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Efficacy of pulsed light for shelf-life extension and inactivation of *Listeria monocytogenes* on ready-to-eat cooked meat products

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ABSTRACT

Pulsed light (PL) was tested for its utility to improve the microbial quality and safety of ready-to-eat cooked meat products. Vacuum-packaged ham and bologna slices were superficially inoculated with *Listeria monocytogenes* and treated with 0.7, 2.1, 4.2 and 8.4 J/cm^2. PL treatment at 8.4 J/cm^2 reduced *L. monocytogenes* by 1.78 cfu/cm^2 in cooked ham and by 1.11 cfu/cm^2 in bologna. The effect of PL on lipid oxidation and sensory properties was also investigated. The 2-thiobarbituric acid values were very low and chromaticity parameters were within the normal values reported for cooked meat products. PL at 8.4 J/cm^2 did not affect the sensory quality of cooked ham, while treatments above 2.1 J/cm^2 negatively influenced the sensory properties of bologna. The combination of PL and vacuum packaging provided ham with an additional shelf-life extension of 30 days compared with only vacuum packaging. The shelf-life of bologna was not extended by PL.
Industrial relevance: The efficacy of pulsed light for the decontamination of surfaces offers excellent possibilities to ensure food safety and to extend shelf-life of ready-to-eat (RTE) products. The results of this study indicate that *Listeria monocytogenes* can be reduced by approximately 2 log cfu/cm^2 in RTE cooked ham and 1 log cfu/cm^2 in bologna using a fluence of 8.4 J/cm^2. This dose does not affect the sensory properties of ham and triples its shelf-life when compared with conventional RTE products. On the contrary, fluences above 2.1 J/cm^2 are not suitable for the treatment of bologna since sensory quality is modified.

1. Introduction

Changes in consumer preferences in recent years have led to the development of a wide variety of ready-to-eat (RTE) foods. Among these foods, the meat industry offers many cooked products prepared in small portions, such as slices, from processed blocks. These blocks are subjected to pasteurization, which provides an adequate microbiological quality. However, RTE meat products are presumed to be contaminated after cooking due to further manipulation (Murphy et al., 2003). Operations such as cutting, slicing and packaging can lead to contamination by a variety of environmental microorganisms present in tools, handlers and mechanical equipment. Concerning food safety, RTE meat products have been reported to be contaminated by *Listeria monocytogenes* (Lin et al., 2006; Ye et al., 2008). This psychrotrophic organism is ubiquitous, can survive in biofilms, and resists diverse environmental conditions, such as low pH and high sodium chloride concentrations, which gives *Listeria* great persistence in the processing environments (Vázquez-Villanueva et al., 2010).

L. monocytogenes is responsible for severe foodborne disease outbreaks, often with fatal consequences. In a research carried out by the FDA and the USDA, 23 categories of RTE foods are considered to be the greatest contributors to listeriosis in the USA (CFSAN/FSIS, 2003). Processed meats are well documented to be a potential vehicle for human listeriosis. Products such as pork rillettes, chicken-based frankfurters and deli meats have caused outbreaks with a significant number of deaths in France, Canada and the USA (de Valk et al., 2001; Gombas et al., 2003; Warriner & Namvar, 2009).

The pH and a_w of cooked RTE meat products allow the growth of *L. monocytogenes* (EFSA, 2007). These products include sodium chloride and nitrite in their formula, but both compounds are not completely effective in controlling the growth of that pathogen if a post-processing contamination occurs on the surface EFSA (2003, 2007). Different emerging technologies are being studied for the control of pathogens in RTE foods, such as high hydrostatic pressure, natural antimicrobials (Jofré et al., 2008) and e-beam irradiation (Cabeza et al., 2007). Another interesting novel technology for this purpose is pulsed light (PL). This is a FDA-approved non-thermal treatment (FDA, 1996) consisting of the emission of short flashes of a broadband spectrum light (200–1100 nm). The decontamination effect of PL is mainly due to photochemical changes on nucleic acids by UV-C, combined with photothermal and photophysical damage to cells due to water vaporization and membrane disruption (Takeshita et al., 2003; Gómez-López et al., 2007). All of these changes occur at a superficial level due to limited light penetration. Moraru and Uesugi (2009) reported a PL penetration depth of 2.3 mm in Vienna sausages. This is not an inconvenience in the case of RTE foods, since post-heat

