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Physicochemical characteristics and quality parameters of a beef product subjected to chemical preservatives and high hydrostatic pressure

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ABSTRACT

The use of high hydrostatic pressure (HHP) on fresh beef causes a deleterious effect on red colour. A beef product subjected to HHP exhibiting acceptable colour and microbiological stability was developed; the process requires as a first step the immersion in a preservative solution containing ascorbic acid, sodium nitrite, and sodium chloride. Desirability functions were used to optimise the composition of this solution in order to maintain the colour attributes minimising the concentration of sodium nitrite. The product was packed in low gas permeability film before HHP treatment. The effect of the applied pressure (300, 600 MPa) on quality parameters (colour, texture) was analysed. The stability of the product during storage at 4 °C was determined by microbial counts, colour, texture, and exudate. The combination of treatments provided acceptable colour and microbiological stability during four and six weeks of refrigerated storage after the product has been subjected to 300 and 600 MPa, respectively.

1. Introduction

High hydrostatic pressure (HHP) processing is a non-thermal technology for food preservation that meet consumer demands for minimally processed products and environmentally friendly technologies (Toepfl, Mathys, Heinz, & Knorr, 2006). 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 (Campus, Flores, Martinez, & Toldrá, 2008; Fulladosa, Serra, Gou, & Arnau, 2009).

The application of high hydrostatic pressure on meat and meat products has been focused mainly on studying its effect on microorganisms as a treatment to improve the microbiological safety of the final product (Aymerich, Jofré, Garriga, & Hugas, 2005; Cheftel, Carlez, & Veciana-Nogues, 1995; Garriga, Grébol, Aymerich, Monfort, & Hugas, 2004; Tanzi et al., 2004; Vaudagna et al., 2012). Nevertheless, high-pressure treatment can also be used to develop new meat products.

This technology can be applied in packaged foods, avoiding possible recontamination after the treatment (Toepfl et al., 2006). This together with the possibility of treating products that cannot be preserved by heating, such as fresh meats and cured products, makes HHP a useful tool to preserve for example sliced packaged dry-cured ham during refrigerate storage (Clariana, Guerrero, Sárraga, & Garcia-Regueiro, 2012).

High pressure treatment has been used for the preservation of chicken, pork, meat products, surimi gels as well as salmon products, with considerable positive effects on protease activity, textural properties, taste and flavour (Bajovic, Bolumar, & Heinz, 2012; Carballo, Cofrades, Solas, & Jiménez-Colmenero, 2000; Garriga, Aymerich, & Hugas, 2002; Knorr, 1993; O'Brien & Marshall, 1996; Ohshima, Ushio, & Koizumi, 1993). The use of HPP for the microbial decontamination has been extensively reviewed but complete microbial inactivation is currently not possible (Knorr, 1995; Smelt, 1998).

Pressures in the range of 100 to 800 MPa are applied on meat products (Cheftel & Culioli, 1997), although commercial pressure vessels have a limit at 700 MPa (Torres & Velazquez, 2005). Pressures above 300 MPa help to inactivate microorganisms, making the product microbiologically safe (Davidson, 2001). Meat products are mainly pasteurized, which is generally done in the range of 300–600 MPa, inactivating vegetative cells (Aymerich, Picouet, & Monfort, 2008; Chung, Vurma, Turek, Chism, & Yuosef, 2005). However, the effect is dependent on temperature. The degree of microbial inactivation is lower at the optimum growth temperatures than at higher or lower temperatures (Hugas, Garriga, & Monfort, 2002). Furthermore, HHP preserves micronutrients better than thermal treatment (Aymerich et al., 2008); however, HHP can affect colour, texture, and flavour of meat products, which is not always accepted by the consumers (Bak et al., 2012; Cheftel & Culioli, 1997; Fulladosa et al., 2009). Nevertheless, HHP treatment induces undesirable changes on meat and meat product characteristics increasing lipid oxidative reactions, which decline the acceptability, especially in some protein-rich foods treated at pressures of >400 MPa (Cheftel & Culioli, 1997).

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For meat products, colour is one of the most important quality features (Feiner, 2006; Risvik, 1994; Young & West, 2001). HHP commercial application in fresh bovine meat has been underutilized due to severe discolouration of the meat to pressure levels necessary for inactivation of pathogenic and spoilage microorganisms (Fernández et al., 2007). Meat discolouration is produced at pressure levels higher than 300 MPa, which are required for inactivation of vegetative cells. In the range of 200–350 MPa discolouration occurs due to meat myoglobin denaturation and the displacement or loss of haem-iron. In the range of 300–600 MPa the characteristic red colour of meat is lost (Szerman et al., 2011).

Colour problems on HHP beef products may be reduced by applying short chemical pre-treatment using salts that are commonly applied in the curing process. In cured meat ascorbic acid favours the decomposition of nitrite ion to nitrous oxide that also reacts with myoglobin producing nitrosomyoglobin, conferring the characteristic red colour of beef. Besides, ascorbic acid is a strong inhibitor, decreasing the formation of nitrosamines (Ohshima & Bartsch, 1981; Stich, Hornby, & Dunn, 1984).

The application of HHP on red meats has been studied on fresh and salted samples (Realini, Guàrdia, Garriga, Pérez-Juan, & Arnau, 2011) and in cured and cooked products (Aymerich et al., 2008; Bover-Cid, Belletti, Garriga, & Aymerich, 2011; Hugas et al., 2002; Szerman et al., 2011). The addition of nitrites improved myoglobin stability after HHP treatments, however the colour was modified (Rubio, Martinez, Garcia-Cachan, Rovira, & Jaime, 2007). Other researchers reported that topical addition of ascorbic acid inhibits discolouration on the surface of cut beef (Grobbel et al., 2006; Mancini, Hunt, Hachmeister, Kropf, & Johnson, 2004; Mancini et al., 2007). The reducing activity of ascorbic acid improves muscle colour stability via metmyoglobin reduction (Lee, Hendricks, & Cornforth, 1999). However, because ascorbic acid acts as both an antioxidant and a pro-oxidant, the appropriate levels for preventing muscle discolouration are not straightforward and depend on a number of factors (Lee & Hendricks, 1997; Lee et al., 1999; Shivas et al., 1984). This behaviour could be attributed to the presence and concentration of metals within a food (Decker, 1998). Yamamoto, Takahashi, and Niki (1987) suggested that ascorbic acid pro-oxidant nature could be due to the production of ferrous haem proteins, which may be more reactive and more oxidative than ferric derivatives.

As HHP treatment modifies the colour of the tissue, it is important to include a pre-treatment in which the meat is immersed in a preservative solution. The composition of this solution must be optimised to maintain colour attributes and to minimise the concentration of sodium nitrite.

The objectives of the present work were:

1) To develop a red beef product subjected to high hydrostatic pressure including a short chemical pre-treatment by dipping in preservative solutions containing sodium nitrite, ascorbic acid and sodium chloride.
2) To analyse the influence of applied pressure and the effect of vacuum packaging in low permeability films on the quality parameters of the product.
3) To optimise the composition of the preservative solution in order to maintain the colour attributes minimising the concentration of sodium nitrite by using desirability functions.
4) To assess the stability of the developed meat product by determining microbial counts, colour, texture, and exudate production during refrigerated storage at 4 °C.

2. Materials and methods

2.1. Raw materials

Beef muscles were obtained from the local retail market. The used commercial cut was top inside round (top side); this cut is integrated by the following muscles: *adductor femoris* and *semimembranosus*. The average weight of the cuts was about 3.9 kg. 25 cuts from different animals were used in the experiments. The muscles were separated after 48 h post-mortem and all visible fat was removed. pH values ranging between 5.4 and 5.7 were measured in the raw beef muscles from the different animals ($n = 25$) used in the experiments. These pH values were consistent with the requirements of SENASA (National Control Service for Animal Sanitary Status — Argentina, 2013) that establish a pH < 5.9 for raw beef.

