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Effect of high hydrostatic pressure treatments on physicochemical properties, microbial quality and sensory attributes of beef *carpaccio*

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Abstract

The application of high hydrostatic pressure (HHP) to fresh and marinated beef has not had the same development as cooked or cured meat products. The limited commercial application of HHP on these products is due to the significant discoloration observed. In the present work, it was studied the application of HHP treatments on frozen or thawed *carpaccio* samples at three pressure levels (400, 500 and 600MPa) during 5 min at refrigeration temperatures (0 and 5°C) and room temperature (20°C). Carpaccio was prepared using *Semitendinosus* beef muscle marinated with antioxidant and preservative additives. Analyses performed in all samples were: expressible moisture, pH, shear force, work of shearing, CIELab chromatic parameters, sensory appearance and aerobic total count (ATC) at 30ºC. There was no temperature effect on HHP processing of thawed (5 and 20°C) or frozen (0 and 5°C) samples. Pressure level effect was only observed on ATC and work of shearing of thawed treated samples. Frozen conditioning of *carpaccio* previous to HHP treatments reduced the harmful effects of pressure on chromatic parameters (L*, a* and b*) and water holding capacity. This could evidence a minimization of the denaturation of sarcoplasmic and myofibrillar proteins. It was observed a lower effectiveness of HHP treatments on microorganisms' inactivation for frozen *carpaccio* than for thawed one.

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Keywords: High hydrostatic pressure; beef; carpaccio; frozen; thawed; chromatic parameters

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1. Introduction

During the last two decades, high hydrostatic pressure (HHP) technology has achieved a greater industrial application in comparison to others non-thermal preservation technologies. HHP technology has been successfully applied for the processing of cured meat products -cooked or dried- and cooked ready-to-eat meats [1]. In the case of ready-to-eat cured fresh meat (i.e. *carpaccio*), the HHP technology could be an alternative for product pasteurization, assuring food safety and extending shelf-life. However, the application of HHP on fresh pigmented meats causes an important discoloration, particularly at pressure levels above 300 MPa, which are required for vegetative cells inactivation [2]. In the range 200-350MPa, lightness increases as a consequence of the denaturation of globin and/or displacement or loss of heme iron. Besides, in the range 300-600MPa redness decreases, probably due to the oxidation of ferrous myoglobin to metmyoglobin [2,3]. In order to avoid or reduce those problems, some authors studied the addition of antioxidant compounds (i.e. sodium nitrite) to beef [2,4] and pork [4] or the effect of cooking on sensory quality of beef meat treated by HHP [3]. Moreover, the application of HHP at subzero temperature (-30ºC) to previously frozen beef minimized the effect of pressure on chromatic parameters of fresh beef meat [5,6]. However, in these studies the reduction of microorganism counts was lower in comparison to unfrozen samples pressurized at moderate temperature [6]. The study of the effect of HHP treatment at refrigeration temperatures to previously frozen meats is required since these temperatures instead of subzero ones could be feasible to apply in industrial scale systems.

The aim of this study was to evaluate the effect of sample conditioning (frozen or thawed) and HHP treatments (different pressure levels at refrigeration or moderate temperature) on physicochemical properties, microbial quality and sensory attributes of beef *carpaccio*.

2. Materials and Methods

For each *carpaccio* conditioning (frozen or thawed), a factorial randomized block (2) design was applied with temperature (two levels: 0°C and 5ºC -frozen samples- and 5°C and 20ºC -thawed samples-) and pressure (four levels: 0, 400, 500 and 600MPa) as main factors. Fresh *carpaccio* non-submitted to HHP treatment (0MPa) was used as control samples. In each block, three experimental units were used for each treatment applied. The experimental unit was a half of a *Semitendinosus* beef muscle. From each whole muscle, one half was used for trials with frozen *carpaccio* and the other half for thawed *carpaccio*. A total number of forty two (42) *Semitendinosus* muscles were used for the complete design.