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treatment contamination takes place on the surface. Therefore, in environments where *Listeria* cannot be removed, PL is an alternative to provide food safety. Some countries such as the USA CFSAN/FSIS (2003), Australia and New Zealand ANZFA (2001) have adopted the zero-tolerance policy for *L. monocytogenes* in RTE meat products in which this organism can grow, which means a Food Safety Objective (FSO) of 0.04 cfu/g. However, the EU establishes a FSO of 100 cfu/g for these products, although if the manufacturer cannot demonstrate the achievement of this objective, then the criterion "not detected in 25 g" is applied before the product leaves the plant (European Commission, 2005).

The purpose of this study was to assess the efficacy of PL for the inactivation of *L. monocytogenes* on cooked ham and bologna slices in view of a future application of this technology for the shelf-life extension of these products.

2. Materials and methods

2.1. Microorganisms and growth conditions

L. monocytogenes Scott A (CIP 103575 serotype 4b, Institut Pasteur, Paris, France) was used in these experiments. The strain was maintained by freezing (−20 °C) in trypticasein soy broth (TSB, Condalab, Madrid, Spain) with 10% glycerol added as a cryogenic agent. For each experiment, fresh cultures were prepared by inoculating a piece of frozen culture within 9 ml of TSB, then incubating at 32 °C for 24 h. Afterwards, the culture was plated on trypticasein soy agar (TSA, Condalab, Madrid, Spain) and incubated under the same conditions. One colony was transferred into 10 ml of TSB and incubated for 24 h at 32 °C to give an initial inoculum of 10^8 cfu/ml (stationary growth phase).

2.2. Sample preparation

Packaged heat-processed cooked ham (10.5 cm diameter) and bologna (5.3 cm diameter) cylinders were purchased in a local supermarket. The composition of ham included 2.2% NaCl and 100 ppm $NaNO_2$. Bologna was made from lean pork and lard and contained 2.5% NaCl and 100 ppm $NaNO_2$. Blocks were cut with an electric slicing machine whose blade and contact surfaces were previously disinfected with 70% ethanol and washed with sterile distilled water. Slices (1 mm thickness) were surface-inoculated, using a Digralsky handle, with 100 µl of the *L. monocytogenes* inoculum on each side to provide an approximate concentration of 7 log cfu/cm^2. Then, slices were left to dry for 1 h and afterwards they were individually vacuum packaged in plastic bags made of 48 µm polyamide/polyethylene/vinyl acetate copolymer (Cryovac, Sealed Air, Barcelona, Spain). Previous *in vitro* experiments indicated that PL could easily penetrate this film (Fernández et al., 2009).

For the shelf-life study, non-inoculated samples were used. These samples were packaged under both aerobic and vacuum conditions and stored at 4 °C.

2.3. Pulsed light treatment

Pulsed light treatment was applied using a desktop Steribeam SBS-XeMatic-2L-A device (SteriBeam Systems, Kehl am Rhein, Germany). The apparatus consists of a metal housing surrounding a treatment chamber made of polished stainless steel (20 cm wide × 14 cm deep × 12 cm high) and equipped with two (upper and lower) xenon lamps and a quartz table located in the centre. The system has a timer for multiple pulsing and a switch for adjusting the energy level. Each pulse is delivered in 250 µs and corresponds to a fluence of 0.7 J/cm^2 at the level of the quartz table. The system delivers 3–4 pulses per minute. The spectral output of the lamp corresponds to 30% UV light (12% UV-C, 10% UV-B and 8% UV-A), 30% infrared radiation and 40% visible light. A vacuum pump was connected to the chamber purge to extract the ozone produced by the lamp.

