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Pulsed light treatment for the reduction of *Salmonella* Typhimurium and *Yersinia enterocolitica* on pork skin and pork loin

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ABSTRACT

The aim of the presented study was to investigate the impact of pulsed light on the reduction of *Salmonella* Typhimurium and *Yersinia enterocolitica* on pork skin and loin. Fluences of 0.52 to 19.11 J/cm^2 were applied to the pathogen-inoculated products to perform microbiological studies, as well as analyses of color, temperature, lipid peroxidation and odor. Reductions on pork skin ranged from 1.73 to 3.16 log for *Salmonella* and from 1.48 to 4.37 log for *Yersinia*. Microbial reduction was significantly lower on pork loin, varying between a minimum of 0.4 and a maximum of 1.7 log for both pathogens. Treatments ≥7.36 J/cm^2 modified the color parameters of pork skin and fluences ≥9.66 J/cm^2 rendered pork loin samples less red. All studies with pulsed light resulted in odor changes, except for the experiment on pork skin at 0.52 J/cm^2. Despite significant microbiological reduction on pork skin, further studies should be carried out to optimize this promising technology.

1. Introduction

Salmonella and pathogenic *Yersinia* ssp. are well-known causes of human food-borne illnesses. In 2016 they were the second and third most frequent causes of bacterial gastroenteritis in Germany and indeed throughout Europe. The Robert Koch Institute (RKI) reported 12.881 cases of salmonellosis and 2.759 cases of yersiniosis in Germany (RKI, 2017) while the European Food Safety Authority (EFSA) confirmed 94.625 cases of salmonellosis and 7.202 cases of yersiniosis in Europe in 2015 (EFSA, 2016). Both diseases can become life-threatening for YOPIs (young, old, pregnant, immunodeficient) and yersiniosis may evoke long-term repercussions, such as reactive arthritis or erythema nodosum. 38.6% of all salmonellosis cases and 21.4% of all *Salmonella*-caused outbreaks originate from *Salmonella* Typhimurium (Federal Institute for risk assessment (BfR, 2016). Yersiniosis is predominantly (81%) caused by *Yersinia enterocolitica* O:3 (BfR, 2016). Both microorganisms are closely associated to the consumption of raw pork meat and products thereof (BfR, 2016; de Boer and Nouws, 1991). Subclinically infected pigs are the main carriers of the bacteria (Bonardi et al., 2013; Fredrikkson-Ahomaa, 2000; Nesbakken et al., 2006; Vanantwerpen et al., 2017). Premortal stress can lead to the translocation of microorganisms from contaminated regions (e.g. the intestines) into usually germ-free organs and muscles by a rising permeability of the intestinal barrier. Usually, serum bactericidal activity is supposed to limit enrichment of undesirable microorganisms. However, stress injures the serum bactericidal activity and promotes dissemination of germs after translocation (Fehlhaber and Alter, 1999) and can also be followed by increased excretion of *Salmonella* ssp. (Ferrer-Savall et al., 2016; Leyman et al., 2012; Seidler et al., 2001) and contamination of so far negative animals during transport or in the lairage (Berends et al., 1996; Ferrer-Savall et al., 2016).The prevalence of positive carcasses in the abattoir can fluctuate depending on the slaughterhouse, sampling day and pig herd. *Salmonella* and *Yersinia* ssp. were detected in up to 65.2% (Berends et al., 1997; Botteldoorn et al., 2003; Fehlhaber et al., 1996; Ludewig et al., 2001; Methner et al., 2011) and up to 55.3% (Bonardi et al., 2013; Fredriksson-Ahomaa et al., 2007; Vanantwerpen, 2014; van Damme et al., 2015) of all tested samples respectively and were mostly isolated from feces, lymph nodes, carcass surfaces and, concerning *Yersinia*, also from tonsils. It is not always observed that carcass contamination stems from the animal itself, since 30% of the positive results are attributable to cross-contamination (Borch et al., 1996; Laukkanen, 2009; Vanantwerpen et al., 2017) occurring during the slaughter process or following steps. The numbers mentioned above indicate that safety of products might not be ensured, despite high hygiene standards in European slaughterhouses, which is the reason for taking additional decontamination measures into account. Pulsed light (PL) has proven to be a promising method for reducing spoilage and pathogenic bacteria, fungi, protozoa and viruses on the surface of several food matrices (Aron-Maftei et al., 2013; Fernández et al., 2016; Ferrario et al., 2015; Gómez-López et al., 2007;

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Huffman et al., 2000; Kramer et al., 2015). Microorganisms are killed by short pulses (μs) of broad spectrum light produced by Xenon or Kryton flash lamps. The light consists of wavelengths from 200 to 1100 nm, particularly rich in UV-C (Gómez-López et al., 2007) and is considered to be an improvement to continuous mode treatments conducted in the past, due to the possibility of reducing processing time significantly (Gómez-López et al., 2012). The germicidal effect of PL is commonly explained by two coexisting mechanisms. Firstly, the so-called photochemical effect is due to the absorption of the UV-C part of the spectrum by the DNA and subsequent formation of pyrimidine dimers, primarily thymine dimers, inhibiting cell replication and, thus, leading to clonogenic death. The second mechanism is a photothermal effect, leading to damage of cell membrane, proteins and expansion of vacuoles (Elmnasser et al., 2007; Gómez-López et al., 2007). PL and its decontamination efficiency has been tested in a number of studies on different food matrices, such as fluids (Chaine et al., 2012; Krishnamurthy et al., 2007), fruits (Bialka and Demirci, 2007, 2008; Huang and Chen, 2014) and vegetables (Aguiló-Aguayo et al., 2013; Kramer et al., 2015). Bacterial reduction was also investigated on several meat products, for example, chicken breast and frankfurter sausages (Keklik et al., 2009, 2010), cooked ham and bologna sausage (Hierro et al., 2011), beef carpaccio (Hierro et al., 2012), and pork meat (Nicorescu et al., 2014) where reductions for *Listeria monocytogenes*, *Salmonella* Typhimurium, *E. coli*, *Pseudomonas fluorescens* and aerobic flora of between 1 and 3.4 log-units were achieved. However, to our knowledge, there is no scientific data on the reduction kinetics of *Salmonella* Typhimurium and *Yersinia enterocolitica* on pork skin and loin in the literature so far. Hence, the objective of this study was to generate basic data on the bactericidal effect of PL on both microorganisms combined with an evaluation of product quality including color, odor and lipid peroxidation.

