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High pressure carbon dioxide on pork raw meat: Inactivation of mesophilic bacteria and effects on colour properties

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ABSTRACT

The objective of the present study was the evaluation of the effectiveness of High Pressure Carbon Dioxide ($HPCO_2$) as a non-thermal technology for the pasteurization of porcine raw meat. The inactivation kinetics of mesophilic bacteria were determined at 25, 35 and 40 °C, from 6 to 16 MPa and from 5 to 60 min. The effects of $HPCO_2$ on the colour parameters in terms of L^*, a^*, b^* and ΔE during and after the treatment were investigated by means of a spectroscopic apparatus. Data demonstrated that $HPCO_2$ treatment assured at least 2 Log (CFU/cm^2) reduction of mesophilic microorganisms, but induced significant colours alterations: lightness increased and redness decreased and the samples turned whitish and looked like "cooked". The technology could hardly be exploited at industrial scale for raw meat pasteurization due to the colour modifications induced to the product.

1. Introduction

Raw meat is one of the most perishable foods: its rich physic-chemical composition favours the microbial growth to unacceptable levels (Gram et al., 2002). In an intact piece, microorganisms are usually on the surface, while the interior is sterile. The initial bacterial load of meat depends on the physiological status of the animal and the spread of contamination into slaughterhouses and during processing (Bae et al., 2010). The survival and growth rates of specific spoilage microorganisms can be affected by different factors, including meat constituents and enzymes, temperature, pH, oxygen, light, moisture and other competing microbes (Nychas et al., 2008).

Traditionally, methods of meat preservation may be grouped into three broad categories based on temperature, moisture and, more directly, inhibitory processes (bactericidal and bacteriostatic) (Zhou et al., 2010). With the increased demand for high quality, convenience, safety, fresh appearance and extended shelf life of fresh meat products, alternative non-thermal preservation technologies have been deeply explored.

Among others, High Pressure Carbon Dioxide ($HPCO_2$) has been increasingly investigated as a technique able to induce the inactivation of the natural microbial flora but also pathogens in solid and liquid matrices. Theories explaining the inactivation mechanism of $HPCO_2$ were described in many previous works (Garcìa-Gonzalez et al., 2008). The process has been applied to liquid substrates with promising results, but few papers reported the effects of $HPCO_2$ processing on solid foods due to the complexity of the matrix that makes CO_2 bactericidal action more difficult (Ferrentino and Spilimbergo, 2011). Under supercritical conditions, CO_2 exhibits interesting physical properties having the ability to diffuse through solids like a gas and dissolve materials like a liquid thus enhancing its pasteurization efficiency (Garcìa-Gonzalez et al., 2008).

Sirisee et al. (1998) tested the tolerance of *Escherichia coli* and *Staphylococcus aureus* in both ground beef and liquid phosphate buffer to $HPCO_2$ treatment (42.5 °C and 31.03 MPa): 1 Log reduction in ground beef took 178 min, but only 1.7 min was needed in the liquid substrate. The presence of carbohydrates, fats and proteins could play an essential role in protecting microorganisms from CO_2 bactericidal action. According to these authors, the colour of ground beef changed after the process and looked like cooked ground beef. Wei et al. (1991) applied the treatment to chicken meat strips to inhibit the pathogens *Salmonella* and *Listeria*: samples treated at 13.7 MPa, 35 °C for 2 h presented an inactivation of 94–98% and 79–84%, respectively, but also in this case the samples turned whitish and seemed to be cooked. Choi et al. (2008) demonstrated that the $HPCO_2$ process (7.4 MPa, 31 °C for 10 min) led to a sarcoplasmic protein denaturation of porcine muscle with a higher lightness and a lower redness. To our knowledge no studies have been performed neither on $HPCO_2$ pasteurization of raw pork meat or on the measurement of the colour changes of meat during the pasteurization process.

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Based on this scenario, the main goals of the present work are: (i) to investigate the feasibility of $HPCO_2$ on fresh porcine meat for the inactivation of mesophilic bacteria as a function of the main process parameters (temperature, pressure and time) and (ii) to monitor the colour of the sample before, during and after the treatment by using an on-line spectroscopic technique.

2. Materials and methods

2.1. Sample preparation

Slices of raw pork meat were purchased daily from a local market. Pieces of 2 cm^2 and $3.5 \times 3.5 \times 1$ cm were cut for microbiological and colour analyses, respectively.

2.2. High pressure apparatus

Liquid CO_2 (Carbon Dioxide 4.0, purity 99.990%; Messer Group GmbH, Bad Soden, Germany) was pumped by using a volumetric pump (mod. LCD1/M910s; LEWA GmbH, Leonberg, Germany) with a maximum flow rate of 11 L/h into 10 identical stainless steel reactors, connected in parallel (total volume 15 mL), where the samples were previously loaded. Each reactor was connected to an on–off valve which could be used to depressurize it independently from the others. The reactors were submerged in a single temperature-controlled water bath to keep a constant and uniform temperature. Before the compression, the gas was cooled down to about 3 ± 1 °C by means of a cooling system. A thermo-controller, connected with a temperature probe (Pt 100Ω; Endress + Hauser, Milan, Italy) inside the water bath, allowed to keep the temperature at a constant set-up value. The operating parameters (temperature, pressure and time) were continuously recorded by a real-time data acquisition system (field point FP-1000 RS 232/RS 485; National Instruments, Austin, TX, USA) and monitored by specific software (LabVIEW™ 5.0). More details of the procedure for pasteurization were described in previous works published by the authors (Ferrentino et al., 2012b).

