$	$_{a}4.84^{B}$	$_{a}3.83$	$_{a}4.59^{B}$	$_{a}3.75^{B}$	$_{a}4.38$	$_{a}4.16$	$_{a}3.02^{A}$
HP	$_{b}5.15^{A}$	$_{a}2.17^{A}$	ND	$_{a}2.27^{A}$	$_{a}2.27^{A}$	ND	ND	$_{a}2.69^{A}$
Micrococcaceae								
CO	$_{a}5.32^{A}$	$_{a}5.08^{B}$	$_{a}3.90^{A}$	$_{a}4.91^{B}$	$_{a}4.47$	$_{a}4.07^{A}$	$_{a}4.61^{B}$	$_{a}4.04^{B}$
HP	$_{b}5.32^{A}$	$_{a}3.12^{A}$	$_{ab}3.98^{A}$	$_{a}2.99^{A}$	ND	$_{ab}3.51^{A}$	$_{a}2.29^{A}$	$_{a}2.55^{A}$
Yeasts and moulds								
CO	$_{ab}3.71^{A}$	$_{a}3.18^{B}$	$_{a}2.64$	$_{a}2.60^{B}$	$_{ab}3.67^{B}$	$_{b}4.67^{B}$	$_{ab}3.61$	$_{ab}3.86^{B}$
HP	$_{c}3.71^{A}$	$_{a}1.07^{A}$	ND	$_{a}1.12^{A}$	$_{b}2.33^{A}$	$_{b}2.68^{A}$	ND	$_{bc}3.30^{A}$

ND: not detected.
a–d: averages with different letters in the same row are different ($p<0.05$).
A–B: averages with different letters in the same column for each microorganism group are different ($p<0.05$).

were just above detection limit and decreased throughout storage being no detectable after 60 days in CO samples and after 15 days in HPP samples. Pseudomonad counts, after treatment and during complete storage period, were below the detection threshold, both in treated samples (HP) and CO samples. LAB, *Micrococcaceae* and yeasts and moulds, the typical microflora of "Cecina de León" according to García et al. (1995), showed a significant reduction ($p<0.05$) of 2 log cycles after treatment. At the end of storage, the counts were also lower in HP samples ($p<0.05$) than in untreated samples except for LAB.

Results in "Cecina de León" slices (Table 2) had a similar tendency to those found in "Cecina de León" cuts. Besides, in "Cecina de León" slices the HPP effect was observed on enterobacteria and pseudomonad counts. Many authors (Carlez, Rosec, Richard, & Cheftel, 1993; Cheftel & Culioli, 1997; Hugas et al., 2002; Saccani et al., 2004; Shigehisa, Ohmori, Saito, Taji, & Hayashi, 1991) pointed out that Gram-negative bacteria are more sensitive to pressure than Gram-positive bacteria. This fact is clearly observed in our results, especially in sliced "Cecina de León" due to that a wider product surface is exposed to the high pressure.

After HPP most of surviving microorganisms were maintained at low levels during the whole storage period, according to the agreed fact that although low water activity protects cells against pressure, microorganisms injured by pressure are more sensitive to low water activity (Cheftel & Culioli, 1997; Garriga et al., 2004). In this sense, Rubio (2006) have reported markable stability of microorganisms along storage in "Cecina de León", due to the characteristics of this product (low a_w) as well as the low temperature storage and the anoxic environments. Garriga et al. (2004) observed a similar behaviour of aerobic bacteria, LAB and psychrotrophs in dry cured ham.

However, results related to moulds and yeasts found in dry cured ham treated with HPP (600 MPa and 6 min) by Garriga et al. (2004) do not agree with the behaviour observed in "Cecina de León", since initial yeast counts were lower than in "Cecina de León", but particularly HPP showed a greater effect and after treatment the number of survivors was kept under detection limit during the whole storage time. In "Cecina de León", especially in slices, moulds and yeasts were notably inhibited by HPP but a subsequent recovery of survivor cells

Table 2
Microbial evolution (log cfu/g) (mean values) of "Cecina de León" slices control (CO) and treated with high pressures (HP) along of storage time

