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Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin

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ABSTRACT

The effect of high pressure on sliced and vacuum packaged commercial dry-cured pork loin was determined by analysing the induced colour changes, the residual enzymatic activities of cathepsins, aminopeptidases and dipeptidilpeptidases and the changes in the content of free amino acids and also its effect on lipid oxidation, as TBARS (Thiobarbituric reactives substances) and volatile flavour compounds. High pressure treatments above 300 MPa affected the colour of dry cured pork loins producing an increase in lightness and decreased redness and these differences were detected during all vacuum storage. High pressure produced a reduction in the activity of aminopeptidases and dipeptidylpeptidases. The untreated samples showed an increase in free amino acid content during vacuum storage while the pressurised samples showed no significant increases probably due to the reduction in aminopeptidase activity. The oxidative stability of the pressurised dry-cured loins was not affected as observed by the absence of differences in TBARS values and in the abundance of volatile compounds from the lipid oxidation. However, the pressurised treatment produced a reduction of several flavour compounds, particularly those derived from Maillard reactions, although they are regenerated during vacuum storage. In summary, high pressure treatment after the ripening of dry-cured loin affects its quality but the differences can be minimised by vacuum storage.

1. Introduction

High-pressure processing (HPP) is a preservation technology for the decontamination of meat products that allows reduction of the microbial load at low or moderate temperatures, being a good alternative to thermal methods for its application to ready to eat foods (Cheftel, 1995; Smelt, 1998). The inactivation of spoilage and pathogen microrganisms has been studied in several meat products, and depending on the type of product different effects of pressure, temperature and holding time of the high pressure treatment have been determined (Aymerich, Jofre, Garriga, & Hugas, 2005; Aymerich, Picouet, & Monfort, 2008; Cheftel & Culioli, 1997; Chung, Vurma, Turek, Chism, & Yuosef, 2005; Garriga, Grebol, Aymerich, Monfort, & Hugas, 2004; Tanzi et al., 2004;). Important aspects to be taken into account are those concerning the effect of the pressure treatment on the quality characteristics of the food product, since treatments can affect texture, colour, external appearance (Cheftel & Culioli, 1997), and, potentially, the aroma and taste.

Recent studies on dry cured meat products have been focused on the effect of HPP treatment on dry-cured Iberian ham and its effect during storage in modified atmosphere (Andres, Adamsen, Møller, Ruiz, & Skibsted, 2006; Andres, Adamsen, Møller, & Skibsted, 2004). Aspects taken into account were mainly lipid oxidation, that was promoted by high pressure treatment, and colour. Other studies have been carried out on frozen hams used for the manufacture of dry-cured ham where high pressure treatment was applied at different stages of the process. These studies were focused on physicochemical parameters and a few endoprotease and antioxidant enzyme activities (Serra, Sarraga, et al., 2007), and on sensory attributes and colour characteristics (Serra, Grebol, et al., 2007). However, there are no studies dealing with the effect of high pressure treatment on quality features of other dry-cured meat products such as dry-cured loin.

The pressurization of meat causes drastic colour changes, basically lightness increases and redness decreases due to protein denaturation or modification. Although several authors have determined the effect of high pressure treatment on several enzymes it is essential to know all the biochemical changes that occur in a product after high pressure treatment.

Enzyme activity is essential in dry-cured products for the development of flavour components or their precursors (Toldrá & Flores, 1998).

In general, high pressure treatment produces a certain degree of enzyme inactivation although the composition of food has a

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protective effect on the pressure inactivation (Seyderhelm, Boguslawski, Michaelis, & Knorr, 1996). On the other hand, Homma, Ikeuchi, and Suzuki (1994) reported an increase in protease activity in high pressurized muscle due to a release of enzymes from lysosomes even though other proteolytic enzymes such as aminopeptidase B were inactivated. Therefore, the effect of high pressure treatment on other proteolytic enzymes such as aminopeptidases and dipeptidylpeptidases should be elucidated to determine its effect on flavour development and in the final products.

The aim of the present work was to evaluate the effect of high pressure on sliced and vacuum packaged commercial dry-cured loin by studying the induced colour changes, the residual enzymatic activities of cathepsins, aminopeptidases and dipeptidilpeptidases and to verify if these changes affect the content of free amino acids. The effect of pressurization on lipid oxidation, as TBARS (Thiobarbituric reactives substances) and on volatile compounds was also evaluated.

2. Materials and methods

2.1. Samples and high pressure treatment

Seven dry-cured pork loins were purchased from a local market and transported to the laboratory in chilled storage. All the loins were from the same batch having an aging time of 3 months and a water content of 48.0 ± 0.4%. The loins were mechanically sliced (3 mm in thickness) to give a minimum of 72 slices per loin. The slices were immediately vacuum-packed individually in plastic bags (co-extruded PA/PE-20/70; O_2 transmission rate: 30–50 cm^3/m^2-24h-atm; water vapour transmission rate: 2.6 g/m^2 atm; CO_2 transmission rate 150 cm^3/m^2-24 h-atm; N_2 transmission rate 10 cm^3/m^2-24 h-atm, at 23 °C and 50% HR). Samples were then divided into four groups. One group (not pressurized) was kept at +4 °C, the remainder were subsequently pressurized. Prior to high pressure treatment packaged samples were kept on ice (0–2 °C). At each time, 42 slices (six slices from each loin) were high pressure treated, repeating the treatment three times.

