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The effects of high hydrostatic pressure at subzero temperature on the quality of ready-to-eat cured beef *carpaccio*

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ABSTRACT

We compared the application of high hydrostatic pressure (HHP) on unfrozen *carpaccio* (HHP at 20 °C) and on previously-frozen *carpaccio* (HHP at −30 °C). HHP at 20 °C changed the color. The pressure increase from 400 to 650 MPa and the time increment from 1 to 5 min at 400 MPa increased L* and b*. a* decreased only with 650 MPa for 5 min at 20 °C. The prior freezing of the *carpaccio* and the HHP at −30 °C minimized the effect of the HHP on the color and did not change the shear force, but increased expressible moisture as compared to the untreated *carpaccio*. HHP at 20 °C was more effective in reducing the counts of microorganisms (aerobic total count at 30 °C, *Enterobacteriaceae*, psychrotrophs viable at 6.5 °C and lactic acid bacteria) than HHP at −30 ° C. With HHP at 20 °C, we observed a significant effect of pressure and time on the reduction of the counts.

1. Introduction

In developed countries, there is an increasing interest in the consumption of minimally processed products (Gómez-Estaca, López-Caballero, Gómez-Guillén, López de Lacey, & Montero, 2009). An example of this type of product is *carpaccio*, which has been considered traditionally as a dish prepared with raw meat – i.e. pork, beef, veal, salmon, tuna – thinly sliced and served with a dressing containing olive oil, Parmesan cheese and seasonings. The meat industry is currently preparing ready-to-eat *carpaccio* by curing pieces of meat, that are then frozen, sliced, packaged under vacuum or in a modified atmosphere and marketed at refrigeration temperature (Realini, Guàrdia, Garriga, Pérez-Juan, & Arnau, 2011). The main concern associated with its consumption is food safety, because during its preparation no treatment is applied to ensure significant reductions of food-borne pathogens. In this way, HHP processing is an alternative to increase safety and extend the shelf life of fresh and salted red meats (Garriga, Grébol, Aymerich, Monfort, & Hugas, 2004; Fernández et al., 2007; Realini et al., 2011). However, the application of HHP at temperature above 0 °C on fresh red meats and uncooked meat products induces an important discoloration, particularly at pressure levels above 300 MPa, which are required for the inactivation of vegetative cells (Carlez, Veciana-Nogues, & Cheftel, 1995; Goutefongea, Rampon, Nicolas, & Dumont, 1995; Jung, Ghoul, & de Lamballerie-Anton, 2003; Marcos, Kerry, & Mullen, 2010). In addition, other undesirable quality changes in meats and meat products treated by HHP have been reported involving texture (Jung, de Lamballerie-Anton, & Ghoul, 2000) and lipid oxidation (Cheah & Ledward, 1996; McArdle, Marcos, Kerry, & Mullen, 2010).

In order to avoid or at least reduce the discoloration of red meats treated by HHP, some authors have evaluated the incorporation of oxygen scavengers, sodium nitrite or antioxidant compounds to beef (Carlez et al., 1995; Goutefongea et al., 1995) and pork (Goutefongea et al., 1995). Other works were also concerned with the effect of cooking on the sensory quality of beef treated by HHP (Jung et al., 2003). Moreover, the application of HHP at subzero temperature to previously frozen beef pieces (Fernández et al., 2007) or cured pork *carpaccio* (Realini et al., 2011) minimized the discoloration of red meats. Fernández et al. (2007) evaluated the effect of air blast freezing plus HHP at subzero temperature (−35 °C) on the physical properties, microbial quality and frozen storage stability of fresh and salt-added beef pieces from the *Longissimus dorsi* muscle. They concluded that freezing is able to protect beef color from the detrimental effect of high pressure. Meat recovers its original color after thawing and therefore, it could be marketed refrigerated without inducing consumer rejection.

Several studies have been carried out looking at the application of HHP in meats and meat products at refrigeration or moderate temperatures (Montero & Gómez-Guillén, 2005; Campus, 2010). However, the effect of HHP at subzero temperatures on the quality of raw cured red meats (slices or whole pieces) has been scarcely evaluated.