Microorganisms	Initial value	1 days	15 days	30 days	60 days	90 days	150 days	210 days
Aerobic mesophilic bacteria								
CO	$_{ab}6.11^{A}$	$_{c}7.31^{B}$	$_{c}7.18^{B}$	$_{bc}6.56^{B}$	$_{a}5.48^{B}$	$_{ab}6.14^{B}$	$_{ab}6.11^{B}$	$_{ab}5.70^{B}$
HP	$_{b}6.21^{A}$	$_{a}4.19^{A}$	$_{a}4.54^{A}$	$_{a}3.89^{A}$	$_{a}3.57^{A}$	$_{a}4.24^{A}$	$_{a}4.83^{A}$	$_{a}3.70^{A}$
Psychrotrophic bacteria								
CO	$_{a}5.46^{A}$	$_{a}5.79^{A}$	$_{a}5.94^{B}$	$_{a}3.65^{A}$	$_{a}3.48^{A}$	$_{a}6.06^{B}$	$_{a}5.96^{B}$	$_{a}5.77^{B}$
HP	$_{b}5.46^{A}$	$_{ab}3.77^{A}$	$_{ab}2.65^{A}$	$_{a}1.50^{A}$	$_{ab}2.38^{A}$	$_{ab}3.06^{A}$	$_{ab}4.09^{A}$	$_{ab}4.24^{A}$
Anaerobic bacteria								
CO	$_{ab}6.02^{A}$	$_{c}7.35^{B}$	$_{bc}7.03^{B}$	$_{ab}5.94^{B}$	$_{a}5.23^{B}$	$_{ab}6.09^{B}$	$_{abc}6.76^{A}$	$_{ab}5.83^{B}$
HP	$_{d}6.02^{A}$	$_{bc}4.16^{A}$	$_{bc}4.26^{A}$	$_{ab}3.40^{A}$	$_{a}2.70^{A}$	$_{bc}4.09^{A}$	$_{c}5.00^{A}$	$_{ab}3.48^{A}$
Enterobacteria								
CO	$_{a}1.86^{A}$	$_{b}3.01$	ND	ND	ND	ND	ND	ND
HP	$_{a}1.86^{A}$	ND	ND	ND	ND	ND	ND	ND
Enterococci								
CO	$_{ab}3.53^{A}$	$_{b}4.52^{B}$	$_{a}2.41$	$_{a}2.57$	ND	$_{a}2.54$	ND	ND
HP	$_{b}3.53^{A}$	$_{a}2.19^{A}$	ND	ND	ND	ND	ND	ND
Pseudomonads								
CO	$_{a}2.29^{A}$	$_{a}3.16$	$_{a}2.57$	$_{ab}3.65$	ND	$_{b}5.25$	$_{a}3.11$	$_{a}2.30$
HP	$_{a}2.29^{A}$	ND	ND	ND	ND	ND	ND	ND
Lactic acid bacteria								
CO	$_{ab}6.35^{A}$	$_{b}7.46^{B}$	$_{ab}6.48^{B}$	$_{ab}6.06^{B}$	$_{ab}6.71^{B}$	$_{ab}6.49^{B}$	$_{ab}6.91^{B}$	$_{a}5.54^{A}$
HP	$_{c}6.35^{A}$	$_{abc}4.44^{A}$	$_{ab}3.15^{A}$	$_{a}2.64^{A}$	$_{ab}3.19^{A}$	$_{abc}4.62^{A}$	$_{bc}5.46^{A}$	$_{abc}4.30^{A}$
Micrococcaceae								
CO	$_{abc}6.38^{A}$	$_{c}7.36^{b}$	$_{bc}7.21^{B}$	$_{ab}5.95^{B}$	$_{ab}5.91^{B}$	$_{ab}6.11^{B}$	$_{abc}6.71^{B}$	$_{a}5.55^{B}$
HP	$_{b}6.38^{A}$	$_{a}4.16^{A}$	$_{ab}4.56^{A}$	$_{a}3.85^{A}$	$_{a}3.76^{A}$	$_{a}4.06^{A}$	$_{a}4.80^{A}$	$_{a}3.73^{A}$
Yeasts and moulds								
CO	$_{ab}4.14^{A}$	$_{a}3.74^{B}$	$_{abc}4.90^{B}$	$_{bcd}5.21^{B}$	$_{cde}6.24^{B}$	$_{e}6.71^{B}$	$_{e}6.66^{B}$	$_{de}6.58^{B}$
HP	$_{b}4.14^{A}$	$_{a}1.57^{A}$	$_{a}1.52^{A}$	$_{a}1.15^{A}$	$_{a}1.76^{A}$	$_{b}4.19^{A}$	$_{b}5.32^{A}$	$_{b}5.00^{A}$