2.1.1. Physicochemical analyses of the raw material

Moisture, ash, protein, and lipid contents were determined according to AOAC methods 24.003, 24.009, 24.027, and 24.005, respectively (AOAC, 1980) in triplicates. Fat content was determined on samples previously dried with sodium sulphate anhydrous (SO_4Na_2) by Soxhlet method, using ethyl ether as extraction solvent. pH was measured using a spear tip glass electrode (Cole-Palmer, cat. U-05998-20) on a pH meter (Hach Sension pH 3, Loveland USA).

2.2. Experiments

The general procedure consisted of: a) sectioning the meat samples in cylindrical sections (3 mm thickness and 6 cm diameter); b) immersion of the samples in preservative solutions modifying the concentration of the components in order to optimise their composition (Tests A and B that are described in the following sections) c) vacuum packaging of the samples in Cryovac BB4L films (Sealed Air Co., Buenos Aires, Argentina, PO_2: 35 (cm^3/m^2 day bar) at 23 °C); d) High pressure treatment of the samples in a Stansted Fluid Power Equipment, model FPG9400:922 with a cylindrical vessel (2 l capacity, maximum working pressure 900 MPa, operating temperature range −20 to 120 °C) located at the ITA Institute (INTA Castelar, Argentina). Pressurization rate was 300 MPa/min and de-pressurization was instantaneous. Experiments were carried out at different pressures 150, 300, and 600 MPa and a temperature of 20 ± 5 °C; the samples were maintained at the working pressures for 5 min. In all cases control samples (not submitted to HHP) were also analysed. Quality attributes (colour and texture) were determined in all cases to find adequate processing conditions.

2.3. Instrumental colour measurements

The colorimetric measurements were carried out using a tristimulus colorimeter (Minolta Chroma Meter Measuring Head CR-400 Minolta, New Jersey, USA). Each measurement was performed on 3 slices with 6 replicates per slice.

The CIE-L*a*b* scale was used in terms of L*(lightness), a* (redness), and b* (yellowness).

2.4. Texture analyses

Textural analyses were performed in a TAXT2i Texture Analyser (Stable Micro Systems, UK) at 25 °C, using the Texture Expert Exceed software supplied by Texture Technologies Corp. The Volodkevich bite jaws (HDP/VB) probe, which simulates the action of an incisor tooth (Wen-Ching, Wen-Chian, Yu-Ting, & Chang-Wei, 2007), was used in measuring the texture of the meat product, because it has been observed that the first bite of the product is done with the fore teeth. Maximum breaking force (F, N) was determined when compression is done until 30% of the specimen. A minimum of three slices were used for each formulation; measurements were done in triplicates for each slice and mean values were reported.

2.5. Test A: selection of additives to be incorporated in the dipping solution

Different experiments were performed to find the preservative solution that meets the appropriate requirements for the development of the meat product.

According to the existing regulations the amount of residual nitrite in the meat final product should not exceed 125 mg/kg (Codex Alimentarius, 1981). Furthermore, an acceptable consumer concentration of sodium chloride (NaCl) in beef products is 30–40 mg/g of meat (Pearson & Gilett, 1996). The concentrations of sodium chloride and sodium nitrite in the meat product were determined to ensure that they are below the permissible limits.

Preliminary tests were performed to establish whether the addition of ascorbic acid (5 g/l) in a solution containing NaCl 60 g/l and $NaNO_2$ 1 g/l. improved the colour of the meat submitted to HHP. Appropriate immersion times to meet the limits for sodium nitrite were established from preliminary experiments in which different dipping times were tested and the average concentrations of nitrite were measured.

The samples were divided into 3 groups:

Group C: fresh meat samples, not subjected to chemical treatment.

Group A: samples of fresh meat, immersed in solution containing ascorbic acid (NaCl 60 g/l, $NaNO_2$ 1 g/l, ascorbic acid 5 g/l).

Group WA: samples of fresh meat, immersed in a solution that does not contain ascorbic acid (NaCl 60 g/l, $NaNO_2$ 1 g/l).

Samples were submitted to HHP procedure (0, 150, 300, and 600 MPa), using muscles from at least two different animals. In each sample concentrations of chloride, nitrite, colour and texture were determined.

2.6. Analytical techniques

To measure the amount of NaCl present in the tissue after each immersion period and HHP process, meat tissue was homogenized in an Omnimixer equipment with bi-distilled water at 90 °C. The suspension was stirred, diluted with water, and filtered. NaCl was determined by measuring chloride content, using a previously calibrated ion-selective electrode (Cole–Parmer 27502-12) with 2 ml of $NaNO_3$ solution (5 M) added to regulate the ionic strength (Graiver, Pinotti, Califano, & Zaritzky, 2006). Similar extraction procedures were followed for nitrite determination using Hach kit Nitriver 3 (method 371) by Graiver et al. (2006). These determinations were made in triplicate.

2.7. Test B: effect of the concentrations of ascorbic acid and $NaNO_2$ in the dipping solution on the colour and texture of the meat samples

The concentrations of ascorbic acid and $NaNO_2$ were optimised (keeping the concentration of NaCl in 60 g/l), in order to achieve an acceptable colour in the product.

Different tests were carried out using concentrations ranging between 0.52 and 1.10 g $NaNO_2$/l and 0–10 g ascorbic acid/l.

A Central Composite Design (DCC) with 9 formulations and 2 replicates in the centre was applied; 60 meat slices were used in the different dipping solutions; 6 samples were used as control (untreated bovine fresh tissue). In all cases colour and texture were measured and the concentrations of nitrite and chloride were tested to verify that the product does not exceed the permitted levels.

The test was repeated at two pressures: 300 MPa and 600 MPa taking into account that according to literature (Tellez, Ramirez, Perez, Vazquez, & Simal, 2001) pressures higher than 300 MPa are suitable for the inactivation of microorganisms that would alter the product during refrigerated storage.

Response surface methodology (RSM) was used to estimate the main effects of the independent variables: $NaNO_2$ and ascorbic acid concentrations on colour, and texture of the products. The following second order polynomial model was fitted to the data of the variables where significant differences were found:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{12} X_1 X_2 \quad (1)$$

where Y is the corresponding response variable (colour or texture); β_0, β_1, β_2, β_{11}, β_{22}, and β_{12} are the regression coefficients; and X_1 and X_2 are the concentrations of $NaNO_2$ and ascorbic acid, respectively. By using a stepwise backward elimination procedure non-significant terms ($P > 0.05$) were eliminated from the initial model. After model fitting was performed the model adequacies were checked by R^2 and lack of fit test (Walpole, Myers, & Myers, 1998); residual analysis was conducted to validate the assumptions used in the ANOVA.

The desirability function approach is one of the most widely used methods for the optimisation of multiple response processes. The method finds operating conditions that provide the "most desirable" response values.

For each response Y(x), a desirability function d(Y) assigns numbers between 0 and 1 to the possible values of Y, with $d(Y) = 0$ representing a completely undesirable value of Y and $d(Y) = 1$ representing a completely desirable or ideal response value.

Depending on whether a particular response Y is to be maximised or minimised d(Y) can be used (Derringer & Suich, 1980). In our case colour parameters were analysed; redness (a*) was maximised and luminosity (L*) was minimised.

After the individual desirability functions (di) are known, the desirability index (D, overall desirability) can be calculated using the geometrical mean as follows (Derringer & Suich, 1980; Harrington, 1965):

$$D = \left(\prod_{i=1}^{k} d_i(Y) \right)^{1/k} \quad (2)$$

with k denoting the number of responses, in our case two.

In order to apply the desirability approach the following steps were carried out:

a) Experiments were conducted and mathematical models were fitted for all k responses; in our case we selected the colour parameters L* and a* as the responses to be optimised due to the known effects of the high pressure on the colour of beef; b) Individual desirability functions were defined for each response; c) The overall desirability D with respect to the controllable factors was maximised establishing the optimal concentrations of $NaNO_2$ and ascorbic acid that lead to an adequate colour in terms of L* and a*.