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In this way, Realini et al. (2011) evaluated the effect of HHP (0, 400, and 600 MPa; holding time 6 min) and freezing temperature (−15° vs. −35 °C) on the quality and microbial inactivation of cured pork *carpaccio* using an industrial high pressure processing system. The authors concluded that HHP in combination with low freezing temperature (−35 °C) can be used successfully to deliver long-lasting and high quality pork *carpaccio* to the minimally processed ready-to-eat market.

The aim of this study was to evaluate the effect of HHP processes (at different levels of pressure and holding times) at room temperature (20 °C) and at subzero temperature (−30 °C) on physical properties and microbial quality of cured beef *carpaccio*.

2. Materials and methods

2.1. Experimental design

Treatments evaluated in this study were fresh *carpaccio* (control), fresh *carpaccio* frozen in an air blast freezer at −35 °C, fresh *carpaccio* treated with HHP at 20 °C, and finally, fresh *carpaccio* frozen in an air blast freezer at −35 °C and then treated with HHP at −30 °C. HHP was assayed at two pressure levels (400 and 650 MPa) and two holding times at working pressure (1 and 5 min). Six bags containing four *carpaccio* slices each (prepared according to Sections 2.2 and 2.3) were processed in each treatment. Three bags were used for the determination of chromatic parameters, expressible moisture, shear force and work of shear. The three remaining bags were used for microbial analysis. A total of 240 *carpaccio* slices were used for the full assay.

2.2. Preparation of carpaccio samples

The *carpaccio* samples were prepared according to an industrial procedure. They were prepared with *Semitendinosus* beef muscles (mean weight 2303.1 ± 369.7 g) and supplied by the company Esteban Espuña SA (Spain): muscles were massaged with additives (listed below) by tumbling. Specifically, muscles were tumbled (in a Metalquimia tumbler, Spain) intermittently for 60 min (7 rpm—1 min on/2 min off) at 1 °C and 85% vacuum. The used additives were: sodium chloride (12.0 g/kg muscle), sodium tripolyphosphate (1.0 g/kg muscle), sodium citrate (0.5 g/kg muscle), sodium nitrite (0.15 g/kg muscle) and sodium isoascorbate (0.5 g/kg muscle). After tumbling, muscles were vacuum-packed in linear polyethylene sealed air bags and stored at 1 °C for 7 days. After this, those muscles were kept frozen at −18 °C for 4 days in a cold room and then sliced transversely to the fibers (slice thickness: 2.5 mm) with a meat slicer model TGE 300 from OMS SRL and vacuum-packed (in groups of 24 slices each) in a Cryovac BB4L plastic bag (transmission rates: O_2 30 $cm^3.m^{-2}.24\ h^{-1}.bar^{-1}$ at 23 °C and 0% RH; CO_2 150 $cm^3.m^{-2}.24\ h^{-1}.bar^{-1}$; water vapor 20 $g.24\ h^{-1}.m^{-2}$, Grace S.A., Barcelona) by using a Multivac A300 packaging machine. Then, vacuum-packed samples were stored at 1 °C for 48 h until processing. The untreated *carpaccio* presented a pH mean value of 5.72 ± 0.02, a moisture content of 71.6 ± 1.1 (%), a total protein content of 23.5 ± 0.3 (%), a fat content of 2.4 (%) and an ash content of 2.2 ± 0.2 (%).

2.3. Preparation of carpaccio samples for air blast freezing and/or treatment with HHP

Samples with almost a rectangular shape (about 55 mm × 75 mm) of *carpaccio* were obtained from each 2.5 mm-thick slice, using a scalpel. Then, samples were stacked in groups of four and each group was vacuum-packed in a Cryovac BB4L 200 × 300 plastic bag (transmission rates: O_2 30 $cm^3.m^{-2}.24\ h^{-1}.bar^{-1}$ at 23 °C and 0% RH; CO_2 150 $cm^3.m^{-2}.24\ h^{-1}.bar^{-1}$; water vapor 20 $g.24\ h^{-1}.m^{-2}$, Grace S.A., Barcelona) by using a laboratory vacuum machine.

