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

学位名	博士(海洋科学)
学位授与機関	東京海洋大学
学位授与年度	2021
学位授与番号	12614博甲第620号
URL	http://id.nii.ac.jp/1342/00002409/

[課程博士・論文博士共通]

博士学位論文要約
Summary

専　攻 Major	Applied Marine Biosciences (応用生命科学)	氏　名 Name	馬 慶保 (バ　ケイホウ)
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)		

◇ここから記述してください。(Please write from here.)

The purpose of this study was to investigate the recrystallization behavior of ice crystals in model food systems, sugar-based food with proteins or salt, containing AF(G)Ps. This research revealed the effect of presence of AF(G)Ps on the ice growth behaviors of sucrose solution such as ice recrystallization, ice shaping, and thermal hysteresis. Meanwhile, the effect of other food ingredient such as other proteins and NaCl on antifreeze activity of AF(G)Ps was carried out. And the possible mechanisms causing these effects had also been discussed. This study can help us gain a deeper understanding of the factors and underlying mechanisms that affect the antifreeze activity of AF(G)Ps. The results were expected to facilitate development of AF(G)P applications for food-related industries and for biological cryopreservation.

The ice recrystallization rates of samples at -10 ºC were determined by an equation based on the theory of Ostwald ripening. It was expected that studies of antifreeze properties of AF(G)Ps were applicable for more excellent cryopreservation and extension of shelf life of frozen desserts. Despite the extent of the research, the solvent, incubation temperature, protein sources and the experimental method were not uniform. In addition, there was little studies about the thermostability (95ºC) of AF(G)Ps examined from the point of view of ice recrystallization rate change and ice shaping ability after heat treatment of AF(G)Ps. Therefore, it was necessary to value the capabilities of AFP I, AFP III, and AFGP to inhibit IR in a comparable and unified manner. The concentration dependence of IRI activity of native AF(G)Ps was demonstrated, which can be fitted well with sigmoid function. A fitting procedure, a new index of AF(G)P IRI activity (C_{50}), and the cooperative coefficient a were proposed. The cooperativity of the AF(G)P concentration effect on IRI efficiency for AFGP was the smallest. Based on the data of C_{50}, the order of IRI activity was inferred as AFGP>AFP III > AFP I. After 95 °C heat treatment for 10 min, AFP III lost its ice crystal recrystallization inhibitory activity the most: AFP I was less affected; AFGP was almost entirely unaffected. These different thermal treatment effects might reflect a lower degree of protein aggregation because of hydrophobic interaction after heat-treatment or might reflect the simplicity and flexibility of the higher order structures of AFP I and AFGP by DSF. Another possible reason might be the lower denaturation temperature of AFP III compared with AFP I or AFGP by micro-DSC. Thermal treatment of these AF(G)Ps had negligible influence on its ability of modifying the conformation of ice crystals. There was no correlation between ice shaping ability and IRI activity of type I AFPs, which meant that ice shaping ability was not necessarily strong at the concentration that exhibited high IRI activity.

The destruction of food structure and quality deterioration caused by ice recrystallization has received increasing attention in the frozen food industry. the recrystallization behavior of ice crystals in sucrose solutions containing type I, III AFPs and AFGPs had been studied in the temperature of -7 °C, -10 °C, -15 °C and -20 °C. The isothermal recrystallization rate constant was evaluated using the technique of image analysis by LTS 120 Linkam cold stage based on the Ostwald ripening principle. And the lowest concentration that showed IRI activity was 10, 20 and 0.1 μg/ml for type I, III AFPs and AFGPs compared with 40% sucrose solutions at -10 °C. The temperature had a greater influence on the recrystallization of ice crystals. AF(G)Ps

showed good IRI activity at any incubation temperature. 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 of the sucrose solution. The trend of IR 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 from -7°C to -20°C. The dielectric relaxation of all samples was well fitted well by the two Cole-Cole relaxations which there exist fast (free water) and slow (solute-coupled water) relaxation process. All dielectric relaxation parameters did not show significantly different when adding AF(G)P into sucrose solutions, indicating that similar water mobility in freeze-concentrated phase. This suggested that there was no correlation between water mobility and suppression of IR by the addition of AF(G)P molecules.

Protein was important in human diet every day to nutrient body needs to grow and repair cells and can be found in a wide range of food. Some studies had reported that endogenous hemolymph proteins sufficiently enhance the THA of beetle AFPs. Because milk and eggs are important raw materials for making ice cream, the ingredients in these raw materials may affect the antifreeze activity of AFPs in ice cream, such as protein (BSA, ALB, α-la, β-lg). Therefore, it is necessary to study the influence of these proteins on AFPs. when adding these 4 types of proteins into AF(G)P solutions, for the AFGP, 4 types of proteins have 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, lowest concentration that shows IRI activity drops to 1 and 2 μg/ml, respectively. As for AFP III, all four kinds of proteins improve AF(G)Ps IRI activity, especially α-la and β-lg, the effect is dramatic which lowest concentration that shows IRI activity all drops to 1 μg/ml. As for the reason, there might be two possible mechanisms: AFPs worked by binding to ice crystal surface, Other proteins made AF(G)Ps easier to adsorb to ice crystals planes, then more AF(G)Ps bind to ice surface; another was other proteins formed complexes with AF(G)Ps to cover larger ice crystals planes. AFP I have the highest TH activity, and the activity of the AFP III is lower. For the AFP III, 4 added proteins increase its TH activity. As to the AFP III and AFGP, the added proteins have weak effect on its TH activity. α-La or β-Lg existed will make the ice crystal irregular that formed in 10 μg/mL AFP I solution.

Sodium chloride (NaCl) is added to many frozen foods for the purpose of flavoring the food and improving its shelf life. 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, which was related to its carbohydrate chains and hydrophobic amino acid residue. Some possible hypotheses (hydration of Na^+ or Cl^-; “salting-in” and “salting-out” effect.) 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.