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

S.R. Vaudagna [a,b], C.B. Gonzalez [a,b], B. Guignon [c], C. Aparicio [c], L. Otero [c], P.D. Sanz [c,*]

[a] Instituto Tecnología de Alimentos, Centro de Investigación de Agroindustria, Instituto Nacional de Tecnología Agropecuaria (INTA), CC77 (B1708WAB), Morón, Buenos Aires, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, CP C1033AAJ, Buenos Aires, Argentina
[c] Malta-Consolider Team, Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN), Consejo Superior de Investigaciones Científicas (CSIC), C/José Antonio Nováis 10, 28040 Madrid, Spain

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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 º C. With HHP at 20 °C, we observed a significant effect of pressure and time on the reduction of the counts.

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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.

* Corresponding author. Tel.: +34 91544507; fax: +34 915493627.
E-mail address: pedro.sanz@csic.es (PD. Sanz).

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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 prechilled 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

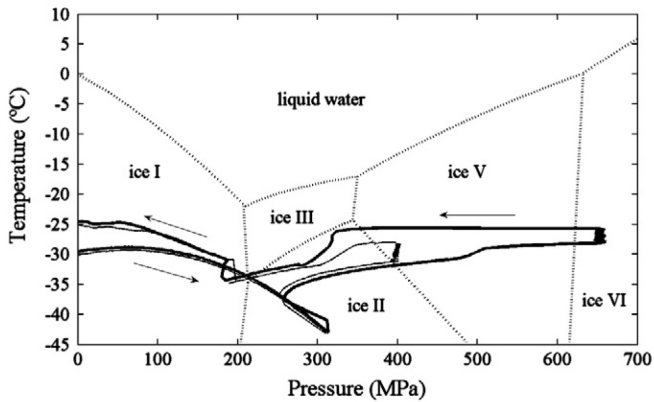

Fig. 1. Temperature versus pressure for a typical treatment of *carpaccio* processed in its frozen state (thin line: 1 min at 400 MPa, thick line: 1 min at 650 MPa) over the water phase diagram (dotted lines). Arrows indicate the kinetic way of the treatment.

analysis. The frozen samples were thawed in a chamber at 1 °C for 24 h before analysis.

2.4.1. Instrumental color

The chromatic parameters were determined in the CIEL*a*b* space using a Konica Minolta CM-3500d spectrophotometer. Because only differences between samples submitted to the same process were looked for, a D_{65} illuminant, 8° viewing angle and a black capsule as a light trap were used. Readings were taken in disks (40 mm diameter) obtained from the rectangular *carpaccio* samples. Also, in order to look for differences between samples and also to prevent weight losses of the sample due to the radiation heat transfer, each disk was placed on a Petri plate with the same diameter. The measurements were made at five positions of the same disk (one central and four lateral positions) and values were averaged for each disk. The chromatic parameters were measured in three disks per treatment evaluated. In addition, hue angle ($\tan^{-1}$ (b*/a*)) and chroma ($\sqrt{a*^2+b*^2}$) were calculated from the a* and b* results.

2.4.2. Expressible moisture (EM)

Disks of 25 mm in diameter were obtained from the rectangular *carpaccio* samples. Each disk was weighed and placed on a metal mesh covered with filter paper. Then it was introduced into centrifuge tubes with a volume of 500 ml. A Sorvall RC-5B 50,000 *g* centrifuge (Dupont Instruments, USA) equipped with a 3 l maximum volume rotor (six tubes) was used. The centrifugation time was 10 min on each side of the disk, using a rotational speed of 3500 rpm and a temperature of 4 °C. After centrifugation, each disk was reweighed and EM was calculated as follows:

$$EM = \frac{W_o - W_F}{W_o} \times 100\,(\%)$$

where: W_o = weight of the disks before centrifugation and W_F = weight of the disks after centrifugation. The determination of EM was performed in triplicate (three disks were separated from each rectangular *carpaccio* sample). EM parameter was measured in three *carpaccio* samples per treatment evaluated.

2.4.3. Instrumental texture

The shear force and work of shear were determined using a five-bladed Kramer cell (HDP/KS5) attached to a texture analyzer Stable Micro Systems Model TA.HD*plus* with a load cell of 500 kg. The following head speed values were applied for the analysis: pre-test 1 mm s^{-1}, test 1 mm s^{-1} and post-test 10 mm s^{-1}. For the measurements, two rectangular *carpaccio* samples (55 mm×75 mm×2.5 mm) were used, which were placed overlapping at the base of the Kramer cell (Taylor, Fjaera, & Skjervold, 2002). Before analysis, the samples were wrapped in aluminum foil and stored in a chamber at 1 °C for 4 h. The shear force was determined as the maximum strength value corresponding to the strength–distance profile, expressing it in Newtons (N). In addition, the work of shear (N mm) was calculated, defined as the area under the strength–distance profile from start to the maximum peak height.

