



## SHORT COMMUNICATION

# Pressure Induced Structural Changes of Proteins Affecting the Ice Nucleation Temperature of Pork Loins

Youngjae Cho<sup>1</sup>, Eun-Jung Lee<sup>2</sup>, Jiseon Lee<sup>1</sup>, SangYoon Lee<sup>1</sup>, Young-Chan Yun<sup>2</sup>, and Geun-Pyo Hong<sup>2,\*</sup>

<sup>1</sup>Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Korea

<sup>2</sup>Department of Food Science and Biotechnology, Sejong University, Seoul 05006, Korea



OPEN ACCESS

Received November 7, 2019

Revised November 18, 2019

Accepted November 19, 2019

\*Corresponding author : Geun-Pyo Hong  
Department of Food Science and  
Biotechnology, Sejong University,  
Seoul 05006, Korea  
Tel: +82-2-3408-2914  
Fax: +82-2-3408-4319  
E-mail: [gphong@sejong.ac.kr](mailto:gphong@sejong.ac.kr)

\*ORCID  
Youngjae Cho  
<https://orcid.org/0000-0002-2811-9703>  
Eun-Jung Lee  
<https://orcid.org/0000-0002-0601-7490>  
Jiseon Lee  
<https://orcid.org/0000-0001-9985-6770>  
SangYoon Lee  
<https://orcid.org/0000-0001-6561-7587>  
Young-Chan Yun  
<https://orcid.org/0000-0002-2527-2737>  
Geun-Pyo Hong  
<https://orcid.org/0000-0002-6343-3407>

**Abstract** This study investigated the effects of pressure-mediated protein changes on the ice nucleation temperature of pork loins. To variate chemical state of meat proteins, pork loin was pressurized at varying pressure levels (100–500 MPa) for 3 min, and moisture content, expressible moisture (EM) and differential scanning calorimetry (DSC) were analyzed. Although, all treatments showed similar moisture content, EM and degree of protein unfolding of pork loin showed different features as of 300 MPa. At moderate pressure treatments (100–200 MPa), all protein fractions were detected in DSC experiments, and pork loin had lower EM than control ( $p < 0.05$ ). Meanwhile, myosin and actin of pork loin treated at greater than 300 MPa were completely unfolded, and the treatments showed high EM compared to control ( $p < 0.05$ ). Unfolding of meat proteins was a factor suppressing ice nucleation, and the ice nucleation temperature tended to decrease with increasing applied pressure level. The ice nucleation characteristics of pressurized pork loin exhibited a potential application in freezing storage of pressurized meat with less tissue damage comparing to freeze fresh meat, and further exploration regarding the quality change after freezing of fresh and pressurized meat was warranted.

**Keywords** high pressure, protein structure, ice nucleation temperature, freezing, pork loin

## Introduction

A phase transition of liquid water to solid ice is a spontaneous phenomenon at subzero temperature. Nevertheless, water is often supercooled to low subzero temperature unless ice nucleation is occurred. Ice nucleation is a complex phenomenon which is yet completely understood, but it is an important factor affecting freezing behavior of meat. Up to date freezing techniques such as a pressure-shift freezing, an electromagnetic freezing or an ultrasound wave freezing are based on arbitrary control of ice nucleation (Espinosa et al., 2016; Zhan et al., 2018; Zheng and Sun, 2006).

Nevertheless, stochasticity of ice nucleation has been regarded as the main problem in developing an advanced freezing technology (Niedermeier et al., 2011).

In general, ice nuclei cannot grow up to crystals unless the nuclei are larger than critical size. From the thermodynamic point of view, the critical size of ice nuclei is proportional to ice-water surface tension, freezing point, amount of latent heat of fusion, while inversely to ice nucleation temperature (Pereyra et al., 2011). Theoretically, ice nuclei formed in meat has lower critical size than those in pure water which indicates that ice nucleation of meat is occurred at near below freezing point of meat. Therefore, supercooling phenomenon was rarely observed during meat freezing, and our previous study revealed that artificially lowering ice nucleation temperature had potential advantages of not only reducing freezing time but also minimizing tissue damage of pork loin (Kim and Hong, 2016).