Once the optimal concentrations were found an experimental validation was carried out. A preservative solution containing the theoretical optimal concentrations of nitrite and ascorbic acid was prepared. Triplicate experiments were conducted by immersing meat samples in this solution. Then the samples were submitted to HHP (300 and 600 MPa) and finally colour and texture measurements were performed.

2.8. Differential scanning calorimetry measurements on the optimised samples

Differential scanning calorimetry (DSC) was used to evaluate changes of the myofibrillar proteins after the meat product was submitted to an immersion stage in the optimised preservative solution with and without a subsequent high pressure treatment.

Analysis of thermal behaviour during warming of meat was performed using Differential Scanning Calorimetry (DSC Q100, TA Instruments, New Castle, Delaware, USA) controlled by a TA 5000 module, with a quench-cooling accessory, under a N_2 atmosphere (nitrogen flow, 0.33 ml/s).

The samples (about 9–10 mg) were placed in aluminium pans, and hermetically sealed; a good contact between the sample and the bottom of the pan was assured. A heating rate of 10 °C/min was applied to change the temperature from 20 to 100 °C using an empty aluminium pan as reference. After differential scanning calorimetry (DSC) analysis, pans were punctured and the sample dry weight was determined at

105 °C until constant weight. Heating thermograms were analysed for the determination of total enthalpy changes; each assay was done using at least three samples.

2.9. Test C: refrigerated shelf life

In order to evaluate the shelf life of the meat product during the period of refrigerated storage, the samples were immersed in the optimised solution (as described in test B), vacuum-packed and subjected to HHP process (300 or 600 MPa).

Throughout the storage period, colour, texture, water activity, exudate and microbial counts were measured weekly for 50 days at 4 °C.

2.10. Exudate production during storage

Exudate analysis was performed in order to study the effect of high pressure on water losses and to analyse its relationship with texture.

Exudate measurements were determined in triplicates by evaluating the difference between the sample weight after a storage period and the corresponding weight at initial storage time after the HHP treatment; it was expressed as grammes of water lost per 100 g of meat sample.

2.11. Water activity

Water activity (aw) was determined during storage of the samples. Aqua Lab Series 3 Instrument (Decagon Devices, USA) calibrated with a K_2SO_4 solution (aw = 0.979 ± 0.005) and double distilled water (aw = 1) was used. The measurement is based on the dew point determination; an infrared beam focused on a tiny mirror determines the precise dew point temperature of the sample. That dew point temperature is then translated into water activity. Measurements were performed at 25 °C in duplicate samples.

2.12. Microbiological analysis

In order to control the microbiological safety of the product and also to determine the shelf life during refrigerated storage microbial counts were performed at different times during refrigerated storage at 4 °C on: i) samples dipped in the preservative solutions and then submitted to 300 or 600 MPa; ii) beef samples without any treatment.

Approximately 10 g meat samples were taken from each package, diluted with 90 ml of 0.1 g/100 ml peptone solution, and homogenized in a Stomacher 400 (Seward, USA) for 120 s at the maximum speed. Serial dilutions were performed using tubes with peptone 0.1 g/100 ml and samples were plated on suitable culture media. After incubation, the number of bacterial colonies grown on each plate were counted and used to calculate colony forming units (CFU) per gramme of beef for each sample.

The samples were tested for total aerobic mesophilic microorganisms (Plate Count Agar PCA, 30 °C, 2 days), total psychrotrophic aerobic counts (PCA, 4 °C, 7 days), Enterobacteriaceae (AVRB, 37 °C, 24 h), lactic acid bacteria (MRS, 30 °C, 2 days), yeasts and moulds (YGC, 5 days, 30 °C). These determinations were made weekly in triplicates.

2.13. Statistical analyses

Statistical analyses were done using SYSTAT version 10.0 (SYSTAT Inc., USA, 1996). Analysis of variance (ANOVA) was carried out to analyse significant effects. Least significant difference tests (LSD) were used to perform pair-wise comparisons between means. Differences in means and F-tests were considered statistically significant when $P < 0.05$. Average values ± standard error of the mean are given throughout the text.

Surface response methodology was applied to model the effect of solution composition on colour and texture variables. The desirability function approach was used for optimising multiple responses, to find the adequate combination of sodium nitrite and ascorbic acid that produced the best colour in the products expressed in terms of L* and a*. These procedures were carried out using the Design Expert v.7 software (State-Ease, Inc., MN, USA).

3. Results and discussion

The protein, lipid, ash and moisture contents of the beef tissue (*adductor femoris* and *semimembranosus*), expressed as g/100 g of meat were: 21.56 ± 0.34, 1.42 ± 0.12, 0.63 ± 0.09, and 75.64 ± 0.34 respectively.

3.1. Test A: selection of chemical preservatives for the immersion solution

This test was conducted to determine whether the incorporation of ascorbic acid in the immersion solution might improve the overall appearance of HHP-treated meat. Fresh samples were used as control (C), and the treated samples were immersed in a solution of 60 g/l NaCl, 1 g/l $NaNO_2$ with (A) or without (WA) ascorbic acid. The colour and texture analysis revealed that L* and a* depended significantly ($P < 0.05$) on the pressure levels applied and on the pre-treatment, as well as on the interaction between both factors. Across all samples, luminosity (L*) did not vary significantly in those samples treated with 0 and 150 MPa, while at pressures higher than 150 MPa the pressure increase resulted in an increase of L* (Fig. 1a). This might be due to the fact that high pressure treatments produce changes in the structure of the myofibrillar proteins (Cheftel & Culioli, 1997; Suzuki, Kim, Tangi, Nishiumi, & Ikeuchi, 2006). These structural changes might explain the increase in the luminosity as an increase in the ratio of light reflected and absorbed by the material (Campus et al., 2008). The comparison of L* across the samples pre-treated with the different preservatives and the control sample revealed that, when pressure levels of 300 and

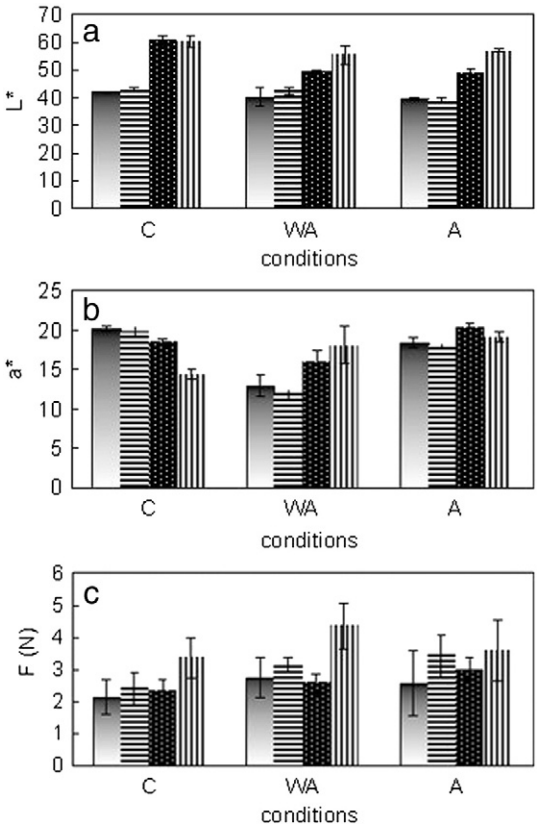

Fig. 1. Effect of pretreatment conditions (immersion solutions with (A) and without (WA) ascorbic acid) and high pressures on colour parameters and texture of meat samples. a) Luminosity (L*), b) parameter a*, and c) maximum strength. □ 0 MPa, ▨ 150 MPa, ▩ 300 MPa, ▥ 600 MPa. Bars indicate standard errors of the means.

600 MPa were applied, the control samples showed higher L* values than all the others ($P < 0.05$), which proves the need to implement the immersion stage in order to reduce the undesired luminosity increase in HHP-treated samples.