Samples were placed on the quartz table and flashed with different fluences (incident light energy per unit of surface area). Fluences tested were 0.7, 2.1, 4.2 and 8.4 J/cm^2. Untreated slices were also analyzed as controls.

To assess the efficacy of PL for the inactivation of *L. monocytogenes* for each fluence tested, three batches of inoculated and PL-treated slices were enumerated: 1) immediately after treatment, 2) after being kept 4 h at 4 °C wrapped with aluminum foil immediately after flashing, and 3) after maintaining them for 4 h at 4 °C under a fluorescent light. The two latter batches were prepared in order to assess possible cell repair by a photoreactivation mechanism (Gómez-López et al., 2005). Ten slices were assayed per batch. The results reported in this study are the mean data obtained with samples from two independent experiments carried out with the same commercial products.

2.4. Microbiological analysis

To enumerate *L. monocytogenes* survivors on ham and bologna, the slices were aseptically removed from the package and mixed with 10 ml of sterile saline solution in a Stomacher bag. The mixture was homogenized in a Stomacher AESAP 1066 (AES Chemunex, Barcelona, Spain) for 2 min and serial dilutions were prepared. Counts were determined by the spread plate method on Palcam agar with polymyxin B, acriflavine hydrochloride and ceftazidime, and egg yolk emulsion (Oxoid, Basingstoke, UK) after incubation at 37 °C for 48 h. Shelf-life was evaluated by enumeration on TSA at 32 °C for 24 h. A spiral plate system (Eddy Jet, IUL Instruments, Barcelona, Spain) was used for plating. Colonies were enumerated with an automatic counter (Countermat Flash, IUL Instrument, Barcelona, Spain). The detection limit of the enumeration technique was <0.5 log cfu/cm^2. Analyses were performed in triplicate.

2.5. Colour measurement

Colour was measured on the surface of untreated and PL-treated non-inoculated slices using a tristimulus colorimeter (Minolta ChromaMeter CR300, Minolta Corporation, NJ, USA). The colorimeter uses the following parametres: L^* indicates the lightness and a^* and b^* are the chromaticity coordinates (a^* indicates redness, b^* yellowness). Three randomly selected spots were analyzed on each sample and the resulting average was calculated. Analyses were performed in triplicate.

2.6. Lipid oxidation

The 2-thiobarbituric acid (TBARs) method described by Salih et al. (1987) was used. Samples (5 g) were homogenized in 15 ml of 0.38 M $HClO_4$ for 3 min using a Polytron PT 10–35 GT probe (Kinematica, Lucerne, Switzerland) in a glass vessel immersed in an ice bath. A control sample was made by replacing the 5 g of sample with 5 ml of distilled water. To avoid further oxidation, 0.5 ml of a 0.19 M BHT ethanolic solution was added. The homogenate was centrifuged (3000 × *g*, 5 min, 5 °C) and filtered through Whatman paper No. 54. An aliquot (0.7 ml) was mixed with the same volume of 0.02 M TBA solution in an eppendorf punctured on the top and heated at 100 °C for 30 min. After cooling in an ice water bath, the mixture was centrifuged at 3000 × *g* for 15 min at 5 °C. Finally, the absorbance was measured at 532 nm using a spectrophotometer (Hitachi, Tokyo, Japan). A stock solution of 1×10^{-7} M 1,1,3,3-tetraethoxypropane (TEP) in distilled water was used to prepare dilutions ranging from 1×10^{-8} to 8×10^{-8} mol TEP. Two ml of each TEP dilution was mixed with 2 ml of 0.02 M TBA solution and then incubated, and absorbance at 532 nm was used to plot a standard curve. Results were expressed

as μg malondialdehyde/g sample. Measurements were done in triplicate.

2.7. Sensory analysis

Samples were evaluated by a panel of 13 tasters of the Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, who had previously been trained in the sensory assessment of meat products. The evaluations were conducted in individual booths built according to the criterion of the International Standards Organization DP 6658 ISO (1981). The evaluation was performed between meals. White fluorescent light was used during tests.

