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EFFECT OF HIGH PRESSURE PROCESSING ON QUALITY OF MEAT AND MEAT PRODUCTS

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Abstract: *The application of high hydrostatic pressure (HHP) to fresh 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, beef samples were pressurised at 200, 400 and 600 MPa during 5 min at refrigeration temperatures (0 and 5°C) and room temperature (20°C). Both pressure and temperature regimes had significant effects on colour, pH, moisture content, and cook loss and lipid oxidation. Pressurisation at 600 MPa had a lower impact on colour parameters than lower pressurisation levels. Cook loss also increased when higher levels of pressure were applied. Across all pressure conditions, lower cook loss was observed at 25 °C compared to 0°C and 5°C. An increase in TBARS values was observed at the higher pressure levels (400, 600 MPa). it was studied the application of HHP treatments on frozen or thawed carpaccio samples at three pressure levels (400, 500 and 600MPa). 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 beef samples than for thawed one.*
Keywords: *High hydrostatic pressure, beef, frozen, thawed, chromatic parameters, shelf life and lipid oxidation.*

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 readyto-eat meats [1]. In the case of ready-to-eat cured fresh meat, 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 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 aim of this study was to evaluate the effect of sample conditioning and HHP treatments (different pressure levels at refrigeration or moderate temperature) on physicochemical properties, microbial quality and sensory attributes of beef meat.

Materials and Methods

Beef meat samples were collected from topside of the beef with the help of scribble blade (SS Blade). Muscles were cut into steaks 2.5 cm in thickness (block). The design was applied with temperature (three levels: 0°C, 5ºC and 20ºC samples) and pressure (four levels: 0, 200, 400 and 600MPa) as main factors. Fresh beef meat non-submitted to HHP treatment (0MPa) was used as control samples. In each block, three experimental units were used for each treatment applied. 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 5.75 ± 0.14. After samples were vacuum packaged (Cryovac BB4L, Sealed Air Co., Argentina) they were stored at 1.5 ± 0.5°C for 12 days. The proximate composition of meat samples was as follows: moisture, 75.0%; protein, 21.1%; total fat, 2.4%; ash, 1.2% and carbohydrate, 0.4%. The a_w of meat samples was 0.981±0.005. After chill storage, cured muscles were frozen and stored at -40ºC during one day in a conventional freezer. Then, samples were stored at -40°C until HHP treatment.

Samples packed under vacuum frozen (-40°C) were submitted to HHP treatments, according to the experimental design, in a High Pressure Iso-Lab System Stansted Fluid Power Ltd. with a vessel working volume of $2 dm^3$and a sample canister with a internal working diameter of 80mm. The rate of pressurization was $300 MPa.min^{-1}$ 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], cook loss (%) [8], CIELab chromatic parameters measured with a Minolta colorimeter model CR 400, sensory appearance (triangular test) under controlled conditions, shelf life analysis by microbial plate count method and aerobic total count (ATC) at 25ºC [9]. Before analysis all frozen samples were thawed in a storage chamber at 4.0 ±1.0°C [10].

Results and Discussion

Regarding the results of the experimental work performed, there was no significant temperature effect on all the treated and non treated meat samples. However, pressure effect was significant for some parameters evaluated on treated samples. The results corresponding to each measured parameter on beef samples treated by HHP and control ones are presented in the following paragraphs.

Effect on Quality of Treated and Non Treated Meat: The effect of High Hydrostatic Pressure on the meat quality is evaluated on the basis of different pressures i.e. 200 MPa, 400 MPa and 600 MPa for each processed meat sample respectively. Table 3.1 shows variation in Moisture Content, pH and Cook loss in accordance with variable Temperature and Pressure conditions over beef meat.

Table 3.1 Effect of pressurizing conditions (pressure and temperature) on quality parameters of beef meat

Temperature (°C)	Pressure (MPa)	Moisture Content (%)	pH	Cook loss (%)
0	**200**	25.67	5.87	38.21
0	**400**	26.37	5.89	38.27
0	**600**	26.89	5.89	38.35
5	**200**	27.17	5.86	36.36
5	**400**	28.57	5.79	36.65
5	**600**	28.91	5.76	36.91
25	**200**	19.11	5.85	36.59
25	**400**	19.77	5.91	36.62
25	**600**	20.13	5.93	36.67
Control		19.5	5.75	38.83

From table 3.1 it was observed that Moisture Content, pH and Cook Loss was the maximum at the pressure of 600 MPa and temperature of 0°C while it was the minimum at pressure of 200 MPa for the same temperature. It was observed that Moisture Content and Cook Loss was maximum at the pressure of 600 MPa and temperature of 5°C while it was minimum at pressure of 200 MPa for the same temperature. While pH was the maximum at 200 MPa pressure while it was minimum at 600 MPa pressure. It was observed that Moisture Content, pH and Cook Loss was the maximum at the pressure of 600 MPa and temperature of 25°C while it was minimum at pressure of 200 MPa for the same temperature.

