Doctoral Dissertation

RATIONAL DESIGN OF STABLE FREEZE-DRIED ENZYME FORMULATIONS FOR THE USE IN FISH FRESHNESS DETERMINATION METHOD

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博士学位論文内容要旨

Abstract

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論文題目 Title	Rational Design of Stable Freeze-dried Enzyme Formulations for the Use in Fish Freshness Determination Method		

Evaluation of freshness is important for quality control of fish and marine products. K value, which is the most popular index of fish freshness, can be evaluated simply by the use of enzyme mixtures, consisting of alkaline phosphatase (ALP), nucleoside phosphorylase (NP) and xanthine oxidase (XOD). The enzymes are, however, thermally unstable and become almost completely inactive during freeze-drying and storage.

To improve activity of the freeze-dried enzymes, the effects of sugar (sucrose and trehalose), polymer (bovine serum albumin [BSA] and dextran) and their mixtures on the loss of XOD activity during freeze-drying and subsequent storage were initially investigated in Chapter 2. All samples were amorphous solid. Sucrose, trehalose and BSA protected XOD from the activity loss during freeze-drying. Dextran, however, showed no stabilizing effect. In addition, the mixture of sugar (sucrose or trehalose) and BSA improved the XOD activity synergistically. It was noted that despite the lower glass transition temperature (T_g) of sucrose, it had stabilizing effects higher than had trehalose. During storage, the XOD activity of all samples decreased gradually at a temperature range of between 25 and 60°C. Samples stored at temperatures below the T_g showed a lower loss of the XOD activity than those stored at just the T_g. In addition, the Kohlrausch-Williams-Watts (KWW) equation, that has been used to characterize

molecular relaxation processes within amorphous systems, was applied to predicting storage stability of the amorphous freeze-dried XOD. The results demonstrated that the non-exponential degradation of the dried XOD could be described by the KWW equation based on the estimated parameters of degradation time constant (τ) and its distribution (β).

In Chapter 3, stabilities of the two enzyme mixtures, NP-XOD and ALP-NP-XOD, freeze-dried with sucrose and sucrose-polymer (BSA, gelatin, dextran, polyethylene glycol [PEG], and polyvinylpyrrolidone [PVP]) were further investigated. The T_g values of sucrose-polymer formulations, with the exception of sucrose-PEG, were higher than that of sucrose alone formulation. Sucrose-protein (BSA or gelatin) showed more excellent stabilizing effects on the enzyme mixtures than did sucrose. Using a non-protein polymer (dextran, PEG or PVP) with sucrose, on the other hand, did not improve the enzymes stabilities.

According to the previous results, in Chapter 4 the NP-XOD and ALP-NP-XOD freeze-dried with sucrose-gelatin (the most effective additive) were then applied to developing a colorimetric method for evaluation of fish freshness based on the K_i value. As a result, a linear relationship was obtained between K_i values determined by the developed colorimetric method and a conventional high-performance liquid chromatography with a high correlation coefficient of 0.997. In addition, the developed method showed the potential for measuring K_i value accurately even after storage of the enzyme mixtures at 40°C for up to 172 days.