ND: not detected.
a–d: averages with different letters in the same row are different ($p<0.05$).
A–B: averages with different letters in the same column for each microorganism group are different ($p<0.05$).

was observed during storage (Table 2). A similar behaviour, although less evident, was also observed in other groups of microorganisms, so mesophilic aerobic, anaerobe and psychrotroph counts decreased until 60 days and then increased again. It is well known, that vegetative forms of eukaryotes, such as yeasts and moulds, are more sensitive to pressure than prokaryotic microorganisms (Garriga et al., 2004; Hoover et al., 1989; Smelt, 1998). However, some species of yeasts and moulds are xerophiles microorganisms and had a greater growth capacity on low a_w foods than bacteria (Christian, 1997).

Finally, *L. monocytogenes* was absent in all samples (HP and CO) and during the whole storage period.

3.2. pH and a_w

Evolution of pH and a_w values during the storage period of "Cecina de León" cuts and "Cecina de León" slices are showed in Tables 3 and 4, respectively. In general, no differences ($p>0.05$) in pH and a_w were found between HP and CO samples after treatment with high pressure and during storage period. The values obtained for both parameters in this study and their evolution are in agreement with those obtained by Rubio et al. (2006) and Rubio (2006) in vacuum packaged of "Cecina de León" cuts and slices. Besides, authors such as Gutiérrez, Domínguez and Zumalacárregui (1988) and Molinero, Rubio, González-Fernández, Martínez and García-Cachán (2004) reported, in studies on unpacked "Cecina de León", pH and a_w values similar to those found in this work.

3.3. Instrumental colour

Results of colour parameters measurement are shown in Table 3 for "Cecina de León" cuts and in Table 4 for "Cecina de León" slices. High pressure treatment did not determine differences ($p>0.05$) in lightness (L^*), redness (a^*) and yellowness (b^*) at any storage time, in both cuts and slices of "Cecina de León". Carlez, Veciana-Nogues and Cheftel (1995) and Cheftel and Culioli (1997) reported that the dark red colour of dry cured ham is fairly resistant to HPP, unlike in fresh meat, where this treatment induces drastic changes in the colour of red muscle due to the oxidation of oxymyoglobin to metmyoglobin. The resistance of the nitrosylmyoglobin pigment to oxidation might be probably the explanation to the colour stability of cured meat products. Nevertheless, and in disagreement with this hypothesis, Andrés-Nieto et al. (2004) and Cava et al. (2005) found a significant increase in L^* and a significant decrease in a^* when Iberian dry cured ham slices were treated with HPP (400 MPa and 15 min or combinations of 200–300 MPa and 15–30 min).

The colour parameters evolution observed during of storage time in all samples was similar to the reported by Rubio et al.

Table 3
Effect of high-pressure treatment and storage time on pH, a_w, different colour parameters (L^*, a^*; b^*) and on texture parameters measured on "Cecina de León" cuts