The samples corresponding to *carpaccio* submitted only to freezing and *carpaccio* frozen and then treated with HHP, were both frozen at −35 °C in a Lab Freezer Frigoscandia 010 (AGA Frigoscandia, Helsingborg, Sweden) using an air temperature and speed of −35 °C and 5.5 m s^{-1}, respectively. During freezing, the temperature–time evolution was measured in the freezing chamber and at the center of two samples using T-type thermocouples and recorded (scanning time of 3 s) with a Fluke Helios I data-logger (John Fluke Mfg. Co. Inc., Everett, USA) connected to a personal computer. After freezing, the samples were stored at −30 °C in a conventional freezer (Liebherr Economy model) until analysis (*carpaccio* only frozen) or until they were subjected to HHP.

The HHP was applied at 20 °C in fresh *carpaccio* samples or at −30 °C in *carpaccio* previously frozen at −35 °C in air blast freezer. In both cases, two pressure levels (400 and 650 MPa) and two holding times (1 and 5 min) were applied. For this, a U111 high pressure equipment from UNIPRESS (High Pressure Research Center, Warsaw, Poland), as described in Guignon, Otero, Molina-García, and Sanz (2005), was used. The vessel has an internal diameter of 30 mm and a working volume of 45 ml. Due to the reduced capacity of the vessel, several identical runs for each HHP treatment were necessary to get enough samples for analytical determinations. Silicone oil M40.165.10 (Peter Huber Kältemaschinenbau GmbH, Offenburg, Germany) was used as pressure-transmitting medium. The pressurization rate was 6 MPa s^{-1} (up to 400 MPa) and 8 MPa s^{-1} (up to 650 MPa). By contrast with the pressurization rate, the decompression rate was carried out by hand in order to get a lower rate than in compression. It resulted in decompression rate values ranging from 3 to 4 MPa s^{-1}. During the HHP treatment, pressure was monitored with a pressure transducer (EBM6045, Erich Brosa Mesgerate GmbH/KGT Kramer, Germany). T-type thermocouples installed into the vessel gave the temperature variation due to the adiabatic heat compression. This heat has been offset by plunging the high pressure vessel into a thermostatic bath. Ethanol was used as the refrigeration fluid due to its low temperature freezing point. To cool down the ethanol an accordingly powerful refrigeration system was used. It was possible to reach −30 °C inside the pressure vessel since the silicone oil used as the pressure-transmitting medium does not freeze at this temperature. In addition, in the treatments at −30 °C, the samples and the silicone oil were loaded at that temperature and the temperature inside the high-pressure vessel was kept at −30 °C by means of the ethanol bath described above. In the treatments at 20 °C, samples of fresh *carpaccio* and silicone oil pre-chilled at 0 °C were loaded into an ice bath. This pre-cooling was performed to reduce the increase in temperature caused by the heat of compression.

Temperatures of two *carpaccio* samples during HHP treatments were also monitored using T-type thermocouples. In all the cases, the data were recorded every 0.5 s using a data-logger (Yokogawa DC100 Data Collector, Tokyo, Japan) connected to a personal computer. Fig. 1 shows the temperature versus pressure for a typical treatment of *carpaccio* processed in its frozen state. The initial values of temperature are transiently distorted due the combined contributions of the adiabatic compression heat and the thermoregulation system.

After HHP treatment, samples treated at 20 °C were stored in a cold chamber at 1 °C until analysis (24 h after the HHP treatment), whereas the samples treated at −30 °C were stored at that temperature in a conventional freezer (Liebherr Economy model) until analysis.

2.4. Analysis of samples

Six bags containing four *carpaccio* slices each (prepared according to Sections 2.2 and 2.3) were processed in each treatment. Three of them were used for the determination of chromatic parameters, expressible moisture (loss of water by centrifugation), shear force and work of shear. The three remaining bags were used for microbial