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Effects of pulsed light on the organoleptic properties and shelf-life extension of pork and salmon

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ABSTRACT

The aim of our study was to assess the impacts of a pulsed light (PL) technology on the shelf-life, microbial inactivation, chemical and sensorial qualities of raw pork roast (RPR), roast pork (RP) and raw salmon (RS). In the first step, the effects of PL on aerobic flora and *Pseudomonas fluorescens* were investigated. Afterwards samples were evaluated in terms of their colour, shelf-life and lipid peroxidation. A maximum rate of microbial inactivation, 3.4 log (CFU/g), was found in the case of RP samples and was accompanied by improved shelf-life. Significant colour changes were found for the RP and RPR samples exposed to PL dose of 30 J cm^{-2}. The PL treatments at 3 and 10 J cm^{-2} did not induce the lipid peroxidation. Malondialdehyde (MDA) content substantially increased in RS and RP samples submitted to 30 J cm^{-2}, i.e. by 39.3% and 25.5%, respectively. In conclusion, PL technology has a potential for inactivation of both, aerobic flora and *P. fluorescens*, however moderate fluencies has to be applied in order to obtain satisfying level of decontamination and high product quality.

1. Introduction

Meat and fish are rich sources of high quality nutrients characterized by high digestibility. They are well known as an important source of omega-3 fatty acids, vitamin B12, protein and highly bioavailable iron (Verma & Banerjee, 2010). These rich nutrients provide an ideal environment for the growth of spoilage microorganisms and food-borne pathogens (Shekhar & Kumar, 2005). Indeed, meat and fish are highly perishable products (Kumar, Sharma, & Kumar, 2010) and microbial spoilage lead to the wastage of precious biological material as well as disease outbreaks. Therefore prevention of the microbial growth in meat and fish is essential for the safety of the consumers (Aymerich, Picoue, & Monfort, 2008).

In the recent years, different non-thermal technologies have emerged for the decontamination of food products. Among them, pulsed light (PL) process showed to be effective in inactivating a wide range of microorganisms (vegetative bacteria, moulds, bacterial, fungal spores…) involved in food products spoilage (Arrowood, Xie, Rieger, & Dunn, 1996; Gómez-López, Devlieghere, Bonduelle, & Debevere, 2005; Nicorescu et al., 2013; Rajkovic, Smigic, & Devlieghere, 2010; Turtoi & Nicolau, 2007). Indeed, recent studies have demonstrated the efficacy of PL to inactivate microorganisms on the surface of several food products, such as seafood (Dunn, Ott, & Clark, 1995), salmon filets (Ozer & Demirci, 2006), ready-to eat sausages (Uesugi & Moraru, 2009) and chicken breast (Keklik, Demirci, & Puri, 2010). Ozer and Demirci (2006) reported 1 log reduction of *Escherichia coli* O157:H7 or *Listeria monocytogenes* in PL treated salmon fillets at fluence of 5.6 J cm^{-2}. Higher rates of the microbial reductions were achieved in the case of meat samples. For instance Dunn et al., (1995) reported 2 log reductions in microbial count in beef steaks submitted to PL treatment at 5 J cm^{-2}. Same authors obtained 2 log cycle reduction of *Listeria innocua* in PL-treated frankfurters at 30 J cm^{-2}. Keklik et al., (2010) achieved 2 log reduction of *Salmonella Typhimurium* on boneless chicken breast. Despite these contributions, informations concerning the PL effects on the sensorial properties of raw meat and fish are still very scarce (Hierro et al., 2011; Hierro, Ganan, Barroso, & Fernández, 2012).

Therefore the aim of our study was to investigate the PL effects on the decontamination of both aerobic flora and *Pseudomonas fluorescens* inoculated on pork and salmon and also monitoring the modification of their organoleptic properties in time.

2. Materials and methods

2.1. Samples description

Raw pork roast (RPR), roast pork (RP) and raw salmon (RS) were purchased in a local supermarket. Once in the laboratory, samples were stored at 4 °C until performance of the experiments.

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2.2. Bacterial strain and cultivation conditions

P. fluorescens MF37 strain was used in this study to inoculate the surface of fish and meat samples. The MF37 strain is a spontaneous rifampicin-resistant mutant of the strain MF0 which was isolated from raw milk (Guyard-Nicodème et al., 2008). *P. fluorescens* MF37 was grown at 28 °C for 24 h in M17 medium (Merck, Germany) supplemented with 0.5% glucose, under vigorous shaking (180 rpm). Cultures were centrifuged at 10,000 *g* for 20 min at 20 °C and then bacterial pellet was re-suspended with 0.9% saline solution in order to give a concentration of 10^6 log CFU/mL. The concentration of the stock solution was previously checked plating and counting on PCA medium.

2.3. Samples inoculation with P. fluorescens vegetative cells

Squares of RPR (2 × 2 × 0.5 cm), RP (2 × 2 × 0.5 cm) and RS (2 × 2 × 0.5 cm) were placed on an aluminium foil in sterile conditions. Samples were inoculated with *P. fluorescens* MF37 by spraying their surface with stock solution (10^6 log CFU/mL). Sprays were applied over the entire surface of the samples without soaking them. A plastic stencil was used in order to avoid contamination of the lateral sides of the sample. The inoculated samples were transferred to sterile Petri dishes and immediately submitted to pulsed light treatment. The initial concentration of *P. fluorescens* MF37 on samples surface was of 5 log CFU/g.