2.4.4. Microbial analysis

Counts were analyzed in untreated *carpaccio* and in the processed *carpaccio* (three samples per treatment). Samples were prepared according to ISO 6887-1, 1999. Aerobic total count (ATC) at 30 °C, psychrotrophs count at 6.5 °C, *Enterobacteriaceae* and lactic acid bacteria (LAB) counts were determined following AFNOR NF V-08-051, 1999, ISO 17410, 2001, AFNOR NF V-08-054, 1999 and ISO 13721, 1995 respectively. In the untreated *carpaccio*, the presence/absence of *Listeria monocytogenes* was detected in 25 g (ISO 11290–1, 1996). All analyses were performed in duplicate for each sample. Data were expressed as $\log_{10}$ CFU g^{-1} with a detection limit of 1 $\log_{10}$ CFU g^{-1}.

2.5. Statistical analysis

One-way variance analysis (ANOVA) was performed to compare the effect of the treatments (combination of pressure, holding time and temperature) on the parameters evaluated. Tukey's multiple range test was used to determine differences among means ($P<0.05$). SAS 8.0 (SAS Inst. Inc, Cary, NC) software was applied.

3. Results and discussion

3.1. Instrumental color

Table 1 shows the effect of HHP treatments evaluated in this study on the chromatic parameters of cured beef *carpaccio*.

In the samples treated at 20 °C, a significant ($P<0.05$) increase in lightness (L*) was observed as a result of the increment of pressure from 400 to 650 MPa for a holding time of 1 min. However, the L* value in the samples treated at 400 MPa and 20 °C did not differ from that of samples treated at 650 MPa and 20 °C, when the holding time was 5 min. In addition, the increase in pressure (400 to 650 MPa) in the treatment for 1 min induced an increment in L* similar to that observed when the holding time was raised from 1 to 5 min at 400 MPa. L* values of untreated *carpaccio* (40.24 ± 0.58; data not shown in Table 1) and *carpaccio* frozen at −35 °C (40.28 ± 1.67; data not shown in Table 1) were similar to those of *carpaccio* treated at 20 °C and 400 MPa for 1 min (Table 1). Several studies have pointed out the loss of the original color of meat when HHP was applied at a temperature above 0 °C for beef pieces (Jung et al., 2003, Marcos et al., 2010), minced beef (Carlez et al., 1995; Goutefongea et al., 1995) and fresh and cured minced pork (Goutefongea et al., 1995). The obtained results on fresh cured beef *carpaccio* pressurized at 20 °C are supported by the findings described in previous paragraphs. Nevertheless, a less studied issue was the effect of holding time at working pressure on chromatic parameters or visual appraisal of meat. In this way, Montero and Gómez-Guillén (2005) reported that the whitening and redness decrease phenomena appear to depend more on critical pressure thresholds than on pressurization time. However, in this study a significant effect of holding time (1 or 5 min) was observed on the lightness and yellowness of cured beef *carpaccio* treated at 400 MPa and 20 °C.

It is commonly accepted that no variation in the original color appears in meat after air blast freezing. This fact has also been assessed here for cured beef *carpaccio* as was described above. When this sample was then treated with HHP at −30 °C, no effect of pressure and holding time increase was observed (Table 1). In turn, there were no significant differences ($P>0.05$) of these L* values as compared

Table 1
Effect of HHP on CIEL*a*b*, hue angle and chroma parameters of cured beef carpaccio.

Chromatic parameter	Pressure (MPa)	Holding time (min)			
		1		5	
		Temperature (°C)			
		−30	20	−30	20
L*	400	39.64 ± 2.44 b	42.66 ± 0.76 b	39.39 ± 0.78 b	51.23 ± 0.39 a
	650	40.50 ± 1.56 b	50.46 ± 1.17 a	42.70 ± 1.87 b	52.65 ± 1.58 a
a*	400	4.38 ± 0.82 a	4.23 ± 0.14 ab	4.46 ± 0.60 a	3.64 ± 0.65 ab
	650	3.65 ± 0.48 ab	3.42 ± 0.55 ab	4.04 ± 0.97 ab	2.47 ± 0.54 b
b*	400	8.60 ± 0.78 bc	8.02 ± 0.41 c	7.91 ± 0.24 c	9.82 ± 0.33 ab
	650	8.32 ± 0.29 bc	10.09 ± 0.15 a	10.19 ± 1.01 a	9.76 ± 0.84 ab
Hue angle	400	1.09 ± 0.14 b	1.09 ± 0.01 b	1.06 ± 0.07 b	1.21 ± 0.06 ab
	650	1.16 ± 0.06 ab	1.25 ± 0.06 ab	1.19 ± 0.05 ab	1.33 ± 0.04 a
Chroma	400	9.72 ± 0.80 b	9.06 ± 0.43 b	9.09 ± 0.09 b	10.48 ± 0.28 ab
	650	9.09 ± 0.22 b	10.66 ± 0.09 ab	11.71 ± 1.19 a	10.07 ± 0.92 ab

a–c Different letters in the cells corresponding to the same chromatic parameter indicate mean values that are significantly different ($P<0.05$).

