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Abstract

This study investigated the effects of brine injection and high hydrostatic pressure (HHP) on the quality characteristics of pork loin. Brine with ionic strength conditions (0.7% vs 1.5% NaCl, w/v) were injected into pork loins, and the meat was pressurized up to 500 MPa for 3 min. As a quality indicator, moisture content, color, cooking loss and texture profile analysis (TPA) of pork loins were estimated. Based on the results, brine with low ionic strength (0.7% NaCl) resulted in low injection efficiency and high cooking loss, although, it improved tenderness of pork loin at moderate pressure level (~200 MPa). While high ionic strength condition (1.5% NaCl injection) lowered the hardness of pork loins at relatively high HHP level (400-500 MPa), it also caused high cooking loss. To commercialize the brine injected pork loins, it was necessary to regulate brine compositions, which was not evaluated in this study. Nevertheless, the present study demonstrated that brine injection followed by moderate pressure (200 MPa) could improve the tenderness of pork loins without causing other major quality losses.

Keywords pork loin, tenderization, NaCl, high pressure, brine injection

Introduction

Tenderness is the most important quality attribute that influences the palatability of meat. The tenderness is closely related to not only the chemical state of myofibrils but also connective tissue, juiciness as well as marbling condition (Aktaş, 2003; Purslow et al., 2016). Recently, wet-aging and sous-vide processing have been recognized as good ways to tenderize meat (Beldarrain et al., 2020). However, these techniques have some limitations regarding microbial control, energy efficiency and commercialization for mass-production. An alternative method that improves the tenderness and the flavor of meat is marination. In this method, meat quality is affected by the composition of marinade solution, which regulates the pH and ionic strength of meat (Aktaş, 2003). However, demerits associated with marination are long processing time, high moisture exudation during cooking or storage, which thereby require alternative processing techniques.

Injection in meat, as a one of method for salting, has been used to accelerate the attainment of equilibrium between the salt concentration of meat and brine solution. Salt penetrating into meat acts on the protein-ion interactions in the meat fibers and affects the swelling of matrix (Offer et al., 1988). Brine injection has been reported to positively affect the water retention and thereby the tenderness of meat. However, studies on suitable processing conditions are required because brine injection is also known to induce high drip generation (Andersen et al., 2007; McDonnell et al., 2013). Furthermore, brine with low ionic strength is applied for moisture enhancement process of meat which is distinguished from regular ham processing (Pietrasik and Shand, 2005), and strategy to prevent drip loss of injected meat during processing and storage is necessary.

High hydrostatic pressure (HHP) is a non-thermal process that is applied to ensure the microbiological safety and thereby the shelf-life extension of foods (Guillou et al., 2017; Yuste et al., 1999). During the moderate HPP treatment (200~300 MPa), the internal void of the protein could be expanded by HPP induced moisture migration. This causes the reversible denaturation of protein structure leading to a molten globule state (Chen and Makhatadze, 2017). The structural modification of the protein upon depression is manifested in the form of high water-holding capacity (WHC) of HHP treated meat (Boonyaratanakornkit et al., 2002; Hong et al., 2012). However, it is reported that the continuous expansion of the protein due to the application of extreme HHP results in irreversible protein denaturation (Boonyaratanakornkit et al., 2002; Mozhaev et al., 1996). Thus, to produce meat with improved juiciness and stable moisture retention, it is hypothesized that the moisture injection requires an optimal ionic strength, and the stability of moisture retention can be obtained through HHP processing. Therefore, in this study, two NaCl concentrations of brine and various pressure levels were investigated to explore the tenderizing effect of brine injected pork loins.