For each block, twenty one (21) *Semitendinosus* beef muscles (48h post slaughter) were vacuum packaged (Cryovac BB4L, Sealed Air Co., Argentina) and stored for 24h at 1.5 ± 0.5°C until processing. The trimmed raw muscles had an average weight of 1263.6 ± 207.7g and an average pH of 5.84 ± 0.14. Muscles were tumbled intermittently for 60min (5rpm-2min on / 8min off) at 1.5 ± 0.5°C in a Lance Industries tumbler (model LT-15, Allenton, USA) using a drum load of 45kg (half of the maximum working capacity) under constant vacuum (2kPa) with the following additives: 12g/kg NaCl (Dos Anclas, Argentina), 1g/kg sodium tripolyphosphate (Carfosel 991, Tecnoalimenti, Argentina), 0.5g/kg sodium citrate (Polar, Tecnoalimenti, Argentina), 0.15g/kg sodium nitrite (General Chemical, Tecnoalimenti, Argentina) and 0.5g/kg sodium isoascorbate (Tate & Lyle, DGM, Brasil). After tumbling, muscles were vacuum packaged (Cryovac BB4L, Sealed Air Co., Argentina) and stored at 1.5 ± 0.5°C for 12 days. The proximate composition of *carpaccio* samples was as follows: moisture, 75.0%; protein, 21.1%; total fat, 2.4%; ash, 1.2% and carbohydrate, 0.4%. The aw of *carpaccio* samples was 0.981±0.005. After chill storage, cured muscles were frozen and stored at -40ºC during one day in a conventional freezer (Righi, Argentina). Afterward, frozen muscles were sliced transversely to the fibres (slice thickness: 1.5-2mm) with a slicer machine (Berkel Rotterdam Type 834, Netherlands) and vacuum packaged. Then, *carpaccio* samples were stored at -40°C until HHP treatment.

Samples packed under vacuum -frozen (-40°C) or thawed (in a storage chamber at 4°C)- were submitted to HHP treatments, according to the experimental design, in a High Pressure Iso-Lab System Stansted Fluid Power Ltd. (model FPG9400:922, Stansted, UK) with a vessel working volume of $2dm^3$ and a sample canister with a internal working diameter of 80mm. The rate of pressurization was $300MPa.min^{-1}$ and the holding time at working pressure was 5min. After HHP treatment, samples were stored at -40°C until further testing.

Analysis performed in all samples were: expressible moisture [7], pH [7], shear force and work of shearing measured with a 10 blade Kramer shear cell attached to a texture analyzer (Stable Micro Systems Model TA.XTplus) [6], CIELab chromatic parameters measured with a Minolta colorimeter model CR 400 (D65 illuminate and 2º observer), sensory appearance (triangular test) under controlled conditions (Veri-Vide CAC120 cabinet, D65 illuminate and 45º observer) and aerobic total count (ATC) at 30ºC [5,8]. Before analysis all frozen samples were thawed in a storage chamber at 4.0 ± 1.0°C.

Data analysis was performed using SAS software (version 8, SAS Institute Inc., 2004, Cary, NC). ANOVA was performed to evaluate significant ($p=0.05$) effect of main factors (pressure, temperature and its interaction) on all measured parameters on *carpaccio* samples. For each measured parameter, statistical differences between means from *carpaccio* samples (frozen or thawed) treated by HHP and control ones were determined using the Dunnett test ($p=0.05$). Besides, statistical differences among means from samples (frozen or thawed) treated by HHP were analyzed applying Tukey test ($p=0.05$).

3. Results and Discussion

Regarding the results of the ANOVA performed, there was not significant ($p>0.05$) temperature effect (0 and 5°C for frozen samples and 5 and 20°C for thawed samples) on anyone of all measured parameters on *carpaccio* samples treated by HHP and control samples. However, pressure effect was significant ($p<0.05$) for some parameters evaluated on *carpaccio* samples. The results corresponding to each measured parameter on *carpaccio* samples treated by HHP and control ones are presented in the following paragraphs.

3.1. Expressible moisture

Frozen or thawed *carpaccio* treated by HHP presented expressible moisture values (Table 1) significantly ($p<0.05$) higher than their respective controls. Myofibrillar proteins are denatured at pressures above 200-400MPa and consequently, the water holding capacity of meat tissue is reduced [5]. Frozen *carpaccio* treated by HHP presented significantly ($p<0.05$) higher values of water loss during centrifugation stage than thawed ones (data not shown). It would appear that the application of HHP treatment to frozen *carpaccio* samples minimized myofibrillar protein denaturation, reducing water loss during pressurization. Therefore, this water would be available for squeezing during centrifugation stage. Supporting this result, a previous report [6] has indicated a minimization of water loss during HHP processing of frozen beef *carpaccio* treated at subzero temperature (-35°C).

3.2. pH mesurement

A significant increase ($p<0.05$) of pH was observed in thawed samples treated by HHP (Table 1) compared to their control sample. Pressure induces a pH increment, which has been attributed to the redistribution of ions that occurs at elevated pressures [9,10]. Regarding frozen samples (Table 1), non significant differences ($p>0.05$) were observed between pH mean values of pressurised samples and the pH value of control one. This result would evidence that the freezing of *carpaccio* samples before the HHP treatment could minimize myofibrillar protein denaturation.

3.3. Shear force and work of shearing

Shear force values of frozen or thawed samples treated by HHP were higher than their respective controls (Table 1); although, those differences were not significant ($p>0.05$). Regarding work of shearing (Table 1), all frozen samples treated by HHP and thawed ones treated at 600MPa presented significantly higher ($p<0.05$) values than their respective controls. Previous studies also informed an increase in mechanical resistance [11] and shear force and work of shearing values [6] when pressure was incremented at the HHP processing of beef meat. This effect could be associated with a tissue morphology modification (fiber elongation) induced by HHP treatment [12], which would cause an increase in sample toughness.

