



Inactivation of *Listeria monocytogenes* by pulsed light in packaged and sliced *salpicão*, a ready-to-eat traditional cured smoked meat sausage

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ABSTRACT

The efficacy of pulsed light (PL) for the surface decontamination of a sliced ready-to-eat cured meat product, *salpicão*, was studied. The surface of the slices was inoculated with *Listeria monocytogenes* (10^7 cfu/g), and then PL treatment was applied. Microbial analyses (*L. monocytogenes*, coagulase-negative Staphylococci (CNS), Lactic acid bacteria (LAB) and *Enterobacteriaceae*) were performed immediately after the treatment. pH, a_w , colour and volatile profile were also evaluated. Response Surface experimental Design was applied regarding factors voltage (ranging from 1828 to 3000 V) and distance (ranging from 2.6 to 5 cm) to the light source, and a maximum of 16.11 J/cm^2 energy dose was achieved. A reduction of 1.58 log cfu/g in *L. monocytogenes* count was obtained when a fluence of 5.31 J/cm^2 was applied. *Enterobacteriaceae*, LAB and CNS endogenous microbial populations were not influenced by PL treatment. PL application reduced a^* values and influenced b^* values without impacting the sample ΔE or L^* values. The potential oxidative effect of this technology was evaluated by solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS), and no significant changes were verified after its application in this sliced cured meat product.

1. Introduction

Salpicão is a ready-to-eat (RTE) traditional cured and smoked meat sausage typically produced in the region of *Trás-os-Montes* – Portugal, where the natural conditions and specific climate assure a peculiar curing process and unique flavour (Ferreira et al., 2009). It is produced from pork loin, using salt, red wine, garlic, and chilli pepper as ingredients, being naturally fermented for approximately eight days by endogenous bacteria (Todorov et al., 2013). Afterwards, it is stuffed, smoked, and dried until stabilisation. Similar cured loin products, with recipes dependent on the regions, are produced all over Europe and elsewhere. Due to the potential use of contaminated raw ingredients or secondary microbial contamination in processing plants during production, slicing, packaging, and other post-processing steps, pathogenic bacteria like *Listeria monocytogenes*, are occasionally isolated in cured meat products (Cadavez et al., 2016). This pathogen is responsible for listeriosis, a rare foodborne disease with a 20–30% mortality rate that targets high-risk consumers such as immunocompromised individuals, pregnant women, and the elderly (Jamshidi & Zeinali, 2019). Also, *L. monocytogenes* is of particular interest in RTE packaged cured products

with a long shelf-life like *salpicão*, where its survival and growth can occur during storage and because there is no antilisterial treatment between production and consumption (Jordan & McAuliffe, 2018). Another concern in RTE foods is the limitation of shelf-life by the growth of microbial spoilage microorganisms, leading to unsatisfactory quality products. Also, the trend toward a reduction in the use of salt and no addition of nitrites could compromise the safety of these meat products (Fraqueza et al., 2021). Nonthermal processing techniques to improve RTE safety without impact on sensory characteristics are of major interest. Pulsed light (PL) is a non-thermal technology developed for food preservation and decontamination, which uses an inert gas flash lamp to generate and convert very short (μs), high-power electric pulses into broad-spectrum light (from UV to infrared), where the UV region is critical for the successful inactivation of microorganisms (Hefferon et al., 2019; Ren et al., 2021).

The use of PL technology for the production, processing, and handling of foods up to 12 J/cm^2 has been approved by the FDA since 1996 (FDA, 1996). In European Union, according to Regulation (EU) 2015/2283, the application of PL as an emergent technology is seen as a new process method, and consequently, the safety of food considered to

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be novel due to a new production method will be assessed by the European Food Safety Authority (EFSA) as part of an EU-wide authorization process.

PL is considered a green, nonselective, multitarget process that causes the inactivation of microorganisms by photochemical, photo-thermal and photophysical effects, with the formation of DNA thymine dimers being the main germicidal mechanism (Albert et al., 2021). The effectiveness of PL for decontamination depends on food characteristics, pathogen type, and PL dose applied (Bryant et al., 2021). As far as we know, scarce information has been published to date about PL treatment effects on the volatile compounds profile, or microbiological quality of sliced cured meat products like *salpicão*. This study aimed to verify the effect of PL treatment on the overall microbiological quality, *L. monocytogenes* counts reduction, colour changes, and volatile compounds of a sliced and packaged cured meat product.

2. Material and methods

2.1. Strains and preparation of bacteria suspensions for inoculation

Three *L. monocytogenes* strains were used in this experiment: a reference (CECT 934) and two wild strains belonging to the collection of our laboratory, isolated and identified according to the methods described by Henriques et al. (2014) from RTE meat products (strain A4 isolated from sliced poultry ham and strain A5 isolated from packaged roasted piglet). The strains were maintained at $-80\text{ }^{\circ}\text{C}$ in Brain Heart Infusion (BHI, Condalab, Madrid, Spain) with 15% glycerol added. For each experiment, fresh cultures were prepared by inoculating a loop (10 μl) in 4.5 ml of Tryptic Soy Broth with 0.6% yeast extract (TSBYE; Merck, Darmstadt, Germany), and then incubated at $37\text{ }^{\circ}\text{C}$ for 24 h. Afterwards, the culture was plated on Tryptic Soy Agar (TSA, Condalab, Madrid, Spain) and incubated under the same conditions. One colony was transferred into 10 ml of Tryptic Soy Broth (TSB, Condalab, Madrid, Spain) and incubated for 24 h at $32\text{ }^{\circ}\text{C}$ to give an initial inoculum of 10^9 cfu/ml (stationary growth phase). An initial culture suspension was prepared for each *L. monocytogenes* strain in an isotonic solution of NaCl at 0.9% with an optical density adjusted to an OD_{600} of 0.4–0.5. A mixed bacterial suspension was prepared with an equivalent proportion of the three *L. monocytogenes* strains and then used to inoculate the sliced *salpicão* samples at approximately 7 log cfu/g.

2.2. Samples preparation

The cured and smoked meat product *salpicão* was obtained from a local Portuguese manufacturer who elaborated the product with low addition/content of nitrite (30 mg/kg) and the following ingredients: pork meat (98%), wine, garlic, and spices (2%) and large calibre casings (7 cm). All the products used were kept refrigerated at $2 \pm 2\text{ }^{\circ}\text{C}$ until preparation. The *salpicão* was sliced (3 mm thick) with an electric slicing machine, whose blade and contact surfaces were previously disinfected with 70% ethanol and rinsed with sterile distilled water. The edges of the slices were cut using a sterile stencil to obtain squares of 16 cm^2 with an average of 5 g. Two groups of samples were prepared in a food safety cabinet: one was inoculated with a pool of the three *L. monocytogenes* strains and the other was a control group that was not inoculated. Slices were surface inoculated on one side, using a Digralsky handle, with 50 μl of the inoculum providing a concentration of 7 log cfu/g. Then, samples were left to dry for 15 min under the food safety cabinet flow, and afterwards, they were individually vacuum packaged in a low-strength vacuum pack polyamide and polypropylene (PA/PP 90) bags (O_2 permeability: $<70\text{ cm}^3/\text{m}^2/\text{day}$ and 0% RH; CO_2 permeability: $<350\text{ cm}^3/\text{m}^2/\text{day}$ and 75% RH; N_2 permeability: $<17\text{ cm}^3/\text{m}^2/\text{day}$ and 75% RH; water-vapour permeability: $<4.5\text{ g}/\text{m}^2/\text{day}$ at $23\text{ }^{\circ}\text{C}$ and 85% RH; SISTEMCOC-IN, Sistemas d'Embalage ESTUDI GRAF, S.A., Aiguaviva, Girona, Spain), using a vacuum-packer EV-15-2-CD (Tecnoprop, Terrassa, Spain). For physicochemical and volatile analyses, non-inoculated

samples were used. Samples were stored at $4\text{ }^{\circ}\text{C}$ until PL treatment.