Materials and Methods

Material and sample preparation

A total of 36 pork loins (*M. longissimus dorsi*, 74.7% moisture) were purchased at 24 h post-mortem from a local meat market (Seoul, Korea) and were trimmed of connective tissue and visible fat. For injection treatment, all pork loins were put into an ice box with ice and transported (~ 3 h) to Kongju National University (Yesan, Korea). The pork loins were divided into three groups of 12 loins. One group was used as the non-injected control, and the other two groups were injected with brine (0.7% or 1.5% NaCl, w/v) to 125% (targeting 80% moisture content in pork loin) of initial loin weight using a meat injector (Ideal-VA, Vakona GmbH, Lienen, Germany). In this study, only NaCl was used in the formulation of brine to evaluate the interaction effect of ionic strength of brine and HHP. The injected pork loins were vacuum-tumbled for 2 h using a tumbler (VTS-41, Biro Co., Marblehead, OH, USA). After tumbling, all pork loins were individually vacuum-packaged with poly-nylon bags, put into the ice box with fresh ice, and transported (~3 h) to Nano Bio Research Center (Jangseong, Korea) for HHP treatment. HHP treatment was performed using a commercial high-pressure machine (500MPA, Innoway, Anyang, Korea). In the present study, 6 pressure levels (0.1-500 MPa) were adopted to evaluate the influence of HHP on the quality of the injected pork loins. Two loins from each group were compressed by 2.5 MPa/s to the target pressure level. Based on Jo et al. (2014), HHP treatments were holding for 3 min at the target pressure level thereafter being depressed by 20 MPa/s. Following the HHP processing, all samples were kept in chilled ice box and transported (~5 h) to Sejong University (Seoul, Korea) for physicochemical analysis. For experimental replications, the above procedure was repeated three times using a fresh batch of pork loins.

Moisture content

As the weight gain of each injected pork loin could not be measured, the yield of injected pork loin was estimated by moisture content. The moisture content of pork loin was measured by hot air drying (105℃) as described in the AOAC method (2012). The moisture content of each pork loin was tested in triplicate.

Instrumental and visual color

Each loin was cut into slices of 1 cm thickness and oxygenated at ambient for 10 min. The surface color of the meat sample was measured using a color reader (CR-10, Konica Minolta Sensing Inc., Tokyo, Japan) calibrated with white standard board. CIE L* (lightness), a* (redness) and b* (yellowness) were measured from random 4 positions on the sample surface. To compare the color values and visual perception, a photograph of the sample surface was taken with a digital camera (α350, Sony, Tokyo, Japan).

Cooking loss

From the remaining pork loins, four slices of 3 cm thickness were taken and weighed. The loin cuts were separately put into a plastic bag and cooked in a water bath at 75℃ for 30 min. After cooking, surface exudates were gently wiped away and weighed again. Cooking loss was calculated as the difference in sample weight before and after cooking and expressed in percentage (%).

Texture profile analysis (TPA)

After measuring the cooking loss, each cooked slice was cut into strips (1 × 1 × 1 cm). A texture analyzer (CT3, Brookfield Engineering Labs, Stoughton, MA, USA) equipped with a probe (TA4/1000, Brookfield Engineering Labs) was applied to evaluate the TPA of the samples. Each sample was compressed twice under the conditions of 1 g_f trigger load, 1 mm/s test speed, and a compression height of 50%. The TPA was undertaken 15 times for each loin slice.

Statistical analysis

A complete randomized block design was adopted to evaluate the effects of the main factors (NaCl concentration vs pressure levels). The means of the three averages from the entirely repeated experiment (n=3) were analyzed using two-way ANOVA (analysis of variance) using R-statistical software (Ver. 3.6.1, R Studio, Inc., Boston, MA, USA). Duncan's multiple range test was conducted when the main effects were significant ($p<0.05$).

Results and Discussion

Moisture content

Raw pork loin (unpressurized control) contained 72.4% moisture, and the brine injection yielded better moisture content of pork loins (Table 1). However, the yields of injected treatments were dependent on the NaCl concentration of the brine. For brine with 0.7% NaCl concentration, retention of injected brine was not stable and easily released during transportation. The moisture content of 0.7% brine treatment was 73.0% and was not different from the not-injected loin. Contrarily, pork loin injected with 1.5% NaCl concentration exhibited a moisture content of 76.1%, which was higher ($p<0.05$) than the other two loins. Due to the salt-soluble nature of meat proteins, the impact of NaCl concentration on the moisture retention of pork loin is well understood (Desmond, 2006; Hamm, 1986). Since injection treatments were designed to achieve 20% weight extension by moisture (brine), moisture release from the injected meat was still caused even if 1.5% brine was applied.

