

## Cryoprotective Effect of Trehalose on Myofibrillar Proteins and Surimi during Frozen Storage

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## Abstract

The effect of different cryoprotectants (trehalose, sucrose and sorbitol) at various concentrations (0, 2, 4, 6, and 8% (w/v)) on the properties of natural actomyosin (NAM) subjected to 1 and 2 freeze-thaw cycles was investigated. Ca<sup>2+</sup>-ATPase activity, sulfhydryl content, and solubility decreased with increasing freeze-thaw cycles. However, the increase in disulfide bonds content and surface hydrophobicity was generally observed. Higher level (8%) of trehalose, sucrose, and sorbitol could retard physico-chemical changes more effectively than the lower level. To optimize cryoprotective effect of trehalose, sucrose, or sorbitol, the mixture design was used to formulate different formulae under condition that total amount of cryoprotectant was 8%(w/v). Cryoprotectant formulae exhibiting the highest cryoprotective efficiency in NAM stabilization were 8%trehalose (Formula 1), or the blend of 5.34%trehalose, 1.33%sucrose, and 1.33% sorbitol (Formula 2). Those two formulae were found to prevent the protein denaturation effectively as shown by the retarded changes in physico-chemical properties, including disulfide bond formation and hydrophobic exposure.

Solubility and protein pattern of NAM aggregate in various denaturating solutions was studied. Different solutions including 1) 1%SDS, 2) 1%SDS+8M urea and 3) 1%SDS+8M urea+2% $\beta$ ME were used. Control showed the lower solubility in all denaturating solutions, compared to other treatments. The aggregation of NAM with and without cryoprotectant treatments was caused by hydrogen bonds,

hydrophobic interaction and disulfide bonds. Aggregate of NAM added with Formula 1 (8% trehalose) was more dissolved in three solutions than those added with the blends, commercial cryoprotectants and the control. This suggested that 8%trehalose may prevent protein-protein interaction more effectively than other treatments. From the SDS-PAGE, lower bands intensity of myosin heavy chain (MHC) in all treatments were observed under non-reducing condition, however no marked differences in protein pattern were found under reducing condition. Therefore, disulfide bond played an important role in protein aggregation during freeze-thawing process.

Cryoprotective efficacy of different formulae in surimi as compared with commercial cryoprotectants (4% sucrose and 4% sorbitol) during storage at -18 °C for 12 weeks and freeze-thawing for 0, 1, 2, 4 and 6 cycles was investigated. The cryoprotectants Formula 1 and 2 effectively stabilized proteins and maintained the gel-forming ability, comparable to the commercial cryoprotectants during extended frozen storage and after freeze-thawing. From the results, surimi added with cryoprotectant Formula 1 and 2 has the higher breaking force than that added with commercial cryoprotectants after 4 weeks of storage (p<0.05). However, sample added with cryoprotectant Formula 2 had highest deformation when stored for 10 and 12 weeks. The same results in cryoprotective efficacy were observed in samples subjected to freeze-thawing. Therefore, the use of trehalose or trehalose in combination with sucrose and sorbitol effectively prevents the denaturation of myofibrillar and surimi during or frozen storage. It would be an alternative to reduce the sweetness in surimi products.