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Use of pulsed light to increase the safety of ready-to-eat cured meat products

Monica Ganan [a,b], Eva Hierro [a,*], Xavier F. Hospital [a], Elvira Barroso [a], Manuela Fernández [a]

[a] Departamento de Nutrición, Bromatología y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Complutense, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain
[b] CEI Campus Moncloa, UCM-UPM e INIA, Madrid, Spain

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ABSTRACT

Pulsed light (PL) was tested for its efficacy to reduce *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium on the surface of two ready-to-eat (RTE) dry cured meat products (*salchichón* and loin). Maximum log reductions between 1.5 and 1.8 cfu/cm^2 were obtained for both microorganisms when a fluence of 11.9 J/cm^2 was applied. Slight and particular differences in the instrumental color parameters were observed due to the treatment in both products, although no changes in the sensory analysis were detected either immediately after treatment or during 30 days storage in *salchichón*. Panelists perceived some changes in the sensory quality of loin immediately after treatment, but these differences disappeared along storage. PL could be considered a useful alternative to other non-thermal techniques for increasing the safety of RTE dry cured meat products.

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1. Introduction

Ready-to-eat (RTE) foods may pose a safety risk for consumers due to post-processing handling (cutting, slicing, packaging) during which pathogenic bacteria can reach the surface. According to the WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe, nearly 27% of the food-borne outbreaks reported in the period 1993–1998 were related to inadequate handling and environmental contamination (WHO, 2000). Significant contributing factors included equipment (3.7%) and personnel (7%).

The most relevant pathogens that can be present in RTE foods are *Listeria monocytogenes* and *Salmonella enterica* (Cabedo, Picart, Barrot, Teixidó, & Canelles, 2008; CFSAN/FSIS, 2003). These bacteria are commonly isolated from different RTE products and have been associated to several food-related outbreaks (Bohaychuk et al., 2006; Borch & Arinder, 2002; Levine, Rose, Green, Ransom, & Hill, 2001). Different studies (Angelidis & Koutsoumanis, 2006; Di Pinto, Novello, Montemurro, Bonerba, & Tantillo, 2010) report a prevalence of *L. monocytogenes* of 10–20% in sliced dry sausages in the European market, with low contamination levels ($\leq$10 cfu/g) (Angelidis & Koutsoumanis, 2006).

Concerning RTE meat products, *S. enterica* serovar Typhimurium is the most frequent serovar of *Salmonella* reported in pork (EFSA, 2008). On the other hand, *L. monocytogenes* is a ubiquitous organism that shows great persistence in the processing environments (Thévenot, Dernburg, & Vernozy-Rozand, 2006; Vázquez-Villanueva et al., 2010). Nesbakken, Kapperud, and Caugant (1996) reported an increasing prevalence of *L. monocytogenes* at every stage of the fresh pork meat industry from the slaughterhouse to the cutting room.

National and international food regulations establish safety criteria to guarantee that these bacteria are below the limits considered harmful for consumers of RTE products. For instance, the European Union (EU) Regulation 1441/2007 (European Commission, 2007) sets a limit of 100 ufc/g for *L. monocytogenes* in RTE meat products during shelf-life, while the criterion for *Salmonella* spp. in RTE raw meat products is zero tolerance (no organisms found in 25 g). The zero-tolerance policy is also applied for *L. monocytogenes* and *Salmonella* spp. in the USA (CFSAN/FSIS, 2003; USDA/FSIS, 2010). In 1997, the US Food Safety and Inspection Service (FSIS) included both bacteria in the microbiological testing programs for dry and semidry fermented sausages, as a result of foodborne illness outbreaks associated with these products (Levine et al., 2001).

Different non-thermal technologies are being studied for the control of pathogens in RTE cured meats, such as high pressure processing (Porto-Fett et al., 2010; Rubio, Martínez, García-Gachán, Rovira, & Jaime, 2007), irradiation (Cabeza, de la Hoz, Velasco, Cambero, & Ordóñez, 2009; Fu, Sebranek, & Murano, 1995) and pulsed light (PL) (Hierro et al., 2011; Wambura & Verghese, 2011). PL consists on the application of short length flashes (10^{-3}–10^2 ms) of an intense broad-spectrum, rich in UV light. Its bactericidal effect appears to be mainly due to photochemical damage in DNA induced by the UV-C component (Wang, MacGregor, Anderson, & Woolsey, 2005). Nevertheless, as stated by Wuytack et al. (2003), pulsed light

* Corresponding author. Tel.: +34 913943946; fax: +34 913943743.
E-mail address: hierro@vet.ucm.es (E. Hierro).

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inactivation can be regarded as a multitarget process; apart from changes in DNA, physical damages to membrane and other structures can occur (Takeshita et al., 2003; Krishnamurthy, Tewari, Irudayaraj, & Demirci, 2010), together with a photothermal effect due to a temporary overheating of the cells when long PL treatments are applied (Wekhof, Trompeter, & Franken, 2001; Krishnamurthy et al., 2010) or high fluences are used.

