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Property Changes during Frozen Storage in Frozen Soy Bean Curds Prepared by Freezing Accompanied with Supercooling

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Summary

Ice crystals play an important role in the degradation of frozen food quality. Quality degradation occurs even in the frozen storage process owing to recrystallization. On the other hand, it is assumed that ice crystals formed with higher nucleation have the potential to be resistant to ice recrystallization since they have homogenous size distribution of ice crystals when considering the mechanism of ice recrystallization. In this study, recrystallization behavior and quality degradation in soy bean curds prepared by freezing accompanied with supercooling (SCF) were investigated. As a result, ice crystals in SCF maintained the initial character of small particle size and homogenous ice structure after 28 day storage at -5°C, even though the characteristics of ice crystals in Conventional rapid freezing(CRF) and Conventional slow freezing (CSF) were changed dramatically during storage. Additionally it was observed soy bean curds prepared by SCF maintained their softness rather than those prepared by CRF and CSF; furthermore, SCF samples prepared with relative lower resolving temperatures of supercooling maintained the softness rather than the SCF samples prepared with relatively higher resolving temperature of supercooling.The results indicated that the homogenous ice structure determined by SCF is an effective factor in preventing quality degradation as well as recrystallization, even though the storage temperature is higher.

Keywords: Frozen Food, Supercooling, Ice crystals, Frozen storage, Recrystallization

1. Introduction

Freezing has been widely used as an efficient method of food preservation since it can extend the shelf life of foods dramatically. However, the quality of foods such as texture, color, and water holding capacity always declines after freezing and thawing. Since the characteristics of ice crystals play an important role in the degradation of frozen food quality, much attention has been paid towards understanding the mechanism of ice crystallization. It is well understood that higher nucleation rate and lower growing rate, which are determined by the degree of supercooling and the heat and mass transfer rates, lead to smaller-sized and larger number of ice crystals [1)].

Recently, some novel freezing methods, pressure shift freezing (PSF), ultrasound-assisted crystallization, and freezing accompanied with supercooling were investigated. In these methods, higher nucleation was achieved that led to the formation of fine ice crystals and prevented quality degradation after the freezing process. It has been reported that PSF allows food stuffs to reach the supercooling state by application of high pressure and subsequently releasing the pressure under subzero temperatures. As a result, fine ice crystals were formed depending on the supercooling degree [2)]. It has also been reported that ultrasonic waves play a role as the promoter of higher ice nucleation, even though the mechanism underlying improvement in the ice crystal form by ultrasound remains unclarified [3)]. Additionally, several studies have focused on the freezing process accompanying the deeper supercooling state of the entire sample and the process that can form fine and homogenous ice crystals owing to higher ice nucleation rate, depending on the supercooling degree [4)-6)]. Thus, there are possibility that degradation of frozen food can be prevented through novel freezing techniques that can achieve higher ice nucleation rates.

However, the quality degradation of frozen food occurs even during the frozen storage process. It is well known that ice cream loses the creamy texture and obtains the contrary grainy and icy texture owing to recrystallization of ice crystals. It occurs as a result of the recrystallization phenomena that the number, size, shape, and orientation of ice crystals that change in response to the length of storage periods [7)8)]. In other words, the advantages of ice characteristics formed with the higher nucleation rate may be lost by recrystallization during the frozen storage process.

On the other hand, it is assumed that ice crystals formed with higher nucleation have the potential to

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be resistant to ice recrystallization since they have homogenous size distribution of ice crystals when considering the mechanism of ice recrystallization. The mean size of ice crystals increased and the width of the crystal size distribution were spread, since the small ice crystals melted and converted into large ice crystals owing to the difference in thermodynamic equilibrium between crystals of different sizes, as defined by the Gibbs-Thomson equation, which is known as Ostwald ripening[9]. In other words, similar-sized ice crystals are resistant to ripening since ice crystals of the same size show thermodynamic equilibrium.