Parameters	Initial value	1 days	15 days	30 days	60 days	90 days	150 days	210 days
pH								
CO	$_{ab}5.87^{A}$	$_{ab}5.83^{A}$	$_{ab}5.87^{A}$	$_{ab}5.89^{A}$	$_{ab}5.88^{A}$	$_{b}5.91^{A}$	$_{a}5.77^{A}$	$_{ab}5.80^{A}$
HP	$_{ab}5.87^{A}$	$_{ab}5.85^{A}$	$_{ab}5.91^{A}$	$_{ab}5.93^{A}$	$_{ab}5.88^{A}$	$_{b}5.94^{A}$	$_{a}5.80^{A}$	$_{ab}5.85^{A}$
a_W								
CO	$_{de}0.909^{A}$	$_{cd}0.907^{A}$	$_{b}0.896^{A}$	$_{bc}0.898^{A}$	$_{e}0.917^{A}$	$_{b}0.896^{A}$	$_{a}0.877^{A}$	$_{a}0.875^{A}$
HP	$_{cd}0.909^{A}$	$_{bc}0.904^{A}$	$_{b}0.896^{A}$	$_{bc}0.897^{A}$	$_{d}0.918^{A}$	$_{b}0.891^{A}$	$_{a}0.869^{A}$	$_{a}0.872^{A}$
L^*								
CO	$_{a}34.04^{A}$	$_{ab}38.92^{A}$	$_{abc}40.19^{A}$	$_{ab}39.84^{A}$	$_{c}49.94^{A}$	$_{bc}46.32^{A}$	$_{a}35.41^{A}$	$_{abc}42.20^{A}$
HP	$_{a}34.04^{A}$	$_{ab}38.18^{A}$	$_{ab}40.56^{A}$	$_{b}43.15^{A}$	$_{b}42.80^{A}$	$_{ab}38.47^{A}$	$_{ab}38.30^{A}$	$_{b}43.35^{A}$
a^*								
CO	$_{b}7.03^{A}$	$_{b}6.04^{A}$	$_{a}2.14^{A}$	$_{a}1.25^{A}$	$_{a}2.42^{A}$	$_{a}2.02^{A}$	$_{a}2.02^{A}$	$_{a}1.16^{A}$
HP	$_{d}7.03^{A}$	$_{d}6.60^{A}$	$_{ab}1.57^{A}$	$_{ab}1.00^{A}$	$_{bc}3.27^{A}$	$_{c}4.21^{A}$	$_{ab}1.44^{A}$	$_{a}0.56^{A}$
b^*								
CO	$_{a}3.58^{A}$	$_{a}3.20^{A}$	$_{a}1.70^{A}$	$_{a}1.61^{A}$	$_{b}7.54^{A}$	$_{ab}3.84^{A}$	$_{ab}5.48^{A}$	$_{a}2.80^{A}$
HP	$_{a}3.58^{A}$	$_{ab}4.13^{A}$	$_{a}1.34^{A}$	$_{a}1.91^{A}$	$_{b}5.95^{A}$	$_{ab}4.17^{A}$	$_{ab}4.54^{A}$	$_{ab}3.45^{A}$
Hardness								
CO	$_{b}5709.42^{A}$	$_{a}3661.31^{A}$	$_{a}2843.23^{A}$	$_{a}2908.93^{A}$	$_{a}2959.66^{A}$	$_{ab}4391.22^{A}$	$_{a}4119.92^{A}$	$_{ab}4382.64^{A}$
HP	$_{b}5709.42^{A}$	$_{a}3245.72^{A}$	$_{a}4105.77^{A}$	$_{a}3069.30^{A}$	$_{a}3262.91^{A}$	$_{ab}4558.52^{A}$	$_{ab}4916.92^{A}$	$_{ab}4615.57^{A}$
Springiness								
CO	$_{abc}0.43^{A}$	$_{cd}0.49^{A}$	$_{cd}0.51^{A}$	$_{bcd}0.49^{A}$	$_{ab}0.40^{A}$	$_{d}0.52^{A}$	$_{a}0.36^{A}$	$_{bcd}0.48^{A}$
HP	$_{a}0.43^{A}$	$_{ab}0.47^{A}$	$_{ab}0.48^{A}$	$_{ab}0.49^{A}$	$_{ab}0.44^{A}$	$_{b}0.52^{A}$	$_{a}0.42^{A}$	$_{b}0.53^{A}$
Cohesiveness								
CO	$_{bc}0.42^{A}$	$_{bc}0.43^{A}$	$_{bc}0.44^{A}$	$_{abc}0.40^{A}$	$_{abc}0.40^{A}$	$_{c}0.46^{A}$	$_{a}0.36^{A}$	$_{ab}0.39^{A}$
HP	$_{a}0.42^{A}$	$_{a}0.43^{A}$	$_{a}0.44^{A}$	$_{a}0.42^{A}$	$_{a}0.42^{A}$	$_{a}0.46^{A}$	$_{a}0.40^{A}$	$_{a}0.39^{A}$
Chewiness								
CO	$_{a}1031.12^{A}$	$_{a}853.02^{A}$	$_{a}648.95^{A}$	$_{a}583.68^{A}$	$_{a}495.50^{A}$	$_{a}1150.09^{A}$	$_{a}642.80^{A}$	$_{a}817.34^{A}$
HP	$_{a}1031.12^{A}$	$_{a}689.36^{A}$	$_{a}878.36^{A}$	$_{a}646.68^{A}$	$_{a}660.45^{A}$	$_{a}1231.64^{A}$	$_{a}819.26^{A}$	$_{a}941.04^{A}$

Control (CO), treated with high pressures (HP).
a–c: averages with different letters in the same row are different ($p<0.05$).
A–B: averages with different letters in the same column are different ($p<0.05$).