The effect of PL is exerted at the surface level. Nevertheless, this is not a drawback for the hygienization of RTE products since post-processing contamination mainly occurs on the surface. In comparison to continuous UV systems, PL allows a greater energy input and reduces the exposure time. Different studies have demonstrated the sensitivity of bacteria to PL. In relation to this, previous works have shown the efficacy of PL against *L. monocytogenes* and *S. enterica* on agar (Fernández, Manzano, Hoz, Ordóñez, & Hierro, 2009; Rowan et al., 1999) and a number of foods such as RTE cooked meat products (Hierro et al., 2011), salmon fillets (Ozer & Demirci, 2006) or berries (Bialka & Demirci, 2008). It is critical to evaluate the effect of PL on the target matrices, since properties such as topography or transparency may influence the effectiveness of the technique.

The RTE dry cured meat product industry is a highly dynamic sector in Western countries. In Spain, it accounts for about 16% of the total meat product manufacture (Mercasa, 2007) and also shows an increasing trend. PL has not been assessed yet on these products, which, as previously mentioned, have to comply with the corresponding safety criteria. This technology could provide the achievement of these criteria in a rapid and simple way since it can be easily integrated at the production lines, allowing a continuous processing. The purpose of this study was to evaluate the efficacy of PL for the inactivation of *L. monocytogenes* and *S. enterica* on two RTE dry cured meat products (*salchichón* and loin) in view of a future application of this technology to improve their microbiological safety and shelf-life.

2. Materials and methods

2.1. Samples

Two Mediterranean style dry cured products, *salchichón* and loin, were purchased at a local supermarket. *Salchichón* is a dry fermented sausage made from minced pork and back fat. Dry cured loin is manufactured from the whole *Longissimus dorsi* muscle of pigs, which is stuffed after curing.

2.2. Microorganisms

Three strains of *L. monocytogenes* (CECT 4032, CECT 7467, and Scott A) and two strains of *S. enterica* serovar Typhimurium (CECT 7159 and CECT 4371) were used for direct inoculation of the samples. All the strains were obtained from the Spanish Collection of Type Cultures (CECT, Valencia, Spain).

2.3. Inoculum preparation

The strains were stored at −20 °C in Tryptone Soy Broth (TSB) (Pronadisa, Madrid, Spain) containing 20% (v/v) glycerol. Cultures were prepared by inoculating a loop of frozen stock into 10 mL of TSB, then incubating at 37 °C for 24 h; subsequently, a 20 μl inoculum of the grown cultures was transferred to 10 mL broth and incubated under the same conditions. Afterward, each strain was plated on TSA (Pronadisa) and incubated at 37 °C for 24 h. For each organism, one colony was transferred into 10 mL of TSB and incubated at 37 °C to reach the stationary growth phase. For the inoculation, cocktails containing the strains of *L. monocytogenes* or *S.* Typhimurium were prepared to give a concentration of 7–8 log cfu/mL.

2.4. Sample preparation

Slices (1 mm thickness) of *salchichón* and loin were obtained using an electric slicing machine whose blade and contact surfaces were previously disinfected with 70% ethanol and washed with sterile distilled water. Slices were surface-inoculated, using a Digralsky handle, with 100 μl of the corresponding inoculum on each side providing a concentration of 4–5 log cfu/cm^2. Then, samples were left to dry for 15 min and afterward, they were individually vacuum packaged in 60 μm polyamide/polyethylene plastic bags (Plastiñi, La Rioja, Spain). Previous *in vitro* experiments indicated that PL could easily penetrate this copolymer (Fernández et al., 2009). For the color and sensory analyses, non-inoculated slices were used. Samples were packaged under vacuum conditions and stored at 4 °C until PL treatment.

2.5. Pulsed light treatment

A desktop Steribeam SBSXeMatic-2L-A device (SteriBeam Systems, Kehl am Rhein, Germany) was used. The apparatus consists on 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. 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 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: 0.7, 2.1, 4.2, 8.4 and 11.9 J/cm^2. Untreated samples were also prepared as control. Ten slices were assayed per batch.

The temperature of the samples was monitored before and during the treatment using a Testo 720 thermocouple (Testo AG, Lenzkirch, Germany). A temperature increase of approximately 2.5 °C per pulse was recorded. Since samples were PL treated at an initial temperature of 4 °C, the maximum temperature recorded after treatment was approximately 40 °C when the highest fluence was used.

2.6. Microbiological analysis

For bacterial enumeration, PL treated slices were aseptically removed from the package and mixed with 20 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. For determining *L. monocytogenes* counts, samples were plated on PALCAM agar supplemented with 2.5% egg yolk emulsion (Oxoid, Unipath, Basingstoke, Hampshire, UK) and incubated at 37 °C for 48 h. For *S.* Typhimurium, samples were seeded on Salmonella–Shigella agar (Oxoid) and incubated at 37 °C for 48 h. Plates were also examined at 72 h. Analyses were performed in duplicate. The detection limit was 0.7 log cfu/cm^2.

2.7. Color measurement

Color was measured immediately after PL treatment and every week during 28 days of storage at 4 °C using a tristimulus colorimeter (ChromaMeter CR-400, Konica Minolta, Osaka, Japan). Results were expressed using the CIELAB color space: lightness (L^*), redness (a^*) and yellowness (b^*). The instrument was calibrated

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