2. Materials and methods

2.1. Bacterial strains and preparation of Inocula

Salmonella enterica ssp. enterica serovar Typhimurium and *Yersinia enterocolitica* (Biotype 4) were isolated from pork meat and stored in cryogenic tubes containing 2 mL of a hypertonic preservative solution (Cryobank TM-yellow, Mastgroup, Merseyside, UK) at −70 °C.

A pellet of both stock cultures was transferred into 5 mL of nutrient broth (NB I, TN1172, sifin diagnostics GmbH, Berlin, Germany) and incubated at 37 °C (*Salmonella* Typhimurium) or 28 °C (*Yersinia enterocolitica*) for 24 h. A loop of *Salmonella* Typhimurium suspension was spread on brilliant green agar (BGA, TN1111, sifin diagnostics GmbH, Berlin, Germany) and incubated at 37 °C for 24 h. The *Yersinia enterocolitica* suspension was streaked onto CIN agar (CM0653, supplement: SR 0109, Oxoid, Wesel, Germany) and incubated at 28 °C for 24 h.

For each trial, one colony of either *Salmonella* Typhimurium or *Yersinia enterocolitica* was transferred into 5 mL of NB I and grown for 16 h at 37 °C (*Salmonella*) or for 18 h at 28 °C (*Yersinia*) to yield a concentration of 10^8–10^9 cfu/mL, which was determined by plate count.

2.2. Sample preparation and inoculation procedure

Pork skin and loin were obtained from a local retailer, sliced, vacuumed and frozen (−20 °C) in vacuum bags. Samples were sliced in a frozen state to guarantee homogeneous thickness and reproducibility. 24 h in advance, samples were placed in a refrigerator (4 °C) to thaw overnight. Preliminary experiments with thawed and fresh samples resulted in no statistically significant differences in microbial reduction.

A sample size of 25 cm^2 was obtained by using a punch of 5.6 cm in diameter and put into sterile petri dishes. The inoculation was performed under a laminar flow hood (Heraeus, Germany). 100 μL of the initial inoculum were pipetted onto one side of the samples and spread homogeneously with a sterile Drigalsky spatula. This procedure led to a cell concentration of approximately 5×10^6 cfu/cm^2. Inoculated samples were left to dry for 30 min under the laminar flow, allowing bacterial adaptation to the matrix. Two samples per vacuum bag were used as controls. One was inoculated, but not treated and the other one was analyzed with regard to the total aerobic plate count and naturally occurring *Salmonella* or *Yersinia*. They were determined by plating 1 mL of the undiluted suspension onto either three XLD (TN1196, sifin diagnostics GmbH, Berlin, Germany) or three CIN agar plates.

Non-inoculated samples were used for chemical and sensory analysis. For odor analysis fresh samples (skin and loin) were taken to exclude odor changes induced by freezing and thawing.

2.3. Pulsed light treatment

Light pulses were produced by a laboratory scale, benchtop sterilization system (SteriPulse-XL 3000, Model RS-3000C, Xenon Corporation, Wilmington, Massachussetts, USA). As reported by the manufacturer's specifications, the system generated 1.27 J/cm^2 per pulse at a distance of 1.93 cm from the quartz window. Fluences were calculated using the number of flashes, distance and illumination area (measured by means of solar paper). Pulse frequency was set to four pulses in the first second and three pulses in every additional second with a pulse length of 300 μs. The wavelengths emitted by the xenon flash lamp ranged from 200 to 1100 nm.

Pork skin and loin samples were treated in quadruplicate for microbiological analysis ($n = 180$) or in triplicate for color and temperature measurements ($n = 108$) at 8.3 cm, 10.8 cm and 13.4 cm for 1, 5, 10, 15, 20 and 30 s corresponding to fluences between 0.52 and 19.11 J/cm^2 (Table 1) for *Salmonella* and *Yersinia*, respectively. To determine lipid peroxidation, the following fluences were tested in quadruplicate: 0.52, 0.64, 0.84, 5.98, 7.36, 9.66, 11.83, 14.56 and 19.11 J/cm^2. The trials were carried out on the day of the PL treatment (day 0), day 5 and 10 of the pork skin and loin being stored in a refrigerator at 4 °C ($n = 135$). For sensory analysis, assays were chosen where microbial reductions of at least 2 log (99%) were obtained however no color differences ($\Delta E^*ab < 3$) could be identified by potential consumers. In order to represent the impact of low, medium and high fluences on odor 0.52, 4.96 and 12.81 J/cm^2 were selected.

2.4. Microbiological analysis

PL treated samples and controls were instantaneously transferred into stomacher bags (Roth, Karlsruhe, Germany) containing 50 mL of peptone water (51094, bioMérieux, Nürtingen, Germany) and homogenized in a Stomacher®400 *Circulator* (Seward Limited, West Sussex, UK) for one minute at 260 rpm. Serial dilutions of the homogenates were prepared with sodium chloride peptone solution (Roth, Karlsruhe, Germany) and 100 μL of each dilution were plated in duplicate, either onto XLD or onto CIN agar. The XLD or CIN plates were incubated for 24 h at 37 °C or 28 °C and colonies were enumerated. The reduction factor was calculated by using the following equation:

$$\log (cfu_N/cfu_T) \quad (1)$$

where cfu_N indicates the number of bacteria found on the non-treated sample and cfu_T the number of bacteria found on the treated sample.

Trials resulting in a reduction of ≥2 log-units on pork skin and approximately 1.5 log on loin were chosen for enrichment trials, to verify whether a lower bacterial load could lead to higher reduction using a contamination dose of 1.6×10^2 cfu/cm^2 for *Salmonella* and 4.7×10^2 cfu/cm^2 for *Yersinia* ($n = 5$).

Skin samples were treated with 4.03, 7.93, 11.83 J/cm^2 for both pathogens, as those were assays where reductions of ≥2 log were achieved. In the loin meat trials, different fluences for each pathogen had to be selected, as only some specific fluences led to reduction of 1.5

Table 1
Reduction of S. Typhimurium and Y. enterocolitica on pork skin and pork loin using pulsed light treatment.