2.3. Experimental design of pulsed light treatment

A central composite rotatable design (CCRD) as a function of electric potential difference (Voltage) and distance to the source of PL lamp (D) with five levels for each factor was used, allowing to fit of first or second-order polynomials to the experimental data points (Rendueles et al., 2011). Twelve trials were performed testing different combinations of voltage (ranging from 1828 to 3000 V) and distances to the PL lamp (ranging from 2.6 to 5.4 cm), as outlined in Table 1. Four factorial points were considered, resulting from combinations of levels coded as (+1) and (−1) for both Voltage and D, plus four-star points coded as $+\sqrt{2}$ and $-\sqrt{2}$ for combinations of Voltage and D, and three centre points, coded as 5, 6 and 7 (Table 1). A group not submitted to PL treatment was used as a control. For each binomial combination, three replications were used. The samples were treated according to the experimental design, with fixed factors representing the number (3 pulses of 300 μs /each) and frequency (1 Hz) of light pulses in a laboratory-scale PL system, using a PL Tecum Unit (Claranor, Manosque, France) with 15% UV-C light (200–280 nm), 50% visible light (280–780 nm) and 35% proximal IR light (780–1100 nm). By relocating the sample rack upwards or downwards, the distance between the sample and the lamp could be changed. The fluence (J/cm^2) received by samples was measured by a laser power detector (Joulmeter UP17P connected to MAESTRO-monitor, Gentec-EO, Canada). A waiting period of at least 60 s between samples was ensured, to avoid flashlamp overheating. A second assay was carried out using the same experimental design with 9 pulses. Untreated samples were analysed as the control group. The analyses of sample colour and microbiological characteristics were performed immediately after PL treatment. For volatile compounds analyses, the samples were stored at $-18\text{ }^{\circ}\text{C}$ until use.

2.4. Microbial analysis

The preparation of samples, initial suspension, and serial dilutions were performed according to (ISO - ISO 6887-1, 2017). Microbial determinations were carried out for *Enterobacteriaceae* counts (Violet Red Bile Dextrose agar, Scharlau Chemie, Spain) after incubation at $37\text{ }^{\circ}\text{C}$ for 24 h (ISO 21528-2:2017); *Listeria monocytogenes* counts (ALOA Agar, Biomérieux, France) incubated at $37\text{ }^{\circ}\text{C}$ for 24 h; lactic acid bacteria (LAB) counts (Man Rogosa and Sharpe Agar, Scharlau Chemie, Spain) after incubation at $30\text{ }^{\circ}\text{C}$ for 48 h in anaerobiosis (ISO 15214:1998); coagulase-negative staphylococci (CNS) (Mannitol Salt Agar, Scharlau Chemie, Spain) after incubation at $37\text{ }^{\circ}\text{C}$ during 48 h according to procedures described by Fraqueza et al. (2019). All counts were expressed as log cfu/g.

Table 1

Experimental data obtained for the optimization of voltage (V) and distance (cm) to light source applied to *salpicão* samples.

No	Voltage/V	Distance/cm
Control	0	0
1	2414	2.6
2	2000	3
3	2828	3
4	1828	4
5	2414	4
6	2414	4
7	2414	4
8	3000	4
9	2000	5
10	2828	5
11	2414	5.4

2.5. Physicochemical analysis

2.5.1. Temperature determination

Before and after PL treatments, and using an IR-Thermometer (Testo, Spain), the temperature of the samples was measured. The average of three determinations was retained for further data analyses. The temperature difference between samples analysed before and after PL treatment (named ΔT) was calculated.

2.5.2. Colour evaluation

Before and after PL treatment, sample colour was measured using a tristimulus colourimeter CR-300 ChromaMeter (KonikaMinolta Inc., Osaka, Japan). The colourimeter was calibrated with a standard white tile, according to manufacturer instructions. Data were collected in CIELAB $L^*a^*b^*$ colour space and the values of L^* (brightness), a^* (red to green colour), and b^* (yellow to blue colour) were recorded during each determination. The colour of *salpicão* was determined in three randomly chosen spots in the slice surface to be treated or already treated with PL. Three values of $L^* a^* b^*$ were obtained per sample, which were averaged to compute the difference between values before and after PL treatment (ΔL^* , Δa^* and Δb^* , respectively). The overall variation in colour (ΔE) was calculated using ΔL^* , Δa^* , and Δb^* values as: $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}$.

2.5.3. Volatile compounds

The volatile compounds in *salpicão* samples were evaluated by solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) adapted from [Serra et al., 2014](#). Briefly, about 5 g of *salpicão* was minced and weighted to a glass vial and closed with an aluminium cap with a PTFE-septum. Samples were then conditioned at 35 °C for 15 min and then, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS) Stable Flex SPME fibre (50/30 µm; 2-cm long) (Supelco, Bellefonte, PA, USA) was exposed to headspace for 30 min at 35 °C. The fibre was inserted into the injector of the Shimadzu GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) set at 250 °C and maintained in the injector for 30 min to complete its desorption. The GC-MS was equipped with an SPB-5 capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness, Supelco Inc., Bellefonte, PA, USA) and the initial oven temperature was set at 40 °C, held for 8 min, and then increased to 220 °C at 4 °C/min and held for 20 min. Helium was used as the carrier gas at a 1 mL/min flow rate. The GC-MS settings were ion source temperature, 220 °C; interface temperature, 220 °C; ionisation energy, 70 eV; scan, 35–500 atomic mass units. Previously to each analysis, the SPME fibre was conditioned for 30 min at 250 °C in the GC injector. The identification of the volatiles was accomplished by comparison with the mass spectra of the NIST/E-PA/NIH Mass Spectral Database (Version 2008), by comparing with commercial standards and using linear retention indexes (LRI) that were calculated using the retention times of a homologous series of n-alkanes C5–C25. Volatile compounds were expressed as a percentage of total volatile compounds in the chromatograms.

2.5.4. Determination of pH and water activity

The pH and water activity (a_w) were measured on the final product *salpicão* immediately after purchasing. *Salpicão* casings were removed, and a homogenised sample was prepared. Then pH values were measured with a pH-meter (Testo 205, Germany) following the procedures described in [ISO 2917:1999](#). The a_w was determined at 22 °C using Aqualab™ equipment (Ferrer Lab, Spain). These determinations were performed also in samples treated with the higher energy dose applied. Three replicates per sample were obtained for both pH and a_w .

2.6. Statistical analysis

The SAS software package, version 9.4 (SAS Institute, Cary, NC, USA)

was used for statistical analyses. The effect of fluence on the microbiological counts and physicochemical properties was assessed with the GLM procedure of SAS, using a polynomial model including the linear, quadratic and cubic effects of the energy dose fluence (J/cm^2). In addition, the GLM procedure was used to perform analyses of variance, by comparing the results obtained with treated vs. untreated samples, and then the results from the various treatment combinations with each other. The mean \pm standard error (SE) of microbiological counts and physicochemical parameters for the various combinations of V and D were obtained, and differences between treatments were tested using Tukey's post hoc test.

The fluence measured was analysed by multiple regression, with the goal of obtaining a prediction model that can be used to infer fluence in future experiments performed with the same type of equipment. The predictor variables included in the model were voltage, distance, pulse and the two-way interactions between these factors.

