TUMSAT-OACIS Repository - Tokyo

University of Marine Science and Technology

(東京海洋大学)

Recrystallization behavior of ice crystals in sugar-based solutions

メタデータ	言語: English 出版者: 公開日: 2018-01-04 キーワード (Ja): キーワード (En): 作成者: Klinmalai, Phatthranit メールアドレス: 所属:
URL	https://oacis.repo.nii.ac.jp/records/1481

Doctoral Dissertation

RECRYSTALLIZATION BEHAVIOR OF ICE CRYSTALS IN SUGAR-BASED SOLUTIONS

September 2017

Graduate School of Marine Science and Technology

Tokyo University of Marine Science and Technology

Doctoral Course of Applied Marine Biosciences

KLINMALAI PHATTHRANIT

Doctoral Dissertation

RECRYSTALLIZATION BEHAVIOR OF ICE CRYSTALS IN SUGAR-BASED SOLUTIONS

September 2017

Graduate School of Marine Science and Technology

Tokyo University of Marine Science and Technology

Doctoral Course of Applied Marine Biosciences

KLINMALAI PHATTHRANIT

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

専　攻 Major	Applied Marine Biosciences	氏　名 Name	Klinmalai Phatthranit (クリンマライ　パトラニット)
論文題目 Title	Recrystallization behavior of ice crystals in sugar-based solutions (糖系溶液中の氷結晶の再結晶化挙動に関する研究)		

Freezing process is widely used method to preserve food in long term storage. During storage and distribution, an increase in the mean size of ice crystals by recrystallization is a major difficulty leading to quality deterioration such as the damage of cell structure in meat causing drip loss, an icy texture of ice cream. Recrystallization is defined as any change in the size, shape, orientation, or perfection of individual crystals after the completion of solidification. Three recrystallization mechanisms occur in frozen system under constant temperature conditions; accretion, migratory, and isomass recrystallization. Accretion recrystallization is the process by which two or more ice crystals contacting each other merge into one larger crystal by bridging between their surfaces. It is the major recrystallization mechanism affecting early stages of recrystallization. The major recrystallization mechanism at the later process is migratory recrystallization. It is the process by which larger crystals grow in a polycrystal system at the expense of smaller crystals. The water molecules on the surfaces of smaller crystals tend to migrate to the surfaces of larger ice crystals because of their higher specific surface energy. Consequently, the larger ice crystals grow while smaller crystals disappear. Isomass recrystallization is the process by which the rough surface of a single crystal becomes smoother by the surface energy minimization principle. It has been recognized that the recrystallization of ice crystal is an important phenomenon that must be controlled to increase the quality of frozen food. However, only limited number of ice recrystallization rate constant data are available until now and systematic studies of recrystallization of ice crystals have been hardly carried out. Sugar-based solution containing various di- and trisaccharides, polysaccharides and protein can represent simple model food. In this research, the recrystallization of ice crystals in these solutions were investigated to reveal the effect of these component upon recrystallization behavior for systematic understanding of ice recrystallization in frozen foods. Simultaneously, properties of freeze-concentrated matrix were also estimated by dielectric relaxation spectroscopy, NMR, fluorescent microscopy and differential scanning calorimetry (DSC) to understand the mechanism causing the effects. The research was divided in four parts; (1) Correlation between ice recrystallization rate and dielectric relaxation time in the series of saccharide solutions, (2) Ice recrystallization in sucrose, trehalose and raffinose solutions, (3) Ice recrystallization in sucrose solution containing polysaccharide (xanthan gum and locust bean gum), and (4) Ice recrystallization in sucrose solution containing protein (bovine serum albumin; BSA). In general introduction part; the background, purpose, and importance of this research as described above were stated.

In Chapter 1, literature review about water freezing, recrystallization of ice crystals, and the experimental techniques used for estimating molecular mobility was carried out.

In Chapter 2, the dielectric relaxation spectroscopy of series of mono and disaccharide solutions (maltose, sucrose, glucose, and fructose) were investigated and the correlation between the dielectric relaxation time in freeze-concentrated matrix and the ice recrystallization rate constant in these solutions were examined. A broadening of the dielectric relaxation function was described well by double Cole-Cole functions and two dielectric relaxation time were obtained. The fast relaxation time was assigned as that of free water. The difference in ice recrystallization rate can be explained well by the difference of dielectric relaxation time of free water in freeze-concentrated matrix even though the combination effect of solutions such as types of

sugars, temperatures and concentrations. These results suggested that the dielectric relaxation time of free water was a useful parameter to predict and control recrystallization rate of ice crystals.

In Chapter 3, the recrystallization behavior of ice crystals in trehalose and mixture of trehalose and raffinose solutions were investigated at -5, -7, and -10 °C. These saccharides have been expected as an ingredient for frozen food because of their unique characteristics, such as less sweetness, cryoprotective actions, and physiological functions. However, understanding the recrystallization behavior of ice crystals in the presence of these saccharides have not been extensively studied until now. The recrystallization rate constant of trehalose solution tended to be smaller than those of sucrose at the same temperature. The mixture solution of trehalose and raffinose retarded ice recrystallization only at -5 °C and -7 °C. The recrystallization rate constant of ice crystals was correlated well with the dielectric relaxation time of free water in a linear fashion. The correlation between ^{1}H spin-spin relaxation time T_2 of water component in freeze-concentrated matrix and the recrystallization rate constant was also fairly reasonable. These results suggested that difference of recrystallization rate constants among trehalose, sucrose, and mixture of trehalose and raffinose solutions were caused by difference of mobility of water molecules in freeze-concentrated matrix. That is to say, the observed trend of smaller recrystallization rate constants in trehalose solution and mixture solution of trehalose and raffinose were originated from smaller water mobility in freeze-concentrated matrix of these solutions.

Polysaccharides are also important components which are widely used in frozen food especially in ice-cream. However, the knowledge of polysaccharide ability to retard ice recrystallization was not clear until now. In Chapter 4, the recrystallization behavior of ice crystals in sucrose solution containing polysaccharide were investigated by using locust bean gum (LBG) or xanthan gum (XG) as a model polysaccharide. Increasing polysaccharide concentration did not always give smaller recrystallization rate constant of ice crystals. The trend of larger recrystallization rate constant was observed in the sample containing xanthan. The fluorescent microscopy showed that inhomogeneous distribution of xanthan in freeze-concentrated matrix, indicating phase separation by cryogelation during freezing process. From these observation, the following mechanism causing larger recrystallization rate constant may be possible; sucrose molecules were entrapped in the cryogel matrix during initial freezing process. As the result, apparent freezable water content increased and then recrystallization rate constant increased. These mechanisms can be supported by the heterogeneity of water mobility from the shape of dielectric relaxation spectrum curves.

In Chapter 5, the effect of protein addition on the recrystallization rate constant of ice crystals in 20% sucrose solution was investigated by using bovine serum albumin (BSA) as a model protein. Protein is one of important component in food. However, there have been few researches about the ice crystal recrystallization behavior in the presence of protein. Increasing BSA concentration (0 to 6%) reduced freezable water content in the samples. The size of ice crystals decreased with increasing BSA concentration, which can be explained by the reduced freezable water content. However, recrystallization rate constants of all samples were not significantly different. The dielectric relaxation time of free water did not change by increasing BSA concentration from 0 to 4% but in the presence of 6% BSA it took smaller value. This may be caused by loosely bound water in the outer of hydration shell of BSA.

In conclusion, we have demonstrated that the dielectric relaxation measurement was a useful tool to obtain the parameter for predicting and controlling recrystallization rate constant of ice crystals. Furthermore, the effects of existence of basic food components (di and trisaccharides, polysaccharides, and protein) on ice recrystallization rate constant were experimentally revealed and some of the mechanisms causing these effects were also successfully proposed. The results obtained in this study would be helpful to understand, predict and control the recrystallization behavior of ice crystals in frozen foods in a systematic way.

ACKNOWLEDGEMENTS

In the first place, I would like to sincere my grateful gratitude to my thesis advisor Prof. Dr. Tomoaki Hagiwara to give me an opportunity to conduct Ph.D. research, his guidance and encouragement towards the successful completion of my research. It was really a great time working with him. I am truly grateful my research advisor when I was an undergraduate and master student at Kasetsart University, Asst. Prof. Dr. Savitree Ratanasumawong, for her support, knowledge, and uncountable useful suggestions since that time until now. She also gave me opportunity to make some experiments in Japan. It is a good chance to meet Prof. Dr. Tomoaki Hagiwara and continue my Ph.D. study with him.

I would also like to express gratitude my thesis committees, Prof. Dr. Toro Suzuki for his valuable comments and suggestion in fulfillment of Ph.D. study as well as Prof. Dr. Emiko Okazaki and Assoc. Prof. Dr. Mika Fukuoka. Moreover, I also appreciate Prof. Dr. Shingo Matsukawa and Assoc. Prof. Dr. Mika Fukuoka for their teaching and techniques of Nuclear Magnetic Resonance (NMR) measurement. I would like to thank Asst. Prof. Dr. Mario Shibata for his kind support and recommendation in this research. I am also grateful to all the lecturers in bachelor and master studies, department of Food Science and Technology, Kasetsart University, Thailand, for giving me the fundamental knowledge in Food Science and Technology.

I sincerely thank my colleagues at Food Process Engineering laboratory for their kindness, help, support and encouragements as well as Thai friends in Japan and Thailand.

I also appreciate the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government (MEXT) for granting me the Monbukagakusho Scholarship during Ph.D. study.

Finally, I am deeply indebted my parent and younger sister for always supporting my education and being behind me with their unceasing loves, understanding, spirit and unconditional support.

Klinmalai Phatthranit

September 2017

TABLE OF CONTENTS

TABLE OF CONTENTS (Continuted)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

RECRYSTALLIZATION BEHAVIOR OF ICE CRYSTALS IN SUGAR-BASE SOLUTIONS

GENERAL INTRODUCTION

Freezing is regarded as one of widely used method for long term food preservation. Liquid water is converted to ice crystals during freezing process. At the low temperatures used for many frozen food products storage and distribution, an increase in the mean size of ice crystals by recrystallization is a major difficulty leading to quality deterioration such as the damage of cell structure in meat causing from drip loss (Leygonie et al., 2012), an icy texture of ice cream (Miller-Livney et al., 1997; Sutton and Wilcox, 1998; Ndoye and Alvarez, 2015). Recrystallization is defined as any change in the size, shape, orientation, or perfection of individual crystals after the completion of solidification (Fennema, 1973).

Three recrystallization mechanisms occur in frozen system under constant temperature conditions; accretion, migratory, and isomass recrystallization (Donhowe and Hartel, 1996; Goff et al., 1999). Accretion recrystallization is the process by which two or more ice crystals contacting each other merge into one larger crystal by bridging between their surfaces. It is the major recrystallization mechanism affecting early stages of recrystallization. The major recrystallization mechanism at the later process is migratory recrystallization. It is the process by which larger crystals grow in a polycrystal system at the expense of smaller crystals (Pham et al., 1995; Sutton et al., 1996). The water molecules on the surfaces of smaller crystals tend to migrate to the surfaces of larger ice crystals because of their higher specific surface energy. Consequently, the larger ice crystals grow while smaller crystals disappear. Isomass recrystallization is the process by which the rough surface of a single crystal becomes smoother by the surface energy minimization principle (Pham et al., 1995). In addition, temperature fluctuation during storage enhances recrystallization process by melting and refreezing of ice crystals. The melt-refreezing is the primary mechanism of ice recrystallization under temperature changing condition (Goff and Hartel, 2006).

The growth of ice crystals by recrystallization causes quality deterioration. For example, when the ice crystals in ice cream grow so large that they can be detected individually in the mouth, the smooth texture of ice cream is lost, replaced by a coarse and icy texture that degrades the ice cream quality (Leygonie et al., 2012). Therefore, recrystallization is an important phenomenon that must be controlled to increase the quality of frozen food. However, only limited number of ice recrystallization rate constant data are available until now and systematic studies of recrystallization of ice crystals have been hardly carried out.

Sugar solutions and sugar-based solutions containing polysaccharides or proteins can represent simple model food. In this thesis, the recrystallization of ice crystals in these solutions were investigated to reveal the effect of these component upon recrystallization behavior for systematic understanding of ice recrystallization in frozen foods. Simultaneously, properties of freeze-concentrated matrix were also estimated by dielectric relaxation spectroscopy, NMR, fluorescent microscopy and differential scanning calorimetry (DSC) to understand the mechanism causing the effects. Saccharides, polysaccharides and protein are not only used in food industry but also pharmaceutical and biomedical industries. Therefore, the results obtained in this thesis would be also helpful for these industries.

The research was divided in four parts; (1) Correlation between ice recrystallization rate and dielectric relaxation time in the series of saccharide solutions, (2) Ice recrystallization in sucrose, trehalose and raffinose solutions, (3) Ice recrystallization in sucrose solution containing polysaccharide (xanthan gum and locust bean gum), and (4) Ice recrystallization in sucrose solution containing protein (bovine serum albumin; BSA).

In Chapter 1, literature review about water freezing, recrystallization of ice crystals, and the experimental techniques used for estimating molecular mobility was carried out.

In Chapter 2, the dielectric relaxation spectroscopy of series of mono and disaccharide solutions (maltose, sucrose, glucose, and fructose) were investigated and the correlation between the dielectric relaxation time in freeze-concentrated matrix and the ice recrystallization rate constant in these solutions were examined.

In Chapter 3, the recrystallization behavior of ice crystals in trehalose and mixture of trehalose and raffinose solutions were investigated at -5, -7, and -10 ^{o}C by comparing with sucrose solution. Simultaneous estimation of water mobility in the freeze-concentrated matrix was conducted using the ^{1}H spin–spin relaxation time and dielectric relaxation spectroscopy to investigate the mechanism causing the difference of ice crystal recrystallization behaviors among the samples.

Polysaccharides are also important components which are widely used in frozen food. In Chapter 4, the recrystallization behavior of ice crystals in sucrose solutions containing locust bean gum and xanthan gum as a model polysaccharide were investigated. The effects of these polysaccharides on ice recrystallization behavior were discussed by considering the experimental results of water mobility, rheological properties, and microstructure of freeze-concentrated matrix.

Protein is one of important component in food. However, there have been few researches about the ice crystal recrystallization behavior in the presence of protein. In Chapter 5, the effect of protein addition on the recrystallization rate constant of ice crystals in sucrose solution was investigated by using bovine serum albumin (BSA) as a model protein. Simultaneously, properties of freeze-concentrated matrix were also estimated by dielectric relaxation spectroscopy and differential scanning calorimetry (DSC) in order to discuss the mechanism of effects of protein addition on ice recrystallization behavior.

CHAPTER 1

LITERATURE REVIEW

1.1 Freezing Processes

1.1.1 Freezing of water

Water is a major component of foods and biological specimens. It affects food quality and shelf life stability. The chemical formula of water is H_2O with covalent bond (Figure 1). Water can interact not only with other polar molecules but also the other polarity of water molecules. The bond attraction between one of the positive hydrogen atoms of water molecules and negative oxygen atom of another molecule is a hydrogen bond.

To understand the behavior of frozen systems, equilibrium phase diagram of water is useful (Figure 2). Freezing can be described by the removal of heat accompanied with a phase change of liquid water to ice. In the phase diagram, pure liquid water can turn into ice at 0 ^{o}C. Freezing point can define when solid (ice) and liquid (water) phase are in equilibrium. The free energies of two phases (solid and liquid) at that temperature are equal.

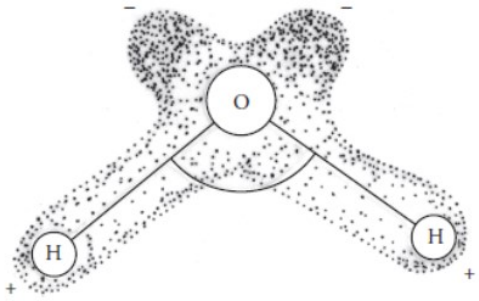


Figure 1 Water molecule (Zaritzky, 2012)

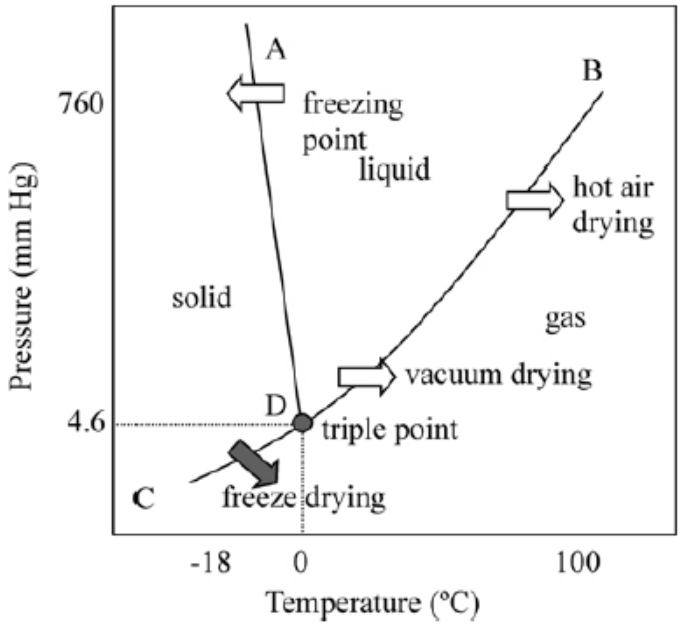


Figure 2 Equilibrium phase diagram of water (Welti-Chanes et al., 2004).

1.1.2 Ice content, freezable water and unfreezable water

Changing in phase of water from liquid to ice results in the thermophysical property changes of foods. The water in frozen food can take different state. It is composed of freezable water, unfreezable water, and ice (Rahman, 2009; Wang and Weller, 2012).

Total water in frozen food = Freezable water + Unfreezable water + ice

Total liquid water in frozen food = Freezable water + Unfreezable water

Unfreezable water is defined as the water that cannot form ice although at low temperature such as -40 ºC or below temperature (Wang and Weller, 2012; Rahman, 2015).

1.1.3 Nucleation and ice crystal growth

Generally, the freezing process includes two main stages namely nucleation (beginning of ice formation) and crystal growth (increasing in ice crystal size) (Zaritzky, 2012).

The interaction between these two stages determines the characteristics of ice crystals such as size, distribution, and morphology of ice crystals (Kiani and Sun, 2011).

1.1.3.1 Nucleation

Nucleation refers to the process by which the combination of molecules is formed with the minimum size. It is called nucleus or seed of crystals. Nucleation is necessary for freezing. Nucleation of pure water needs cooling the temperature below 0 ºC, supercooling (Zaritzky et al., 2000). The latent heat of ice crystals are released leading to aggregation of molecules and then resulting in the particle of sufficient size for further ice crystal growth. Homogeneous nucleation occurs in water free from all impurities. Heterogeneous nucleation (catalytic nucleation) takes place when water molecules aggregate in a crystalline arrangement on nucleating agents such as active surfaces. It is a predominant nucleation process in most of food systems (Zaritzky, 2012). Homogeneous nucleation requires a higher supercooling than heterogeneous nucleation.

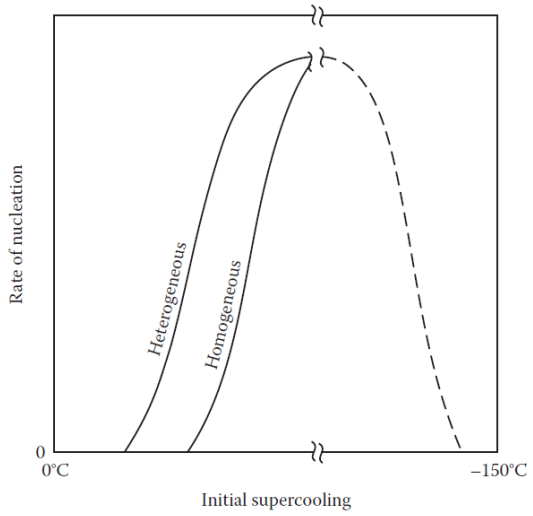


Figure 3 Effect of initial supercooling on homogeneous and heterogeneous rates of ice nucleation (Zaritzky, 2012).

1.1.3.2 Ice crystal growth

After the formation of nuclei, ice crystal growth occurs. Diffusion rate of water to growing ice crystals, the rate of reaction at the ice crystal surface, and the rate of heat removal influence on the ice crystal growth (George, 1997). Water molecules must diffuse to the surface of the growing ice crystal, and at the same time solute molecules must diffuse away from the surface of the growing crystal. When the freezing occurs rapidly, the propagation of ice crystal is insufficient, leading to decreasing in size accompanied with increasing in the number of ice crystals. On the contrary, slowing freezing rate gives larger crystal size and smaller number of crystals as shown in Figure 4.

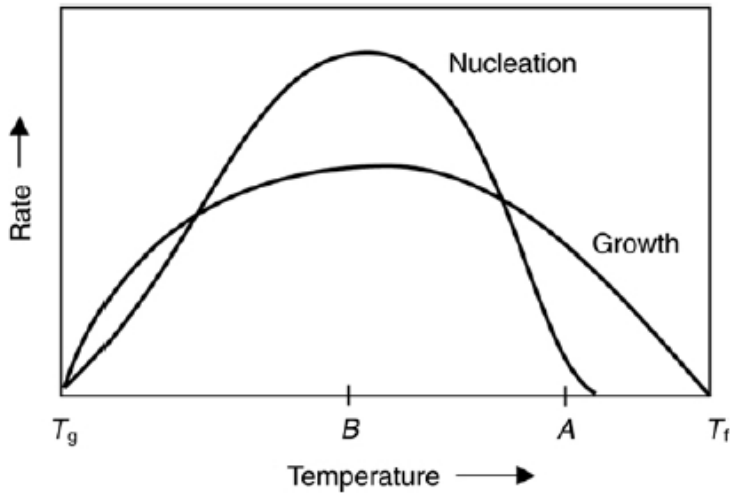


Figure 4 Plots between rates of nucleation and growth with decreasing temperature (Hartel, 1996)

1.2 Ice recrystallization process

Recrystallization is defined as any change in the size, shape, orientation, or perfection of individual crystals after the completion of solidification (Fennema, 1973). In frozen aqueous solutions, recrystallization is usually observed as increasing the mean ice crystals size with time. There are three types of recrystallization under constant temperature; Migratory recrystallization, isomass recrystallization, and accretion recrystallization (Donhowe and

Hartel, 1996; Goff et al., 1999). Moreover, when temperature fluctuation during storage occurs, recrystallization process is enhanced by melt-refreeze recrystallization (Hartel, 2001; Goff and Hartel, 2006; Zaritzky, 2012).

1.2.1. Migratory recrystallization (Ostwald ripening)

Migratory recrystallization, which is also called Ostwald ripening, is caused by the difference in surface energy between ice crystals with different size (Zaritzky, 2012). The water molecules on the surfaces of smaller crystals tend to migrate to the surfaces of larger ice crystals because of higher surface energy. Consequently, the larger ice crystals grow while smaller ice crystals disappear (Pham and Mawson, 1995; Sutton et al., 1996).

1.2.2. Isomass recrystallization

Similar process as migratory recrystallization can occur within single crystal. It is called isomass recrystallization. It is the process by which the rough surface of a single crystal becomes smoother by the surface energy minimization principle (Pham and Mawson, 1995).

1.2.3. Accretion recrystallization

Accretion recrystallization is the process by which two or more ice crystals contacting each other merge into one larger crystal by bridging between their surfaces. It is the major recrystallization mechanism in early stages of ice recrystallization.

1.2.4. Melt-refreeze recrystallization

Melt-refreeze recrystallization is the primary mechanism of ice recrystallization under temperature changing conditions. Temperature fluctuations are often unavoidable during storage and distribution, inducing melting and refreezing behaviors of ice (Zaritzky, 2012). This process is more significant for ice cream texture/shelf-life in practical situation than other ice recrystallization processes at isothermal temperature (Hartel, 2001).

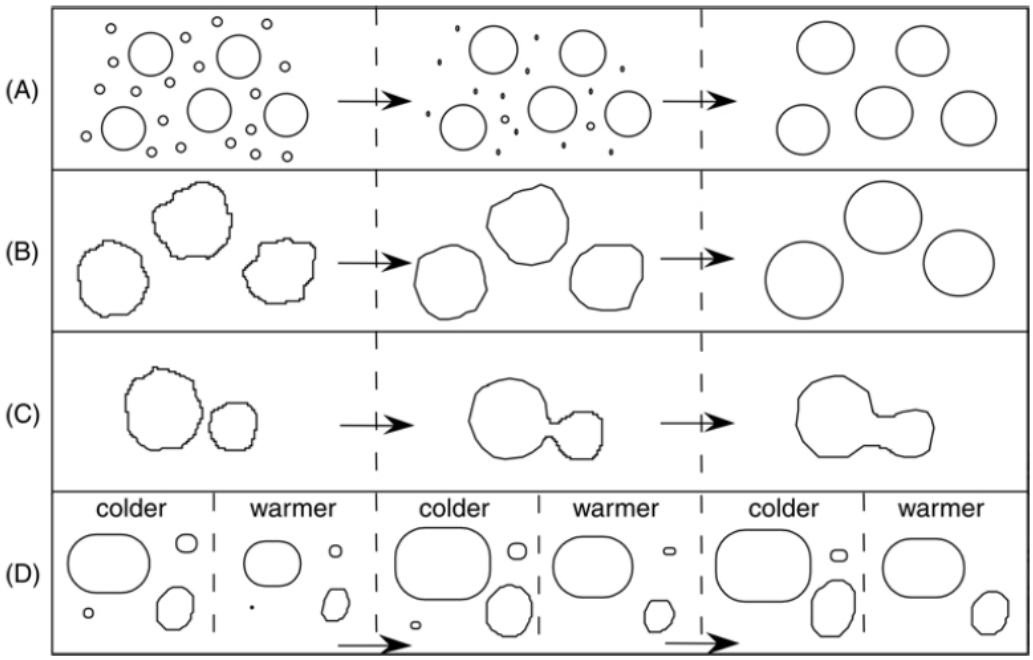


Figure 5 Mechanism of ice recrystallization (A) Migratory recrystallization (Ostwald ripening) (B) Isomass recrystallization (C) Accretion recrystallization (D) Melt-refreeze recrystallization (Goff and Hartel, 2006)

1.3 Recrystallization model equation

The Lifshitz–Slyozov–Wagner (LSW) theory of Ostwald ripening principle (Lifshitz and Slyozov, 1961; Wagner, 1961) has been applied to ice recrystallization studies in frozen food. According to this theory, recrystallization process can be described by the following equation (Sutton et al., 1996; Hagiwara et al., 2006; Budke et al., 2009; Klinmalai et al., 2017)

$$r^3=r_0^3+kt$$

where r stands for the number-based mean crystal radius, r_0 signifies the number-based mean crystal radius at time t=0, and k denotes the isothermal recrystallization rate constant. The value of k is evaluated from the slope of the plot of cube of r versus time t.

1.4 Phase/state behavior in frozen food

Freezing process causes phase transitions of water and, in some case, solute components. The phase transition in freezing process involves the changing of water from liquid to ice and is the key step in determining the efficiency of the process and quality of the frozen foods.

1.4.1 Freezing profile

The time-temperature relationship during freezing process is called freezing profile or freezing curve. In Figure 6, schematic freezing profiles of pure water and aqueous solution are shown. As for pure water, temperature is decreased from point A to S by removing the sensible heat. Note that the temperature is cooled below 0 ºC; supercooling state. When the critical of nuclei is reached, the nucleation occurs at point S and the latent heat is removed by the system surrounding the nuclei. When cooling rate of whole system is not enough to compensate the latent heat, the temperature rise to the equilibrium freezing temperature (point B). Ice crystals continue to grow without changing the temperature (0 ºC) until completion of crystallization (point C). The time from initial ice nucleation (point B) to the complete removal of latent heat (point C) is sometimes defined as freezing time. After crystallization is completed, water temperature decreased to point D as sensible heat is released.

Aqueous solution contains both water and solutes. When it is frozen, similar processes occur as pure water; supercooling, release of latent heat and simultaneous raise of temperature (point S to point B). Freezing point of aqueous solution (point B) or food system are below 0 ºC due to the freezing point depression. The increasing solute concentration of unfrozen phase leads to a continuously decreasing freezing point of solutions (Zaritzky, 2012) as water forms ice. Solute concentration increases during the freezing process and eventually reaches its eutectic temperature. Solute supersaturation is indicated by point C. Latent heat of solute crystallization is released at point C, causing a slight increase in temperature from point C to point D. At point D, the solution assumes the eutectic equilibrium composition that remains constant during eutectic solidification and constant temperature (D–E). From point E to F, the further cooling to complete crystallized or solidified at very low temperature occurs.

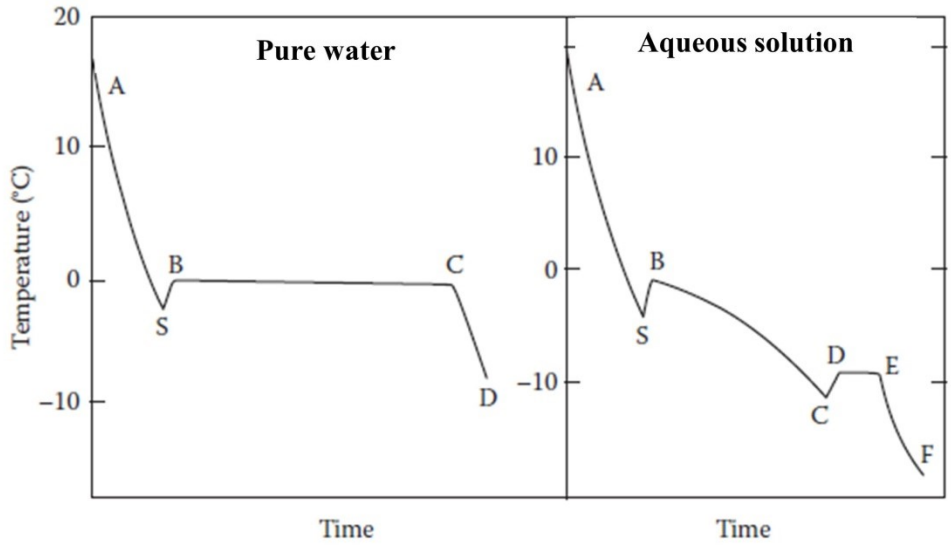


Figure 6 Typical time-temperature relationships during the freezing of pure water and aqueous solution (Zaritzky, 2012)

1.4.2 Freeze-concentration in frozen food

Freeze-concentration is the term to describe the solute redistribution in an aqueous solution and based on the solidification phenomena of water by using freezing temperature - solute concentration diagram (Welti-Chanes et al., 2004) as shown in Figure 7. During ice crystal formation, solutes are rejected by ice crystals which is formed of pure water. The concentrated solution is physically separated by forming ice crystals during freeze-concentration. The concentration of solute increases during the freeze-concentration process. Sample solution can freeze when the temperature of solution is lower below its freezing point. The freezing curve corresponds to the equilibrium between ice crystals and solution. Freezing point depression occur because the solute molecules interact with water and inhibit the ability of water molecules to come together and form ice crystal. The amount of water frozen can be calculated from fitting equation of freezing point depression curve. Moreover, the extend of freezing point depression is based on the size and number of solute molecules such as fructose has a lower freezing point than sucrose at the same concentration because it has a lower molecular weight than sucrose. However, the freezing points of difference saccharides having

same molecular weight at same concentration can take different freezing points, especially at high concentration. (CRC Handbook of Chemistry and Physics 90th Edition, 2009).

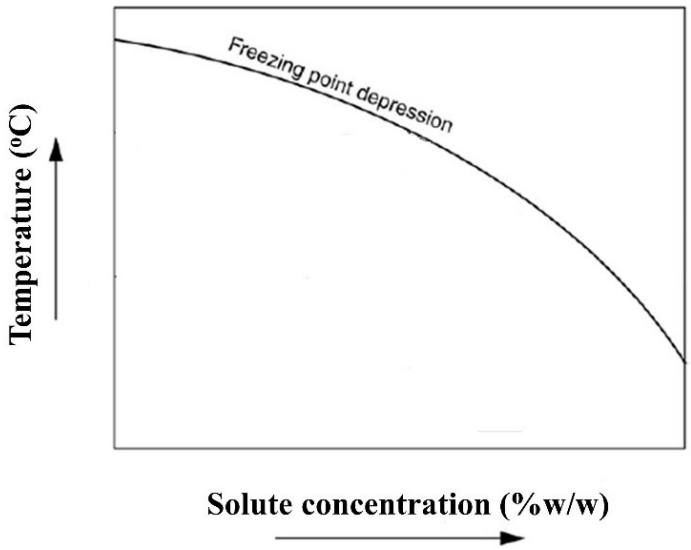


Figure 7 Typical freezing point depression curve

1.5 Physical deterioration in frozen food

At the low temperatures used for many frozen food products storage and distribution, the increase of average ice crystal size by recrystallization can cause physical damage on foods, leading to quality loss such as the damage of cell structure in meat causing drip loss (Leygonie et al., 2012) and an icy texture in ice cream (Miller-Livney and Hartel., 1997; Sutton and Wilcox, 1998; Ndoye and Alvarez, 2015). In order to increase the quality of frozen food, recrystallization process must be controlled. However, only limited number of studies about ice recrystallization in frozen foods have been carried out.

1.6 Ice recrystallization in saccharides system

Hagiwara and Hartel (1996) reported a direct relation between ice recrystallization rate and the freezing point or amount of frozen water in ice cream with different formulations. It was also demonstrated that isothermal ice recrystallization rates in the series of frozen sugar

solutions are strongly correlated with the molecular mobility of water in the freeze-concentrated matrix such as the translational diffusion coefficient (Hagiwara et al., 2006) and ^{1}H spin-spin relaxation time (T_2) (Ablett et al., 2002).

Sutton et al. (1996) revealed ice recrystallization in aqueous fructose solutions at -10 ºC, -15 ºC, and -20 ºC. They reported that ice recrystallization rate depended on the ice phase volume presented in the system. Ice recrystallization rate decreased when ice phase volume decreased. Moreover, decreasing temperature can help decrease ice recrystallization rate.

Whelan et al. (2008) investigated the effects of replacing sucrose with trehalose on the recrystallization of ice crystal in ice cream when it was stored in a heating and cooling cycle condition. Results showed that trehalose cannot retard ice crystal growth in ice cream mixes.

1.7 Ice recrystallization in polysaccharides system

Polysaccharides have no significant effect on freezing point. (Buyong and Fennema, 1988; Herrera et al., 2007), especially in dilute solution (Miller-livney and Hartel, 1997).

Harper and Shoemaker (1983) studied effect of locust bean gum on ice recrystallization rates in sweetener solutions during fluctuating temperature of -9 ºC to -23 ºC. Locust bean gum in the concentration range 0-0.5% did not retard ice recrystallization rate even though the viscosity of these samples increased.

Buyong and Fennema (1988) studied the influence of hydrocolloids (gelatin, guar gum, locust bean gum, and carboxymethyl cellulose (CMC)) on the amount and ice crystals in frozen hydrocolloid-water solutions. The differential scanning calorimetry (DSC) analysis revealed that hydrocolloids (2% (w/w) or less) did not contribute to to a reduction in the amount of freezable water. As for ice recrystallization, ice-cream mix with and without gelatin were investigated. Gelatin did not effect on the amount of ice and ice recrystallization rate in ice cream mix during storage at -15 ºC.

Miller-livney and Hartel (1996) studied the effect of sweetener (20 DE corn syrup solids, 42 DE corn syrup solids, sucrose, and high fructose corn syrup) and stabilizer types (gelatin, locust bean gum, xanthan gum, and carrageenan) on ice recrystallization rate in ice cream during storage at -5.2 ºC, -9.5 ºC, and -15 ºC. Different combination of solutes result in the difference of ice recrystallization rate. The combination effects of sweeteners, stabilizer types, storage temperature on ice recrystallization were complex and there was no systematic trend observed.

Sutton et al. (1997) studied the influence of the range of galactomannan (locust bean gum and guar gum) on ice recrystallization rate in fructose solution. The reduction of ice recrystallization rate was not related well with the viscosity of solution. Both guar gum and LBG retarded ice recrystallization at lower concentration. But increasing the concentration to a certain value resulted in no further reduction. It was suggested that this was caused by phase separation and heterogeneity in freeze-concentrated matrix.