Treatment			Pork skin		Pork loin	
PL fluence (J/cm^2)	Distance (cm)	Time (s)	*Salmonella*	*Yersinia*	*Salmonella*	*Yersinia*
0.52	13.4	1	1.84 ± 0.17^{ac}	1.97 ± 0.46^{a}	0.40 ± 0.13^{a}	0.37 ± 0.05^{a}
2.08		5	1.90 ± 0.30^{ac}	2.53 ± 0.61^{ac}	0.51 ± 0.13^{a}	0.58 ± 0.09^{ab}
4.03		10	2.09 ± 0.21^{ab}	2.43 ± 0.21^{ac}	0.64 ± 0.12^{abh}	0.86 ± 0.09^{bcf}
5.98		15	2.73 ± 0.26^{ab}	3.12 ± 0.63^{abc}	1.03 ± 0.20^{ce}	0.86 ± 0.10^{bcf}
7.93		20	2.90 ± 0.36^{ab}	2.30 ± 0.24^{a}	0.90 ± 0.06^{bcf}	0.95 ± 0.10^{bcfh}
11.83		30	2.29 ± 0.48^{ab}	2.37 ± 0.50^{a}	0.94 ± 0.13^{ch}	0.99 ± 0.17^{cfg}
0.64	10.8	1	1.73 ± 0.35^{a}	1.48 ± 0.41^{a}	0.54 ± 0.23^{af}	0.84 ± 0.13^{bcf}
2.56		5	2.38 ± 0.43^{ab}	2.00 ± 0.36^{a}	0.78 ± 0.08^{ac}	1.06 ± 0.27^{cd}
4.96		10	2.50 ± 0.52^{ab}	2.07 ± 0.27^{a}	1.03 ± 0.12^{ce}	1.16 ± 0.11^{cd}
7.36		15	2.35 ± 0.52^{ab}	2.36 ± 0.33^{a}	1.35 ± 0.21^{de}	1.42 ± 0.24^{de}
9.76		20	3.16 ± 0.57^{b}	3.42 ± 1.49^{ab}	1.26 ± 0.18^{egh}	1.69 ± 0.26^{e}
14.56		30	2.59 ± 1.13^{ab}	2.07 ± 0.32^{a}	1.59 ± 0.18^{dg}	1.37 ± 0.18^{dge}
0.84	8.3	1	1.81 ± 0.24^{ac}	1.99 ± 0.18^{a}	1.00 ± 0.12^{bce}	0.78 ± 0.08^{bc}
3.36		5	2.19 ± 0.61^{ab}	2.54 ± 0.71^{ac}	1.08 ± 0.06^{ce}	1.02 ± 0.10^{cfg}
6.51		10	2.62 ± 0.40^{ab}	1.94 ± 0.50^{a}	1.49 ± 0.10^{dg}	0.84 ± 0.17^{bcf}
9.66		15	2.34 ± 0.50^{ab}	4.37 ± 1.39^{b}	1.67 ± 0.25^{d}	1.14 ± 0.20^{cd}
12.81		20	2.12 ± 0.60^{ab}	4.20 ± 1.32^{bc}	1.71 ± 0.25^{d}	1.18 ± 0.09^{df}
19.11		30	2.97 ± 0.64^{bc}	4.19 ± 1.35^{bc}	0.76 ± 0.10^{ac}	1.33 ± 0.25^{dghe}

Data represent mean log reductions (log CFU/cm^2) of five replicates ± standard deviations.
The mean initial populations of *S*. Typhimurium and *Y. enterocolitica* were 4.72 × 10^6 cfu/cm^2 and 5.34 × 10^6 cfu/cm^2 respectively.
Data in the same column followed by the same lowercase superscript are not significantly different ($p > 0.05$).

log: 9.66, 12.81 and 14.56 J/cm^2 (*Salmonella*); 7.36, 9.76 and 14.56 J/cm^2 (*Yersinia*). Untreated, non-inoculated and untreated, inoculated samples were used as negative and positive controls.

Recovery for *Salmonella* was performed by adding 50 mL of peptone water to the samples and stomaching them for 60 s at 260 rpm. 1 mL of the homogenate was directly plated onto three XLD plates and incubated for 24 h at 37 °C. The enrichment procedure was conducted according to DIN EN ISO 6579-1:2014-08. Thus, the remaining homogenate was incubated for 24 h at 37 °C. Samples, where no presumptive colonies could be found in 1 mL, underwent further analysis. Hence, 100 µL or 1 mL of the enriched peptone water was transferred into 9.9 mL of RVS (Rappaport-Vassiliadis-soja broth, Sifin) or into 9 mL of MKTT (Muller-Kauffmann-tetrathionate broth, Sifin) respectively. 200 µL of iodine-potassium iodide solution (Merck, Darmstadt, Germany) was added to the MKTT before incubating the media for 24 h either at 42 °C (RVS) or at 37 °C (MKTT). Afterwards, three loops of RVS or MKTT were streaked onto a XLT_4 (Xylose-Lysine-Tergitol$_4$, Merck) and a BGA (from RVS) or BGA-N (brilliant-green-agar with novobiocin, Sifin and Sigma Aldrich, Steinheim, Germany) (from MKTT) plate and incubated once more for 24 h at 37 °C. Samples with presumptive colonies were considered as *Salmonella* positive.

Yersinia enrichment followed the instructions described in ISO/TC 34/SC 9 N with slight modifications, omitting enrichment with ITC (Irgasan, ticarcillin and potassium chlorate broth) and CEB (cold enrichment broth). 28 °C was chosen as incubation temperature for the CIN plates aiming at suppressing background flora. Additionally, PSB (peptone, sorbitol and bile salts) broth was vortexed in KOH solution (0.5%, Merck and Roth) for 15 instead of 20 s, to ensure the recovery of *Yersinia*. Presumptive colonies were subcultured on CIN agar and afterwards transferred to urea (TN1143, sifin diagnostics GmbH, Berlin, Germany), (24 h, 30 °C) and CRMOX (congo red-magnesium oxalate, sifin and Merck), (24 h, 37 °C) agar. Positive reactions (urea: color change from orange to pink; CRMOX agar: small red colonies proving presence of virulence plasmid) confirmed the existence of pathogenic *Yersinia*. Once presumptive colonies of naturally occurring *Salmonella* or *Yersinia* on non-inoculated control samples (naturally contaminated samples) were found, trials were repeated with *Salmonella* or *Yersinia* free samples. *Salmonella* was found on 9.6% (up to 6 × 10^2 cfu/cm^2) and *Yersinia* was detected on 1.8% of samples (up to 1.8 × 10^1 cfu/cm^2).