Samples were tested by a descriptive analysis. A rank order test was performed asking the panellists to independently evaluate four sensory characteristics: appearance, colour, odour and flavour of each sample. Samples were graded according to their proximity to the optimum sensory quality of cooked ham and bologna. A 5 point scale was used, in which 1 corresponded to the lowest preference and 5 to the highest. No repetitions were allowed. Results were expressed as the sum of ranks, which corresponds to the sum of the preference scores for a specific sensory attribute, according to the following formula: $[(N1\times1)+(N2\times2)+(N3\times3)+(N4\times4)+(N5\times5)]$. Where N1, N2, N3, N4 or N5 are the number of panellists that ranked the sample in the position 1 (minimum preference), 2, 3, 4 or 5 (maximum preference) in the rank order test. The significance level of data obtained in these tests was determined by Friedman's rank addition according to the model proposed by Joanes (1985), and the tables for multiple comparison procedures for analysis of ranked data (Christensen et al., 2006).

2.8. Shelf-life determination

On the basis of the results obtained in the studies that have been described above, the most adequate fluences were selected for each product. Non-inoculated ham and bologna slices were vacuum and aerobically packaged and then PL-treated. Periodical bacterial enumeration and sensory analysis (odour and appearance) were performed during storage at 4 °C. Non-pulsed vacuum and aerobically packaged slices were used as controls. The end of shelf-life was set when off-odours and/or slime appeared together with bacterial counts higher than 10^7 cfu/cm^2 (Stanbridge & Davies, 1998).

2.9. Electron microscopy

Scanning Electron Microscopy (SEM) of cooked ham and bologna was carried out to evaluate for a possible shadowing effect interfering with PL treatment due to the location of *L. monocytogenes* on the samples.

Inoculated ham and bologna samples were prepared under aseptic conditions. Ham and bologna portions of less than 1 cm^2 were obtained using a scalpel and fixed in 3% glutaraldehyde in phosphate buffer solution (PBS) during 15 h at 4 °C. After washing with PBS, samples were post-fixed with 1% osmium tetroxide during 1 h. Samples were then dehydrated in increasing concentrations of acetone (30, 50, 70, 80, 90 and 100%) for a period of 40 min in each solution. Finally, samples were dried at critical point by introducing them in carbon dioxide, followed by metallisation with gold/graphite. A scanning electron microscope (JEOL, mod. JM-6400, Tokyo, Japan) at the Microscopy and Cytometry Center (UCM) was used to perform the analyses at different magnification rates.

2.10. Statistical analysis

A one-way ANOVA was conducted to compare the results of the different assays, using Statgraphics Plus 5.0 (Manugistics, MD, USA).

3. Results and discussion

3.1. Inactivation of L. monocytogenes

The inactivation effect of PL treatment on ham and bologna slices is shown in Table 1. The maximum reduction when enumerating bacteria immediately after treatment was 1.78 and 1.11 log cfu/cm^2 on ham and bologna, respectively, with a treatment of 8.4 J/cm^2. The lower inactivation obtained on bologna could be explained by the distinct microstructural features of both products. It is well known that the surface topography greatly influences the efficacy of PL treatment (Woodling & Moraru, 2005). A high degree of surface roughness allows some hiding of microbial cells inside surface details. This can be observed in Fig. 1. Although both products show an irregular surface, bologna (Fig. 1b), being a fine emulsion, provides a higher number and variety of crevices which shield bacteria from light.

There are few published works related to the inactivation of pathogenic bacteria by PL on cooked meat products. In relation to *Listeria*, some studies have been conducted on the surface of RTE sausages. The inactivation achieved in the present work with a fluence of 8.4 J/cm^2 in both cooked ham and bologna is higher than the outcome reported by Uesugi and Moraru (2009), which obtained a reduction of 1.37 log cfu per sausage for *Listeria innocua* on the surface of unpackaged Vienna sausages, when treated with 9.4 J/cm^2. According to these authors, *L. innocua* mimics the response of *L. monocytogenes* to PL treatment. Although results on packaged and unpackaged meat products are being compared, previous *in vitro* experiences have shown that PL can easily penetrate films with different compositions and thicknesses (Fernández et al., 2009).