Although cryoprotectants can inhibit either ice nucleation or ice crystallization (Boonsupthip and Lee, 2003), usage of these chemicals is limited in unprocessed meat. Since water in meat is held within meat structure (myofibrillar systems and connective tissue), ice nucleation is related with interactions between water and proteins, and hydrophobicity of meat proteins can influence on the ice nucleation phenomenon (Lupi et al., 2014; Zachariassen and Kristiansen, 2000). However, it has never investigated how chemical state of proteins affect the ice nucleation phenomenon. Typically, thermal process is the representative unit process which affects the protein structure, while compositional changes caused by drip generation can act as an additional factor to interpret ice nucleation phenomenon in meat. In this case, high pressure process may be the best tool to modify chemical state of meat proteins without compositional changes (Rastogi et al., 2007). Consequently, this study explored the relationship between pressure-mediated structural changes of proteins and ice nucleation phenomenon of pork loin.

## Materials and Methods

### Materials and sample preparation

Pork loin (*Musculus longissimus dorsi*) at 48 h post-mortem was purchased from a local market. Visible fat was completely trimmed off, and pork loins were vacuum packaged with poly-nylon pouch. Pork meat was pressurized using a commercial device (IWPM-600MPa-50L, Innoway, Seoul, Korea) from 100 to 500 MPa for 3 min at ambient temperature. Pressure built up and depression rates were 100 and 150 MPa/min, respectively. After treatment, all pork loins were kept at 2°C refrigerator prior to analysis.

### Moisture content and expressible moisture (EM)

Three slices with 20 mm thickness was obtained from middle part of each pork loin for the analyses of moisture content and expressible moisture (EM). From the center of each slices, approximately 1.5 g samples for moisture content and EM analyses were taken and weighed. Moisture content of each treatment was conducted by 105°C hot-air drying method. EM of sample was measured by Boles and Shand (2001) with slight modification. Meat sample was placed on the centrifuge tube with gauze as a moisture absorber and centrifuged at 1,500×g for 10 min under refrigeration mode (4°C). After centrifugation, meat pellet was removed, and tube was weighed. The tube was dried at 105°C for 24 h and weighed again. The EM of treatment was expressed as percent moisture loss from meat weight.

### Differential scanning calorimetry (DSC)

DSC was adopted to measure pressure-induced structural changes and ice nucleation phenomenon of pork loin.

Approximately 20 mg meat was sampled from the inside of pork loin and moved to an aluminum pan. The pan was tightly sealed and placed into a differential scanning calorimeter (DSC200F3, Netsch Geratebau GmbH, Selb, Germany) calibrated with an indium. Thermal scan of the pan was conducted under the condition of equilibrium at 20°C for 4 min thereafter heating to 95°C by 5°C/min. For observation of ice nucleation, meat sampling procedure was conducted as the same way described above. Program setting condition was in the order of equilibrium at 20°C for 4 min and cooling to -20°C by 1°C/min. All tests were determined in triplicate, and an empty pan was used as a reference. Data obtained from each experiment was analyzed using a Proteus analysis software (Netsch Gerätebau GmbH).