Effect on Moisture Content of HPP Treated Beef Meat: Samples at 0°C and 5°C showed significantly higher values than the samples stored at room temperature. This may be due to the denaturation of proteins in beef meat due to the high pressure applied. In (Table 4.1) it may be seen that sample had the most significant effect at 600 MPa pressure. It may be due to the reduced water holding capacity on the effect of high pressure. The moisture content ranged from 25.67-19.11%. Similar effect was seen [10, 11].

Effect of hpp on pH: A significant increase in pH was observed in all the samples stored at different temperature (Table 4.1). The pH range was between 5.87-5.93 and the pH of the control was 5.75. It may be seen that high pressure resulted in protein denaturation which might change the ionic configuration of protein due to which pH alteration occurred. The similar results were seen [10, 12].

Effect on Cook Loss of hpp Treated Beef Meat: The effect of hpp on cook loss can be clearly seen from (Table 4.1.). There was a significant effect on cook loss on high pressure. All the samples treated with hpp showed a slightly high cook loss than the control sample. The cook loss ranged between 38.21-36.59 %. It may be due to the reduced water binding capacity by the effect of high pressure in the samples. Similar results were also showed [12, 8].

Chromatic Parameters (CIEL*a*b*) and Sensory Appearance: Table 3.2 shows change in chromatic parameters i.e. Lightness (L*), Redness (a*) and Yellowness (b*) of beef meat by High Hydrostatic Pressure. The effect of High Hydrostatic Pressure on the meat quality is evaluated on the basis of different pressures i.e. 200 MPa, 400 MPa and 600 MPa for each processed meat sample respectively.

Table 4.2 Effect of chromatic parameters on high pressure processed beef meat

Temperature (°C)	Pressure (MPa)	L*	a *	b*
0	**200**	48.56	24.13	17.65
0	**400**	48.14	25.33	18.03
0	**600**	47.89	25.52	18.13
5	**200**	56.16	23.51	17.32
5	**400**	56.09	24.88	18.56
5	**600**	55.92	21.92	18.38
25	**200**	59.22	23.59	15.50
25	**400**	59.19	23.59	16.65
25	**600**	59.13	23.65	16.72
control		46.71	26.35	18.77

Table 3.2 depicts the change in chromatic parameters i.e. Lightness (L*), Redness (a*) and Yellowness (b*) of processed beef meat. It was observed from these data that Lightness, Redness and Yellowness were greater at the 600 MPa Pressure and 0°C Temperature. Regarding the chromatic parameters on the treated sample stored at different temperatures can be seen in (Table 3.2). The value of L* ranged between 48.56-59.13 which showed a slight increment from the controlled sample, it may be due to the denaturation of globin protein of haemoglobin under the effect of high pressure. Considering a* value ranged from 24.13-23.65, which showed a decline in value from the control sample. Low temperature had a greater effect as it did not show much difference in chromatic parameters from the controlled sample. Modifications were also shown in b* where all samples were having significantly low value from the controlled sample. The values ranged from 15.50-18.56. In addition, HPP over the samples stored at low temperature did not have much significant change on chromatic parameters as compared to the samples stored at room temperature. Similar results were observed [13, 10].

Sensory Evaluation of Pressurized Beef Meat: The processed beef meat was cooked in refined oil and was subjected to sensory evaluation amongst 15 panel members. The mean sensory score for the overall texture is given in (Table 3.3). When texture has been assessed by means of a TPA, values of hardness, of springiness and juiciness and fat sensation decreased as ageing time increased. Hardness was the most variable parameter, and it decreased as time elapsed. The score for hardness ranged from 3.5 – 5.0. The scores for springiness ranged from 2- 4.5 i.e slightly springy to quite springy. The score for juiciness ranges from 2–4.5, i.e. moist to juicy. The score for fat sensation was quite unchanged i.e. it ranged between 3.8 – 3, i.e. between quite fat and fat.

Table 33 Sensory score on TPA of pressurized beef meat during storage

Texture	Storage period	Mean score
Hardness	0	3.5
	30	4
	60	4.5
	90	5
Springyness	0	2
	30	3
	60	3.5
	90	4.5
Juiceness	0	2
	30	2
	60	3.5
	90	4.5
Fat sensation	0	3.8
	30	3.5
	60	3.5
	90	3

Different types of lipid replacements affected the textural properties of the beef meat. Meat prepared with refined oil showed greater hardness, springiness, juiciness and fat sensation values than emulsions made with beef fat. This may be due to the protein gels, with small fat globules (within a narrow particle size distribution), have higher gel strength. This is most probably due to the higher number of small globules present in a given volume and/or high amount of protein content in the creamy phase, both of which can offer more resistance to compression. In our work, differences in TPA sensory scores among the formulations suggest that chemical composition of the lipid phase has a major effect on the resulting meat, and the addition of vegetable oils improved the textural properties of meat batters.

