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A combined high pressure carbon dioxide and high power ultrasound treatment for the microbial stabilization of cooked ham

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ABSTRACT

The feasibility of a combined treatment based on high pressure carbon dioxide and high power ultrasound (HPCD + HPU) technologies was investigated for the microbial stabilization of cooked ham and compared with the one of HPCD alone. Inactivation kinetics of the natural microbiota (mesophilic microorganisms, lactic acid bacteria, yeasts and molds) were determined at 12 MPa, 40 and 45 °C, 10 and 20 W (delivered continuously or every 2 min during the combined treatment) from 2 up to 15 min. Additionally, a shelf life study of the product treated at the optimal process conditions was performed for 4 weeks at 4 °C to evaluate the effects of the combined process on its quality (pH, acidity, color and texture) and long term stability.

At 12 MPa, 45 °C and 10 W delivered every 2 min of treatment, inactivation to undetectable levels of lactic acid bacteria, yeasts and molds was achieved after 15 and 4 min while 4 log reductions of mesophilic microorganisms was obtained after 15 min of the combined treatment. On the contrary, HPCD performed alone induced the same inactivation degree of mesophilic and lactic acid bacteria increasing the treatment time to 60 and 45 min, respectively. The microbial stability of the product was assured up to 3 weeks of storage. The analysis of the quality attributes revealed that the pH, acidity and texture remained stable for the entire storage time while color changes were perceivable after the second week. Overall, the results showed the feasibility and potential of HPCD + HPU as an innovative non-thermal tool for the microbial stabilization of ready-to-eat meat.

1. Introduction

Meat products represent a category with a remarkable health safety risk (Borch and Arinder, 2002).

In cooked ham production, heat treatments during the cooking step enable to control the risk of microbial contamination of the finished product, assuring a satisfactory degree of microbial inactivation. However, for this specific product, the post heat treatment procedures such as cutting, slicing and repackaging in small portions, pieces or slices are considered critical points for the risk of further recontamination and reduction of the product shelf life (Unnevehr and Jensen, 1996).

To meet the food industry demand for technologies enabling to increase the safety of food while fulfilling the demand of consumers for fresh foods with higher quality, in recent decades the scientific research has promoted the investigation of innovative techniques to reduce microbial spoilage of the products prolonging the shelf life and preserving the organoleptic and qualitative attributes.

High pressure carbon dioxide (HPCD) is one of the innovative technologies with noticeable potentials for the microbial stability and at the same time preservation of quality attributes and shelf life extension of foods (Spilimbergo et al., 2002). The mechanism through which CO_2 exerts its bactericidal action is not yet well defined, but is thought to be based on the ability of CO_2 to solubilize in the liquid phase of foods being in contact with the microbes and consequently diffusing through their cell membrane, thus negatively influencing the microbial and enzymatic activity (García-González et al., 2007). Several scientific results demonstrated the potential of the treatment for the microbial stabilization of liquid foods and recently more and more studies are published showing the possibility to apply the treatment also to solid food matrices (Haas et al., 1989; Valverde et al., 2010; Ferrentino et al., 2012a). However, it has been demonstrated that HPCD process parameters,

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necessary to reach the stabilization of solid foods, were considerable and in some cases required long treatment times (up to 30 min) and temperatures affecting their physicochemical attributes (up to 50 °C) (Ferrentino et al., 2012a, 2013). To obtain the safety and microbial stability of the product at less severe processing conditions, the scientific research is moving towards the implementation of a system that provides the possibility to combine HPCD treatment with other innovative techniques such as high hydrostatic pressure (Park et al., 2002), pulsed electrical fields (Spilimbergo et al., 2003; Pataro et al., 2010), high power ultrasound (Ortuño et al., 2012a, 2012b, 2013).

Recently, HPCD treatment has been combined with high power ultrasound (HPU) for the microbial stabilization of liquid (Ortuño et al., 2012a, 2012b, 2013; Cappelletti et al., 2014) and solid foods (Spilimbergo et al., 2014; Ferrentino and Spilimbergo, 2015) with positive results in the reduction of the treatment time and temperature needed to achieve a satisfactory product safety degree compared to HPCD alone. In HPCD + HPU combined treatment, CO_2 is used as a dense fluid through which the ultrasound acoustic waves can be transmitted. In a system pressurized with CO_2, the ultrasound induces micro-stirring and solvent cavitation, with physical consequences as cracking or damaging of microbial cell walls; furthermore, they increase the solvent diffusion and cause interfacial turbulence, thus reducing the external resistance to CO_2 mass transfer (Gao et al., 2009).

From this starting point, the present work has been developed with the aim to assess the effectiveness of HPCD + HPU combined treatment on the inactivation of the microbiota (mesophilic microorganisms, lactic acid bacteria, molds and yeasts) naturally occurring on cooked ham pieces. The effectiveness of the combined treatment was also compared with HPCD performed alone.