Table 1. Expressible moisture (EM), pH, shear force (SF) and work of shearing (WS) of frozen or thawed *carpaccio* samples treated by HHP at different pressures and temperatures.

T (°C)	P (MPa)	EM (%)	pH	SF (N)	WS (J)
Frozen					
0	400	26.56±3.09*	5.87±0.12	232.53±52.56	0.66±0.17*
0	500	28.33±3.46*	5.88±0.08	239.00±54.48	0.69±0.21*
0	600	28.83±1.95*	5.88±0.11	223.32±30.48	0.66±0.12*
5	400	29.71±1.96*	5.90±0.19	247.82±55.57	0.73±0.17*
5	500	28.46±3.08*	5.84±0.05	235.53±57.17	0.64±0.15*
5	600	28.73±3.79*	5.87±0.13	241.48±25.94	0.69±0,09*
Control		20.49±4.18	5.79±0.07	199.05±18.78	0.53±0.04
Thawed					
5	400	25.57±2.62*	6.01±0.12*	215.78±35.31	0.59±0.08
5	500	26.25±2.55*	5.92±0.07*	220.82±59.43	0.59±0.18
5	600	26.34±1.89*	5.97±0.08*	234.78±35.17	0.65±0.12*
20	400	25.96±1.82*	5.98±0.11*	241.85±45.76	0.66±0.12
20	500	26.29±2.34*	5.98±0.06*	222.58±65.09	0.63±0.24
20	600	27.10±1.67*	5.97±0.07*	236.32±56.13	0.69±0.20*
Control		22.33±3.99	5.88±0.07	190.78±38.42	0.51±0.08

*3.4. Chromatic parameters (CIEL*a*b*) and sensory appearance*

Regarding chromatic parameters (Table 2), for all samples (frozen and thawed) treated by HHP it can be seen that L* increased significantly ($p<0.05$) in comparison to their respective control samples. The increment of L* parameter could be associated to globin denaturation and/or to heme displacement at pressures above 300 MPa [2]. Nevertheless, frozen carpaccio samples presented L* values significantly ($p<0.05$) lower than thawed samples ones (data not shown). This result could be related with a minimization of myoglobin irreversible denaturation when high pressure treatments were applied on frozen carpaccio [5,13].

Table 2. Chromatic parameters (CIEL*a*b*) of frozen or thawed *carpaccio* samples treated by HHP at different pressures and temperatures.

T (°C)	P (MPa)	L*	a*	b*
Frozen				
0	400	48.04±0.82*	26.76±2.06*	18.65±1.03
0	500	47.72±2.37*	27.58±2.83*	19.03±1.93
0	600	46.49±3.03*	27.64±1.61*	18.14±0.79
5	400	48.21±1.92*	25.88±1.45*	17.61±0.91
5	500	49.05±2.23*	26.46±2.72*	18.56±0.71
5	600	48.49±2.54*	26.26±1.78*	18.16±1.33
Control		44.50±2.74	29.77±2.24	18.86±1.16
Thawed				
5	400	57.13±3.50*	22.59±2.75*	15.37±1.22*
5	500	57.26±2.43*	22.82±2.20*	16.21±1.21*
5	600	56.56±3.13*	22.59±1.61*	16.38±1.33*
Thawed				
20	400	58.22±0.98*	21.92±0.53*	15.50±0.88*
20	500	55.91±1.17*	23.07±2.38*	14.53±1.79*
20	600	56.88±3.42*	22.64±2.84*	15.65±0.76*
Control		45.33±3.35	29.89±3.18	19.77±0.80

Concerning a* parameter (Table 2), all samples (frozen and thawed) treated by HHP presented significant lower values ($p<0.05$) than their control samples. It was proposed that HHP processing would diminish meat redness as a consequence of an oxidation increase (from ferrous myoglobin to metmyoglobin) [2,3]. Nevertheless, when results of frozen and thawed *carpaccio* treated by HHP were statistically compared at the same processing temperature (5°C), it was found that a* parameter values of thawed samples were significantly lower ($p<0.05$) than frozen ones at all pressure levels (data not shown). This result suggests that the freezing of *carpaccio* samples before the HHP treatment would minimize myoglobin oxidation.

The modification of b* parameter was different for frozen or thawed *carpaccio* treated by HHP. In the case of frozen samples, this parameter did not present significant ($p>0.05$) differences in relation to their control one. However, thawed *carpaccio* treated by HHP showed b* parameter values significantly ($p<0.05$) lower than their control.

