

DETERMINATION OF THE EUTECTIC TEMPERATURE OF TREHALOSE-WATER BINARY SYSTEM BY X-RAY DIFFRACTOMETRY

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Background. The eutectic temperature (T_e) is a critical thermophysical property dictating the primary drying temperature of frozen binary solute-water systems. In order to prevent melt-back, primary drying is conducted below T_e . In trehalose-water systems, since the crystallization of trehalose is not kinetically favored, the solute is retained in the amorphous freeze-concentrate. As a result, the eutectic temperature is usually extrapolated from the solubility curve of trehalose dihydrate. The reported eutectic temperature ranges from -2.5 to -18.8°C .⁽¹⁻³⁾ Based on solubility studies, carried out after long equilibration times, the eutectic temperature was reported to be -2.5°C .⁽²⁾

Purpose. To determine the eutectic temperature of the trehalose-water system by low temperature X-ray diffractometry.

Method. Aqueous trehalose solution (4% w/v) was cooled from room temperature to -30°C at $0.5^\circ\text{C}/\text{min}$ in a custom designed sample holder. The frozen solution was warmed to -18°C and annealed with or without seeding. The XRD patterns were collected both during cooling and annealing. The annealed sample was warmed, at $1^\circ\text{C}/\text{min}$, to 2°C and the XRD patterns were collected periodically. The crystallizing phases were identified by comparing the XRD patterns with Powder Diffraction Files of International Centre for Diffraction Data (ICDD). The frozen solutions were also characterized by differential scanning calorimetry (DSC).

Results. In frozen solutions, trehalose was retained amorphous. However, it crystallized upon annealing. The crystalline phase was identified to be trehalose dihydrate, based on the characteristic lines with d-spacings of 10.1, 7.0 and 6.5 Å. Seeding facilitated the crystallization of trehalose dihydrate. At 3°C , both the solute and ice peaks were observed. When the temperature was increased to 2°C , only the solute peaks disappeared, indicating the eutectic temperature to be $\sim -2^\circ\text{C}$. The endotherm observed in the DSC at -2.5°C could be attributed to the eutectic melt, which was followed by ice melting.

Conclusion. The eutectic temperature of trehalose-water system was determined by low temperature X-ray diffractometry with DSC serving as a complementary technique. The technique is rapid and direct. Unlike the conventional approaches, the eutectic temperature (but not composition) can be determined by analyzing a solution of single composition.

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