On the other hand, Keklik et al. (2009) assayed different PL treatment conditions (time and distance from the lamp) for the inactivation of *L. monocytogenes* Scott A on chicken frankfurters. These authors established optimum parameters as 60 s at 8 cm distance, yielding 1.5 and 1.6 log cfu/cm^2 reductions in unpackaged and vacuum-packaged frankfurters, respectively. When calculating the fluence, these treatments are equivalent to 55.9 and 48.4 J/cm^2, respectively, which exceed, by far, the FDA regulations. According to this administration, the total cumulative PL treatment for foods must not exceed 12 J/cm^2 (FDA, 1996).

Table 1
Inactivation (log cfu/cm²) of *Listeria monocytogenes* in vacuum-packaged cooked ham and bologna slices using different level of PL fluences.

	Fluence (J/cm²)							
	Cooked ham*				Bologna**			
	0.7	2.1	4.2	8.4	0.7	2.1	4.2	8.4
Immediately after treatment	0.75 ± 0.05c	1.01 ± 0.03b	1.12 ± 0.21b	1.78 ± 0.12a	0.41 ± 0.10c	0.67 ± 0.04b	0.75 ± 0.07b	1.11 ± 0.05a
After 4 h exposure to light	0.74 ± 0.03c	1.01 ± 0.11b	1.08 ± 0.11b	1.56 ± 0.08a	0.40 ± 0.05c	0.70 ± 0.02b	0.80 ± 0.02b	1.15 ± 0.04a
After 4 h in the dark	0.79 ± 0.08c	1.07 ± 0.03b	1.09 ± 0.26b	1.66 ± 0.05a	0.37 ± 0.03c	0.67 ± 0.01b	0.73 ± 0.02b	1.07 ± 0.04a

*Initial contamination (mean ± SD) 7.23 ± 0.1 log cfu/cm².
**Initial contamination (mean ± SD) 6.76 ± 0.05 log cfu/cm².
a, b, c: values in the same row (for each product) with different letter are significantly different ($p<0.05$).

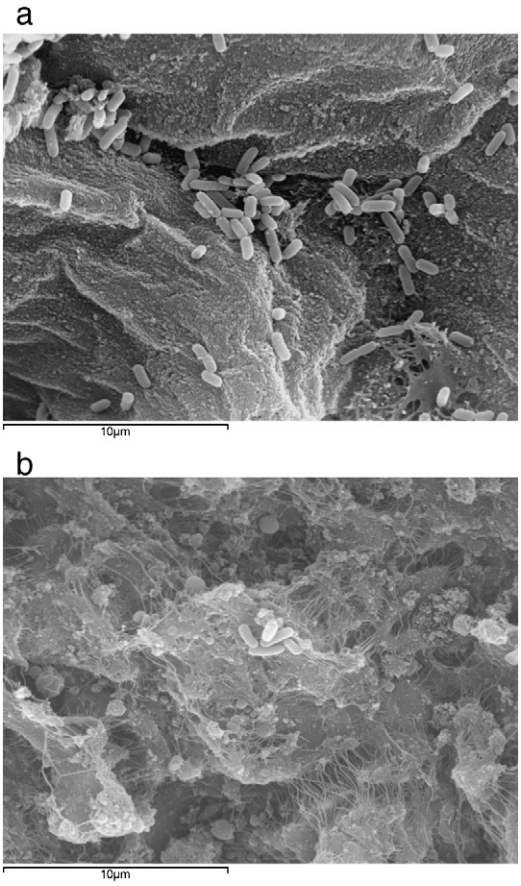

Fig. 1. Scanning Electron Microscopy (SEM) micrographs of cooked ham (a) and bologna (b) inoculated with *Listeria monocytogenes*. Magnification: 5000×.