Redness of the non-treated samples (C) was reduced when a higher hydrostatic pressure was applied and lower a* values were observed (Fig. 1b); because myoglobin (sarcoplasmic protein) – which is responsible for the red colour in meat – is not stable at high pressure, and thus it oxidizes into metmyoglobin (Rubio et al., 2007). However, it must be noted that the value of a* tends to increase when a higher pressure is applied on chemically treated samples (A and WA), reaching maximum values in the samples which contained ascorbic acid as opposed to those which did not (WA). Nitrosomyoglobin is more stable at high pressure because it is more resistant to oxidation than myoglobin and oxymyoglobin (Rubio et al., 2007); however, the increase in a* produced by HHP would indicate that it favours the formation of nitrosomyoglobin, enhancing the redness.

The value of b* was not considered significant to represent the colour variations of the samples. Thus it was concluded that the samples with the best surface colour after applying the HHP treatment were those where the immersion solution contained ascorbic acid (A).

With respect to texture, Fig. 1c shows the maximum force variation for the different pressure levels applied both to C samples and to those where an immersion pre-treatment was applied. The chemical pre-treatments as well as the level of pressure applied had a significant influence ($P < 0.05$) on the maximum force, although there was no interaction between both factors. For the 3 groups of samples – C, A, WA – the maximum force (3.77 ± 0.36 N average of C, A, WA) at 600 MPa was significantly higher than at 0, 150, and 300 MPa (average value: 2.73 ± 0.21 N). Control samples (C) showed the lowest maximum force values in the compression test – 2.56 ± 0.24 N – while those chemically treated with (A) and without (WA) ascorbic acid showed no significant differences (3.32 ± 0.43 N) between each other ($P > 0.05$). These results can be attributed to the partial denaturation of the myofibrillar proteins caused by the chemical pre-treatment (Graiver et al., 2006) and by the high pressure technique. Pressure treatment at temperatures higher than 0 °C can induce changes in meat texture (Sun & Holley, 2010). The effect of HHP at temperatures of >0 °C on the toughness or tenderness of the meat is dependent upon the rigor state of meat (Cheftel & Culioli, 1997), the pressure level (Ma & Ledward, 2004), the working temperature (Beilken, Macfarlane, & Jones, 1990) and the holding time at working pressure (Sun & Holley, 2010). Several authors reported that HHP treatment at low or moderate temperatures caused toughening of post-rigor meat. Jung, de Lamballerie-Anton, and Ghoul (2000) reported that HHP treatment (130 or 520 MPa and 10 °C for 260 s) increased the mechanical resistance of both raw and cooked (65 °C, 1 h) post-rigor beef significantly, compared with control samples. They also reported an effect of the pressure level, finding the highest values of beef mechanical resistance at the highest pressure level evaluated. Jung et al. (2000) suggested that the integrity of myofibrils rather than the connective component appeared to be involved in the effect of HHP on meat texture. They proposed that myofibrillar protein changes could increase the toughness. Ma and Ledward (2004) found that the toughness of beef muscle increased with the increment of the pressure from 200 to 800 MPa at a constant temperature of 20 to 40 °C.

3.2. Test B: effect of the $NaNO_2$ and ascorbic acid concentrations of the immersion solution on the colour and texture of meat

Considering that the chemical composition of the immersion solution has an influence on the colour and texture parameters, the effects of the $NaNO_2$ and ascorbic acid concentrations of the immersion solution on these beef attributes were analysed.

3.2.1. Colour analysis

Fig. 2 a, b, c, and d shows the response surfaces for surface colour parameters L* and a* according to the different sodium nitrite and ascorbic acid concentrations for each HHP level applied (300 and 600 MPa).

Fig. 2a and b shows the effects of the different sodium nitrite and ascorbic acid concentrations on luminosity (L*) in the samples treated at 300 and 600 MPa, respectively. At 300 MPa, L* is shown to go down with the increase of the ascorbic acid concentration, while it has a quadratic dependence on the sodium nitrite content; thus the minimum luminosity value will result from combining 10 g/l of ascorbic acid with 0.85 g/l of sodium nitrite. At 600 MPa, the luminosity (Fig. 2b) was higher than at 300 MPa across the whole range of concentrations under study. At 600 MPa, L* showed a negative correlation with ascorbic acid concentration in the immersion solution; however, this variation of L* with the acid concentration was not significant. Even though the samples treated at 600 MPa showed a lighter colour than those treated at 300 MPa, their overall appearance was acceptable.

Fig. 2c and d shows the dependence of a* on the different sodium nitrite and ascorbic acid concentrations, in samples treated at 300 and 600 MPa, respectively. At 300 MPa (Fig. 2c) above 3.7 g/l ascorbic acid in the immersion solution, a* increased when ascorbic acid concentration was incremented.

In addition, a* was not dependent on sodium nitrite concentration (Fig. 2c). At 600 MPa (Fig. 2d) a* reached its lowest values within a range of 0.70–0.87 g/l sodium nitrite and 5 g/l ascorbic acid. However, across the whole range of ascorbic acid and $NaNO_2$ concentrations under study, the values of a* ranged between 16.2 and 20.6, being similar to the redness parameter ($a^* = 20.20 \pm 0.32$) of the control untreated beef sample.

3.2.2. Texture analysis

The statistical analysis of 132 samples revealed a significant effect ($P < 0.05$) of the pressure level applied in the HHP test on the maximum force (F); the higher the pressure applied, the higher the F value, ranging from 2.98 ± 0.13 at 300 MPa to 3.80 ± 0.18 at 600 MPa. Additionally, the ascorbic acid and sodium nitrite concentrations had no significant influence on F values.

3.2.3. Optimisation of the immersion solution composition based on colour measurements

In order to optimise the immersion solution, the best combination of $NaNO_2$ and ascorbic acid was tested to achieve the following goal: maximum redness (a*) and minimum luminosity (L*). The main purpose was to find the right additive combination to provide the treated samples with the necessary characteristics to be accepted by consumers. Based on the response surface equations of L* and a*, the immersion solution composition was optimised, after finding out that at 300 and 600 MPa the best combination was 0.6 g/l $NaNO_2$, 8.5 g/l ascorbic acid, and 60 g/l NaCl. The overall desirability values obtained were 0.767 at 300 MPa, and 0.726 at 600 MPa.

Once the sodium nitrite and ascorbic acid concentrations which provided the most acceptable colour were determined, the resulting immersion solution composition was experimentally validated. Meat samples were immersed in the optimised solution, and then treated with HHP (300 MPa and 600 MPa). Afterwards, the colour parameters (L*, a*) were measured and compared with those predicted by the equations representing the response surfaces shown in Fig. 2. Table 1 shows the colour parameters predicted and the experimental values. As can be observed there is a good agreement between the experimental colour values obtained for the optimised immersion solution concentration and those predicted by the response surface models.

3.2.4. Denaturation of myofibrillar proteins in HHP-treated samples immersed in the optimised solution

Differential Scanning Calorimetry was used to analyse the effects of the preservative solution and the high pressure treatment on beef

