

# IMPACT OF ICE STRUCTURING PROTEIN ON MYOFIBRILLAR PROTEIN AGGREGATION BEHAVIOUR AND STRUCTURAL PROPERTY OF QUICK-FROZEN PORK PATTY DURING FROZEN STORAGE

Fangfei Li\*, Xiufang Xia and Yihong Bao

College of Life Science, Northeast Forestry University, China

\*Corresponding author email: [lifangfei33@163.com](mailto:lifangfei33@163.com)

## I. INTRODUCTION

Quick-frozen pork patties are popular among consumers due to their satisfactory quality and sensory attributes. Rapid freezing of pork patties at temperatures below  $-18^{\circ}\text{C}$  can effectively extend the shelf life of meat and meat products by preventing microbial spoilage, reducing enzyme activity, and slowing down biochemical reactions. However, frozen storage can lead to some undesirable changes, such as the formation of ice crystals, recrystallization, oxidative reactions, and protein denaturation. These changes can result in the degradation of myofibrillar protein (MP) structure and functionality. To prevent these changes, emerging techniques have been developed that target MP structure. Among these techniques, ice structuring protein (ISP) is well-known for its ability to inhibit ice nucleation and recrystallization [1]. However, there is still limited information available on the impact of ISP on MP aggregation behavior and structure during the frozen storage of meat. The objective of this research was to investigate the cryoprotective effect of ISP on the aggregation behavior and structural changes of MP, and to elucidate the mechanism by which ISP preserves the MP characteristics of quick-frozen pork patties during frozen storage.

## II. MATERIALS AND METHODS

Pork shoulder and neck were trimmed of visible connective. The ISP (purity higher than 95%) were purchased from Nanjing Anfei Bio (China). The meat, ISPs (0% and 0.20%) and 12% ice water were mixed for 5 min. The mixture was made into patties of approximately 100 g each (9 cm diameter and 2 cm thickness) and then kept at  $-18^{\circ}\text{C}$ . Patties were divided into 2 groups: control group (patties with 0% ISP) and ISP group (patties with 0.20% ISP). Each group was randomly divided into 5 treatments and frozen for 0, 30, 60, 90 and 180 d. Samples were thawed at  $4^{\circ}\text{C}$  overnight before analyzing. The effect of ISP and frozen storage on the myofibrillar protein aggregation was assessed by particle size, zeta-potential and surface morphology (measured by atomic force microscopy (AFM)). Changes in primary structure (carbonyl and free amino contents), secondary structure (circular dichroism (CD) far-ultraviolet spectra) and tertiary structural (fluorescence intensity) were used to evaluate the alteration on MP structural property. The data were analyzed using analysis of variance with Statistix 8.1 (Analytical Software, St. Paul, MN). The means  $\pm$  standard deviations were reported at a significance level of  $P < 0.05$ .

## III. RESULTS AND DISCUSSION

The results of the study showed that the addition of ISP to patty led to a more uniform distribution of MP particle size and increased electrostatic repulsive force. Furthermore, the inclusion of ISP was found to decrease the degree of muscle cell damage by inhibiting recrystallization. Additionally, ISP was observed to possess the ability to prevent irreversible mechanical damage to tissues caused by ice crystals, inhibit recrystallization, and maintain protein structure stability by preventing protein oxidation during frozen storage [2]. As a result, the addition of ISP significantly inhibited MP aggregation after freezing ( $P < 0.05$ ).

The results suggested that the mechanical damage to the muscle can be controlled by inhibiting the growth of ice crystals and reducing the carbonyl content of protein through ISP binding. The higher amino acid content in the ISP group indicated that ISP has the ability to impede recrystallization and alter ice crystal morphology, thereby maintaining cell structure integrity and reducing protein oxidation. Additionally, the incorporation of ISP significantly reduced the degree of exposure of hydrophobic residues ( $P < 0.05$ ), inhibited interactions among amino acid side chains, and minimized freezing and oxidation damage. This hindered myofibrillar protein denaturation and weakened hydrogen interactions between the protein molecules.

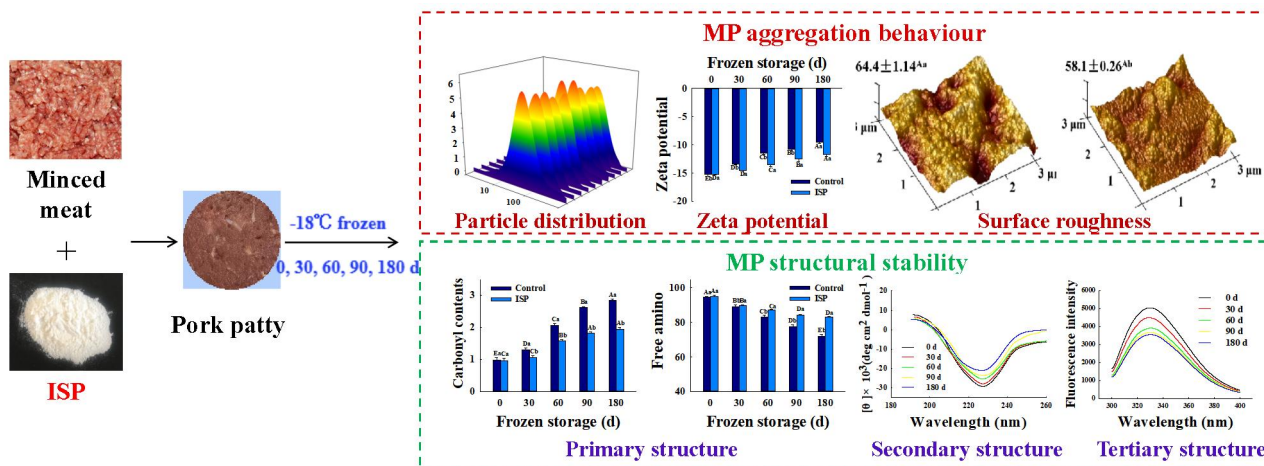


Figure 1. Impact of of ice structuring protein on myofibrillar protein aggregation behaviour structural property of quick-frozen patty during frozen storage

The means for the same frozen storage with different lowercase letters (a-b) and for the same treatment with different uppercase letters (A-E) differ significantly ( $P < 0.05$ ).

#### IV. CONCLUSION

By analyzing the results of MP particle size distribution, zeta-potential, and AFM, it can be concluded that the addition of ISP to frozen pork patty can decrease the degree of protein aggregation. This is further supported by the lower carbonyl contents and higher free amino contents observed in the ISP group, indicating that the primary structure degradation of MP was inhibited by the addition of ISP into the patty. Furthermore, the secondary and tertiary structure of MP in pork patty with ISP exhibited increased stability after frozen storage. Overall, these findings suggest that the cryoprotective effect of ISP can effectively mitigate MP aggregation behavior and structural deterioration during frozen storage. However, further research is needed to explore the mechanism of action between ISP and ice crystals in frozen meat and products.

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#### REFERENCES

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