Goff et al. (1999) visualized the hydrocolloids (locust bean gum and guar gum) structure in frozen sucrose solution with fluorescence microscope. They used the hydrocolloids labelled by rhodamine isothiocyanate for the visualization. Gel-like networks of LBG was observed around ice crystals after temperature cycling. They reported that LBG was more effective to retard ice recrystallization rate than guar gum.

Regand and Goff (2003) studied the effect of stabilizers on the structure and ice recrystallization in frozen sucrose solution at fluctuating temperature. Xanthan gum can retard ice recrystallization. It was suggested that the steric blocking of ice interface or high viscosity of unfrozen phase during freezing-thawing cycles contributed to the reduction of recrystallization. Gel-like network was observed in the solution containing locust bean gum (LBG). Ice recrystallization rate of LBG was not different from sucrose solution as a control sample.

1.8 Ice recrystallization in proteins system

1.8.1 Antifreeze protein (AFP)

Antifreeze proteins can retard ice recrystallization by their ability to adsorb on to the surface of ice. They attach the growing ice front and bind to specific planes in the crystal structure. Therefore, the growth of this ice front is retarded (Kennedy, 2000).

1.8.2 Whey protein

Chun et al. (2012) studied effect of whey protein isolate in the concentration of 0.2%, 1%, 3%, and 5% (w/w) on ice recrystallization in 40% (w/w) sucrose solutions at -6 ^{o}C. The ice crystal shapes were round shape in every concentration. Increasing the concentration of whey protein isolate, the mean size of ice crystals decreased.

1.9 Molecular dynamic analysis

During freezing process, the temperature is lowered, leading to a reduction in the mobility of the liquid phase.

1.9.1 ^{1}H spin-spin relaxation time (T_2)

Nuclear magnetic resonance (NMR) is a powerful technique that can detect the molecular mobility of the components in system by analyzing the relaxation behavior of nuclides (such as proton) and can be used to measure their rotational mobility. The proton spin-spin relaxation time (T_2) is the index of the mobility of mobile protons (Kumagai and Kumagai, 2002).

The magnetic field is generated when the electric current is made to flow in a loop by electron; the electron circulates around the proton such as hydrogen atom (Figure 8A). Moreover, magnetism can also come from spin magnetism of electrons and nuclei, which is not due to a circulating current as shown in Figure 8B. Electron and nuclear magnetic moments

contribute a positive value (Levitt, 2008). They have a spin angular momentum. The angular momentum of a rotating object is a vector and indicates the axis of the rotational motion. The field B is called magnetic field and interact with magnetic moment like a nuclear spin.

If the external magnetic field is present, the spin polarization vectors steadily precess around the magnetic field. The applied frequency pulse in the system is proportional to the magnetic field. As for T_2 spin-spin relaxation, the nuclear spin magnetization along the perpendicular of magnetic field is measured. Thus, T_2 relaxation process occurs in the x-y plane (Figure 9). This net magnetic moment perpendicular to the magnetic field is called transverse magnetization.

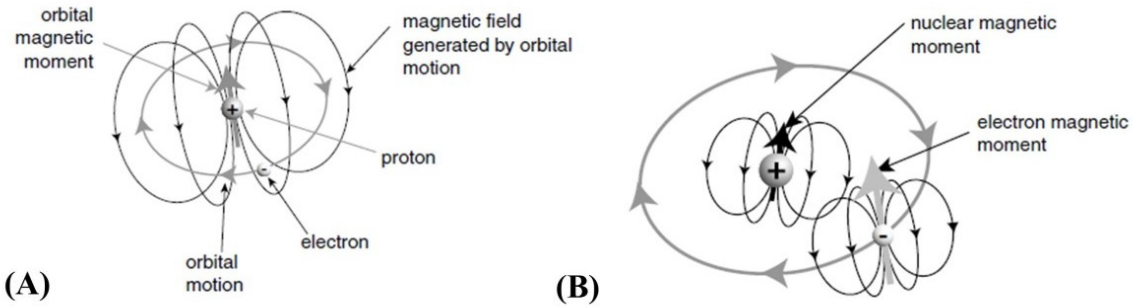


Figure 8 Schematic diagram of (A) orbital magnetism of the electron in a hydrogen atom and (B) electron and proton spin magnetism in a hydrogen atom (Adapted from Levitt, 2008)

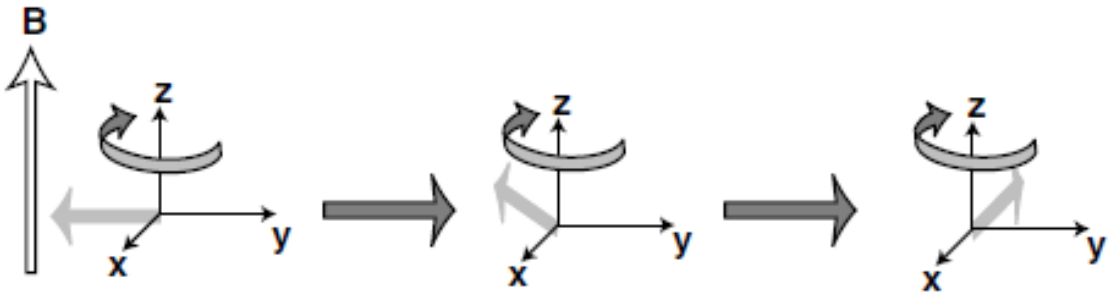


Figure 9 Precession of the transverse magnetization (Levitt, 2008).

The method based on Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion can remove the effect of magnetic gradient on the decay of NMR data, generally CPMG is applied for measurement of T_2 relaxation time (Ronczka and Müller-Petke, 2012; Bakhmutov, 2015). The CPMG sequence applies not only radio frequency pulse but also 90 degree pulse and 180 degree pulse in the system. Each time a pulse is applied the signal decay to the magnetic field is removed and a single data point is obtained. Hence, the decay of the data reflects the sample properties in the exponential manner (Figure 10). The decay constant of the exponential is called the spin-spin relaxation time. Longer T_2 values of protons means larger mobility of proton.

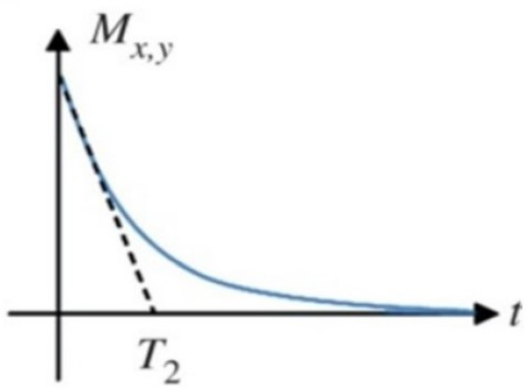


Figure 10 Evolution as a function of time of $M_{x,y}$ with represent T_2 as time constant derived from the initial slope (dashed lines) after removing of the magnetic field (Adapted from Lacroix et al., 2013)

1.9.2 Dielectric relaxation spectroscopy

Dielectric relaxation spectroscopy is one of the effective methods to study dynamics properties of solutions. Molecular or electrolyte (ion) motions in a system are reflected by electrical properties and directly influence on dielectric relaxation processes. Relaxation phenomena are related to molecular fluctuations of dipoles belonging to molecules or parts of them. Moreover, the movement of mobile charge carriers (electrons, ions or charged defects) causes conductive contributions to the dielectric response (Schönhals and Kremer, 2003). Time dependent dielectric function can be measured directly as the time dependent

response caused by a step-like change of electrical field as shown in Figure 11. The complex dielectric function can be measured in the large frequency range (10^{-6}-10^{12} Hz). But in general, only single measurement system cannot access this wide frequency range. In order to cover this frequency range, several methods are employed. Dielectric relaxation is defined as the dielectric constant decreases and dielectric loss shows a peak; they are caused by the delay in dipole moments with increasing frequency (Figure 12).

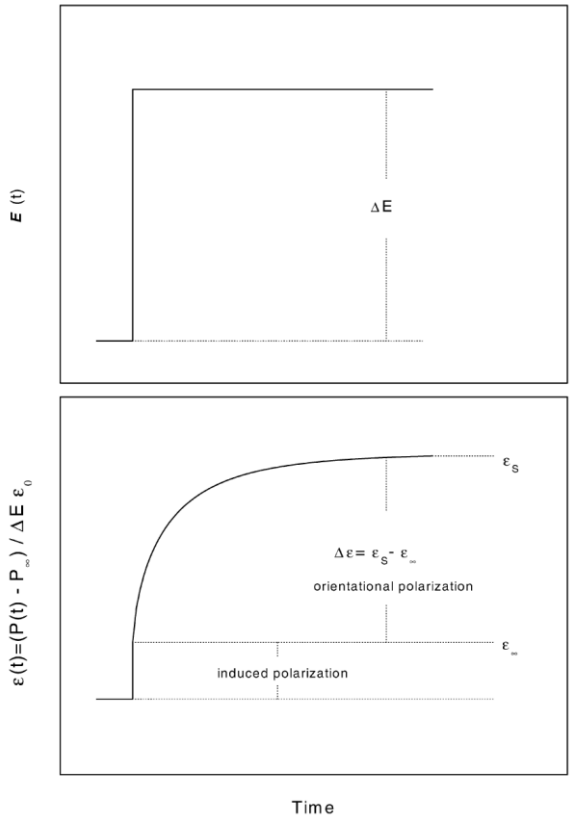


Figure 11 Schematic relationships between the time dependence of the electric field (ΔE), the polarization (P) and time dependent relaxation function (ε (t)) (Schönhals and Kremer, 2003)

Time domain reflectometry (TDR) method is a technique of measuring complex permittivity over a high frequency region. It is a powerful method for determining the dielectric permittivity in the frequency range 10^6-10^{10} Hz. (Kremer and Schönhals, 2003). Since the relaxation process of water molecules is observed around GHz region (Mashimo et al., 1987)

at ambient temperature, TDR can access the dielectric relaxation process of water molecules. The dielectric relaxation process from reorientation of water molecules is described well by the Cole-Cole equation (Shinyashiki et al., 1994).

The dielectric relaxation process can be related to various different processes: (1) microscopic fluctuations of molecular dipoles (rotational diffusion), (2) the propagation of mobile charge carriers (translational diffusion of electrons, cavity, or ions), and (3) the separation of charges at interfaces which gives rise to an additional polarization. The information about molecular dynamics can be obtained by analyzing the dielectric function. Dielectric constant (ε') and dielectric loss (ε") are most often obtained by direct analysis. If the frequency of the applied outer electric field corresponds to reorientation time of molecular dipoles (relaxation time), the complex dielectric function shows a characteristic behaviors (Figure 12). The real part or dielectric constant (ε') is mainly associated with the effects of molecular polarization, and the imaginary part or dielectric loss (ε") represents the loss of energy necessary to force the polarization and conduction state. The phenomena of dielectric relaxation can be defined as dielectric constant (ε′) decreases and the dielectric loss (ε") shows a peak with increasing frequency due to the delay in dipole moments (Kumagai and Kumagai, 2002; Schönhals and Kremer, 2003). In some case, conductivity phenomena is shown as an increase of imaginary part of the dielectric function with decreasing frequency (Schönhals and Kremer, 2003). From dielectric relaxation process, dielectric relaxation time (τ) is obtained. It is a parameter reflecting molecular mobility, corresponds to the time needed for electric dipoles to orient in the direction of an electric field (Kumagai et al., 2000). It can be obtained from the peak position in the imaginary part after fitting the model functions.

Consequently, both nuclear magnetic resonance (NMR) and dielectric relaxation techniques are useful methods to evaluate molecular motions; NMR provides information about the mobility of protons of molecule in sample while dielectric relaxation time (τ) reflects molecular motions in the system.

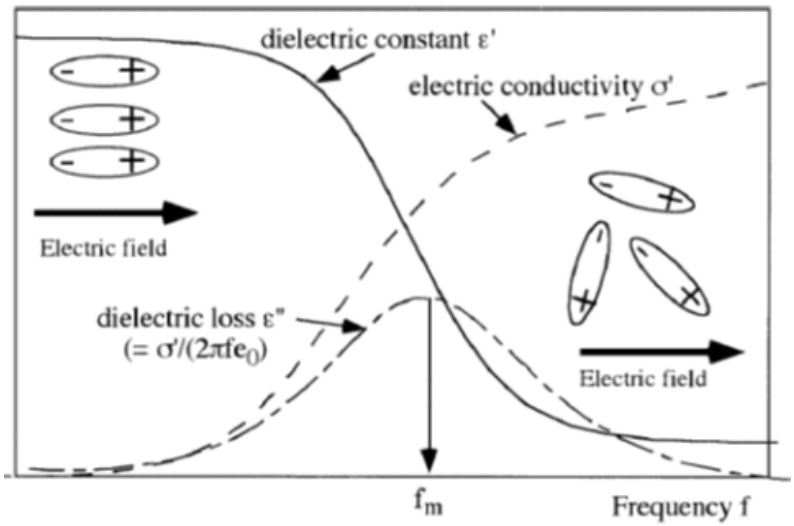


Figure 12 Dielectric constant (ε') and dielectric loss (ε") of complex dielectric function when applied outer electric field (Kumagai and Kumagai, 2002)

References

Ablett, S., Clarke, C. J., Izzard, M. J., & Martin, D. R. (2002). Relationship between ice recrystallization rates and the glass transition in frozen sugar solutions. *Journal of the Science of Food and Agriculture, 82,* 1855-1859.

Budke, C., Heggemann, C., Koch, M., Sewald, N., & Koop, T. (2009). Ice recrystallization kinetics in the presence of synthetic antifreeze glycoprotein analogues using the framework of LSW theory. *The Journal of Physical Chemistry B, 113,* 2865-2873.

Buyong, N., & Fennema, O. (1988). Amount and size of ice crystals in frozen samples as influenced by hydrocolloids. *Journal of Dairy Science, 71,* 2630-2639.

Chun, J. Y., Kim, J. I., & Min, S. G. (2012). Effect of whey protein isolate on ice recrystallization characteristics in whey protein isolate/k-carrageenan matrix. *Korean Journal for Food Science of Animal Resources, 32,* 627-634.

Donhowe, D. P., & Hartel, R. W. (1996). Recrystallization of ice in ice cream during controlled accelerated storage. *International Dairy Journal, 6,* 1191-1208.

Fennema, O. R. (1973). Nature of freezing process. In Fennema, O. R., Powrie, W. D., Marth, E. H. (Eds.), Low-temperature preservation of foods and living matter (p. 151). (1st ed.). New York: Marcel Dekker.

George, R. M. (1997). Freezing systems. In Erickson, M. C., Hung, Y. C. (Eds.), Quality in frozen food (1st ed.). Dordrecht: Springer Science+Business Media B.V..

Goff, H. D., Ferdinando, D., & Schorsch, C. (1999). Fluorescence microscopy to study galactomannan structure in frozen sucrose and milk protein solutions. *Food Hydrocolloids, 13,* 353-362.

Goff, H. D., & Hartel, R. W. (2006). Ice cream and frozen desserts. In Hui, Y.H. (Eds.), Handbook of food science, technology, and engineering (1st ed.). Boca Raton: CRC Press.

Hagiwara, T., & Hartel, R. W. (1996). Effect of sweetener, stabilizaer, and storage temperature on ice recrystallization in ice cream. *Journal of Dairy Science, 79,* 735-744.

Hagiwara, T., Hartel, R. W., & Matsukawa, S. (2006). Relationship between recrystallization rate of ice crystals in sugar solutions and water mobility in freeze-concentrated matrix. *Food Biophysics, 1,* 74-82.

Harper, E. K., & Shoemaker, C. F. (1983). Effect of locust bean gum and sweetening agents on ice recrystallization rate. *Journal of Food Science, 48,* 1801-1806.

Hartel, R. W. (1996). Ice crystallization during the manufacture of ice cream. *Trends in Food Science and Technology, 7,* 315-321.

Hartel, R. W. (2001). In Crystallization in Foods (pp. 284-308). (1st ed.). Gaithersburg: Aspen Publisher.

Herrera, M. L., M'Cann, J. I., Ferrero, C., Hagiwara, T., Zaritzky, N. E., & Hartel, R. W. (2007). Thermal, mechanical, and molecular relaxation properties of frozen sucrose and fructose solutions containing hydrocolloids. *Food Biophysics, 2,* 20-28.

Kennedy, C. J. (2000). Future trends in frozen foods. In C. J. Kennedy (Eds.), Managing frozen foods (pp. 263-278). (1st ed.). Cambridge: CRC Press.

Kiani, H., & Sun, D. W. (2011). Water crystallization and its importance to freezing of foods: a review. *Trends in Food Science & Technology, 22,* 407-426.

Klinmalai, P., Shibata, M., & Hagiwara, T. (2017). Recrystallization of ice crystals in trehalose solution at isothermal condition. *Food Biophysics, https://doi.org/10.1007/s11483-017-9496-1*.

Kremer, E., & Schönhals, A. (2003). Broadband dielectric measurement techniques (10^{-6}Hz-10^{12}Hz). In Kremer, F., Schonhals, A. (Eds.), Broadband dielectric spectroscopy (pp. 35-56). (1st ed.). New York: Springer-Verlag.

Kumagi, H., Sugiyama, T., Iwamoto, S. (2000). Effect of water content on dielectric relaxation of gelatin in a glassy state. *Journal of Agricultural and Food Chemistry, 48,* 2260-2265.

Kumagai, H., & Kumagai, H. (2002). Review analysis of molecular or ion mobility in glassy and rubbery foods by electric and proton-NMR measurements. *Food Science and Technology Research, 8,* 95-105.

Lacroix, L. M., Delpech, F., Nayral, C., Lachaize, S., & Chaudret, B. (2013). New generation of magnetic and luminescent nanoparticles for *in vivo* real-time imaging. *Interface Focus, 3, 20120103.*

Levitt, M. H. (2008). *Spin dynamics: basics of nuclear magnetics resonance*. (2nd ed.). Chichester: John Wiley & Sons, (Part 2).

Leygonie, C., Britz, T. J., & Hoffman, C. (2012). Impact of freezing and thawing on the quality of meat: review. *Meat Science, 91,* 93-98.

Lide, D.R. (2003). CRC Handbook of Chemistry, Physics, 84th Ed. (pp. 8-81). Boca: CRC Press.

Lifshitz, I. M., & Slyozov, V. V. (1961). The kinetics of precipitation from supersaturated solid solutions. *Journal of Physics and Chemistry of Solids, 19,* 35–50.

Mashimo, S., Umehara, T., Ota, T., Kuwabara, S., Shinyashiki, N., & Yagihara, S. (1987). Evaluation of complex permittivity of aqueous solution by time domain reflectometry. *Journal of Molecular Liquids, 36,* 135-151.

Miller-Livney, T., & Hartel, R. W. (1997). Ice recrystallization in ice cream: interactions between sweeteners and stabilizers. *Journal of Dairy Science, 80,* 447-456.

Ndoye, F. T., & Alvarez, G. (2015). Characterization of ice recrystallization in ice cream during storage using the focused beam reflectance measurement. *Journal of Food Engineering, 148,* 24-34.

Pham, Q. T., & Mawson, R. F. (1995). Moisture migration and ice recrystallization in frozen foods. In Erickson, M. C., Hung, Y. C. (Eds.), Quality in frozen food (pp. 67-91). (1st ed.). Dordrecht: Springer Science + Business Media.

Rahman, M. S. (2009). Prediction of ice content in frozen foods. In Rahman, M. S. (Eds.), Food properties handbook (pp.193-204). (2nd ed.). New York: CRC Press.

Rahman, M. S. (2015). Thermal properties measurement of foods. In Nollet, L. M. L., Toldrá, F. (Eds.), Handbook of Food Analysis (p. 44). (3rd ed.). New York: CRC Press.

Regand, A., & Goff, H. D. (2003). Structure and ice recrystallization in frozen stabilized ice cream model systems. *Food Hydrocolloids, 17,* 95-102.

Ronczka, M., & Müller-Petke, M. (2012). Optinization of CPMG sequences to measure NMR transverse relaxation time T_2 in borehole applications. *Geoscientific Instrumentation, Methods and Data Systems, 1,* 197-208.

Schönhals, A., & Kremer, F. (2003). Theory of dielectric relaxation. In Kremer, F., Schonhals, A. (Eds.), Broadband dielectric spectroscopy (pp. 1-33). (1st ed.). New York: Springer-Verlag.

Schönhals, A., & Kremer, F. (2003). Analysis of dielectric spectra. In Kremer, F., Schonhals, A. (Eds.), Broadband dielectric spectroscopy (pp. 59-98). (1st ed.). New York: Springer-Verlag.

Sutton, R. L., Lips, A., Piccirillo, G., & Sztehlo, A. (1996). Kinetics of ice recrystallization in aqueous fructose solutions. *Journal of Food Science, 61,* 741-745.

Sutton, R. L., Cooke, D., & Russell, A. (1997). Recrystallization in sugar/stabilizer solutions as affected by molecular structure. *Journal of Food Science, 62,* 1145-1149.

Sutton, R. L., & Wilcox, J. (1998). Recrystallization in Ice Cream as Affected by Stabilizers. *Journal of Food Science, 63,* 104-107.

Shinyashiki, N., Matsumura, Y., Miura, N., Yagihara, S., & Mashimo, S. (1994). Dielectric study of water structure in polymer solution. *The Journal of Physical Chemistry, 98,* 13612-13615.

Wang, L., & Weller, C. L. (2012). Thermophysical properties of frozen foods. In Sun, D. W. (Eds.), Handbook of frozen food processing and packaging (pp.101-125). (2nd ed.). New York: CRC Press.

Wagner, C. (1961). Theorie der Alterung von Niderschlagen durch Umlösen (Ostwald Reifung). *Z Elektrochem, 65,* 581–591.

Welti-Chanes, J., Bermúdez, D., Valdez-Fragoso, A., Mújica-Paz, H., & Alzamora, S. M. (2004). Principles of Freeze-Concentration and freeze-drying. In Hui, Y.H., Cornillon, P., Legaretta, I.G., Lim, M.H., Murrell, K.D., Nip, W.K. (Eds.), Handbook of Frozen Foods (pp. 22-33). (1st ed.). New York: Marcel Dekker.

Whelan, A. P., Regand, A., Vega, C., Kerry, J. P., & Goff, H. D. (2008). Effect of trehalose on the glass transition and ice crystal growth in ice cream. *International Journal of Food Science and Technology, 43,* 510-516.

Zaritzky, N. E., Cidca, Plata, L. (2000). Factors affecting the stability of frozen foods. In C. J. Kennedy (Eds.), Managing frozen foods (pp. 119-143). Cambridge: CRC Press.

Zaritzky, N. (2012). Physical-chemical principles in freezing. In Sun, D.W. (Eds.). Handbook of Frozen Food Processing and Packaging. (2nd ed.). Boca Raton: CRC Press.

CHAPTER 2

CORRELATION BETWEEN ICE RECRYSTALLIZATION RATE AND DIELECTRIC RELAXATION

2.1 Introduction

Ice crystal growth by recrystallization is the major cause of deterioration in frozen food during storage and distribution. Therefore, understanding ice recrystallization is important to control and predict the quality of frozen food. Ice recrystallization rate can be evaluated quantitatively by observation of ice crystal with cryomicroscope and then determination of ice crystal mean size with image analysis (Donhowe and Hartel, 1996; Miller-Livney and Hartel, 1997; Sutton et al., 1996; Hartel, 2001; Ablett et al., 2002; Hagiwara et al., 2005; Hagiwara et al., 2006; Klinmalai et al., 2017). To obtain the ice recrystallization rate constant, the Lifshitz–Slyozov–Wagner (LSW) theory of Ostwald ripening principle (Lifshitz and Slyozov, 1961; Wagner, 1961) has been used. However, measuring ice crystal mean size has been a headache because it is a time-consuming work. Establishing the methods to provide the results faster and to predict ice recrystallization under various conditions has been required. Comprehension of dynamics of water molecules in a freeze-concentrated matrix may be helpful because it is one of factors affecting recrystallization rate of ice crystal (Ablett et al., 2002; Hagiwara et al., 2005; Hagiwara et al., 2006; Klinmalai et al., 2017). Dynamic of water molecules can be explored by several techniques such as nuclear magnetic resonance (NMR), molecular dynamic (MD) simulation and dielectric measurement.

Diffusion coefficient of water components in freeze-concentrated matrix and molecular dynamic (MD) simulations were proved to have a potential parameter to predict different ice recrystallization rate of sugar solutions in previous studies (Hagiwara et al., 2006; Hagiwara et al, 2009). Ablett et al. (2002) also reported positive good correlation between isothermal ice recrystallization rates in a series of frozen saccharide solutions (glucose (monosaccharide), maltose (disaccharide), maltotriose (trisaccharide), and pullulan (glucose polymer)) and their spin-spin relaxation time (T_2) of water component in freeze-concentrated matrix. Recently,

relationship between spin-spin relaxation time (T_2) values of water component and recrystallization rate of ice crystals in trehalose and sucrose solutions with the various temperatures was proved (Klinmalai et al., 2017). From these researches, the concept of water mobility can be helpful to describe and predict ice recrystallization rate in frozen solutions. Considering the facts that the limit in the expensive experiment of NMR and difficulty to determine water properties in complex systems by molecular dynamics simulation due to time limitation, dielectric relaxation is another method to determine molecular dynamics and will enable it to predict the recrystallization rate of ice crystals.

Dielectric relaxation measurement has been used to determine relaxation time to understand the molecular mechanism of dynamic liquids structure in saccharide, polysaccharide and protein solutions (Shinyashiki et al., 1994; Shinyashiki et al., 1999; Kumagai et al., 2000; Hayashi et al., 2002; Jansson et al., 2005; Yokoyama et al., 2006; Kaminski et al., 2008; Kwon et al., 2010; Wolf et al., 2012; Kishikawa et al., 2013; Yamamoto et al., 2015; Sasaki et al., 2016). Many researchers studied dielectric relaxation in sugar-water mixtures (Jansson et al., 2005; Kaminski et al., 2008; Kwon et al., 2010; Yamamoto et al., 2015). However, the knowledge about relationship between ice recrystallization and dielectric relaxation time was not reported until now. If there exists good correlation, dielectric measurement will be helpful to predict ice recrystallization rate constant in a reduced time. In this chapter, relation between dielectric relaxation time and previous experimental ice recrystallization rate in various saccharide solutions was investigated.

2.2 Materials and methods

2.2.1 Materials

Sucrose, maltose, glucose and fructose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and dissolved in Milli-Q water.

2.2.2 Sample preparations

The details of saccharide solutions investigated in this study were obtained from previous research (Hagiwara et al., 2006) as shown in Table 1. Ice recrystallization rate and diffusion coefficient values of water molecule in a freeze-concentrated matrix (measured by PFGSTE ^{1}H-NMR) were also presented in the same table. To eliminate effect of ice content in this study, ice content of all samples were set almost equal.

Table 1 Details of sample saccharide solutions

Sample	Temperature (°C)	Freeze-concentrated matrix conc. (%)	Ice content (%)	Water frozen (%)	Ice recrystallization rate (μm^3/min)	Diffusion coefficient of water molecules in freeze-concentrated matrix (x10^{-10}m^2/s)
21.8% maltose	-4.4	40.0	45.4	58.1	19.18±0.90	3.75±0.09
22.45% sucrose	-4.6	41.1	45.4	58.4	19.65±2.43	3.69±0.06
18.5% glucose	-5.8	34.0	45.5	55.9	28.18±2.24	4.05±0.06
25.0% sucrose	-5.8	45.8	45.5	60	14.98±1.04	2.83±0.09
22.45% fructose	-8	41.1	45.4	58.6	15.01±2.19	3.29±0.05
28.6% sucrose	-8	52.4	45.4	63.6	5.38±0.28	1.54±0.03
25.0% fructose	-10	45.8	45.5	60.7	9.43±0.15	1.91±0.04
25.0% glucose	-10	45.9	45.4	60.8	8.65±1.39	1.93±0.03

2.2.3 Dielectric relaxation measurement

Time domain reflectometry (TDR) was employed to measure dielectric relaxation in a frequency range from 1 MHz to 3 GHz. A voltage pulse of 230 mV in amplitude was applied to a sample solution through a coaxial probe and the time dependent change in the voltage of the reflected wave was measured using the main frame of digitizing oscilloscope (HP54120B, Hewlett-Packard, USA) and four channel test set (HP54121A, Hewlett-Packard, USA) with the following parameter values; time interval 3 ns/div, delay (time offset relative to trigger) 23.94 ns, and voltage sensitivity 50 mV/div for setting waveform characteristic. A probe with pin diameter d = 1.0 mm and electric length γd = 2.001165 mm was used. The reflected wave was accumulated 2048 times for each measurement. Water was used as the reference sample. During the experiments the sample temperature was monitored with digital thermometer (7563, Yokogawa meters & instruments corp., Japan). A broadening of the dielectric function was fitted by two Cole-Cole equations, which is given by (Schönhals & Kremer, 2003)

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\Delta\varepsilon_s}{1+(i\omega\tau_s)^{\beta s}} + \frac{\Delta\varepsilon_f}{1+(i\omega\tau_f)^{\beta f}} \quad (1)$$

where $\varepsilon^*(\omega)$ is the complex permittivity, ε_∞ is the limiting high frequency permittivity. $\Delta\varepsilon$ is the relaxation strength, τ is the relaxation time, ω is the angular frequency ($\omega = 2\pi f$; f =frequency), and β ($0<\beta\leq 1$) is referred to as the parameter for the symmetrical broadening distribution of the relaxation curve. s and f denote the slow and fast relaxation processes, respectively. The dielectric loss (ε'') for single Cole-Cole equation is described by

$$\text{Imaginary part} = \frac{\varepsilon''(\omega)}{\Delta\varepsilon} \quad (2)$$

$$\varepsilon'' = (\omega\tau)^\beta \sin(\beta\pi/2)\, r^{-1}(\omega) \quad (3)$$

$$r(\omega) = 1+2(\omega\tau)^\beta \cos(\beta\pi/2)+(\omega\tau)^{2\beta} \quad (4)$$

These mathematical relations were used for obtaining dielectric relaxation parameters.

2.2.4 Statistical analysis

Dielectric relaxation measurements were conducted in triplicate. Mean values of dielectric relaxation time were compared by analysis of variance (ANOVA) and Duncan's multiple range test for comparing treatments ($p \leq 0.05$). All calculations were done using the SPSS 17.0 software for windows (SPSS Inc., Chicago, IL, USA).

2.3 Results and discussion

2.3.1 Dielectric relaxation

Dielectric loss spectra (ε'') were fitted well by two Cole-Cole equations as shown in Figure 13 in all samples. This clearly shows that slow and fast relaxation processes were observed. Dielectric relaxation peak of fast relaxation process was observed at the frequency higher than 1 GHz and the lower relaxation appeared around 100 MHz. Considering the preceding report by Mashimo et al. (1987), it was strongly suggested that the fast relaxation came from the orientation of free water molecules (free water) and slow one was reflected by the orientation of water molecules interacting cooperatively with saccharide molecules.

Ice content of all saccharide solutions were almost set equal to eliminate the effect of ice content. Table 2 shows that type of saccharides had effect on dielectric relaxation time both slow and fast water. Dielectric relaxation time (τ) is the parameter that can be calculated from dielectric loss and reflects the mobility of molecular in solution. It corresponds to the time needed for electric dipoles to orient in the direction of an electric field and can be referred to mobility of molecules (Kumagai et al., 2000; Kumagai and Kumagai, 2002). Dielectric strength ($\Delta\varepsilon$) can be determined by the dipole-dipole correlation both self-correlation and cross correlation reflecting different properties of the same underlying motional process (Kremer and Schönhals, 2003) and be referred to the number of water molecules and water content in solutions (Yagihara et al., 2007; Kishikawa et al., 2013). Dielectric strength of free water molecules were not significantly different among sample solutions. The reason for this may be the similarity of ice content.

The relaxation time (τ) of slow and fast water, including dielectric strength (Δε) of slow water for fructose and glucose at -10 ºC were not significantly different whereas these values of sucrose solution are higher than glucose and fructose solutions both at -5.8 ºC and -8 ºC. It was suggested that water mobility of disaccharide solutions were lower than that of monosaccharide solutions at the same temperature due to large number of hydroxyl groups of disaccharide, which affects the dynamic structure of water molecules. In anyway, The results in Table 2 showed that the type of saccharide solution had effect on the water mobility in the solution system.

Table 2 Dielectric strength (Δε) and relaxation time (τ) of saccharide solutions

Sample	Dielectric strength (Δε)		Relaxation time (τ)	
	$\Delta\varepsilon_{Slow\ water}$	$\Delta\varepsilon_{Fast\ water}$	$\tau_{Slow\ water}$	$\tau_{Fast\ water}$
40%maltose -4.4 ºC	7.16 ± 0.70^{a}	48.48 ± 1.57^{a}	1.203 ± 0.127^{d}	0.080 ± 0.002^{b}
41.1% sucrose -4.6 ºC	11.21 ± 1.90^{bc}	49.82 ± 3.61^{a}	0.999 ± 0.136^{cd}	0.083 ± 0.001^{b}
34%glucose -5.8 ºC	9.09 ± 0.58^{ab}	49.61 ± 1.58^{a}	0.437 ± 0.026^{a}	0.068 ± 0.004^{a}
45.8% sucrose -5.8 ºC	12.08 ± 1.87^{c}	48.10 ± 3.22^{a}	1.49 ± 0.322^{e}	0.113 ± 0.003^{d}
41.1% fructose -8 ºC	11.05 ± 1.09^{bc}	50.87 ± 1.06^{a}	0.719 ± 0.084^{b}	0.093 ± 0.005^{c}
52.4% sucrose -8 ºC	16.12 ± 0.66^{d}	47.70 ± 0.57^{a}	1.640 ± 0.082^{e}	0.143 ± 0.007^{f}
45.8% fructose -10 ºC	13.53 ± 0.62^{cd}	50.34 ± 1.63^{a}	0.95 ± 0.038^{bcd}	0.117 ± 0.001^{de}
45.9%glucose -10 ºC	16.24 ± 2.99^{d}	46.69 ± 4.85^{a}	0.915 ± 0.082^{bc}	0.122 ± 0.010^{e}

Different superscript letters indicate a significant difference ($p\leq0.05$) means in the same column.
Values shown are mean ± SD from triplicate measurements

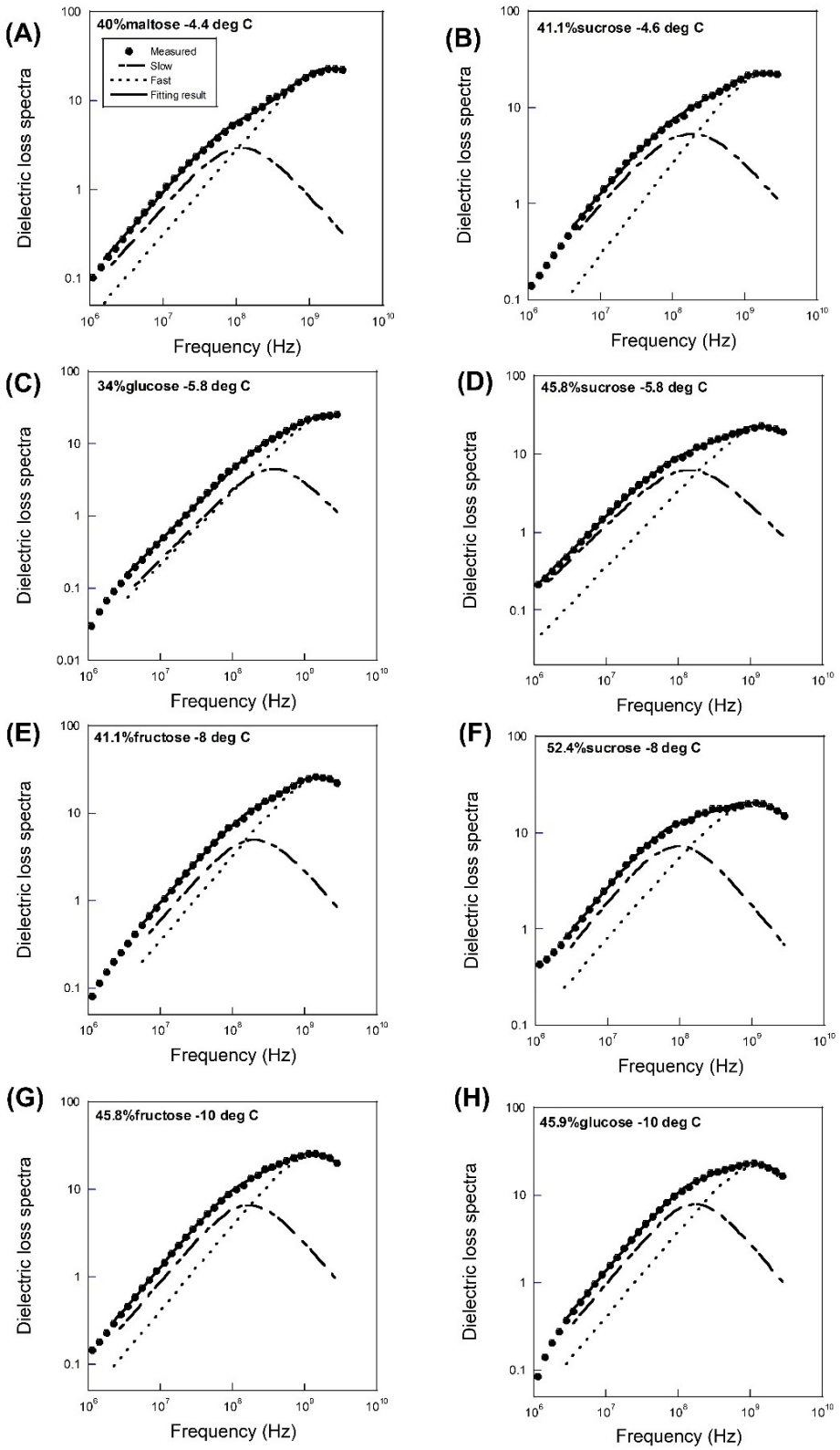


Figure 13 Frequency dependences of dielectric loss spectra for various saccharide solutions at various temperatures. The solid curves were obtained by fitting using Cole-Cole equation (Eq.2-4)

2.3.2 Correlation between recrystallization rate (*k*) and dielectric relaxation time (τ)

It was expected that the fast relaxation process is associated with ice recrystallization behavior because it reflects free water mobility. In order to clarify the relation between recrystallization rate and dielectric relaxation time, the ice recrystallization rate constant was plotted as a function of the measured fast relaxation time. Relationship between ice recrystallization rate (*k*) and relaxation time (τ) of fast water in freeze concentration showed good correlations as in Figure 14. The fitting results by a linear function were in reasonable agreement ($R^2 = 0.90$), which means ice recrystallization rate is depend on relaxation time of fast water.