HHP also caused moisture loss of not-injected loins, and particularly loin treated at 400 MPa showed 67.6% of the lowest moisture content among control group ($p<0.05$). It is known that pressurization causes changes in the meat protein structures and changes vary depending on the applied pressure level (Cheftel and Culioli, 1997). It is also known that HHP up to 300 MPa causes a complete unfolding of myosin and actin, a structural change similar to thermal treatment (Hong et al., 2012). Since protein unfolding under HHP resulted from the moisture migration to the inside of protein, the protein network effectively entrapped the moisture, in turn resulting in improved water-holding capacity (Boonyaratanakornkit et al., 2002; Xue et al., 2017). At 400 MPa, sarcoplasmic protein unfolding occurred, and the water retention ability decreased (Hong et al., 2012), which is in agreement with this study. However, HHP at 500 MPa could have influenced the structure of collagen resulting in higher moisture content compared to loins processed at 400 MPa ($p<0.05$).

As shown in Table 2, both the factors (NaCl and pressure) and interaction (NaCl × pressure) between these factors affected the moisture content of the injected pork loins. For 0.7% injection treatments, the best injection efficiency was found at 100 and 500 MPa, where the moisture content of pork loins ranged from 76.5-77.3%. Two identical pressure levels affecting the moisture content could be explained by the sequential unfolding of meat proteins as also shown in the control groups. The change in quaternary structure was initiated by moderate pressurization, which caused an exposure of hydrophobic residues (Mozhaev et al., 1996). Water molecules could act to form clathrates around the hydrophobic residues, and the presence of low concentration of NaCl could prevent the formation of salt-bridges in meat proteins upon depression (Boonyaratanakornkit et al., 2002). Therefore, moderate pressurization could be favorable for the improvement of the water-binding properties of meat proteins, which accounted for the high moisture content of 0.7% NaCl treatment at 100 MPa. With increasing pressure, irreversible loss of tertiary structure of myofibrillar proteins resulted in lowered water-binding properties as reflected in the lower moisture content at treatments of 300-400 MPa (Boonyaratanakornkit et al., 2002; Mozhaev et al., 1996). Alternately, it is likely that the HHP at 500 MPa could have caused structural changes in connective tissue (Potekhin et al., 2009), in turn affecting the water-binding property of meat. In this study, the pattern of moisture content with pressure level of the 1.5% NaCl treated samples were similar to those injected with 0.7% NaCl until 300 MPa. However, the optimum pressure level for best injection efficiency was 400 MPa. Due to the increased NaCl concentration, the moisture content of pork loin was not different among the 1.5% injection treatments with the exception of 400 MPa treatment. Since NaCl also affected the unfolding of meat proteins, the impact of HHP shown for the 0.7% NaCl treatments could be obtained at a lower pressure level. The lack of high injection efficiency of 1.5% NaCl treatments at lower pressures could be due to salting-out.

Instrumental and visual color

HHP treatment resulted in similar color change of all pork loins, irrespective of the brine concentration (Fig. 1). On the other hand, increasing the pressure level increased the L* of pork loins linearly ($p<0.05$). Both injection treatments exhibited greater L* than control group, while the L* of 0.7% NaCl treatments was higher than those of 1.5% treatment ($p<0.05$). At moderate pressurization (100 MPa), the a* of all pork loins increased, whereas the a* values decreased when the HHP was increased beyond 200 MPa for 0.7% injection treatment ($p<0.05$). This pattern was also similar to b* of pork loins. The change in b* as a function of pressure showed a second-order polynomial pattern, and injected treatments showed higher b* than non- injected. Largely, the pressure mediated color changes of pork loins were in agreement with the values reported in literature (Canto et al., 2012; Carlez et al., 1995; Jung et al., 2003). It is widely recognized that moderate pressurization (100-200 MPa) causes lighter appearance of meat, whereas meat treated with pressure greater than 300-400 MPa exhibits gray discoloration. The lighter appearance is attributed to the globin denaturation, heme displacement and moisture-binding condition on sample surface, and the gray discoloration is mainly due to myoglobin oxidation (Carlez et al., 1995; Goutefongea et al., 1995). These types of meat color changes have been regarded as a major demerit of HHP application for intact meat.

