Recrystallization behavior of ice crystals in model food system containing antifreeze (glyco) proteins (AF(G)Ps)

[課程博士・論文博士共通]

博士学位論文内容要旨
Abstract

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論文題目 Title	Recrystallization behavior of ice crystals in model food system containing antifreeze (glyco)proteins (AF(G)Ps) (不凍タンパク質および不凍糖タンパク質を含むモデル食品系における氷結晶の再結晶化挙動)		

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Frozen foods have become indispensable not only as fresh foods but also as commercial food ingredients in restaurants. However, it is known that ice crystals inside food during freezing and storage repeatedly grow and coarsen as time passes by taking in surrounding water molecules. This phenomenon is called recrystallization, which refers to any change in the size, shape, orientation, or perfection of individual crystals after the completion of solidification. It is an important difficulty leading to qualitative deterioration of frozen foods, such as coarse, grainy, and icy texture of ice cream, drip loss of frozen meat and fish, and damage to the network structure of frozen dough. Therefore, IR must be suppressed during the storage and distribution of frozen foods. Therefore, IR must be suppressed during the storage and distribution of frozen foods. Three recrystallization processes can occur in frozen food under isothermal temperature conditions simultaneously: accretion, migratory and isomass. Accretion recrystallization is the process by which two or more ice crystals in contact with each other merge into one ice crystal. It is the main recrystallization mechanism which contributes to the early stages of recrystallization. The main recrystallization mechanism in the later stages is migratory recrystallization. This is a process in which larger crystals grow at the expense of smaller crystals in a polycrystalline system. The water molecules on the surface of the smaller crystals tend to migrate to the surface of the larger ones due to their higher specific surface energy. As a result, the large ice crystals grow, and the small ice crystals shrink. Isomass recrystallization is a process in which the rough surface of a single crystal becomes smooth due to the principle of surface energy minimization.

Antifreeze proteins (AFPs) or antifreeze glycoproteins (AFGPs) are diverse classes of proteins produced by certain bacteria, fungi, algae, diatoms, plants, insects, and fish that ensure their survival in ice in cold climates. In fact, AF(G)Ps are well-known for their ice recrystallization inhibition (IRI) capability, thermal hysteresis (TH) activity, and ice shaping (IS) ability. It has been expected that the addition of AF(G)Ps to food products would dramatically inhibit ice crystal growth, resulting in superior frozen foods that maintain their quality for extremely long periods. In this study, type I antifreeze protein (AFP I) (from Pleuronectidae family fish; M. W. ～3,200), type III antifreeze protein (AFP III) (from Zoarcidae family fish; M. W. ～6,700), and antifreeze glycoprotein (AFGP) (from Gadidae family fish; M.W. ～2,600-32,000) were used to add to model food to clarify its effect on ice crystal recrystallization behaviors. AFP I has amphiphilic α-helical secondary structures, which consist of three repeats of T-xxxAxxxAxx (x: mostly alanine). Regarding AFGP, it comprises a (Thr-Ala-Ala)n tripeptide repeat where the secondary hydroxyl group of the threonine residue is glycosylated with β-D-galactosyl-(1,3)-α -N-acetyl-D-galactosamine. However, AFP III is a globular protein comprising numerous short β-strands and one turn of α-helix; its amino acid composition is more complex than that of either AFGP or AFP I. The objective of this study was to investigate recrystallization behavior of ice crystals in model food system containing three fish-drived AF(G)Ps (AFGP or AFP I and AFP III).

In Chapter 1, literature review about three fish-drived AF(G)Ps and its ice recrystallization inhibition (IRI) capability, thermal hysteresis (TH) activity, and ice shaping (IS) ability. Also, the experimental techniques used for estimating proteins denaturation point and water mobility was carried out.

In Chapter 2, compared the capabilities of AFP I, AFP III, and AFGP to inhibit IR and quantified concentration dependence of IRI activity of native AF(G)Ps in a comparable and unified manner. Additionally, the AF(G)P thermostability was examined in terms of IR rate change after heat treatment. The concentration dependence of the IR rate constant well fit a sigmoidal function, the order of IRI activity was inferred as AFGP>AFP III > AFP I. By thermal treatment, AFP III lost its IRI activity to the greatest degree, AFP I was less affected, and AFGP was almost unaffected. Also heat-treatment was less influence on IS ability of AF(G)Ps.

In Chapter 3, to understand the effect of AF(G)P on the ice recrystallization behavior stored at a temperature range of -7°C to -20°C. Furthermore, the effect of AF(G)Ps on dynamic state of the freeze-concentrated matrix was investigated by the dielectric measurements to study the correlation between water mobility and suppression of IR by the addition of AF(G)P molecules. The holding temperature had a greater influence on the recrystallization of ice crystals in sucrose solutions containing AF(G)Ps. The trend of ice recrystallization rate with the decreasing of incubation temperature was not linear plot which may be related to the freeze-concentration effect or viscosity of the sucrose solution. The recrystallization of ice crystals growth in different AF(G)Ps solutions fellow Arrhenius behavior well in the temperature range of -7°C to -20°C. By electric relaxation spectrum, there was no correlation between water mobility and IRI activity of AF(G)P solutions.

In Chapter 4, to investigate the effect of addition of other proteins on IRI activity, TH activity and IS ability of AF(G)Ps. Four kinds of other proteins, Bovine serum albumin (BSA), albumin (ALB), α-lactalbumin (α-La) and β-lactoglobulin (β-Lg), were used. For the AFGP, 4 types of proteins had minimal impact on its IRI activity. For the type I AFP, the presence of α-la and β-lg is helpful for its IRI activity. As for AFP III, all four kinds of proteins improve AF(G)Ps IRI activity. AFP I had the highest TH activity, and 4 added proteins increase AFP III TH activity. As for AFP III and AFGP, the added other proteins had no significant effect on its TH activity. α-La or β-Lg existed made the ice crystal irregular that formed in 10 μg/mL AFP I solution.

In Chapter 5, the effect of salt (NaCl) addition on the inhibition of recrystallization of ice crystals in 40% sucrose solution containing AF(G)Ps at -10ºC was studied using the method we established. The addition of salt to the 40% sucrose solution accelerated the recrystallization of ice crystals. However, when AFP I and AFP III were added, the salt enhanced the ability of AFPs to inhibit ice crystal recrystallization. And the inhibitory effect was more obvious with the increase of experimental dosage of NaCl. As for AFGP, salt addition did not improve the inhibition of ice crystal recrystallization. Some possible hypotheses have been discussed, that is, how and why the presence of NaCl affects the adsorption of AF(G)Ps on ice crystals planes in the water-ice interface.

In conclusion, we have quantitatively evaluated the recrystallization rate constant of ice crystals in foods and model foods containing several AF(G)Ps using the method we established, and effects of AF(G)P concentration and heat treatment were examined. And the explanation was concluded from the point of protein aggregation and simplicity and flexibility of structure. Furthermore, the effect of addition of other proteins and NaCl on the IRI activity, TH activity and IS ability of AF(G)Ps was carried out and some of the mechanisms causing these effects were also successfully proposed. These findings might facilitate AF(G)P practical application development for sugar products.