to those of untreated *carpaccio*, *carpaccio* which was only frozen at −35 °C, or *carpaccio* pressurized at 400 MPa/20 °C for 1 min (Table 1). Arnau et al. (2006) proposed that the application of HHP at a low temperature on frozen meat might prevent color degradation. In the same way, Fernández et al. (2007) reported that beef processed by a combination of air blast freezing and high pressure-low temperature showed chromatic parameter values similar to those of fresh beef meat after thawing. Those authors also proposed that irreversible myoglobin denaturation could occur in fresh meat treated with HHP, whereas that protein might recover its native conformation in frozen beef pressurized at subzero temperature upon thawing. Fernández et al. (2007) pointed out that further research should focus on fundamentals of the protection of subzero temperature against the detrimental effect of HHP on meat color. Realini et al. (2011) reported that frozen cured pork *carpaccio* treated with HHP (at 400 MPa or 600 MPa; holding time 6 min) at −15 °C was lighter than control samples. However, the authors did not find differences in L* values when samples were pressurized at −35 °C. In the present study no differences in lightness among cured beef *carpaccio* samples treated with HHP at −30 °C and control samples were observed either, regardless of pressure level and holding time applied. The hypothetical relation of L* value and the pressure-induced protein denaturation is an important topic under deep investigation.

The mean a*-values (green–red) of the untreated *carpaccio* and the *carpaccio* frozen at −35 °C were 4.68 ± 0.20 and 4.00 ± 0.38, respectively. In addition, Table 1 shows the results of the a*-values belonging to HHP treated cured beef *carpaccio*. In general, this parameter was less affected by the HHP treatments than L*. The only treatments that differed were those performed at −30 °C and 400 MPa (1 and 5 min) compared to the treatment at 20 °C and 650 MPa for 5 min. In the last case, the samples presented a significantly lower value of a* ($P<0.05$). An important decrease in redness was reported in minced beef meat after pressurization ranging between 400 and 500 MPa/10 min/10 °C (Carlez et al., 1995). These results were confirmed by Jung et al. (2003) in *Bicep femoris* beef samples after HHP treatment (350–600 MPa/10 °C/20–300 s). They proposed that this decrease could be due to the oxidation of ferrous myoglobin to ferric metmyoglobin resulting in a gray–brown coloration (Carlez et al., 1995; Jung et al., 2003). High pressure treatment of raw cured meat has shown discoloration problems, with an increase in lightness but a minor effect on redness (Realini et al., 2011). In the present study, redness was in general less affected by the HHP treatment than lightness; even fresh *carpaccio* samples pressurized at 20 °C showed a* values similar to those of samples treated with HHP at −30 °C, with the exception of fresh *carpaccio* submitted to the most severe HHP treatment evaluated (650 MPa at 20 °C for 5 min). Concerning cured beef *carpaccio* treated with HHP at −30 °C, all samples presented similar values of a* parameter, regardless of the pressure level and holding time applied. Realini et al. (2011) also reported that the redness of cured pork *carpaccio* treated with HHP (400 MPa or 600 MPa) at low temperature (−15 °C or −35 °C) did not differ from the control samples.