NaCl is also an important factor of meat color change. In general, NaCl improved the water-binding property of meat proteins and caused the dark-red appearance of meat (Ferreira et al., 2013; Puolanne et al., 2001). However, this type of color change can be mainly found in comminuted meat products (Cheftel and Culioli, 1997). In this study, a large amount of brine injection caused drip generation during transportation, indicating loss of soluble myoglobin, which could account for the high L* of injected treatments. In addition, NaCl is also known to promote lipid oxidation thereby leading to metmyoglobin oxidation (Mariutti and Bragagnolo, 2017) as demonstrated by the high b* of brine injected pork loins. With the exception of a*, which was not affected by brine concentration, the color changes in pork loins is attributed to NaCl, pressure, and their interactions (Table 2).

Although, slight lighter appearance was found in the 0.7% NaCl treated samples, the appearance was distinguished by pressure levels rather than by brine concentration (Fig. 2). In terms of visual appearance, the color of pork loin remained unchanged for samples treated at 200 MPa, whereas 300 MPa treatment initiated discoloration, particularly in injected treatments. At > 400 MPa, all meat could be distinguished from fresh pork loin (unpressurized control), but the injected treatments appeared light-pinkish compared to the gray or brownish color of the non-injected pork. The diluting effect of metmyoblobin by brine could be involved in the injected treatments. Based on the result, HHP at > 300 MPa leads to meat discoloration, which is major disadvantage of HHP treatment of brine injected meat. However, pork loin is consumed after cooking and the cooked color of HHP processed pork could compensate for the disadvantage of discoloration.

Cooking loss

Juiciness is regarded as an important characteristic for meat tenderness, and the juiciness is predicted by cooking loss. As shown in Table 3, the control showed cooking loss of 28.1%, whereas control group remained unaffected. It was reported that moderate pressurization caused less cooking loss of meat (Sikes et al., 2009), and the rack of consistency was probably be resulted from the long standing for processing and transportation of samples before analysis. Alternately, cooking loss of injected pork loins tended to increase with increasing pressure levels. For 0.7% brine injected treatments, increasing pressure up to 400 MPa did not affect the cooking loss of pork loin, while 500 MPa treated loin had greater cooking loss than that unpressurized ($p<0.05$). The cooking loss of 1.5% brine injection showed similar pattern to those of 0.7% treatments. In particular, the impact of HHP on cooking loss of pork loin was profoundly occurred in 1.5% brine treatments. In comparison of brine concentrations, 1.5% brine treatments tended to show higher cooking loss than those of 0.7% treatments reflecting that higher cooking loss of pork loin was caused by higher moisture content before cooking. Based on the results, best moisture retention of cooked pork loin could be obtained by brine injection and HHP at 100-200 MPa. It has been reported that juiciness could be improved by brine injection followed by HHP (Pingen et al., 2016). Hence, to obtain juicy meat, HHP at a moderate pressure level, particularly ~200 MPa was more advantageous than > 400 MPa. However, it should be noted that this study did not consider the usage of curing agents such as phosphates. Since a small addition of phosphates could improve the moisture-binding of pork loins, this could result in different characteristics of HHP treated products (Long et al., 2011). Further investigations are required for the enhancement of water-protein interactions in brine injection of meat.