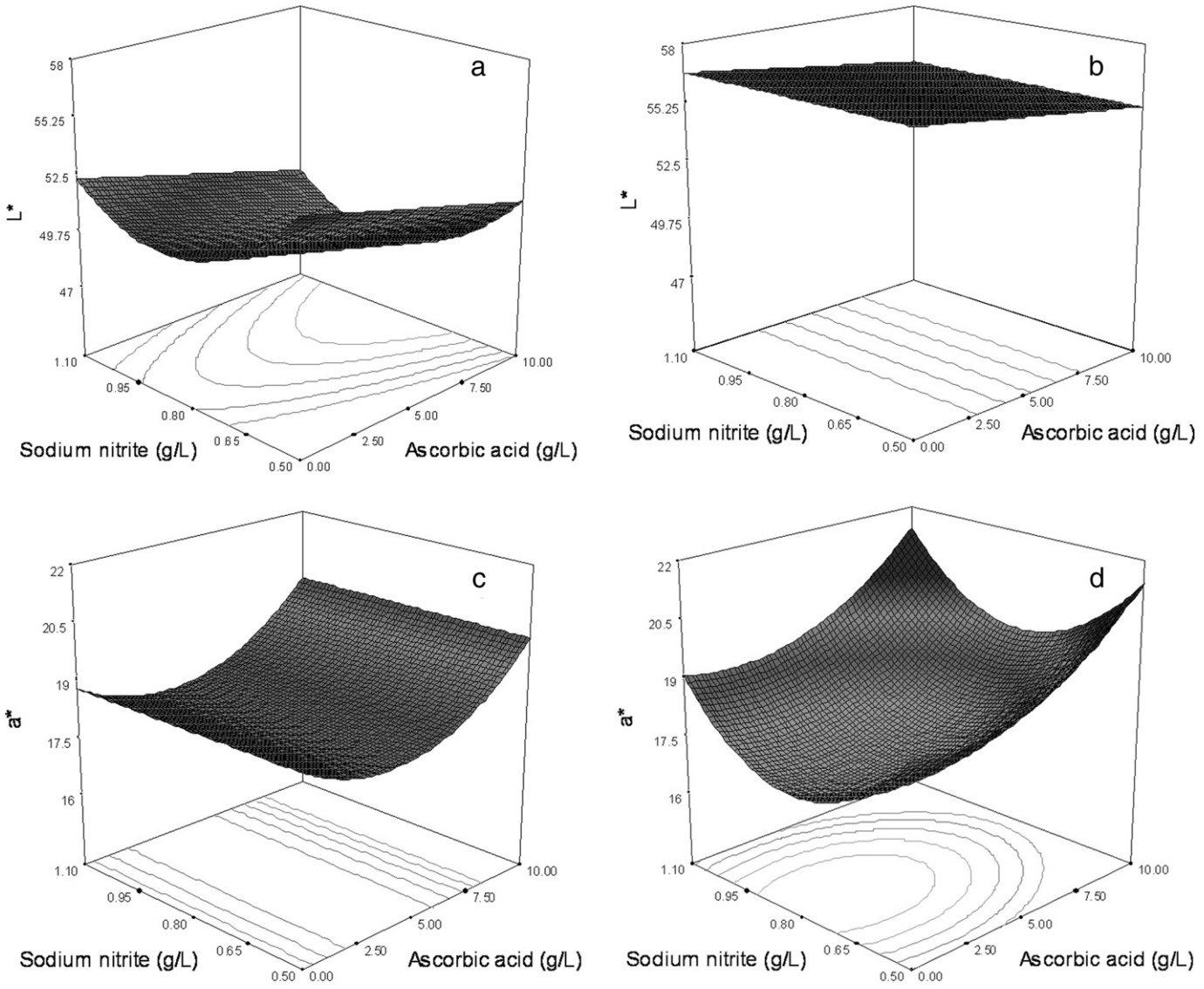

Fig. 2. Effect of sodium nitrite and ascorbic acid concentrations on colour parameters L* and a* for the different tested pressures (300 and 600 MPa). a) Parameter L* at 300 MPa, b) parameter L* at 600 MPa, c) parameter a* at 300 MPa and d) parameter a* at 600 MPa.

myofibrillar proteins. Thermograms of the control samples (fresh meat) and of the samples immersed in the optimised solution, both treated and non-treated with HHP were obtained and total enthalpy changes were estimated in each case. The immersion in the preservative solution of samples that were not subjected to HHP decreased the protein denaturation enthalpy by 38.5% (from 22.1 ± 1.4 W/g in fresh beef to 13.5 ± 1.2 W/g for beef samples immersed in the optimised solution). The decrease in the DSC thermogram peak is attributed to the action of salts, especially NaCl, on myofibrillar protein denaturation.

Different authors have analysed the effect produced by NaCl and other salts on protein denaturation in different types of meat. Kijowski and Mast (1988) studied the thermal transitions of chicken and its isolated myofibrils (water washed), particularly the effect produced by adding NaCl to different concentrations: 10–40 g/l. The analysis revealed that an increase in the concentration of NaCl led to a decrease of the total enthalpy. Barbut and Findlay (1991) studied the effect of adding different concentrations of NaCl, KCl, or $MgCl_2$ (12.5, 25, and 50 g/l) to beef, and established that an increase in the salt concentration affects the thermal stability of myosin and actin. Graiver et al. (2006) found that when pork was immersed into brine prepared with 5 to 50 g/l of NaCl, the salt triggered a protein denaturation process.

Table 1
Experimental and predicted colour parameters of beef samples immersed in the optimised preservative solution and high pressure treated at 300 and 600 MPa.

HPP (MPa)	Parameter	Experimental	Predicted
300	L*	47.46 ± 0.79	49.7
300	a*	19.74 ± 0.56	19.3
600	L*	56.26 ± 0.60	55.30
600	a*	19.31 ± 0.42	17.98

± Standard error of the mean.

The thermograms of the samples subjected to the optimum preservative solution and then to HHP (300 and 600 MPa) showed the absence of the endothermic typical peaks corresponding to myofibrillar proteins in samples treated with pressure of ≥300 MPa (data not shown). At low to moderate pressure levels (<100 MPa), the formation of hydrogen bonds allows to maintain the helical structure of proteins and minimise the effects on them. A pressure of 100–300 MPa leads to a reversible denaturation, while levels higher than 300 MPa cause an irreversible denaturation of proteins (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989).

Ikeuchi, Tanji, Kim, and Suzuki (1992) observed that the remarkable increase in the storage modulus of pressurized actomyosin at low and high KCl concentrations seemed to arise from pressure-induced denaturation of actin in actomyosin. Muscle proteins including myofibrillar

proteins are unfolded up to a pressure of 300 MPa. Pressures above this level result in increased denaturation, gel formation and agglomeration of proteins. Pressure induces structural changes in the main constituent of muscle filaments, which are possibly caused by increased ATPase activity at 30 MPa as well as with an increase of soluble materials from the myofibrils enhanced by pressurization above 150 MPa (Bajovic et al., 2012).

3.3. Test C: refrigerated storage of the samples under optimal conditions

Once the most appropriate immersion solution formulation was determined, the changes in the colour, texture, and exudate were studied for the immersed, vacuum-packed, and HHP-treated product during refrigerated storage at 4 °C for 50 days. Such findings, along with the microbial counts, allowed determining the shelf life of the product.

3.3.1. Production of exudate

Fig. 3 shows the exudate results throughout the refrigerated storage period for both levels of the HHP treatment. The pressure applied to the samples significantly affected ($P < 0.05$) the production of exudate, with higher values in samples treated with 600 MPa. In addition, the production of exudate increased significantly throughout the refrigerated storage period.

3.3.2. Water activity

Neither the pressure levels applied, nor the refrigerated storage time had a significant influence on the water activity of the product, with a mean value of 0.950 ± 0.005.

3.3.3. Colour parameters

Both the pressure levels applied and the refrigerated storage time had a significant effect on L*, while there was no significant interaction between these two parameters. Fig. 4a shows that L* values throughout the refrigerated storage period were higher at 600 MPa than at 300 MPa, with a similar pattern throughout the storage time. The storage time did not have a significant influence on L* values; at both pressure levels, after the third week of storage, L* tended to increase. In samples treated at 600 MPa, L* values varied between 52.33 at the beginning of the storage period, and 57.62 at the end. There was a significant dependence of a* ($P < 0.05$) on the pressure level applied, the storage time, and the interaction between these two factors. Fig. 4b shows that at both pressure levels, a* reached its maximum values after two weeks of storage, and then began to decrease. In the samples treated at 600 MPa, a* reached its lowest value (12.91 ± 0.80) after seven weeks of storage; however, it remained above 12 throughout the whole period. In the samples treated at 300 MPa, a* remained stable at 16.47 ± 0.34 as of the fourth week. Similarly, Campus et al. (2008) found that at refrigeration temperatures the colour underwent severe changes during vacuum storage. In these tests, the values of a* and L* in the HHP-treated samples showed a significant reduction after two days of storage, while a* values remained constant during the rest of the period. Jung, Ghoul, and de Lamballerie-Anton (2003), performed HHP experiments using vacuum packed meat in polyethylene film; as this plastic film material has a high oxygen gaseous permeability a significant decrease in a* values between the 1st and 7th days of storage was reported (a* < 9) after a treatment at 520 MPa. The authors assumed that the variation of a* values could be correlated to the accumulation of metmyoglobin in the samples. These results contrast with the obtained in the present work because in our case a barrier film (Cryovac BB4L) was used avoiding the formation of metmyoglobin and leading to a* values that remained >9 during storage.

