PROTEIN STRUCTURE CHANGES IN PORCINELONGISSIMUS MUSCLE AS INFLUENCED BY MULTIPLE FREEZE-THAW CYCLES

Xiufang Xia*, Mingcheng Zhang, Fangfei, Li, Baohua Kong

College of Food Science, Northeast Agricultural University, Harbin, Heilongjiang, China;

*Corresponding author email: kongbh63@hotmail.com

Abstract – This study investigated the effects of multiple freeze-thaw (F-T) cycles on protein structure changes in porcine longissimus muscle. In this study, myofibrillar protein (MP) was extracted form muscles which were subjected 1-5 F-T cycles, free amine group, circular dichroism (CD) spectropolarimeter and UV absorption spectra could use to reveal the change in structure . Results show that multiple F-T cycles caused protein cross-linking and oxidation. In addition, multiple F-T cycles could cause α-helix structure disruption, hydrophobic domain exposure.

Key Words – Circular dichroism spectra, Dityrosine, Myofibrillar proteins.

I. INTRODUCTION

Myofibrillar proteins (MPs)denaturation induced by frozen storage can be linked to some factors, such as decreased ATPase activity, protein oxidation, and increased intracellular ionic strength following the migration of water [1]. The repeated crystallization of water during multiple F-T cycles could cause changes in the secondary and tertiary structures of protein .

The objective of this study was to investigate the influence of multiple FT cycles on moisture migration, microstructure damage, and MP structure changes of porcine longissimus muscle.

II. MATERIALS AND METHODS

1. Sample preparation and preparation of myofibrillar protein

The method of muscle F-T cycles as described by Xia *et al* [2]. MP was prepared according to the procedure of Xia *et al*. [3].

2. Free amine group and Dityrosine measurement

Free amine group (NH_2) content was measured as described by Adler-Nissen [4]. The dityrosine content was measured by the method of Davies*et al*.[5].

3.CD spectra measurement

The CD was measured according to the modified method of Liu *et al*. [6].

4. UV second-derivative spectroscopy

UVsecond-derivative spectroscopy was measured according to the modified method of Zhao *et al*. [3].

5. Statistical analysis

All the data were analyzed statistically using the General Linear Models procedure of the Statistix 8.1 software package.

III. RESULTS AND DISCUSSION

1. Free amino groups and dityrosine

Free amine content in the control was 96.7 nmol/mg protein, which decreased by 4.3%, 21.031%, and 11.9% after 1, 3, and 5 F-T cycles, respectively. Meanwhile, multiple F-T cycles caused a significant increase (P <0.05) in dityrosine content(Fig. 1).

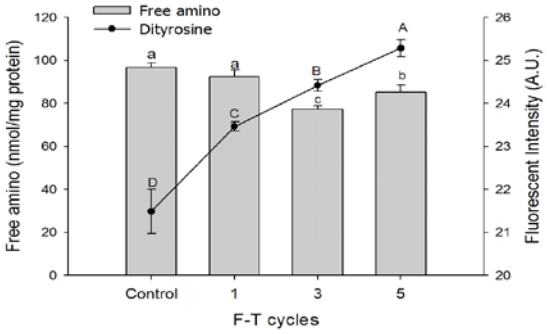

Figure 1. Influence of different freeze-thaw cycles on the content of free amino and dityrosine of myofibrillar protein (MP).

2. Circular dichroism spectra analysis

When MP was subjected to multiple F-T cycles, the distinct helical pattern underwent remarkable attenuation in the 220 nm negative band region(Fig 2A). The α-helix content of MP in the control was 55.62%, which decreased to 48.71%, 43.31%, and 37.91% after 1, 3, and 5 F-T cycles, respectively (Fig 2B), suggesting that α-helix structures were lost heavily in MP when subjected to multiple freeze-thaw cycles.

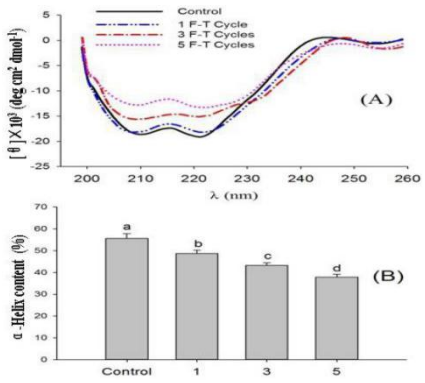

Figure 2. Influence of different freeze-thaw (F-T) cycles on secondary structure changes of myofibrillar protein (MP).

3. UV second derivative absorption spectra

The value of 'r' and the positions of the peaks and troughs are a function of the relative amounts of the tryptophans and tyrosines and of the average polarity of the environments of the two amino acids. It was noted that F-T cycles caused an obvious increase in 'r' values from 1.13 (control) to 1.50 (5 F-T cycles)(Fig. 3). Which indicated that the tyrosine residues buried inside MP were gradually exposed to the surface due to protein unfolding.

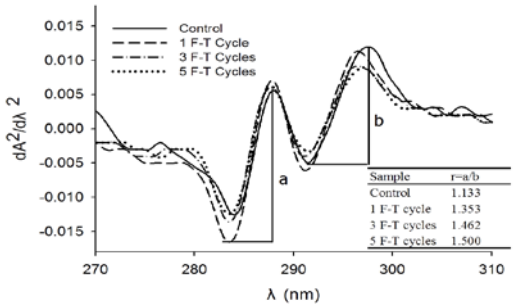

Figure 3. Influence of different freeze-thaw (F-T) cycles on the second derivative spectrum of myofibrillar protein (MP).

IV. CONCLUSION

F-T cycles caused a decrease in free amino groups and an increase in dityrosine. The MP secondary, tertiarystructures were changed.Moreover, analysis of the conformational changes of protein during F-T cycles revealed a decrease in the α-helical content of MP and unfolding of the tertiary structure.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation (3157101503) and the Foundation of Science and Technology Major Projects in Heilongjiang (grant no. GA15B302).

REFERENCES

1. Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: review. Meat Science 91: 93-98.

2. Xia, X. F., Kong, B. H., Liu, Q., & Liu, J. (2009). Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze-thaw cycles. Meat Science 83: 239-245.
3. Zhao, J. Y., Dong, F. J., Li, Y. Y., Kong, B. H., & Liu, Q. (2015). Effect of freeze-thaw cycles on the emulsion activity and structural characteristics of soy protein isolate. Process Biochemistry 50: 1607-1613.
4. Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. Journal of Agricultural and Food Chemistry 27: 1256-1262.
5. Davies, K. J., Delsignore, M. E., & Lin, S. W. (1987). Protein damage and degradation by oxygen radicals. II. Modification of amino acids. Journal of Biological Chemistry 262: 9902-9907.
6. Liu, Q., Lu, Y., Han, J. C., Chen, Q., & Kong, B. H. (2015). Structure-modification by moderate oxidation in hydroxyl radical-generating systems promote the emulsifying properties of soy protein isolate. Food Structure6: 21-28.