Treatments were carried out with a Pilot scale Warm Isostatic Press (Engineered Pressure System International, Walgoedstraat, Belgium). Internal diameter of the vessel was 100 mm, inside length 300 mm, internal volume 2.35 l, the pressure transmitting medium was monoethylene glycol/distilled water solution 30/70-v/v. Pressures used were 300, 350, 400 MPa with thermostatic bath set at 20 °C, for 10 min. The pressure come-up time was 5 MPa/s, pressure release time 10 s. Control and pressurized samples were stored at +4 °C in the dark and analysed for colour, enzyme activity, amino acid content, TBARS and flavour compounds) after 1 d and 45 d of storage. Also the colour was measured at day 7, 9 and 11. The results were expressed as the average with the standard error.

2.2. Microbiological analysis

Samples were analysed for aerobic total count 1 d after treatment and 45 d after vacuum storage. The product was sampled aseptically (5 g), stomached for 2 min in 0.1% sterile peptone water. 10-fold serial dilutions of the samples were made using 0.1% sterile peptone water and 0.1 ml aliquots of the appropriate dilutions were plated on Plate Count Agar (Merk). Plates were incubated for 72 h at 30 °C.

2.3. Colour

Surface colour of the slices was measured three times at each sampling using a tristimulus colorimeter (Minolta Chroma Meter Measuring Head CR-410 Minolta, Osaka, Japan), with D65 illuminant and 10° CIE standard observers angle. $L^*a^*b^*$ scale coordinates were obtained: L^*(lightness), a^* (redness) and b^* (yellowness).

Before measurement, the colorimeter was calibrated against the white tile supplied with the instrument. Measurements were performed directly on the surface of the samples, which were repacked under vacuum and kept in chilled storage and darkness, immediately after the measurements (approx. 5 s). Measures were taken at 0, 2, 7, 9, 11 and 45 days after treatment.

2.4. Preparation of enzyme extracts for cathepsin/peptidases assays

2.5 g of muscle were homogenised in 25 ml of 50 mM sodium citrate buffer containing 1 mM EDTA and 0.2% (v/v) Triton X-100 at pH 5.0 (for cathepsins) or 4 g of muscle in 20 ml of 50 mM disodium phosphate buffer, pH 7.5, containing 5 mM EGTA (for peptidases). In both cases, the extracts were homogenised (3 × 10 s at 27.000 rpm on ice) with a polytron (Kinematica, Switzerland), centrifuged at 10,000*g* for 20 min at 4 °C and the supernatants filtered through glass wool and used for the enzyme assays.

2.5. Assay of enzyme activities

Cathepsins were assayed as described by Toldrá and Etherington (1988) using N-CBZ- L-arginyl-L-arginyl-7-AMC, N-CBZ-L-phenylalanyl-L-arginine-7-AMC (Sigma, St. Louis, MO), at pH 6.0, for cathepsins B and B+L. For each assay, 50 μl of extract was diluted with 250 μl of reaction buffer (40 mM sodium phosphate at pH 6.0, containing 0.4 mM EDTA, 10 mM cysteine, and 0.05 mM substrate) and then incubated for 15 min at 37 °C.

Muscle aminopeptidase activities were measured by fluorometric assays using aminoacyl-7-amido-4-methyl coumarin as substrates (aa-AMC) (Sigma Chemical Co., St. Louis, MO) (Toldrá & Flores, 2000). Alanyl aminopeptidase (AAP) was assayed using 0.1 mM alanine-AMC as substrate in 100 mM phosphate buffer, pH 6.5, with 2 mM 2-mercaptoethanol. Arginyl aminopeptidase (RAP) was assayed using 0.1 mM arginine-AMC in 50 mM phosphate buffer, pH 6.5, with 0.2 M NaCl. Leucyl aminopeptidase activity (LAP) was assayed using 0.25 mM leucine-AMC in 50 mM borate-NaOH buffer, pH 9.5, with 5 mM magnesium chloride. Pyroglutamyl aminopeptidase (PGAP) was assayed with 0.1 mM pyroglutamic-AMC in 50 mM borate-HCl, pH 8.5, containin 1 mM DTT. Methionyl aminopeptidase (MAP) by using 0.15 mM Ala-AMC as substrate, in 100 mM phosphate buffer, pH 7.5 containing 10 mM ditiothreitol (Flores, Marina, & Toldrá, 2000).

Dipeptidyl peptidases (DPP) I, II, III and IV were assayed as previously described by Sentandreu and Toldrá (2001), using AMC (Bachem, Switzerland, Sigma, St. Louis, MO), as fluorescent substrates. DPP I was measured using 0.5 mM Gly-Arg-AMC in 50 mM sodium acetate/acetic acid buffer, pH 5.5,containing 5 mM DTT; DPP II: 0.5 mM Lys-Ala-AMC or Gly-Pro-AMC in 50 mM sodium acetate/acetic acid buffer, pH 5.5, containing 0.04 mM bestatin; DPP III: 0.5 mM Arg-Arg-AMC in 50 mM sodium tetraborate/potassium phosphate buffer, pH 8.0, containing 0.05 mM $CoCl_2$. DPP IV: 0.25 mM Gly-Pro-AMC in 50 mM tris-base buffer, pH 8.0, containing 5 mM DTT. Then 50 μl of each enzyme preparation were added to 250 μl of the respective substrate solution.

In all cases, the reaction was incubated at 37 °C and the fluorescence continuously monitored at 355 nm and 460 nm as excitation and emission wavelengths, respectively for AMC derivatives, using a Fluoroskan Ascent fluorimeter (Thermo Electron Co, Finland). Three replicates were performed for each enzyme assay. One unit (U) of proteolytic activity was defined as the amount of enzyme capable of hydrolysing 1 nmol of substrate per hour or per minute at 37 °C.