2.5. Color measurements

Before and immediately after treatment, the color of each sample was measured by using the spectrophotometer CM 600 d (Konica Minolta, Germany). Measurements were performed in three random locations on each sample. The color was described by three parameters of the CIELAB system: L (lightness), a (redness) and b (yellowness).

2.6. Temperature measurements

The temperature of samples was monitored using an infrared thermometer (104-IR, Testo, Germany). Differences in surface temperature (ΔT) were calculated and temperature profiles were generated by depicting the mean value ± standard deviation.

2.7. Lipid peroxidation

The thiobarbituric-acid reactive substances (TBARS) method was applied for evaluating lipid peroxidation. The analysis was performed according to Botsoglou et al. (1994) with some modifications. 5 g samples were acidified with 10 mL of 5% aqueous trichloroacetic acid (TCA; Roth). The hexane – containing homogenate was centrifuged for 5 min at 3000*g* (Centrifuge 5804 R, Eppendorf, Germany). The aqueous phase (2 mL) was filtered by using a syringe filter (0.45 µm for pork skin; 0.2 µm for pork loin, Sartorius, Germany). Subsequently, 1 mL of the filtrate and 0.6 mL 0.8% TBA (AppliChem, Darmstadt, Germany) were filled into a tube for the following heating process. Absorbancies were recorded against a blank reaction mixture at 532 nm by means of a spectrophotometer (Novaspec II, GE Healthcare Life Sciences, Freiburg, Germany). The malondialdehyde (MDA) content was determined by using a standard curve established by serially diluting 1, 1, 3, 3-tetraethoxypropane (TEP, Sigma Aldrich, Steinheim, Germany) according to Rosenbauer (2002).

2.8. Sensory analysis

Sensory analysis comprised evaluation for a rancid odor induced by PL treatments and was conducted by ten experienced food appraising panelists, certified by DLG (German Agricultural Society). The following assays were chosen based on high microbial reductions, minimal

color differences (ΔE*ab < 3) and in order to represent the impact of low, moderate and high fluences on product quality: 0.52, 4.96 and 12.81 J/cm^2. Ten samples per assay (treated and untreated) and matrix (pork skin and pork loin) were analyzed and equally distributed over three independent sessions (n = 80). After treatment, all samples were transferred into screw-capped glass bottles, blinded by a three-digit numerial code and offered to the panelists in random order. At the beginning of each session all ten panelists were asked to evaluate the odor of four reference samples per matrix (one untreated and one per assay) together. They were required to form a consensus on the magnitude of odor deviation on a scale from 0 to 5, 0 meaning no sensory deviation and 5 meaning a strong off-odor Those reference samples should serve as orientation for the following sensory analysis. Afterwards, panelists formed three groups of two or three members and evaluated blinded samples by means of consensus profiling using the above-mentioned scores (0–5) which led to three different scores per sample (since there were three groups). Those three scores were averaged and their mean values were used for statistical analysis (ANOVA).

2.9. Statistical analysis

GraphPad software version 4.00 for Windows (GraphPad Software, San Diego, USA) was used for statistical analysis. The Kolmogorov-Smirnov-test was performed to ensure the normal distribution of samples. One-way analysis of variance (ANOVA) was carried out to determine the impact of energy input (fluence) on bacterial reduction, lipid peroxidation as well as color and odor change. Where significant differences (p < 0.05) between assays were found, a Bonferroni *post-hoc* test was added to determine which parameter combinations exactly differed significantly from each other. For evaluation of the effect of bacterial species and matrix on decontamination success a *t*-test was conducted. Results are averages of five (microbiological and lipid peroxidation trials), three (temperature and color measurements) or ten (sensory analysis) independent samples and reported as a mean ± standard deviations.

3. Results and discussion

3.1. Microbiological analysis

3.1.1. Effect of PL treatment on the reduction of Salmonella Typhimurium and Yersinia enterocolitica on pork skin

A reduction of 1.73 to 3.16 log and 1.48 to 4.37 log was obtained for *Salmonella* and *Yersinia* on pork skin, respectively. 90–99% of the bacteria were already inactivated within the first second of PL treatment (Table 1). Minimally higher reductions could be achieved by increasing the fluence. Statistically significant differences in reduction rates between fluences could be determined only occasionally due to fluctuation and high standard deviations. Similar treatments were only carried out with lower energy, other matrix (Haughton et al., 2011) or different light sources (Wong et al., 1998) impeding direct comparison with known literature values.

Analogically to *Salmonella*, only in some cases there were significant differences between assays in trials with *Yersinia* on pork skin. *Yersinia* was better reduced than *Salmonella* on pork skin, but statistical significance could only be detected in two cases (9.66, 12.81 J/cm^2). This might be due to higher susceptibility of *Yersinia* to UV light or ozone. Standard deviations are remarkably high (up to 1.39) and were not reported before in scientific literature, leading to the assumption that it is related to the matrix. Samples had different numbers of pores and crevices as well as traces of bristles, which might have resulted in different shading effects.

At a contamination dose of 10^2 cfu/cm^2 no *Salmonella* could be recovered from pork skin. Those samples were enriched and then proved to be *Salmonella*-positive. *Yersinia* could solely be recovered from three pork skin samples treated with 19.11 J/cm^2 which could be due to photoreactivation occurring during storage meaning the recovery of culturability by a photolyase-dependent DNA repair mechanism triggered by visible light. This phenomenon leads to the assumption that PL may cause sublethal damage to bacterial cells inducing a viable but non culturable (VBNC) state (Kramer et al., 2017).VBNC cells are characterized by their incapability to reproduce but they are still able to maintain a membrane potential, metabolic activity, esterase activity, ATP maintenance, glucose uptake activity or pump activity (Kramer and Muranyi, 2014) and might limit the application of PL since there is no data showing if those survivors still pose a risk to human health. Further studies in other food commodities are needed in order to identify new methods of enumeration and identification of VBNC cells other than conventional growth dependent techniques on agar plates which might bring about radical reappraisal of processing parameters and detection limits (Rowan et al., 2015).