No significant differences ($p>0.05$) were found among the counts obtained immediately after PL treatment and those recorded on samples exposed to light and kept in the dark for 4 h (Table 1), which means that *L. monocytogenes* did not show the capability of photoreactivation on the products assayed. Few data are available in the literature concerning this repairing mechanism (Cook, 1970) after PL treatment. Only a slight photoreactivation for *L. monocytogenes* has been reported by Gómez-López et al. (2005). The main lethal effect of PL has been extensively attributed to photochemical damage on DNA, which is indeed the case of continuous wave UV (CW-UV). However, PL involves a much greater energy input, as high as 35 MW, compared to a peak power emission of 100–1000 W in CW-UV (MacGregor et al., 1998; Demirci, 2002). This energy input over a very short time span produces higher fluences on the food surface. Some authors (Dunn et al., 1989; Wekhof et al., 2001) hypothesize that the absorption of UV by microorganisms may cause cell disruption due to overheating, which could also have as a contributing factor the infrared component of the pulse. This overheating would also inactivate the DNA photorepairing mechanisms (Dunn et al., 1995; McDonald et al., 2000), therefore preventing photoreactivation.

3.2. Colour analysis

The L^*, a^* and b^* values of cooked ham and bologna were determined immediately after PL treatment (Table 2). Pulsed light lightened cooked ham; the L^* values of all treated samples were significantly higher ($p<0.05$) than that of the control, although no differences were observed among the fluences assayed. The a^* value gradually decreased as fluence increased. No significant differences ($p>0.05$) were appreciated between untreated samples and those pulsed with the lowest fluence (0.7 J/cm^2). On the other hand, only the highest fluences assayed (4.2 and 8.4 J/cm^2) significantly affected the b^* value ($p<0.05$), which increased in these samples. In any case, all the measurements recorded are within the normal values reported for commercial cooked ham (Sindelar et al., 2007).

In relation to bologna, treatments equal to or higher than 4.2 J/cm^2 significantly decreased L^* and increased b^* ($p<0.05$). No significant differences ($p>0.05$) were found for the a^* value when comparing samples treated with 2.1 J/cm^2 or lower fluences to the control samples (Table 2).

As far as the authors know, there is only one study (Keklik et al., 2009) reporting colour quality of meat products treated by PL. These authors, when pulsing chicken frankfurters with a fluence of 2.7 J/cm^2 found significant differences for the a^* value, which decreased in treated samples. In the present work, when applying a similar energy level (2.1 J/cm^2), this behaviour was observed in ham but no significant differences were found for this parameter in bologna. Keklik et al. (2009) did not report significant differences for L^* and b^* values with this mild treatment. These authors also tested much higher fluences than 2.7 J/cm^2 which cannot be compared with the other conditions assayed in the present work.

Table 2
L^*, a^* and b^* values of cooked ham and bologna slices after PL treatment with different fluences.

	Fluence (J/cm²)									
	Cooked ham					Bologna				
	0	0.7	2.1	4.2	8.4	0	0.7	2.1	4.2	8.4
L^*	61.64 ± 4.14b	65.34 ± 3.39a	65.11 ± 1.49a	65.88 ± 3.49a	66.62 ± 2.42a	58.86 ± 1.18a	58.64 ± 0.76a	58.41 ± 2.31a	55.89 ± 0.94b	54.92 ± 1.04b
a^*	17.82 ± 2.26a	16.28 ± 2.94a,b	15.51 ± 1.26b,c	14.49 ± 2.85c,d	12.98 ± 1.84 d	22.75 ± 0.95a	22.30 ± 1.09a,b	21.92 ± 1.48a,b	21.55 ± 0.94b	21.58 ± 0.76b
b^*	9.82 ± 0.67c	10.22 ± 0.80b,c	9.97 ± 0.58b,c	10.53 ± 1.03a,b	10.91 ± 1.18a	8.80 ± 0.31b	8.63 ± 0.53b	8.65 ± 0.59b	9.71 ± 0.28a	10.06 ± 0.28a

L^*: lightness, a^*: redness, b^*: yellowness. a, b, c, d: values at the same row (for each product) with different letter are significantly different ($p<0.05$).

Table 3
TBARs values (μg malondialdehyde/g product) for cooked ham and bologna using different PL fluences.