The mean b*-values (blue–yellow) belonging to the untreated *carpaccio* and the *carpaccio* frozen at −35 °C were 7.65 ± 0.78 and 7.94 ± 0.88, respectively. As regards the b* parameters of the *carpacci*o treated with HHP at 20 °C, an effect similar to that described for L* was observed (Table 1). There was a significant increase ($P<0.05$) of b* when time increased from 1 to 5 min at 400 MPa and also when pressure increased from 400 to 650 MPa for 1 min. An increase of yellowness induced by high pressure was also observed by Goutefongea et al. (1995) in fresh minced beef and pork treated at 600 MPa/20 °C/30 min and by Fernández et al. (2007) in fresh and salt-added beef pressurized at 650 MPa/20 °C/10 min. Besides, in the present study, most of the *carpaccio* samples treated with the different HHP treatments at −30 °C presented no differences, showing b* values similar to that of the untreated *carpaccio* (7.65 ± 0.78; data not shown in Table 1) and that of the *carpaccio* frozen at −35 °C (7.94 ± 0.88; data not shown in Table 1). The only exception to this finding was the *carpaccio* treated with 650 MPa at −30 °C for 5 min, which showed a significantly higher value of b* ($P<0.05$). The result for *carpaccio* samples treated at 650 MPa/−30 °C/5 min differ from that reported by Fernández et al. (2007), who observed that frozen beef (raw or salt-added) pressurized at 650 MPa/−35 °C/10 min presented, after thawing, similar yellowness values to those of raw or salt-added control samples. However, present results are supported by those reported by Realini et al. (2011), who found that b* values were higher for cured pork *carpaccio* treated at 600 MPa at −15 °C or −35 °C for 6 min, compared with control samples.

Discoloration (fading) decreases a*-values and increases b*-values (with or without changes in L*). For this reason, the ratio of these parameters is more sensitive in detecting shifts from pink to tan or from red to maroon or red to brown (AMSA, 1991). Hue angle is the development of color from red to yellow and larger angles indicate a less red product (Tapp, Yancey, & Apple, 2011). Regarding hue angle values (Table 1) it can be seen that this parameter was in general only slightly affected by the HHP treatment; even fresh *carpaccio* samples pressurized at 20 °C showed hue angle values similar to those of samples treated with HHP at −30 °C, with the exception of fresh *carpaccio* treated at 650 MPa and 20 °C for 5 min. In this case, samples presented significantly higher ($P<0.05$) hue angle values than the samples treated at 400 MPa and −30 °C (1 or 5 min) or 400 MPa and 20 °C for 1 min, and consequently, a more yellow and less red product was obtained. According to Goutefongea et al. (1995), the changes in yellowness could be related to myoglobin oxidation.

A general view of the results described in previous paragraphs would indicate that the combination of conventional freezing and HHP treatment at subzero temperature prevents color degradation

of cured beef *carpaccio*. In these conditions, no effects of pressure level or holding time were observed on lightness and redness of raw cured beef. In addition, the *carpaccio* samples processed under HHP at −30 °C presented no differences in yellowness compared to controls, with the exception of the samples treated at 650 MPa for 5 min, which showed a significantly higher value of b*.

3.2. Expressible moisture and instrumental texture

The water holding capacity (WHC) of cured beef *carpaccio* was measured by means of the expressible moisture (EM). The mean EM value of untreated *carpaccio* was 15.93 ± 2.71. Regarding the HHP treated *carpaccio*, it was observed that samples treated at 20 °C and 400 or 650 MPa for either 1 or 5 min showed no significant differences ($P>0.05$) among them (Table 2). Thus, neither pressure level nor holding time changed WHC of fresh cured beef *carpaccio* pressurized at 20 °C. Marcos et al. (2010) reported EM increment in beef pieces treated at 400 or 600 MPa for 20 min at different temperatures (10, 20 or 30 °C), without differences between both pressure levels. They observed no changes in EM of samples treated at 200 MPa compared with control samples. Kim, Lee, Lee, Kim, and Yamamoto (2007) reported a reduction in WHC of beef *Semitendinosus* muscle treated between 200 and 500 MPa, although they observed no differences in WHC of samples treated at 100 MPa compared with control samples. In addition, Fernández et al. (2007) reported EM increase in beef pieces treated at 650 MPa and 20 °C for 10 min, although they did not find changes of EM in salt-added beef pieces pressurized at the same conditions. Myofibrillar proteins, mainly myosin and actin, are the major water-binding components in muscular tissue (Offer & Knight, 1988). It is generally accepted that pressure above 200–400 MPa (at temperature > 0 °C) induces native myofibrillar protein aggregation and denaturation (Ledward, 1998; Ma & Ledward, 2004), the extent of it depending on pressure, temperature and ionic strength conditions (Montero & Gómez-Guillén, 2005). In addition, Marcos et al. (2010) suggested that pressure-induced denaturation of sarcoplasmic proteins could influence to some extent the loss of WHC in pressurized meats. Fernández et al. (2007) suggested that the salt effect upon EM of pressurized salt-added beef pieces could be explained in terms of water-binding increment because of myofibril swelling. Those authors inferred that myofibril swelling could be enough to bind water and, consequently, to reduce its loss during HHP treatment.