3. Results and discussion

3.1. Pulsed light energy dose on samples

The various combinations of electric potential difference (ranging from 1828 to 3000 V) and distance to the source UV lamp (ranging from 2.6 to 5 cm) originated different radiation energy received by samples, corresponding to the energy dose applied named fluence (J/cm^2). This means that the energy dose (fluence J/cm^2) given to the *salpicão* samples ranged from 1.74 to 16.11 J/cm^2 ([Table 2](#)). Regarding the effect on fluence of the voltage applied and the distance of the light source, it was observed that higher voltage and lower distances increase fluence values by 16.11 J/cm^2 , assuming a fixed number and frequency of pulses ([Table 2](#)). Also, [Hsu and Moraru \(2011\)](#) stated that fluence decays exponentially with increasing vertical distance from the lamp. When considering the pulse number, the effect was very pronounced, resulting in a significant increase in the energy dose ([Table 2](#)). The prediction model obtained to infer fluence from combinations of voltage, distance and pulse resulted in an $r^2 = 0.978$, and the corresponding regression coefficients are shown in [Supplementary Table 1](#).

Given the broad-spectrum energy provided by the lamp during the PL treatment, the surface temperature of the samples was measured before and immediately after treatment, to observe the influence of energy on sample temperature, and the difference between measurements was expressed as ΔT and is presented in [Fig. 1](#). The initial surface temperature on the cured meat product slices was 2.98 ± 1.44 °C. As expected, the smallest variation (2.1 °C) was observed when the minimum fluence of 2 J/cm^2 was applied and the largest (19.4 °C) was obtained for the maximum fluence of 16.11 J/cm^2 . Thus, based on the energy dose received by samples, a significant ($P < 0.05$) increase in their surface temperature from 2.1 to 19.4 °C was observed, with greater ΔT obtained when higher fluences were applied, with a regression equation as follows: $\Delta T = 0.37 + 1.31x(\text{Fluence}) - 0.01x(\text{Fluence})^2$ ([Fig. 1](#)). Also, sample temperature was found to increase with an increase in the number of pulses and with a decrease in the sample distance from the lamp as fluence increased ([Table 2](#)). [Cassar et al. \(2021\)](#) stated that PL treatment significantly increases the surface temperature of samples, and this effect could be minimised if open PL equipment is used. The pilot equipment used in this experiment was closed and, on an industrial scale, the use of open equipment will be a good option to reduce the observed effect of temperature increase in the samples treated. According to [Bhavaya and Umesh Hebbar \(2017\)](#), a significant temperature increase caused by longer PL treatments have an extra effect on microbial reductions, depending on the matrix properties. Nevertheless, the increased temperature observed in this study was not expected to be sufficient to add this extra effect as described in the work condition of [Bialka & Demirci, 2008](#).

Table 2
Mean \pm SE for microbial and colour parameters of *salpicão* samples treated with PL.

Treatment	Voltage (V)	Distance (cm)	Pulses (no)	Fluence (J/cm ²)	L. monocytogenes Log cfu/g	LAB Log cfu/g	CNS Log cfu/g	Enterobacteriaceae Log cfu/g	L*	a*	b*	ΔE^*ab	ΔT (°C)
1	0	0	0	0.00 \pm 0.00m	7.04 \pm 0.11a	7.34 \pm 0.33abcde	2.57 \pm 0.58abcd	0.17 \pm 0.27b	52.35 \pm 2.36 ab	13.22 \pm 0.76a	11.63 \pm 0.54bcdefg		
2	2000	3	3	2.65 \pm 0.23jk	6.15 \pm 0.16bcd	7.42 \pm 0.47abcde	4.12 \pm 0.82a	0.00 \pm 0.38b*	57.10 \pm 2.36a	5.27 \pm 0.76 fg	11.14 \pm 0.54defg	0.00 \pm 0.86c	3.40 \pm 0.92hi
3	2000	3	9	6.81 \pm 0.23f	5.72 \pm 0.16def	8.42 \pm 0.47a	2.25 \pm 0.82abcd	0.89 \pm 0.38 ab	52.24 \pm 2.36 ab	7.97 \pm 0.76cde	11.24 \pm 0.54cdefg	0.86b	8.87 \pm 0.92ef
4	2000	5	3	1.74 \pm 0.23l	6.32 \pm 0.16b	6.31 \pm 0.47ef	3.68 \pm 0.82 ab	0.00 \pm 0.38b*	56.99 \pm 2.36a	4.56 \pm 0.76 fg	10.90 \pm 0.54g	2.34 \pm 0.86bc	4.93 \pm 0.92gh
5	2000	5	9	5.49 \pm 0.23g	5.88 \pm 0.16cdef	8.16 \pm 0.47abc	2.45 \pm 0.82abcd	0.00 \pm 0.38b*	51.43 \pm 2.36 ab	11.40 \pm 0.76 ab	12.59 \pm 0.54abcde	2.76 \pm 0.86b	9.20 \pm 0.92e
6	2828.50	3	3	5.31 \pm 0.23g	5.61 \pm 0.16ef	7.52 \pm 0.47abcde	1.26 \pm 0.82cd	0.00 \pm 0.38b*	55.16 \pm 2.36 ab	4.38 \pm 0.76 fg	11.02 \pm 0.54 fg	2.73 \pm 0.86b	6.37 \pm 0.92 fg
7	2828.50	3	9	16.11 \pm 0.23a	5.83 \pm 0.16cdef	8.25 \pm 0.47 ab	2.67 \pm 0.82abcd	0.00 \pm 0.38b*	53.07 \pm 2.36 ab	9.29 \pm 0.76bcd	12.67 \pm 0.54abcd	3.45 \pm 0.86b	19.37 \pm 0.92a
8	2828.50	5	3	2.94 \pm 0.23j	5.87 \pm 0.16cdef	6.98 \pm 0.47bcde	4.15 \pm 0.82a	0.00 \pm 0.38b*	55.37 \pm 2.36 ab	4.72 \pm 0.76 fg	11.06 \pm 0.54efg	3.41 \pm 0.86b	3.90 \pm 0.92ghi
9	2828.50	5	9	10.85 \pm 0.23c	5.82 \pm 0.16cdef	7.78 \pm 0.47abcd	1.76 \pm 0.82bcd	0.68 \pm 0.38 ab	53.50 \pm 2.36 ab	10.53 \pm 0.76b	12.86 \pm 0.54 ab	3.12 \pm 0.86b	13.90 \pm 0.92dc
10	2414.25	4	3	3.55 \pm 0.13i	6.00 \pm 0.09bcde	5.82 \pm 0.27f	3.79 \pm 0.48 ab	0.00 \pm 0.22b*	55.32 \pm 1.36 ab	4.35 \pm 0.44g	10.89 \pm 0.32g	2.34 \pm 0.86bc	4.58 \pm 0.53gh
11	2414.25	4	9	9.82 \pm 0.13d	5.69 \pm 0.09ef	8.18 \pm 0.27 ab	2.80 \pm 0.48abcd	0.47 \pm 0.22b	51.02 \pm 1.36b	10.88 \pm 0.44b	12.64 \pm 0.32abcd	5.68 \pm 0.50a	12.41 \pm 0.53d
12	2414.25	2.59	3	4.53 \pm 0.23h	5.71 \pm 0.16ef	7.61 \pm 0.47abcde	1.00 \pm 0.82d	0.00 \pm 0.38b*	51.89 \pm 2.36 ab	6.45 \pm 0.76fef	11.74 \pm 0.54bcdefg	3.47 \pm 0.50b	5.30 \pm 0.92gh
13	2414.25	2.59	9	13.21 \pm 0.23b	5.80 \pm 0.16cdef	8.01 \pm 0.47abcd	1.87 \pm 0.82abcd	1.35 \pm 0.38a	51.73 \pm 2.36 ab	7.68 \pm 0.76de	12.54 \pm 0.54bcdef	3.74 \pm 0.86 ab	16.10 \pm 0.92bc
14	2414.25	5.41	3	2.19 \pm 0.23 kl	6.02 \pm 0.16bcde	6.72 \pm 0.73def	3.55 \pm 0.82abc	0.00 \pm 0.38b*	54.38 \pm 2.36 ab	5.31 \pm 0.76 fg	10.69 \pm 0.54g	3.86 \pm 0.86 ab	2.73 \pm 0.92hi
15	2414.25	5.41	9	7.68 \pm 0.23e	5.79 \pm 0.16cdef	7.32 \pm 0.47abcde	2.49 \pm 0.82abcd	0.57 \pm 0.38 ab	52.20 \pm 2.36 ab	10.06 \pm 0.76bc	13.60 \pm 0.54a	3.81 \pm 0.86 ab	8.50 \pm 0.92ef
16	1828.41	4	3	2.00 \pm 0.23 kl	5.90 \pm 0.16cdef	6.88 \pm 0.47cdef	3.83 \pm 0.82 ab	0.00 \pm 0.38b*	54.80 \pm 2.36 ab	5.17 \pm 0.76 fg	10.43 \pm 0.54g	3.38 \pm 0.86b	2.07 \pm 0.92i
17	1828.41	4	9	4.99 \pm 0.23gh	5.98 \pm 0.16bcde	8.06 \pm 0.47abcd	2.58 \pm 0.82abcd	0.49 \pm 0.38 ab	53.19 \pm 2.36 ab	11.32 \pm 0.76 ab	12.77 \pm 0.54abc	3.20 \pm 0.86b	8.50 \pm 0.92ef
18	3000.09	4	3	5.37 \pm 0.23g	6.17 \pm 0.16bc	7.08 \pm 0.47abcde	2.53 \pm 0.82abcd	0.00 \pm 0.38b*	54.11 \pm 2.36 ab	4.42 \pm 0.76 fg	11.35 \pm 0.54bcdefg	4.23 \pm 0.86 ab	5.30 \pm 0.92gh
19	3000.09	4	9	15.87 \pm 0.23a	5.47 \pm 0.16f	7.51 \pm 0.47abcde	2.67 \pm 0.82abcd	0.72 \pm 0.38 ab	53.78 \pm 2.36 ab	9.51 \pm 0.76bcd	13.52 \pm 0.54a	3.84 \pm 0.86 ab	17.63 \pm 0.92 ab

