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The effect of pulsed UV light on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Staphylococcus aureus* and staphylococcal enterotoxin A on sliced fermented salami and its chemical quality

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ABSTRACT

Pulsed UV light (PL) applied at a fluence of 3 J/cm^2 was effective to reduce *Listeria monocytogenes*, *Escherichia coli* 0157:H7, *Salmonella* Typhimurium and *Staphylococcus aureus* for 2.24, 2.29, 2.25 and 2.12 log CFU/g on the surface of dry fermented salami. Further increase in the fluence of PL treatment did not increase levels of microbial inactivation. However, the time interval between the contamination and PL treatment was found to have a significant impact on the efficacy of PL treatment and should be kept as short as possible. After initial PL treatment slices of fermented salami were packed in vacuum or in 80% CO_2/20%N_2 modified atmosphere and stored at 4 °C to investigate the effect of PL treatment on protein and lipid oxidation as the shelf life of fermented salami is not usually limited by microbial deterioration, but by chemical and sensory alterations. In this study observed lipid oxidation values for PL treated vacuum and modified atmosphere packed fermented salami slices fall within the acceptable threshold for the rancid odor, except for the sample treated with the highest fluence tested (15 J/cm^2), packed in modified atmosphere and kept in cold storage for 9 weeks (1.23 mg MDA/kg). All values were below the threshold for rancid flavor, too. The significant rise in protein oxidation of PL treated fermented salami slices, perceived as 28% increase of carbonyl content compared to untreated samples, was observed only after 9 weeks of cold storage in both vacuum and modified atmosphere packed samples. The results of chemical analysis are in agreement with previously published results of sensory analysis. Current results show the applicability of PL to improve microbial safety of sliced fermented salami that are prone to cross-contamination without affecting quality attributes by lipid and protein oxidation.

1. Introduction

The primary reason for fermentation of meats was to extend the shelf life of these highly valued, often traditional, but also perishable foods. A variety of procedures for producing stable, fermented meat sausages have been developed around the world. In addition to fermentation, sausage processing may also include curing, smoking, drying, and aging to improve both the flavor and the shelf life. Fermented sausages are prepared by mixing ground meat with various combinations of spices, flavorings, salt, sugar, additives, and frequently, starter cultures. The meat is generally used raw, with no heat processing, and fermented sausages are ready-to-eat, eaten without prior cooking (Rahman, 2007). The later fact, combined with modern consumer demand for convenience food products, such as sliced and packaged Ready-to-Eat (RTE) fermented sausage (Cabeza, de la Hoz, Velasco, Cambero, & Ordóñez, 2009) opens new contamination routes for foodborne pathogens. Processing operations, such as cutting, slicing and packing increase the (surface) (cross) contamination risks. Pathogenic microorganisms originating from the processing environment, food contact materials and personnel can contaminate the final product (Gil-Díaz, Santos-Delgado, Rubio-Barroso, & Polo-Díez, 2009; Uyttendaele et al.,

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2004). Depending on intrinsic and extrinsic product characteristics, as well as the properties of contaminating organisms the later may multiply or remain at risk-posing levels during the shelf-life. Applying usual inactivation technologies to decontaminate sliced and packed RTE dry fermented sausages is not possible and therefore shelf life extension, together with microbial safety, requires additional hurdles to be implemented. To match these demands in the last decades alternative non-thermal preservation technologies such as light pulses, high hydrostatic pressure, and natural biopreservatives have been proposed and further investigated (Aymerich, Picouet, & Monfort, 2008). Packaging in modified atmospheres (vacuum and modified gas packaging) allows increases in the shelf life as well as attractive commercialization formats such as packaged sliced fermented meat products (Rubio, Martínez, García-Cachán, Rovira, & Jaime, 2008). Recommendation of storage temperature for dry fermented sausages is up to 10 °C (Kamenik et al., 2012). The shelf life of packaged sliced dry fermented sausage, limited by sensory changes rather than microbiological spoilage, was even under 100% nitrogen inert atmosphere (gas with no direct antimicrobial properties) longer than 120 days while in vacuum it was 95 days when stored at 4 °C. The shelf-life was reduced to 30 and 40 days at 22 °C for the samples packaged in vacuum and N2 atmosphere, respectively (Ščetar, Kovačić, Kurek, & Galić, 2013). In the extended shelf life even a minor growth of highly virulent foodborne pathogens may pose a risk to public health, without producing sensory warning to the consumer.