Once the inactivation kinetics have been obtained as a function of the pressure, temperature, time and power, the optimal process conditions were identified in order to assess the microbial and quality (color, pH, acidity and texture) stability of the product as a function of the storage time.

2. Materials and methods

2.1. Cooked ham preparation

Slices of cooked ham (10 mm thickness) were purchased from a local market and manually cut in cubic pieces with a surface area of about 100 mm^2.

2.2. HPCD apparatus

HPCD treatments were carried out in a multi-batch apparatus. The vessels consisted of ten 15 mL cylinders (used to process the food for the investigation of the effect of temperature and time on microbial inactivation) and of two 310 mL cylinders (used to process the food for the analyses of the storage stability). The cylinders were connected in parallel, so that each experimental run provided a set of experimental data taken at identical process conditions but different treatment times. Each reactor was connected to an on-off valve that could be used to depressurize it independently from the others. The reactors were submerged in a single temperature-controlled water bath. Liquid CO_2 (Messer, Carbon dioxide 4.0, purity 99.990%) was fed into the reactors by a volumetric pump (LEWA, Milano, Italy) which increased the pressure to the desired processing levels, with a rate of about 6 MPa/min. The apparatus was provided with a transducer (Hendress + Houser GmbH, Maulburg, Germany) to control the pressure values while two cover lids, one of the ten 15 mL reactors and one of the two 310 mL reactors, were equipped with a fixed thermocouple (Pt 100 Ω) to control the product temperature. At the end of the process, two micrometric valves and one on-off valve were used to depressurize and release CO_2 from the apparatus occurred over approximately 1 min. After the treatment, the reactors were disconnected from the pressurization line, opened in a laminar flow hood to avoid any microbial contamination and the processed samples collected in sterile containers and cooled down immediately at 4 °C until further use. The operating parameters (temperature, pressure and time) were continuously recorded by a real time acquisition data system (National Instruments, Milano, Italy) and monitored by a specific software (LabVIEW™ 5.0, National Instruments, Milano, Italy). The process parameters chosen for the determination of the natural microbiota inactivation kinetics were: pressure of 8 and 12 MPa, temperature of 40 and 45 °C, and treatment times from 2 to 15 min.

2.3. HPCD + HPU combined apparatus

The apparatus consisted of a sapphire high pressure visualization cell (Separex S.A.S., Champigneulles, France) with an internal volume of 50 mL, designed to withstand up to 40 MPa and 100 °C. The plant included a CO_2 tank, kept at room temperature, a chiller reservoir, a HPLC pump, and a thermostatic bath to keep the inactivation vessel at the desired temperature. The cell was equipped with a safety device calibrated for operations up to 40 MPa, a thermocouple and a manometer to measure the temperature and pressure inside the cell. The apparatus was equipped with an ultrasound system (Aktive Arc Sarl, La Vue-des-Alpes, Switzerland) designed on purpose and embedded in the HPCD plant (Fig. 1). The HPU system consisted in a transducer (40 KHz), a buster, a special retainer (M36 × 1.5), a flat sonotrode and a power generator unit. For the experiments, the ultrasound unit was turned on (time zero) when the desired HPCD pressure and temperature were reached in the vessel. As the power output was found to be a strong function of the applied pressure, the amplitude of the generator was modulated for each condition of pressure and temperature to provide the desired applied power. HPCD pressure and temperature were kept constant during the combined process through the pump and the thermostatic bath, respectively. During the opening and sampling operations, a Bunsen burners flame was placed near the vessel to avoid any microbial contamination. After the treatment, the samples were collected in individual sterile tubes for microbial analyses. The vessel was cleaned and disinfected with ethanol (96% v/v) after each sampling. Experiments were performed to investigate the synergistic effect of HPCD + HPU combined treatment in terms of lower treatment time/lower temperature assuring the same inactivation level or a higher inactivation of HPCD performed alone.

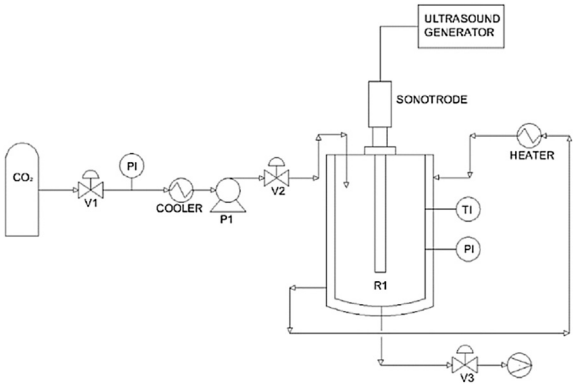

Fig. 1. Combined HPCD + HPU apparatus.