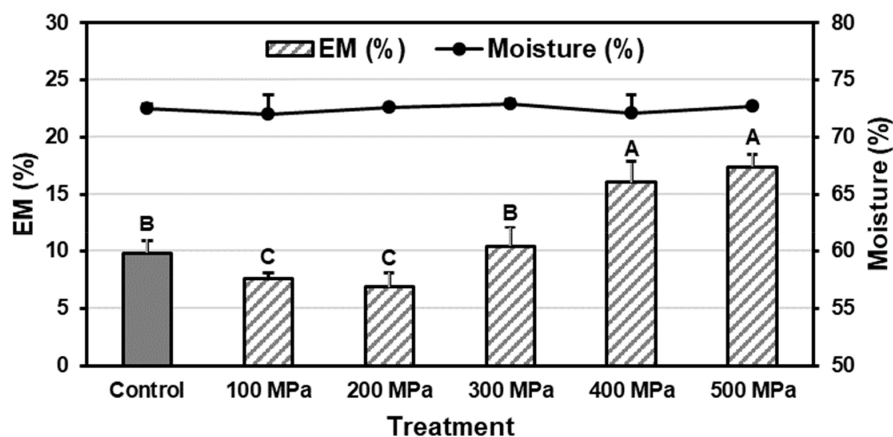
### Statistical analysis

From each experiment, triplicated determination was conducted, and the whole experiment was repeated three times on three different days using new batches (n=9). Data were analyzed using R software (ver. 3.6.1, The R Foundation, Vienna, Austria). One-way analysis of variance (ANOVA) was performed to evaluate the effect of pressure level ( $p < 0.05$ ), and Duncan's multiple range test was conducted as a post-hoc procedure.

## Results and Discussion

### Moisture content and EM

There was no difference in moisture content among treatments with different pressure level, and all treatments contained 72.0%–72.9% of identical moisture content (Fig. 1). Meanwhile, pressure level affected the EM of pork loin. Pork treated at moderate pressure level (100–200 MPa) showed 6.83%–7.54% of lower EM comparing to 9.82% of control ( $p < 0.05$ ). Further increasing pressure level (>200 MPa) caused a gradual increase in EM, and pork pressurized at greater than 400 MPa had 16.05%–17.41% of lower EM than that of control ( $p < 0.05$ ). The former decrease in EM of pork could be explained by structural changes of meat protein under high pressure processing below 200 MPa (Hong et al., 2012). It was reported that proteins underwent reversible unfolding as known to molten globule state under moderate pressurization. Upon depression, unfolded protein was refolded but not recover to its origin which accounted for the improve the water-binding properties of proteins (Boonyaratanakornkit et al., 2002). Alternately, protein unfolding was irreversible when applied pressure level is greater



**Fig. 1.** Moisture content and expressible moisture (EM) of pressurized pork loin. Vertical bars indicate standard deviations. <sup>A-C</sup> Means with different letters are significantly different ( $p < 0.05$ ).

than 300 MPa, and the irreversible unfolding of meat proteins would attribute to high EM of pork loin (Boonyaratankornkit et al., 2002; Knorr et al., 2006).

### Thermal stability

DSC thermal scan profiles revealed that fresh meat had three typical peaks of which maximum endothermic peak temperatures were 57.5°C, 63.8°C, and 80.0°C, respectively (Fig. 2). It was broadly known that the first and third peaks from DSC data were associated with myosin and actin, respectively, while the second peak was related with sarcoplasmic protein and/or connective tissue (Fernandez-Martin et al., 2000; Kazemi et al., 2011). Maximum first peak temperature slightly decreased to 56.3°C after 100 or 200 MPa pressurization, reflecting that myosin structure was affected even by moderate pressurization. The peak was disappeared in pork pressurized at greater than 300 MPa, reflecting that the complete unfolding of myosin structure was occurred at 300 MPa. Second peak showed irregular change in peak temperature depending on applied pressure level. The maximum peak temperature tended to increase with increasing pressure up to 200 MPa, thereafter decreasing with further increasing pressure level. Based on the report of Fernandez-Martin et al. (2000), maximum denaturation temperatures of sarcoplasmic protein and collagen were separated to 60°C and 67°C, respectively. In addition, collagen was stable comparing to other meat proteins against high pressure treatment (Cheftel and Culioli, 1997). Up to 200 MPa of pressurization, therefore, pressure-labile sarcoplasmic fractions would undergo unfolding without changing collagen structure, whereas the collagen structure was affected by pressurization greater than 300 MPa. The third peak was not affected by moderate pressurization (100–200 MPa), while it also completely denatured by pressurization at greater than 300 MPa.