	Fluence (J/cm²)				
	0	0.7	2.1	4.2	8.4
Cooked ham	0.035 ± 0.005b	0.031 ± 0.008b	0.037 ± 0.007b	0.095 ± 0.012a	0.105 ± 0.010a
Bologna	0.589 ± 0.060	0.542 ± 0.089	0.626 ± 0.092	0.652 ± 0.114	0.584 ± 0.071

a, b: values in the same row with different letter are significantly different ($p<0.05$).

Table 4
Sensory evaluation of bologna slices after PL treatment with different fluences.

Fluence	Sum of ranks*			
(J/cm²)	Appearance	Colour	Odour	Flavour
0	53a	53a	61a	63a
0.7	53a	56a	65a	63a
2.1	52a	52a	53a,b	51a,b
4.2	36a	39a,b	29b,c	31b,c
8.4	27b	25b	17c	17c

*Sum of ranks: $[(N1\times1)+(N2\times2)+(N3\times3)+(N\times4)+(N5\times5)]$ where N1, N2, N3, N4 or N5 are the number of panellists that ranked the sample in the position 1 (minimum preference), 2, 3, 4 or 5 (maximum preference) in the rank order test. a, b, c: values in the same column with different letter are significantly different ($p<0.01$).

3.3. Lipid oxidation

The malondialdehyde (MDA) content of samples, expressed as μg MDA/g of product, was determined before and after PL treatment. The results are shown in Table 3. Although significant differences ($p<0.05$) were found in hams when PL treatments above 2.1 J/cm^2 were applied, TBARs values were low in any case, reflecting very low lipid oxidation in the product, even at the highest fluences tested. All of the values recorded can be considered as normal for commercial cooked ham (Sindelar et al., 2007). On the other hand, PL treatment did not increase TBARs values in bologna, since no significant differences ($p>0.05$) were found among samples. Similar values to those obtained for bologna (0.5–0.6 μg MDA/g) have been reported by other authors in emulsion-type meat products such as commercial and PL-treated frankfurters (Sindelar et al., 2007; Keklik et al., 2009).

Ultraviolet light is well known to accelerate oxidative changes in lipids. It has been reported, however, that when applied in pulsed form, the short duration of the pulse limits these reactions due to the short half-life of the π-bonds, which prevents efficient coupling with oxygen (Fine & Gervais, 2004). This fact could explain the low TBARs numbers observed in the present work for both PL cooked ham and bologna.

3.4. Sensory analysis

The testing panellists did not find significant differences ($p>0.01$) in any of the parameters evaluated among pulsed and non-pulsed ham slices (data not shown). In relation to bologna (Table 4), the sensory test reflected differences ($p<0.01$) for odour and flavour in the samples treated with fluences of 4.2 J/cm^2 or higher, while appearance and colour only differed in the slices pulsed with 8.4 J/cm^2. Most of the literature concerning the application of PL for the preservation of foods mainly deals with microbiological inactivation and few data are reported on sensory analysis. The results obtained in the present work are promising, especially in ham, since apart from achieving a reasonable level of inactivation of *L. monocytogenes*, PL preserved the sensory quality of the product.

3.5. Shelf-life determination

The maximum fluence tested (8.4 J/cm^2) was selected for treating cooked ham. Table 5 shows the shelf-life of cooked ham slices stored under aerobic and vacuum conditions. The initial microbial counts in untreated slices were approximately 2 log cfu/cm^2, while no microorganisms were detected in the samples immediately after PL treatment (the detection limit was <0.5 log cfu/cm^2). Untreated ham packaged in air resulted organoleptically unacceptable after 8 days of storage, although bacterial counts were lower than 6 log cfu/cm^2 on that day of sampling. In pulsed slices this condition was reached 4 days later.