^{abc} within columns means with the same letter are not significantly different. Sig. NS = not significant, * = $P < 0.05$. *Where is "0" refers to "not quantified at 10^{-1} "

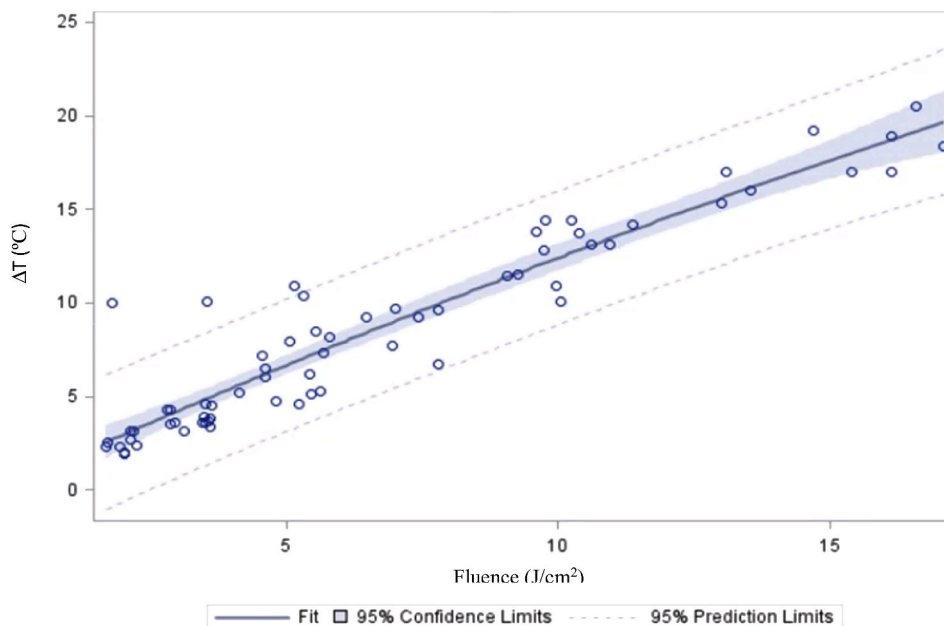


Fig. 1. Effect of the PL energy dose on *salpicão* samples ΔT ($^{\circ}C$).

3.2. Inactivation of *Listeria monocytogenes* by pulsed light treatment

Samples of the packaged sliced cured and smoked meat product, inoculated with *L. monocytogenes*, were microbiologically analysed before and after PL treatments, and the reduction of this pathogen was assessed, and the corresponding results are in Table 2. To mitigate the influence of protective shading effects on *L. monocytogenes*, the target of initial microbial population density was fixed at ≤ 7 log cfu/g, thereby the initial counts of this pathogen in sliced meat product samples were 7.04 ± 0.13 log cfu/g. After PL treatment, a significant reduction of *L. monocytogenes* counts was observed ($P < 0.05$). The minimum and maximum fluences applied (1.74 and 16.1 J/cm²) achieved reductions of *L. monocytogenes* counts of 0.72 and 1.21 log cfu/g, respectively.

Nevertheless, the highest significant reduction of counts found (1.58 log cfu/g) was achieved in samples treated with a fluence of 5.31 J/cm², obtained with a 2828.50 V at 3 cm of distance from the light source and 3 pulses of 300 μs and 1 Hz of frequency. Indeed, as shown by polynomial model adapt to data in Fig. 2, for fluences below 5 J/cm² the reduction of *L. monocytogenes* obtained was dose-dependent and the relationship seemed to be linear, while for fluences above 5 J/cm² a tailing effect in the reduction of *L. monocytogenes* counts was observed. Uesugi et al. (2007) also observed a tailing effect line for *L. innocua*, as a characteristic of this microorganism in response to PL treatment when applied in transparent liquids. In the present matrix under study, characterised by opaque, rough, and rose-coloured surfaces contaminated with a high inoculum, a similar behaviour was observed for

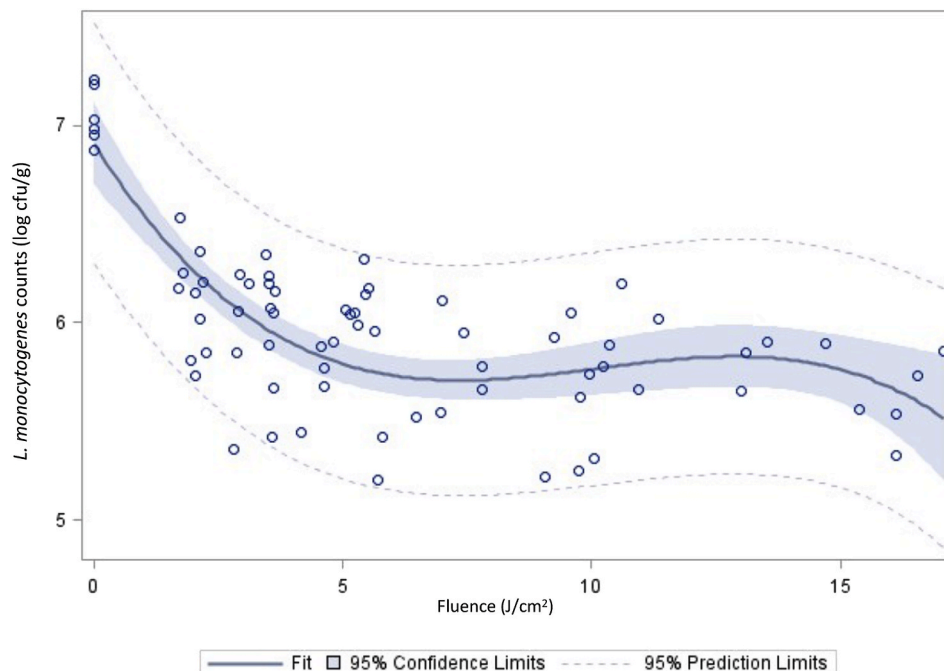


Fig. 2. Effect of fluence on *Listeria monocytogenes* counts (log cfu/g), polynomial model obtained.