Frozen *carpaccio* samples treated with HHP at −30 °C tended to show higher EM values than the ones submitted to HHP treatment at 20 °C. However statistical differences were only confirmed for some treatments (Table 2). It would appear that the application of HHP treatment to frozen *carpaccio* samples reduced the water loss during pressurization, probably due to the inhibited mobility of frozen water. Therefore, this water would be available for squeezing during centrifugation of thawed samples. Fernández et al. (2007) reported that the application of HHP at 650 MPa and −35 °C for 10 min to the previously frozen beef reduced water loss due to a minimization of pressure induced myofibrillar proteins denaturation. However, in the present work this effect would not have been observed.

Table 2 also shows the values of shear force and work of shear of cured beef *carpaccio* samples submitted to the HHP processes evaluated in this study. For both parameters, it was observed that the only samples that differed significantly ($P<0.05$) were those treated at 400 MPa and 20 °C for 1 min and 650 MPa and 20 °C for 5 min. The former showed the lowest values of both parameters whereas the latter showed the highest ones. The values of shear force and work of shear of untreated *carpaccio* were 228.5 ± 38.7 N and 644.4 ± 59.7 N mm, respectively (data not shown in Table 2).

A large body of research has shown that pressure treatment at temperatures higher than 0 °C can induce changes in meat texture (Sun & Holley, 2010). The effect of HHP at temperatures > 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). In general, low pressure (<200 MPa) treatment can tenderize pre-rigor meat, whereas tenderization post-rigor with HHP can only be achieved at temperatures between 40 and 80 °C. Besides, some authors reported that HHP treatment at low or moderate temperatures caused toughening of post-rigor meat. Thus, Jung et al. (2000) reported that HHP treatment (130 or 520 MPa and 10 °C for 260 s) significantly increased the mechanical resistance of both raw and cooked (65 °C, 1 h) post-rigor beef 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 and/or neutralize the effect of HHP on post-rigor tenderization in the absence of heat treatment. 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, and with further increases at increased temperature and ambient pressure. In the present study, regarding fresh cured beef *carpaccio* pressurized at 20 °C, an increment in the shear force and work of shear values of those samples treated at the most intense conditions (650 MPa for 5 min) were also observed.

Concerning conventional freezing plus high pressure-low temperature treatment, it can be noted that there are a few studies regarding its effect on meat texture. Fernández et al. (2007) reported no effect of HHP on air blast frozen beef (fresh and salt-added pieces) at 650 MPa/−35 °C/10 min on Warner–Bratzler shear force values. However, Realini et al. (2011) reported that cured pork loin treated with HHP at 400 or 600 MPa and −15 or −35 °C for 6 min showed higher values of Warner Bratzler shear force than control samples,

Table 2
Effect of HHP on expressible moisture (EM), shear force and work of shear of cured beef carpaccio.

Parameter	Pressure (MPa)	Holding time (min)			
		1		5	
		Temperature (°C)			
		−30	20	−30	20
EM (%)	400	16.17 ± 0.35 c	11.63 ± 1.31 c	22.12 ± 2.07 ab	18.70 ± 0.40 bc
	650	22.97 ± 1.85 ab	15.60 ± 1.15 c	23.23 ± 0.84 a	19.30 ± 3.12 bc
Shear force	400	271.53 ± 85.39ab	136.84 ± 7.54 b	246.79 ± 89.44 ab	190.48 ± 60.35 ab
(N)	650	239.09 ± 33.82 ab	270.47 ± 28.54 ab	230.54 ± 28.49 ab	289.21 ± 43.28 a
Work of shear	400	727.52 ± 139.28 ab	399.02 ± 30.61 b	699.19 ± 228.35 ab	529.84 ± 180.66 ab
(N mm)	650	635.82 ± 19.70 ab	803.74 ± 124.28 a	628.29 ± 71.60 ab	870.73 ± 162.45 a

a–c Different letters in the cells corresponding to a same parameter (EM, shear force or work of shear) indicate mean values that are significantly different ($P<0.05$).

with that toughness increment being independent of the pressure level. In the present study, the shear force and the work of shear of the cured beef *carpaccio*, treated at 400 or 650 MPa and at −30 °C for 1 or 5 min did not differ significantly (Table 2). These results suggest that the previous freezing process and the HHP treatment at −30 °C allowed for minimizing the effect of the HHP on the microstructure of the *carpaccio* samples. In this way, Realini et al. (2011) reported that cured pork loin treated at −15 °C tended to show higher shear force values than samples treated at −35 °C, and they suggested a protective effect of the lower temperature from the HHP effects on muscle structure. These findings would be associated with a reduction of the pressure-induced muscle fiber elongation (Gonzalez et al., 2009), which would cause an increase in meat toughness.