(2006) and Rubio (2006) in "Cecina de León" packed. Besides, presence of white film on surface of "Cecina de León" cuts was observed in treated and untreated samples. However this fact was not observed on surface of "Cecina de León" slices. According to Butz, Blumer, Christian and Swaisgood, (1974) and Arnau (1996), white film is a cloak of whitish substance formed on product surface whose main component is tyrosine. The white film formation depending on sample thickness and higher white film formation is produced when higher sample thickness is presented (higher tyrosine concentration/ cutting surface) (Arnau, García-Regueiro, Hugas, & Monfort, 1987).

Table 4
Effect of high-pressure treatment and storage time on pH, a_W and on different colour parameters (L*, a*; b*) measured on "Cecina de León" slices

Parameters	Initial value	1 days	15 days	30 days	60 days	90 days	150 days	210 days
pH								
CO	$_{ab}5.94^{A}$	$_{bc}6.00^{A}$	$_{d}6.09^{A}$	$_{d}6.09^{A}$	$_{a}5.87^{A}$	$_{bc}5.98^{A}$	$_{bc}5.99^{A}$	$_{cd}6.05^{A}$
HP	$_{a}5.94^{A}$	$_{ab}5.99^{A}$	$_{d}6.07^{A}$	$_{d}6.08^{A}$	$_{ab}5.97^{A}$	$_{ab}5.95^{A}$	$_{cd}6.06^{A}$	$_{bc}6.01^{A}$
a_W								
CO	$_{b}0.897^{A}$	$_{b}0.897^{A}$	$_{ab}0.890^{A}$	$_{ab}0.888^{A}$	$_{ab}0.884^{A}$	$_{ab}0.879^{A}$	$_{ab}0.879^{A}$	$_{a}0.870^{A}$
HP	$_{a}0.897^{A}$	$_{a}0.893^{A}$	$_{a}0.890^{A}$	$_{a}0.888^{A}$	$_{a}0.885^{A}$	$_{a}0.879^{A}$	$_{a}0.874^{A}$	$_{a}0.875^{A}$
L^*								
CO	$_{b}34.70^{A}$	$_{ab}33.36^{A}$	$_{ab}33.24^{A}$	$_{ab}31.83^{A}$	$_{ab}32.48^{A}$	$_{ab}32.72^{A}$	$_{ab}30.01^{A}$	$_{a}28.12^{A}$
HP	$_{ab}34.70^{A}$	$_{ab}31.66^{A}$	$_{ab}34.67^{A}$	$_{ab}33.56^{A}$	$_{b}35.71^{A}$	$_{b}35.64^{A}$	$_{a}30.72^{A}$	$_{ab}31.81^{A}$
a^*								
CO	$_{d}15.60^{A}$	$_{abc}10.44^{A}$	$_{abc}10.60^{A}$	$_{cd}13.00^{A}$	$_{abc}10.54^{A}$	$_{ab}7.76^{A}$	$_{bcd}11.79^{A}$	$_{a}6.29^{A}$
HP	$_{d}15.60^{A}$	$_{bcd}11.31^{A}$	$_{cd}12.43^{A}$	$_{cd}14.26^{A}$	$_{abc}11.10^{A}$	$_{ab}7.73^{A}$	$_{ab}7.84^{B}$	$_{a}6.85^{A}$
b^*								
CO	$_{b}8.73^{A}$	$_{ab}6.50^{A}$	$_{ab}5.61^{A}$	$_{ab}6.34^{A}$	$_{ab}6.47^{A}$	$_{ab}7.24^{A}$	$_{ab}6.12^{A}$	$_{a}4.24^{A}$
HP	$_{b}8.73^{A}$	$_{ab}6.03^{A}$	$_{ab}6.21^{A}$	$_{ab}6.85^{A}$	$_{ab}6.84^{A}$	$_{a}4.88^{A}$	$_{a}4.53^{A}$	$_{ab}6.42^{A}$

Control (CO), treated with high pressures (HP).
a–c: averages with different letters in the same row are different ($p<0.05$).
A–B: averages with different letters in the same column are different ($p<0.05$).