Evaluation of Shelf Life of Meat and Meat Products: The shelf life of meat was analyzed through microbial count of treated sample in equal amount using 25 g. The results obtained in this study with an extensive cell inactivation ratio (<3 log cycles) without reactivation capacity, starting with an important initial contamination level of 6 log CFU g^{-1} agree with the fact that the higher the pressure, the higher the inactivation obtained. HPP is a powerful tool to control risks associated with Salmonella spp. and *L. monocytogenes* in raw or marinated meats as shown from the data obtained in (Table 3.4). Most of the untreated samples (NT) showed the presence of one or both pathogens in 25 g, whereas all pressurized samples (HPP) when treated with 600mPa for 5 mins showed the absence of these pathogens in 25 g of sample. it may be cleared that HPP treated sample had a shelf life of 90 days (due to the reduced risk of activation of pathogens). These values shown in Table 3.4 and also the presence of *Salmonella* and *L. monocytogenes* in 25gm in most untreated samples, it may be pointed out that slaughterhouse operations, handling, or chilled storage before processing have been inappropriate. Similar results were seen [14].

Microbial Analysis of Treated (HPP) and Non-treated (NT) Beef Meat: The vaccum packed beef meat treated at different pressures with a constant time of 5 mins showed significant results (Table 3.5). The samples treated at 200 mPa pressures showed the microbial reduction of 3-4 log cycles from the non treated samples. The pressurized beef meat at 400MPa also showed the similar results with a reduction of 3-4 log cycles. The samples treated with 600MPa pressure had the significant effect in comparison to the above two pressure applied. The samples showed the reduction of 5 log cycles from the non treated samples. In HPP samples the number of survivors in almost all the samples remains unchanged except for the samples treated at 600mPa with greater significant effect. The microbial count of all the samples were below the detection limit during the whole storage investigation of 90 days, while untreated sample reached <10^8 CFU/g after only 30 days of storage.

Table 3.4 Effect of HPP on the attacking pathogens (salmonella spp and *L.monoctytogenes*) on beef meat

Time (days)	*L. monocytogenes*		*Salmonella* spp.	
	Non treated	HPP	Non treated	HPP
0	2/3	0/3	3/3	0/3
30	2/3	0/3	2/3	0/3
60	3/3	0/3	1/3	0/3
90	3/3	0/3	1/3	0/3
120	1/3	1/3	1/3	1/3

HPP was effective in reducing the microbial load for aerobic total count, lactic acid bacteria, yeasts and Enterobacteriaceae. For aerobic total count and LAB the reduction was 3-4 log cycles for 200-400 MPa pressure and 5 log cycles for 600MPa pressure during the entire storage period and for untreated sample the counts were >10^6 CFU/g^{-1} for first 30 days of storage which increased to 10^8 CFU/g^{-1} at 90 days of storage for both ATC and LAB. In case of yeasts the reduction was 2 log cycles for 200MPa and 400 MPa and 600MPa pressures. The untreated samples showed the microbial growth reduced significantly throughout the storage period and ranged between 10^3 – 10^2 CFU/g^{-1} of the sample. The yeasts count so obtained can be considered negligible as the growth was <10^1 CFU/g^{-1}. For Enterobacteriaceae the reduction was 1 log cycle for 200 MPa and 400MPa pressure treatments. While, for the pressure at 600MPa the growth was constant throughout storage i.e.10^1 CFU/g^{-1}. Thus it can also be considered negligible, as all the count was below the detection level. The untreated samples ranged from 3.76-6.23 log cycles. Thus HPP was found to be very effective in reducing the Enterobacteriaceae count in beef meat samples. Similar results were shown [14, 10, 15]. 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 log10 cycles at 600MPa for 10min at 20°C [16]. ATC reductions achieved in the present work were greater than those informed [5, 16] probably due to the higher counts of control *carpaccio* (~ 6 log10 CFU g^{-1}) observed in the former

Table 3.5 Microbial count (CFU/g^{-1}) in vaccum packed beef for treated & non treated samples during storage at 5°C

Time (days)	Pressure (MPa)	ATC		LAB		Yeasts		Enterobacter	
		Non treated	HPP	Non treated	HPP	Non treated	HPP	Non treated	HPP
0	**200**	6.76	<3	6.4	<3	3.80	<1	3.76	<2
	400	6.72	<3	6.56	<3	3.78	<1	3.67	<2
	600	5.58	<2	6.12	<2	3.31	<1	3.89	<1
30	**200**	7.74	<3	7.67	<3	4.09	<1	3.87	<2
	400	7.35	<3	7.63	<3	3.98	<1	4.01	<2