### Ice nucleation behavior

According to the results, pork loin had different water-binding properties without difference in composition (Fig. 1), and the pressure-mediated structural change of meat proteins would be the main factor influencing on the ice nucleation phenomenon (Fig. 3). Upon cooling to subzero temperature, exothermic enthalpy of all treatments did not differ from one another (mean 684 kJ/kg) which would be resulted from the identical moisture content of treatments. However, ice nucleation

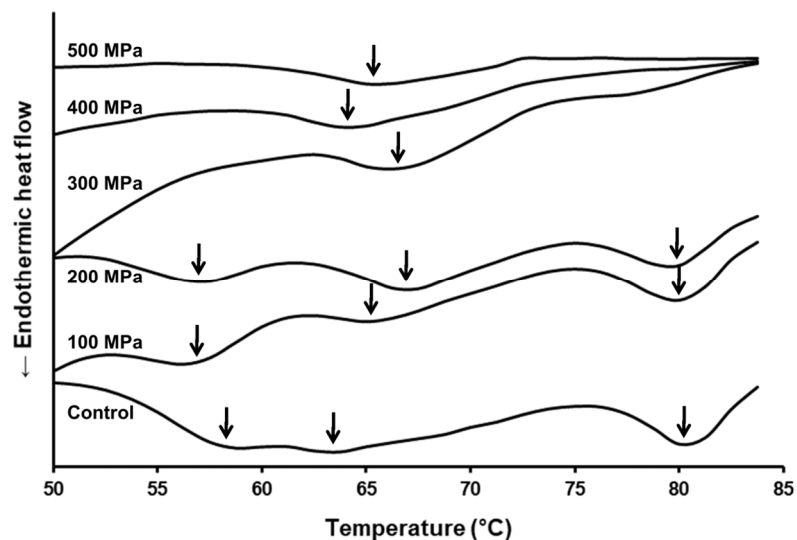
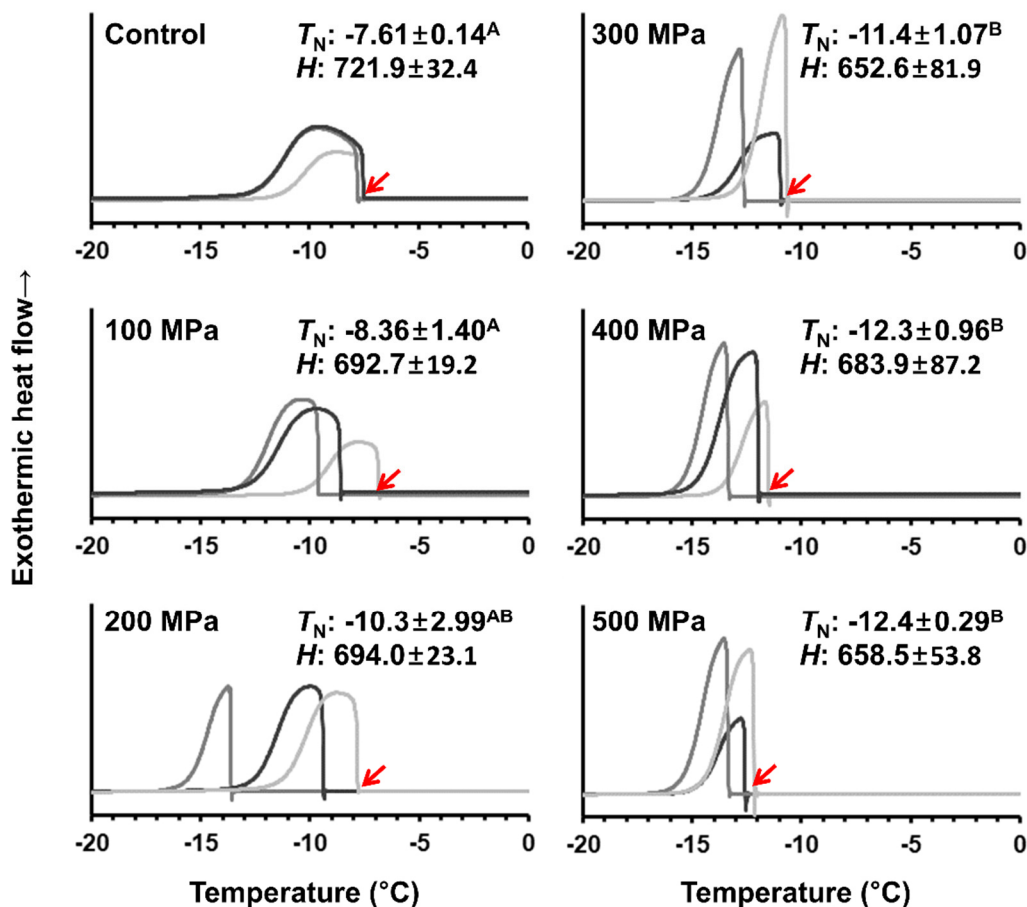


Fig. 2. Thermal stability of meat proteins from pressurized pork loin. Lower arrows indicate maximum peak temperatures.