Previous studies on recrystallization behavior showed that ice crystals prepared by PSF in NaCl solution retain the round shape after storage; however, ice crystals prepared by conventional freezing do not retain the round shape[10]. In addition, a previous study investigated the behavior of ice recrystallization in salmon fillets prepared by PSF under -20 °C storage for 6 months and reported that ice crystals prepared with 100 MPa pressure have more homogenous size distribution and retain their initial shape than those prepared with 200 MPa pressure[11].

As the mentioned above, there is the possibility that the initial size distribution of ice crystals has a potential to control the recrystallization behavior and subsequent quality degradation during the storage process. However, there are limited studies on this behavior, on the other hands there are several studies on the effect of some factors, such as storage temperature, storage period, and food additives, e.g., anti-freezing proteins or polysaccharides on ice recrystallization behavior[12]-[14]. Therefore, in the present study, the recrystallization behavior of ice crystals formed by freezing accompanied with the deeper supercooling state was investigated Furthermore, quality degradation during frozen storage such as lipid oxidation, color change, and protein denaturation occurring in the freeze-concentrated phase are affected by ice recrystallization directly or indirectly[15]-[17]. Therefore, changes in the texture of frozen-thawed soy bean gels prepared by freezing accompanied with the deeper supercooling state during the storage period were investigated. In this study, soy bean curd were used as a sample of frozen food models, since soy bean curds have been used as frozen food models in some previous studies about freezing accompanied supercooling or PSF[18][19]. Furthermore, soy bean curds were mainly consisted protein and lipid, therefore some results from experiment using soy bean curds have potential to provide some discussion about frozen degradation of some protein based food.

2. Material and Method

2.1 Sample preparation

Soy bean curds ("Kinugoshi-tofu", MORINAGA MILK INDUSTRY Co., Ltd., Japan) were cut into cubes (20 mm × 20 mm × 20 mm), subsequently each cube of soy bean curd was wrapped by plastic film. In order to measure the freezing curves during freezing, Type-T thermocouple (φ 0.254 mm, T-T-30, Ishikawasangyo Co., Ltd., and Japan) was inserted into the center of each soy bean curd. Temperature hysteresis was logged with a date logger (MEMORY HiLOGGER LR8431 and LR8432, HIOKI Co., Ltd. Japan). The soy bean curds were cooled as mentioned below. In order to freeze the samples accompanying supercooling, a temperature-programmable cooling incubator (SMU-0541, SIBATA SCIENTIFIC TECHNOLOGY LTD., Japan) was used. The samples were kept in a chamber of the incubator and pre-cooled in +4 °C air for 1 h before freezing. Subsequently, samples were gradually cooled down to approximately -8.0 °C in the same chamber, with an air cooling rate set at 0.0375 °C/min. During this cooling process, some samples were in the supercooling state. When sample temperature in supercooling state reached -4°C or -6°C, samples were then moved into a chest freezer set at approximately -80 °C (DW-86L490J, EBARA CORPORATION, Japan) to resolve the supercooling state and freeze the sample completely. This freezing process is called SCF hereafter. Conventional rapid freezing and the conventional slow freezing were performed using a chest freezer set at -80 °C (DW-86L490J, EBARA CORPORATION, Japan) and at -30 °C (GS-3120HC, NIHON FREEZER CO., LTD., Japan) (hereafter, these freezing processes are called CRF and CSF). After the freezing process, all samples were transferred into other cool chambers, set at -30 °C (GS-3120HC, NIHON FREEZER CO., LTD., Japan) and set at -5 °C (MIR-254-PJ, Panasonic Healthcare Holdings Co., Ltd.) for 0, 4,7,14, and 28 days, respectively.