3.3. Microbial analysis

The untreated *carpaccio* and *carpaccio* only frozen at −35 °C presented the following counts: ATC at 30 °C: 5.65 ± 0.39 and 5.48 ± 0.02 $\log_{10}$ (CFU g^{-1}) respectively; psychrotrophs: 6.21 ± 0.37 and 6.46 ± 0.04 $\log_{10}$ (CFU g^{-1}) respectively; LAB: 5.22 ± 0.31 and 5.24 ± 0.37 $\log_{10}$ (CFU g^{-1}) respectively; *Enterobacteriaceae*: <1 $\log_{10}$ (CFU g^{-1}), in both cases. Besides, *Listeria monocytogenes* was found to be absent from the 25 g of untreated *carpaccio*.

Table 3 shows the counts of ATC at 30 °C, psychrotrophs at 6.5 °C and LAB corresponding to samples of *carpaccio* submitted to the HHP processes. The *Enterobacteriaceae* counts were not included in Table 3 because all the samples had counts below the detection limit (<1 $\log_{10}$ CFU g^{-1}). ATC from samples of fresh *carpaccio* treated at 650 MPa and 20 °C for 1 or 5 min differed significantly ($P<0.05$) from the counts of the samples submitted to other treatments, with the exception of ATC from samples treated at 400 MPa and 20 °C for 5 min. ATC from these samples did not differ significantly ($P>0.05$) from counts from *carpaccio* pressurized at 650 MPa and 20 °C for 1 min. Thus, cycle reductions of at least 2.9 and 4.4 $\log_{10}$ were observed after treatment at 650 MPa and 20 °C for 1 and 5 min, respectively. ATC reductions after the application of the other HHP treatments at 20 °C were 1.7 $\log_{10}$ cycles (400 MPa for 1 min) and 2.6 $\log_{10}$ cycles (400 MPa for 5 min). Regarding psychrotrophs and LAB counts, a similar effect to that for ATC was observed. Concerning psychrotrophs, at least 4.2 and 5.5 $\log_{10}$ cycle reductions were found after treatment at 650 MPa and 20 °C for 1 and 5 min, respectively. In turn, the other HHP treatments at 20 °C reduced 2.8 $\log_{10}$ cycles (400 MPa for 1 min) and 3.9 $\log_{10}$ cycles (400 MPa for 5 min) for psychrotroph counts. In addition, LAB counts were reduced by at least 3.6 and 4.2 $\log_{10}$ cycles after treatment at 650 MPa and 20 °C for 1 and 5 min, respectively. Besides, HHP at 400 MPa and 20 °C reduced LAB counts in 1.5 and 2.9 $\log_{10}$ cycles for 1 and 5 min, respectively. According to the present results, it can be pointed out that HHP treatment at 20 °C reduced significantly bacterial counts of fresh cured beef *carpaccio*. In general, these reductions were affected by pressure level and holding time at working pressure (mainly at 650 MPa). The count reductions observed after treatments at 650 MPa and 20 °C in cured beef *carpaccio* were similar to those reported by Franceschini, Gola, Rovere, and Frustoli (2005) for beef *carpaccio* treated at 600 MPa and 20 °C for 10 min. These authors observed 3 $\log_{10}$ cycle reductions for aerobic plate counts. As well, Garriga et al. (2004) observed at least 4 $\log_{10}$ cycle reductions (aerobic, psychrotrophic and LAB) after HHP treatment (600 MPa and 31 °C for 6 min) for sliced, vacuum-packaged marinated beef loin.

In the present study, treatments carried out at 650 MPa and 20 °C on fresh *carpaccio* were more effective in reducing bacterial counts than the application of HHP at 650 MPa and −30 °C for 1 or 5 min on frozen *carpaccio*. In addition, cured beef *carpaccio* treated by HHP at −30 °C showed similar microbial counts for 400 and 650 MPa, in spite of the holding time applied (1 or 5 min). Thus, no additional advantage in the inactivation of bacterial counts at higher pressure and/or longer holding time was observed. After HHP processing at −30 °C, the highest reductions achieved were 1.9, 2.6 and 2.4 $\log_{10}$ cycles for ATC, psychrotrophs and LAB counts, respectively. Fernández et al. (2007) reported reductions for ATC (>2 $\log_{10}$ cycles) and LAB (>2.4 $\log_{10}$ cycles) with a count below the detection limits after HHP treatment (650 MPa and −35 °C for 10 min) in frozen beef samples. Realini et al. (2011) reported 0.5 and 1.5 $\log_{10}$ cycle reductions for LAB and psychrotroph counts after HHP treatment (400 or 600 MPa and −15 or −35 °C for 6 min) of cured pork *carpaccio*, respectively. In addition, they reported that pork samples treated with HHP showed similar microbial counts for 400 and 600 MPa and −15 or −35 °C. The reduction in the effect of HHP on the inactivation of microorganisms observed in frozen meats seems to be due to their lower water activity (Luscher, Sunderhoff, Urrutia-Benet, & Knorr, 2005; Realini et al., 2011). Moreover, Realini et al. (2011) suggested an additional effect of the curing mixture used in their study (sodium chloride, sodium nitrite, dextrose and sucrose).