In Figure 15, plots of ice recrystallization rate and temperatures for these saccharide solutions were also presented for comparison. Ice recrystallization rate of these saccharide solutions did not depend on temperature in linear line. Therefore, it was suggested that the difference in ice recrystallization rate was explained better by using relaxation time (τ) of samples rather than temperature even if the combination effect of solutions such as type of saccharides, temperatures and concentrations existed. In the previous study, ice recrystallization rate in frozen saccharide solutions were correlated well with diffusion coefficient of water molecules in freeze-concentrated matrix ($R^2 = 0.90$) (Hagiwara et al., 2006) as shown in Figure 16. It showed that diffusion coefficient of water had an impact factor to predict ice recrystallization rate. Thus, correlations between diffusion coefficient and dielectric relaxation time of water mobility in freeze-concentrated matrices were also evaluated in Figure 17. The results showed good correlation between fast dielectric relaxation time and diffusion coefficient of water component (R^2=0.94). These results confirmed that water mobility in freeze-concentrated matrix is an important factor that affect ice recrystallization process in frozen food even if different method to determine water mobility is used.

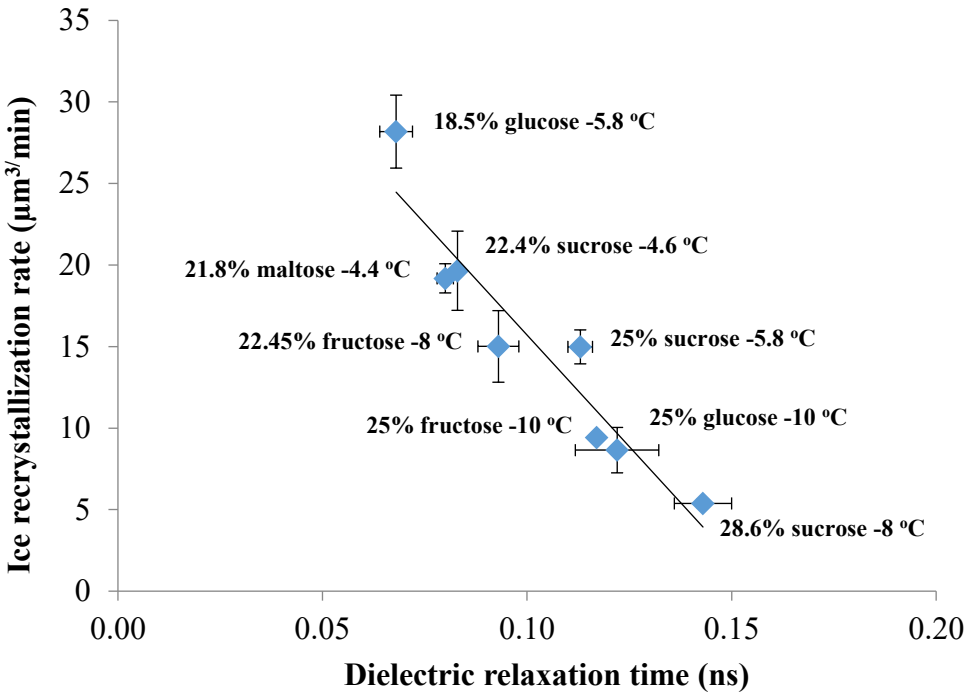


Figure 14 Correlation between ice recrystallization rate and dielectric relaxation time. The solid line represents result of linear fitting

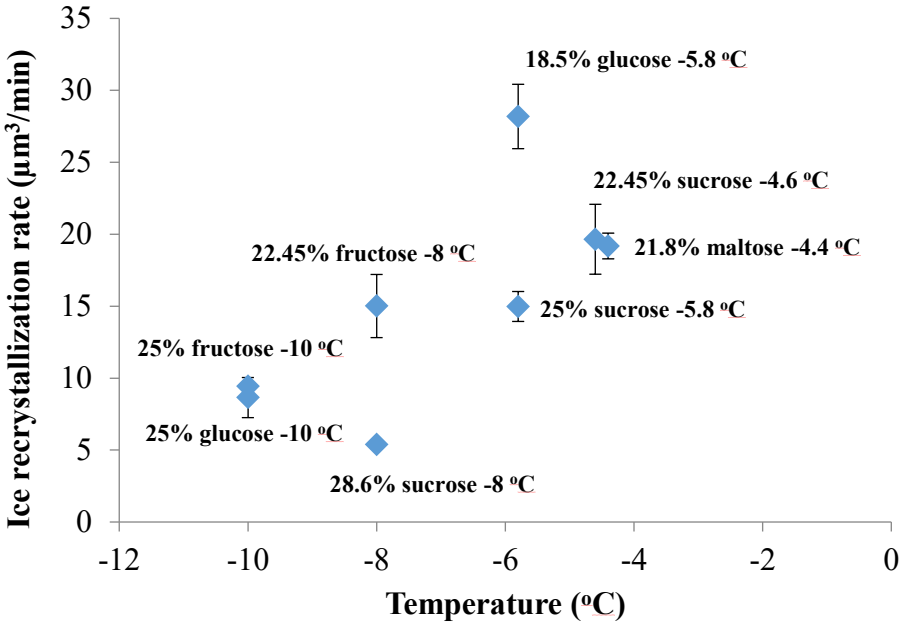


Figure 15 Correlation between ice recrystallization rate and temperatures

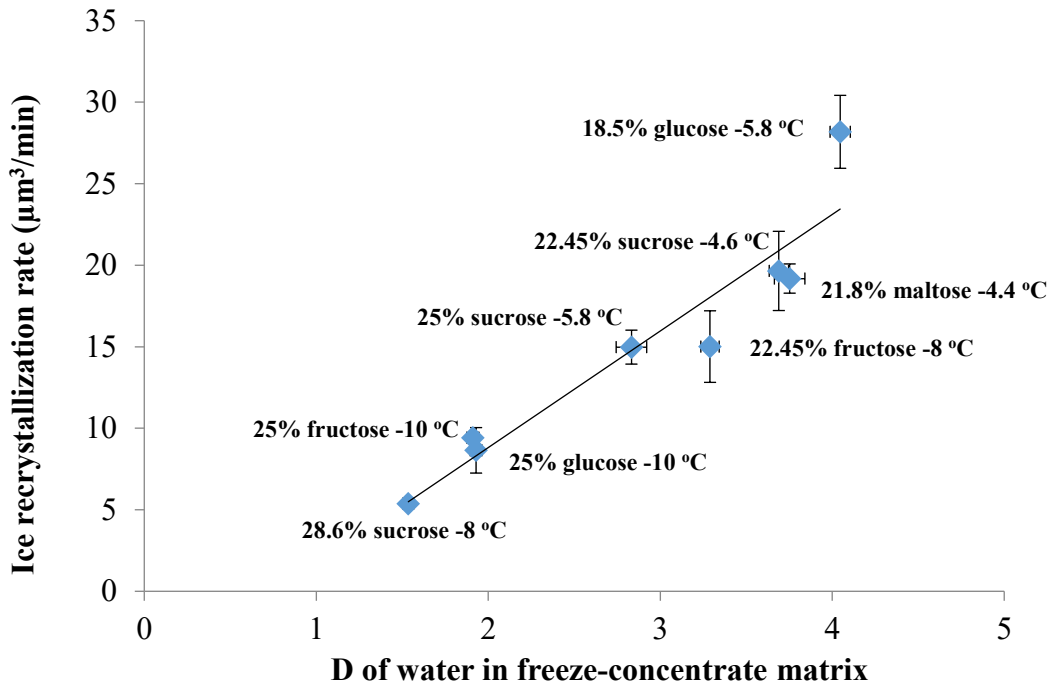


Figure 16 Correlation between ice recrystallization rate and diffusion coefficient of water component. The solid line represents result of linear fitting

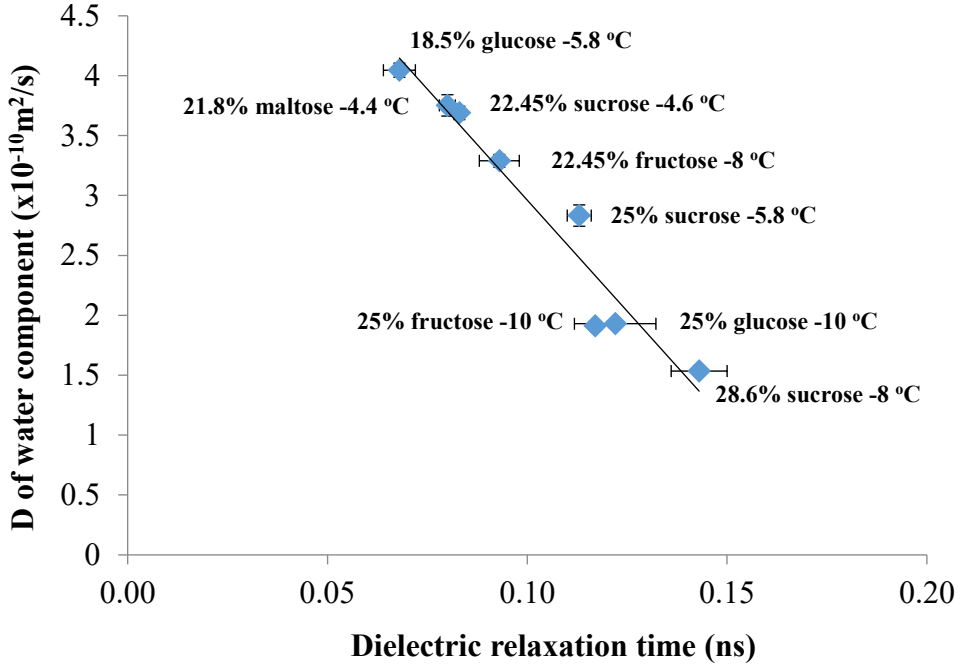


Figure 17 Correlation between diffusion coefficient of water component and dielectric relaxation time. The solid line represents result of linear fitting

2.4 Conclusion

Ice recrystallization of these saccharide solutions did not depend on temperature in linear fashion. The dielectric relaxation time of fast water was a useful parameter to predict and control recrystallization rate of ice crystals. The difference in ice recrystallization rate of saccharide solutions was explained well by the fast relaxation time of all samples even though the combination of solutions such as types of sugars, temperatures and concentrations existed.

References

Ablett, S., Clarke, C. J., Izzard, M. J., & Martin, D. R. (2002). Relationship between ice recrystallisation rates and the glass transition in frozen sugar. *Journal of the Science of Food and Agriculture, 82,* 1855-1859.

Donhowe, D. P., & Hartel, R. W. (1996). Recrystallization of ice in ice cream during controlled accelerated storage. *International Dairy Journal, 6,* 1191-1208.

Hagiwara, T., Hartel, R.W., & Matsukawa, S. (2006). Relationship between recrystallization rate of ice crystals in sugar solutions and water mobility in freeze-concentrated matrix. *Food Biophysics, 1,* 74-82.

Hagiwara, T., Mao, J., Suzuki, T., & Takai, R. (2005). Ice recrystallization in sucrose solutions stored in atemperature range of -21^{o}C to -50^{o}C. *Food Science and Technology Research, 11,* 407-411.

Hagiwara, T., Sakiyama, T., & Watanabe, H. (2009). Estimation of water diffusion coefficients in freeze-concentrated matrices of sugar solutions using molecular dynamics: correlation between estimated diffusion coefficients and measured ice-crystal recrystallization rates. *Food Biophysics, 4,* 340-346.

Hartel, R.W. (2001). Crystallization in foods. In Myerson, A. S. (Eds.), Handbook of industrial crystallization (pp.287-303). (1st ed.). Oxford: Elsevier science & technology.

Hayashi, Y., Shinyashiki, N., Yagihara, S., Yoshiba, K., Teramoto, A., Nakamura, N., Miyazaki, Y., Sorai, M., & Wang, Q. (2002). Ordering in aqueous polysaccharide solutions. I. dielectric relaxation in aqueous solutions of triple-helical polysaccharide schizophyllan. *Biopolymers, 63,* 21-31.

Jansson, H., Bergman, R., & Swenson, J. (2005). Dynamics of sugar solutions as studied by dielectric spectroscopy. *Journal of Non-Crystalline Solids, 351,* 2858-2863.

Kaminski, K., Kaminska, E., Wlodarczyk, P., Paluch, M., Ziolo, J., & Ngai, K.L. (2008). Dielectric relaxation study of the dynamics of monosaccharides: D-ribose and 2-deoxy-D-ribose. *Journal of Physics: Condensed Matter, 20,* 335104-335111.

Kishikawa, Y., Seki, Y., Shingai, K., Kita, R., Shinyashiki, N., & Yagihara, S. (2013). Dielectric relaxation for studying molecular dynamics of pullulan in water. *The Journal of Physical Chemistry, 117,* 9034-9041.

Klinmalai, P., Shibata, M., & Hagiwara, T. (2017). Recrystallization of ice crystals in trehalose solution at isothermal condition. *Food Biophysics, https://doi.org/10.1007/s11483-017-9496-1*.

Kremer, E., & Schönhals, A. (2003). The scaling of the dynamics of glasses and supercooled liquids. In Kremer, F., Schonhals, A. (Eds.), Broadband dielectric spectroscopy (pp. 99-129). (1st ed.). New York: Springer-Verlag.

Kumagi, H., Sugiyama, T., Iwamoto, S. (2000). Effect of water content on dielectric relaxation of gelatin in a glassy state. *Journal of Agricultural and Food Chemistry, 48,* 2260-2265.

Kumagai, H., & Kumagai, H. (2002). Review analysis of molecular or ion mobility in glassy and rubbery foods by electric and proton-NMR measurements. *Food Science and Technology Research, 8,* 95-105.

Kwon, H. J., Seo, J. A., Kim, H. K., & Hwang, Y. H. (2010). A study of dielectric relaxations in galactose-water mixtures. *Journal of Non-Crystalline Solids, 356,* 2836-2841.

Mashimo, S., Umehara, T., Ota, T., Kuwabara, S., Shinyashiki, N., & Yagihara, S. (1987). Evaluation of complex permittivity of aqueous solution by time domain reflectometry. *Journal of Molecular Liquids, 36,* 135-151.

Miller-livney, T., & Hartel, R. W. (1997). Ice recrystallization in ice cream: Interactions between sweeteners and stabilizers. *Journal of Dairy Science, 80,* 447-456.

Sasaki, K., Matsui, Y., Miyara, M., Kita, R., Shinyashiki, N., & Yagihara, S. (2016). Glass transition and dynamics of the polymer and water in the poly(vinylpyrrolidone)-water mixtures studied by dielectric relaxation spectroscopy. *The Journal of Physical Chemistry B, 120,* 6882-6889.

Schönhals, A., & Kremer, F. (2003). Analysis of dielectric spectra. In Kremer, F., Schonhals, A. (Eds.), Broadband dielectric spectroscopy (pp. 59-98). (1st ed.). New York: Springer-Verlag.

Shinyashiki, N., Matsumura, Y., Miura, N., Yagihara, S., & Mashimo, S. (1994). Dielectric study of water structure in polymer solution. *The Journal of Physical Chemistry, 98,* 13612-13615.

Shinyashiki, N., Sakai, T., Yamada, G., & Yagihara, S. (1999). Dielectric study on the dynamics of an queous solution of gellan gum. *Progress in Colloid and Polymer Science, 114,* 36-40.

Sutton, R., Lips, A., Piccirillo, G., & Sztehlo, A. (1996). Kinetics of ice recrystallization in aqueous fructose solutions. *Journal of Food Science, 4,* 741-745.

Wolf, M., Gulich, R., Lunkenheimer, P., & Loidl, A. (2012). Relaxation dynamics of a protein solution investigated by dielectric spectroscopy. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 1824,* 723-730.

Yagihara, S., Oyama, M., Inoue, A., Asano, M., Sudo, S., & Shinyashiki, N. (2007). Dielectric relaxation measurement and analysis of restricted water structure in rice kernels. *Measurement Science and Technology, 18,* 983-990.

Yamamoto, W., Sasaki, K., Kita, R., Yagihara, S., & Shinyashiki, N. (2015). Dielectric study on temperature-concentration superposition of liquid to glass in fructose-water mixtures. *Journal of Molecular Liquids, 206,* 39-46.

Yokoyama, A., Ishikawa, H., Shinohara, M., Shinyashiki, N., Yagihara, S., & Hayashi, Y. (2006). Broadband dielectric spectroscopy of albumin-water mixture in frozen state. *AIP Conference Proceedings, 832,* 163-166.

CHAPTER 3

RECRYSTALLIZATION OF ICE CRYSTALS IN DI-AND TRISACCHARIDE SOLUTIONS

3.1 Introduction

The concept of water mobility effects on ice recrystallization rate in saccharide systems was clarified in Chapter 2. Recently, trehalose and raffinose have been attracting attention for their unique properties. Trehalose is a natural non-reducing disaccharide forming a α, α-1,1-glucoside bond between two α-glucose units. Its molecular weight is almost equal to that of sucrose. It is about 45% as sweet as sucrose, with a nice finish (Aidoo et al., 2013). Raffinose is a non-reducing trisaccharide containing galactose, glucose, and fructose (α-D-galactopyranosyl-(1-6)-α-D-glucopyranosyl-(1-2)-β-D-fructofuranoside). Raffinose has only 20 % of the sweetening intensity of sucrose. Low sweetness of trehalose and raffinose are one of the advantages for application these sugars in many food products. Reportedly, trehalose has been used as a cryoprotectant in foods, namely frozen dough (Gélinas et al., 1989), surimi (Osako et al., 2005), frozen tofu (Fuchigami et al., 2002), and ice cream (Whelan et al., 2008). Trehalose suppresses deterioration reactions of food components such as protein denaturation and lipid oxidation (Zhou et al., 2006; Ma et al., 2015). Furthermore, trehalose can prevent fatty liver disease in mice by inhibiting glucose transporters in hepatocyte plasma membrane from entering the liver cells and triggering autophagy of high-fat liver cells (Debosch et al., 2014; Debosch et al., 2016). Therefore, it might be interesting to use trehalose as a sweetener and ingredient in frozen food because of the functions described above. Additionally, trehalose is useful in many areas not only in food manufacturing but also in the field of medicine and pharmaceutical due to its efficiency in preserving biological molecules (Buchanan et al., 2004; Jain and Roy, 2009; Paul and Paul, 2015; Li et al., 2016). Raffinose was used for cryopreservation in mouse sperm (Storey et al., 1998; Tada et al., 1990). Moreover, raffinose is, as a functional food, intended to improve intestine health (Nagura et al., 1999). Many preceding researches about trehalose and raffinose focused on their cryoprotective actions.

However, understanding the recrystallization behavior of ice crystals in the presence of trehalose and raffinose are fundamentally important and have not been extensively studied yet.

Whelan et al. (2008) investigated the effects of replacing sucrose with trehalose on the recrystallization of ice crystal in ice cream when it was stored in a heating and cooling cycle condition. They reported no clear difference in recrystallization behavior of ice crystals. However, no information was available for the isothermal ice recrystallization rate constant in a trehalose and raffinose solution. To elucidate the potential of trehalose and raffinose as a sweetener and ingredient in frozen food, the isothermal ice recrystallization rate constant in a trehalose and raffinose solutions were measured and compared to that in a sucrose solution in this study because isothermal frozen storage condition is practically utilized in most of frozen storage. Simultaneous estimation of water mobility in the freeze-concentrated matrix was conducted using the ^{1}H spin–spin relaxation time and dielectric relaxation time (τ) to investigate the mechanisms underlying the mutually different ice crystal recrystallization behaviors of sucrose, trehalose, and raffinose.

3.2 Materials and Methods

3.2.1 Materials

Sucrose and trehalose dihydrate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and from Hayashibara Co., Ltd. (Okayama, Japan), respectively. Raffinose pentahydrate was purchased from Sigma Aldrich (St Louis, Missouri, USA). They were used without further purification.

3.2.2 Sample preparation

Powder samples were dissolved in distilled water at room temperature. The final sucrose and trehalose concentrations were set to 30% (w/w; anhydride basis). Due to a limit of raffinose solubility, 20% (w/w) trehalose and 10% (w/w) raffinose mixture solution was used for examination of raffinose effects.

3.2.3 Freezing point depression curve

Freezing point depression curve of trehalose, trehalose and raffinose mixture are fundamentally important information for calculating the concentration in a freeze-concentrated matrix and ice contents at certain subzero temperatures. Regarding sucrose, numerical data are available in the literature (Reiser et al., 1995; Miyawaki et al., 1997; Lide et al., 2003). However, few numerical data exist for freezing point depression of trehalose, although several researchers have investigated it (Nicolajsen and Hvidt, 1994; Miller et al., 1997; Sei and Gonda, 2006; Whelan et al., 2008). In this study, the freezing points of 10-60% (w/w) sucrose solutions and 10–55% (w/w) trehalose solutions were investigated. As for the freezing point of trehalose and raffinose mixture solutions, the solutions different total solutes concentrations with fixed ratio between trehalose and raffinose (2:1) were prepared, namely, 10% (w/w) trehalose and 5% (w/w) raffinose, 14% (w/w) trehalose and 7% (w/w) raffinose, 20% (w/w) trehalose and 10% (w/w) raffinose, 24% (w/w) trehalose and 12% (w/w) raffinose, 30% (w/w) trehalose and 15% (w/w) raffinose, 34% (w/w) trehalose and 17% (w/w) raffinose, 36% (w/w) trehalose and 18% (w/w) raffinose, 40% (w/w) trehalose and 20% (w/w) raffinose.

Freezing point measurements were carried out by the method of Miyawaki et al. (1997). Five milliliters of sample solutions were poured into a plastic tube (16 mm in diameter) and then the platinum resistance thermometer probe (SE012 PT100, Pico Technology, United Kingdom) was inserted through a silicon stopper into the solution. The silicon stopper was used for connecting the probe with the tube to fix the position of the temperature sensing part of the probe in the sample. Then, the tube was stored in conventional freezer at -80 ºC until the sample solution was completely frozen. After that, the sample tube was warmed at room temperature with a vortex mixer (Scientific industries, USA). During the warming process, the temperature was recorded at every 1 second using thermometer probe connected to a data logger (PT-104, Cambridgeshire, United Kingdom) to identify the melting point of samples. Measurements of the freezing points of sucrose solutions were also obtained and the measured data were compared to the numerical data in the literature (Reiser et al., 1995; Miyawaki et al., 1997; Lide, 2003) to validate the method used. The measured freezing point depression curve of trehalose, trehalose and raffinose mixture solutions were used for additional recrystallization

experiments and for ^{1}H NMR spin–spin relaxation time (T_2) and dielectric relaxation measurements.

3.2.4 Isothermal recrystallization rate constant

Isothermal recrystallization of ice crystals in sucrose, trehalose, trehalose and raffinose mixture solutions were observed at -5 °C, -7 °C, and -10 °C using a similar method that described in reports of previous studies (Sutton et al., 1996; Hagiwara et al., 2006). A light microscope (BX53; Olympus Corp., Tokyo, Japan) equipped with a cold stage (THMS 600; Linkam Scientific Instruments Ltd., Surrey, UK) and a microscopic digital camera (DS-L3; Nikon Corp., Tokyo, Japan) were used. Two microliters of sample solution enclosed between two microscope coverslips (16 mm diameter) was placed on the cold stage. Then the stage temperature was set to 30 °C at 90 °C/min. After 10 min, the sample was cooled to -30 °C at 90 °C/min to be frozen. After 10 min, the sample temperature was elevated to the observation temperature (-5 °C, -7 °C, or -10 °C; temperature variability was ±0.05 ºC) at 10 ºC/min. Immediately after the observation temperature was reached, recording of ice crystal images using the digital camera was started. Depending on the sample solution and observation time, the maximum observation time varied from 2 h to 6 h to obtain ice crystal images suitable for image analysis. When the ice crystals were so small and stuck together that no detailed structure could be observed, extension of the observation time was necessary to observe the ice crystals, which were large and sufficiently separated. Numbers of ice crystal observation per one image were more than 100. However, if the ice crystals were so large that the ice crystals in the observation view were too few (<100 ice crystals), a shorter maximum observation time was needed. The size of each ice crystal was calculated by using image analysis software (PopImaging; Digital being Kids Corp., Japan) and image handling software (Photoshop Elements 13; Adobe Systems Inc., USA) as the radius of a circle having the same projected area as the ice crystal. Then, the number-averaged radius was evaluated. To obtain the isothermal recrystallization rate constant, the Lifshitz–Slyozov–Wagner (LSW) theory of Ostwald ripening principle (Lifshitz and Slyozov, 1961; Wagner, 1961) was used (Sutton et al., 1996; Hagiwara et al., 2006; Budke et al., 2009). According to the theory, the following equation holds.

$$r^3 = r_0^3 + kt \quad (5)$$

Therein, r stands for the number-based mean crystal radius, r_0 signifies the number-based mean crystal radius at time t=0, and k denotes the isothermal recrystallization rate constant. The value of k was evaluated as the slope of the cube of r versus time t.

3.2.5 ^{1}H NMR spin–spin relaxation time T_2 of water component

For this study, we used a benchtop low-field magnetic resonance analyzer (Maran 23 ultra; Oxford Instruments plc., United Kingdom) equipped with thermo digital indicating controller (TC-500, DB 1000; Chino Corp., Tokyo, Japan) and optical fiber thermometer (FTC-DIN-ST-HA; Photon Control Inc., Canada) for samples. The sample concentration was set equivalent to the freeze-concentrated concentration of saccharide solutions at the temperature used in the recrystallization experiment (-5 °C, -7 °C, and -10 °C). Two milliliters of a sample solution was put into an NMR glass tube of 8 mm inner and 10 mm outer diameter. The NMR tube was set to the analyzer. The sample temperature was controlled by the temperature control unit. During the experiment, the sample temperature was measured continuously using a thermometer. After 20 min, the sample temperature reached the observation temperature (-5 °C, -7 °C, or -10 °C; temperature variability was ±0.05 ºC), proton relaxation function was measured by the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence that was used to obtain two exponential spin-spin relaxation decay curves. According to the method described by Ablett et al. (2002) and Ribeiro et al. (2014), the measured spin–spin relaxation function was fitted by two exponential functions using software (RI Winfit, ver. 2.5; Oxford Instruments plc.). A longer relaxation time from water component T_2 was obtained. ^{1}H spin-spin relaxation time T_2 value is given by

$$I_{(t)} = I_{0(s)} \exp \frac{-t}{T_{2,s}} + I_{0(l)} \exp \frac{-t}{T_{2,l}} \quad (6)$$

Where, s and l are the shorter and longer spin-spin relaxation times, respectively. I_0 is the proton intensity, t is time after the pulse have the form and T_2 is spin-spin relaxation time constant.

3.2.6 Dielectric relaxation measurement

Time domain reflectometry (TDR) was employed to measure dielectric relaxation in a frequency range from 1 MHz to 3 GHz. Saccharide solutions were prepared by dissolving in deionized water. The sample concentration was set equivalent to the freeze-concentrated matrix concentrations of saccharide solutions at the temperature used in the recrystallization experiment (-5 °C, -7 °C, and -10 °C; temperature variability was ±0.1 ºC). A voltage pulse of 230 mV in amplitude was applied to a sample solution through a coaxial probe and the time dependent change in the voltage of the reflected wave was measured using the main frame of digitizing oscilloscope (HP54120B, Hewlett-Packard, USA) consisting four channel test set (HP54121A, Hewlett-Packard, USA) and digital thermometer (7563, Yokogawa meters & instruments corp., Japan) and used with the following parameter values: time interval 3 ns/div, delay (time offset relative to trigger) 23.94 ns, and voltage sensitivity 50 mV/div for setting waveform characteristic. A probe with pin diameter d = 1.0 mm and electric length γd = 2.001165 mm was used. The reflected wave was accumulated 2048 times for each measurement. Frequency dependences of dielectric loss spectra was fitted by double Cole-Cole function (Schönhals and Kremer, 2003) using Eq. 2-4 in Section 2.2.3 for evaluating the dielectric relaxation parameters.

3.2.7 Statistical analysis

All measurements were conducted in triplicate. Mean values were compared by analysis of variance (ANOVA) and Duncan's multiple range test for comparing treatments ($p \leq 0.05$). All calculations were done using the SPSS 17.0 software for windows (SPSS Inc., Chicago, IL, USA).

3.3 Results and discussion

3.3.1 Freezing point depression

Table 3 presents the measured freezing point of sucrose, trehalose, trehalose and raffinose mixture solutions. The sucrose solution values obtained from the literature are also shown in the same table. The freezing point of the measured sucrose solution agreed well with the values from the literature. This agreement indicated that the method used in this study to measure freezing point was valid. Freezing point of saccharide solutions as a function of saccharide concentrations are presented in Figure 18. The measured values of the melting point of ice decreased with increasing in the concentration of saccharide solutions. The freezing points of trehalose solution at same concentration were slightly different from those of sucrose solution at same concentration, especially at high concentration even though they have same molecular weight. This may be from the different hydration properties between trehalose and sucrose. The solid line represents the results of fitting by the quartic polynomial function. The measured freezing point was approximated well by the cubic polynomial function with the correlation R^2>0.995. The fitting equation of these saccharide solutions were following equations:

$$\text{Sucrose: } y = -0.00000137x^4 + 0.000101x^3 - 0.00421x^2 - 0.02002x - 0.02157$$

$$\text{Trehalose: } y = -0.000006115x^4 + 0.0005528x^3 - 0.01733x^2 + 0.1002x - 0.06505$$

Trehalose and raffinose:

$$y = -0.0000007188x^4 + 0.000009103x^3 - 0.000795x^2 - 0.0425X + 0.01599$$

By using the fitting by the quartic polynomial function, sucrose, trehalose, trehalose and raffinose mixture concentrations in freeze-concentrated matrix at -5 ºC, -7 ºC, and -10 ºC were calculated freeze-concentrated matrix concentration in Table 4. The ice contents (weight percent of ice against total sample solution) of saccharides agreed within 3.6% at the same temperature. These values were used for the preparation of sample solutions for ^{1}H spin–spin relaxation time (T_2), dielectric relaxation measurement and for calculating the ice contents of the sample solutions at -5 °C, -7 °C, and -10 °C.

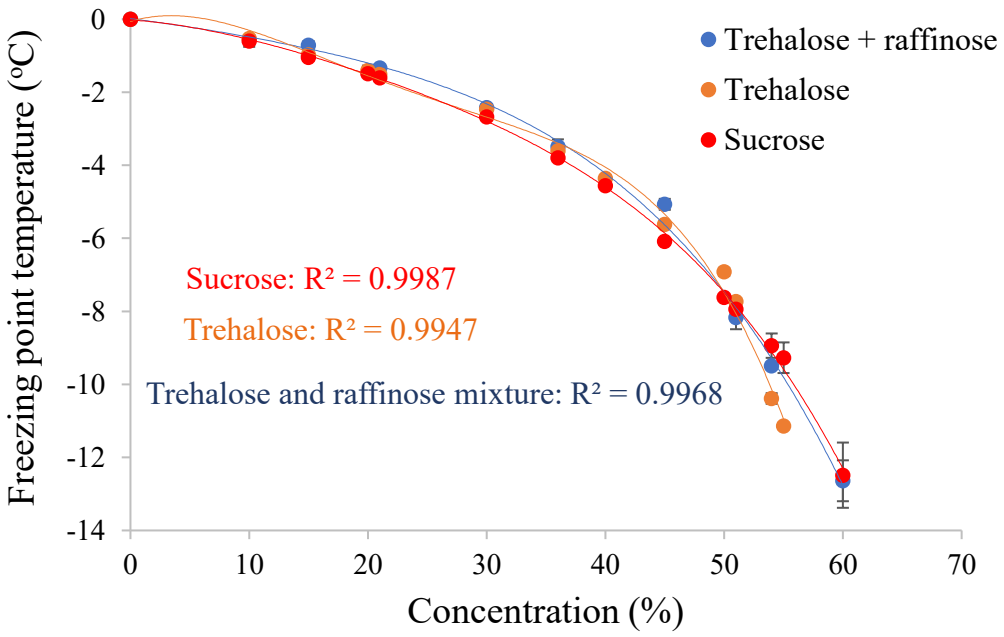


Figure 18 Freezing point depression curve of saccharides. The solid line represents the results of fitting by the quartic polynomial function.

Table 3 Freezing point depression (ºC) of sucrose, trehalose, and trehalose-raffinose mixture solutions (% w/w)

Concentration (w/w)	Sucrose	Sucrose[1]	Sucrose[2]	Trehalose	Trehalose + Raffinose (2:1)
10	0.60±0.16[a]	-0.63	-0.63	0.53±0.06[a]	n/a
15	1.05±0.08[ab]	-1.03	n/a	0.97±0.09[b]	0.71±0.11[a]
20	1.49±0.01[bc]	-1.49	-1.47	1.40±0.13[c]	n/a
21	1.61±0.01[c]	n/a	n/a	1.51±0.12[c]	1.33±0.08[b]
30	2.67±0.03[d]	-2.71	-2.64	2.47±0.02[d]	2.42±0.08[c]
36	3.80±0.06[e]	-3.72	-3.63	3.60±0.04[e]	3.49±0.20[d]
40	4.56±0.09[f]	-4.58	-4.45	4.35±0.07[f]	n/a
45	6.09±0.05[g]	n/a	n/a	5.62±0.09[g]	5.07±0.15[e]
50	7.61±0.03[h]	-7.61	n/a	6.88±0.11[h]	n/a
51	7.94±0.08[h]	n/a	n/a	7.73±0.09[i]	8.17±0.32[f]
54	8.94±0.33[i]	-9.28	n/a	10.39±0.15[j]	9.49±0.06[g]
55	9.27±0.42[i]	n/a	n/a	11.14±0.06[k]	n/a
60	12.49±0.89[j]	-12.45	n/a	n/a	12.64±0.56[h]

An asterisk in the same raw indicates that the value is significantly different from that for the sucrose ($p \leq 0.05$). The data having the same grouping letter in the same column indicate that the differences were not statically significant ($p \leq 0.05$).