2.2 Indirect observation of ice crystals using scanning electron microscopy (SEM)

Indirect observation of ice crystals was performed using low vacuum scanning electron microscopy (Miniscope TM-1000, Hitachi High-Tech Solutions Corporation, Japan). After freeze-storage, frozen soy bean curds were cooled once in a chest freezer set at -80 °C to fix the characteristics of ice crystals and subsequent frozen samples were cut into rectangular solids (5 mm × 5 mm × 10 mm). Thereafter, the samples were freeze-dried for 2 days under approximately 5 Pa using a freeze-drying machine (KYOWAC RLE-103, Kyowa Vacuum Engineering. Co., Ltd., Japan) for sublimation of ice crystals. Freeze-dried soy bean curds were cut into small pieces, and were coated with vapor deposition techniques. The samples were scanned at 200x magnification with a low energy of 15 kV.

2.3 Texture measurement

The texture of thawed tofu was measured by the compression test using a texture analyzer (CR-3000EX; SUN SCIENTIFIC CO., LTD., Japan), equipped with a 200 N load cell. The samples were pressed with a plate-like plunger (φ 20 mm) at a 1.0 mm/sec rate until it pressed 80% of the sample thickness. The breaking strength and the initial elastic modulus were calculated from the first maximum strength of the stress–strain curve and the slope of the initial section of the stress–strain curve, respectively.

3. Result and Discussion

3.1 Effect of supercooling on changing behavior of ice crystal and texture of frozen soy bean curds during frozen storage under -30°C

Fig. 1 shows the typical freezing curves of soy bean curds by freezing accompanied supercooling (SCF). A sudden increase in temperature was observed in the SCF curve, due to the resolving of the supercooling state and the nucleation of ice crystals. In the SCF curve, the resolving of the supercooling state and ice nucleation occurred at -4.3 °C. Fig 2 shows the SEM images of frozen-storage tofu that were frozen by SCF and CRF method and subsequently stored at -30 °C for 0 and 28 days, respectively. The SCF samples were prepared with the two different resolving temperatures of the supercooling state, which is -4 °C (SCF4) and -6 °C (SCF6). In the figure, the darker part represents the ice crystals and the brighter part represents the structure of unfrozen parts. The ice crystals in CRF showed a needle-like shape, whereas the ice crystals in SCF showed a round shape. These characteristics of ice crystals were maintained for 28 days during storage under -30 °C air, regardless of the freezing method.

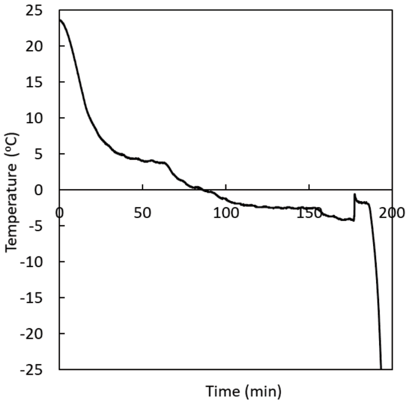

Fig.1 The typical freezing curves of soy bean curds by the freezing accompanied supercooling.