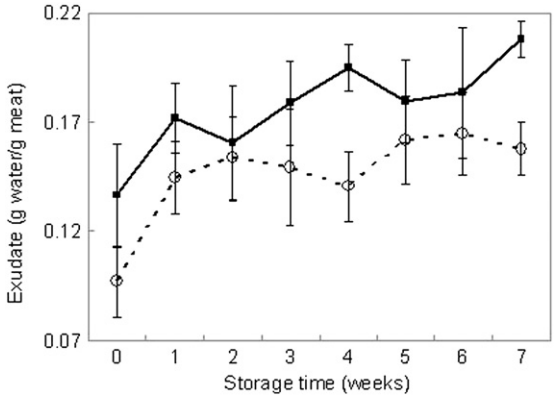

Fig. 3. Exudate results throughout the refrigerated storage at 4 °C of meat samples immersed in the optimised preservative solution, vacuum packaged, and submitted to HHP treatment: (--o--) 300 MPa and (—■—) 600 MPa. Bars indicate standard errors of the means.

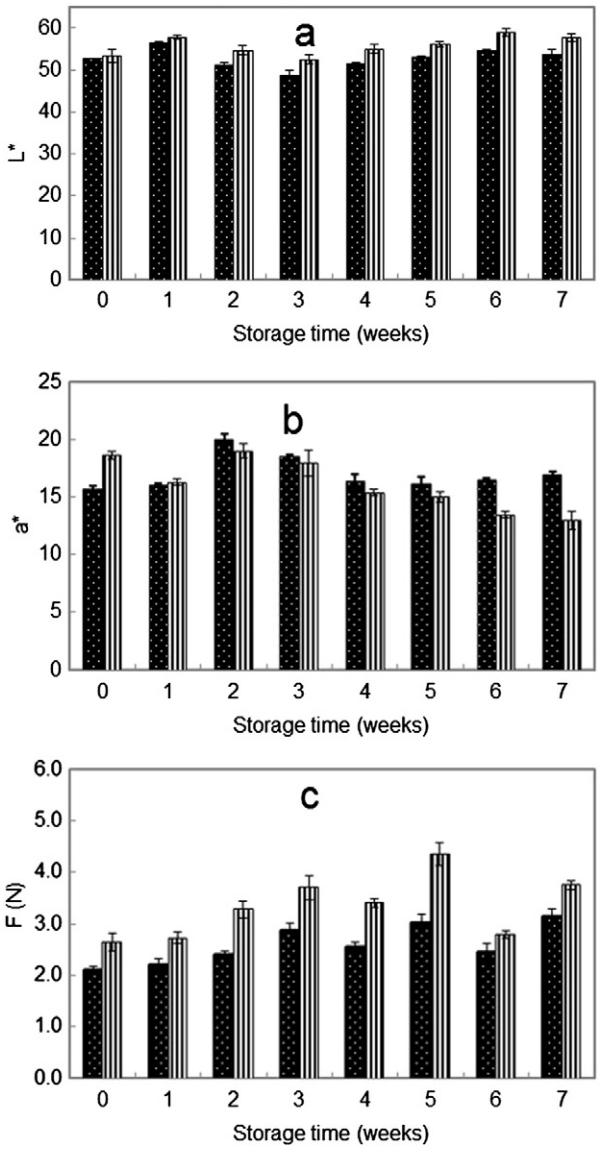

Fig. 4. Colour parameters and texture results throughout the refrigerated storage at 4 °C of meat samples immersed in the optimised preservative solution, vacuum packaged, and submitted to HHP treatment. a) L*, b) a*, c) maximum force ▩ 300 MPa, and ▥ 600 MPa. Bars indicate standard errors of the means.

3.3.4. Texture

The maximum force revealed by shear tests conducted with Volodkevich bite jaws depended significantly ($P < 0.05$) on the pressure level applied and on the storage time, but not on the interaction

between these two factors. The samples treated at 600 MPa showed a higher hardness value throughout the storage period (3.33 ± 0.13 N) than those treated at 300 MPa (2.58 ± 0.08 N). High pressure affected the microstructure of the tissue, increasing its hardness, which would be added to the decrease in the humidity content of the samples throughout the storage period caused by the higher production of exudate explained above. Fig. 4c shows that the maximum force tended to increase throughout the refrigerated storage period at both pressure levels.

3.3.5. Microbial counts

Fig. 5 shows the microbial growth in vacuum-packed HHP-treated meat (at 300 and 600 MPa) and fresh meat samples (FM) during refrigerated storage at 4 °C for a) mesophilic bacteria and b) lactic acid bacteria. According to Articles 255 bis (Res. 712, 25.4.85) and 286 (Joint Resolution of the Secretariat of Sanitary Policies and Regulations No. 104/2005 and the Secretariat of Agriculture, Livestock, Fisheries, and Food No. 414/2005) of the Argentine Food Code, in agreement with the Codex Alimentarius, the microbiological requirements for a brine-cured raw beef, are as follows:

- Mesophilic bacteria (count at 35 °C) < 10^6 CFU/g.
- Enterobacteriaceae < 100 CFU/g.
- Fungi and yeasts < 1000 CFU/g.
- No limit for acid lactic bacteria.

These values can be considered as a reference for our chemical pre-treated and HHP beef product.

In the case of fresh meat counts of mesophilic and lactic acid bacteria were higher than the legally permitted levels at the third week of refrigerated storage and Enterobacteriaceae counts were higher than 100 CFU/g after the second week.

The lactic acid bacteria and the mesophilic bacteria were the main constituents of non-treated fresh meat's flora during the refrigerated storage at 4 °C.

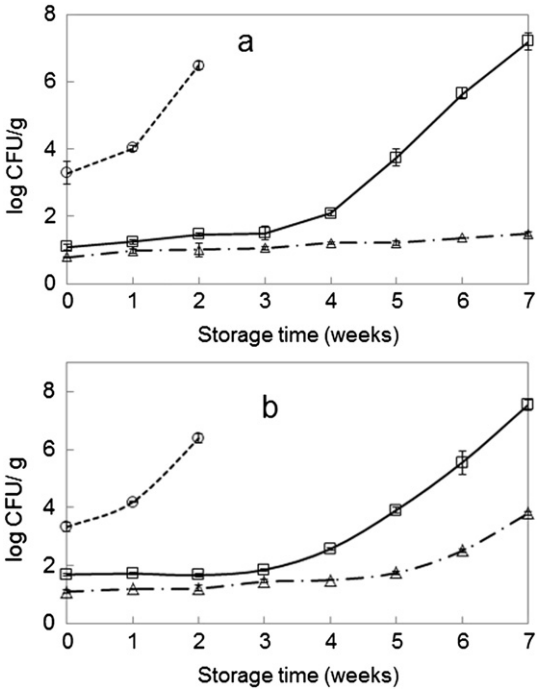

Fig. 5. Microbial growth: a) mesophilic bacteria and b) lactic acid bacteria during refrigerated storage at 4 °C of meat samples immersed in the optimised preservative solution, vacuum packaged, and submitted to HHP treatment, 300 (—) and 600 (–·–·–) MPa. Control sample corresponds to fresh meat without high pressure treatment (FM, – – – – –). Bars indicate standard errors of the means.

After a pressure of 300 MPa was applied, both the mesophilic bacteria and the lactic acid bacteria counts were under 2 log CFU/g until the fourth week of storage increasing to around 4 log CFU/g at the fifth week, and traversed two logarithmic cycles over the following week. The samples treated at 600 MPa showed a slight increase in the mesophilic bacteria counts throughout the seven weeks of storage, while the lactic acid bacteria increased at the fifth week.