[1] Sucrose properties and application (Reiser et al., 1995) p. 214

[2] CRC Handbook of chemistry, Physics, 84th Ed. pp. 8-81

Table 4 Details of saccharide solutions

Temp (°C)	Sample (w/w)	Freeze-concentrated matrix conc. (% w/w)	Ice content (weight percent of ice against total sample solution)	Frozen water (weight percent of ice against total water)
-5	30% sucrose	41.72	28.09	40.13
	30% trehalose	43.87	31.62	45.17
	20% trehalose +10% raffinose	42.91	30.09	42.99
-7	30% sucrose	48.64	38.32	54.74
	30% trehalose	49.04	38.83	55.47
	20% trehalose +10% raffinose	48.85	38.59	55.13
-10	30% sucrose	55.84	46.28	66.11
	30% trehalose	53.82	44.26	63.23
	20% trehalose +10% raffinose	55.37	45.82	65.46

3.3.2 Ice recrystallization behavior

Typical ice crystals images of sucrose, trehalose, trehalose and raffinose mixture solutions are shown in Figure 19, 20, 21 at -5 °C, -7 °C, and 10 °C, respectively. The ice crystals grew with increasing time for all sample examined, demonstrated that ice recrystallization occurred. Figure 22, 23 and 24 are the plots of the cube of the number-averaged radius of ice crystal r^3 vs. time (t). The plots show a tendency and from the fitting by Equation 5, the isothermal rate constant k was evaluated. Values of k from various saccharide solutions at -5 °C, -7 °C, and -10 °C were compared in Table 5. At lower temperatures, a smaller isothermal recrystallization rate constant was obtained for saccharide solutions. The recrystallization rate constants of trehalose solution tended to be smaller than those of sucrose at the same temperature. Moreover, trehalose and raffinose mixture solution had a potential to retard ice recrystallization only at -5 °C and -7 °C.

Whelan et al. (2008) investigated the effects of replacing sucrose with trehalose on the ice crystal recrystallization in an ice cream mix stored in a heating and cooling cycle condition. They reported no clear difference in recrystallization behavior. The present study observed the recrystallization behaviors of sucrose and trehalose solution in isothermal conditions. Results showed that the isothermal recrystallization rate constant was the same level although the tendency of a slightly lower value was observed in a trehalose solution. Results reported by Whelan et al. (2008) and those of the present study suggested that replacing sucrose with trehalose will not deleteriously affects on ice cream including frozen desserts by recrystallization of ice crystals during a heating–cooling cycle or during constant temperature storage. Moreover, Kimizuka and Suzuki (2007) reported that trehalose has a larger supercooling ability than sucrose. Therefore, the degree of supercooling before formation of ice nucleus in trehalose solution may be larger than that of sucrose if the concentration is same. This may attribute to the smaller initial ice crystal size in trehalose solution. It was reported that a larger degree of supercooling tended to induce smaller crystals (Kobayashi et al., 2014; Kobayashi et al., 2015) because of more ice nucleus formation (Fennema, 1973). Therefore, the initial ice crystal size in trehalose solution can be smaller than that in sucrose solution by more ice nucleus formation caused by the larger degree of supercooling.

Sutton et al. (1996) investigated the effects of ice contents on recrystallization rates of ice crystals in fructose solutions at -10 °C, -15 °C and -20 °C. They reported the influence of ice phase volume on ice recrystallization rate. A markedly higher recrystallization rate between samples was observed when ice contents increased more than 8.4%. However, the same trend was not observed clearly in this study for isothermal recrystallization rates at the same temperatures: In spite of having almost identical ice contents, the isothermal recrystallization rate of the trehalose and raffinose mixture solution at -5 °C was lower than that of the sucrose solution and also ice recrystallization rate of the trehalose solution at -7 °C was lower than that of the sucrose solution. These facts suggested that the level of ice fraction difference between trehalose, mixture of trehalose and raffinose, and sucrose solution at the same temperature observed in this study was so small (less than 3.6%) that it did not have a marked effect on the ice crystal recrystallization behavior.

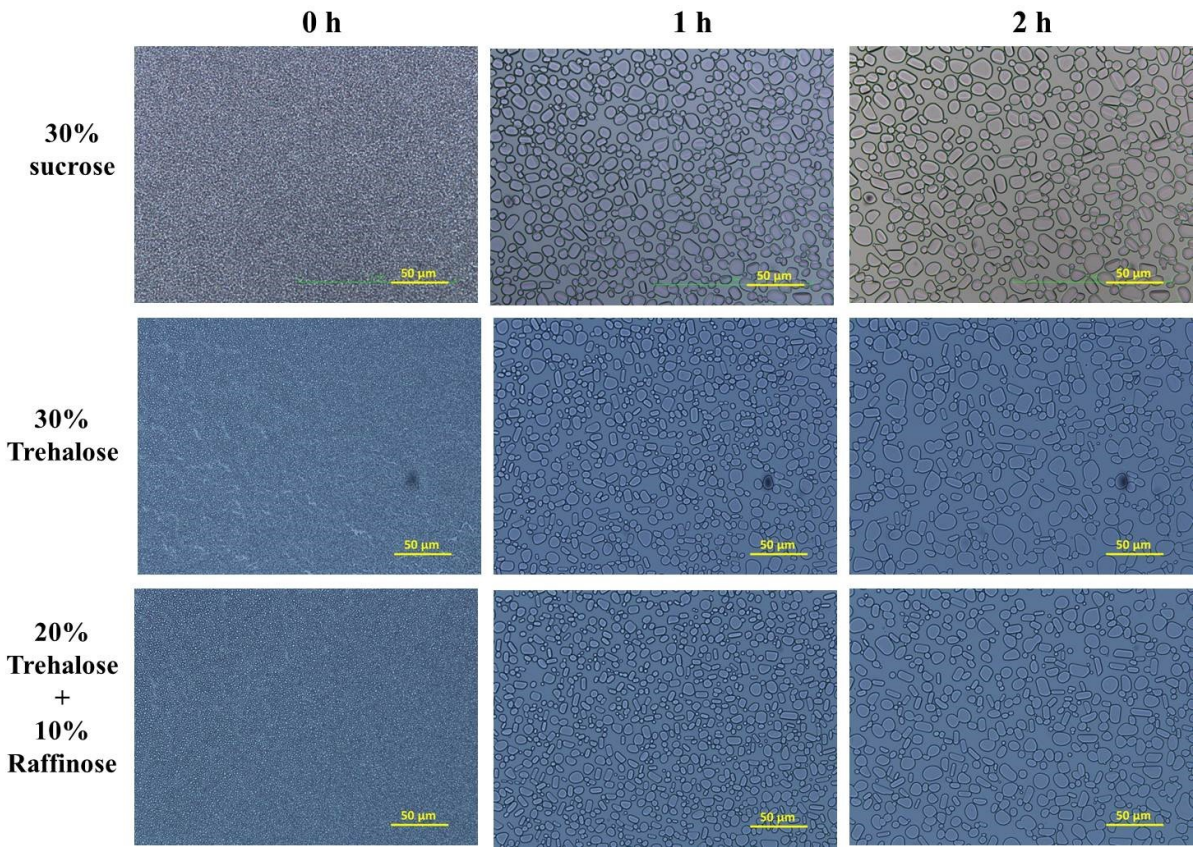


Figure 19 Typical images of ice crystals at -5 ºC. Bar = 50 µm (All images)

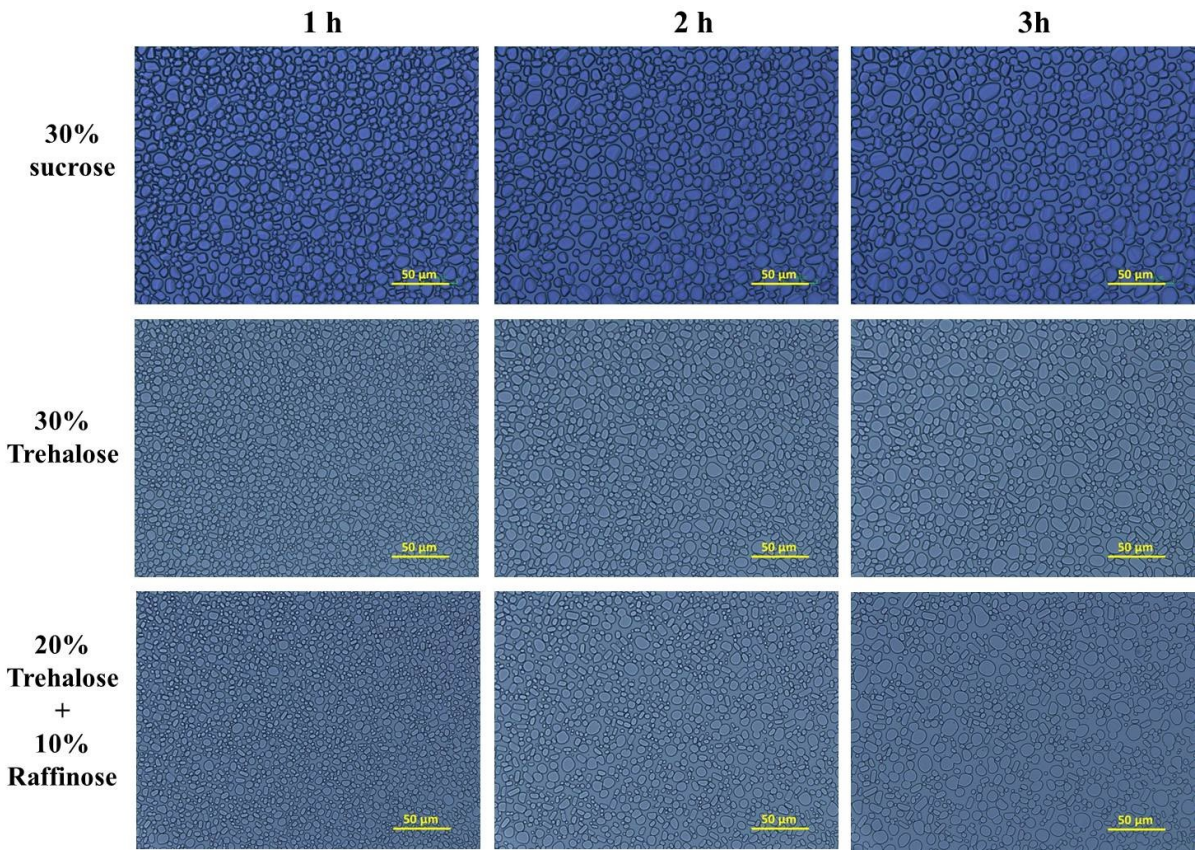


Figure 20 Typical images of ice crystals at -7 ºC. Bar = 50 µm (All images)

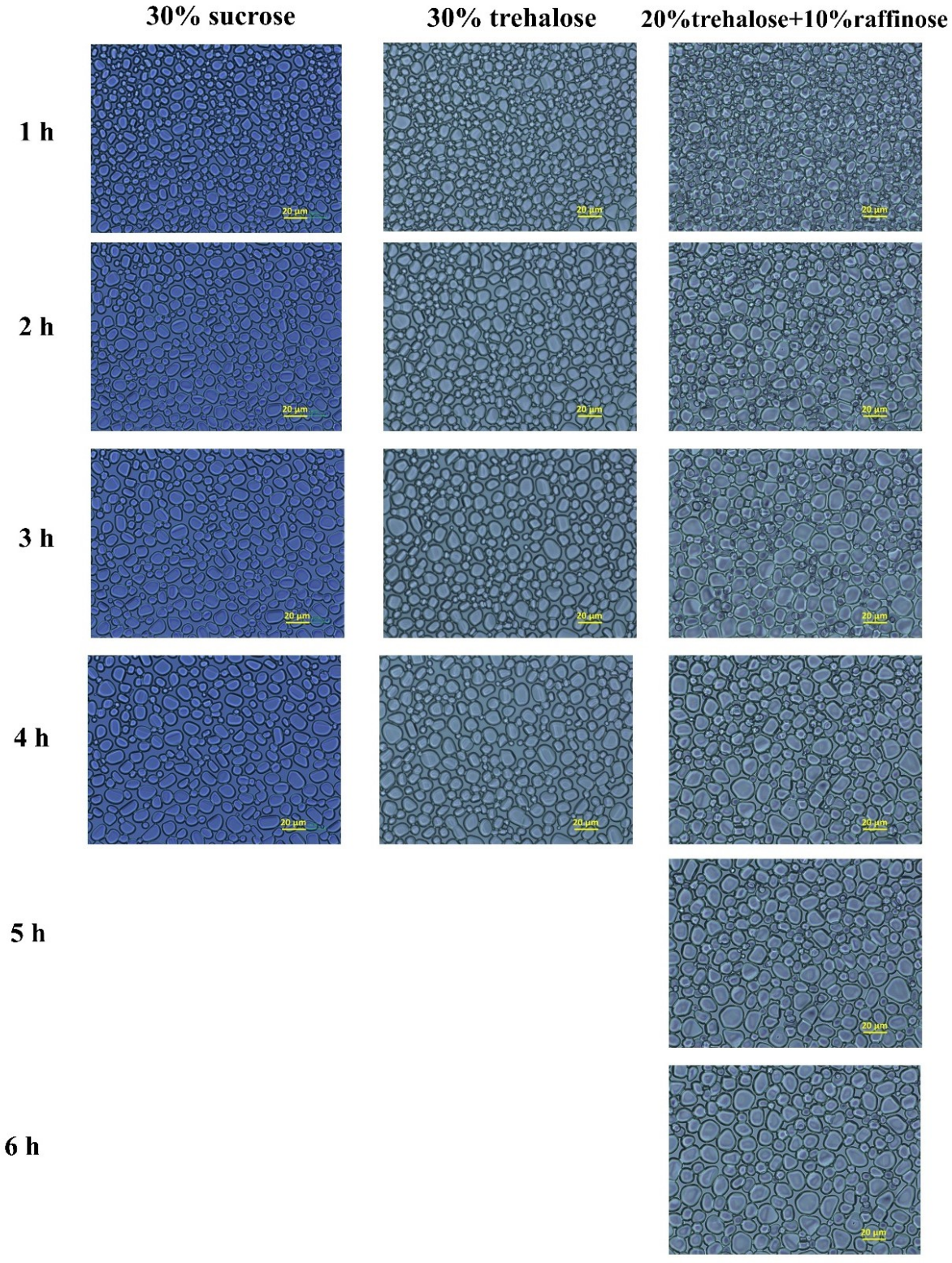


Figure 21 Typical images of ice crystals at -10 ºC. Bar = 20 µm (All images)

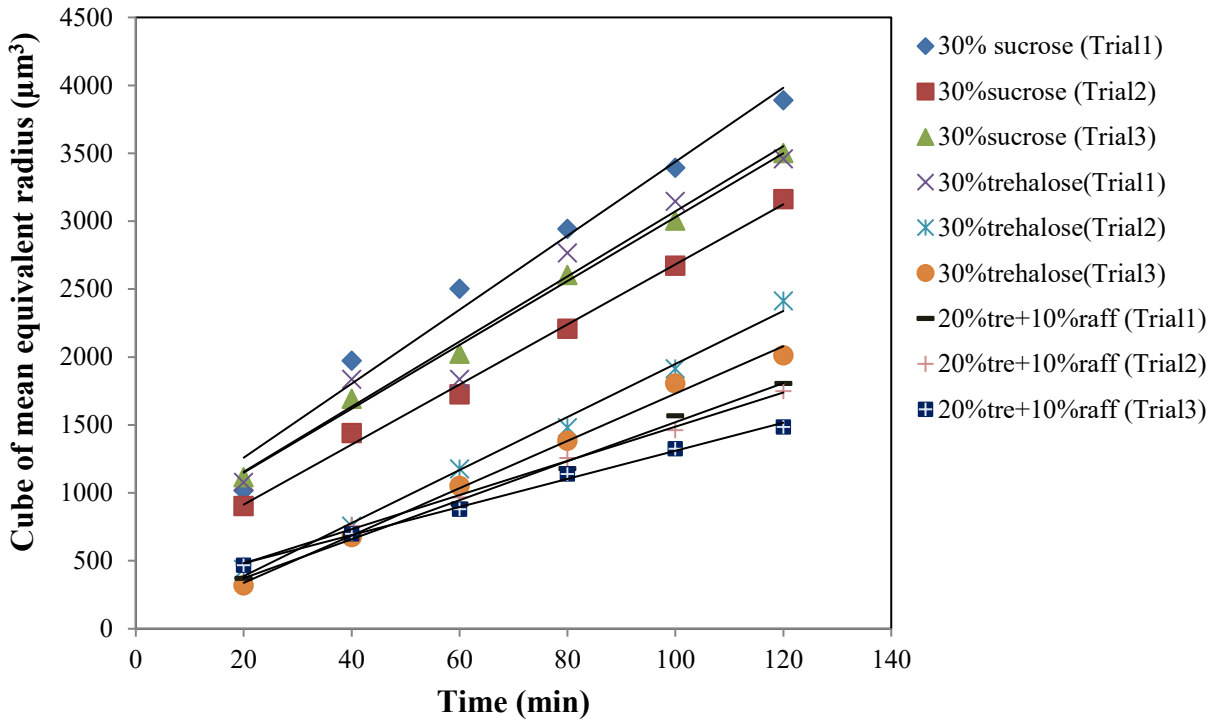


Figure 22 Plots between cube of mean equivalent radius and time during storage ice crystals for saccharide solutions (% w/w) at -5 °C

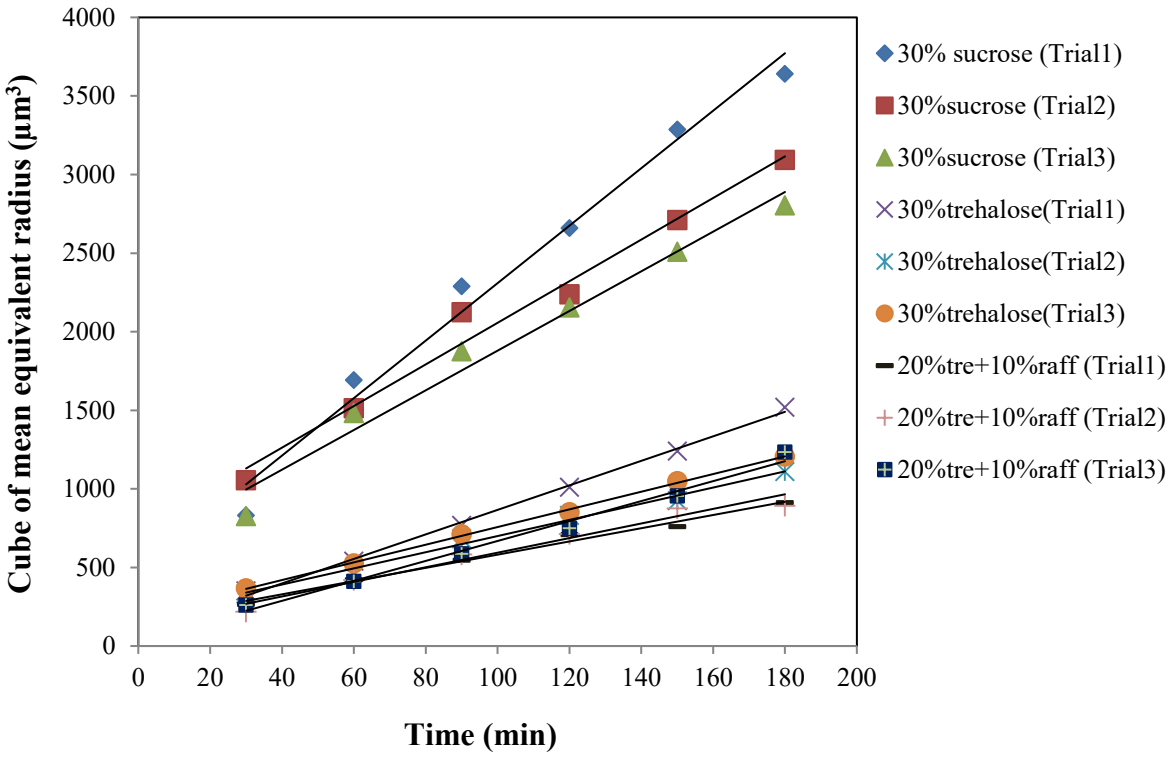


Figure 23 Plots between cube of mean equivalent radius and time during storage ice crystals for saccharide solutions (% w/w) at -7 °C

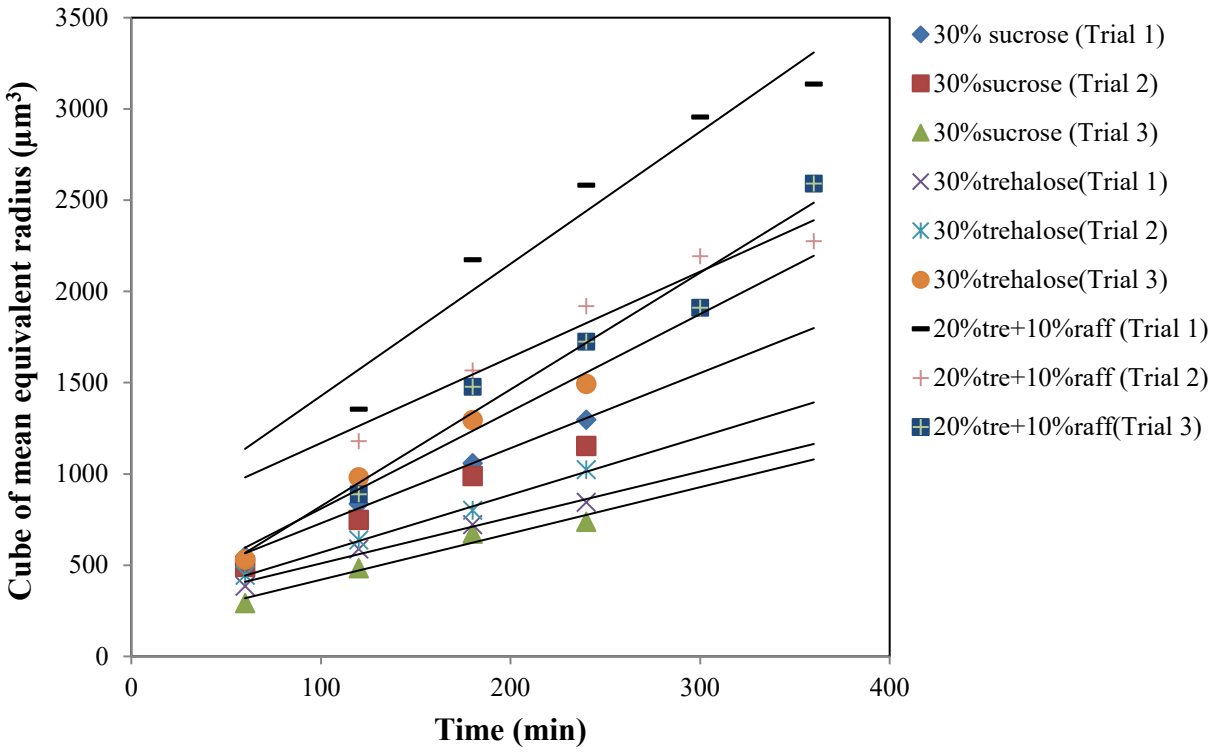


Figure 24 Plots between cube of mean equivalent radius and time during storage ice crystals for saccharide solutions (% w/w) at -10 °C

Table 5 Ice recrystallization rate of saccharide solutions

Temp (°C)	Sample (w/w)	Ice recrystallization rate (*k*) (μm³/min)
-5	30% sucrose	24.28±2.65[b]
	30% trehalose	20.30±3.35[b]
	20% trehalose +10% raffinose	12.43±2.01[a]
-7	30% sucrose	14.72±3.11[b]
	30% trehalose	6.19±1.42[a]
	20% trehalose +10% raffinose	5.07±1.30[a]
-10	30% sucrose	3.46±0.82[a]
	30% trehalose	3.67±1.47[ab]
	20% trehalose +10% raffinose	6.11±1.30[b]

Different superscript letters indicate a significant difference ($p \leq 0.05$) between saccharide solutions at the same temperature within the same column at each temperature.
Values shown are mean ± SD from triplicate measurements

3.3.3 Molecular dynamic analysis

3.3.1 ^{1}H spin-spin relaxation time (T_2)

^{1}H spin–spin relaxation times of proton were measured with the CPMG sequence in various saccharide solutions in freeze-concentrated matrix concentration at -5 °C, -7 °C, and -10 °C. The different relaxation curves of solutions are shown. T_2 is an indicator of proton mobility in the freeze-concentrated matrix in saccharide solutions. Various type of proton belonging to water and solute can contribute to the relaxation curves. The plots were described well by two exponential decay function (Eq. 6) and two T_2 values were obtained, indicating that at least two type of proton with different mobility existed in these samples. Decreasing of relaxation times can be associated with decreasing of proton mobility in the

unfrozen matrix (Herrera et al., 2007). The longer T_2 values correspond to faster proton mobility. We assumed that the longer T_2 was originated from water because it is expected to have a larger mobility than saccharide molecules.

Decreasing temperature resulted in reduction of T_2 of water component (faster mobility) when the solute was same. This may be caused by increasing interaction among water molecules and between water molecules and saccharide molecules. Thus, protons in matrixes with saccharide solutions at -5 ºC were more mobile than protons in matrixes at -7 ºC and -10 ºC, respectively. These results can be related to ice recrystallization rate of sample solutions containing same saccharide (Table 5); ice recrystallization rate at -5 ºC were higher than -7 ºC and -10 ºC, respectively.

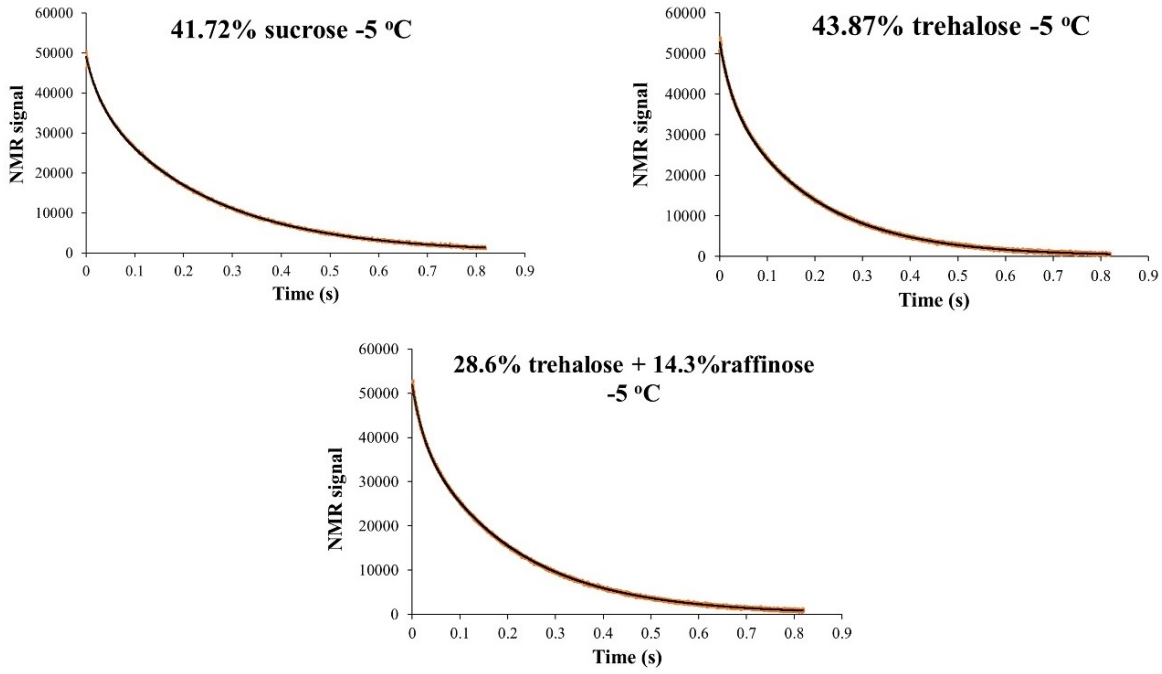


Figure 25 Spin-spin relaxation time of saccharide solutions at -5 ºC. The solid line represents the fitted results by the two exponential functions (Eq. 6).

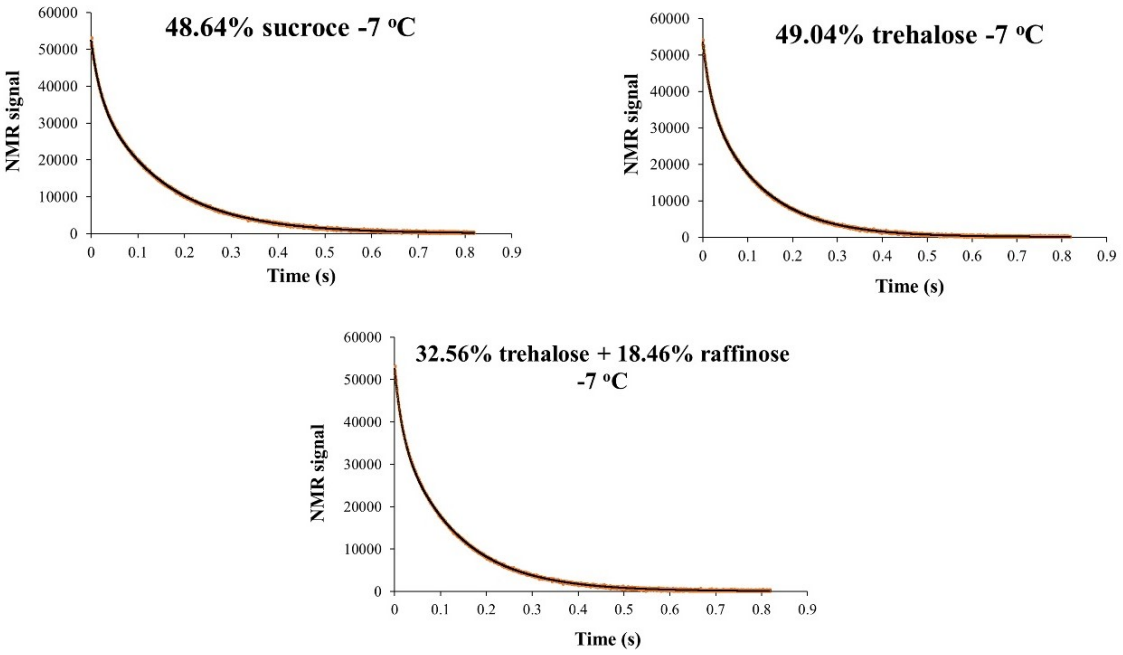


Figure 26 Spin-spin relaxation time of saccharide solutions at -7 ºC. The solid line represents the fitted results by the two exponential functions (Eq. 6).

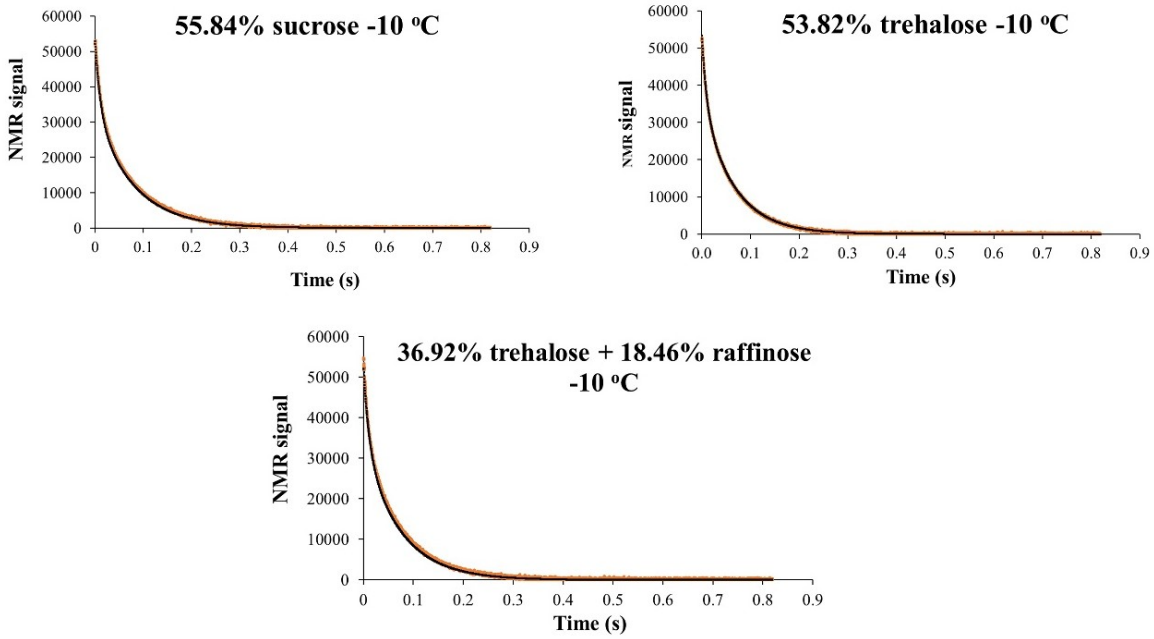


Figure 27 Spin-spin relaxation time of saccharide solutions at -10 ºC. The solid line represents the fitted results by the two exponential functions (Eq. 6).

The ^{1}H spin-spin relaxation time T_2 water component in freeze-concentrated matrix for saccharide solutions at -5 °C, -7 °C, and -10 °C are shown in Table 6. The values of T_2 obtained from trehalose solutions tended to be smaller than those from sucrose solutions at the same temperature while the smaller of T_2 in trehalose and raffinose mixture solutions is obtained only at -5 ºC and -7 ºC. It can be related with the results of ice recrystallization rate in Table 5. Hence, it was suggested that ice recrystallization rate decreased due to decreasing of water mobility in freeze-concentrated matrix.

Table 6 ^{1}H spin–spin relaxation time T_2 of water component, percentage of saccharides and water in freeze-concentrated matrix concentration for saccharide solutions at -5 °C, -7 °C, and -10 °C

Temp (ºC)	**Sample (w/w)**	**T_2 of water component in freeze-concentrated matrix conc. (ms)**	**Saccharides (%)**	**Water (%)**
-5	41.72% sucrose	233.77±1.90^{c}	19.99±1.20^{a}	80.01±1.20^{b}
	43.87% trehalose	188.83±4.53^{a}	22.48±0.56^{b}	77.52±0.56^{a}
	28.6% trehalose +14.3% raffinose	211.87±4.87^{b}	21.30±0.39^{ab}	78.70±0.39^{ab}
-7	48.64% sucrose	144.43±4.40^{c}	26.59±1.20^{a}	73.41±0.56^{a}
	49.04% trehalose	118.37±8.58^{a}	27.38±0.51^{a}	72.62±0.51^{a}
	32.56% trehalose +16.28% raffinose	131.82±2.62^{b}	27.50±0.52^{a}	72.50±0.52^{a}
-10	55.84% sucrose	78.65±3.97^{b}	36.78±0.82^{b}	63.22±0.82^{a}
	53.82% trehalose	65.81±6.82^{a}	33.75±1.00^{a}	66.25±1.00^{b}
	36.92% trehalose +18.46% raffinose	76.62±6.61^{ab}	35.20±1.59^{ab}	64.80±1.59^{ab}

Different superscript letters indicate a significant difference ($p \leq 0.05$) between saccharide solutions at the same temperature within the same column at each temperature.
Values shown are mean ± SD from triplicate measurements

3.3.2 Dielectric relaxation

The dielectric relaxation was measured only at -5 ºC and -7 ºC because trehalose and raffinose were precipitated from the solutions when decreasing temperature to -10 ºC. Slow and fast relaxation process of dielectric loss spectra (ε'') were observed of all samples in this study and were fitted well by the double Cole-Cole equation as shown in Figure 28 and Table 7. Dielectric relaxation time (τ) is the parameter that reflects the mobility of dipole in solution. As same as in Chapter 2, we assumed that the fast relaxation came from the orientation of free water molecules (free water) and slow one was reflected by the orientation of water molecules interacting cooperatively with saccharide molecules. The fast relaxation time (τ) of free water for sucrose, trehalose, trehalose and raffinose mixture solutions were not significant difference (p>0.05) at same temperature -5 ºC and -7 ºC even though the trend of increase in fast relaxation time of trehalose and raffinose mixture, trehalose, and sucrose were observed. The dielectric strength ($\Delta\varepsilon$) of fast relaxation were also not significantly different (p>0.05). This suggested that the number of water molecules contributing to the fast relaxation were not significantly different.

