Effect of High Pressure Processing on Quality, Sensory Acceptability and Microbial Stability of Marinated Beef Steaks and Pork Chops during Refrigerated Storage

Haihong Wang[1]*, Jimmy Yao[1] Mindy Gerlat[1] and Michael Gänzle [2]

[1]Food Processing Development Centre, Food and Bio Processing Branch, Alberta Agriculture and Forestry, Leduc, AB, T9E 7C5, Canada

[2]Department of Agricultural, Food and Nutritional Science, University of Alberta, 410 Agriculture/Forestry Centre, Edmonton, AB T6G 2P5, Canada

*Email: haihong.wang@gov.ab.ca

The growing demand by consumers for safe, natural, minimally processed and convenient food products has stimulated food industry interest in high pressure processing (HPP). HPP is a cold pasteurization technique where food, previously sealed in flexible and water-resistant packaging, is subjected to high levels of hydrostatic pressure (up to 600 MPa) for several minutes. Generally, the application of high pressure at low or moderate temperature causes the inactivation of vegetative microbial cells, without markedly altering the taste and flavour of cooked foods. Fresh muscle foods are susceptible to pressure-induced colour change which has limited the adoption of HPP for raw meat applications. Marinating is commonly used by the meat industry to enhance moisture and improve the texture of meat products. We proposed that the colour imparted by marinating may mask undesirable discolouration caused by the HPP treatment. Marinated beef steaks and pork chops were evaluated in this project.

HPP is an approved processing method in the USA; however, in Canada, high pressure processing was considered novel processing. Based on the data generated from this project, a letter entitled, "Notification to Health Canada for selling high pressure processed marinated pork chops and beef steaks in Canada" was submitted to Health Canada. A letter of non-novelty for these HPP-treated foods was issued by Health Canada on April 8, 2016, indicating industry can start producing and selling marinated beef and pork products in the marketplace immediately.

On Dec. 22, 2016, Health Canada announced that based on the number of HPP-related assessments conducted by the Department, the scientific literature currently available regarding HPP and the breadth of food products that are known to be treated with HPP, it is Health Canada's position that HPP is no longer considered a novel process. More detailed information can be found a link below to Health Canada official position regarding HPP: http://www.hc-sc.gc.ca/fn-an/legislation/guide-ld/hpp-phph-eng.php

Materials and Methods

Each beef *semitendinosus* muscle (eye of round) was cut into eight steaks with 1 inch thickness; one steak was reserved as a fresh steak (no further treatment). The remaining seven steaks were marinated with sweet teriyaki. The marinated steaks were subjected to high pressure processing at 300, 350, 400, 450, 500, or 600 MPa for 3 min at 8°C. One treatment after marination and packaging was not subjected to HPP and served as control. Eight replicates were conducted for each treatment. The colour of steaks was digitally taken after HPP treatment. The colour (CIE *L*, a*, b**), pH, water binding capacity, expressible moisture (EM), cook loss and Warner-Bratzler shear force (WBSF) were measured. Data were subjected to analysis of variance using the General Linear Model procedure of SAS. The Tukey test was used to compare the differences ($p \leq 0.05$) between the treatments. Microbiological analyses (aerobic colony count, lactic acid bacteria, yeast, mold, coliforms, *E. coli, Listeria monocytogenes, Salmonella spp.*) were conducted after 7, 15, 28, 42, 57 and 85 days of refrigerated storage and enumerated according to Health Canada protocols.

Consumer sensory evaluation was conducted at the Consumer Product Testing Centre (106, 10030-107 Street, Edmonton, Alberta). Five treatments were evaluated: 1. Fresh steaks (without HPP or marination); 2. Control (with teriyaki marination but non HPP); 3. Steaks with teriyaki marinade and treated with 400 MPa, stored at 2°C for 31 days; 4. Marinated beef steaks with 450 MPa, stored for 31 days; 5. Marinated beef steaks with 450 MPa, stored for 61 days. A total of 85 panelists evaluated the cooked steaks for appearance, internal colour, flavour, tenderness, juiciness, aftertaste and overall acceptability using 9-point hedonic scales. Data were exported from Compusense (*Five* version 5.2 software) and analyzed in SAS (v. 9.3, SAS Institute Inc., Cary, NC) using a mixed model analysis of variance with day, treatment and panelist as effects. Panelist was treated as a random variable. A Tukey's test ($p \leq 0.05$) was used for means separation.