There was an increase of Enterobacteriaceae (ABRV) in HHP non-treated vacuum-packed fresh meat during the first week of storage (3.83 ± 0.13 log CFU/g), with a relatively high concentration in the second week (4.39 ± 0.25 log CFU/g), while in the HHP-treated samples (at both pressure levels) the Enterobacteriaceae concentration was below the detection limit of the Most Probable Number (MPN) method throughout the storage period.

No fungi or yeasts were detected, and there was no increase of the psychrotrophic bacteria in the fresh meat or in the HHP-treated samples. An *Escherichia coli* detection test was also conducted in MacConkey broth using the Most Probable Number method, with negative results across all samples.

The high hydrostatic pressure treatment applied guaranteed the microbiological innocuity of the products. Based on these findings, it can be concluded that at 300 MPa the samples were microbiologically stable during 4 weeks and at 600 MPa during 6 weeks.

In order to ensure that the legally permitted concentration limits for preservatives were not exceeded, the sodium nitrite and sodium chloride concentrations of the HHP-treated meat samples stored at 4 °C were analysed. The values obtained at the beginning of the storage period – which remained stable until the end – were as follows: 0.048 ± 0.004 g $NaNO_2$/kg meat tissue and 33.4 ± 1.80 g NaCl/kg meat tissue, which complies with the regulations of the Codex Alimentarius for $NaNO_2$.

4. Conclusions

- The use of high hydrostatic pressure on fresh beef without a previous immersion in a preservative solution caused a deleterious effect on the distinctive red colour of beef as it affected the myoglobin.
- The presence of ascorbic acid in the preservative solution containing sodium nitrite and sodium chloride was essential to obtain the desired colour in the meat after hydrostatic pressure treatment.
- Pressure treatment significantly affected colour, texture, and water holding capacity; colour changes, maximum force and exudate values increased at the highest assayed pressure (600 MPa).
- The composition of the dipping solution was optimised (minimum L* and maximum a*) and experimentally validated.
- During the refrigerated storage at 4 °C, the beef product immersed in the optimised preservative solution, vacuum packed in low gas permeability film, and subjected to high pressure, showed that colour and texture attributes remained at acceptable consumer standards.
- The use of HPP decreased the initial microbial load by two logarithmic cycles and kept sanitary conditions for four and six weeks during refrigerated storage at 4 °C in samples subjected to 300 MPa and 600 MPa, respectively. In contrast, fresh beef (without immersion treatment or application of high hydrostatic pressure) lost its suitable sanitary condition for consumption after the second week of storage.

In conclusion, a HHP beef product exhibiting good colour and microbiological stability was developed; the process requires as a first step immersion in a preservative solution with an optimised composition based on colour parameters followed by vacuum packaging in low gas permeability film before the high pressure treatment.

Conflict of interest

The authors have declared no conflict of interest.

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References

AOAC (1980). *Official methods of analysis (13th ed)*. Washington, DC USA: Association of Official Analytical Chemists.

Aymerich, T., Jofré, A., Garriga, M., & Hugas, M. (2005). Inhibition of *Listeria monocytogenes* and *Salmonella* by natural antimicrobials and high hydrostatic pressure in sliced cooked ham. *Journal of Food Protection*, *68*, 173–177.

Aymerich, T., Picouet, P.A., & Monfort, J.M. (2008). Decontamination technologies for meat products. *Meat Science*, *78*, 114–129.

Bajovic, B., Bolumar, T., & Heinz, V. (2012). Quality considerations with high pressure processing of fresh and value added meat products. *Meat Science*, *92*, 280–289.

Bak, K.H., Lindahl, G., Karlsson, A.H., Lloret, E., Ferrini, G., Arnau, J., & Orlien, V. (2012). High pressure effect on the color of minced cured restructured ham at different levels of drying, pH, and NaCl. *Meat Science*, *90*, 690–696.

Barbut, S., & Findlay, C. (1991). Influence of sodium, potassium and magnesium chloride on thermal properties of beef muscle. *Journal of Food Science*, *56*, 180–182.

Beilken, S.L., Macfarlane, J.J., & Jones, P.N. (1990). Effect of high pressure during heat treatment on the Warner–Bratzler shear force values of selected beef muscles. *Journal of Food Science*, *55*(1), 15–18.

Bover-Cid, S., Belletti, N., Garriga, M., & Aymerich, T. (2011). Model for *Listeria monocytogenes* inactivation on dry-cured ham by high hydrostatic pressure processing. *Food Microbiology*, *28*, 804–809.

Campus, M., Flores, M., Martinez, A., & Toldrá, F. (2008). Effect of high pressure treatment on colour, microbial and chemical characteristics of dry cured loin. *Meat Science*, *80*, 1174–1181.

Carballo, J., Cofrades, S., Solas, M.T., & Jiménez-Colmenero, F. (2000). High pressure/thermal treatment of meat batters prepared from freeze-thawed pork. *Meat Science*, *54*, 357–364.

Cheftel, J.C., Carlez, A., & Veciana-Nogues, T. (1995). Changes in color and myoglobin of minced beef meat due to high pressure processing. *Lebensmittel Wissenschaft und Technologie*, *28*(5), 528–538.

Cheftel, J.C., & Culioli, J. (1997). Effects of high pressure on meat: A review. *Meat Science*, *46*, 211–236.

Chung, Y.K., Vurma, F., Turek, E.J., Chism, G.W., & Yuosef, A.E. (2005). Inactivation of barotolerant *Lysteria monocytogenes* in sausage by combination of high pressure processing and food-grade additives. *Journal of Food Protection*, *68*, 744–750.

Clariana, M., Guerrero, L., Sárraga, C., & Garcia-Regueiro, J.A. (2012). Effects of high pressure application (400 and 900 MPa) and refrigerated storage time on the oxidative stability of sliced skin vacuum packed dry-cured ham. *Meat Science*, *90*, 323–329.

Codex (1981). Standard for cooked cured ham, Codex stan 96-1981. http://www.codexalimentarius.org

Davidson, P.M. (2001). *Chemical preservatives and natural antimicrobial compounds*. (In M.P.).

Decker, E.A. (1998). Antioxidant mechanisms. In C.C. Akoh, & D.B. Min (Eds.), *Food lipids* (pp. 397–421). New York: Marcel Dekker.

Derringer, G., & Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology*, *12*(4), 214–219.

Feiner, G. (2006). Colour in fresh meat and in cured meat products. *Meat products handbook: Practical science and technology* (pp. 142–157). Cambridge: Woodhead Publishing.

Fernández, P.P., Sanz, P.D., Molina-García, A.D., Otero, L., Guignon, B., & Vaudagna, S.R. (2007). Conventional freezing plus high pressure-low temperature treatment: Physical properties, microbial quality and storage stability of beef meat. *Meat Science*, *77*, 616–625.

Fulladosa, E., Serra, X., Gou, P., & Arnau, J. (2009). Effects of potassium lactate and high pressure on transglutaminase restructured dry-cured hams with reduced salt content. *Meat Science*, *82*, 213–218.

Garriga, M., Aymerich, M.T., & Hugas, M. (2002). *Effect of high pressure processing on the microbiology of skin-vacuum packaged sliced meat products: Cooked pork ham, dry cured pork ham and marinated beef loin (Profit Final Project Report FIT060000200066)*.

Garriga, M., Grébol, N., Aymerich, M.T., Monfort, J.M., & Hugas, M. (2004). Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science and Emerging Technologies*, *5*, 451–457.

Graiver, N., Pinotti, A., Califano, A., & Zaritzky, N. (2006). Diffusion of sodium chloride in pork tissue. *Journal of Food Engineering*, *77*, 910–918.