L. monocytogenes. Relatively low reductions of *L. monocytogenes* were also obtained by Ganan et al. (2013), who described a reduction of 0.89 and 1.81 log cfu/cm² for *salchichon* and dry-cured loin, respectively, when 11.9 J/cm² of fluence was applied. Also, Kramer et al. (2019) found a 1.14 log cfu/cm² *L. monocytogenes* reduction in boiled ham samples with fluences of 3.6 J/cm². Similar or even lower reductions were described by Proulx et al. (2015) in their work with different food matrices such as smooth surfaces of cheese. In fact, the reduction of 3 log cfu/slice obtained by Proulx et al. (2015) was less than the reduction obtained in the present study, which using the same method of expression of results corresponds to 7.9 log cfu/slice of *salpicão*. Light penetration in solid foods is superficial and surface irregularities, where microorganisms can hide, thus limiting their exposure to PL (Fernández et al., 2020; Hilton et al., 2017). Accordingly, Mandal et al. (2020) also stated that PL cannot be used to sterilise food products due to their non-uniform surfaces and opacity, except to reduce their microbial load. Moreover, microorganisms can use protective mechanisms against UV light. *L. monocytogenes* is a Gram-positive bacteria with a thick cell peptidoglycan wall layer that could shield the DNA located in the cell's nucleus from PL lethal light discharges (Proulx et al., 2015); Gram-positive are therefore more resistant to the effects of UV light than Gram-negative bacteria.

The final product *salpicão* used in this study was a low nitrite (30 mg/kg) cured meat product with a pH of 5.06 (± 0.04) and an a_w of 0.96 (± 0.004), thus with physicochemical parameters allowing potential growth of *L. monocytogenes* as considered in Regulation (EU) 2073/2005. No changes in pH were observed, even when the highest dose energy of 16.1 J/cm² was applied. The pH value of the *salpicão* products used in this study was slightly lower than those published by Ferreira et al. (2009), Campelos, Silva, Gibbs, and Teixeira (2016a) and Patarata et al. (2022), who reported for the same product, pH values ranging from 5.3 to 5.6 and an a_w ranging from 0.87 to 0.94. The differences encountered in the values of pH and a_w of the *salpicão* products used in the different studies reflect differences in curing times and environment processing conditions in this traditional cured and smoked Portuguese product. The dry curing and smoking process, together with the growth of LAB producing lactic acid, reduce a_w and pH (Bis-Souza et al., 2019; Leroy et al., 2022). The combination of all these technological factors enters within the “hurdle concept” and contributes to the microbial

stability of cured sausages like *salpicão* (Leroy et al., 2022). In fact, the proper pH/ a_w binomial to avoid *L. monocytogenes* is frequently not achieved in the practice of the meat industry (Patarata et al., 2022) and this meat cured sausage is prone to *L. monocytogenes* development if contaminated, since it fails to achieve the binomial requirements of pH ≤ 5.0 and $a_w \leq 0.94$, as stated in EC Regulation 2073/2005. If initial contamination occurs, both from ingredients or due to slicing procedures (Henriques et al., 2014), a limit of 2.0 log cfu/g is required by current EU legislation for RTE products (Regulation 2073/2005) until the end of the shelf-life, in order to minimize any potential hazards for public health. Hence, the use of PL treatments as performed in this study, allowing a maximum of 1.58 log reduction of *L. monocytogenes* counts, may facilitate the accomplishment of this criterion.

3.3. Effect of pulsed light treatment on *salpicão* microbial quality

High counts of LAB and CNS are often present in traditional cured sausages (Laranjo et al., 2015). In our study, *Salpicão* samples presented counts of 7.34 ± 0.33 , 2.57 ± 0.58 and 0.17 ± 0.27 log cfu/g for endogenous populations/communities of LAB, CNS and *Enterobacteriaceae*, respectively (Table 2). LAB counts in final product *salpicão* were high (7.34 ± 0.33 log cfu/g) and in accordance with other authors who obtained similar results (Silva et al., 2020; Cadavez et al., 2016; Fraqueza et al., 2020). The statistical model indicated that PL treatment had a significant effect on LAB counts ($P < 0.05$); however, only a slight reduction in LAB counts was observed (± 1.2 log cfu/g) for low energy doses but not for higher doses (Fig. 3). CNS counts of final products were 2.57 ± 0.58 log cfu/g and were not affected by the PL treatment. Therefore, this microbial group was resistant to PL treatment in the range of energy doses under study. Due to their psychrotrophic nature and excellent adaptation to cured sausages, LAB and CNS dominate the product's microbiota and contribute to avoiding the multiplication of unwanted microorganisms, pathogens, or those responsible for spoilage, mainly by the production of acid lactic and other antimicrobials by LAB (Fraqueza, 2015). They also contribute to colour stability and maintenance of sensorial characteristics, mainly by CNS proteolytic and lipolytic and nitrate-reductase activity, being implicated in the catabolism of free amino acids, and inhibiting the oxidation of unsaturated free fatty acids (Alfaia et al., 2018; Talon et al., 2000). However, if the counts of

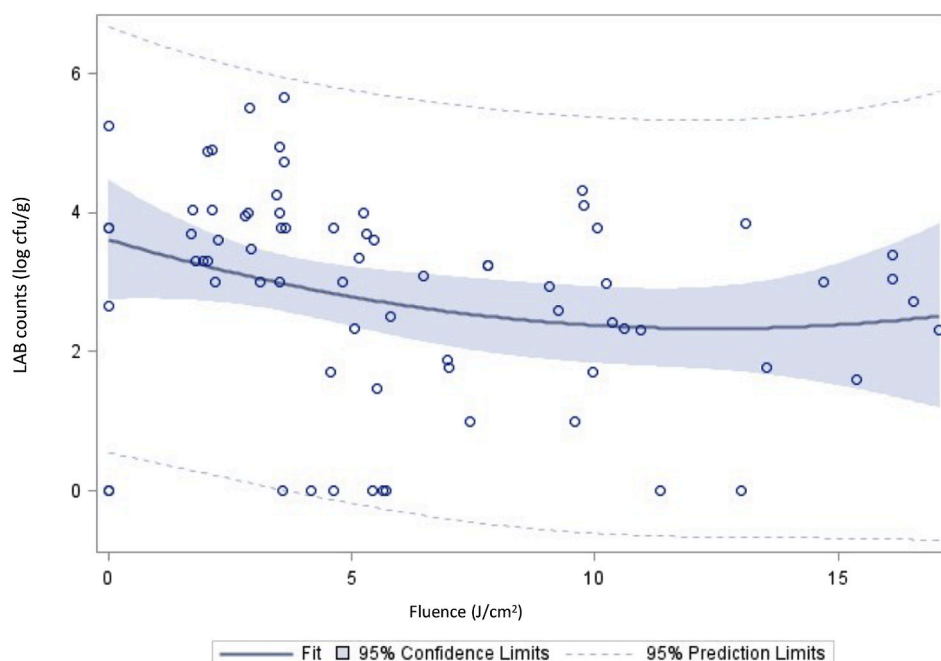


Fig. 3. Effect of fluence on LAB counts (log cfu/g), polynomial model obtained.

these bacterial groups change, this could have implications for the sausage's shelf-life (Flores, 2018; Laranjo et al., 2015). Thus, the PL treatment is preserving this important microbiota and thus *salpicão* properties and shelf life are maintained after treatment.