Each pork loin (m. *longissimus dorsi*) was cut into eight 2 cm thick pork chops and one treatment was reserved as fresh pork chop. The remaining seven treatments were arranged for marination and HPP. After tumbling with honey garlic marinade and marinating 20 hours at 2°C, packaged samples were immediately subjected to HPP at 350, 400, 450, 500, 550 or 600 MPa for 3 min at 8°C. One treatment after marination and packaging was not subjected to HPP and served as control. Eight replicates were conducted for each treatment. Instrumental colour (CIE L^*, a^*, b^*), pH, thaw loss, water binding capacity, expressible moisture, cook loss, and Warner-Bratzler shear force were measured. Microbiological testing was conducted on the duplicate samples after 7, 21, 31, 49, 70, 86 days of storage in the 2 °C cooler. Aerobic colony count, lactic acid bacteria, yeast, mold, coliforms, *E. coli*, *Listeria monocytogenes*, and *Salmonella spp.* of the samples were tested.

Consumer sensory evaluation was conducted by 90 panelists. Four samples were evaluated: 1. Fresh (without HPP or marinade), 7 days refrigerated storage; 2. Control (marinated without HPP), 7 days storage; 3. 450 MPa HPP, 7 days storage; 4. 450 MPa HPP, 31 days storage.

Results

The instrumental colour of marinated steaks in the package indicated that steaks treated with pressures of 450 and 600 MPa were paler (higher L^*) than the control steaks and steaks treated with pressures of 300 and 350 MPa. The redness (a^*) values remained unchanged ($p > 0.05$) among the marinated steaks. The yellowness (b^*) of steaks treated with 500 and 600 MPa was higher than that of the control steaks and steaks treated with 300 MPa. As shown in Fig. 1, digital images of the teriyaki marinated steak indicate that there were no noticeable differences in colour when pressure below 350 MPa was applied. With increased pressure, the colour of marinated steaks had a cooked-like appearance.

The pH of marinated steaks treated with 400, 450 and 600 MPa was significantly ($p < 0.05$) higher than the control steaks. As expected, the pH of the seven marinated steaks was 0.2-0.3 units higher than the fresh steaks due to the pH of the sweet teriyaki marinade. The thaw loss was the highest for 600 MPa treated steaks with a value of 7.07% compared to the control steaks that had a thaw loss of 5.53%. The fresh steaks had the lowest thaw loss with a value of 3.14%. The fresh steak had higher expressible moisture compared to the seven marinated steaks. Steaks treated with 300 and 350 MPa had the lowest expressible moisture. The control steaks had lower cook loss compared to steaks treated with 500 and 600 MPa, indicating that the cook loss increased with increasing pressure.

FIGURE 1. The fresh or marinated beef steak treated with and without HPP. The first row from left to right were fresh steak, marinated steak without HPP, marinated beef steak treated with 300 MPa, and 350 MPa. The second row from left to right were marinated beef steaks treated with 400 MPa, 450 MPa, 500 MPa, and 600 MPa, respectively.

As shown in Fig. 2, the control steaks had the lower WBSF value compare to steaks treated with 500, 600 MPa and fresh. Marinated steaks treated with 450, 400, 350 and 300 MPa had similar WBSF values with control steaks.

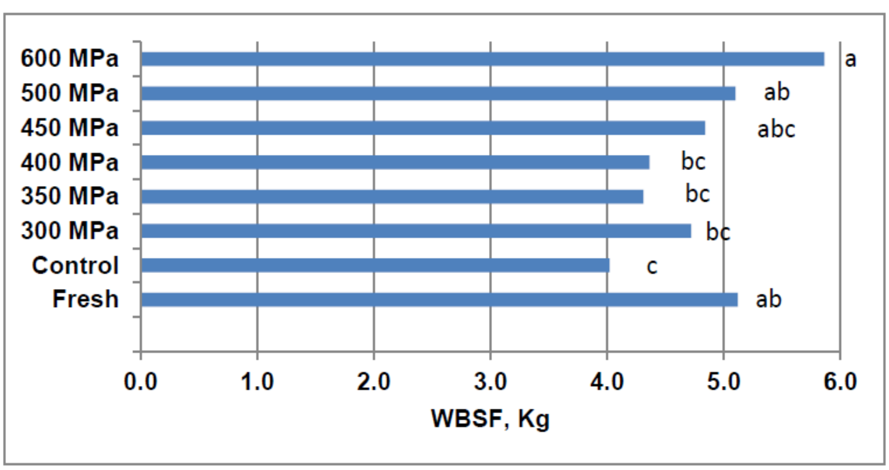

FIGURE 2. The tenderness (WBSF, Kg) of beef steaks from fresh, marinated steak without HPP (Control), marinated beef steak treated with 300 MPa, 400 MPa, 450 MPa, 500 MPa, and 600 MPa, respectively. Means with different letters (abc) in the Figure are significantly different ($p < 0.05$).