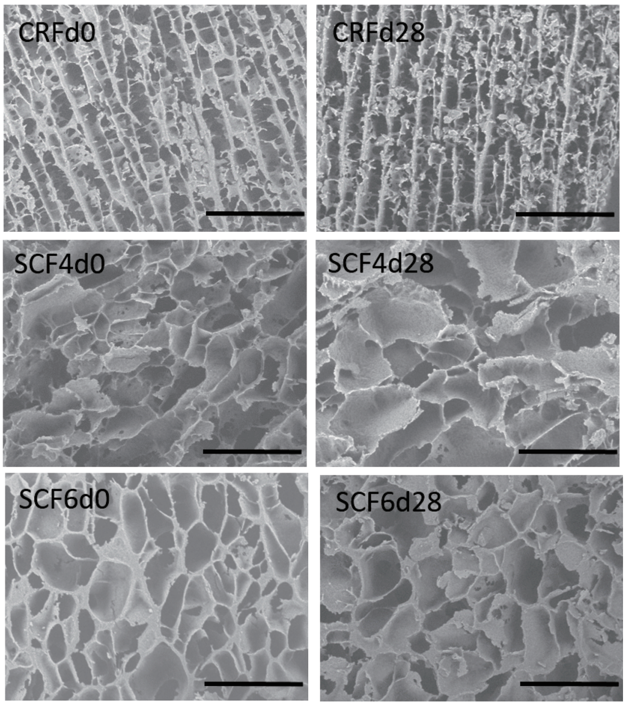

Fig.2 The SEM images of freeze-dried soy bean curds which were frozen with different freezing method and stored for different period under -30°C, CRF and SCF show the conventional rapid freezing and the freezing with supercooling, d0 and d28 means stored for 0 day, and 28 day , respectively. Bar shows 200 μm.

Fig. 3 shows the result of the texture measurement of the thawed tofu after frozen storage under -30 °C. Fig. 3-(i) and Fig. 3-(ii) show the changing behavior of the breaking strength and the initial elastic modulus of soy bean curds frozen by different freezing methods and storage under -30 °C.

Both breaking strength and initial elastic modulus value of soy bean curds were high in the order SCF6 > SCF4 > CRF, and they showed a constant value during all storage periods. This result indicated that storage temperature of -30 °C was too low to observe the ice recrystallization behavior in soy bean curds at approximately 1 month. The glass transition temperature of frozen concentrated phase (Tg') of soy bean curds used in this experiment was shown as -28.8 °C by DSC measurement (data not shown). It has been reported that ice recrystallization behavior in sugar solution and the changing behavior of phenolic compound amount in frozen berries during storage periods were determined by whether storage temperature is higher than the Tg' or not [20)21)]. Namely, our result was due to the lower storage temperature of -30 °C than the glass transition temperature of the frozen concentrated phase (Tg') of soy bean curd samples.

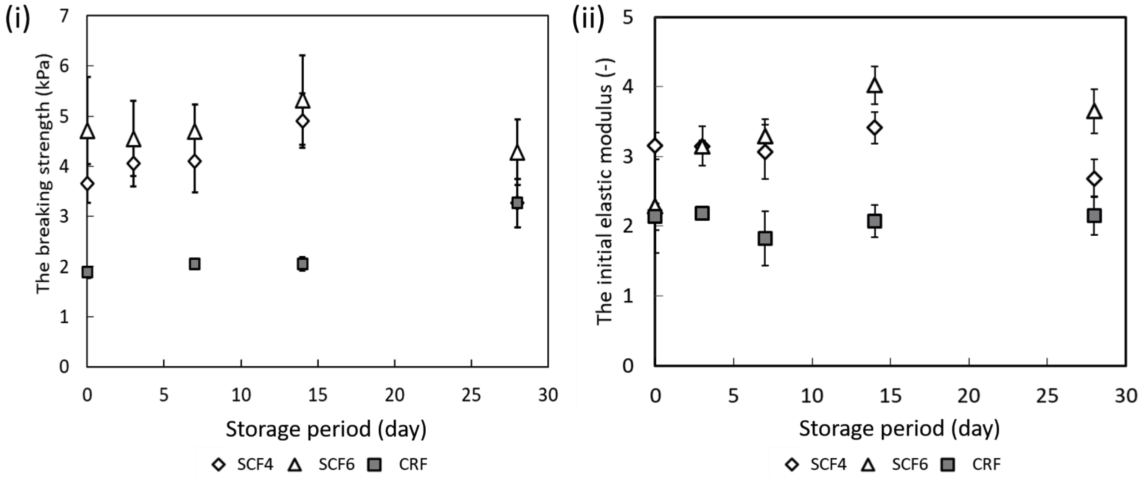

Fig.3 The changing behavior of the breaking strength (i) and the initial elastic modulus (ii) of soy bean curd during storage at -30°C, sample were prepared by the freezing accompanied supercooling with resolving of supercooling state at -4°C (SCF4),-6°C (SCF6) and by the conventional rapid freezing(CRF).