The effect of PL treatment on *Enterobacteriaceae* was also evaluated. The initial counts of *Enterobacteriaceae* in *salpicão* samples were very low ($0.17 \pm 0.27 \log \text{ cfu/g}$), which may indicate low contamination of raw materials and careful observation of the hygiene rules during the manufacturing process. *Enterobacteriaceae* in cured sausages may come from raw materials, spices, and cross-contamination from the environment and workers (Henriques et al., 2014; Talon et al., 2007). As the counts of *Enterobacteriaceae* were low for all the samples analysed, it was not possible to demonstrate a PL's real effect in this group of bacteria. Nevertheless, it is known that smoking and ripening processes develop the hurdles of acidification, dehydration, competition with LAB, and smoke compounds (like short-chain fatty acids and aldehydes) that, in addition to the presence of salt, were sufficient to control *Enterobacteriaceae* growth in cured products like *salpicão* (Cadavez et al., 2016; Patarata et al., 2022).

3.4. Effect of pulsed light treatment on *salpicão* colour

The effect of PL treatment on meat colour should be assessed since it can promote colour changes due to oxidation, which depreciates the product by consumers. The L^* , a^* and b^* values of *salpicão* samples were measured before and immediately after treatment, ΔE was calculated, and the results are shown in Table 2. The use of PL significantly decreased ($P < 0.05$) redness (a^* values), independently of the energy dose applied and also influenced yellowness (b^* values) in sliced meat product samples. However, no changes were observed for ΔE nor for lightness (L^*) between treated and untreated samples. Maximum L^* values were observed in PL treatments where lower energy doses were applied and where lower ΔT were observed. Overall, a decrease in a^* values was observed when PL treatment was applied, and there was a tendency to register a higher decrease of a^* values where less energy was applied and, therefore, a lower ΔT resulted. Lower b^* values were obtained for treatments with lower fluences ($1\text{--}4 \text{ J/cm}^2$) and higher b^* values when extreme treatments ($13\text{--}16 \text{ J/cm}^2$) with ΔT above 8°C were applied. These results agree with those reported in the literature, indicating that more extreme PL treatments negatively affect the colour of cured meat products, where discolouration occurs as a reduction in a^* values and a rise in b^* values, with or without a change in L^* (Cacace & Palmieri, 2014; Ganan et al. (2013)). In this study, the same behaviour was observed for *salpicão* samples, with a decrease in a^* and a rise in b^* values when extreme treatments were applied ($13\text{--}16 \text{ J/cm}^2$). PL potentially affects food products due to free radical generation, via a wide variety of organic photochemical reactions, being able, among other effects, to change product colour, including brown discolouration, as it encourages metmyoglobin formation in meat (Lázaro et al., 2014). Other authors hypothesised that changes in the colour of meat tissue are possibly due to local temperature increases, due to exposure to PL or oxidation of lipids, leading to modified sensory attributes of meat (Guyon et al., 2016). Nevertheless, in this study, the temperatures never raised above the ambient temperature.

3.5. Effect of pulsed UV light treatment in volatile compounds profile

PL is a broad-spectrum light composed of UV-C, and its application can be associated with an increase in sample temperature, thus promoting lipid oxidation. The volatile compounds of the cured sausage *salpicão* were analysed in both treated and non-treated samples. As far as is known, this is the first time that the volatile compounds profile of *salpicão* is described. A total of 56 compounds were identified: 2 acids, 5 alcohols, 2 aldehydes, 15 aromatics compounds, 15 esters, 4 furans, 4 hydrocarbons, 7 ketones, and 2 other compounds (Table 3). An attempt to perceive the origin of the volatile compounds was made, to

Table 3

Volatil compounds of PL treated *salpicão* samples (% of total volatile compounds in the chromatograms).

^{a-c} Means without a common superscript are significantly different ($P < 0.05$)

¹the position of the methoxy group may vary;

²the position of the methyl group may vary.

Volage (V)	2000	2828.5	2414.25	1828.41	
Distance (cm)	3	3	2.59	4	
Fluence	6.81	16.11	13.21	4.99	
Treatment	Control (1)	A (2)	B (3)	C (4)	D (5)
Mean \pm SDE					
Acids					
n-Hexadecanoic acid	0.13 \pm 0.02a	0.07 \pm 0.02b	0.10 \pm 0.02 ab	0.11 \pm 0.02 ab	0.08 \pm 0.02 ab
Octadecanoic acid	0.06 \pm 0.01	0.04 \pm 0.01	0.04 \pm 0.01	0.06 \pm 0.01	0.04 \pm 0.01
Total acids	0.19 \pm 0.03	0.11 \pm 0.03	0.14 \pm 0.03	0.17 \pm 0.03	0.12 \pm 0.03
Alcohols					
1-Butanol, 3-methyl-	26.80 \pm 3.02	24.09 \pm 3.02	29.74 \pm 3.02	25.76 \pm 3.02	25.45 \pm 3.02
1-Butanol, 3-methyl-, acetate	2.95 \pm 0.58	2.50 \pm 0.58	3.08 \pm 0.58	2.55 \pm 0.58	2.67 \pm 0.58
Benzyl Alcohol	1.48 \pm 0.22	1.84 \pm 0.22	1.42 \pm 0.22	1.83 \pm 0.22	1.60 \pm 0.22
1-Decanol, 2-methyl-	0.36 \pm 0.08 ab	0.49 \pm 0.08a	0.25 \pm 0.08b	0.28 \pm 0.08 ab	0.41 \pm 0.08 ab
1-Hexadecanol, 2-methyl-	0.27 \pm 0.17	0.16 \pm 0.17	0.15 \pm 0.17	0.03 \pm 0.17	0.22 \pm 0.17
Total alcohols	31.85 \pm 3.13	29.08 \pm 3.13	34.64 \pm 3.13	30.45 \pm 3.13	30.36 \pm 3.13
Aldehydes					
Benzaldehyde	0.69 \pm 0.14	0.63 \pm 0.14	0.64 \pm 0.14	0.68 \pm 0.14	0.69 \pm 0.14
Benzeneacetaldehyde	0.71 \pm 0.24	1.14 \pm 0.24	0.87 \pm 0.24	1.13 \pm 0.24	1.20 \pm 0.24
Total aldehydes	1.40 \pm 0.26	1.77 \pm 0.26	1.51 \pm 0.26	1.81 \pm 0.26	1.89 \pm 0.26
Esters					
Propanoic acid, ethyl ester	3.05 \pm 0.32	2.25 \pm 0.32	3.07 \pm 0.32	3.06 \pm 0.32	3.09 \pm 0.32
Butanoic acid, ethyl ester	6.60 \pm 1.17	6.42 \pm 1.17	5.88 \pm 1.17	5.75 \pm 1.17	6.12 \pm 1.17
Lactic acid, ethyl esters	8.26 \pm 1.12	6.42 \pm 1.12	7.99 \pm 1.12	7.83 \pm 1.12	7.15 \pm 1.12
Butanoic acid, 2-methyl-, ethyl ester	0.44 \pm 0.12	0.69 \pm 0.12	0.33 \pm 0.12	0.47 \pm 0.12	0.39 \pm 0.12
Pentanoic acid, ethyl ester	0.41 \pm 0.05	0.38 \pm 0.05	0.40 \pm 0.05	0.36 \pm 0.05	0.40 \pm 0.05
2-Butenoic acid, 2-methyl-, ethyl ester ²	0.42 \pm 0.05	0.41 \pm 0.05	0.44 \pm 0.05	0.34 \pm 0.05	0.39 \pm 0.05
2-Butenoic acid, 2-methyl-, propyl ester, (E)-	0.20 \pm 0.02	0.16 \pm 0.02	0.16 \pm 0.02	0.19 \pm 0.02	0.17 \pm 0.02
Iso-Hexanoic acid, ethyl ester	1.43 \pm 0.09	1.22 \pm 0.09	1.31 \pm 0.09	1.42 \pm 0.09	1.43 \pm 0.09
Hexanoic acid, ethyl ester	2.72 \pm 0.18	2.95 \pm 0.18	2.70 \pm 0.18	2.78 \pm 0.18	2.76 \pm 0.18
Butanedioic acid, diethyl ester	0.15 \pm 0.02	0.18 \pm 0.02	0.18 \pm 0.02	0.19 \pm 0.02	0.18 \pm 0.02
Octanoic acid, ethyl ester	0.81 \pm 0.16	1.02 \pm 0.16	0.78 \pm 0.16	0.94 \pm 0.16	0.89 \pm 0.16
Decanoic acid, ethyl ester	0.04 \pm 0.02	0.07 \pm 0.02	0.06 \pm 0.02	0.06 \pm 0.02	0.06 \pm 0.02
Butyric acid, ester with citronellol	0.02 \pm 0.03	0.03 \pm 0.03	0.07 \pm 0.03	0.07 \pm 0.03	0.05 \pm 0.03
Dodecanoic acid, propyl ester	0.64 \pm 0.05	0.53 \pm 0.05	0.50 \pm 0.05	0.50 \pm 0.05	0.51 \pm 0.05
Hexadecanoic acid, ethyl ester	0.05 \pm 0.01	0.06 \pm 0.01	0.05 \pm 0.01	0.05 \pm 0.01	0.05 \pm 0.01
Total esters	25.25 \pm 2.38	22.79 \pm 2.38	23.91 \pm 2.38	23.99 \pm 2.38	23.64 \pm 2.38