Pathogen counts (*E. coli, Listeria monocytogenes, Salmonella spp*.) were below the detection limit in all beef steak samples during the storage period. In Fig. 3, aerobic colony count results showed that HPP can significantly extend shelf life of marinated steaks from 7 days to: 15 days for marinated steaks treated with 350 MPa; 42 days for marinated steaks treated with 400 MPa; and up to 85 days for marinated steaks treated with 450 MPa.

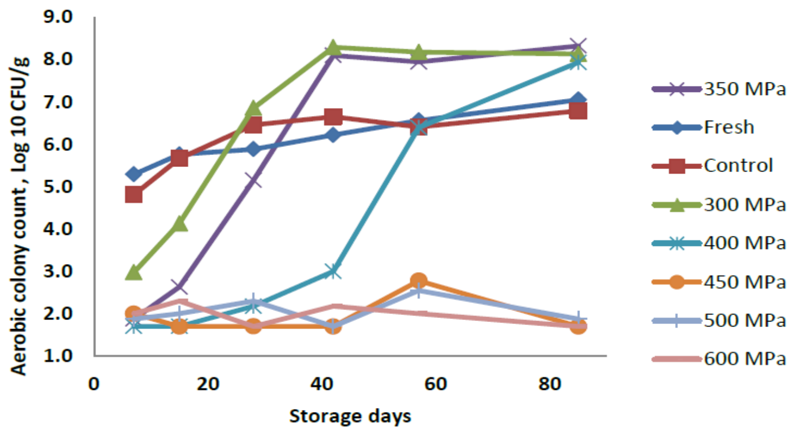

FIGURE 3. The aerobic colony count (log 10 CFU/g) of steak samples with different treatments and stored at refrigerated temperature at different days.

Consumer sensory evaluation (Table 1) indicated that HPP treated marinated steaks had lower scores on overall, appearance, flavour, tenderness, juiciness and aftertaste acceptability than the control samples, but higher scores than the fresh steaks on those sensory attributes. There were no differences on sensory acceptability scores between 450 MPa treated samples stored 31 days and 61 days. There were no differences in all sensory scores among the three HPP treated samples. Scores for the colour acceptability of packaged raw beef steaks treated with 400 MPa stored for 31 days and 450 MPa stored for 61 days were significantly higher compared to control and fresh steaks.

Table 1. Mean acceptability scores of sensory attributes from different treated samples with different storage times of teriyaki beef steaks (n=85)

Acceptability Attribute	p-value	Beef steak treatments				
		Fresh	Control	400 MPa, 31 days	450 MPa, 31 days	450 MPa, 61 days
Overall	<0.001	5.2 c	7.2 a	6.0 b	6.5 b	6.4 b
Appearance	<0.001	5.6 c	7.3 a	6.4 b	6.6 b	6.5 b
Internal Colour	<0.001	6.0 c	7.1 a	6.5 b	6.6 b	6.2 bc
Flavour	<0.001	5.0 c	7.0 a	5.9 b	6.4 b	6.2 b
Tenderness	<0.001	4.7 c	6.9 a	5.5 b	5.7 b	6.0 b
Juiciness	<0.001	4.4 c	6.7 a	5.7 b	5.8 b	6.0 b
Aftertaste	<0.001	5.0 c	6.7 a	5.8 b	6.1 b	5.8 b
Raw Overall	<0.001	4.7 b	5.4 a	5.9 a	5.5 a	5.9 a
Raw Colour	<0.001	4.6 c	5.2 b	5.9 a	5.6 ab	5.9 a

* Hedonic scale 1=dislike extremely, 2=dislike very much, 3=moderately dislike, 4=slightly dislike, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely

Samples sharing the same letter are not significantly different from each other (p>0.05)

Conclusions from instrumental and microbiological analysis: HPP can significantly extend shelf life of marinated steaks. The shelf life of marinated steaks treated with 400 or 450 MPa pressure for 3 min can be extended from 7 days to more than 42 days. The tenderness and protein functionality (WBC, EM and cook loss) of steaks treated with 400 or 450 MPa were similar compared to control and fresh steaks.