TPA

The hardness of unpressurized control was 82.1 N, which is higher than both brine injection and HHP treated pork loins (Table 4). For the control group, hardness decreased to 57.1 N at 200 MPa ($p<0.05$), thereafter gradually increasing again with pressure. The pattern of hardness change with pressure was not in agreement with a previous study (Hong et al., 2012). This variation in the result could be due to the processing conditions such as holding time or processing temperature. It was reported that HHP improved the activity of proteases, which could have led the enzymatic tenderization during 5 h of transportation (Maresca and Ferrari, 2017). The hardness of pork loin tended to be lower when brine was injected, particularly 0.7% brine treatments showed lower hardness compared to 1.5% treatments at < 200 MPa. NaCl solubilizes meat proteins, and the protein-protein interactions could have intensely occurred at 1.5% brine treatments during cooking. Since NaCl was essential to improve moisture-protein and protein-protein interactions, the hardness of 1.5% brine treatment was not affected by HHP processing. Moreover, the highest hardness (103.9 N) was observed for the 0.7% brine treated samples, which could be due to the low injection efficiency and increased loss of moisture during cooking.

Cohesiveness of pork loins was not affected by NaCl concentration, but pressure ($p<0.001$) and interaction of NaCl × pressure ($p<0.001$) influenced the cohesiveness of pork loins (Table 2). However, the springiness of pork loins increased for brine injected samples. Furthermore, springiness tended to increase in 0.7% brine treatments than those of 1.5% treatments, although the differences were not statistically significant. The TPA data indicated that the moisture fortification by brine injection produced different textural properties of pork loins. Considering the hardness data, the results showed that brine injection followed by HHP was beneficial in producing tender pork loin. However, excessively high pressure (500 MPa) is not recommended as it hindered the tenderization of meat, which is associated with the water-binding property of pork loins.

Conclusion

This study demonstrated that brine injection followed by moderate pressurization (< 200 MPa) could tenderize pork loins. The increased moisture content of pork loins affected the textural properties of the pork loins. For brine composition, two NaCl concentrations were used to evaluate the ionic strength of the brine. Since the juiciness of pork loin might be changed by brine compositions, it warrants further research for the commercialization of the moisture fortified pork loin products. The HHP treatment is an important factor and the appropriate pressure condition must be selected for the stabilization of the injected moisture and the regulation of the chemical interactions of meat proteins. Based on the results, the best HHP condition to produce a tender pork loin was 200 MPa at which the pork loin had a high injection efficiency and tenderness. The eating quality of meat can be further improved by regulating the brine composition and brine injection weight. This study suggested that the application of HHP has potential benefits in the production of moisture enhanced meat products.

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Figure Captions

Fig. 1. Effects of NaCl concentration and pressure levels on (A) CIE L*, (B) a* and (C) b* of pork loins. Vertical bars indicate standard deviations.

Fig. 2. Effects of NaCl concentration and pressure levels on visual appearance of pork loins.

Table 1. Effects of NaCl concentration and pressure levels on the moisture content of pork loins

Pressure (MPa)	Control	Brine injection treatments	
		0.70% NaCl	1.50% NaCl
0.1[1)]	$72.36 \pm 0.55^{abB,\ 2)}$	72.97 ± 0.24^{bB}	76.05 ± 0.29^{bA}
100	70.72 ± 0.09^{cC}	77.30 ± 0.67^{aA}	74.98 ± 1.52^{bB}
200	71.86 ± 1.16^{bcB}	73.93 ± 0.92^{bAB}	76.94 ± 2.66^{bA}
300	73.29 ± 0.83^{a}	74.39 ± 0.87^{b}	74.79 ± 1.39^{b}
400	67.62 ± 0.66^{dC}	74.16 ± 0.91^{bB}	80.32 ± 0.46^{aA}
500	72.91 ± 0.32^{abB}	76.48 ± 0.91^{aA}	77.11 ± 1.83^{bA}

[1)] Without high pressure treatment

[2)] The lowercase (a-d) and uppercase (A-C) superscripts indicate significant difference among pressure and NaCl concentration, respectively ($p<0.05$).