3.2 Effect of supercooling on changing behavior of ice crystal and texture of frozen soy bean curds during frozen storage under -5°C

The ice recrystallization was promoted by storing samples at relatively higher temperature, at -5 °C, in order to observe the ripening behavior of ice crystals clearly. Fig.4 shows the SEM images of soy bean curds that were frozen with SCF, CRF, and CSF, and subsequently stored at -5 °C for 0, 7, or 28 days, respectively. In this SCF freezing process, the supercooling state was resolved at approximately -6 °C (SCF6). In CRF and CSF, the size of ice crystals were increased depending on the storage period, and as a result, the coarse structure was formed at the end of the storage period. In particular, the characteristics of ice crystals in CRF were changed dramatically during storage.

In contrast, ice crystals in SCF maintained the initial character of small particle size and homogenous ice structure, regardless of the storage period, even though the storage temperature was relatively high at -5 °C.

Fig.5 shows the SEM images of freeze-dried soy bean curds which were prepared by the freezing accompanied with the supercooling state, with two different resolving temperatures of the supercooling state, -4 °C (SCF4) and -6 °C (SCF6). These samples were stored for 3 or 7 days before freeze-drying. Comparing the SEM images of SCF4 and SCF6 after 7 days of storage, the shapes of ice crystals in SCF6 were retained relatively. In other words, the samples that had relative homogenous structure of ice crystals at the initial period of storage could prevent ice recrystallization.

The results of the texture measurements of thawed tofu are shown in Fig.6. Fig.6-(i) shows the breaking strength of thawed tofu after being frozen with different freezing methods and subsequent storage for selected periods of time. The breaking strength of thawed tofu was increased depending on the storage period, regardless of freezing method. The value of breaking strength of SCF was kept slightly lower than that of CRF. In addition, SCF4 and SCF6 showed similar value of the breaking strength value. However, the thawed tofu frozen by conventional slow freezing was not ruptured since the sample structure became extremely tough during storage. Furthermore, it was observed that the longer the storage period, the lower the occurrence of ruptured samples. The rates of ruptured samples (%) by different freezing methods are shown in Fig.6-(ii). It was observed that during the -5 °C storage, soy bean curds prepared by SCF maintained their softness rather than those prepared by CRF and CSF; in addition, SCF samples prepared with relative lower resolving temperatures of supercooling (SCF6) maintained the softness rather than the SCF samples prepared with relatively higher resolving temperature of supercooling (SCF4).

The initial elastic modulus by different freezing methods are shown in Fig.6-(iii). The initial elastic modulus value of tofu was high in the order SCF6 > SCF4 > CRF > CSF. In addition, the initial elastic modulus value at any freezing method showed little changes during all storage periods even at -5 °C.

From the result of changing behavior in ice crystal of frozen soy bean curds during frozen storage under -5°C, it was investigated that freezing with supercooling inhibited ice recrystallization even in relative higher storage temperature. It is because that freezing with supercooling could form the ice crystal having a large curvature, which show relative low melting point owing to the Gibbs-Thomson effect. Therefore, it was supposed that freezing with supercooling inhibit ice recrystallization by same mechanism with Anti frozen protein.

Furthermore, it was observed that freezing with supercooling also prevented texture changes of frozen thawed soy bean curds during frozen storage under -5 °C. Soy bean curds during frozen storage under -5 °C were becoming hard, therefore, the breaking strengths of soy bean curds which were measured by the compression test increased and the soy bean curds which cannot be ruptured also increased depending on storage period. The rate of ruptured sample in soy bean curd prepared by freezing with supercooling was higher than soy bean curd prepared by conventional rapid and slow freezing. It was assumed that hardening of soy bean curds were owing to protein denaturation drawn by dehydration from soy bean curd matrix associated with ice recrystallization. As mentioned above, recrystallization of ice crystal formed by freezing with supercooling was suppressed since it have the lower melting temperature due to a high curvature of itself. Namely, it was supposed that dehydration from unfrozen matrix and subsequent protein denaturation were also suppressed in soy bean curd frozen with supercooling.