Table 5
Sensorial parameter evolution (means) of the control "Cecina de León" cuts (CO) and of the "Cecina de León" cuts treated with high pressures (HP) along storage time

Parameters	Initial value	1 days	15 days	30 days	60 days	90 days	150 days	210 days
Colour								
CO	$_{b}4.9^{A}$	$_{b}4.5^{A}$	$_{b}4.4^{A}$	$_{ab}4.3^{A}$	$_{a}3.9^{A}$	$_{a}4.0^{A}$	$_{a}3.9^{A}$	$_{a}4.1^{A}$
HP	$_{b}4.9^{A}$	$_{ab}4.4^{A}$	$_{a}4.3^{A}$	$_{b}4.6^{A}$	$_{a}4.1^{A}$	$_{a}4.1^{A}$	$_{ab}4.4^{A}$	$_{a}4.3^{A}$
Odour								
CO	$_{c}4.8^{A}$	$_{bc}4.4^{A}$	$_{bc}4.4^{A}$	$_{abc}4.1^{A}$	$_{abc}3.8^{A}$	$_{a}3.6^{A}$	$_{ab}3.7^{A}$	$_{abc}4.0^{A}$
HP	$_{b}4.8^{A}$	$_{a}4.2^{A}$	$_{ab}4.4^{A}$	$_{a}4.1^{A}$	$_{a}4.0^{A}$	$_{a}4.1^{A}$	$_{a}4.1^{A}$	$_{a}4.1^{A}$
Taste								
CO	$_{c}4.8^{A}$	$_{bc}4.1^{A}$	$_{ab}3.9^{A}$	$_{abc}4.1^{A}$	$_{ab}3.4^{A}$	$_{a}3.4^{A}$	$_{a}3.1^{A}$	$_{ab}3.4^{A}$
HP	$_{b}4.8^{A}$	$_{a}4.0^{A}$	$_{a}3.7^{A}$	$_{a}3.9^{A}$	$_{a}4.0^{A}$	$_{a}3.4^{A}$	$_{a}3.8^{A}$	$_{a}3.6^{A}$
Hardness								
CO	$_{c}4.9^{A}$	$_{ab}4.2^{A}$	$_{bc}4.5^{A}$	$_{a}3.6^{A}$	$_{ab}4.0^{A}$	$_{ab}3.9^{A}$	$_{ab}4.1^{A}$	$_{ab}4.0^{A}$
HP	$_{c}4.9^{A}$	$_{b}4.3^{A}$	$_{b}4.3^{A}$	$_{a}3.5^{A}$	$_{ab}4.0^{A}$	$_{ab}3.6^{A}$	$_{ab}4.1^{A}$	$_{ab}4.0^{A}$
Juiciness								
CO	$_{c}4.9^{A}$	$_{b}4.3^{A}$	$_{ab}4.2^{A}$	$_{a}3.4^{A}$	$_{ab}4.0^{A}$	$_{a}3.4^{A}$	$_{ab}3.9^{A}$	$_{ab}3.9^{A}$
HP	$_{d}4.9^{A}$	$_{c}4.3^{A}$	$_{bc}4.1^{A}$	$_{a}3.3^{A}$	$_{bc}4.2^{A}$	$_{ab}3.5^{A}$	$_{bc}4.1^{A}$	$_{abc}3.7^{A}$
Acceptability								
CO	$_{c}4.8^{A}$	$_{bc}4.1^{A}$	$_{bc}4.2^{A}$	$_{ab}4.0^{A}$	$_{ab}3.7^{A}$	$_{a}3.3^{A}$	$_{a}3.3^{A}$	$_{ab}3.7^{A}$
HP	$_{b}4.8^{A}$	$_{ab}4.1^{A}$	$_{a}4.0^{A}$	$_{a}3.9^{A}$	$_{a}4.1^{A}$	$_{a}3.5^{A}$	$_{a}3.8^{A}$	$_{a}3.7^{A}$

a–c: averages with different letters in the same row are different ($p<0.05$).
A–B: averages with different letters in the same column are different ($p<0.05$).