Pulsed UV light (PL) has been shown to be an effective way to eliminate pathogens in various types of foods (Condón, Álvarez, & Gayán, 2014; Gómez-López, Ragaert, Debevere, & Devlieghere, 2007; Keklik, Krishnamurthy, & Demirci, 2012; Rajkovic, Smigic, & Devlieghere, 2010), but its antimicrobial efficacy or effects on quality attributes of meat (Hierro, Ganan, Barroso, & Fernández, 2012; Keklik, Demirci, & Puri, 2010), meat products (Hierro et al., 2011; Wambura & Verghese, 2011) and meat contact surfaces reported by Rajkovic et al. (2010) were investigated only on few occasions. Ganan, Hierro, Hospital, Barroso, and Fernández (2013) reported reductions of up to 1.5 and 1.8 log CFU/cm^2 for *Listeria monocytogenes* and *Salmonella* Typhimurium when a fluence of 11.9 J/cm^2 was applied on ready-to-eat cured meat products. Only limited differences in the instrumental color parameters were observed, although no significant and lasting changes in the sensory analysis were detected. Also RTE cooked meat products, such as vacuum-packaged ham and bologna slices were treated with 8.4 J/cm^2 PL resulting in reduction of inoculated *L. monocytogenes* by 1.78 CFU/cm^2 in cooked ham and by 1.11 CFU/cm^2 in bologna. The PL treatment of 8.4 J/cm^2 had no significant impact on sensory properties of cooked ham, while treatments above 2.1 J/cm^2 negatively influenced theory properties of bologna (Hierro et al., 2011).

Different types of fermented sausages have been implicated in alerts of Rapid Alert System for Food and Feed of European Commission including pathogens like *L. monocytogenes*, *Salmonella* spp., *Escherichia coli* VTEC, *Clostridium botulinum* (RASFF https://webgate.ec.europa.eu/rasff-window/portal, accessed on 02.09. 2016), but also in a number of foodborne outbreaks, such as those caused by *E. coli* VTEC (Jureidini et al., 1997; Paton et al., 1996; Sartz et al., 2008), *Salmonella spp.* (Bremer et al., 2004), and *Staphylococcus aureus* (Lücke, 1997). *S. aureus, which* is a common bacterium found in chopped meat mixes and often transmitted as cross contamination agent due to the fact that it is carried by many healthy individuals, is also salt and nitrite tolerant and therefore able to grow and produce enterotoxin during initial stages of fermentation (Akkaya, Gok, Kara, & Yaman, 2014; Diez & Patarata, 2013; Marcy, Kraft, Olson, Walker, & Hotchkiss, 1985). The identification of dry-fermented sausages as a vehicle of infection for verotoxigenic *Escherichia coli* (VTEC) was acknowledged on numerous previous occasions (Muthukumarasamy & Holley, 2007; Riordan et al., 1998; Ross, Zhang, & McQuestin, 2008), while microbiologically safe dry-fermented sausages produced without a processing step to eliminate VTEC have been difficult to obtain. Growth and enterotoxin production of *S. aureus* have been reported in different fermented sausages (Akkaya et al., 2014). However, most staphylococcal strains initiated growth and produced detectable enterotoxin aerobically, while anaerobically most strains failed to produce detectable enterotoxin below pH 5.7 (Barber & Deibel, 1972). Considering reported prevalence, pathogen and product characteristics, exposure to the agent, severity of the disease, and epidemiological data, the main risks to the consumer of fermented sausages are salmonellosis, infections caused by enterohemorrhagic *E. coli*, and *S. aureus* enterotoxicosis. Although there is no epidemiological evidence for the involvement of fermented sausages in outbreaks of listeriosis (B. M. Lund & Hunter, 2008), *L. monocytogenes* is a pathogen of concern in fermented meats due its wide distribution on meat and its ability to grow at low temperatures and relatively low water activity (>0.93) (De Cesare, Mioni, & Manfreda, 2007).

Implementation of strategies that provide effective reduction of these pathogens in the manufacturing process of RTE dry fermented sausages is therefore essential. This study represents an investigation of the effects of PL on microbial safety of RTE dry fermented sausage in the type of salami, as well as on quality attributes of lipid and protein oxidation changes during the cold storage (+4 °C) under vacuum and modified atmosphere conditions during period of 60 days.

2. Materials and methods

2.1. Pulsed UV light (PL) equipment

The PL unit used was a Tecum—Mobile Decontamination Unit (Claranor, Manosque, France). Light pulse duration of 300 μs and pulse intensity of 3 J/cm^2 were used for an input voltage of 3000 V. The lamps were 20 cm cylindrical xenon flash lamps (Flashlamps Verre & Quartz, Bondy, France), with the spectral distribution of the light as reported in Rajkovic et al. (2010) and the arc length produced by the flash lamp of 175 mm. PL unit was laboratory scale four-lamp batch system. Top and bottom lamp were set at 6 cm and left and right hand lamp at 10 cm from the treated surface of the fermented salami. Both side lamps were elevated 5° above the horizontal line to allow wider side-beam distribution over the treated fermented salami surface. The setup of the experimental equipment is visualized in Fig. 1. The delivered energy was measured with SOLO 2 — Power and Energy Meter (Gentec Electro-Optics, Inc., Quebec, Canada) where the certified corrections are very low: for a broadband spectrum and are always inferior to 5%. Particularly the overestimation in the UV range is lower than 5%. This error is inferior to the energy variation produced (Rajkovic et al., 2010).

2.2. Bacterial strains and culture conditions

At least four strains from an in-house culture collection of each species were initially tested for their growth at room temperature mimicking conditions of temperature abuse, (22 °C, 24 h, initial inoculum 3 log CFU/ml in TSB). As no statistical difference in final counts achieved by each tested strain ($p > 0.05$, ANOVA) for further PL experiments strains implicated in foodborne outbreaks were selected. The microorganisms screened and used in this study are summarized in Table 1 together with typical incubation