(continued on next page)

Table 3 (continued)

Voltage (V)		2000	2828.5	2414.25	1828.41
Distance (cm)		3	3	2.59	4
Fluence		6.81	16.11	13.21	4.99
Treatment	Control (1)	A (2)	B (3)	C (4)	D (5)
Aromatics compounds					
Benzene, 1,2-dimethoxy-	0.13 ± 0.04	0.18 ± 0.04	0.20 ± 0.04	0.19 ± 0.04	0.15 ± 0.04
Benzene, 1,2,3-trimethoxy-5-methyl-3,4-	1.58 ± 0.55	1.57 ± 0.55	0.82 ± 0.55	0.01 ± 0.55	0.87 ± 0.55
Dimethoxytoluene ¹	0.01 ± 0.01	± 0.01	0.01	0.01	0.01
1,2,4-Trimethoxybenzene	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	0.06 ± 0.02
Phenol	4.06 ± 0.36	4.73 ± 0.36	3.92 ± 0.36	4.53 ± 0.36	4.39 ± 0.36
Phenol, 3-methyl-	2.52 ± 0.26	3.06 ± 0.26	2.39 ± 0.26	2.88 ± 0.26	2.79 ± 0.26
Phenol, 2-methoxy-	5.68 ± 0.55	6.30 ± 0.55	5.76 ± 0.55	6.51 ± 0.55	6.07 ± 0.55
Phenol, 2,3-dimethyl ²	0.41 ± 0.09	0.56 ± 0.09	0.32 ± 0.09	0.52 ± 0.09	0.46 ± 0.09
Phenol, 2,6-dimethyl ²	0.48 ± 0.08	0.60 ± 0.08	0.41 ± 0.08	0.57 ± 0.08	0.53 ± 0.08
2-Methoxy-5-methylphenol	0.36 ± 0.06	0.41 ± 0.06	0.30 ± 0.06	0.45 ± 0.06	0.39 ± 0.06
2-Methoxy-5-methylphenol	0.09 ± 0.02	0.10 ± 0.02	0.12 ± 0.02	0.13 ± 0.02	0.10 ± 0.02
Phenol, 2-methoxy-4-methyl-	2.40 ± 0.34	2.95 ± 0.34	2.34 ± 0.34	3.00 ± 0.34	2.64 ± 0.34
Phenol, 2,3,5-trimethyl-	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Phenol, 4-ethyl-2-methoxy-	1.08 ± 0.19	1.43 ± 0.19	1.03 ± 0.19	1.30 ± 0.19	1.21 ± 0.19
Phenol, 2,6-dimethoxy-	0.54 ± 0.15	0.73 ± 0.15	0.83 ± 0.15	0.60 ± 0.15	0.50 ± 0.15
Total aromatics	19.50 ± 2.48	22.80 ± 2.48	18.56 ± 2.48	20.85 ± 2.48	20.28 ± 2.48
Furans					
2-Furanmethanol	6.43 ± 0.75	5.24 ± 0.75	5.59 ± 0.75	6.10 ± 0.75	6.30 ± 0.75
Butyrolactone	4.55 ± 0.14	4.75 ± 0.14	4.69 ± 0.14	4.32 ± 0.14	4.58 ± 0.14
2(3H)-Furanone, dihydro-5-methyl-	0.33 ± 0.04	0.20 ± 0.04	0.24 ± 0.04	0.22 ± 0.04	0.23 ± 0.04
non-identified furan	0.48 ± 0.03	0.48 ± 0.03	0.50 ± 0.03	0.53 ± 0.03	0.48 ± 0.03
Total furans	11.79 ± 0.75	10.68 ± 0.75	11.03 ± 0.75	11.18 ± 0.75	11.59 ± 0.75
Hydrocarbons					
Decane	0.89 ± 0.15	1.05 ± 0.15	0.77 ± 0.15	0.93 ± 0.15	1.13 ± 0.15
Decane, 4-methyl-	1.24 ± 0.16	1.47 ± 0.16	1.11 ± 0.16	1.25 ± 0.16	1.46 ± 0.16
Pentadecane	0.02 ± 0.01b	0.04 ± 0.01	0.01 ± 0.01b	0.05 ± 0.01a	0.01 ± 0.01b
1-Methylcycloheptene	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Total hydrocarbons	1.33 ± 0.16	1.59 ± 0.16	1.21 ± 0.16	1.38 ± 0.16	1.55 ± 0.16
Ketones					
2-Cyclopenten-1-one, 2-methyl ²	1.71 ± 0.09	1.78 ± 0.09	1.77 ± 0.09	1.72 ± 0.09	1.66 ± 0.09
2-Cyclopenten-1-one, 3,4-dimethyl-	0.36 ± 0.10	0.49 ± 0.10	0.47 ± 0.10	0.46 ± 0.10	0.48 ± 0.10
2-Cyclopenten-1-one, -dimethyl ²	0.53 ± 0.06	0.62 ± 0.06	0.55 ± 0.06	0.60 ± 0.06	0.57 ± 0.06
2-Cyclopenten-1-one, -dimethyl ²	0.61 ± 0.06	0.58 ± 0.06	0.55 ± 0.06	0.60 ± 0.06	0.59 ± 0.06
1,2-Cyclopentanedione, 3-methyl-	1.08 ± 0.13	1.21 ± 0.13	1.33 ± 0.13	1.18 ± 0.13	1.09 ± 0.13