2.4. Pulsed light treatment

The PL treatments were performed using pilot-scale experimental set-up provided by Claranor (France). The system was composed of a power supply unit and a treatment chamber (Nicorescu et al., 2013). The treatment chamber housed four cylindrical xenon lamps that emitted light flashes in a broad range of wavelengths from 200 to 1100 nm.

The RPR, RP and RS samples were exposed to PL in order to evaluate the light effects on microbial population (naturally occurring aerobic flora, inoculated *P. fluorescens* MF37), physical and chemical properties. In each test type square of a given sample (RPR, RP, RS) was placed on the Petri dish at 3 cm beneath the xenon lamps. During treatment sample was exposed to 3, 10 or 30 pulses of light, at a pulse repetition rate of 1 Hz and a pulse width of 300 μs. The energy levels of PL light for each treatment conditions were measured using a calorimeter Solo-2 (Gentec-EO, Canada). The exposures to 3, 10 and 30 pulses resulted in fluencies of 3, 10 and 30 J cm^{-2}, respectively. All treatment conditions for each test type were done in triplicate.

2.5. Microbiological analysis

a) PL effects on aerobic flora

To analyse the PL effects on aerobic flora naturally present on RPR, RP and RS, squares of 5 g of each non-treated and treated sample were re-suspended in peptone salt (PS) medium (AES, France) and shaken for 1 min to reconstitute the cellular suspension. Decimal dilutions were spread in triplicate on Plate Count Agar (PCA) medium (AES, France). Plates were incubated for 72 h at 30 °C before colonies enumeration.

b) PL effects on *P. fluorescens* MF37

Non-treated and PL-treated samples inoculated with *P. fluorescens* MF37 were homogenized for 1 min in a stomacher (Lab-Blender 400, Bioblock, UK) with peptone salt medium (AES, France). Decimal dilutions were spread in triplicate on PCA medium (AES, France) in presence of 20 μg/mL rifampicin. Plates were incubated for 24 h at 28 °C and then colonies were counted.

2.6. Lipid peroxidation (TBARS test)

To ensure the chemical stability of samples, thiobarbituric acid-reactive substances (TBARS) method (Ouattara, Giroux, Smoragiewicz, Saucier, & Lacroix, 2002) was used to determine the effects of PL treatments on lipid peroxidation. The principle of this method is based on the reaction between 1 molecule of malondialdehyde (MDA) and 2 molecules of thiobarbituric acid (TBA). Red coloured malondialdehyde-TBA complex is formed as product, which can be quantified by spectrophotometric assay. The following procedure was used for this test:

10 g of non-treated and PL treated samples were homogenized for 1 min in a stomacher (Lab-Blender 400, Bioblock, UK) with 50 mL distilled deionized water and 10 mL of 15% (w/v) trichloroacetic acid (VWR Intl., Belgique). The homogenate was filtered using Whatman filter paper n°4 (VWR, France) and obtained filtrate was filtered again through a 0.45 μm syringe filter (Millipore, Whatman, Germany). 8 mL of the final filtrate was then added to the test tubes containing 2 mL of 0.06 M TBA (Labosi, France). The test tubes were incubated at 80 °C for 90 min in a water bath and then cooled to room temperature. Assays absorbencies were read at 520 nm using a spectrophotometer (Thermo Spectronic™, USA).

The malondialdehyde (MDA) content was calculated from a standard curve prepared by using serial dilutions of 1, 1, 3, 3-tetraethoxypropane (TEP) (Sigma – Aldrich, China) (Lawlor, Sheehy, Kerry, Buckley, & Morrissey, 2000) and expressed as μgMDA/10 g meat. First, 3.5 × 10^{-5} M solution was prepared by diluting 3.5 × 10^{-4} M stock solution of 1, 1, 3, 3-TEP. Next, aliquots of the solution (0, 0.3, 0.6, 1.0, 1.3 and 1.6 mL) were diluted to 10 mL with deionized water. Five milliliters of each dilution were then added to screw-capped test tubes containing 5 mL of 0.02 M TBA in 10% (v/v) glacial acetic acid. To initiate the reaction leading to the formation of TBA_2-MDA complex, tubes were placed in boiling water for 15 min. Reaction was stopped by cooling to the room temperature, and then absorbencies were read at 520 nm. The calibration curve was plotted as absorbance in function of working solution concentration.

2.7. Colour measurements

Colour measurements of non-treated and PL-treated samples were performed using a CR-200 Minolta colorimeter (Tokyo, Japan). Analyses were performed immediately after PL treatment (d_0) and during the storage period at 4 °C. The instrument was calibrated against a ceramic reference prior to use. The sample colour was described by three CIELAB parameters: L^* (lightness), a^* (redness), and b^* (yellowness) (Hunter, 2006). On the basis of experimentally obtained parameters, the total colour difference (ΔE^*) between non-treated and PL-treated samples was calculated according to the following equation:

$$\Delta E^* = \sqrt{\left(L^*_{\text{treated}} - L^*_{\text{nontreated}}\right)^2 + \left(a^*_{\text{treated}} - a^*_{\text{nontreated}}\right)^2 + \left(b^*_{\text{treated}} - b^*_{\text{nontreated}}\right)^2}$$