In summary, the combination of frozen conditioning and a curing process would limit the effect of high pressure at subzero temperature on beef *carpaccio*, restricting the reduction of bacterial counts to 2 or 2.5 $\log_{10}$ cycles. Therefore, the high bacterial counts observed in untreated *carpaccio* and the limited reduction achieved after HHP treatment at subzero temperature, would reduce markedly the shelf life of cured beef *carpaccio* during chilled storage. It should also be noted that cells could be able to recover activity during refrigerated storage, overcoming the sub lethal damage induced by HHP at low temperature (Realini et al., 2011).

4. Conclusions

The application of HHP at low temperature (−30 °C) to previously frozen cured beef *carpaccio* minimized the discoloration occurring in unfrozen *carpaccio* pressurized at 20 °C. In these conditions, no effects of pressure level or holding time at working pressure were observed on lightness, redness and yellowness of raw cured beef, with the exception of the samples treated at 650 MPa for 5 min, which showed a significantly higher b* value. Moreover, the HHP treatment at

Table 3
Effect of HHP on microbial counts of cured beef carpaccio.

Counts $\log_{10}$ (CFU g^{-1})	Pressure (MPa)	Holding time (min)			
		1		5	
		Temperature (°C)			
		−30	20	−30	20
Aerobic total count at 30 °C	400	4.06 ± 0.30 a	3.82 ± 0.61 a	3.57 ± 0.83 a	2.90 ± 0.41 ab
	650	4.42 ± 0.15 a	2.56 ± 0.42 b	3.81 ± 0.87 a	1.10 ± 0.17 c
Psychrotrophs	400	4.87 ± 0.55 a	3.66 ± 0.47 abc	4.28 ± 1.23 ab	2.48 ± 0.44 bc
	650	4.85 ± 0.37 a	2.26 ± 0.24 c	3.87 ± 0.71 abc	<1
Lactic acid bacteria	400	3.38 ± 0.02 ab	3.78 ± 0.56 a	2.99 ± 0.89 ab	2.35 ± 0.10 ab
	650	3.05 ± 0.53 ab	1.67 ± 0.78 b	2.85 ± 1.35 ab	<1

a–c Different letters in the cells corresponding to the same type of count indicate mean values that are significantly different ($P<0.05$).

−30 °C did not affect the shear force of the *carpaccio* respecting the untreated sample but was less effective than the HHP treatment at 20 °C in reducing the counts of the microorganisms analyzed. The freezing process would limit the effect of high pressure on microbial inactivation.

The optimum industrial use of the results presented in this paper should involve the use of untreated samples with a reduced initial microbial load. Consequently, for the actual development of the product, further studies including the addition of antimicrobial compounds during *carpaccio* preparation (the tumbling stage) or before HHP treatment at subzero temperatures should be needed to ensure safety and extend the shelf life of cured beef *carpaccio*.

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References

AFNOR NF V-08-051 (1999). Microbiology of food and animal feeding stuffs. *Enumeration of microorganisms by colony-count technique obtained at 30 °C. Routine method*.

AFNOR NF V-08-054 (1999). Microbiology of food and animal feeding stuffs. *Enumeration of the Enterobacteriaceae by colony-count technique at 30 °C. Routine method*.

American Meat Science Association (AMSA) (1991). Guidelines for meat color evaluation. *Originally published in the 1991 proceedings of the reciprocal meat conference, vol. 44*. (pp. 9–17) (9–12 June 1991, Kansas, USA.).

Arnau, J., Gou, P., Monfort, J. M., Sanz, P. D., Molina-García, A. D., Otero, L., et al. (17/03/2006). *Procedimiento para la protección y estabilización del color de carnes y productos elaborados de carne, frescos, marinados o parcialmente deshidratados, tratados por alta presión. Spanish application number: ES20060000735*.

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 Food Science*, *55*(1), 15–18.

Campus, M. (2010). High pressure processing of meat, meat products and seafood. *Food Engineering Review*, *2*, 256–273.

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

Cheah, P. B., & Ledward, D. A. (1996). High pressure effects on lipid oxidation in minced pork. *Meat Science*, *43*, 123–134.

Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat: A review. *Meat Science*, *46*(3), 211–236.