Table 2. The physicochemical properties of pork loins as affected by NaCl concentration and pressure levels

Property	NaCl		Pressure		NaCl × Pressure	
	F-value	P-value	F-value	P-value	F-value	P-value
Moisture content	107.26	***	2.70	*	12.88	***
L*	42.21	***	182.31	***	3.05	***
a*	2.72	NS	366.86	***	9.57	***
b*	3.34	*	22.89	***	3.81	***
Cooking loss	72.49	***	19.23	***	9.87	***
Hardness	9.57	***	6.49	***	5.18	***
Cohesiveness	1.04	NS	9.33	***	8.10	***
Springiness	30.02	***	8.57	***	3.99	**

*p<0.05; ** p<0.01; *** p<0.001; NS, not significant

Table 3. Effects of NaCl concentration and pressure levels on the cooking loss of pork loins

Pressure (MPa)	Control	Brine injection treatments	
		0.70% NaCl	1.50% NaCl
0.1[1)]	28.07±3.39^{B}[2)]	35.51±2.01^{bcA}	29.75±2.25^{eB}
100	29.33±2.50^{B}	36.51±1.95^{abcA}	32.89±1.93^{dAB}
200	30.89±0.67^{B}	32.86±1.32^{cB}	37.48±0.42^{cA}
300	31.28±1.18^{B}	37.01±2.65^{abA}	34.43±0.90^{cdAB}
400	29.58±2.01^{C}	38.03±2.19^{abB}	47.52±2.21^{aA}
500	31.84±1.13^{B}	39.96±2.30^{aA}	41.84±0.77^{bA}

[1)]Without high pressure treatment

[2)]The lowercase (a-c) and uppercase (A-C) superscripts indicate significant difference among pressure and NaCl concentration, respectively ($p<0.05$).

Table 4. Effects of NaCl concentration and pressure levels on the textural properties of pork loins

Pressure (MPa)	Control	Brine injection treatments	
		0.70% NaCl	1.50% NaCl
Hardness (N)			
0.1[1)]	82.10±11.32$^{aA,2)}$	54.50±6.19^{cB}	61.93±10.72^{B}
100	73.52±14.35^{abA}	53.26±5.25^{cB}	66.87±11.77^{AB}
200	57.07±7.05^{b}	44.71±8.65^{c}	49.57±10.97
300	59.71±9.76^{bB}	80.32±6.21^{bA}	54.86±10.99^{B}
400	64.40±15.86^{b}	52.13±4.79^{c}	52.35±3.75
500	82.87±13.06^{aB}	103.88±8.63^{aA}	49.65±12.60^{C}
Cohesiveness			
0.1	0.53±0.02^{a}	0.51±0.03^{ab}	0.50±0.01^{a}
100	0.41±0.02^{cB}	0.52±0.01^{aA}	0.50±0.02^{aA}
200	0.47±0.03^{bA}	0.42±0.02^{cB}	0.51±0.04^{aA}
300	0.48±0.01^{bA}	0.47±0.06^{abA}	0.41±0.02^{bB}
400	0.50±0.02^{b}	0.49±0.01^{ab}	0.48±0.03^{a}
500	0.50±0.03^{bA}	0.46±0.03^{bcAB}	0.43±0.02^{bB}
Springiness (mm)			
0.1	2.56±0.12^{B}	2.93±0.05^{abA}	2.94±0.07^{aA}
100	2.41±0.14^{C}	3.05±0.14^{aA}	2.85±0.14^{abB}
200	2.30±0.14^{B}	2.60±0.08^{cdA}	2.76±0.18^{bA}
300	2.53±0.23	2.80±0.19^{bc}	2.47±0.13^{c}
400	2.51±0.12^{B}	2.82±0.15^{bcA}	2.71±0.09^{bA}
500	2.42±0.20	2.54±0.27^{d}	2.38±0.07^{c}

[1)]Without high pressure treatment

[2)]The lowercase (a-c) and uppercase (A-C) superscripts indicate significant difference among pressure and NaCl concentration, respectively ($p<0.05$).