	600	6.6	<2	7.24	<2	3.92	<1	5.62	<1
60	**200**	8.01	<3	7.76	<3	3.23	<1	4.56	<2
	400	8.11	<3	7.56	<3	3.01	<1	4.54	<2
	600	7.75	<2	7.64	<2	2.96	<1	5.96	<1
90	**200**	8.22	<3	8.21	<3	2.94	<1	4.89	<2
	400	8.45	<3	8.57	<3	2.68	<1	4.79	<2
	600	8.16	<2	8.34	<2	2.50	<1	6.23	<1

Effect on the Physicochemical Properties during Storage of HPP Treated Beef Meat: The HPP treated beef meat was stored for 90 days and its physicochemical properties such as pH, moisture content, protein solubility and TBARS was monthly analyzed. The pH ranged from 5.26 - 5.86 for all pressures during storage (Table 3.6). The alteration in pH may be the result of denaturation of protein due to ionic dislocation on the application of high pressure. Thus samples treated with 600 MPa pressure were more favorable during storage in comparison to other samples. This may also be due to the heat generated during processing resulting in unfolding of the acidic groups leading to increase in pH. Similar analysis was shown [13]**.**

It can be seen clearly that sample at 600 MPa pressure showed the maximum rise in pH due to inactivation of protein due to high pressure. Thus preserving the animal protein during storage was favorable at this pressure. The moisture content ranged from 27.17 – 28.95 % during the storage period. Table 3.6 showed that the moisture content increased in all samples with the increasing storage time. It was also significant to the different pressure applied to the sample. The pressure of 600 MPa showed higher increment than other samples. It may be due to the reduced water holding capacity which is inversely proportional to the applied pressure. Similar conditions were seen [13].

The total protein solubility ranged from 50.12 - 84.32 µg/g. The effect on protein solubility with pressure was inversely proportional where at high pressure the protein solubility reduces. This may be due to the denaturation of protein (Table 3.6). Thus the sample with 600 MPa showed the least solubility making it favorable for storage without altering the overall protein quality of beef meat. Similar results were showed [13].

The TBARS ranged from 1.22 - 2.21 mgMDA / kg of the sample. A decrease in oxidative stability was observed for all the samples during storage at 4°C. Higher the TBARS value led to high susceptibility for oxidation of fatty acids. It is directly proportional to pressure applied means higher the pressure more will be the chances for lipid oxidation. It may be due to the conformational changes in hemoprotein which result in greater exposure to catalytic group to unsaturated fatty acids. Thus the variability observed in the TBARS value as shown in (Table 3.6) did not have much significant effect on the nutritional quality of meat during storage.

Samples pressurised at 25°C at 600 MPa showed a tendency towards higher TBARS values when compared to samples pressurised at 400 MPa at the same temperature at each time point analysed (Table 3.6). It was found an increase of TBARS values of pressurised beef meat, as the pressure increased from 200 to 600 MPa. Similar evaluations were made [13, 17].

Table 3.6 Effect of high pressure on physicochemical properties on beef meat during storage at 5°C

Time (days)	Pressure (MPa)	pH	Moisture content (%)	Total protein solubility (µg/g)	TBARS (mg MDA/kg)
0	**200**	5.86	27.17	84.32	1.22
	400	5.79	28.57	78.16	1.32
	600	5.76	28.91	50.87	1.37
30	**200**	5.71	27.22	84.06	1.24
	400	5.63	28.59	77.82	1.33
	600	5.72	28.91	50.43	1.41
60	**200**	5.48	27.32	83.51	1.52
	400	5.47	28.61	77.63	1.49
	600	5.68	28.93	50.27	1.57
90	**200**	5.26	27.54	83.19	1.72
	400	5.34	28.70	77.43	1.89
	600	5.65	28.95	50.12	2.21

Conclusion: The pressure treatment at 600 MPa showed minimum effect on meat quality parameters while improving meat hygiene. HPP higher than 600 MPa might promote lipid oxidation. However, HPP in the range 200-600 MPa did not alter the fatty acid profile of beef meat. The importance of the pressurisation temperature has been highlighted in terms of colour, water binding properties, lipid oxidation and FAA composition and TPA. Further research on temperature control during HPP would be necessary to understand the exact mechanisms influencing HPP effects in combination with temperature. These results suggest that HPP is a mild processing technology in terms of its effect on nutritional meat content and its use could be considered as a pre-treatment for low value muscles such as M. pectoralis profundus.

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