In relation to vacuum-packaged ham, as this technique is a very useful tool to extend the shelf-life on its own, the lag phase was extended in the control samples more than one week, and the product was acceptable until day 19 of storage. These results are in accordance with previous findings in vacuum-packaged ham, for which a shelf-life of 15–28 days has been reported, depending on the initial microbial load and the properties of the packaging material Silla & Simonsen, 1985; Stojanowic & Flemming, 1988; Ahvenainen et al., 1989; Ambrosiadis & Georgakis, 1993. Pulsed light treatment prolonged the lag phase up to 26 days and samples were organoleptically and microbiologically acceptable for 49 days (Table 5). Therefore, PL

Table 5
Effect of PL on the shelf-life (microbial counts, odour and appearance) of cooked ham slices.

	Aerobic packaging		Vacuum packaging	
Fluence	Untreated samples	8.4 J/cm^2	Untreated samples	8.4 J/cm^2
	log cfu/cm^2	log cfu/cm^2	log cfu/cm^2	log cfu/cm^2
Days	Odour	Odour	Odour	Odour
0	2.2	<0.5*	2.0	<0.5*
	Acceptable	Acceptable	Acceptable	Acceptable
5	3.4	2.1	2.2	<0.5*
	Acceptable	Acceptable	Acceptable	Acceptable
8	5.6	3.7	2.8	<0.5*
	Light hot culture medium	Acceptable	Acceptable	Acceptable
12	6.1	5.7	3.1	<0.5*
	Light hot culture medium	Light hot culture medium	Acceptable	Acceptable
15	7.4	6.7	3.3	<0.5*
	Off-odour/slime			
	Marked hot culture medium	Acceptable	Acceptable	
19		7.5	4.3	<0.5*
		Off-odour/slime	Acceptable	Acceptable
26			5.7	<0.5*
			Light hot culture medium	Acceptable
33			6.2	2.2
			Light sour	Acceptable
40			7.6	3.4
			Spoiled strong sour	Acceptable
49				4.2
				Acceptable
54				5.1
				Light hot culture culture medium

* Detection limit <0.5 log cfu/cm^2.

Table 6
Effect of PL on the shelf-life (microbial counts, odour and appearance) of bologna slices.

	Aerobic packaging		Vacuum packaging	
Fluence	Untreated samples	2.1 J/cm^2	Untreated samples	2.1 J/cm^2
	log cfu/cm^2	log cfu/cm^2	log cfu/cm^2	log cfu/cm^2
Days	Odour	Odour	Odour	Odour
0	3.5 Acceptable	3.1 Acceptable	3.5 Acceptable	3.1 Acceptable
5	6.4 Acceptable	5.6 Acceptable	6.6 Acceptable	6.0 Acceptable
8	6.7 Off-odour	6.2 Off-odour	6.9 Off-odour	7.0 Strong sour off-odour/slime
12	7.1 Light sour Off-odour	6.6 Light sour Off-odour/slime	7.2 Strong sour Off-odour/slime	7.4 Strong sour Off-odour/slime
15		7.5 Strong sour Off-odour/slime		

provided an additional shelf-life extension of 30 days in vacuum-packaged ham slices when compared to untreated vacuum-packaged samples.

As PL modified to a greater extent the sensory properties of bologna, a fluence of 2.1 J/cm^2 was selected for the application of this treatment (Table 6). The initial microbial numbers in the untreated samples were 3.5 log cfu/cm^2, providing a shelf-life of 5 days either under aerobic or vacuum conditions. As the PL dose was very low in order to keep the sensory quality of the product, the inactivation rate achieved by the treatment was not sufficient to delay microbial growth and therefore, no shelf-life extension was observed.

4. Conclusion

Pulsed light provided a reasonable reduction of the population of *L. monocytogenes* Scott A on the surface of RTE cooked ham and bologna, demonstrating to be more effective on ham due to its microstructural features. In RTE cooked ham, a PL treatment of 8.4 J/cm^2 gave an inactivation level of *L. monocytogenes* of approximately 1.8 log cfu/cm^2 without changing the quality of the product, and extended shelf-life of up to 49 days under vacuum packaging. Since bologna resulted to be more sensitive to pulsed light, milder treatments had to be applied and no shelf-life extension was subsequently observed.

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