Yersinia-negative pork skin samples were enriched and the following amount of samples contained presumptive pathogenic *Yersinia enterocolitica*. This could be confirmed by positive reactions on urea and CRMOX agar: 3/5 at 9.66 J/cm^2, 1/5 at 12.81 J/cm^2 and 4/5 at 19.11 J/cm^2. Survival of pathogenic *Salmonella* and *Yersinia* in real food systems is probable if they are sheltered or protected in crevices.

3.1.2. Effect of PL treatment on the reduction of Salmonella Typhimurium and Yersinia enterocolitica on pork loin

Reduction on pork loin ranged from 0.40 to 1.71 log-units for *Salmonella* and from 0.37 to 1.69 log for *Yersinia* (Table 1). Comparable to pork skin, reduction barely increased with fluence leading to a tail shaped curve. Fluctuation, as well as standard deviations, led to only rarely occurring significant differences in reduction rates between various energy inputs. In contrast to pork skin, standard deviations were much smaller (0.05–0.25) and comparable to those obtained by Nicorescu et al. (2013) when inactivating aerobic flora and *Pseudomonas fluorescens* on raw pork roast (0.1–0.24) confirming the assumption that standard deviations are matrix-dependent, as mentioned above. Our decontamination results for *Salmonella* on loin are consistent with results by Rajkovic et al. (2017), who reduced *Salmonella* Typhimurium on sliced fermented salami by 2.3 log (pork). Other authors who treated different meat products and raw meat with PL obtained comparable reduction rates. Keklik et al. (2010), Gudelis and Luksiene (2010) and Pasceviciute et al. (2011) reduced *Salmonella* Typhimurium by 2 log on raw chicken, Hierro et al. (2011) by 1 log on beef carpaccio and Ganan et al. (2013) by 1.5 and 1.7 log on salchichón and dry - cured loin.

Reduction efficacy on pork loin was significantly lower for almost every distance/time combination compared to that on skin. Possible reasons for this observation may be differences in contour, porosity and surface roughness between the skin and muscle which, allows PL to interact differently with the microorganisms present on each surface (Wong et al., 1998; Yaun et al., 2004). The smoother the surface, the greater the decontamination efficiency (Yaun et al., 2004) which was proven by comparing reduction rates and roughness of different fruits (Syamaladevi et al., 2015). It has also been shown that surface hydrophobicity has an influence on decontamination efficiency, which was investigated more profoundly by Syamaladevi et al. (2013). Less hydrophobic surfaces showed better reductions, most likely because of the broader spatial distribution of bacterial cells, which resulted in a higher exposure to PL. These findings are contradictory to our results, since pork skin, mainly consisting of fat, is obviously more hydrophobic than loin. When inoculating our samples, we were able to observe how the inoculum on pork skin formed small beads on the surface while on loin it was almost immediately absorbed. This shows that bacteria on loin are transported into deeper layers by diffusion, where they might be sheltered from the PL whereas remaining microorganisms on skin surface can be reached more easily (Nicorescu et al., 2014). Few samples were *Salmonella*-negative when using an inoculation level of 2 log (9.76, 14.56 J/cm^2). Lower inoculation levels should avoid formation of

Table 2
Fluence and temperature change corresponding to parameter-combinations.

Distance (cm)	Time (s)	Fluence (J/cm²)	Temperature change (ΔT)	
			Pork skin	Pork loin
8.3	1	0.84	1.4 ± 0.7	1.5 ± 0.2
	5	3.36	5.7 ± 0.3	4.5 ± 0.9
	10	6.51	10.2 ± 0.1	8.1 ± 1.9
	15	9.66	12.9 ± 0.5	10.8 ± 1.9
	20	12.81	15.6 ± 3.6	13.7 ± 2.7
	30	19.11	24.1 ± 1.6	17.2 ± 3.1
10.8	1	0.64	1.0 ± 0.6	0.9 ± 0.5
	5	2.56	4.3 ± 1.2	2.8 ± 0.4
	10	4.96	8.5 ± 0.8	5.4 ± 0.8
	15	7.36	12.5 ± 1.7	7.4 ± 0.5
	20	9.76	15.3 ± 1.8	9.2 ± 0.4
	30	14.56	20.3 ± 2.8	13.9 ± 0.8
13.4	1	0.52	0.4 ± 0.4	0.6 ± 0.2
	5	2.08	3.0 ± 0.9	2.6 ± 0.3
	10	4.03	5.2 ± 0.8	4.5 ± 0.7
	15	5.98	7.4 ± 1.6	6.0 ± 0.2
	20	7.93	10.0 ± 2.2	7.2 ± 0.9
	30	11.83	13.2 ± 2.8	10.2 ± 1.7

Initial temperature of samples was 23 °C.

multi-layers and prevent shadowing, leading to the expectation that almost all microorganisms should be destroyed by PL (Gomez-Lopez et al., 2005). However, where no *Salmonella* could be recovered, an enrichment was performed which turned out positive in both cases. *Yersinia* could be recovered after all three tested assays, which rendered an enrichment unnecessary. Hence, no complete elimination could be obtained. According to Uesugi et al. (2007) there is a certain number of crevices on a surface where microorganisms are able to hide. The remaining upper layers of bacteria are exposed to PL. Thus, no matter how high the initial inoculum was, the amount of survivors remains constant which is why enrichment procedures were always positive.

3.2. Temperature measurements

Table 2 presents the temperature rise on pork skin and loin resulting from PL. The initial temperature was 23 °C and increased with longer treatment time and smaller distance to the PL source. Skin heated up faster than loin under same treatment conditions. This phenomenon is reported in the literature as being attributed to the higher amount of water in pork meat (75%) (Kim et al., 2008) than pork skin (65%) (Schnaeckel et al., 2014). Thus, much of the energy input is used for water vaporization resulting in lower product heating. The highest temperatures were registered after 30 s at a distance of 8.3 cm from the light source with a ΔT of 24.1 °C on skin and 17.2 °C on loin resulting in product temperatures of 47.1 °C and 40.2 °C, respectively. It is generally known that *Salmonella* and *Yersinia* ssp. start dying at temperatures > 45 °C (Doherty et al., 1998; Doyle and Mazzotta, 2000; Gurman et al., 2016). As only one assay (pork skin: 8.3 cm, 30 s) reached this threshold and this did not lead to significantly higher reduction, microbial reduction was not attributable to heating. Our findings in temperature rise are comparable with those reported by Keklik et al. (2009, 2010).