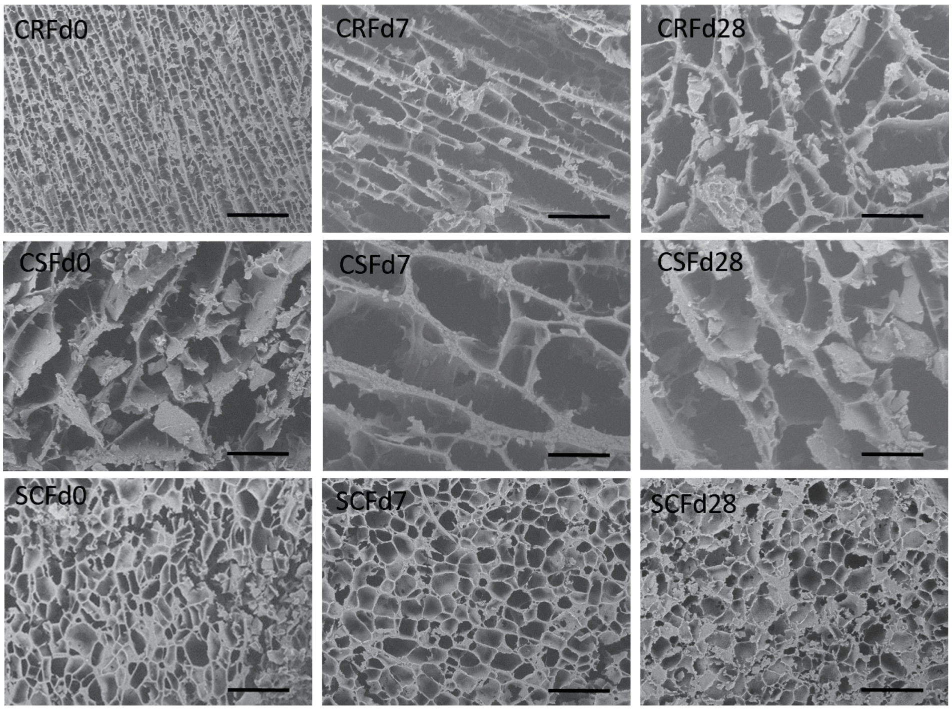

Fig.4 The SEM images of freeze-dried soy bean curds which were frozen with different freezing method and stored for different period under -5°C, SCF, CRF, and CSF show the freezing with supercooling, the conventional rapid freezing , and the conventional slow freezing, and d0,d7,and d28 is stored for 0 day, 7 day, and 28 day , respectively. Bar shows 250 μm.

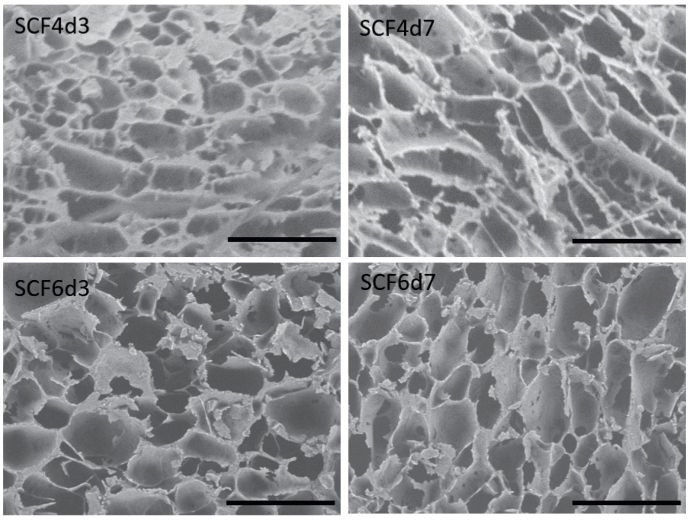

Fig.5 The SEM images of freeze-dried soy bean curds which were frozen accompanied supercooling with different resolving temperature of supercooling at -4°C(SCF4) or -6°C (SCF6) , and stored at -5°C for 3 day (d3) and 7 day(d7), respectively. Bar shows 200 μm.

