

Purification and Study of Mechanism of Action of Apoplastic Ice Structuring Proteins from Cold-Acclimated Winter Wheat Leaves¹

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Ice structuring proteins (ISPs) naturally exist in many foods consumed as part of the human diet including plants or fish. ISPs from winter wheat grass have gained interest in the pharmaceutical and food industries as a non-toxic, natural and cost-effective product, which is easy to prepare as a crude extract. However, they have not been purified reproducibly and studied in detail to elucidate their structures, mechanism of actions and difference(s). ISPs from the apoplast region of cold-acclimated winter wheat leaves were extracted through vacuum infiltration and purified using heat and ethanol precipitations, size exclusion and anionic exchange fast protein liquid chromatography techniques. The ISPs showed both inhibition of ice growth and thermal hysteresis activities. The non-acclimated apoplastic extracts from winter wheat leaves contained similar proteins without any abovementioned activities, perhaps due to compositional differences (e.g., post translation modification). The ISPs were highly disulfide linked, similar to thaumatin-like proteins (TLPs). ISPs remained active after extensive thermal treatment at 75, 85 or 95°C and over a wide range of pH (3-12).

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