3.3. Color measurements

Table 3 illustrates the effect of PL on the color of pork skin and loin. Positive values for ΔL, Δa and Δb imply that pork skin and loin turned lighter, redder or yellower, respectively; negative values signify that samples became darker, greener or bluer, respectively. Cserhalmi et al. (2006) investigated whether a color difference is visually perceivable and divided the values for ΔE*ab (total color difference) into the following categories: not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0) and highly visible (6.0–12.0).

The L*value of pork skin only changed minimally during assays. A significant difference ($p < 0.05$) could only be observed in two cases (0.64/7.36 vs. 14.56 J/cm²). Furthermore, skin samples turned significantly less red after being treated at 7.36, 9.76, 12.81 and 19.11 J/cm². Fluences ≥7.36 J/cm² (except for 9.66 J/cm²) led to well visible color changes (ΔE*ab ≥3). The b*value was not significantly different between treatments for either of the investigated matrices.

The L*value of the PL treated loin was not affected by any of the selected parameter combinations.

Loin samples became significantly less red when treated at 9.66, 12.81 and 19.11 J/cm². ΔE*ab did not exceed the value of 3 on either of the tested fluences. Loss of redness of treated samples could be explained by destruction of several chromophores, such as hemoglobin (pork skin) and myoglobin (pork loin) (Lim et al., 2014; Nicorescu et al., 2014) and seemed to be dependent on red color distribution before the PL treatment. Skin samples were quite heterogeneous with respect to color. Poor exsanguination or ranking fights amongst fattening pigs might have resulted in petechiae or maculae. Other samples were beige with no traces of red and did not change their color visibly whereas red samples turned noticeably paler. Loin, on the other hand, was homogeneous in all samples tested.

Our findings, namely a decrease in L*and a*values, are in line with the results of Keklik et al. (2009) who also obtained decreasing L* and a* values in chicken frankfurters.

A number of other research groups have made the same observations regarding redness. Almost all tested meat types showed a decrease in a*values except for chicken and fish (Hierro et al., 2011; Nicorescu et al., 2014; Tomasevic, 2015; Tomasevic and Rajkovic, 2015; Wambura and Verghese, 2011) which might be attributable to only small amounts of chromophores in chicken and fish.

3.4. Lipid peroxidation

Tarlagdis et al. (1960) reported a threshold range of 0.5–1.0 μg MDA/g pork for sensory detection of rancidity as determined by trained panelists. The MDA levels of all samples tested in our study were below that threshold (even after a storage at 4 °C for 10 days, data not shown) as shown in Table 4. Generally, susceptibility of food matrices to lipid peroxidation depends on fat content and amount of (poly)-unsaturated fatty acids as well as the antioxidative potential of the matrix. Pork loin possesses relatively low proportions (10%) of polyunsaturated fatty acids (Castaneda Rocha de Kopp, 2013; Nicorescu et al., 2014), which might be the reason why, even at high fluences, the above-mentioned threshold for MDA was not exceeded.

In pork skin, the MDA concentration was significantly higher ($p < 0.05$) at fluences of 0.84, 9.66 and 14.56 J/cm² in comparison to the control and the other fluences applied, but clearly remained beneath the threshold concentration of 0.5 μg/g. In loin, there is no statistically significant difference between the control and treated samples. Consequently, PL treatment did not induce accelerated lipid peroxidation in loin. These results are in agreement with the work of Rajkovic et al. (2017) who did not find any significant differences in the concentration of MDA between the control (0.33 mg MDA/kg), 3 J/cm² (0.31 mg MDA/kg) and 15 J/cm² treated salami samples (0.49 mg MDA/kg). Nicorescu et al. (2014) conducted TBARS trials concerning raw pork roast and found MDA concentrations between 30 and 125 times lower than ours. This considerable disparity may be caused by different fat content and fatty acid patterns in pork meat used in the different studies.

3.5. Sensory analysis

The results of the sensory analysis for pork skin are presented in Fig. 1 and Table 5. Panelists assessed even untreated samples as deviant (a score of 0.75) and described them as slightly chlorine-like (pork

Table 3
Effect of PL on the color of pork skin and pork loin.