HPP marinated pork chops and marinated control pork chops, in the package, had similar $L*$ values. However, they were all paler (higher $L*$) than the fresh pork chop due to both the marinade and HPP. There were no significant differences among pressure treated samples for $a*$ (redness) and $b*$ (yellowness) values although the control marinated samples had lower $a*$ and $b*$ values. As shown in Fig. 4, the honey garlic marinade partially masked meat discolouration due to HPP.

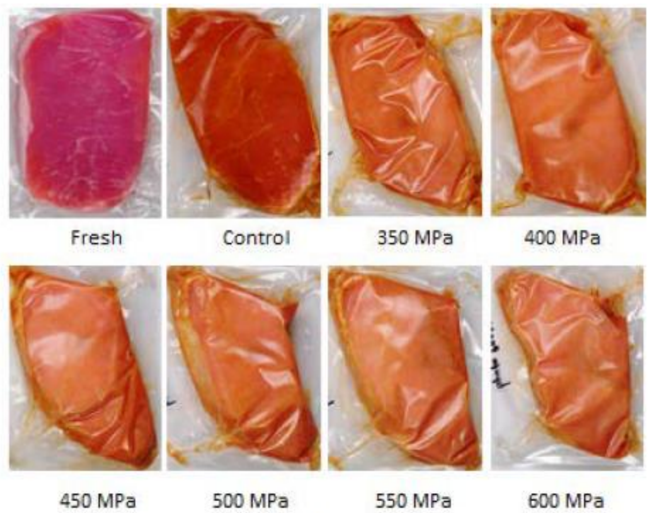

FIGURE 4. Picture of pork chops in the package with 8 treatments.

The pH of marinated pork chops treated with 450, 500, 550 and 600 MPa was significantly ($p < 0.05$) higher than the control pork chops. The pH of the seven marinated pork chops was 0.2-0.3 units higher than the fresh steaks, likely due to the marinade. The thaw loss was the highest for 600 MPa treated pork chops with a value of 4.8%. Samples treated with 400 MPa had the lowest thaw loss at 2.5%. Surprisingly, HPP marinated pork chops had a higher water binding capacity than the fresh and control samples. Pork treated with 600 MPa bound 28.8% extra water, as compared to the fresh pork which bound 10.5% of extra water. Fresh pork chops had the highest EM at 18.3%, while control and 350 MPa treated samples had the lowest expressible moisture at 5.8% and 5.5%, respectively. The control pork chops had a lower cook loss compared to all other treatments except for the samples treated with 400 MPa. As shown in Fig. 5, the fresh pork and control pork had the highest total protein solubility at 14.1% and 13.6%, respectively. The total protein solubility decreases with increasing pressure. The sarcoplasmic protein solubility had a similar trend as total protein solubility (data not shown). It is generally understood that higher protein solubility corresponds to less protein denaturation.

Fig. 6 shows the WBSF of pork chops treated with marination and HPP. The control and 400 MPa treated samples had the lower WBSF values at 2.75 and 2.70 kgf compared to the fresh pork chop. Marination can improve the tenderness of the pork chops. HPP did not affect the tenderness of the marinated pork chops.

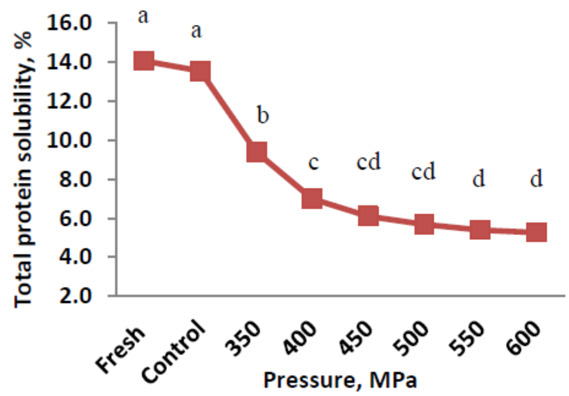

FIGURE 5. Total protein solubility of the pork chops treated with margination and high pressure. a-d, the different letters in the figure are significantly different (p <0.05).

