Effect of Controlled Ice Nucleation on Protein Product Quality

Kazunari Tanaka

Vamsi Mudhivarthi; Robin Bogner; Michael J. Pikal

University of Connecticut, 69 North Eagleville Road, Storrs, CT 06269 USA

Objective: Several controlled ice nucleation techniques are being developed to improve the product quality and to increase the process efficiency. Especially, those techniques are considered to be useful for protein products that are inactivated in freezing process. The objective of this study is to evaluate the effect of a controlled ice nucleation on the protein product stability.

Methods: Beta-galactosidase (b-gal) in 5% sucrose with varied concentration of sodium phosphate buffer (2-100mM) was frozen with two different manners, a) controlled nucleation at -5 deg C and b) uncontrolled nucleation plus annealing, and was freeze-dried. Then, the activity of b-gal was measured by increase in absorbance of specific substrate (ONPG) at 405 nm. The remaining b-gal tetramer content was also measured by SEC. In addition, pH change during each freezing process was monitored using low temperature electrode.

Results: With the annealing step, a buffer concentration dependent activity reduction, decrease of tetramer content and a remarkable pH shift (7.0 to 4.5) were observed. On the other hand, there were no decrease of activity/tetramer content and a small pH change with controlled nucleation at -5 deg C.

Conclusion: The results suggest that the controlled nucleation technique unlike annealing could improve the batch homogeneity without inducing crystallization of buffer components.