Table 7 Dielectric strength ($\Delta\varepsilon$) and relaxation time (τ) of saccharide solutions

Sample	Dielectric strength ($\Delta\varepsilon$)		Relaxation time (τ)	
	$\Delta\varepsilon_{Slow\ water}$	$\Delta\varepsilon_{Fast\ water}$	$\tau_{Slow\ water}$	$\tau_{Fast\ water}$
41.72%sucrose -5 ºC	10.27±1.37^{a}	43.04±2.42^{a}	1.18±0.17^{ab}	0.111±0.007^{a}
43.87%trehalose -5 ºC	11.14±0.39^{a}	42.17±3.31^{a}	0.95±0.11^{a}	0.116±0.003^{ab}
28.6%trehalose+ 14.3%raffinose -5 ºC	9.71±2.19^{a}	41.68±1.41^{a}	1.21±0.10^{ab}	0.122±0.004^{ab}
48.64%sucrose -7 ºC	16.22±2.10^{b}	43.09±2.69^{a}	1.34±0.02^{b}	0.129±0.007^{bc}
49.04%trehalose -7 ºC	15.49±1.78^{b}	38.44±4.46^{a}	1.15±0.27^{ab}	0.138±0.012^{cd}
32.56%trehalose+ 16.28%raffinose -7 ºC	12.05±0.99^{a}	44.59±4.29^{a}	1.41±0.04^{b}	0.144±0.002^{d}

Different superscript letters indicate a significant difference ($p\leq$0.05) means in the same column.

Values shown are mean ± SD from triplicate measurements

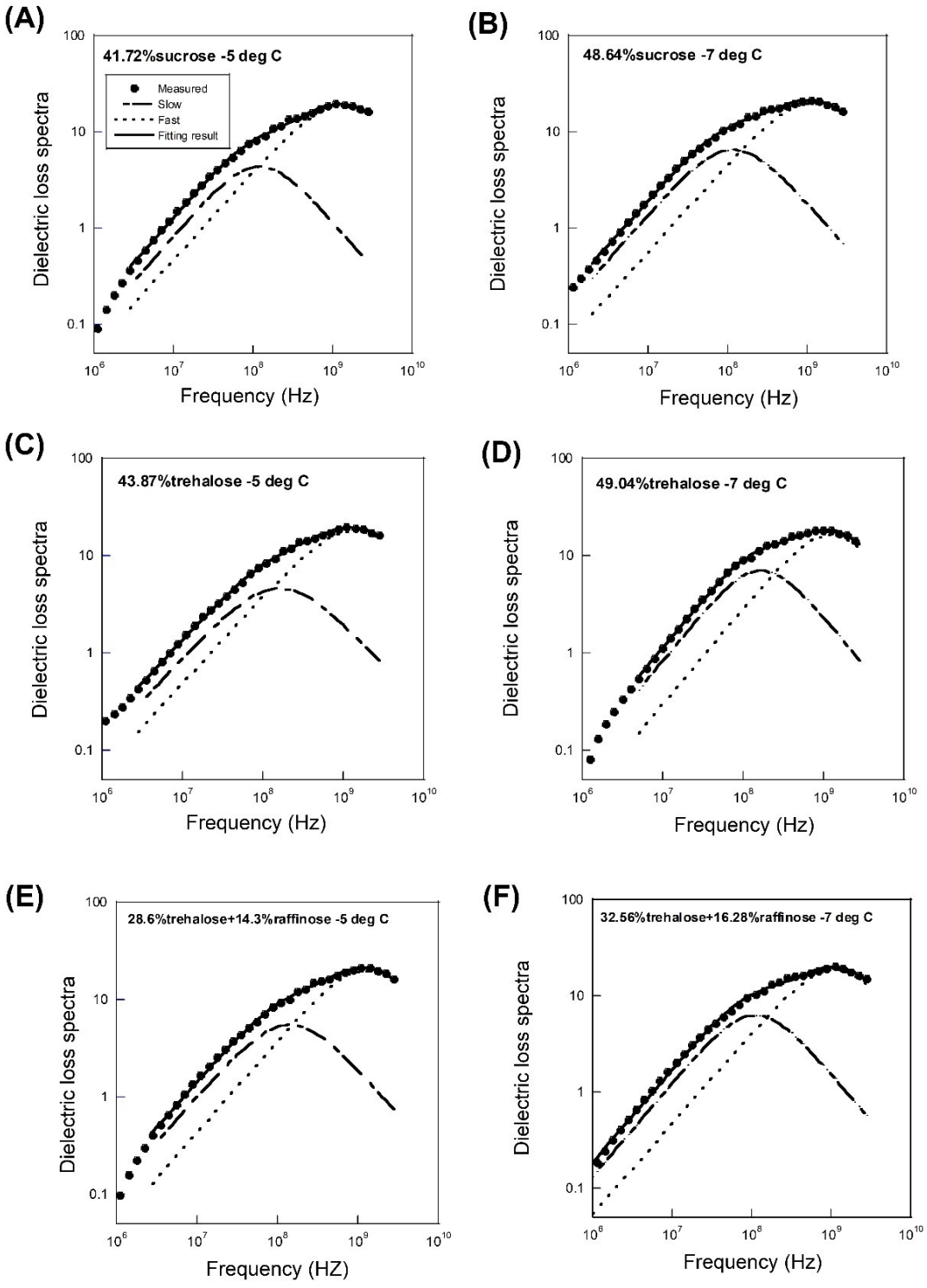


Figure 28 Frequency dependences of dielectric loss spectra for various saccharide solutions at various temperatures. The solid curves were obtained by fitting using Cole-Cole equation (Eq.2-4)

3.3.4 Correlation between ice recrystallization rate (*k*), ^{1}H spin-spin relaxation time (T_2), and dielectric relaxation time (τ)

Figure 29 shows plot between the recrystallization rate of ice crystal and T_2 of water component of sucrose, trehalose, trehalose and raffinose mixture solutions. Direct correlations between ice recrystallization rate and T_2 water component was not observed (R^2=0.76). The correlation between ice recrystallization rate and dielectric relaxation time of free water is also presented in Figure 30. The results showed better linear correlation (R^2 = 0.91). Moreover, plot between T_2 water component and dielectric relaxation of free water is also comparison in Figure 31. The correlation coefficient (R^2) was 0.82. The results showed better correlations only between ice recrystallization rate and dielectric relaxation. This may be caused by the fact that dielectric relaxation measurement estimated the molecular mobility of free water in solution better than ^{1}H NMR spin-spin relaxation measurement, especially the mixture of trehalose and raffinose solution. The higher of T_2 in mixture solutions may be due to the exchangeable between labile solute hydroxyl protons (OH) and water protons of raffinose. Then, it provided the contribution of relaxation time in mixture solutions (Fabri et al., 2005).

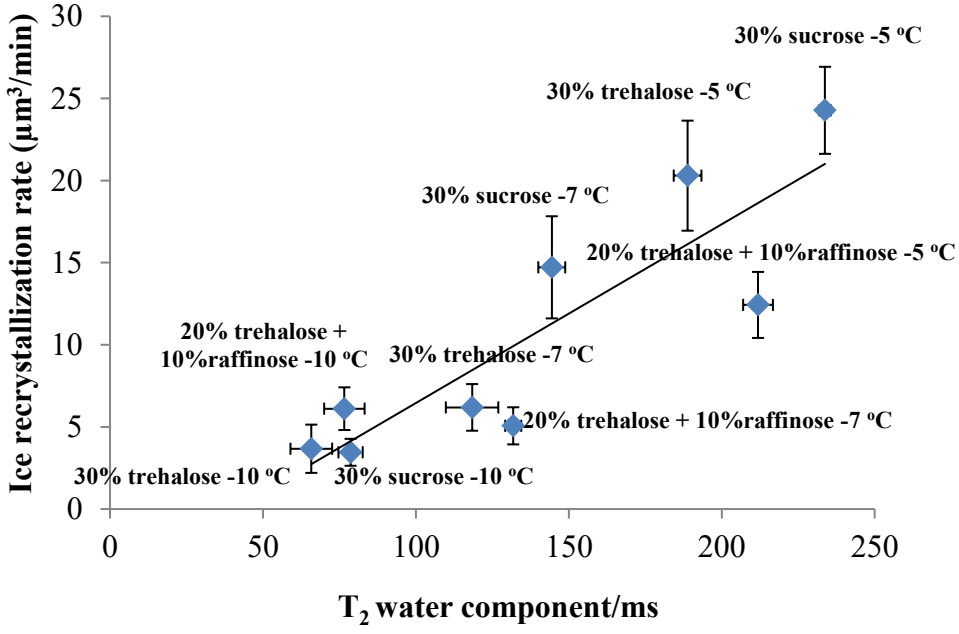


Figure 29 Correlation between ice recrystallization rate of various saccharide solutions and T_2 of water component at -5 ºC, -7ºC, and -10 ºC. The solid line represents result of linear fitting.

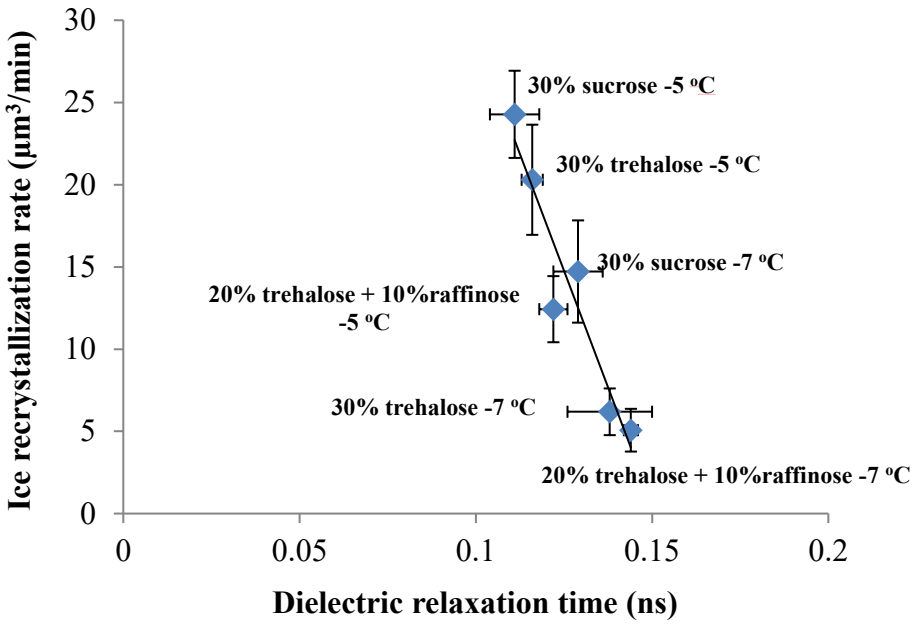


Figure 30 Correlation between ice recrystallization rate and dielectric relaxation time of various saccharide solutions in freeze-concentrated matrix at -5 °C and -7 °C. The solid line represents result of linear fitting.

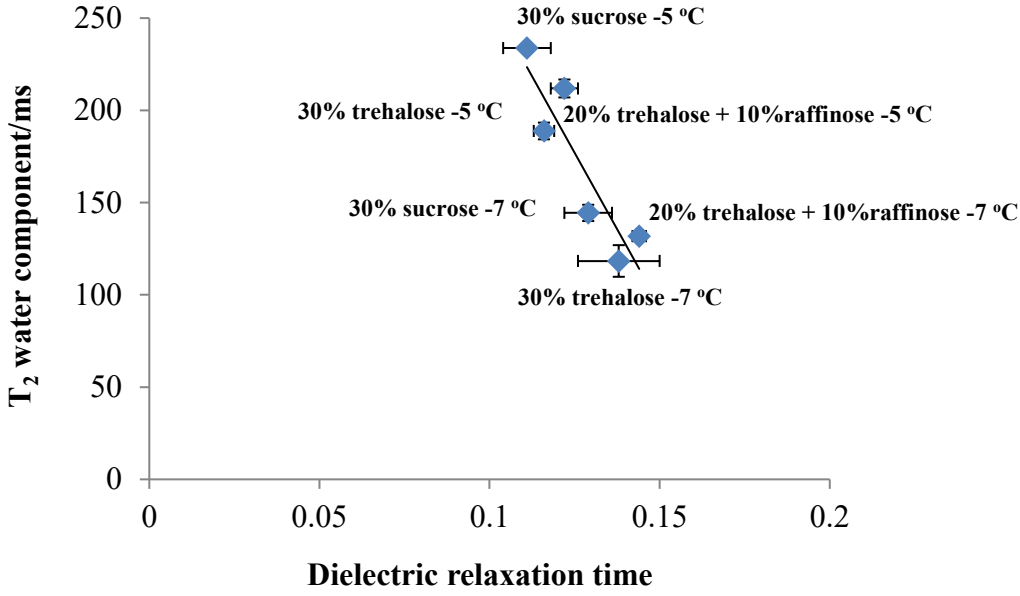


Figure 31 Correlation between T_2 of water component and dielectric relaxation time of various saccharide solutions at -5 °C and -7 °C in freeze-concentrated matrix. The solid line represents result of linear fitting.

Moreover, the correlation between water mobility values (T_2 water component and dielectric relaxation time of free water) and ice recrystallization rate in trehalose and sucrose solutions were also calculated. Figure 32 shows plot between the recrystallization rate of ice crystal and T_2 of water component in sucrose and trehalose solutions. Direct correlations between ice recrystallization rate and T_2 of water component was obtained with R^2=0.95. The correlation between ice recrystallization rate and dielectric relaxation was also presented in Figure 33. The results showed excellent linear correlation (R^2 = 0.97). Moreover, plot between T_2 water component and dielectric relaxation was also comparison in Figure 34. Results showed good correlation between T_2 water component and dielectric relaxation. The correlation coefficient (R^2) was 0.95. Therefore, these results suggested that ice recrystallization rate difference between trehalose and sucrose solution was explained well by the difference of both both T_2 of water component and the faster dielectric relaxation time.

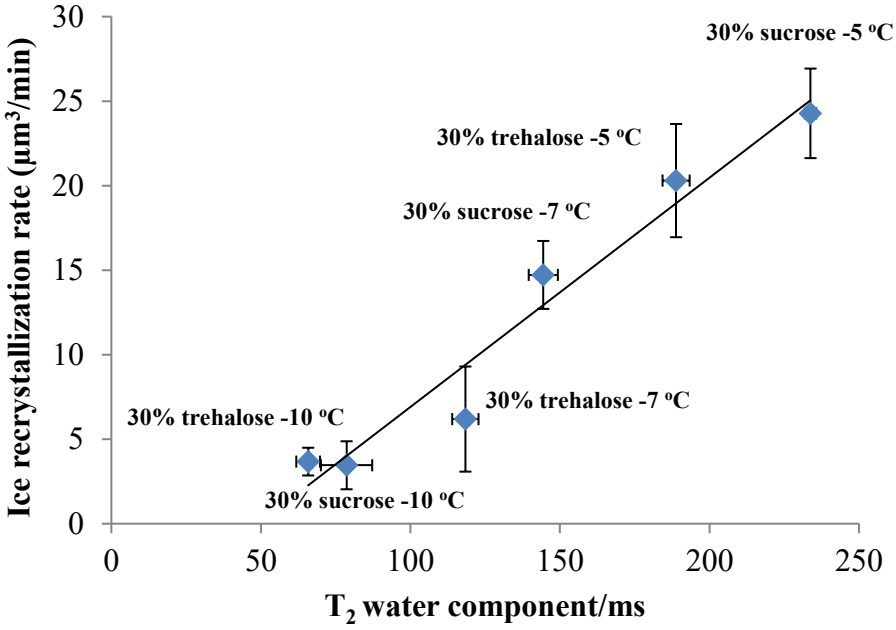


Figure 32 Correlation between ice recrystallization rate of sucrose and trehalose solutions and T_2 of water component in freeze-concentrated matrix at -5 ºC, -7ºC, -10 ºC. The solid line represents result of linear fitting.

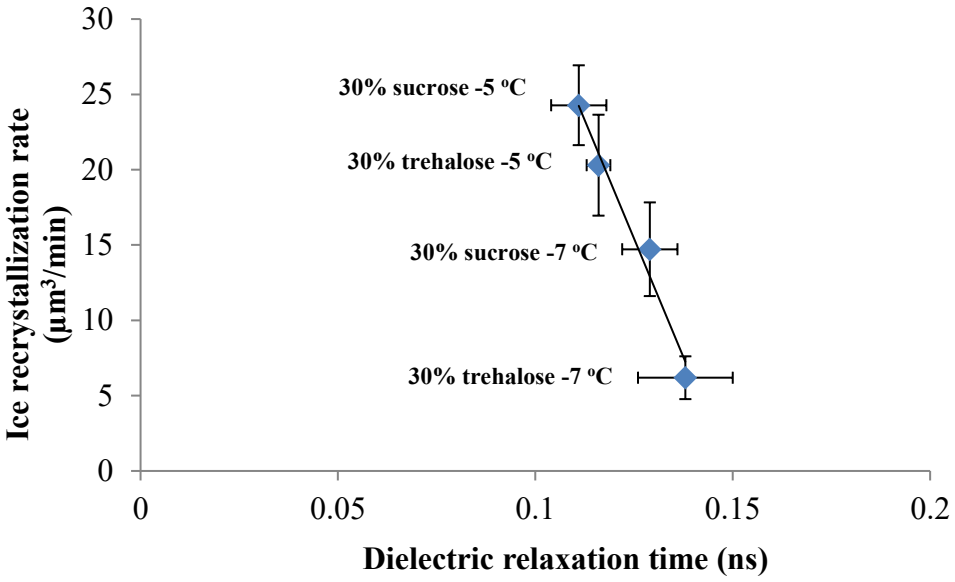


Figure 33 Correlation between ice recrystallization rate and dielectric relaxation time of sucrose and trehalose solutions in freeze-concentrated matrix at -5 ºC and -7 ºC. The solid line represents result of linear fitting.

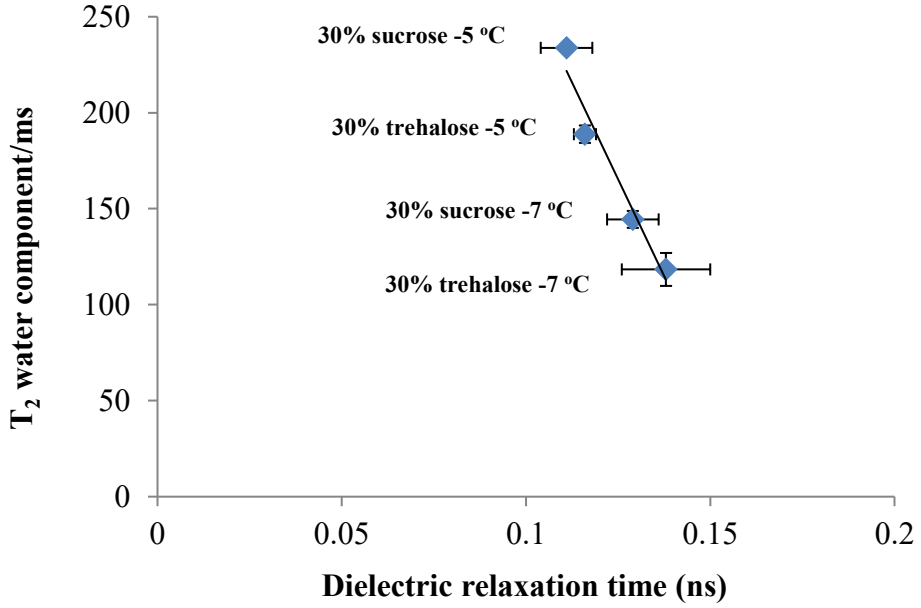


Figure 34 Correlation between T_2 of water component and dielectric relaxation time of sucrose and trehalose solutions in freeze-concentrated matrix at -5 ºC and -7 ºC. The solid line represents result of linear fitting.

The smaller ice recrystallization rate of a mixture trehalose and raffinose solution was observed at -5 ºC and -7 ºC. As for trehalose solution, although significant clear difference of ice recrystallization rate (*k*) between sucrose and trehalose at -5 ºC and -10 ºC was not appeared, the trend of a smaller ice recrystallization rate constant of trehalose solution and a mixture of trehalose and raffinose solution were observed as shown in Table 5. The trend of a smaller ice recrystallization rate constant of the trehalose solution against sucrose solution at same temperature might be explained by water mobility (Ablett et al., 2002; Hagiwara et al., 2006) both T_2 spin-spin relaxation time and dielectric relaxation time, as shown in Figure 32 and 33. During the ice crystal recrystallization process, water molecules on the surfaces of smaller ice crystals or more curved surface areas migrate translationally to the surfaces of larger ice crystals or less curved surfaces through the freeze-concentrated matrix because of greater surface energy, thereby markedly increasing the mean size of ice crystals occurs. Therefore, the translational mobility of water in freeze-concentrated matrix is an important factor determining the ice crystal recrystallization rate (Hagiwara et al., 2006). Although the ^{1}H spin–spin relaxation time (T_2) itself reflects the rotational mobility of component having hydrogen atoms (Ablett et al., 1973; Ablett et al., 2002), it can also be used as an index of translational mobility in malto-oligomer solutions (Ablett et al., 1973). Actually, Ablett et al. (2002) reported positive good correlation between isothermal ice recrystallization rates in a series of frozen saccharide solutions (glucose, maltose, maltotriose, and pullulan) and their T_2 values of water component in freeze-concentrated matrix. A smaller T_2 value was associated with a lower recrystallization rate constant. The observed trend of a smaller ice recrystallization rate constant in trehalose solutions and mixture of trehalose and raffinose solutions (Table 5) and smaller T_2 (Table 6) at the same temperature against sucrose solutions agrees with the results reported by Ablett et al. (2002) which suggested that the trend of smaller ice recrystallization rate constant of saccharide solutions was caused by different water mobilities between these saccharide solutions.

The mechanism causing slower water mobility in trehalose solution is the difference of their hydration number that effect on changing of the hydrogen bonding structure in the solution (Lerbret et al., 2005). It may be reasonable to assume that the water molecules in saccharide solution is categorized in 2 groups; water molecules having little interaction with saccharide molecules and those associating strongly with saccharide molecule through

hydrogen bond. Since the interaction between saccharide and water is comparable to those of water-water hydrogen bonds (Fennema, 1996), the exchange between free water and associating water occurs (Okada et al., 2002; Ramadugu et al., 2009). The net water mobility will be affected by both of water mobilities and the ratio of associating water to free water. It was reported that rotational correlation time of water molecules around trehalose molecule was larger than that of water molecules around sucrose molecules (Uedaira et al., 1989; Uedaira et al., 1990). This means that the mobility of associating water in trehalose solution was slower than that in sucrose solution. Furthermore, it was also reported that trehalose has larger effective hydration number than sucrose (Branca et al., 1999; Branca et al., 2000; Lerbret et al., 2005; Lerbret et al., 2011), suggesting the ratio of associating water to free water for trehalose solution is larger than that for sucrose solution. As the results both two different hydration properties will act to retard net water mobility in trehalose solution, although trehalose has the same molecular weight as sucrose.

3.4 Conclusion

The different ice recrystallization rate of trehalose and sucrose was explained well by the differences in dielectric relaxation time, based on the concept of water mobility in freeze-concentrated matrix. The ^{1}H spin-spin relaxation time T_2 of water component had also fairly reasonable correlation to the ice recrystallization rate. Trehalose showed a tendency to have a lower ice recrystallization rate during storage at isothermal temperatures. However, the mixture of trehalose and raffinose retarded ice recrystallization only at -5 ^{o}C and -7 ^{o}C. It was suggested that the observed trend of smaller ice recrystallization rate constants in trehalose solutions and mixture of trehalose and raffinose solutions were originated from smaller water mobility in freeze-concentrated matrix of these solutions. Results indicated that trehalose and raffinose can be useful as food ingredient in frozen food during storage at constant temperature without deleterious effects from ice crystal recrystallization.

References

Ablett, S., Darke, A. H., Izzard, M. J., & Lillford, P. J. (1993). Studies of the glass transition in malto-oligomers. In Blanshard, J. M. W., Lillford, P. J. (Eds.), The glassy state in foods (pp. 189-206). (1st ed.). Nottingham: Nottingham University Press.

Ablett, S., Clarke, C. J., Izzard, M. J., & Martin, D. R. (2002). Relationship between ice recrystallization rates and the glass transition in frozen sugar solutions. *Journal of the Science of Food and Agriculture, 82,* 1855-1859.

Aidoo, R. P., Depypere, F., Afoakwa, E. O., & Dewettinck, K. (2013). Industrial manufacture of sugar free chocolates-Applicability of alternative sweeteners and carbohydrate polymers as raw materials in product development. *Trends in food science & technology, 32,* 84-96.

Branca, C., Magazù, S., Maisano, G., & Migliardo, P. (2000). Experimental study of the hydration properties of homologous disaccharides. *Journal of Biological Physics, 26,* 295-306.

Branca, C., Magazù, S., Maisano, G., & Migliardo, P. (1999). Anomalous cryoprotective effectiveness of trehalose: raman scattering evidences. *The Journal of Chemical Physics, 111,* 281-287.

Buchanan, S. S., Gross, S. A., Acker, J. P., Toner, M., Carpenter, J. F., & Pyati, D. W. (2004). Cryopreservation of stem cells using trehalose: evaluation of the method using a human hematopoietic cell Line. *Stem Cells and Development, 13,* 295-305.

Budke, C., Heggemann, C., Koch, M., Sewald, N., & Koop, T. (2009). Ice recrystallization kinetics in the presence of synthetic antifreeze glycoprotein analogues using the framework of LSW theory. *The Journal of Physical Chemistry B, 113,* 2865-2873.

Debosch, B. J., Chen, Z., Saben, J. L., Finck, B. N., & Moley, K. H. (2014). Glucose transporter 8 (GLUT 8) mediates fructose-induced de Novo lipogenesis and macrosteatosis. *The Journal of Biological Chemistry, 16,* 10989-10998

Debosch, B. J., Heitmeier, M. R., Mayer, A. L., Higgins, C. B., Crowley, J. R., Kraft, T. E., Chi, M., Newberry, E. P., Chen, Z., Finck, B. N., Davidson, N. O., Yarasheski, K. E., Hruz, P. W., & Moley, K. H. (2016). Trehalose inhibits solute carrier 2A (SLC2A) proteins to induce autophagy and prevent hepatic steatosis. *Science Signaling, 9,* ra21.

Fabri, D., William, M. A. K., & Halstead, T. K. (2005). Water T_2 relaxation in sugar solutions. *Carbohydrate Research, 340,* 889-905.

Fennema, O. R. (1996). Water and Ice. In Food chemistry (pp. 18-94). (3rd ed.). New York: Marcel Dekker.

Fennema, O. R. (1973). Nature of freezing process. In Fennema, O. R., Powrie, W. D., Marth, E. H. (Eds.), Low-temperature preservation of foods and living matter (pp. 160-161). (1st ed.). New York: Marcel Dekker.

Fuchigami, M., Ogawa, N., & Teramoto, A. (2002). Trehalose and hydrostatic pressure effects on the structure and sensory properties of frozen tofu (soybean curd). *Innovative Food Science and Engineering Technologies, 3,* 139-147.

Goff, H. D., & Hartel, R. W. (2006). Ice cream and frozen desserts. In Hui, Y.H. (Eds.), Handbook of food science, technology, and engineering. (1st ed.). Boca Raton: CRC Press.

Gélinas, P., Fiset, G., Leduy, A., & Goulet, J. (1989). Effect of growth conditions and trehalose content on cryotolerance of baker's yeast in frozen dough. *Applied and Environmental Microbiology, 55,* 2453-2459.

Hagiwara, T., Hartel, R.W., & Matsukawa, S. (2006). Relationship between recrystallization rate of ice crystals in sugar solutions and water mobility in freeze-concentrated matrix. *Food Biophysics, 1,* 74-82.

Herrera, M. L., M'Cann, J. I., Ferrero, C., Hagiwara, T., Zaritzky, N. E., & Hartel, R. W. (2007). Thermal, mechanical, and molecular relaxation properties of frozen sucrose and fructose solutions containing hydrocolloids. *Food Biophysics, 2,* 20-28.

Jane, N. K., & Roy, I. (2009). Review: effect of trehalose on protein structure. *Protein Science, 18,* 24-36.

Kimizuka, N., & Suzuki, T. (2007). Supercooling Behavior in Aqueous Solutions. *The Journal of Physical Chemistry B, 111,* 2268-2273.

Kobayashi, R., Kimizuka, N., Watanabe, M., & Suzuki, T. (2014). Effect of supercooled freezing methods on ice structure observed by X-ray CT. *Proceedings of 3rd IIR International Conference on Sustainability and the Cold Chain, London, UK.* 174 of reference number

Kobayashi, R., Kimizuka, N., Watanabe, M., & Suzuki, T. (2015). The effect of supercooling on ice structure in tuna meat observed by using X-ray computed tomograohy. *International Journal of Refrigeration, 60,* 270-277.

Lerbret, A., Bordat, P., Affouard, F., Guinet, Y., Hédoux, A., Paccou, L., Prévost, D., & Descamps, M. (2005). Influence of homogeneous disaccharides on the hydrogen-bond network of water: complementary raman scattering experiments and molecular dynamics simulations. *Carbohydrate Research, 340,* 881-887.

Lerbret, A., Affouard, F., Bordat, P., Hédoux, A., Guient, Y., & Descamps, M. (2011). Slowing down of water dynamics in disaccharide aqueous solutions. *Journal of Non-Crystalline Solids, 357,* 695-699.

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science, 91,* 93-98.

Li, Y. Q., Hu, R., Zhong, L. H., Sun, Q., & Yan, Y. P. (2016). Synergistic effect of trehalose and saccharose pretreatment on maintenance of lyophilized human red blood cell quality. *Tropical Journal of Pharmaceutical Research, 15,* 527-533.

Lide, D.R. (2003). CRC Handbook of Chemistry, Physics, 84th Ed. (pp. 8-81). Boca: CRC Press.

Lifshitz, I.M., & Slyozov, V.V. (1961). The kinetics of precipitation from supersaturated solid solutions. *Journal of Physics and Chemistry of Solids, 19,* 35–50.

Ma, L., Zhang, B., Deng, S., & Xie, C. (2015). Comparison of the cryoprotective effects of trehalose, alginate, and its oligosaccharides on peeled shrimp (Litopenaeus Vannamei) during frozen storage. *Journal of Food Science, 80,* 540-546.

Miller-livney, T., & Hartel, R. W. (1997). Ice recrystallization in ice cream: Interactions between sweeteners and stabilizers. *Journal of Dairy Science, 80,* 447-456.

Miyawaki, O., Saito, A., Matsuo, T., & Nakamura, K. (1997). Activity and activity coefficient of water in aqueous solutions and their relationships with solution structure parameters. *Bioscience, Biotechnology, and Biochemistry, 61,* 466-469

Nagura, T., Muraguchi, H., Uchino, K., Aritsuka, T., & Benno, Y. (1999). Effects of ingestion of raffinose-rich soup on the fecal flora and daily defecation in humans. *Journal of Intestinal Microbiology, 13,* 1–7.

Nicolajsen, H., & Hvidt, A. (1994). Phase behavior of the system trehalose-NaCl-water. *Cryobiology, 31,* 199-205.

Okada, R., Matsukawa, S., & Watanabe, T. (2002). Hydration structure and dynamics in pullulan aqueous solution based on ^{1}H NMR relaxation time. *Journal of Molecular Structure, 602-603,* 473-483.

Osako, K., Hossain, M. A., Kuwahara, K., & Nozaki, Y. (2005). Effect of trehalose on the gel-forming ability, state of water and microfibril denaturation of horse mackerel Trachurus japonicas surimi during frozen storage. *Fisheries Science, 71,* 367-373.

Paul, S., & Paul, S. (2015). Molecular insights into the role of aqueous trehalose solution on temperature-induced protein denaturation. *The Journal of Physical Chemistry B, 119,* 1598-1610.

Ramadugu, S. K., Chung, Y. H., Xia, J., & Margulis, C. J. (2009). When sugars get wet. A comprehensive study of the behavior of water on the surface of oligosaccharides. *The Journal of Physical Chemistry B, 113,* 11003-11015.

Reiser, P., Birch, G. G., & Mathlouthi, M. (1995). Physical properties. In Mathlouthi, M., Reiser, P. (Eds.), Sucrose properties and application (p. 214). Dordrecht: Springer Science+Business Media, Dordrecht.

Ribeiro, R. O. R., Máralém, E. T., Carneiro, C. S., Monteiro, M. L. G., Júnior, C. C., & Jesus, E. F. O. (2014). Detection of honey adulteration of high fructose corn syrup by Low Field Nuclear Magnetic Resonance (LF ^{1}H NMR). *Journal of Food Engineering, 135,* 39-43.

Schönhals, A., & Kremer, F. (2003). Analysis of dielectric spectra. In Kremer, F., Schonhals, A. (Eds.), Broadband dielectric spectroscopy (pp. 59-98). (1st ed.). New York: Springer-Verlag.

Sei, T. & Gonda, T. (2006). Melting point of ice in aqueous saccharide solutions. *Journal of Crystal Growth, 293,* 110-112 (2006)

Sutton, R., Lips, A., Piccirillo, G., & Sztehlo, A. (1996). Kinetics of ice recrystallization in aqueous fructose solutions. *Journal of Food Science, 4,* 741-745.

Storey, B. T., Noiles, E. E., & Thompson, K. A. (1998). Comparison of glycerol, other polyols, trehalose, and raffinose to provide a defined cryoprotectant medium for mouse sperm cryopreservation. *Cryobiology, 37,* 46-58.

Tada, N., Sato, M., Yamanoi, J., Mizorogi, T., Kasai, K., & Ogawa, S. (1990). Cryopreservation of mouse spermatozoa in the presence of raffinose and glycerol. *Journals of Reproduction & Fertility Ltd, 89,* 511-516.

Uedaira, H., Ikura, M., & Uedaira, H. (1989). Natural-abundance oxygen-17 magnetic relaxation in aqueous solutions of carbohydrates. *Bulletin of the Chemical Society of Japan, 62,* 1-4.

Uedaira, H., Ishimura, M., Tsuda, S., & Uedaira, H. (1990). Hydration of oligosaccharides. *Bulletin of the Chemical Society of Japan, 63,* 3376-3379.

Wagner, C. (1961). Theorie der Alterung von Niderschlagen durch Umlösen (Ostwald Reifung). *Z Elektrochem, 65,* 581–591.

Whelan, A. P., Regand, A., Vega, C., Kerry, J. P., & Goff, H. D. (2008). Effect of trehalose on the glass transition and ice crystal growth in ice cream. *International Journal of Food Science and Technology, 43,* 510-516.

Zhou, A., Benjakul, S., Pan, K., Gong, J., & Liu, X. (2006). Cryoprotective effects of trehalose and sodium lactate on tilapia (Sarotherodon nilotica) surimi during frozen storage. *Food Chemistry. 96,* 96-103.