3.4. Instrumental texture

No differences ($p>0.05$) were found in texture parameters of "Cecina de León" cuts, between treated and untreated samples (Table 3). According to Pandrangi and Balasubramaniam (2005) the effect of HPP on texture varies according to commodity and intensity of pressure applied. High pressure processing can lead to reversible or irreversible changes in textural

Table 6
Sensorial parameter evolution (mean) of the control "Cecina de León" slices (CO) and of the "Cecina de León" slices treated with high pressures (HP) along storage time

Parameters	Initial value	1 days	15 days	30 days	60 days	90 days	150 days	210 days
Colour								
CO	$_{d}5.0^{A}$	$_{cd}4.6^{A}$	$_{bc}4.3^{A}$	$_{ab}3.8^{A}$	$_{bcd}4.3^{A}$	$_{ab}3.6^{A}$	$_{ab}3.9^{A}$	$_{a}3.6^{A}$
HP	$_{d}5.0^{A}$	$_{cd}4.7^{A}$	$_{abc}4.0^{A}$	$_{ab}3.7^{A}$	$_{bcd}4.3^{A}$	$_{ab}3.6^{A}$	$_{ab}3.7^{A}$	$_{a}3.3^{A}$
Odour								
CO	$_{c}4.7^{A}$	$_{c}4.8^{A}$	$_{bc}4.3^{A}$	$_{abc}3.9^{A}$	$_{ab}3.6^{A}$	$_{a}3.1^{A}$	$_{a}3.2^{A}$	$_{a}3.2^{A}$
HP	$_{c}4.7^{A}$	$_{c}4.6^{A}$	$_{bc}4.2^{A}$	$_{abc}3.8^{A}$	$_{abc}3.8^{A}$	$_{a}3.2^{A}$	$_{ab}3.3^{A}$	$_{a}3.1^{A}$
Taste								
CO	$_{c}4.6^{A}$	$_{c}4.5^{A}$	$_{bc}3.8^{A}$	$_{bc}3.6^{A}$	$_{bc}3.9^{A}$	$_{ab}3.2^{A}$	$_{ab}3.1^{A}$	$_{a}2.6^{A}$
HP	$_{c}4.6^{A}$	$_{c}4.3^{A}$	$_{c}4.1^{A}$	$_{abc}3.4^{A}$	$_{bc}3.8^{A}$	$_{ab}3.0^{A}$	$_{ab}3.1^{A}$	$_{a}2.6^{A}$
Hardness								
CO	$_{b}4.7A$	$_{b}4.8^{A}$	$_{ab}4.1^{A}$	$_{ab}4.2^{A}$	$_{ab}4.2^{A}$	$_{a}3.6^{A}$	$_{a}3.9^{A}$	$_{a}3.7^{A}$
HP	$_{b}4.7^{A}$	$_{b}4.6^{A}$	$_{ab}4.1^{A}$	$_{ab}3.9^{A}$	$_{ab}4.1^{A}$	$_{a}3.4^{A}$	$_{ab}4.1^{A}$	$_{ab}3.9^{A}$
Juiciness								
CO	$_{c}4.9^{A}$	$_{c}4.6^{A}$	$_{bc}4.3^{A}$	$_{bc}4.3^{A}$	$_{bc}4.2^{A}$	$_{a}3.4^{A}$	$_{ab}3.8^{A}$	$_{ab}3.7^{A}$
HP	$_{c}4.9^{A}$	$_{c}4.8^{A}$	$_{b}4.1^{A}$	$_{b}4.1^{A}$	$_{ab}3.8^{A}$	$_{a}3.5^{A}$	$_{ab}3.2^{A}$	$_{ab}3.7^{A}$
Acceptability								
CO	$_{c}4.6^{A}$	$_{c}4.5^{A}$	$_{c}4.1^{A}$	$_{bc}3.8^{A}$	$_{bc}3.8^{A}$	$_{ab}3.3^{A}$	$_{ab}3.2^{A}$	$_{a}2.8^{A}$
HP	$_{d}4.6^{A}$	$_{d}4.5^{A}$	$_{cd}4.0^{A}$	$_{bc}3.6^{A}$	$_{cd}4.1^{A}$	$_{ab}3.1^{A}$	$_{ab}3.3^{A}$	$_{a}2.8^{A}$

a–c: averages with different letters in the same row are different ($p<0.05$).
A–B: averages with different letters in the same column are different ($p<0.05$).

properties of meat products. Mor-Mur and Yuste (2003) found that HP treated cooked sausages (500 MPa during 5 min) were more cohesive than untreated ones; however, springiness, adhesiveness and hardness did not differ significantly. According to these authors, HPP did not affect most texture characteristics because the previous industrial cooking (in the manufacturing process) caused protein gelation. Although no previous studies on HPP "cecina" have been found, Hugas et al. (2002), reported that HPP (600 MPa during 6 min) did not determine significant differences in the nonproteic nitrogen fraction in dry cured ham and these results agree with a lack of protein breakdown due to HPP.