Grobbel, J.P., Dikeman, M.E., Yancey, E.J., Smith, J.S., Kropf, D.H., & Milliken, G.A. (2006). Effects of ascorbic acid, rosemary, and Origanox in preventing bone marrow discoloration in beef lumbar vertebrae in aerobic and anaerobic packaging systems. *Meat Science*, *72*, 47–56.

Harrington, J. (1965). The desirability function. *Industrial Quality Control*, *21*, 494–498.

Hoover, D.G., Metrick, C., Papineau, A.M., Farkas, D.F., & Knorr, D. (1989). Biological effects of high hydrostatic pressure on food microorganisms. *Food Technology*, *43*, 99–107.

Hugas, M., Garriga, M., & Monfort, J.M. (2002). New mild technologies in meat processing: High pressure as a model technology. *Meat Science*, *62*, 359–371.

Ikeuchi, Y., Tanji, H., Kim, K., & Suzuki, A. (1992). Dynamic rheological measurements on heat-induced pressurized actomyosin gels. *Journal of Agricultural and Food Chemistry*, *40*, 1751–1755.

Jung, S., de Lamballerie-Anton, M., & Ghoul, M. (2000). Textural changes in bovine meat treated with high pressure. *High Pressure Research*, *19*, 69–74.

Jung, S., Ghoul, M., & de Lamballerie-Anton, M. (2003). Influence of high pressure on the color and microbial quality of beef meat. *Lebensmittel Wissenschaft und Technologie*, *36*, 625–631.

Kijowski, J. M., & Mast, M. G. E. (1988). Effect of sodium chloride and phosphates on the thermal properties of chicken meat proteins. *Journal of Food Science*, *53*(2), 367–370 (387).

Knorr, D. (1993). Effects of high hydrostatic pressure processed on food safety and quality. *Food Technology*, *47*, 156–161.

Knorr, D. (1995). Hydrostatic pressure treatment of food: Microbiology. In G. W. Gould (Ed.), *New emerging technologies for food preservation*. Glasgow: Blackie Academic and Professional.

Lee, B.J., & Hendricks, D.G. (1997). Metal-catalyzed oxidation of ascorbate, deoxyribose and linoleic acid as affected by phytic acid in a model system. *Journal of Food Science*, *62*, 935–938.

Lee, B.J., Hendricks, D.G., & Cornforth, D.P. (1999). A comparison of carnosine and ascorbic acid on color and lipid stability in a ground beef patty model system. *Meat Science*, *51*, 245–253.

Ma, H.J., & Ledward, D.A. (2004). High pressure/thermal treatment effects on the texture of beef muscle. *Meat Science*, *68*, 347–355.

Mancini, R.A., Hunt, M.C., Hachmeister, K.A., Kropf, D.H., & Johnson, D.E. (2004). Ascorbic acid minimizes lumbar vertebrae discoloration. *Meat Science*, *68*, 339–345.

Mancini, R. A., Hunt, M. C., Seyfert, M., Kropf, D. H., Hachmeister, K. A., Herald, T. J., & Johnson, D. E. (2007). Comparison of ascorbic acid and sodium erythorbate: Effects on the 24 h display colour stability of beef lumbar vertebrae and longissimus lumborum packaged in high-oxygen modified atmospheres. *Meat Science*, *75*, 39–43.

O'Brien, J.K., & Marshall, R.T. (1996). Microbiological quality of raw ground chicken processed at high isostatic pressure. *Journal of Food Protection*, *59*, 146–150.

Ohshima, H., & Bartsch, H. (1981). Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. *Cancer Research*, *41*, 3658–3662.

Ohshima, T., Ushio, H., & Koizumi, C. (1993). High pressure processing of fish and fish products. *Trends in Food Science and Technology*, *4*, 370–375.

Pearson, A., & Gilett, T. (1996). *Curing in processed meats* (3rd ed.). New York: Editorial Chapman & Hall.

Realini, C.E., Guàrdia, M.D., Garriga, M., Pérez-Juan, M., & Arnau, J. (2011). High pressure and freezing temperature effect on quality and microbial inactivation of cured pork carpaccio. *Meat Science*, *88*, 542–547.

Risvik, E. (1994). Sensory properties and preferences. *Meat Science*, *36*, 67–77.

Rubio, B., Martinez, B., Garcia-Cachan, M.D., Rovira, J., & Jaime, I. (2007). Effect of high pressure preservation on the quality of dry cured beef "Cecina de Leon". *Innovative Food Science and Emerging Technologies*, *8*(7), 102–110.

SENASA (2013). National Control Service for Animal Sanitary Status, Buenos Aires, Argentina. Resolución 82/2013. http://www.senasa.gov.ar/contenido.php?to=n&in=1592&io=22814 (accessed April 2013)

Shivas, S.D., Kropf, D.H., Hunt, M.C., Kastner, C.L., Kendall, J.L.A., & Dayton, A.D. (1984). Effects of ascorbic acid on display life of ground beef. *Journal of Food Protection*, *47*, 11–15.

Smelt, J. P. P. M. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science and Technology*, *9*, 152–158.

Stich, H.F., Hornby, A.P., & Dunn, B.P. (1984). The effect of dietary factors on nitrosoproline levels in human urine. *International Journal of Cancer*, *33*, 625–628.

Sun, X.D., & Holley, R.A. (2010). High hydrostatic pressure effects on the texture of meat and meat products. *Journal of Food Science*, *75*(1), R17–R23.

Suzuki, A., Kim, K., Tangi, H., Nishiumi, T., & Ikeuchi, Y. (2006). Application of high hydrostatic pressure to meat and meat processing. In M. Noller, & F. Toldra (Eds.), *Advanced technologies for meat processing* (pp. 193–217).

Szerman, N., Barriob, Y., Schroeder, B., Martínez, P., Sancho, A.M., Sanow, C., & Vaudagna, S.R. (2011). Effect of high hydrostatic pressure treatments on physicochemical properties, microbial quality and sensory attributes of beef carpaccio. *Procedia Food Science*, *1*, 854–861.

Tanzi, E., Saccani, G., Barbuti, S., Grisenti, M.S., Lori, D., & Bolzoni, S. (2004). High-pressure treatment of raw ham. Sanitation and impact on quality. *Industria Conserve*, *79*, 37–50.

Tellez, S.J., Ramirez, J.A., Perez, C., Vazquez, M., & Simal, J. (2001). Aplicación de la alta presión hidrostática en la conservación de alimentos. *Ciencia y Tecnologia Alimentaria*, *3*, 66–80.

Toepfl, S., Mathys, A., Heinz, V., & Knorr, D. (2006). Review: Potential of high hydrostatic pressure and pulsed electric fields for energy efficient and environmentally friendly food processing. *Food Reviews International*, *22*(4), 405.

Torres, J.A., & Velazquez, G. (2005). Commercial opportunities and research challenges in the high pressure processing of foods. *Journal of Food Engineering*, *67*, 95–112.
Vaudagna, S.R., Gonzalez, C.B., Guignon, B., Aparicio, C., Otero, L., & Sanz, P.D. (2012). The effects of high hydrostatic pressure at subzero temperature on the quality of ready-to-eat cured beef carpaccio. *Meat Science*, *92*, 575–581.
Walpole, R.E., Myers, R.H., & Myers, S.L. (1998). *Probability and statistics for engineers and scientists* (6th ed.). Upper Saddle River, NJ: Prentice Hall.
Wen-Ching, K., Wen-Chian, L., Yu-Ting, T., & Chang-Wei, H. (2007). Kinetics of winter mushrooms (*Flammulina velutipes*) microstructure and quality changes during thermal processing. *Journal of Food Engineering*, *81*, 587–598.
Yamamoto, K., Takahashi, M., & Niki, E. (1987). Role of iron and ascorbic acid in the oxidation of methyl linoleate micelles. *Chemical Letter*, *6*, 1149–1152.
Young, O.A., & West, J. (2001). Meat color. In Y.H. Hui, W. -K. Nip, R.W. Rogers, & O.A. Young (Eds.), *Meat science and applications* (pp. 39–69). New York: Marcel Dekker, Inc.