CHAPTER 4

RECRYSTALLIZATION OF ICE CRYSTALS IN SUCROSE SOLUTION CONTAINING POLYSACCHARIDES

4.1 Introduction

Polysaccharides are important components which widely used in frozen foods including ice cream. They are high molecular weight polymers (>10^6 Da) which can improve the rheological and texture properties in food. Locust bean gum is a galactomannan composed of a (1-4)-linked β-D-mannopyranose backbone with (1-6)-linked α-D-galactose side groups as shown in Figure 35A. Xanthan gum is a branched anionic heteropolysaccharide. The primary structure is glucose units and the side chain is a trisaccharide that consists of α-D-mannose which contains an acetyl group, β-D-glucuronic acid, and a terminal β-D-mannose unit linked with a pyruvate group (Figure 35B). Many studies reported that stabilizers are added to ice cream mix. They can retard ice crystal growth. However, the potential of polysaccharides to retard ice crystal growth are not equal even though the same level of viscosity. It depends on type of polysaccharides (Harper and Shoemaker, 1983; Sutton et al., 1997). Polysaccharides used in ice cream formulations are added in relatively small amounts around 0 to 0.50% (Flores and Goff, 1999). Several potential mechanisms were explained both gel stabilizer and non-gelling stabilizers to retard ice crystal, however the exact mechanism was still not clear until now.

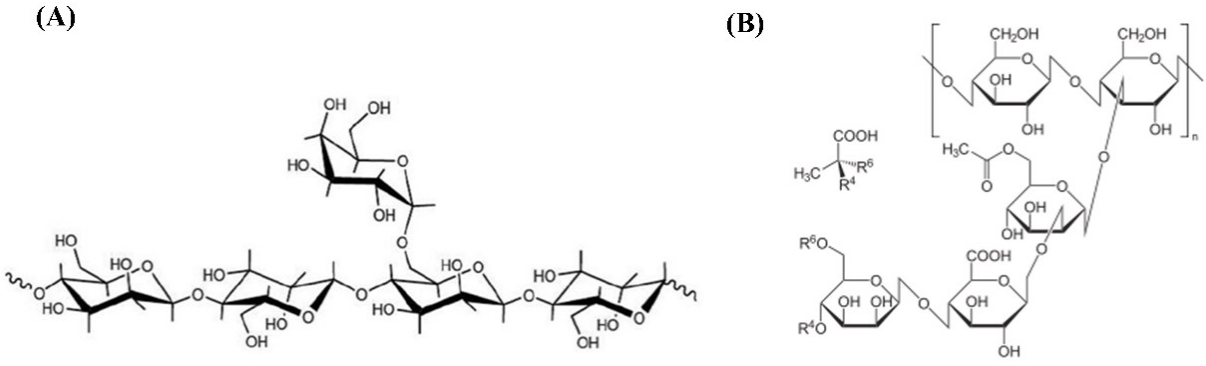


Figure 35 Structure of locust bean gum (A) and xanthan gum (B)

The ability to form cryogel or gel like intermolecular interaction of locust bean gum had effectiveness against ice recrystallization (Sutton et al., 1997). Locust bean gum can form gel-like network around ice crystals after freezing and temperature cycling (Goff et al., 1999; Regand and Goff, 2002). However, a firm gel can be also more fragile and more easily ruptured by the ice front leading to be easily ice crystal growth. In this case, a firm gel cannot be effective to retard ice recrystallization. Moreover, some non-gelling stabilizers such as xanthan can retard ice recrystallization due to steric blocking of ice interface or high viscosity. It had an effective to retard ice recrystallization higher than LBG at the fluctuating temperature (Regand and Goff, 2002). Conversely, Sutton and Wilcox (1998) reported that the effective retarding ice recrystallization of non-gelling such as guar gum, methoxy pectin were not as effective as LBG due to phase separation leading to lack of homogeneity in the system. Various hydrocolloids impart different cryoprotective effects to food products depending on rheological properties and structure. Mostly, the structural of polysaccharides was observed after repeated temperature cycling (Goff et al., 1999; Regand and Goff, 2002). However, no report of the relevant literature described the knowledge of location of polysaccharides in unfrozen phase during freezing at constant temperature by using fluorescence microscope.

In this chapter, the recrystallization behavior of ice crystals in sucrose solution containing polysaccharide was investigated by using locust bean gum (LBG) and xanthan gum (XG) as a model polysaccharide at constant temperature -7 ^{o}C. The effect of polysaccharide on ice recrystallization was discussed by using the measured physical properties in freeze-concentrated matrix such as water mobility, rheological properties, including the microstructure that revealed location of polysaccharides in sample solutions during storage at low temperature.

4.2 Materials and Methods

4.2.1 Materials

Locust bean gum (LBG), xanthan gum (XG), and rhodamine B isothiocyante were purchased from Sigma Aldrich (St Louis, Missouri, USA). Sucrose was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

4.2.2 Sample preparation

Sucrose was dissolved in distilled water at room temperature. The final sucrose concentration was set to 20% (w/w) and 30% (w/w) sucrose as control sample. The following aqueous solutions were prepared; 20% (w/w) sucrose, 20% (w/w) sucrose + 0.30% (w/w) LBG or XG, 20% (w/w) sucrose + 0.50% (w/w) LBG or XG, 30% (w/w) sucrose, 30% (w/w) sucrose + 0.30% (w/w) LBG or XG, 30% (w/w) sucrose + 0.50% (w/w) LBG or XG. The solutions were heated at 40 ºC for 3 h before measuring experiments to ensure complete dissolution of polysaccharides.

4.2.3 Isothermal recrystallization rate constant

Isothermal recrystallization of ice crystals in sucrose solution containing polysaccharide was observed at -7 °C; temperature variability was ±0.05 ºC and using the same method as described in Section 3.2.4. Observation time of each sample solutions was 3 h to obtain ice crystal images suitable for image analysis. Numbers of ice crystal observation per one image were more than 100 ice crystals. To obtain the isothermal recrystallization rate constant, the Lifshitz–Slyozov–Wagner (LSW) theory of Ostwald ripening principle (Lifshitz and Slyozov, 1961; Wagner, 1961) was used (Sutton et al., 1996; Hagiwara et al., 2006; Budke et al., 2009) as in Chapter 3.

4.2.4 Rheological characteristic

The viscoelastic properties of sample solutions containing LBG or XG were measured by using rheometer in oscillatory test (HAAKE MARS II model, Thermo Fisher Scientific Inc., Newington, Germany) with plate-plate (35 mm; gap between plates 1 mm). Mechanical spectra were recorded at a frequency of 1 Hz by stress sweep experiments; a constant deformation γ = 0.01 and measured depending on time (30 min) at -7 ºC. Temperature was set at -7 ºC 10 min before measurement.

4.2.5 Fluorescence microscopy

4.2.5.1 Rhodamine b isothiocyanate labeling of locust bean gum and xanthan gum

Locust bean gum and xanthan gum were labelled with rhodamine b isothiocyanate (RITC) by covalent bond labelling according to the method of Goff et al. (1999). Locust bean gum (LBG) or xanthan gum (XG) (320 mg) was dissolved in 3.2 ml dimethylsulfoxide (DMSO) containing a few drops of pyridine. Then, rhodamine b isothiocyanate (RITC, 32 mg) was added. Then dibutylin dilaurate (as a catalyst) was added 9.6 mg. After that, the solutions were heated at 90 ºC for 2 h. During the heating, sample temperature was measured so that it kept 90 ºC. Polysaccharides were precipitated and filtered by using filter paper and ethanol several times until no further free dye was visible (ethanol after filtering was clear). The precipitated RITC-LBG or RITC-XG was dried overnight at 80 ºC.

4.2.5.2 Sample preparation

Sample solutions were prepared; (1) 20% (w/w) sucrose (dissolved in deionized water), (2) 20% (w/w) sucrose + 0.30% (w/w) RITC-LBG or RITC-XG, (3) 20% (w/w) sucrose + 0.50% (w/w) RITC-LBG or RITC-XG (4) 30% (w/w) sucrose (dissolved in deionized water), (5) 30% (w/w) sucrose + 0.30% (w/w) RITC-LBG or RITC-XG, (6) 30% (w/w) sucrose + 0.50% (w/w) RITC-LBG or RITC-XG. The solutions were heated at 40 ºC for 3 h before measuring experiment.

4.2.5.3 Microscopy and image analysis

Two microliters of sample solution enclosed between two microscope coverslips (16 mm diameter) was placed on the cold stage and loaded on the cold stage (THMS 600; Linkam Scientific Instruments Ltd., Surrey, UK) of the BX53 Digital Fluorescence Microscope (Olympus American, Inc., Center Valley, PA, USA). The method for observing ice crystal and structure of unfrozen phase was similar to that in Section 4.2.3. The temperature was started 30 ºC at 90 °C/min. After 10 min, the sample was cooled to -30 °C at 90 °C/min to be frozen. After 10 min, the sample temperature was elevated to the observation temperature at -7 ºC; temperature variability was ±0.05 ºC. Both the brightfield and fluorescence images of the same field were collected at 20 min.

4.2.6 Dielectric relaxation

Time domain reflectometry (TDR) was employed to measure dielectric relaxation in a frequency range from 1 MHz to 3 GHz. Sucrose solution was prepared by dissolving in deionized water. The sample temperature was set at -7 °C; temperature variability was ±0.1 ºC). A voltage pulse of 230 mV in amplitude was applied to a sample solution through a coaxial probe and the time dependent change in the voltage of the reflected wave was measured using the main frame of digitizing oscilloscope (HP54120B, Hewlett-Packard, USA) and four channel test set (HP54121A, Hewlett-Packard, USA) with the following parameter values: time interval 900 ps/div, delay (time offset relative to trigger) 26.00 ns, and voltage sensitivity 50 mV/div for setting waveform characteristic. A probe with pin diameter d = 1.0 mm and electric length γd = 2.001165 mm was used. The reflected wave was accumulated 2048 times for each measurement. During the experiments, the sample temperature was monitored with digital thermometer (7563, Yokogawa meters & instruments corp., Japan). Frequency dependences of dielectric loss spectra were fitted by double Cole-Cole function (Schönhals & Kremer, 2003) using Eq. 2-4 in Section 2.2.3.

4.2.7 Statistical analysis

All measurements were conducted in triplicate. Mean values were compared by analysis of variance (ANOVA) and Duncan's multiple range test for comparing treatments ($p \leq 0.05$). All calculations were done using the SPSS 17.0 software for windows (SPSS Inc., Chicago, IL, USA).

4.3 Results and discussion

4.3.1 Ice recrystallization behavior

Figure 36 and 37 show typical ice crystals images in 20% (w/w) sucrose and 30% (w/w) sucrose in the presence of locust bean gum (LBG) and xanthan gum at both the concentration of 0.30% (w/w) and 0.50% (w/w). The 20% (w/w) and 30% (w/w) sucrose solution were used as a control. Ice crystals grew with increasing time for all sample examined, demonstrated that ice recrystallization occurred. High concentration of LBG (0.50% LBG in 20% sucrose solution) led to heterogeneous ice crystal during freezing as shown in Figure 36. It may be due to the ability of LBG to form a gel-like network (Regand and Goff, 2003). Therefore, it was hard to calculate the recrystallization rate of ice crystal at this condition. Moreover, the ice crystals size of 20% (w/w) sucrose solution in the presence of xanthan gum was larger when compared with 20% (w/w) sucrose solution with and without LBG.

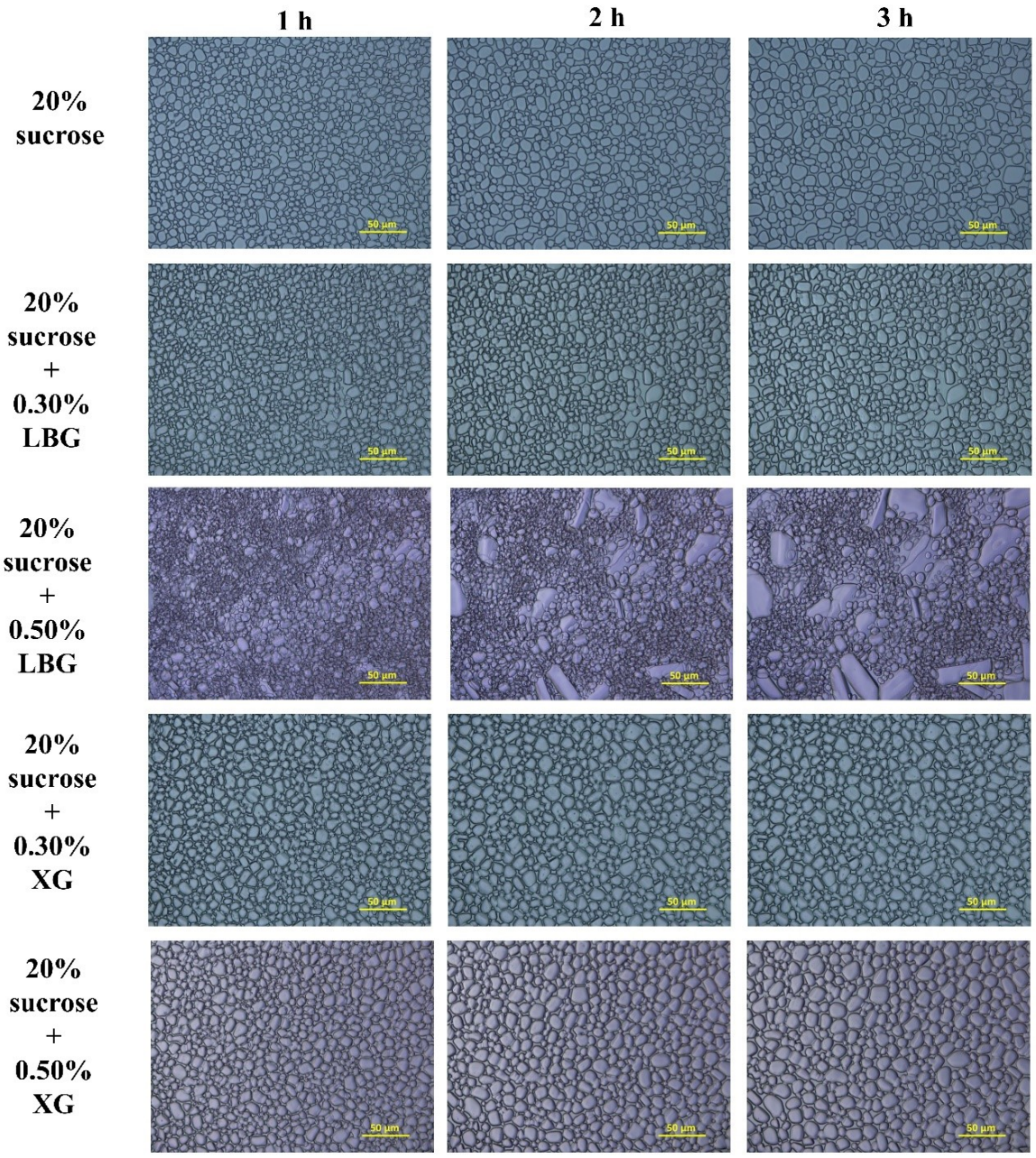


Figure 36 Typical images of ice crystals of 20% (w/w) sucrose with and without the addition of LBG and xanthan at -7 ºC. Bar = 50 µm (All images)

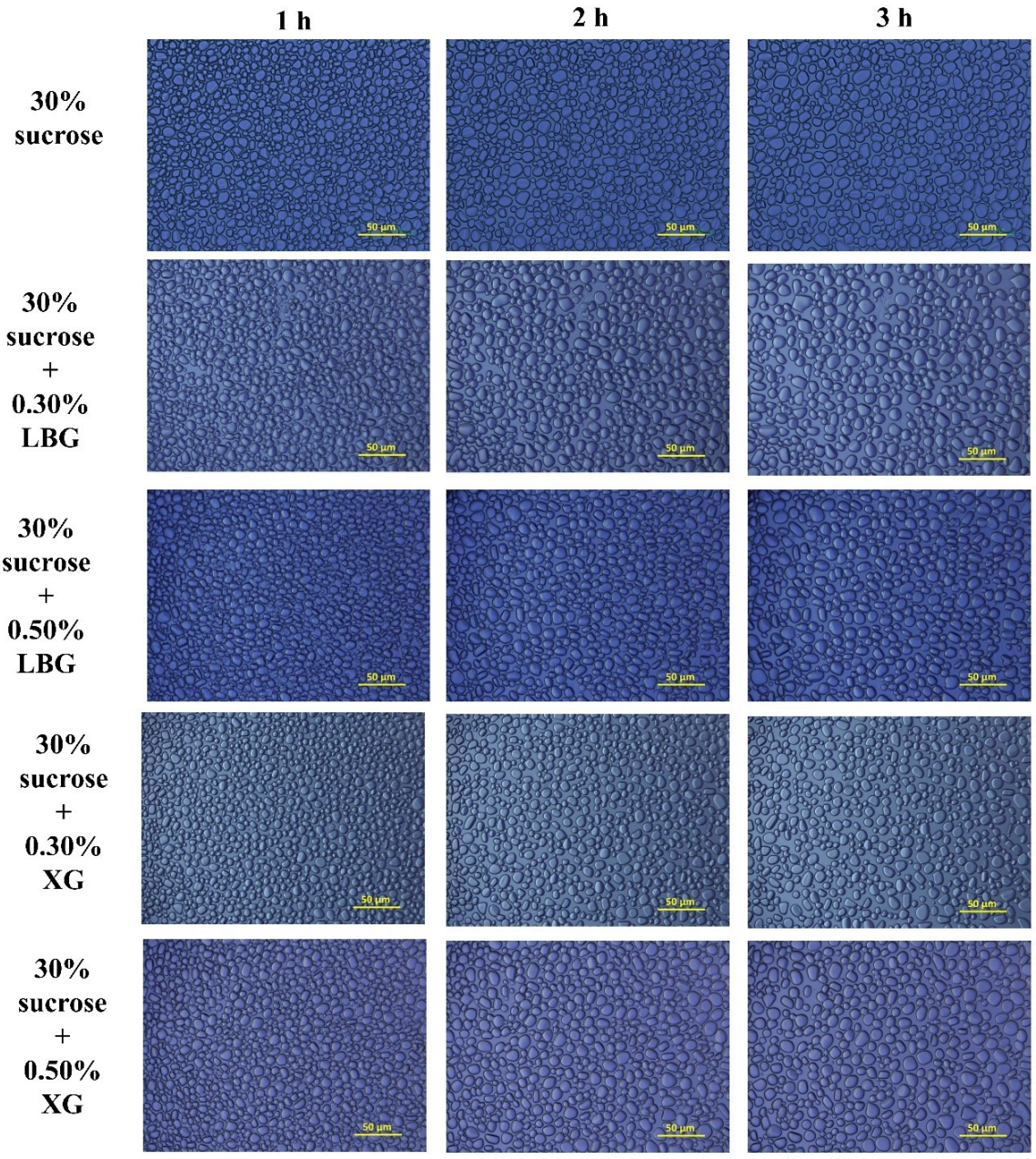


Figure 37 Typical images of ice crystals of 30% (w/w) sucrose with and without the addition of LBG and xanthan at -7 ºC. Bar = 50 µm (All images)

Figure 38 and 39 show plots of the cube of the number-averaged radius of ice crystal r^3 vs. time (t). The plots show a linear tendency and the isothermal rate constant k was evaluated by using Equation 5 (Section 3.2.4). Values of k from 20% (w/w) sucrose and 30% (w/w) sucrose in the presence of LBG and xanthan at both the concentrations of 0.30% (w/w) and 0.50% (w/w) were compared in Table 8. Results showed that LBG and xanthan did not retard ice recrystallization rate in 30% (w/w) sucrose solution at isothermal temperature. Harper and shoemaker (1983) also reported that locust bean gum in the concentration range of 0-0.5% was not effective to retard ice crystal growth at fluctuating temperature. From Table 8, the initial of ice crystal size at 60 min of all sample solutions were not different except 20% (w/w) sucrose solution containing xanthan gum. The presence of xanthan gum led to the highest ice recrystallization rate in 20% (w/w) sucrose solution system. It may be due to the presence of gel structure in this condition. However, in 30% (w/w) sucrose solution system, unfrozen phase of 30% sucrose (unfrozen water 41.91%) are higher than that of 20% sucrose (unfrozen water 27.94%) when calculated from freezing point depression curve (Figure 16). Homogeneity of ice crystal size in 30% (w/w) tended to be larger than that of 20% (w/w) sucrose system. Therefore, a tendency of increasing the ice crystal size of xanthan gum in 30% (w/w) sucrose were smaller than in 20% (w/w) sucrose solution. The results revealed that increasing polysaccharides did not always give smaller ice recrystallization rate constant.

Polysaccharides had not influence on the freezing properties such as freezing point depression, enthalpy of total ice melting, resulting in not different the amount of freezable water in the system (Harper and Shoemaker, 1983; Miller-livney and Hartel, 1997; Flores and Goff, 1999; Herrera et al., 2007). However, the difference of polysaccharides at same concentration influence on the differences of ice recrystallization rate. The structural and elastic behavior properties including water mobility in freeze-concentrate matrices may play an important role in affecting the ice recrystallization behavior in polysaccharide system.

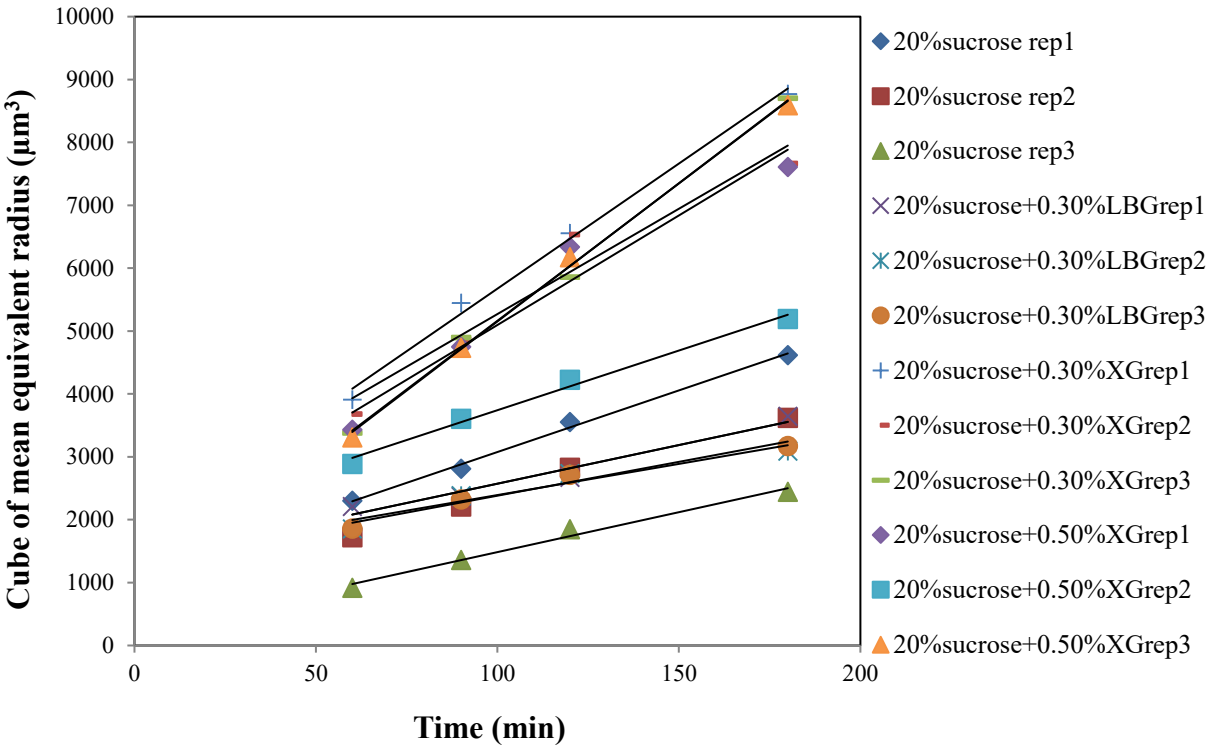


Figure 38 Plots between cube of mean equivalent radius and time during storage ice crystals for 20% (w/w) sucrose solution with and without the addition of LBG and xanthan at -7 °C

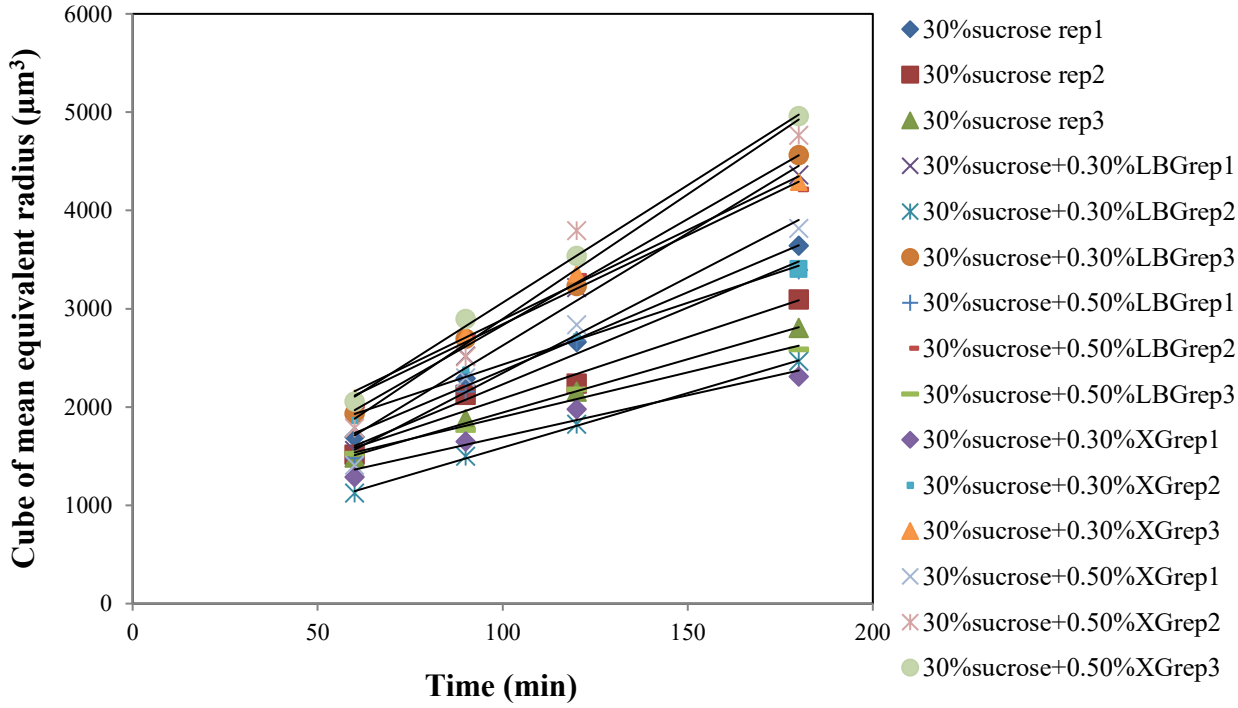


Figure 39 Plots between cube of mean equivalent radius and time during storage ice crystals for 30% (w/w) sucrose solution with and without the addition of LBG and xanthan at -7 °C

Table 8 Ice recrystallization rate of sucrose solution containing locust bean gum and xanthan gum at -7 ºC

Treatment	60 min	180 min	Ice recrystallization rate (μm³/min)
20%sucrose	11.64±1.76	15.16±1.60	16.07±3.45^{ab}
20%sucrose+0.30%LBG	12.54±0.43	14.88±0.45	10.98±1.20^{a}
20%sucrose+0.50%LBG	heterogeneous	heterogeneous	heterogeneous
20%sucrose+0.30%XG	15.40±0.38	20.30±0.51	38.98±5.14^{d}
20%sucrose+0.50%XG	14.74±0.44	19.16±1.64	32.59±12.64^{cd}
30%sucrose	11.60±0.28	14.69±0.65	14.72±3.11^{ab}
30%sucrose+0.30%LBG	11.48±1.03	15.47±1.71	18.52±6.46^{ab}
30%sucrose+0.50%LBG	11.84±0.68	14.97±1.21	14.27±4.69^{ab}
30%sucrose+0.30%XG	11.98±0.99	14.84±1.53	13.06±4.93^{ab}
30%sucrose+0.50%XG	12.02±0.76	16.50±0.77	22.91±3.07^{bc}

Different superscript letters indicate a significant difference ($p \leq 0.05$) between samples at same column.
Values shown are mean ± SD from triplicate measurements

4.3.2 Rheological characteristic

The concentration of sample solutions in freeze-concentrate matrices were obtained from freezing point depression curve in Chapter 3 (Figure 18). The ratio between sucrose and polysaccharide in solutions were set as same as the sample preparation for observing ice crystal growth because the addition of stabilizers did not effect on freezing point of the solution (Harper and Shoemaker, 1983; Miller-livney and Hartel, 1997; Sutton et al., 1997; Flores and Goff, 1999; Herrera et al., 2007). Oscillation time sweep was used to observe how the materials change over time.

Table 9 shows storage (G') and loss (G") modulus of sucrose solutions containing locust bean gum and xanthan gum at -7 ºC 30 min in freeze-concentrated concentration. Storage modulus (G') is a useful parameter to measure the elasticity or solid-like character of the system. The values of G' and G" of locust bean gum and xanthan gum enhanced when the polysaccharide concentration increased. Both LBG and xanthan formed weak gel at high concentration. Elastic behavior 0.50% (w/w) LBG in 20% (w/w) sucrose solution was the highest. High elastic behavior of solution in freeze-concentrated matrix led to heterogeneity of ice crystal. The location of polysaccharides during freezing were also clarified by using

fluorescence microscopy in microstructure analysis part and water mobility in these systems were also clarified by dielectric relaxation measurement.

Table 9 Storage (G') and loss (G") modulus of sucrose solution containing locust bean gum and xanthan gum at -7 ºC 30 min in freeze-concentrated matrix

Sample (Freeze-concentration)	Sample	Storage modulus G'(Pa)	Loss modulus G" (Pa)
48.64% sucrose	20% or 30% sucrose	0.05±0.01[a]	0.37±0.01[a]
48.64%sucrose+0.73%LBG	20%sucrose+0.3%LBG	10.93±1.18[c]	13.46±1.02[d]
48.64%sucrose+1.22%LBG	20%sucrose+0.5%LBG	43.90±1.73[g]	36.89±1.50[g]
48.64%sucrose+0.73%XG	20%sucrose+0.3%XG	19.27±1.57[d]	15.59±3.41[de]
48.64%sucrose+1.22%XG	20%sucrose+0.5%XG	40.28±3.61[f]	28.51±2.84[f]
48.64%sucrose+0.49%LBG	30%sucrose+0.3%LBG	3.11±0.62[b]	5.77±0.66[b]
48.64%sucrose+0.81%LBG	30%sucrose+0.5%LBG	11.10±1.42[c]	13.60±1.32[d]
48.64%sucrose+0.49%XG	30%sucrose+0.3%XG	12.42±1.29[c]	9.44±1.05[c]
48.64%sucrose+0.81%XG	30%sucrose+0.5%XG	23.68±2.51[e]	18.73±2.16[e]

Different superscript letters indicate a significant difference ($p \leq 0.05$) between samples at same column. Values shown are mean ± SD from triplicate measurements

4.3.3 Microstructural analysis

Fluorescence microscopy images showed clear different microstructure between sample solutions during freezing process**.** Locust bean gum and xanthan gum were labeled with rhodamine b isothiocyanate, which enables us to visualize the location of polysaccharides in the unfrozen phase (red regions). Brightfield (left images) and fluorescence (right images) of frozen sucrose solution with the addition of LBG and xanthan are shown in Figure 40 and 41, respectively.

LBG was freeze-concentrated during the first freezing process at -30 ºC. At the high concentration of LBG, namely 0.50% (w/w) LBG in 20% (w/w) sucrose condition, the freeze-concentrated matrix was gelled and high elastic behavior when the concentration of LBG increased beyond a gelation threshold concentration. Then, the sucrose was entrapped inside the network structure of the gel. However, the gel network distribution was inhomogeneous in freeze-concentrated solution. Locust bean gum - gel like network structure (red region) was located surrounding the ice crystals as shown in Figure 40 and it may have effect on the ice

crystal growth due to preventing the freely mixing between melted ice and solute. The concentration was not equilibrium in the system leading to heterogeneity of ice crystal size. Three phases, namely, ice, gel matrix containing highly concentrated sucrose, and the liquid phase with lower concentrated sucrose may existed in the system when the temperature increased to observation temperature at -7 ºC. The ice phase content of sucrose solution with the present of LBG was larger than that without LBG.

To calculate ice phase content of 20% (w/w) sucrose solution with and without gelling stabilizers (LBG and xanthan), schematic diagram is shown in Figure 42(A), (B) and (C). In the case of sucrose solution without gelling stabilizer (Figure 42A), freeze-concentration of 20% sucrose at -30 ºC was 78.016 % that was calculated from fitting equation by the quartic polynomial function of freezing point depression curve for sucrose in Figure 18 ($y = -0.00000137x^4 + 0.000101x^3 – 0.00421x^2 – 0.02002x – 0.02157$). Ice phase content was 74.36% (w/w). After increasing temperature to -7 ºC, freeze-concentration of sucrose was 48.64% (w/w) and ice phase content was 58.8% (w/w). As for 20% (w/w) sucrose solution containing gelling stabilizer, we assumed 0.50% locust bean gum (Figure 42B) because the gelation of freeze-concentrated matrix occurred in this concentration. Freeze-concentration of solutions at -30 ºC was 78.016 %. The concentration of solution in freeze-concentrated matrix was the same as solution without stabilizer because the addition of stabilizers did not effect on freezing point of the solution. Ice phase content was 73.96% (w/w). After increasing temperature to -7 ºC and ice started melting, assuming 80% of sucrose is still entrapped inside the gel matrix. The sucrose entrapped inside the gel matrix continued to move to the liquid phase according to the concentration difference between two phases. Therefore, sucrose content in the liquid phase increased (60.80% (w/w)). Assuming, sucrose can be released on 20% from gel matrix. Ice phase content will be increased (65.06% (w/w)). The larger ice phase content resulted in more frequent accretion of ice crystals. This result promoted the growth of ice crystals. As for the sucrose entrapped inside the gel matrix, it continued to move to the liquid phase according to the concentration difference between two phases. Therefore, sucrose content in the liquid phase increased. Heterogeneity of ice crystal occurred due to the difference of sucrose concentration in the system. In order to keep phase equilibrium between the liquid phase and ice phase, ice continued to melt even after the temperature reached to -7 ºC for a significant long period. This melting also promoted the growth of ice crystals.

Moreover, gel-like network structure of LBG was also observed after freezing and more clarify when temperature cycling (Regan and Goff, 2002). These may be the reasons why heterogeneous of ice crystals were observed in the condition of 20% (w/w) sucrose containing 0.50% (w/w) LBG. From Figure 39, homogeneity of unfrozen phase containing LBG (red region) can be found in 20% (w/w) sucrose + 0.30% (w/w) LBG, 30% (w/w) sucrose + 0.50% (w/w) LBG, and 30% (w/w) sucrose + 0.30% (w/w) LBG. Similarity of ice crystals (black regions) in solutions were observed and can be related to not significantly different of ice recrystallization rate among these samples in Table 8.

Figure 41 shows fluorescence micrograph of 20% (w/w) and 30% (w/w) sucrose solution containing xanthan gum. The black regions represented ice crystal and red regions represented the location of xanthan gum. Ice crystals of 20% (w/w) sucrose solution containing xanthan gum were larger than those of 30% (w/w) sucrose solution relate to the increasing of ice recrystallization rate in 20% (w/w) sucrose solution containing xanthan gum. It may be caused by the larger ice phase content due to phase separation by cryogelation during freezing process. The mechanism causing larger ice recrystallization rate constant may be possible as follows; sucrose molecules were entrapped in the cryogel matrix during initial freezing process. As the results, the frequent accretion of ice crystals increased and then promoted the ice crystal growth as schematic diagram shows in Figure 42C. In this case, elastic structure of 20% (w/w) sucrose solution containing 0.5% (w/w) XG was lower than 20% (w/w) sucrose solution containing 0.5% (w/w) LBG. Assuming, 70% of sucrose is still entrapped inside the gel matrix. Homogeneity of ice crystals in this sample was higher than that of 20% (w/w) sucrose solution containing 0.5% (w/w) LBG because the difference between higher and lower concentrated sucrose solutions were not large. Therefore, phase equilibrium between two phases were not long period when compared with 20% (w/w) sucrose solution containing 0.5% (w/w) LBG. As for 30% (w/w) sucrose solution, unfrozen phase was higher than that of 20% (w/w) sucrose solution. Hence, the smaller of ice phase content (black regions) in 30% (w/w) sucrose solution containing xanthan gum was observed in this study.