Regarding the results described in the preceding paragraphs it would appear that the freezing of beef *carpaccio* prior to HHP treatments helps to maintain its chromatic parameters. In addition, the different pressure levels applied during HHP treatments of frozen or thawed *carpaccio* did not have a significant effect ($p>0.05$) on chromatic parameters (Table 2). This could be a consequence of sodium nitrite addition, which allowed a color stabilization of *carpaccio* samples. Even at pressures above 400 MPa, nitrosomyoglobin could be more stable than oxymyoglobin to oxidation into ferric form [2], and consequently a* values remained almost constant when pressure was increased.

Sensory appearance of all pressurised samples (frozen and thawed) resulted significantly different ($p<0.01$) of control samples one.

3.5. Microbiological results

Aerobic total counts (ATC) at 30ºC of *carpaccio* samples treated by HHP and control samples were presented in Figure 1. Concerning ATC of thawed *carpaccio* treated by HHP, count reductions at 500 and 600MPa were higher than at 400MPa, indicating an important effect of pressure level on ATC of thawed samples. ATC reductions obtained in the present study for thawed samples were greater than those observed by [6] and [14] for beef *carpaccio* treated with similar HHP treatments. In this work, count reductions for thawed *carpaccio* treated by HHP ranged from 5.24 $\log_{10}$ cycles (600MPa-5ºC-5min) to 3.79 $\log_{10}$ cycles (400MPa-5ºC-5min) (Figure 1), whereas those authors observed that ATC reductions were 4.5 log10 cycles for *carpaccio* samples treated at 650MPa for 5min at 20°C [6] and 3 $\log_{10}$ cycles at 600MPa for 10min at 20°C [12]. ATC reductions achieved in the present work were greater than those informed by [6] and [12] probably due to the higher counts of control *carpaccio* (~ 6 $\log_{10}$ CFU g^{-1}) observed in the former.

Regarding frozen *carpaccio* treated by HHP, ATC reductions ranged from 4.11 $\log_{10}$ cycles (500MPa-0ºC) to 2.74 $\log_{10}$ cycles (400MPa-0ºC). Consequently, it was observed a lower effectiveness of HHP treatments on inactivation of microorganisms for frozen *carpaccio* samples than for thawed ones. This effect was associated with the reduction of food water activity by freezing [5,15]. However, the use of refrigeration temperatures (0 and 5ºC) during HHP processing of frozen samples, allowed to reach higher reductions than those obtained applying HHP at subzero temperatures, as reported by [6], where ATC reduction was 1.7 $\log_{10}$ cycles for frozen *carpaccio* samples treated at 650MPa for 5 min at -30°C.

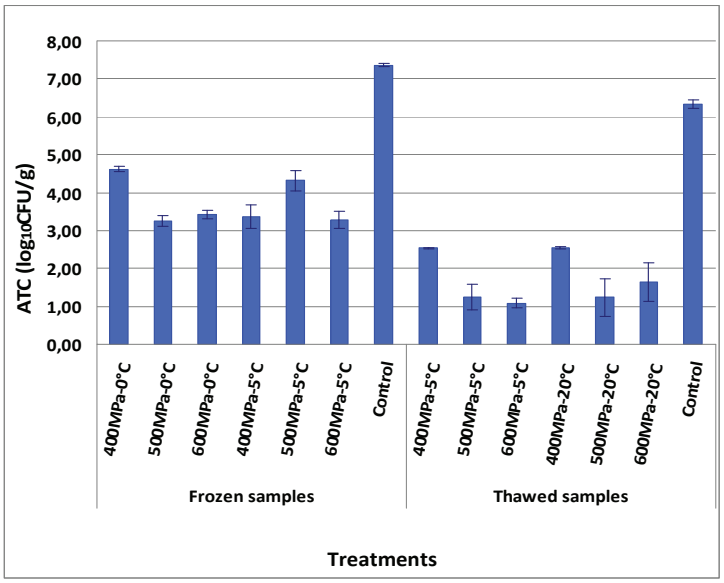

Fig. 1. Aerobic total count (ATC) at 30ºC of frozen or thawed *carpaccio* samples treated by HHP and *carpaccio* control samples

4. Conclusion

Temperature applied during HHP processing of thawed (5 and 20°C) or frozen (0 and 5°C) *carpaccio* did not have a significant effect on the measured parameters. Pressure level effect was only observed on

ATC and work of shearing of thawed samples. Frozen conditioning of *carpaccio* previous to HHP treatments reduced the harmful effects of high pressure on chromatic parameters (L*, a* and b*) and water holding capacity. This could be due to a minimization of the denaturation of sarcoplasmic and myofibrillar proteins induced by pressure. Besides, it was observed a lower effectiveness of HHP treatments on microorganisms' inactivation for frozen *carpaccio* than for thawed one, probably as consequence of the lower water activity of the former.

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