Table 3 (continued)

Voltage (V)		2000	2828.5	2414.25	1828.41
Distance (cm)		3	3	2.59	4
Fluence		6.81	16.11	13.21	4.99
Treatment	Control (1)	A (2)	B (3)	C (4)	D (5)
2-Cyclopenten-1-one, 3-ethyl-	0.31 ± 0.06	0.38 ± 0.06	0.33 ± 0.06	0.40 ± 0.06	0.44 ± 0.06
Cyclohexanone, 3-ethenyl-	0.28 ± 0.06	0.27 ± 0.06	0.20 ± 0.06	0.29 ± 0.06	0.28 ± 0.06
Total ketones	4.88 ± 0.39	5.33 ± 0.39	5.20 ± 0.39	5.25 ± 0.39	5.11 ± 0.39
Others					
1-Propene, 3,3'-thiobis-	2.15 ± 0.93	3.89 ± 0.93	2.45 ± 0.93	3.35 ± 0.93	3.45 ± 0.93
1-Decene, 2,4-dimethyl-	0.76 ± 0.14	0.89 ± 0.14	0.59 ± 0.14	0.65 ± 0.14	0.87 ± 0.14

understand how generation pathways were affected by the meat production process, namely the fermentation and smoking steps. However, some of the compounds enumerated may have more than one origin, and the interpretation must be cautious.

Alcohols were detected as the most abundant group, representing 29.39–32.14% of the volatile profile total area. Five compounds were detected in this group: 3-methyl-1-butanol, acetate 3-methyl-1-butanol, benzyl alcohol, 2-methyl-1-decanol, and 2-methyl-1-hexadecanol, contributing, respectively, with approximately 84, 9, 5, 1, and 1% for total alcohols area. From these, only 2-methyl-1-decanol differ among PL treated samples, but its abundance in *Salpicão* was relatively low. In cured sausages, lipid oxidation, carbohydrate metabolism, and amino acid catabolism could be the most important pathways accounting to produce volatile alcohols (Kaban, 2010). However, ingredients present in *salpicão*, such as wine, can also be considered a source of these compounds and may have an impact on product flavour (Ilc et al., 2016). The meat product *salpicão* used in this study has a pH of 5.06 (±0.04), a slightly acid product, providing better conditions for staphylococci survival and thus 3-methyl-1-butanol production, which was the highest volatile compound detected in *Salpicão* samples (Górska et al., 2017). This compound contributes to sausage flavours and can be originated either from the leucine catabolism of staphylococci, such as *S. xylosum* and *S. carnosus*, commonly present in dry fermented sausage or by lactic acid bacteria, such as *Lactobacillus sakei*, *L. plantarum*, *L. curvatus*, *L. casei* and *L. rhamnosus*, (Ravyts et al., 2009). Like aldehydes and ketones, the presence of alcohol compounds is considered a marker of secondary oxidation of unsaturated fatty acids that may impart off-flavours, limit shelf-life, and shorter storage stability in some products and their levels should therefore be controlled (Clarke et al., 2020; Karwowska et al., 2021).

In *salpicão*, esters were the second most abundant group of volatiles and represented between 23.04 and 25.47% of the total volatile compounds in the chromatogram, being mostly ethyl esters, but no differences were observed among PL-treated *salpicão* samples. The most abundant ethyl esters detected were ethyl lactate, butanoate, propanoate, and hexanoate. Usually, these compounds originated from the esterification of alcohols and carboxylic acids by microbial metabolism (Olivares et al., 2015), or from carbohydrate metabolism. The ethyl esters have low odour threshold values, impart a wide variety of fruity notes, and result in the masking of rancid odours, thus contributing to the sausage aroma (Bis-Souza et al., 2019; Hierro et al., 2004; Olivares et al., 2015).

As expected in smoked meat products like *salpicão*, typical wood smoke compounds were observed as part of the product's volatile profile. In the production of *salpicão*, a process of cold smoking for 3–4 days was used, promoting a smoke flavour that contributes to the authenticity of its organoleptic characteristics. Some authors (Wang & Chambers, 2018) have demonstrated that phenols are the primary contributors to

the smoke that provide flavour and aroma. In our *salpicão* samples, the phenolic compounds were the third most common on the volatile profile, representing 17.61–21.18%, which indicates that this product underwent a smoking process (values presented are relative percentages of the total amount of volatile compounds; Table 3 represents only absolute values). Besides phenolic compounds, other compounds characteristic of wood smoke were also detected, such as furans (10.80–11.89%), ketones (4.92–5.39%), and aromatic hydrocarbons (1.22–1.57%) (Chang et al., 2021; Merlo et al., 2021).

In this study, the relative amount of volatile compounds was also used to assess how the PL treatment affected lipid oxidation in cured-smoked meat products. Aldehydes are the major volatiles produced during lipid oxidation and have been used to successfully follow the development of lipid oxidation in several foods, including muscle foods (Ross & Smith, 2006). Hexanal is the dominant aldehyde produced during oxidation, indicating lipid oxidation of meat more effectively than any other volatile component (Ross & Smith, 2006). The volatile profile of *salpicão* did not present dominant aldehydes, such as hexanal, octanal, nonanal, and pentanal, and the total content varied between 1.42 and 1.91%, suggesting that this product did not have suffered lipid oxidation reaction in great extension during its processing. Other components related to lipid oxidation, like aromatic compounds, aliphatic hydrocarbon, and acid content, were quite low, representing respectively, 1.10–1.86%, 1.22–1.57%, and 0.11–0.19% of *salpicão* total volatile compounds, indicating that, in this product, lipid oxidation might have occurred but in a small extension. Although this result does not agree with the results observed by other authors, who found that this group of volatiles is one of the most important in fermented sausages, they agree with those published by Bis-Souza et al. (2019), where the aldehyde content of a low-fat fermented sausage at the end of ripening represented <1% of the total volatile content.

4. Conclusion

PL treatment was able to inactivate *L. monocytogenes* from the surface of a packaged sliced cured meat product *salpicão*, with a maximum log reduction of 1.58 cfu/g obtained with a fluence of 5.31 J/cm², and may facilitate the accomplishment of the criterion required by current EU legislation for RTE products. The application of higher PL fluence did not result in a higher reduction of *L. monocytogenes*. Thus, based on our results, PL could be recommended to be used as a non-thermal technology to increase the safety of sliced cured meat products like *salpicão*, without promoting relevant changes in quality indicators, such as colour and volatile compounds characteristics. The change of temperature observed in PL-treated samples was under the same range of ambient temperature, a very slight impact product colour. Nevertheless, further research should be conducted to evaluate if other quality traits, including sensory and physical characteristics, are also not affected by the PL treatment proposed when applied to this type of product.

CRedit authorship contribution statement

A. Borges: Conceptualization, Formal analysis, Data curation, Writing – original draft, preparation, Writing – review & editing, Supervision. **E. Baptista:** Conceptualization, Data curation, Writing – original draft, preparation, Writing – review & editing. **T. Aymerich:** Conceptualization, Formal analysis, Writing – original draft, preparation, Supervision. **S.P. Alves:** Conceptualization, Data curation, Writing – review & editing. **L.T. Gama:** Conceptualization, Formal analysis, Writing – original draft, preparation, Supervision. **M.J. Fraqueza:** Conceptualization, Formal analysis, Writing – original draft, preparation, Writing – review & editing, Supervision, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2023.114641>.

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