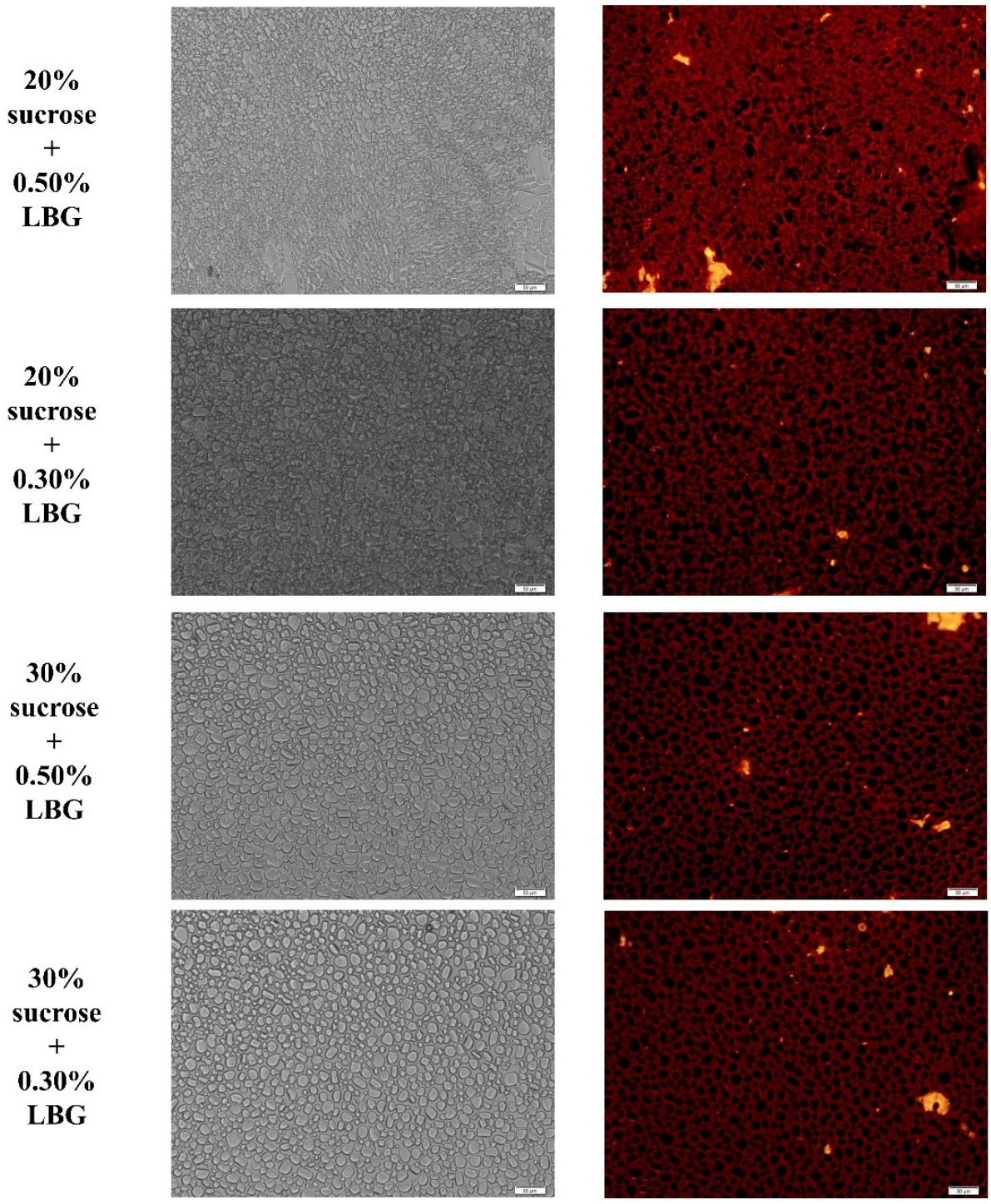


Figure 40 Brightfield (left images) and fluorescence (right images) of frozen sucrose solution with the addition of locust bean gum (LBG) at -7 ºC 20 min from the same field of sample. Bar = 50 µm (All images)

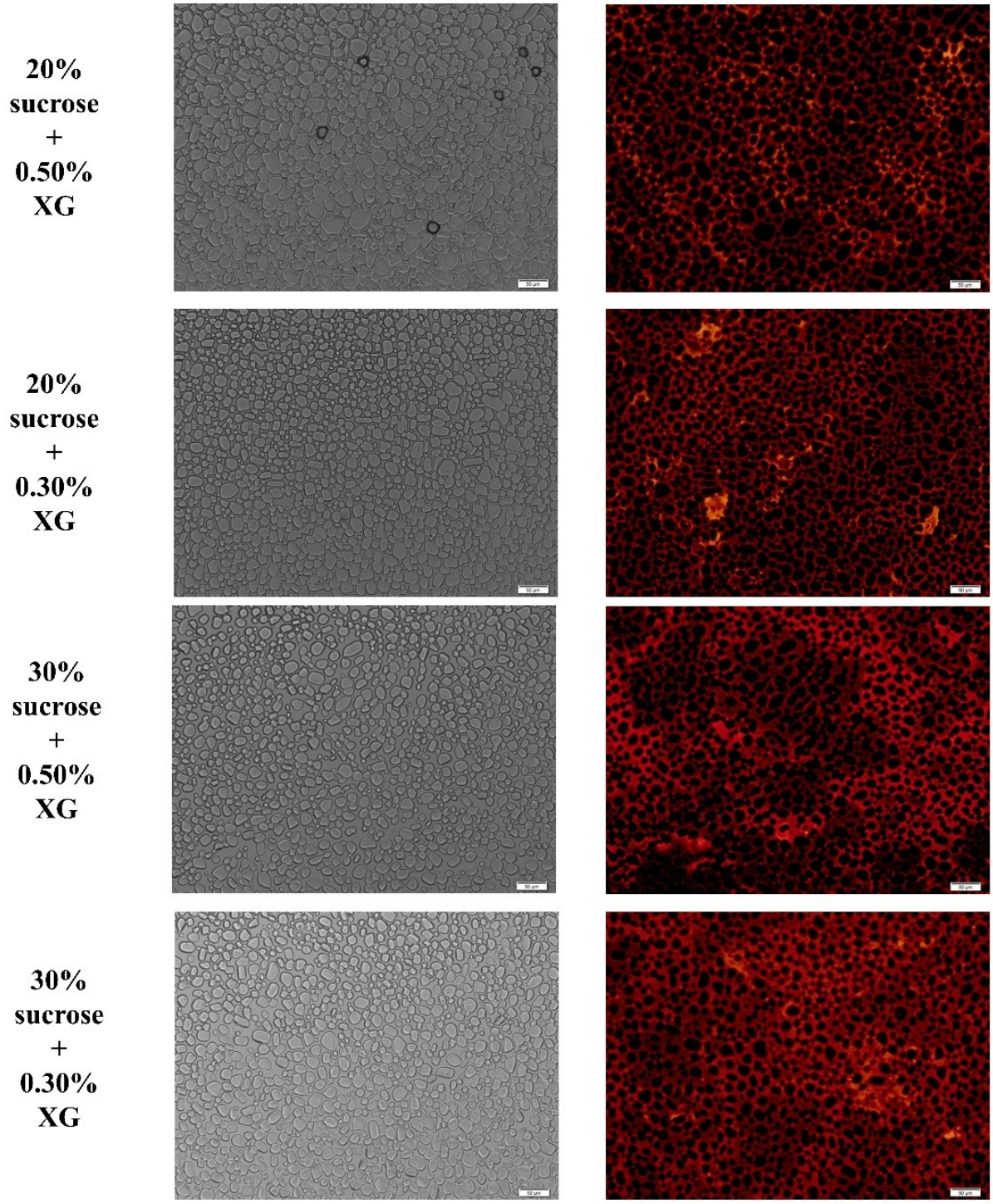
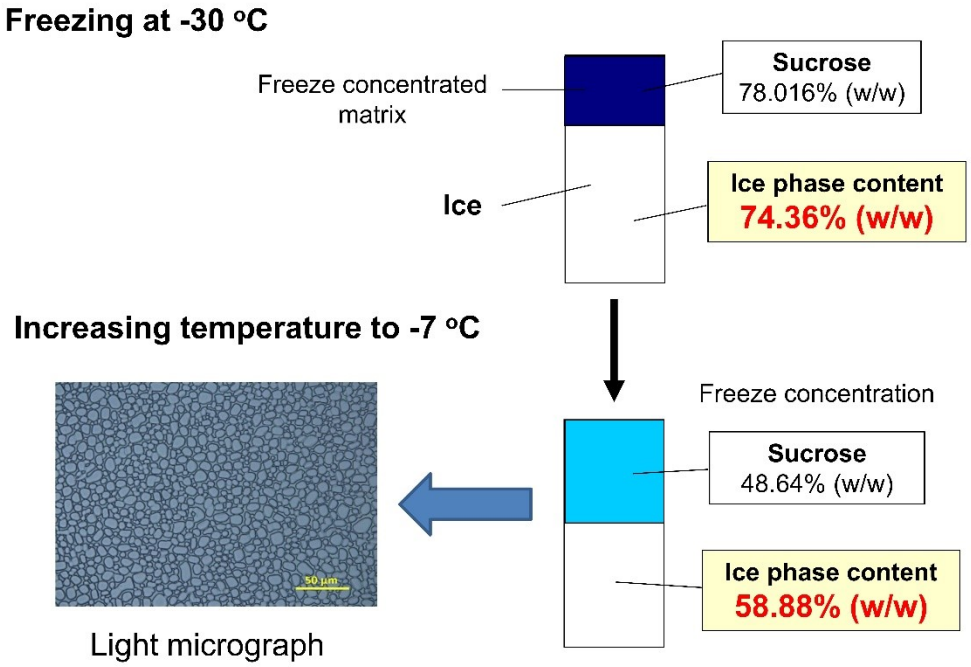
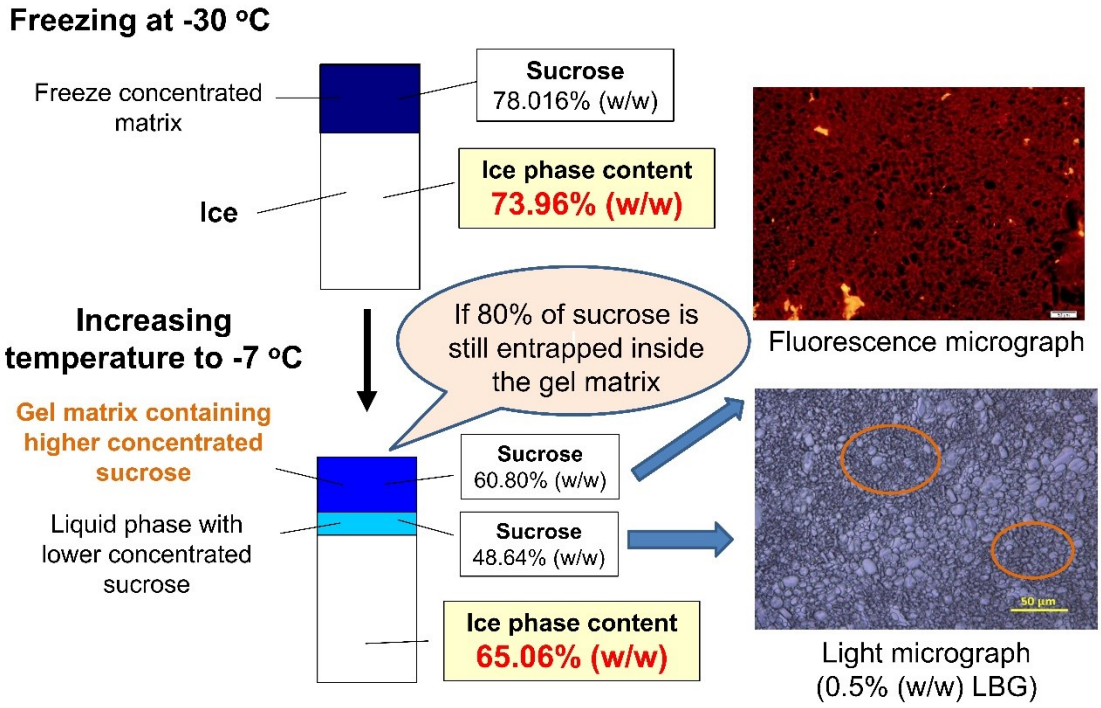


Figure 41 Brightfield (left images) and fluorescence (right images) of frozen sucrose solution with the addition of xanthan gum (XG) at -7 ºC 20 min from the same field of sample. Bar = 50 µm (All images)



(A) Sample: 20% (w/w) sucrose solution, no stabilizer



(B) Sample: 20% (w/w) sucrose solution containing 0.50% (w/w) locust bean gum

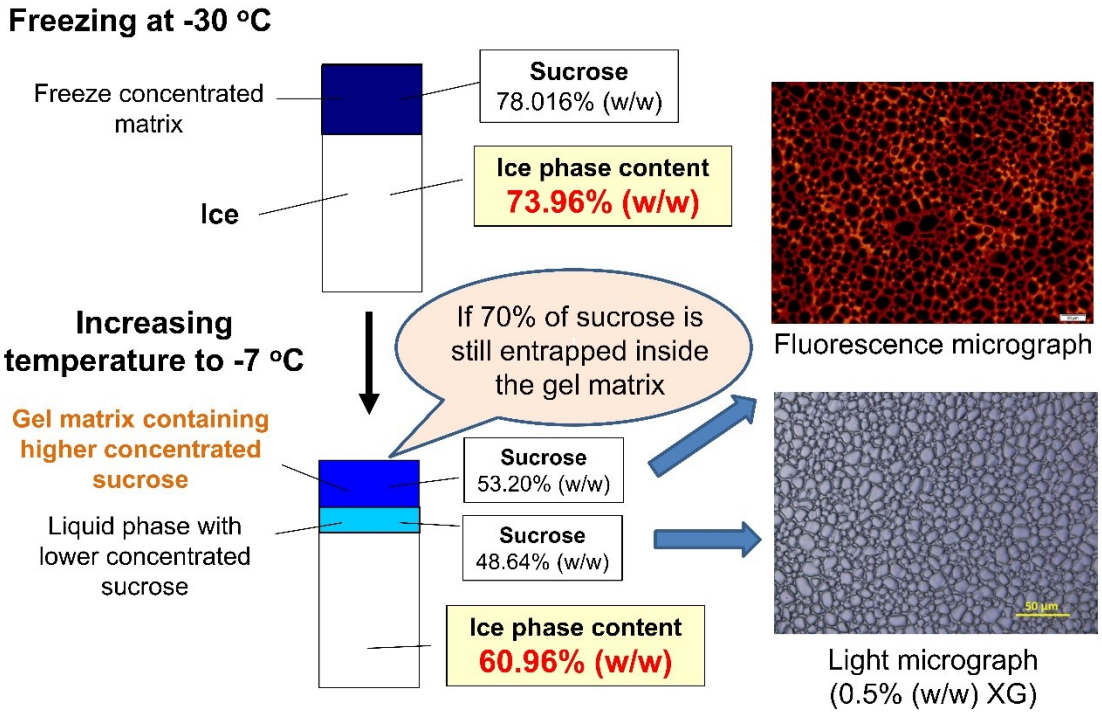


Figure 42 Schematic diagram shows ice phase content of sucrose solution with and without the addition of gelling stabilizer in freeze-concentrated matrix during freezing process (A) 20% (w/w) sucrose solution (B) 20% (w/w) sucrose solution containing 0.50% (w/w) locust bean gum and (C) 20% (w/w) sucrose solution containing 0.50% (w/w) xanthan gum

4.3.4 Dielectric relaxation

Figure 43 and 44 show dielectric relaxation spectrum curves of 20% sucrose with and without polysaccharides. Dielectric relaxation spectrum of sample solutions were not similar between the replications in experiments. This suggested that the heterogeneity inside sample solutions affected the shape of dielectric relaxation spectrum curves. It means that heterogeneous samples occurred in solution. It can be related with the results of heterogeneity microstructure of LBG and xanthan in 20% sucrose solution (Figure 40 and 41). Moreover, freezing rate during decreasing temperature of dielectric relaxation measurement was very slow when compared with the rate of ice crystal observation experiments. Therefore,

cryogelation may be easily occurred in samples. The concentration of sample solution was not equilibrium in the system leading to the heterogeneity of ice phase content in solution.

As for 30% sucrose solution, smooth dielectric relaxation spectrum curves were observed of all samples as shown in Figure 45. Two peaks of double Cole-Cole function were revealed. The low-frequency absorption is probably due to associating water with sucrose molecule while the high-frequency absorption came from free water. Direct current (dc) conductivity was observed in the solutions containing xanthan gum because of an ionic heteropolysaccharide (Bordi et al., 1995). However, the relaxation curves did not display any change with the frequency even though dc conductivity was observed. Relaxation times of free water for all samples were not difference and had no clear correlation to the ice recrystallization rate (Table 10). The structural homogeneity in 30% sucrose solution both with and without polysaccharides may be higher than that of 20% sucrose solutions because unfrozen phase of 30% sucrose (unfrozen 41.91%) were higher than that of 20% sucrose (unfrozen 27.94%), based on the calculation from freezing point depression curve (Figure 16) and observed ice crystal image (Figure 36 and 37).

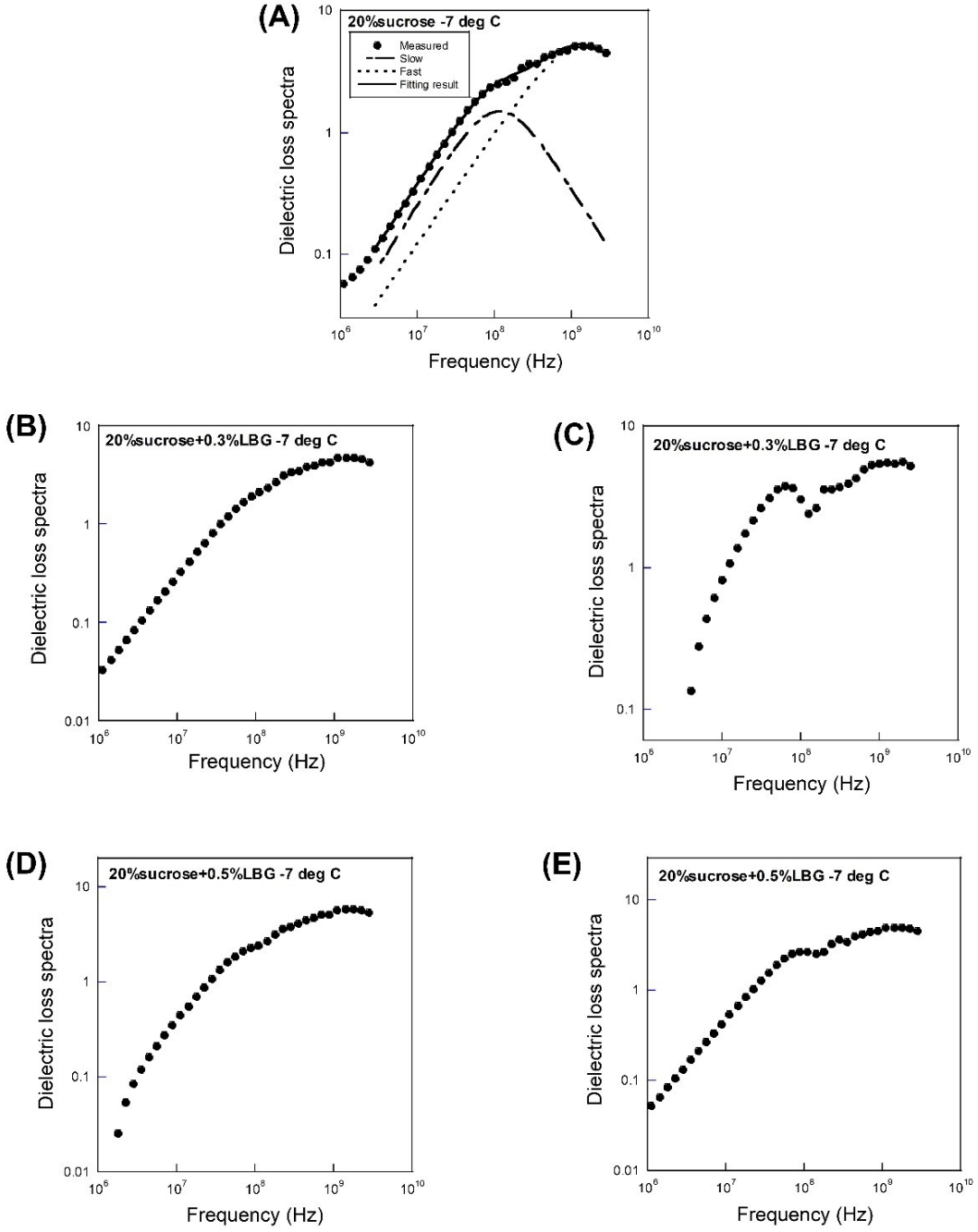


Figure 43 Frequency dependences of dielectric loss spectra for 20% (w/w) sucrose solution containing 0.3% (w/w) and 0.5% (w/w) LBG at -7 ºC.

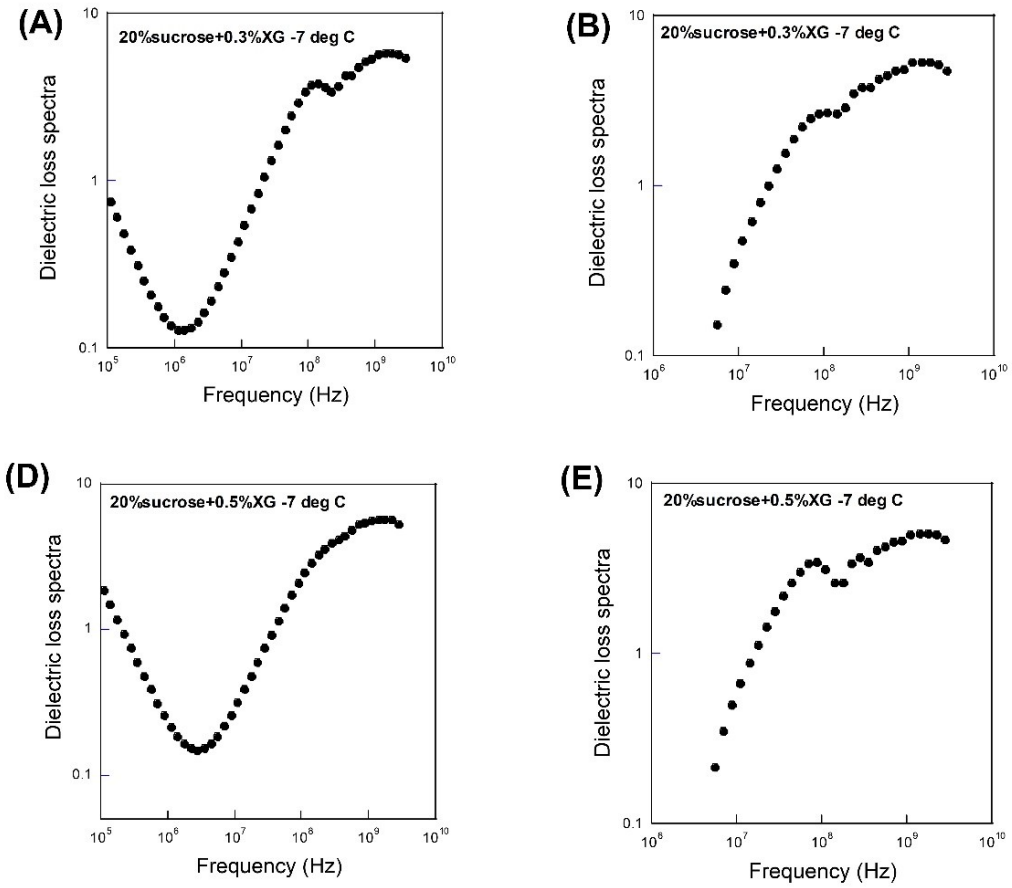


Figure 44 Frequency dependences of dielectric loss spectra for 20% (w/w) sucrose solution containing 0.3% (w/w) and 0.5% (w/w) xanthan gum at -7 ºC.

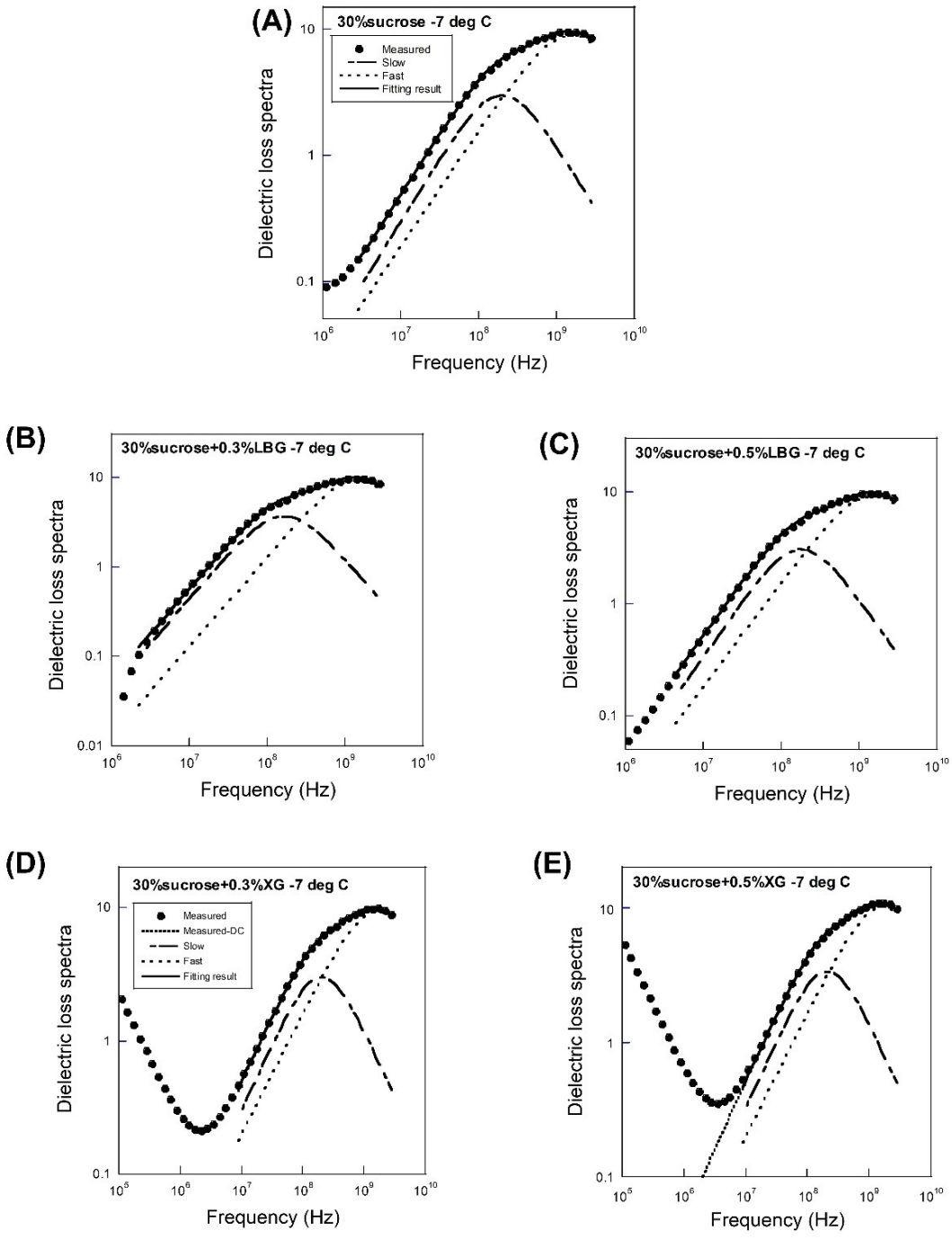


Figure 45 Frequency dependences of dielectric loss spectra for 30% (w/w) sucrose solution containing 0.3% (w/w) and 0.5% (w/w) LBG and xanthan gum at -7 ºC. The solid curves were obtained by fitting using Cole-Cole equation (Eq.2-4)

Table 10 Dielectric strength (Δε) and relaxation time (τ) of 30 %(w/w) sucrose solution containing 0.3% (w/w) and 0.5% (w/w) LBG or xanthan gum

Sample	Dielectric strength (Δε)		Relaxation time (τ)	
	$\Delta\varepsilon_{Slow\ water}$	$\Delta\varepsilon_{Fast\ water}$	$\tau_{Slow\ water}$	$\tau_{Fast\ water}$
30% sucrose	6.05±0.40[a]	20.48±0.84[ab]	0.831±0.112[a]	0.101±0.002[a]
30%sucrose + 0.3%LBG	6.99±0.29[b]	19.71±1.68[a]	0.827±0.133[a]	0.101±0.008[a]
30%sucrose + 0.5%LBG	6.01±0.11[a]	21.06±0.65[ab]	0.782±0.093[a]	0.098±0.005[a]
30%sucrose + 0.3%XG	6.21±0.38[a]	21.83±0.73[b]	0.854±0.065[a]	0.099±0.001[a]
30%sucrose + 0.5%XG	6.23±0.46[a]	21.78±0.97[b]	0.866±0.094[a]	0.100±0.004[a]

Different superscript letters indicate a significant difference ($p \leq 0.05$) between samples at same column. Values shown are mean ± SD from triplicate measurements

4.4 Conclusion

Increasing polysaccharide concentration did not always give smaller recrystallization rate constant of ice crystals. Unfrozen phase of solution had influence on homogeneity of ice crystals when the polysaccharides were added. As for 20% sucrose solution, heterogeneity of ice crystals with high concentration of locust bean gum were caused by phase separation due to gel matrix in freeze-concentrated matrix. High concentration of xanthan gum also increased ice phase content when observed by fluorescence microscope led to increasing in ice recrystallization rate. Moreover, the heterogeneity of water mobility from dielectric relaxation spectrum curves supported the observed heterogeneity of ice crystals in 20% sucrose containing locust bean gum and xanthan gum. In the case of 30% sucrose solution containing polysaccharides, homogeneous ice crystals were observed. Ice recrystallization rate of 30 % sucrose solution containing polysaccharide were not significantly different from that of control, which agreed well to the fact that the fast water mobility did not change so much when the polysaccharide was added.

References

Bordi, F., Cametti, C., & Paradossi, G. (1995). Radiowave dielectric properties of xanthan in aqueous solutions. *The Journal of Physical Chemistry, 99,* 274-284.

Budke, C., Heggemann, C., Koch, M., Sewald, N., & Koop, T. (2009). Ice recrystallization kinetics in the presence of synthetic antifreeze glycoprotein analogues using the framework of LSW theory. *The Journal of Physical Chemistry B, 113,* 2865-2873.

Flores, A. A., & Goff, D. (1999). Recrystallization in ice cream after constant and cycling temperature storage conditions as affected by stabilizers. *Journal of Dairy Science, 82,* 1408-1415.

Goff, H. D., Ferdinando, D., & Schorsch, C. (1999). Fluorescence microscopy to study galactomannan structure in frozen sucrose and milk protein solutions. *Food Hydrocolloids, 13,* 353-362.

Hagiwara, T., Hartel, R. W., & Matsukawa, S. (2006). Relationship between recrystallization rate of ice crystals in sugar solutions and water mobility in freeze-concentrated matrix. *Food Biophysics, 1,* 74-82.

Harper, E. K., & Shoemaker, C. F. (1983). Effect of locust bean gum and sweetening agents on ice recrystallization rate. *Journal of Food Science, 48,* 1801-1806.

Herrera, M. L., M'Cann, J. I., Ferreeo, C., Hagiwara, T., Zaritzky, N. E., & Hartel, R. W. (2007). Thermal, mechanical, and molecular relaxation properties of frozen sucrose and fructose solutions containing hydrocolloids. *Food Biophysics, 2,* 20-28.

Miller-livney, T., & Hartel, R. W. (1997). Ice recrystallization in ice cream: interactions between sweeteners and stabilizers. *Journal of Dairy Science, 80,* 447-456.

Lifshitz, I. M., & Slyozov, V. V. (1961). The kinetics of precipitation from supersaturated solid solutions. *Journal of Physics and Chemistry of Solids, 19,* 35–50.

Regand, A., & Goff, H. D. (2002). Effect of biopolumers on structure and ice recrystallization in dynamically frozen ice cream model systems. *Journal of Dairy Science, 85,* 2722-2732.

Regand, A., & Goff, H. D. (2003). Structure and ice recrystallization in frozen stabilized ice cream model systems. *Food Hydrocolloids, 17,* 95-102.

Schönhals, A., & Kremer, F. (2003). Analysis of dielectric spectra. In Kremer, F., Schonhals, A. (Eds.), Broadband dielectric spectroscopy (pp. 59-98). (1st ed.). New York: Springer-Verlag.

Sutton, R. L., Cooke, D., & Russell, A. (1997). Recrystallization in sugar/stabilizer solutions as affected by molecular structure. *Journal of Food Science, 62,* 1145-1149.

Sutton, R. L., & Wilcox, J. (1998). Recrystallization in ice cream as affected by stabilizers. *Journal of Food Science, 63,* 104-107.

Wagner, C. (1961). Theorie der Alterung von Niderschlagen durch Umlösen (Ostwald Reifung). *Z Elektrochem, 65,* 581–591.

CHAPTER 5

RECRYSTALLIZATION OF ICE CRYSTALS IN SUCROSE SOLUTION CONTAINING PROTEIN

5.1 Introduction

Apart from saccharides and polysaccharides, protein is one of important component in food, not only as components in food but also as a cryoprotectant in biomedical field. Protein can contribute to development of the structure in food. Ice recrystallization behavior of protein system especially general proteins such as whey protein isolate and bovine serum albumin were rarely investigated, though many studies about antifreeze protein (AFP) (Regand and Goff, 2006; Du and Betti, 2016) have been done. Bovine serum albumin is a globular water soluble protein constituent of blood plasma. It has a single polypeptide chain with a molecular weight of 66 kDa and consists of 583 amino acid residues (Quinlan et al., 2005). The structure of BSA is similar with other protein namely, β-lactoglobulin and ovalbumin (egg white protein). BSA has been widely used as a model protein and common additive in foods due to its high resistance to degradation. Moreover, it is used as cryoprotective for frozen storage of microorganisms and biological cell. (Hubálek, 2003; Amidi et al., 2010; EI-Shahat and Hammam, 2014). Chun et al. (2012) reported that ice crystal size was decreased when increasing whey protein isolate (0.2, 1.0, 3.0 and 5.0% (w/w)) in 40% (w/w) sucrose solution at -6 ºC. However, the effect of general protein on ice recrystallization and its mechanism have not been understood well. In this chapter, effects of BSA addition on ice recrystallization behavior was investigated and the mechanism causing the effects was discussed by using the water mobility and thermal properties.

5.2 Materials and Methods

5.2.1 Materials

Bovine serum albumin (BSA) was purchased from Sigma Aldrich (St Louis, Missouri, USA). Sucrose was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

5.2.2 Sample preparation

Protein solutions were prepared by dissolving BSA in 20% (w/w) sucrose solution. The sample solution was stirred 3 h to ensure complete dissolution of bovine serum albumin (BSA) at room temperature. The following aqueous solutions were prepared: 20% (w/w) sucrose, 20% (w/w) sucrose + 0.4% (w/w) BSA, 20% (w/w) sucrose + 4% (w/w) BSA, 20% (w/w) sucrose + 6% (w/w) BSA.

5.2.3 Isothermal recrystallization rate constant

Isothermal recrystallization of ice crystals in sucrose solution containing bovine serum albumin was observed at -7 °C (temperature variability: ±0.05 ºC) as described in chapter 3 and 4. Observation time of each sample solution was set to 3 h to obtain ice crystal images suitable for image analysis. Numbers of ice crystal observation per one image were more than 100 ice crystals. To obtain the isothermal recrystallization rate constant, the Lifshitz–Slyozov–Wagner (LSW) theory of Ostwald ripening principle (Lifshitz and Slyozov, 1961; Wagner, 1961) was used (Sutton et al., 1996; Hagiwara et al., 2006; Budke et al., 2009) as explained in Section 3.2.4.