Treatment			Color change							
			Pork skin				Pork loin			
Distance (cm)	Time (s)	Fluence (J/cm^2)	ΔL	Δa	Δb	ΔE*ab	ΔL	Δa	Δb	ΔE*ab
13.4	1	0.52	0.5 ± 0.8^{a}	−0.2 ± 0.2^{a}	−0.4 ± 0.7^{a}	1.0 ± 0.3^{ae}	0.7 ± 3.3^{a}	0. 8 ± 0.7^{a}	1.0 ± 0.9^{a}	2.9 ± 1.5^{a}
	5	2.08	0.2 ± 0.5^{ab}	−0.2 ± 0.5^{ac}	−0.0 ± 1.3^{a}	1.2 ± 0.3^{ae}	0.7 ± 0.7^{a}	0.2 ± 0.1^{ab}	−0.1 ± 0.4^{a}	1.0 ± 0.3^{a}
	10	4.03	−0.2 ± 0.3^{ab}	−0.5 ± 0.4^{ac}	0.1 ± 0.7^{a}	0.9 ± 0.1^{ae}	0.0 ± 1.5^{a}	0.4 ± 0.7^{a}	0.3 ± 0.8^{a}	1.5 ± 0.4^{a}
	15	5.98	0.6 ± 0.6^{a}	−0.9 ± 0.6^{acd}	0.6 ± 2.7^{a}	2.4 ± 1.2^{acde}	−0.9 ± 0.8^{a}	0.2 ± 0.6^{ab}	0.9 ± 1.1^{a}	1.6 ± 1.1^{a}
	20	7.93	0.0 ± 0.4^{ab}	−0.6 ± 0.3^{ac}	0.3 ± 1.4^{a}	1.4 ± 0.4^{ade}	−0.0 ± 0.6^{a}	−0.2 ± 0.3^{abc}	0.8 ± 0.1^{a}	1.0 ± 0.1^{a}
	30	11.83	−0.9 ± 0.9^{ab}	−0.6 ± 0.5^{ac}	−0.2 ± 1.9^{a}	2.1 ± 0.7^{acde}	0.4 ± 0.7^{a}	−0.1 ± 0.5^{abc}	0.3 ± 0.3^{a}	0.8 ± 0.5^{a}
10.8	1	0.64	0.4 ± 0.3^{a}	−0.4 ± 0.5^{ac}	0.2 ± 0.4^{a}	0.8 ± 0.2^{e}	0.5 ± 0.9^{a}	0.4 ± 0.2^{a}	0.6 ± 0.1^{a}	1.1 ± 0.3^{a}
	5	2.56	−0.6 ± 0.4^{ab}	−0.4 ± 0.1^{ac}	0.7 ± 0.3^{a}	0.9 ± 0.5^{ae}	0.1 ± 0.8^{a}	0.4 ± 0.3^{a}	0.6 ± 0.3^{a}	0.9 ± 0.5^{a}
	10	4.96	−1.3 ± 1.1^{ab}	−0.1 ± 0.5^{a}	0.9 ± 1.2^{a}	2.1 ± 0.4^{aecd}	1.1 ± 0.2^{a}	−0.3 ± 0.3^{abc}	−0.3 ± 0.4^{a}	1.2 ± 0.2^{a}
	15	7.36	1.1 ± 1.5^{a}	−4.8 ± 0.9^{bcde}	−0.5 ± 0.2^{a}	5.1 ± 1.2 ^{b}	0.4 ± 1.1^{a}	−0.4 ± 0.1^{abc}	−0.2 ± 0.1^{a}	0.9 ± 0.6^{a}
	20	9.76	−0.7 ± 1.1^{ab}	−2.6 ± 1.2^{ce}	−1.1 ± 0.5^{a}	3.1 ± 1.3^{ab}	0.3 ± 0.5^{a}	−0.1 ± 0.5^{abc}	0.3 ± 0.4^{a}	0.8 ± 0.4^{a}
	30	14.56	−2.6 ± 1.8^{b}	−0.9 ± 2.1^{acd}	−0.8 ± 0.7^{a}	3.7 ± 0.6^{cb}	0.9 ± 0.5^{a}	−0.7 ± 0.2^{abcd}	0.1 ± 0.2^{a}	1.2 ± 0.5^{a}
8.3	1	0.84	−0.6 ± 0.5^{ab}	0.2 ± 0.6^{a}	0.2 ± 0.9^{a}	1.1 ± 0.6^{ae}	0.1 ± 0.3^{a}	0.4 ± 0.5^{a}	0.4 ± 1.1^{a}	1.0 ± 0.7^{a}
	5	3.36	−0.9 ± 0.6^{ab}	−1.2 ± 0.5^{acde}	−0.5 ± 0.3^{a}	1.7 ± 0.3^{aecd}	−1.1 ± 0.6^{a}	0.1 ± 0.2^{ab}	0.6 ± 0.2^{a}	1.3 ± 0.6^{a}
	10	6.51	−0.4 ± 1.2^{ab}	−1.2 ± 0.4^{acde}	−0.1 ± 0.6^{a}	2.4 ± 0.1^{aecd}	−1.9 ± 1.4^{a}	0.2 ± 0.5^{ab}	0.7 ± 0.7^{a}	2.1 ± 1.5^{a}
	15	9.66	−1.7 ± 0.9^{ab}	−1.0 ± 0.8^{adc}	0.3 ± 0.4^{a}	2.2 ± 0.4^{aecd}	−0.7 ± 1.3^{a}	−1.2 ± 0.3^{bcd}	0.1 ± 0.3^{a}	1.7 ± 0.7^{a}
	20	12.81	−0.5 ± 0.9^{ab}	−3.3 ± 0.7^{dce}	−0.5 ± 0.4^{a}	3.5 ± 0.7^{dbc}	0.2 ± 0.3^{a}	−1.6 ± 0.4^{bcd}	−0.4 ± 0.2^{a}	1.7 ± 0.4^{a}
	30	19.11	−1.2 ± 0.5^{ab}	−3.5 ± 0.3^{ec}	−0.4 ± 0.4^{a}	3.8 ± 0.2^{cbd}	0.4 ± 0.4^{a}	−1.9 ± 0.9^{bcd}	−0.8 ± 1.3^{a}	2.4 ± 1.1^{a}

ΔL (lightness), Δa (redness), Δb (yellowness) and ΔE*ab were obtained by subtracting the mean value of three color measurements after PL treatment from the mean value of three color measurements before PL treatment
Data in the same column followed by the same lowercase superscript are not significantly different ($p > 0.05$).

Table 4
Effect of PL treatment on lipid peroxidation in pork skin and pork loin.

Treatment			Lipid peroxidation	
Distance (cm)	Time (s)	Fluence (J/cm^2)	Pork skin	Pork loin
			Day 0	Day 0
Control			0.044 ± 0.01^{ab}	0.014 ± 0.001^{ab}
13.4	1	0.52	0.062 ± 0.01^{ad}	0.020 ± 0.01^{a}
	15	5.98	0.042 ± 0.01^{ab}	0.017 ± 0.003^{ab}
	30	11.83	0.065 ± 0.01^{ad}	0.016 ± 0.003^{ab}
10.8	1	0.64	0.034 ± 0.01^{b}	0.012 ± 0.002^{ab}
	15	7.36	0.064 ± 0.01^{ad}	0.016 ± 0.001^{ab}
	30	14.56	0.110 ± 0.02^{c}	0.017 ± 0.001^{ab}
8.3	1	0.84	0.077 ± 0.01^{d}	0.015 ± 0.01^{ab}
	15	9.66	0.073 ± 0.01^{d}	0.013 ± 0.002^{ab}
	30	19.11	0.044 ± 0.01^{a}	0.011 ± 0.001^{b}

Data represent mean MDA concentrations (μg/g) of five replicates ± standard deviations.
Data in the same column followed by the same lowercase superscript are not significantly different ($p > 0.05$).