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

Franceschini, B., Gola, S., Rovere, P. P., & Frustoli, M. (2005). Application of high hydrostatic pressure to increase the safety and the shelf-life of ready-to-eat (RTE) traditional meals. *Industria Conserve*, *80*(4), 391–409.

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.

Gómez-Estaca, J., López-Caballero, M. E., Gómez-Guillén, M. C., López de Lacey, A., & Montero, P. (2009). High pressure technology as a tool to obtain high quality carpaccio and carpaccio-like products from fish. *Innovative Food Science and Emerging Technologies*, *10*, 148–154.

Gonzalez, C. B., Mendoza, F., Vaudagna, S. R., Guignon, B., Aparicio, C., Otero, L. & Sanz, P. D. (2009, October 7–9). Efecto de la aplicación de la tecnología de altas presiones hidrostáticas a temperatura subcero sobre la microestructura de *carpaccio* preparado en base a carne vacuna. In Asociación Argentina de Tecnólogos Alimentarios (Ed.). *XII Congreso Argentino de Ciencia y Tecnología de los Alimentos* (paper 7.27.). Concordia, Entre Ríos, Argentina: Asociación Argentina de Tecnólogos Alimentarios (AATA).

Goutefongea, R., Rampon, V., Nicolas, N., & Dumont, J. P. (1995). Meat colour changes under high pressure treatment. In American Meat Science Association (Ed.). *41st International Congress of Meat Science and Technology* (Vol. II, pp. 384–385). San Antonio, TX, USA. San Antonio, TX: American Meat Science Association (AMSA).

Guignon, B., Otero, L., Molina-García, A. D., & Sanz, P. D. (2005). Liquid water-ice I phase diagrams under high pressure: sodium chloride and sucrose models for food systems. *Biotechnology Progress*, *21*, 439–445.

ISO 11290–1 (1996). Microbiology of food and animal feeding stuffs. *Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 1: Detection method*.

ISO 13721 (1995). Meat and meat products. *Enumeration of lactic acid bacteria colony-count technique at 30 °C*.

ISO 17410 (2001). Microbiology of food and animal feeding stuffs. *Horizontal method for the enumeration of psychrotrophic microorganisms*.

ISO 6887–1 (1999). Microbiology of food and animal feeding stuffs. *Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Part 1: General rules for the preparation of the initial suspension and decimal dilutions*.

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 Wissenschat und-Technologie*, *36*, 625–631.

Kim, I. J., Lee, E. J., Lee, N. H., Kim, Y. H., & Yamamoto, K. (2007). Effects of hydrostatic pressure treatment on the physicochemical, morphological and textural properties de bovine semitendinosus muscle. *Food Science and Biotechnology*, *16*(1), 49–54.

Ledward, D. A. (1998). High pressure processing of meat and fish. In K. Autio (Ed.), *Fresh novel foods by high pressure* (pp. 165–175). Helsinki, Finland: VTT Biotechnology and Food Research.

Luscher, C., Sunderhoff, J. K., Urrutia-Benet, G., & Knorr, D. (2005). Pasteurization of meat in frozen conditions by high pressure. In P. Fito, & F. Toldrá (Eds.), *INTRADFOOD 2005 innovation in traditional foods (vol. II, pp. 1379–1382). Valencia, Spain.* : Editorial de la UPV and REPROVAL SL. Printed in behalf of Elsevier.

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

Marcos, B., Kerry, J. V. P., & Mullen, A. M. (2010). High pressure induced changes on sarcoplasmic protein fraction and quality indicators. *Meat Science*, *85*, 115–120.

McArdle, R., Marcos, B., Kerry, J. P., & Mullen, A. M. (2010). Monitoring the effects of high pressure processing and temperature on selected beef quality attributes. *Meat Science*, *86*, 629–634.

Montero, P., & Gómez-Guillén, M. C. (2005). High-pressure applications on myosystems. In G. Barbosa-Cánovas, M. S. Tapia, & M. P. Cano (Eds.), *Novel food processing technologies* (pp. 311–342). Boca Raton: CRC Press.

Offer, G. W., & Knight, P. (1988). The structural basis of water-holding in meat. In R. Lawrie (Ed.), *Part 1: General principles and water uptake in meat processing. Developments in meat science*, *vol. 4*. (pp. 63–171) London: Elsevier Applied Science Publishers Ltd.

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*(3), 542–547.

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.

Tapp, W. N., Yancey, J. W. S., & Apple, J. K. (2011). How is the instrumental color of meat measured? *Meat Science*, *89*, 1–5.

Taylor, R. G., Fjaera, S. O., & Skjervold, P. O. (2002). Salmon fillet texture is determined by myofiber–myofiber and myofiber–myocommata attachment. *Journal of Food Science*, *67*(6), 2067–2071.