No statistical differences ($p>0.05$) were found in any parameter during the storage period with regarding those observed at the packaging moment. Taking into account the normal variability found for these parameters, all values obtained agree with previous studies on "Cecina de León" packaging and characterisation (Rubio et al., 2006; Rubio, 2006; Molinero, Rubio, González-Fernández, Martínez, & García-Cachán, 2004).

3.5. Sensory analysis

Tables 5 and 6 show the results of the sensory evaluation of "Cecina de León" cuts and slices respectively. Results demonstrated that all sensory attributes evaluated did not vary significantly ($p>0.05$) due to HPP in either "Cecina de León" cuts or "Cecina de León" slices. On the contrary, Saccani et al. (2004) reported that the HPP (600 MPa during 3, 6 or 9 min) on dried hams of 14 and 18 month ripening, modified the sensory parameters (colour intensity decrease, salty taste and firmness increase).

Sensory parameters evaluated on "Cecina de León" (on cuts and slices) presented progressive changes ($p<0.05$) throughout refrigerated storage. The decrease of sensorial quality was faster in slices, where acceptability reached values close to limit at 90 days of storage. The lost of quality was mainly due to the modification of odour and taste. Judges principally detected a remarkable odour and taste decrease in all samples, besides this also they made comments about of presence of anomalous odour and taste in "Cecina de León" slices. Cilla, Martínez, Beltrán and Roncalés (2006) observed a marked odour and flavour decrease in whole dry cured ham, due to long-term vacuum packaging. Rubio (2006) provided similar results during the packaging of "Cecina de León" cuts. Nielsen and Jägerstad (1994) described that sorption of food constituents by the packaging material had detrimental effects on the quality of the product; thus the sorption of aroma compounds might result in loss of flavour intensity.

On the other hand, Rubio et al. (2006) detected anomalous taste in the sliced "Cecina de León" after 60 days of vacuum packaging due to presence of high microbiological counts. In this work, high microbiological numbers were found in "Cecina de León" slices untreated but the treated HP samples presented lower bacterial counts. Compounds that had influence on odour and taste of long-term packed food due to the contact between the food and the packaging film could be formed in HP samples. Hugas et al. (2002) pointed that when using new preservation technologies involving the use of packaging techniques, it is very important to study the safety of the material, the possible formation of compounds that influence smell and taste of packed food and the mechanical and physical properties like strength and barrier properties. Although studies carried out in Japan showed that monolayer or multilayer films currently used in the agrofood industries are not modified in their barrier properties and migration rates after HPP from 400 to 600 MPA, further investigations should be made in order to study the possible compounds formation. In "Cecina de León" cuts, although acceptability had a minimum at 90 days, in general panellists give higher scores to odour and taste than in slices. This probably was because panellists could not detect the anomalous taste due to the fact that slices of the "Cecina de León" evaluated cuts were obtained removing the two first slices of cut, and therefore the "cecina" surface in contact with the packaging film was not present.

According to these results, the high pressure treatment does not have a negative effect on sensorial quality of "Cecina de León", but it did not improve sensorial properties of product along storage, despite the lower microbial counts obtained.

4. Conclusions

After high pressure treatment at 500 MPa for 5 min, physicochemical and sensorial properties of "Cecina de León" vacuum packed did not substantially change with respect to the same untreated products.

In summary, high pressure processing at 500 MPa for 5 min is an efficient method for delaying the growth of spoliage microorganisms in vacuum packed "Cecina de León" and this fact is very notable in vacuum packed "Cecina de León" slices. In this case, the shelf life was increased to 210 days of refrigerated storage from microbiological point of view. However, the treatment did not avoid sensorial changes along storage, restricting the optimum storage time to 90 days, especially when product was sliced.

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