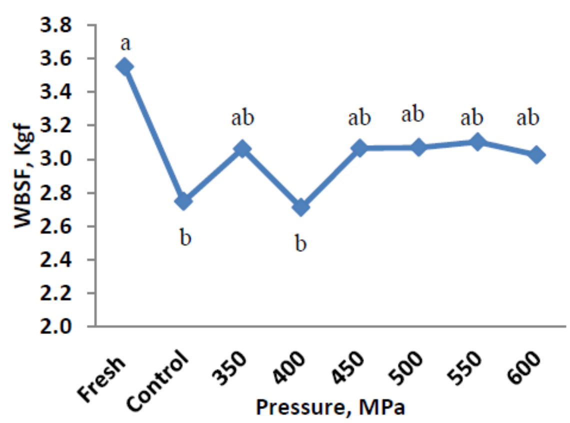

FIGURE 6. WBSF of the pork chops treated with margination and high pressure. a-b, the different letters in the figure are significantly different (p <0.05).

Pathogen counts (*E. coli, Listeria monocytogenes, Salmonella spp*.) were below the detection limit in all pork chop samples. The level of coliforms in 350 MPa treated honey garlic pork chops remained at 2 – 3 log10 (CFU/g) throughout the storage period, however, coliforms, yeast and mold were not detected (limit of detection: 0.17 log 10 CFU/g) in the samples treated with 400 MPa/3min or higher at any storage days tested. After 31 days of refrigerated storage, the aerobic colony count reached 5 log 10 CFU/g, while lactic acid bacteria remained below the limit of detection (1.7 log 10 CFU/g). Microbiological results showed that HPP can significantly extend shelf life of marinated pork chops from 10 days to 31 days with pressure at 450 MPa or higher.

Results from consumer sensory evaluation (Table 2) indicate that 450 MPa/3min HPP treated honey garlic pork chops stored for 7 days and 31 days had similar scores on overall, aroma, internal colour, flavour, tenderness, texture and aftertaste acceptability compared to the control samples. Control and HPP processed pork chops had higher scores than fresh pork chops on all sensory attributes. The 450 MPa, 7 days pork chop had a lower juiciness score than control; however, the 450 MPa, 31 days sample was similar in juiciness acceptability compared to control samples. 450 MPa, 7 days sample had a similar appearance score compared to control, while 450 MPa/3min, 31 days samples had a lower appearance score than both control and 450 MPa 7 days samples. Except for cooked appearance, 450 MPa, 31 days sample had a similar score to that from control sample.

Table 2. Mean scores derived from consumer panelists' ratings of acceptability attributes for honey garlic pork chops (n=90)

Acceptability Attribute*	p-value	Pork Chop Treatments			
		Fresh	Control	450 MPa, 7 days	450 MPa, 31 days
Overall	<0.01	5.3 b	6.8 a	6.6 a	6.7 a
Aroma	<0.01	6.1 b	6.8 a	6.9 a	6.5 ab
Appearance	<0.01	6.2 b	6.9 a	6.6 a	6.3 b
Internal Colour	<0.01	6.5 b	7.1 a	7.1 a	6.9 ab
Flavour	<0.01	5.0 b	6.7 a	6.7 a	6.8 a
Tenderness	<0.01	5.0 b	6.8 a	6.3 a	6.4 a
Juiciness	<0.01	4.5 c	6.7 a	5.9 b	6.0 ab
Texture	<0.01	5.1 b	6.8 a	6.3 a	6.4 a
Aftertaste	<0.01	5.4 b	6.4 a	6.6 a	6.4 a

* Hedonic scale 1=dislike extremely, 2=dislike very much, 3=moderately dislike, 4=slightly dislike, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely

Samples sharing the same letter are not significantly different from each other ($p>0.05$)

Conclusion: HPP can extend shelf life of honey garlic pork chops with pressure ranging from 400 - 450 MPa with minimal effect on meat quality.

ACKNOWLEDGEMENTS

Financial support for this study by the Alberta Livestock and Meat Agency (ALMA), Beef Cattle Research Council (BCRC) and Freybe Gourmet Foods Ltd is gratefully acknowledged. Thanks are expressed to personnel from the Food Processing Development Centre for meat processing, instrumental analyses and sensory evaluation. Microbiological analysis was performed by Agri-Food Laboratories, Alberta Agriculture and Forestry.

For more information regarding this project, please feel free to contact Haihong Wang or Jimmy Yao. They can be reached by haihong.wang@gov.ab.ca or jimmy.yao@gov.ab.ca.