**Fig. 3.** Ice nucleation temperature ( $T_N$ , °C) and exothermic enthalpy ( $H$ , kJ/kg) of pressurized pork loin ( $n=9$ ). Samples with maximum (gray), medium (black) and minimum ice nucleation temperatures (bright gray) are presented. Mean $\pm$ SD with different superscripts are significantly different ( $p<0.05$ ). Arrows indicate the first occurrence of ice nucleation.

temperature was depended on applied pressure levels ( $p<0.05$ ). Fresh control showed  $-7.61^\circ\text{C}$  of reproducible ice nucleation temperature ( $\pm 0.14^\circ\text{C}$ ) which was likely due to finely controlled experimental condition. Increasing pressure level up to 200 MPa seemed to decrease the ice nucleation temperature, however, the result was not significantly difference due to high standard deviation ( $\pm 2.99^\circ\text{C}$ ) of 200 MPa treatment. Compared to control, significantly low ice nucleation temperature ( $-11.4^\circ\text{C}$ ) was observed in pork pressurized at greater than 300 MPa ( $p<0.05$ ). As mentioned, moderate pressurization mediated structural changes of meat proteins provided improved protein-water interactions as shown by low EM, but the change was not uniform within sample matrix probably due to short holding time (3 min). Although, holding time is an important variable in moderate pressurization, the impact of holding time on the protein structure was lesser than that of pressure level (Lee and Hong, 2016). For that reason, reproducibility of ice nucleation was increased again with further increasing pressure level ( $>300$  MPa). According to Okamoto and Suzuki (2002), meat pressurized at 300 MPa lost the integrity of sarcomere structure, hence the completely unfolded contractile systems would form a gel-like structure which retained moisture during pressurization with reducing protein-water interactions. It was supposed that capillary force and surface tension were enhanced by entrapping water molecules within gel matrix, and the force fields would suppress the structuring of ice nuclei (Hruby et al., 2014). Another important feature of ice nucleation phenomenon was the pattern of heat removal from meat. The exothermic heat of sample with ice nucleation temperature higher than  $-10^\circ\text{C}$  was low at the beginning of ice nucleation, and

the heat is gradually and slowly removed during cooling. Contrarily, initial release of heat was steep in sample with lower ice nucleation temperature than  $-10^{\circ}\text{C}$ , thereby showing rapid heat removal during cooling. Since the rapid heat removal indicated rapid phase transition (Kim and Hong, 2016), eventually the result indicated that freezing rate of pork meat was proportional to applied pressure levels. Until now, combined effect of high pressure and freezing of meat was focused on pressure-shift freezing, hence there was no comparable study which estimated quality characteristics of meat pressurized followed by freezing. This study would be first trial to provide information regarding the effect of high pressure on the overall freezing characteristics of meat.

## Conclusion

This study demonstrated that ice nucleation phenomenon could be affected by chemical state of proteins, and pressure-mediated structural change (unfolding) of meat proteins suppressed ice nucleation, thereby lowering the ice nucleation temperature of pork loin. However, this study could not explain the actual mechanisms involving how unfolding of proteins suppressed the ice nucleation, findings of this study could suggest useful strategies to produce better quality frozen meat. Eventually, further explorations regarding either the holding time at moderate pressurization or quality comparison after freezing of fresh and pressurized meat were warranted.

## Conflict of Interest

The authors declare no potential conflict of interest.

## Acknowledgements

This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. NRF-2017R1D1A1B03031795), and the KU Research Professor Program of Konkuk University.

## Author Contributions

Conceptualization: Hong GP. Data curation: Cho Y, Lee EJ, Hong GP. Formal analysis: Lee EJ, Lee J, Lee SY, Yun YC. Methodology: Cho Y, Lee EJ. Software: Lee J, Lee SY. Validation: Cho Y, Lee EJ, Hong GP. Investigation: Lee J, Lee SY, Yun YC. Writing - original draft: Cho Y. Writing - review & editing: Cho Y, Lee EJ, Lee J, Lee SY, Yun YC, Hong GP.

## Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

## References

Boles JA, Shand PJ. 2001. Meat cut and injection level affects the tenderness and cook yield of processed roast beef. *Meat Sci* 59:259-265.

- Boonsupthip W, Lee TC. 2003. Application of antifreeze protein for food preservation: Effect of type III antifreeze protein for preservation of gel-forming of frozen and chilled actomyosin. *J Food Sci* 68:1804-1809.
- Boonyaratanakornkit BB, Park CB, Clark DS. 2002. Pressure effects on intra- and intermolecular interactions within proteins. *Biochim Biophys A* 1595:235-249.
- Cheftel JC, Culioli J. 1997. Effects of high pressure on meat: A review. *Meat Sci* 46:211-236.
- Espinosa JR, Zaragoza A, Rosales-Pelaez P, Navarro C, Valeriani C, Vega C, Sanz E. 2016. Interfacial free energy as the key to the pressure-induced deceleration of ice nucleation. *Phys Rev Lett* 117:135702.
- Fernandez-Martin F, Fernandez P, Carballo J, Jimenez-Colmenero F. 2000. DSC study on the influence of meat source, salt and fat levels, and processing parameters on batters pressurisation. *Eur Food Res Technol* 211:387-392.
- Hong GP, Chun JY, Lee SK, Choi MJ. 2012. Modelization and optimization of quality characteristics of pork treated various hydrostatic pressure conditions. *Korean J Food Sci Anim Resour* 32:274-284.
- Hruby J, Vins V, Mares R, Hykl J, Kalova J. 2014. Surface tension of supercooled water: No inflection point down to  $-25^{\circ}\text{C}$ . *J Phys Chem Lett* 5:425-428.
- Kazemi S, Ngadi MO, Garipey C. 2011. Protein denaturation in pork *longissimus* muscle of different quality groups. *Food Bioprocess Technol* 4:102-106.
- Kim Y, Hong GP. 2016. Effects of artificial supercooling followed by slow freezing on the microstructure and qualities of pork loin. *Korean J Food Sci Anim Resour* 36:650-655.
- Knorr D, Heinz V, Buckow R. 2006. High pressure application for food biopolymers. *Biochim Biophys A* 1764:619-631.
- Lee YK, Hong GP. 2016. Optimization of hydrostatic pressure processing to extending shelf-life with minimal quality changes of refrigerated abalone. *Food Sci Technol Res* 22:419-428.
- Lupi L, Hudait A, Molinero V. 2014. Heterogeneous nucleation of ice on carbon surfaces. *J Am Chem Soc* 136:3156-3164.
- Niedermeier D, Shaw RA, Hartmann S, Wex H, Clauss T, Voigtlander J, Stratmann F. 2011. Heterogeneous ice nucleation: Exploring the transition from stochastic to singular freezing behavior. *Atmos Chem Phys* 11:8767-8775.
- Okamoto A, Suzuki A. 2002. Effects of high hydrostatic pressure-thawing on pork meat. *Prog Biotechnol* 19:571-576.
- Pereyra RG, Szleifer I, Carignano MA. 2011. Temperature dependence of ice critical nucleus size. *J Chem Phys* 135:034508.
- Rastogi NK, Raghavarao KS, Balasubramaniam VM, Niranjana K, Knorr D. 2007. Opportunities and challenges in high pressure processing of foods. *Crit Rev Food Sci Nutr* 47:69-112.
- Zachariassen KE, Kristiansen E. 2000. Ice nucleation and antinucleation in nature. *Cryobiol* 41:257-279.
- Zhan X, Sun DW, Zhu Z, Wang QJ. 2018. Improving the quality and safety of frozen muscle foods by emerging freezing technologies: A review. *Crit Rev Food Sci Nutr* 58:2925-2938.
- Zheng L, Sun DW. 2006. Innovative applications of power ultrasound during food freezing processes: A review. *Trends Food Sci Technol* 17:16-23.