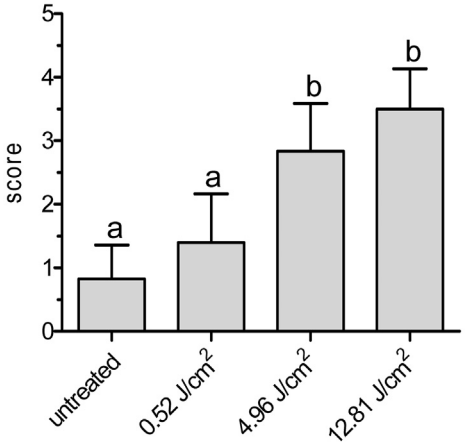

Fig. 1. Sensory analysis for pork skin.

skin). Treated samples, however, were always distinctly recognized, regardless of the treatment intensity, because the odor quality changed to chemical (ozone-like), pungent and unpleasant. However, treatment at 0.52 J/cm^2 rendered samples less fatty or less porky. As these values only indicate a slight odor change and score (1.4) and were minimally higher than that of untreated controls this could not be confirmed by statistical analysis where scores were compared using ANOVA. A statistically significant difference could only be found between controls and samples treated at 4.96, 12.91 J/cm^2 but not between the controls and 0.52 J/cm^2. When comparing samples treated at 4.96 and 12.81 J/cm^2, off-odor was more unpleasant at 4.96 J/cm^2, but at 12.81 J/cm^2 panelists regarded the off-odor as more complex and somewhat sweet.

Our results for loin (Fig. 2 and Table 5) are mostly comparable to those for skin except for untreated samples, which were described as slightly acidic. Odor at 0.52 J/cm^2 was already unpleasant and chemical. Again no statistical difference could be found between controls (score: 1.4) and samples treated at 0.52 J/cm^2 (score: 1.8): as scores were too similar.

Other authors having treated meat matrices with PL made diverse observations concerning odor. Hierro et al. (2011) and Ganan et al. (2013) who treated ready-to-eat cooked and cured meat either did not find any significant differences or only above fluences of 4.2 and 11.9 J/cm^2, respectively. Thus, much higher fluences were required to induce off-odor than in our study. Ganan et al. (2013) postulate a reason for this observation could be that processed meat products are less susceptible to PL, due to the stronger flavor induced by the addition of fat, salt, nitrite/nitrate and spices.

Panelists evaluating PL treated raw meat described samples as having a loss of freshness, but still acceptable (Hierro et al., 2012) or by a strong cooked smell (Nicorescu et al., 2014), but this also required higher fluences than in our trials (8.4 J/cm^2; 30 J/cm^2). Pasceviciute et al. (2011) noticed moderate odor changes in raw chicken meat. An extensive study concerning odor change in raw meat by PL was conducted by Tomasevic (2015) resulting in decreased odor scores for beef, chicken, turkey, deer and rabbit after one pulse. Pork and kangaroo

Table 5
Effect of PL treatment on odor of pork skin and pork loin.

Odor	Fluence (J/cm^2)			
	Untreated	0.52	4.96	12.81
Pork skin	Characteristically porky, greasy, fatty, slightly like chlorine	Less porky, less fatty	Unpleasant, chemical, pungent, sharp, ozoneous	Less unpleasant but more complex than after 4.96 J/cm^2, sweet, strongly chemical, strongly ozoneous
Pork loin	Characteristically like pork meat, slightly acidic	Less porky, slightly chemical	Chemical, pungent, musty, ammoniacal	Strong off-odor, strongly chemical, slightly roasted or burnt, sweet, faecal, pungent

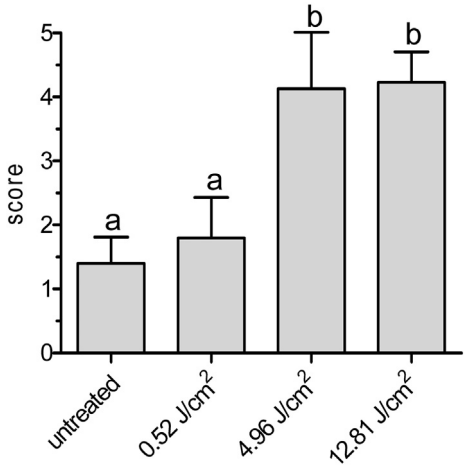

Fig. 2. Sensory analysis for pork loin.

were less sensitive to PL. This is in contrast to our study where pork loin smelled unpleasant even at a low fluence.

Possibly, these disparate findings are caused by the usage of different PL systems and, thus, different lamp systems and ozone formation. Since odor changes were highly undesirable, measures should be taken to avoid their occurrence. Several ways are conceivable to achieve this goal; one way of preventing ozone formation is the use of PL systems with a minimum wave-length of 200 nm, as only very short wave-lengths (< 200 nm) possess enough energy to turn oxygen molecules into ozone.

4. Conclusions

The impact of PL technology on the reduction of *Salmonella* Typhimurium and *Yersinia enterocolitica* on pork skin and loin, as well as on chemical and sensorial properties was investigated. Treatments did not cause temperatures higher than 45 °C (except for 8.3 cm, 30 s on pork skin), so that microbial reductions can solely be associated with photochemical effects. The tested parameter combinations resulted in pathogen reductions on loin of approximately 1 log, but this did not prove to be significant, probably due to its surface topography. In contrast, significant reductions (~2–4.37 log) were achieved on pork skin, however, the high standard deviations may lead to the assumption that PL could only be used to reduce microbial load but not to guarantee full product safety as reductions cannot be reliably predicted. The applied fluences were not satisfying from a sensorial point of view and could therefore not be applied on a real industrial scale at this point. Only a fluence of 0.52 J/cm^2 led to a less fatty and less porky smell in pork skin, which was perceived as pleasant However, since there are very high standard deviations concerning microbial reductions this might still not be the optimal setting. Fluences ≥7.36 J/cm^2 resulted in visible change in color parameters in pork skin and energies ≥9.66 J/cm^2 provoked a decrease of red color in pork loin samples. A significant rise in lipid peroxidation could not be observed.

According to the data PL is not a suitable treatment for pork loin, but could be an option for pork skin decontamination. As contact- and residue free, fast and water-saving technology it might be an interesting alternative to the decontamination methods currently approved in the EU (water and lactic acid) as its reduction results are comparable. Low fluences (0.52 J/cm^2) should be used for an appropriate product quality At the moment the high investment costs and shadow effects on opaque nature foods as well as undesirable odor deviations are major downsides of the PL treatment. Further studies need to be performed in order to investigate optimal parameter combinations, nutritional and toxicological aspects as well as the risk of VBNC cells for human health.

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Conflict of interest

None.

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