5.2.4 Differential scanning calorimetry (DSC)

The heat of fusion of water in 20% (w/w) sucrose solution with and without BSA were determined by using a differential scanning calorimetry (Perkin Elmer DSC 7, USA) and analyzed with a Pyris 7 DSC software (Perkin Elmer, USA). DSC was calibrated with indium and deionized water standards before analysis. Approximately 15.5-16.0 mg of sample solution

was sealed into aluminum pans and placed in the DSC. An empty pan was used as a reference. Samples were cooled from 25 ºC to −60 °C before measurement and then heating at 5 °C/min from −60 °C to 10 °C. The changes in heat flow of sample solutions during melting process were recorded. The amount of water able to freeze was determined as the area under the endothermic peak.

5.2.5 Dielectric relaxation

Time domain reflectometry (TDR) was employed to measure dielectric relaxation in a frequency range from 1 MHz to 3 GHz. The sample temperature was set at -7 °C (temperature variability was ±0.1 ºC). A voltage pulse of 230 mV in amplitude was applied to a sample solution through a coaxial probe and the time dependent change in the voltage of the reflected wave was measured using the main frame of digitizing oscilloscope (HP54120B, Hewlett-Packard, USA) and four channel test set (HP54121A, Hewlett-Packard, USA) with the following parameter values; time interval 900 ps/div, delay (time offset relative to trigger) 26.00 ns, and voltage sensitivity 50 mV/div for setting waveform characteristic. A probe with pin diameter d = 1.0 mm and electric length γd = 2.001165 mm was used. The reflected wave was accumulated 2048 times for each measurement. During the experiments the sample temperature was monitored with digital thermometer (7563, Yokogawa meters & instruments corp., Japan). Frequency dependences of dielectric loss spectra can be fitted well by double Cole-Cole function (Schönhals & Kremer, 2003) using Eq.2-4 in Section 2.2.3.

5.2.6 Statistical analysis

All measurements were conducted in triplicate. Mean values were compared by analysis of variance (ANOVA) and Duncan's multiple range test for comparing treatments ($p \leq 0.05$). All calculations were done using the SPSS 17.0 software for windows (SPSS Inc., Chicago, IL, USA).

5.3 Results and discussion

5.3.1 Differential scanning calorimetry analysis

Increasing the concentration of bovine serum albumin (0 to 6% (w/w) BSA) decreased the heat of ice melting in samples. Therefore, the amount of water able to freeze in BSA were less than that in sample without BSA (Table 11). Ice content and freezable water decreased when increasing BSA in the system.

Table 11 Percentage of ice content, unfreezable and freezable water of 20% (w/w) sucrose solution containing bovine serum albumin (BSA)

Sample	Heat of ice melting ΔH (J/g)	Ice content (weight percent of ice against total sample solution)	Unfreezable water (weight percent of total water-ice content)	Freezable water (weight percent of ice against total water)
20%sucrose	216.88±0.66^{d}	64.90±0.22^{d}	15.10±0.22^{a}	81.13±0.28^{c}
20%sucrose+0.4%BSA	212.47±1.02^{c}	63.92±0.30^{c}	16.22±0.20^{b}	79.92±0.38^{b}
20%sucrose+4%BSA	199.10±1.64^{b}	59.61±0.49^{b}	17.19±0.49^{c}	78.43±0.64^{a}
20%sucrose+6%BSA	194.95±1.54^{a}	58.37±0.46^{a}	16.83±0.46^{bc}	78.87±0.62^{a}

Latent heat of ice melting ΔH_m=334 J/g
Different superscript letters indicate a significant difference ($p \leq 0.05$) between samples at same column.
Values shown are mean ± SD from triplicate measurements

5.3.2 Ice recrystallization behavior

Typical images of ice crystals of 20% (w/w) sucrose with and without the addition of bovine serum albumin (BSA) are shown in Figure 46. The shape of ice crystals in sucrose solution containing BSA were similar with sucrose solution as a control sample. In the case of antifreeze protein, the shape and size of ice crystal are controlled through an adsorption-inhibition mechanism (Regand and Goff, 2006). Therefore, it was strongly suggested that BSA had no antifreeze ability as antifreeze protein. Figure 47 is a plot of the cube of the number-averaged radius of ice crystal r^3 vs. time (t). The plots show a linear tendency and from fitting by Eq. 5 the isothermal rate constant k was evaluated. Values of k from 20% (w/w) sucrose

with and without the addition of bovine serum albumin (BSA) at -7 ºC are compared in Table 12. Ice recrystallization rate of 20% (w/w) sucrose containing 0.4% (w/w), 4% (w/w), and 6% (w/w) BSA were not significantly different (p>0.05) from control sample. However, the initial ice crystal size decreased when increasing the concentration of BSA. This may be caused by decreasing of ice content as suggested by the results of DSC.

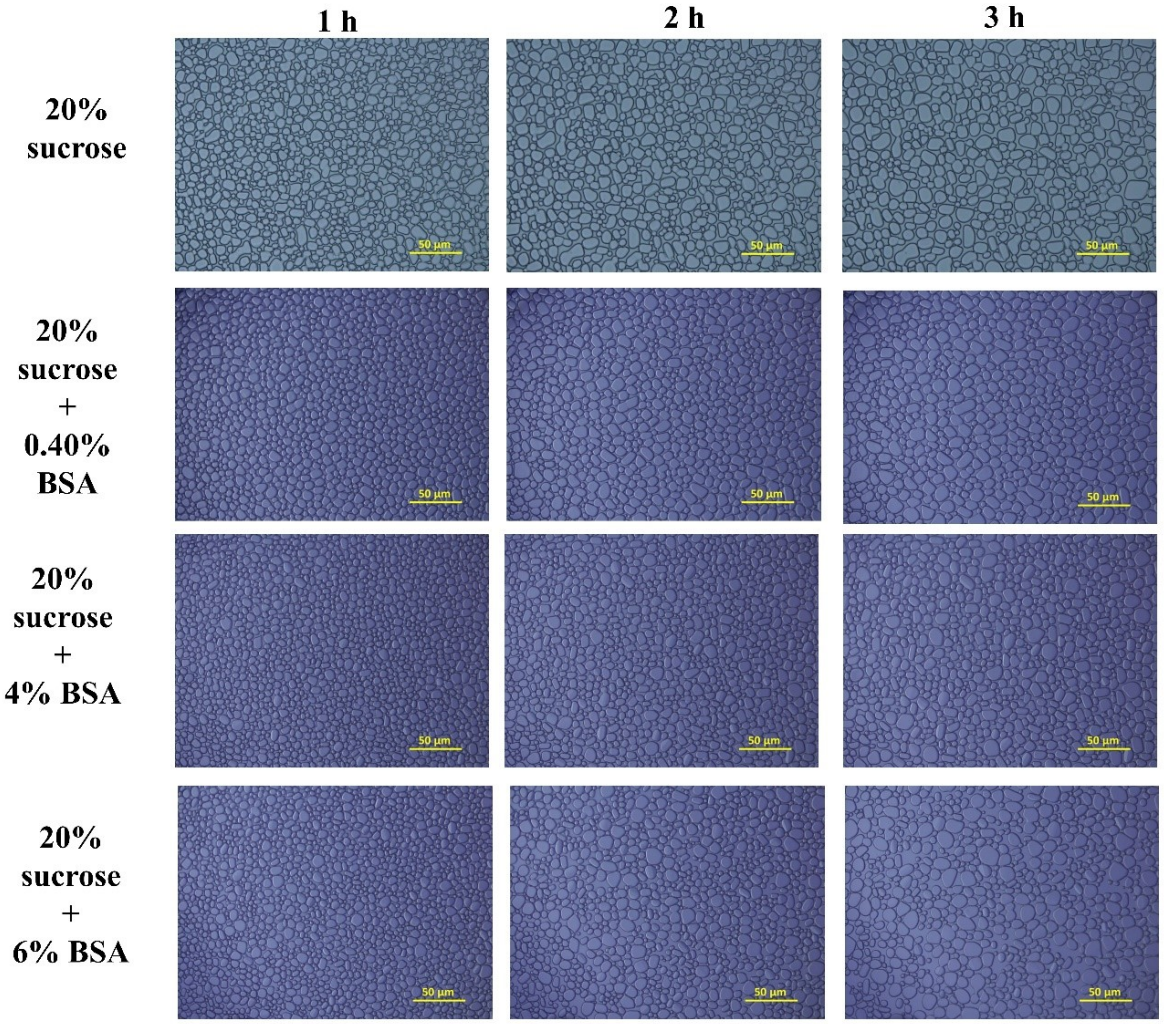


Figure 46 Typical images of ice crystals of 20% (w/w) sucrose solution with and without the addition of bovine serum albumin (BSA) at -7 ºC. Bar = 50 µm (All images)

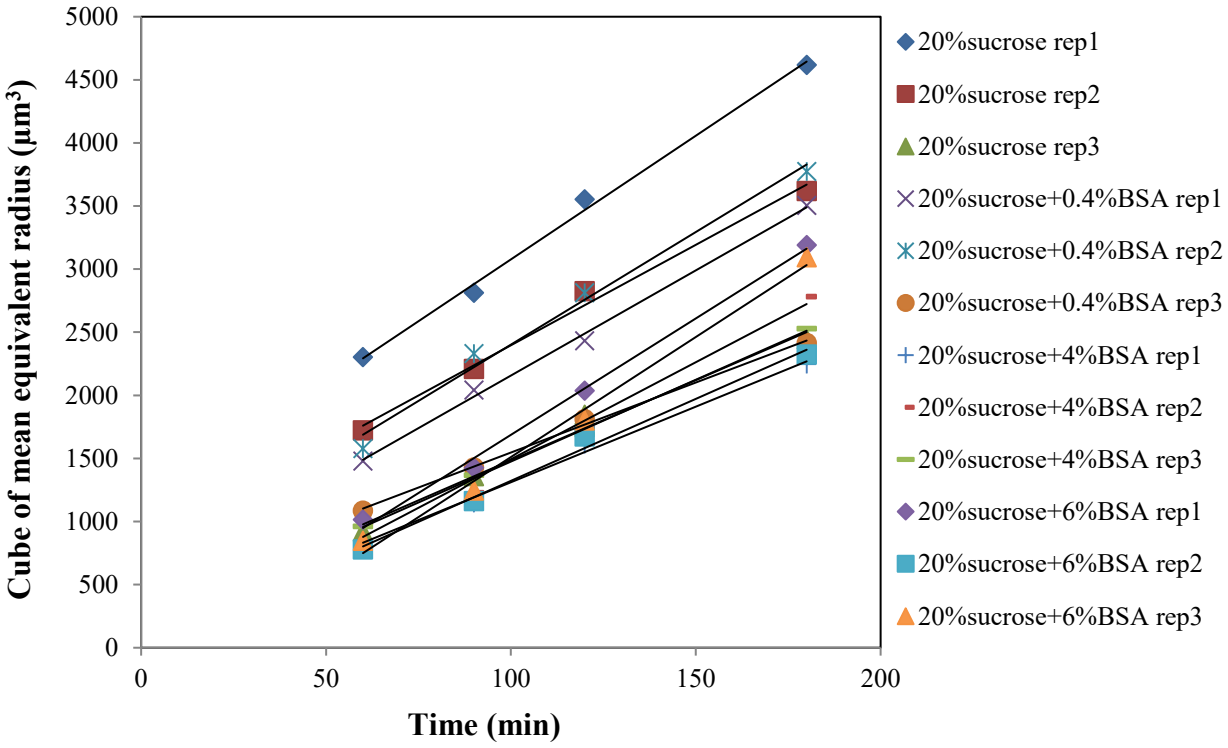


Figure 47 Plots between cube of mean equivalent radius and time during storage ice crystals for 20% (w/w) sucrose solution with and without the addition of bovine serum albumin (BSA) at -7 °C

Table 12 Ice recrystallization rate of sucrose solution containing bovine serum albumin (BSA) at -7 °C

Treatment	60 min	180 min	Ice recrystallization rate (µm³/min)
20%sucrose	11.64±1.76^{b}	15.16±1.60^{a}	16.07±3.45^{a}
20%sucrose+0.4%BSA	11.11±0.73^{ab}	14.72±1.15^{a}	15.20±3.61^{a}
20%sucrose+4%BSA	9.75±0.30^{ab}	13.60±0.48^{a}	13.44±1.75^{a}
20%sucrose+6%BSA	9.57±0.43^{a}	14.18±0.81^{a}	16.82±3.33^{a}

Different superscript letters indicate a significant difference ($p \leq 0.05$) between samples at same column.
Values shown are mean ± SD from triplicate measurements

5.3.3 Dielectric relaxation

Figure 48 show dielectric relaxation spectrum curves of 20% sucrose with and without BSA. Two peaks of double Cole-Cole function were revealed. Direct current (dc) conductivity was observed in the solutions because BSA is an ionic substance. Relaxation times of free water for all samples were not difference except 6% BSA (Table 13).

It is known that the hydration shell surrounding a protein molecule comprises different types of water. In the frequency range from 100 kHz to 10 GHz, three kinds of reorientational motions of water molecules in globular protein solution were identified at room temperature; free water molecules around 10 GHz, unfreezable water which constructs a shell layer around the protein molecule around 1 GHz, and bound water which attaches directly to the protein surface around 100 MHz (Mijović et al. 2005). Moreover, most researchers observed three relaxation processes in protein solution; the rotation of the polar of protein molecules, bound water (loosely and strongly bound water), and the reorientation motion of free water molecules (Miura et al., 1994; Miura et al., 1995; Shinyashiki et al., 2009; Wolf et al., 2012). However, loosely bound water can be incorporated into the highest relaxation process (free water) at a few GHz by using Cole-Cole equation (Wolf et al., 2012). In this study, the temperature was lower than those study. So the frequency of dielectric response would shift to lower frequency. Considering these, the observed fast relaxation in this study may be originated from free water (Figure 49). The slow relaxation process is probably due to the water molecules associating sucrose and BSA (Miura et al., 1994). It may be affected by the amount of water molecules being associating with BSA surface (Wolf et al., 2012). This may explain the fact that the dielectric strength of slow relaxation in the presence of 6% (w/w) BSA was the highest. The dielectric strength of free water of all sample solutions were not significantly different. Relaxation time of fast relaxation and slow relaxation in 20% (w/w) sucrose containing 0.4% (w/w) and 4% (w/w) BSA were not significantly different from 20% (w/w) sucrose solution. The ice recrystallization rate was not significantly different (p>0.05) between sucrose solution with and without BSA. In the case of 6% (w/w) BSA, the direct current (dc) conductivity was observed and it was necessary to deduce it before the fitting procedure (Figure 48). The large contribution of dc conductivity or electrode polarization was shown in the low frequency region of 6% (w/w) BSA in 20% (w/w) sucrose solution. The dc conductivity came from

protein molecules releasing ions. Interestingly, the relaxation time of fast relaxation of 6% (w/w) BSA was the lowest, which means the water mobility of this concentration was highest. Decreasing of relaxation time in higher protein concentrations were also found in lysozyme. Relaxation time of 5 mmol of lysozyme solutions is faster than that of 3 mmol solution (Wolf et al., 2012). The water molecules in the outer hydration shells of protein may be more loosely bound than those in the inner hydration shell (Figure 49). The researches of Wolf et al. (2012) and Cametti et al. (2013) suggested that increasing the protein concentration beyond a certain threshold decreased hydration number of protein because the aggregation process forming protein clusters tends to remove the loosely bound water. As the result removed loosely bound waters are incorporated to the free water, resulting in increasing free water mobility. However, ice recrystallization rate of 20% sucrose solution containing 6% BSA was not significantly different (p>0.05) with 20% sucrose solution with and without 0.4% and 4% BSA. It may be caused by the counter effect of decreasing ice phase content in 20% sucrose solution containing 6% BSA, leading to no clear impact on the recrystallization rate of ice crystals.

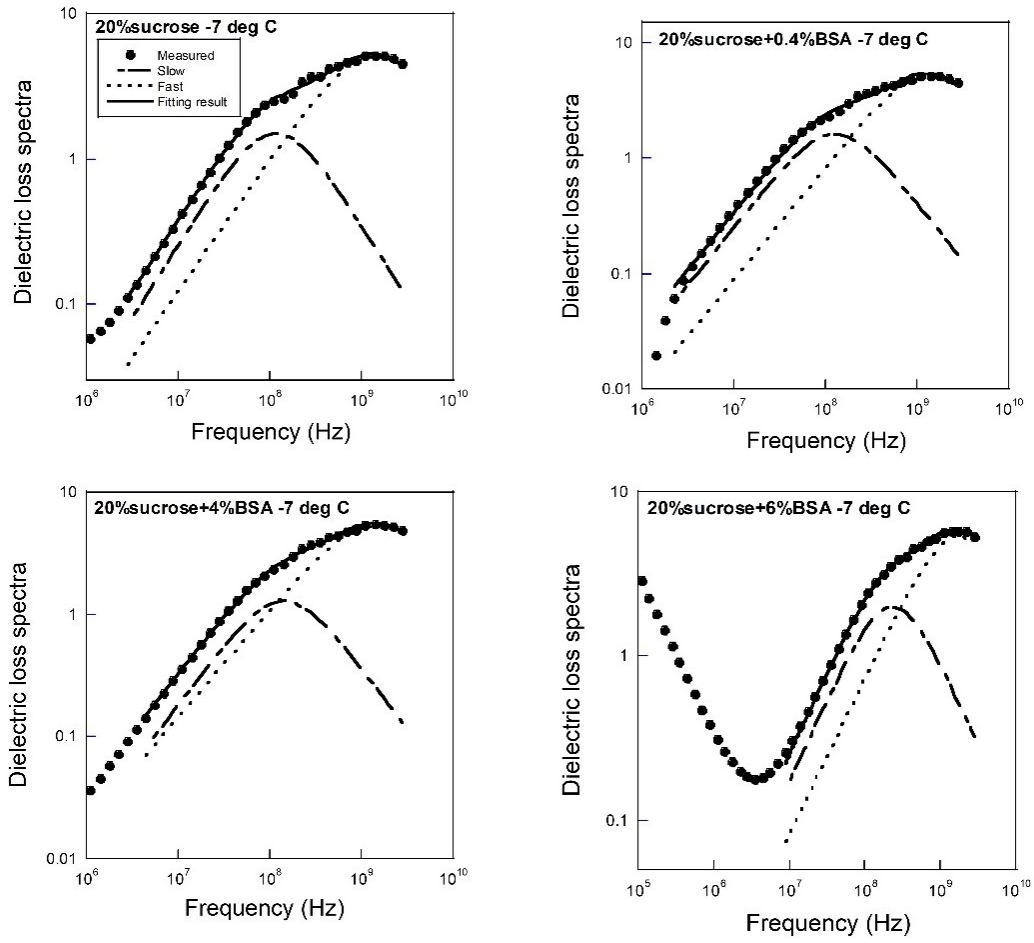


Figure 48 Frequency dependences of dielectric loss spectra for 20% (w/w) sucrose solution containing 0.4% (w/w), 4% (w/w), and 6% (w/w) BSA at -7 ºC. The solid curves were obtained by fitting using Cole-Cole equation (Eq.2-4)

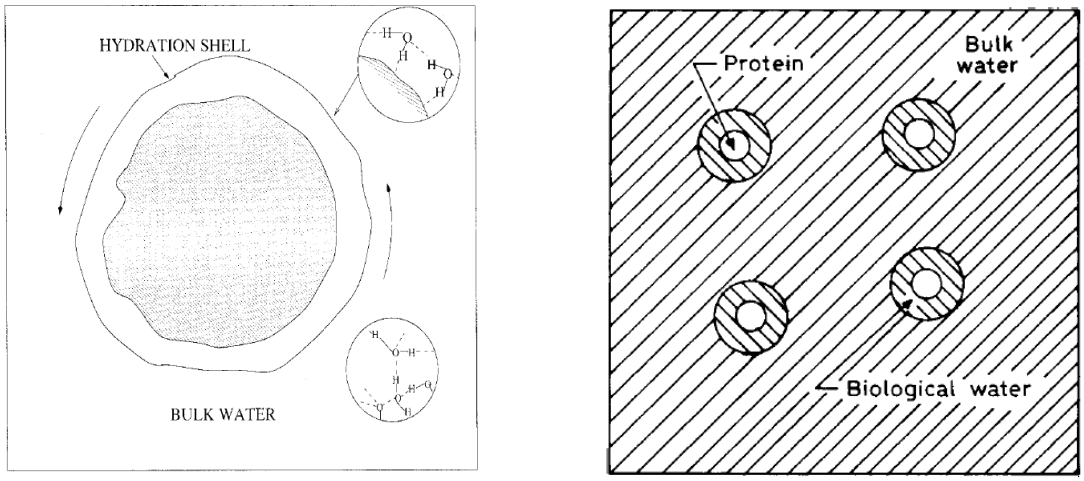


Figure 49 Schematic diagram of water molecules hydrogen bonded with the protein molecule present in the hydration shell as well as the water molecules in the bulk hydrogen bonded network (Nandi and Bagchi, 1997; Nandi et al., 2000).

Table 13 Dielectric strength (Δε) and relaxation time (τ) of 20% (w/w) sucrose solution containing bovine serum albumin (BSA)

Sample	Dielectric strength (Δε)		Relaxation time (τ)	
	$\Delta\varepsilon_{Slow\ water}$	$\Delta\varepsilon_{Fast\ water}$	$\tau_{Slow\ water}$	$\tau_{Fast\ water}$
20% sucrose	2.70 ± 0.25^{a}	12.29 ± 0.69^{a}	1.44 ± 0.09^{bc}	0.107 ± 0.005^{b}
20% sucrose+ 0.4% BSA	2.50 ± 0.71^{a}	11.65 ± 1.97^{a}	1.69 ± 0.46^{c}	0.109 ± 0.009^{b}
20% sucrose+ 4% BSA	3.18 ± 0.53^{ab}	11.61 ± 1.06^{a}	1.18 ± 0.06^{b}	0.107 ± 0.009^{b}
20% sucrose+ 6% BSA	3.96 ± 0.47^{b}	10.80 ± 0.91^{a}	0.64 ± 0.07^{a}	0.087 ± 0.002^{a}

Different superscript letters indicate a significant difference ($p\leq0.05$) between samples at same column. Values shown are mean ± SD from triplicate measurements

5.4 Conclusion

Increasing of bovine serum albumin concentration (0 to 6%) decreased ice content and increased unfreezable water in solutions. However, the addition of BSA reduced ice crystal size only at the initial freezing process; ice recrystallization rate of samples present with BSA were not significantly different from sucrose solution without BSA as a control sample. The free water mobility in BSA system did not change by increasing BSA concentration from 0 to

4% in sucrose solutions. However, water mobility in sucrose solution containing 6% BSA was higher than other sample solutions. It may be caused by the incorporation of loosely bound water in the outer of hydration shell of BSA into free water molecules. The reason why the recrystallization rate constant did not change in spite of increased water mobility may be the decreasing the ice content by the addition of BSA.

References

Amidi, F., Farshad, A., & Khor, A. K. (2010). Effects of cholesterol-loaded cyclodextrin during freezing step of cryopreservation with TCGY extender containing bovine serum albumin on quality of goat spermatozoa. *Cryobiology, 61,* 94-99.

Budke, C., Heggemann, C., Koch, M., Sewald, N., & Koop, T. (2009). Ice recrystallization kinetics in the presence of synthetic antifreeze glycoprotein analogues using the framework of LSW theory. *The Journal of Physical Chemistry B, 113,* 2865-2873.

Cametti, C., Marchetti, S., & Onori, G. (2013). Lysozyme hydration in concentrated aqueous solutions. effect of an equilibrium cluster phase. *The Journal of Physical Chemistry B, 117,* 104-110.

Chun, J. Y., Kim, J. I., & Min, S. G. (2012). Effect of whey protein isolate on ice recrystallization characteristics in whey protein isolate/k-carrageenan matrix. *Korean Journal for Food Science of Animal Resources, 32,* 627-634.

Du, L., & Betti, M. (2016). Identification and evaluation of cryoprotective peptides from chicken collagen: ice-growth inhibition activity compared to that of type I antifreeze proteins in sucrose model systems. *Journal of Agriculture and Food Chemistry, 64,* 5232-5240.

EI-Shahat, K. H., & Hammam, A. M. (2014). Effect of different types of cryoprotectants on developmental capacity of vitrified-thawed immature buffalo oocytes. *Animal Reproduction Science, 11,* 543-548.

Hagiwara, T., Hartel, R.W., & Matsukawa, S. (2006). Relationship between recrystallization rate of ice crystals in sugar solutions and water mobility in freeze-concentrated matrix. *Food Biophysics, 1,* 74-82.

Hubálek, Z. (2003). Protectants used in the cryopreservation of microorganisms. *Cryobiology, 46,* 205-229.

Lifshitz, I. M., & Slyozov, V. V. (1961). The kinetics of precipitation from supersaturated solid solutions. *Journal of Physics and Chemistry of Solids, 19,* 35–50.

Miura, N., Asaka, N., Shinyashiki, N., & Mashimo, S. (1994). Microwave dielectric study on bound water of globule proteins in aueous solution. *Biopolymers, 34,* 357-364.

Miura, N., Hayashi, Y., Shinyashiki, N., & Mashimo, S. (1995). Observation of unfreezable water in aqueous solution of globule protein by microwave dielectric measurement. *Biopolymers, 36,* 9-16.

Mijović, J., Bian, Y., Gross, R. A., & Chen, B. (2005). Dynamics of proteins in hydrated state and in solution as studied by dielectric relaxation spectroscopy. *Macromolecules, 38,* 10812-10819.

Nandi, N., Bhattacharyya, K., & Bagchi, B. (2000). Dielectric relaxation and solvation dynamics of water in complex chemical and biological systems. *Chemical Reviews,* 100, 2013-2045.

Panagopoulou, A., Kyritsis, A., Shinyashiki, N., & Pissis, P. (2012). Protein and water dynamics in bovine serum albumin-water mixtures over wide ranges of composition. *The Journal of Physical Chemistry B, 116,* 4593-4602.

Quinlan, G. J., Martin, G. S., & Evans, T. W. (2005). Albumin: Biochemical properties and therapeutic potential. *Hepatology, 41,* 1211-1219.

Regand, A., & Goff, H. D. (2006). Ice recrystallization inhibition in ice cream as affected by ice structuring proteins from winter wheat grass. *Journal of Dairy Science, 89,* 49-57.

Schönhals, A., & Kremer, F. (2003). Analysis of dielectric spectra. In Kremer, F., Schonhals, A. (Eds.), Broadband dielectric spectroscopy (pp. 59-98). (1st ed.). New York: Springer-Verlag.

Shinyashiki, N., Yamamoyo, W., Yokoyama, A., Yoshinari, T., Yagihara, S., Kita, R., Ngai, K. L., Capaccioli, S. (2009). Glass transitions in aqueous solutions of protein (Bovine serum albumin). *The Journal of Physical Chemistry B, 113,* 14448-14456.

Sutton, R., Lips, A., Piccirillo, G., & Sztehlo, A. (1996). Kinetics of ice recrystallization in aqueous fructose solutions. *Journal of Food Science, 4,* 741-745.

Wagner, C. (1961). Theorie der Alterung von Niderschlagen durch Umlösen (Ostwald Reifung). *Z Elektrochem, 65,* 581–591.

Wolf, M., Gulich, R., Lunkenheimer, P., & Loidl, A. (2012). Relaxation dynamics of a protein solution investigated by dielectric spectroscopy. *Biochimica et biophysica acta (BBA)-Proteins and Proteomics, 1824,* 723-730.

GENERAL CONCLUSION

The aim of this work was to investigate the systematic understanding of ice recrystallization rate in sugar solutions, sugar-based solution containing polysaccharides and protein that are basic food component and represent simple model food. Ice crystals growth by recrystallization causes quality deterioration such as degrading the smooth texture in ice cream and increasing the damage of cell structure causing from drip loss in meat. Therefore, the controlling and predicting ice recrystallization is an important phenomenon to increase the quality of frozen food. The study revealed some of mechanisms causing effects on ice recrystallization rate constant by saccharides, polysaccharides, and protein.

It was shown that dielectric relaxation spectroscopy was a useful method to obtain parameter for predicting and controlling ice recrystallization rate constant in the solution of mono and disaccharide such as maltose, sucrose, glucose, and fructose saccharide. The difference of ice recrystallization rate constant was explained well by the difference of dielectric relaxation time of free water even though the combination effects of solutions such as types of solutes, temperatures, and concentrations. Moreover, the difference of ice recrystallization rate between di and trisaccharide solutions (trehalose, sucrose, and mixture of raffinose and trehalose) was explained well and predicted by dielectric relaxation time of free water in freeze-concentrated matrix. ^{1}H spin-spin relaxation time of water molecules also explain in fairly good manner and predict the difference of ice recrystallization rate between sucrose and trehalose.

Ice recrystallization behavior in sucrose solutions containing locust bean gum (LBG) and xanthan gum (XG) was investigated as a model of polysaccharide system. The effects of LBG and XG on ice recrystallization behavior were discussed by considering the difference of water mobility, rheological properties, and microstructure of freeze-concentrated matrix. Increasing the concentrations of polysaccharide did not always give smaller ice recrystallization rate constant. Moreover, initial sucrose concentration (20% and 30% sucrose solutions) had effect on ice recrystallization behavior in polysaccharide system. High concentration of LBG and XG caused the cryogelation in freeze-concentrated matrix. Elastic behavior of 20% sucrose containing 0.50% LBG was higher than that of 20% sucrose

containing 0.50% XG. Therefore, the content of sucrose in LBG solution entrapped inside gel network may be higher than that in XG solution. The difference of recrystallization rate between the higher and lower concentrated sucrose solution in the presence of LBG was larger than that of XG. These results led to the heterogeneity of ice crystals in LBG solution and increasing ice crystals size and ice recrystallization rate in XG solution when compared with sucrose solution as a control sample. Moreover, the difference of structural heterogeneity in freeze-concentrated matrix agreed to the shape of dielectric relaxation spectra and the fitting results of dielectric relaxation time, respectively.

The effect of protein addition on ice recrystallization rate constant in 20% sucrose solution was examined by using bovine serum albumin (BSA) as a model of protein. The properties of freeze-concentrated matrix were estimated by dielectric relaxation spectroscopy and differential scanning calorimetry (DSC) in order to disucuss the mechanism causing effect of protein addition on ice recrystallization behavior. The addition of BSA reduced initial ice crystal size but ice recrystallization rate of these samples were not significantly changed. The mobility of free water in BSA system did not change by increasing BSA concentration from 0 to 4% in sucrose solutions. Conversely, the free water mobility in sucrose solution containing 6% BSA was higher than other samples. It was suggested that this was caused by the incorporation of loosely bound waters in the outer of hydration shell of BSA into free waters. However, ice recrystallization rate constants of 20% sucrose solution containing 6% BSA was not significantly different with other solutions. The reason why the recrystallization rate constant did not change in spite of increased water mobility may be the decreasing the ice content by the addition of BSA.

The knowledge presented herein would be helped as a guidance for developing application of saccharide, polysaccharides, and proteins in not only food freezing but also pharmaceutical and biomedical fields.

RECOMMEDATION

For further investigation, clarification of the effect of raffinose on retarding ice recrystallization rate in raffinose and sucrose mixture solution should be discussed. Due to the limitation of the solubility in raffinose, it cannot be dissolved at high concentration. To achieve in high concentration of solutions, the mixture solution is necessary. A mixture of raffinose and trehalose was investigated in this study. However, the effect of raffinose on retarding ice recrystallization rate was still not clear. It can only retard ice recrystallization rate at -5 ^{o}C and -7 ^{o}C. Conversely, when decreasing temperature to -10 ^{o}C, retarding ability did not appear. A better understanding of the retarding ability of raffinose in a mixture of raffinose and sucrose should also investigate because sucrose is widely used in the basic component in many foods.

CURRICULUM VITAE

Personal information

Name Miss Phatthranit Klinmalai
Birth Date December 21, 1988
Birth Place Bangkok, Thailand

Education

2014	Kasetsart University, Thailand	M.S. (Food Science)
2011	Kasetsart University, Thailand	B.S. (Food Science and Technology) with second class honors

Scholarships

2014 - 2017	Monbukagakusho (MEXT) Scholarship from the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government for Ph.D. study
2013	The Capacity Building of Kasetsart University Students on International for funding international research aboard
2011- 2013	Tuition Scholarship for Master's study from department of Food Science and Technology, Kasetsart University, Thailand

List of publications

- **Klinmalai, P.**, Shibata, M., & Hagiwara, T. 2017. Recrystallization of ice crystals in trehalose solution at isothermal condition, *Food Biophysics, https://doi.org/10.1007/s11483-017-9496-1*.
- **Klinmalai, P.**, Shibata, M., & Hagiwara, T. 2017. Correlation between dielectric relaxation time in freeze-concentrated matrix and recrystallization rate of ice crystals in sugar solutions, *Japan Journal of Food Engineering* (Under submission)
- **Klinmalai, P.**, Hagiwara, T., Sakiyama, T., & Ratanasumawong, S. 2017. Chitosan effects on physical properties, texture, and microstructure of flat rice noodles. *LWT-Food Science and Technology, 76,* 117-123.

- Rachatanapun, C., Tantala, J., **Klinmalai, P.**, & Ratanasumawong, S. 2016. Effect of chitosan on Bacillus cereus inhibition and quality of cooked rice during storage. *International Journal of Food Science and Technology, 50,* 2419-2426.

Conference and academic presentation

- **Klinmalai, P.**, Shibata, M., & Hagiwara, T. 2016. Influence of trehalose and raffinose on ice recrystallization with various temperatures. Japan Society for Food Engineering 17, Tokyo University of Marine Science and Technology, Tokyo, Japan. 4-5 August 2016 (Oral presentation)
- **Klinmalai, P.**, Shibata, M., & Hagiwara, T. 2016. Predicting ice recrystallization of trehalose solution by using NMR technique in freeze-concentrated matrix at the various temperatures. 9th Joint Symposium on Food Science and Technology among NUS, TUMSAT and HU, Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Japan. 1-2 December 2016 (Oral presentation)
- **Klinmalai, P.**, Shibata, M., & Hagiwara, T. 2016. Recrystallization of ice crystals in trehalose and raffinose solutions. International Symposium on the Properties of Water (ISOPOW), Olympic Museum, Lausanne, Switzerland. 26-29 June 2016 (Oral presentation)
- **Klinmalai, P.**, Shibata, M., & Hagiwara, T. 2015. Recrystallization behavior of ice crystals in various saccharides solutions. Japan Society for Food Engineering 16, Hiroshima University, Hiroshima, Japan. 10-11 August 2015 (Oral presentation)
- **Klinmalai, P.**, Shibata, M., & Hagiwara, T. 2015. Estimation of ice crystals behavior in trehalose and raffinose solutions with the various temperatures. 8th Joint Symposium on Food Science and Technology between NUS and TUMSAT, National University of Singapore, Singapore. 3-4 December 2015 (Oral presentation)
- **Klinmalai, P.**, Ratanasumawong, S., Sakiyama, T., & Hagiwara, T. 2013. Effect of chitosan on the physico-chemical properties of rice noodle. Japan Society for Food Engineering 14, Kyoto-terrsa, Kyoto, Japan. 9-10 August 2013 (Oral presentation)

- **Klinmalai, P.**, & Ratanasumawong, S. 2013. Effect of acetic acid on the physicochemical properties of rice flour and the quality of rice noodle. The 51st of Kasetsart University Annual Conference, Kasetsart University, Bangkok, Thailand. 5-7 February 2013 (Poster presentation)
- **Klinmalai, P.**, & Ratanasumawong, S. 2012. Effect of chitosan on eating quality of cooked rice. Food Innovation Asia Conference 2012. BITEC, Bangkok, Thailand. 14-15 June 2012 (Poster presentation)

Work experience

- **Research Assistant**

March 2011-May 2011 Research topic: Effect of chitosan on the quality of cooked rice
Supervisor: Asst. Prof. Dr. Savitree Ratanasumawong