2.3. Process conditions

Different values of pressure, temperature and time were screened to investigate the process conditions to assure a satisfactory level of microbial inactivation. The process conditions tested were: temperatures of 25, 35 and 40 °C, pressures of 6, 8, 12 and 16 MPa, and treatment times of 5, 10, 20, 30, 45 and 60 min.

2.4. Microbiological analysis

The standard plate count method was used to determine the initial microbial load and the effectiveness of the treatment in reducing the number of mesophilic microorganisms naturally present on the surface of the meat. The samples were treated, mixed in a sterile vial with 4 ml of Phosphate Buffer Solution (PBS, pH 7.4) and subsequently stomached at 230 rpm for 2 min (Stomacher 400; International P.B.I., Milan, Italy). The homogenate was serially diluted in PBS and plated onto Plate Count Agar medium (Liofilchem, Italy). At the end of the incubation period (48 h, 30 ± 2 °C), the number of colonies was counted and the degree of inactivation was determined by evaluating the Log (N/N_0) versus time, where N_0 (CFU/cm^2) was the number of microorganisms initially present on the untreated sample and N (CFU/cm^2) was the number of survivors after the treatment. Two independent experiments were carried out for each experimental condition and the results were reported as mean values and standard deviations. The initial microbial load of the samples was 5.18 ± 0.83 Log (CFU/cm^2).

2.5. Colour monitoring system

The colour of the samples was measured with a spectroscopic apparatus (Ferrentino et al., 2012a, 2012b). The system consisted of a high-resolution miniature spectrometer (HR2000+; Ocean Optics Inc., Dunedin, FL, USA) to which a fiber optic reflection probe (Ocean Optics Inc.) was connected. The probe transmitted the light from a halogen lamp to the sample by the illuminating fibers, while the reflected light from the sample was acquired by the reading fiber and measured by the spectrometer (Apruzzese et al., 2000). After the calibration of the signal, the sample was placed inside the vessel (internal volume of 310 ml) on a holding device, the reactor tightly closed, and the optic probe fixed vertically onto the cover lid of the high-pressure apparatus. The reflectance spectrum was continuously acquired and monitored during the process by a specific software (Spectra Suite®, Ocean Optics Inc.), providing L^* (lightness), a^* (redness), and b^* (yellowness) parameters. Colour measurements were performed in duplicate and the mean values and standard deviations were evaluated. To quantify colour differences, the ΔE values were analyzed based on the equation of Hunter and Harold (Hunter and Harold, 1975). For the calculation of the on-line ΔE values, colour differences were expressed between the values obtained at 1 min and those acquired at 5, 10, 20, 30, 45 and 60 min, while the off-line ΔE values were evaluated taking into account the L^*, a^*, b^* parameters measured before and after the treatment. More details of the procedure were reported in previous works (Ferrentino et al., 2012a).

3. Results and discussion

3.1. Microbial inactivation kinetics

Fig. 1 showed the inactivation kinetics of mesophilic microorganisms as a function of pressures (6, 8, 12, and 16 MPa) and treatment times (from 5 to 60 min) both at 25 and 35 °C.

In agreement with several studies (Garcìa-Gonzalez et al., 2008), the increase of temperature was found to have a beneficial effect on the inhibition rate: data obtained at 25 °C and 6 MPa showed about 2 Log reductions achieved in 60 min (Fig. 1a), while at 35 °C a shorter treatment time, 45 min, was needed to reach the same inactivation level (Fig. 1b). Besides the impact that the thermal treatment itself has on the inhibition of microorganisms, high temperatures stimulates the diffusion of CO_2 and increases the fluidity of cell membranes to facilitate CO_2 penetration, causing several metabolic alterations responsible for cellular death (Garcìa-Gonzalez et al., 2008).

As concerns the effect of pressure, it was demonstrated that an increase from 6 to 16 MPa allowed to enhance, but not significantly, the microbial inactivation: at a fixed temperature of 35 °C, 60 min were needed to achieve about 3 Log reductions at 8, 12 or 16 MPa (Fig. 1b). It is well known that higher pressures permit a significant increase in density and solvation power of CO_2, which, in turns, promotes the contact with the cells, inducing the removal of vital constituents from cells or cell membranes (Damar et al., 2009). However, this increase is limited by the saturation solubility of CO_2: once the treatment medium is saturated with CO_2, the bactericidal effect did not change significantly. Our results were justified considering that above 10 MPa, CO_2 solubility is a weak function of the pressure (Dodds et al., 1956).

To deeply investigate the effect of temperature, further experiments were performed at 40 °C keeping the pressure at 6 and 8 MPa. Compared to lower temperatures, 20 min at 6 MPa were sufficient to reduce about 2 Log of mesophilic bacteria, while the increase of the pressure from 6 to 8 MPa did not induce any further inactivation (data not shown).