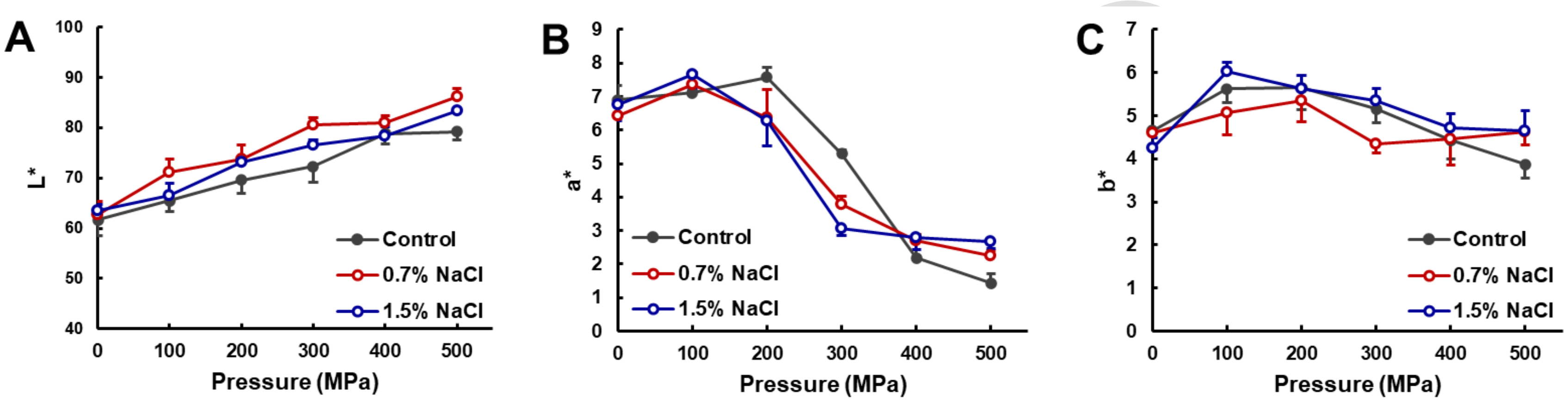
A
L*
100
90
80
70
60
50
40
0
100
200
300
400
500
Pressure (MPa)
Control
0.7% NaCl
1.5% NaCl
B
a*
9
8
7
6
5
4
3
2
1
0
0
100
200
300
400
500
Pressure (MPa)
Control
0.7% NaCl
1.5% NaCl
C
b*
7
6
5
4
3
2
1
0
0
100
200
300
400
500
Pressure (MPa)
Control
0.7% NaCl
1.5% NaCl

Fig. 1.

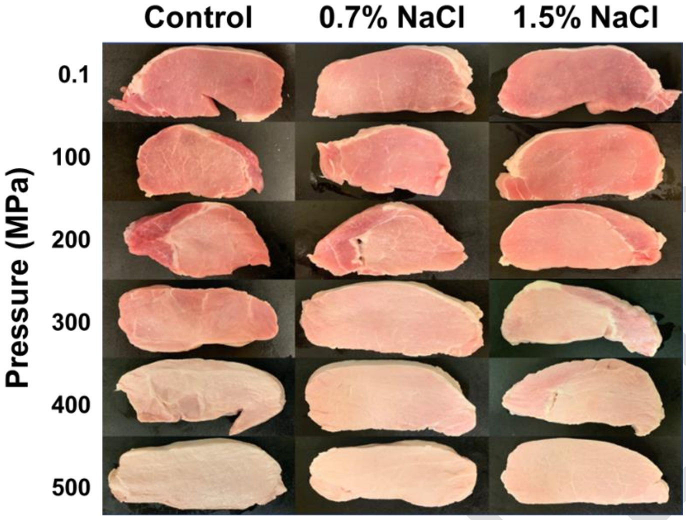

Fig. 2.