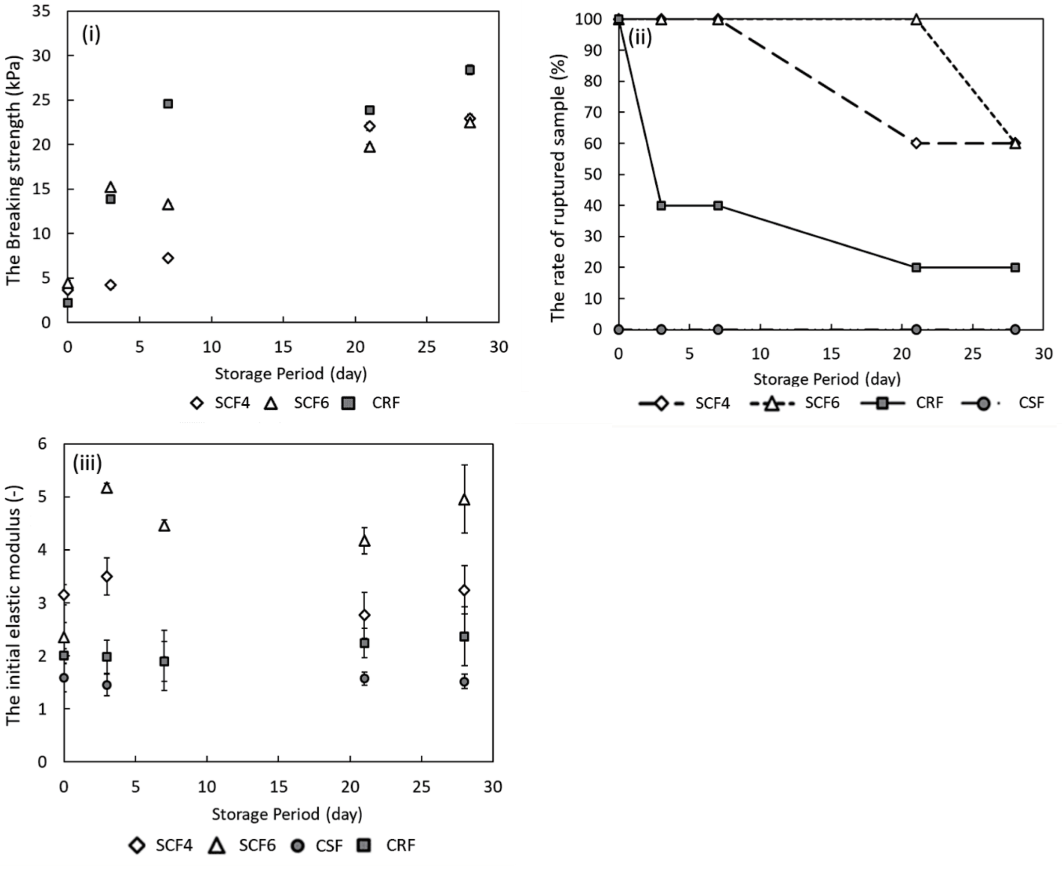

Fig.6 The changing behavior of the breaking strength (i) , the rate of ruptured sample (ii), and the initial elastic modulus (iii) of soy bean curd during storage at -30°C, sample were prepared by the freezing accompanied supercooling with resolving of supercooling state at -4°C (SCF4),-6°C (SCF6) and by the conventional rapid freezing(CRF) and the conventional slow freezing (CSF).

Additionally, it was considered that soy bean curd frozen with supercooling resolved at -6 °C showed the higher ruptured rate than it at -4 °C since the lower resolving temperature of supercooling can let the higher curvature of ice crystals.

4. Conclusion

In this study, the effect of initial ice structure determined by freezing with supercooling (SCF) on the recrystallization behavior and the texture changes as quality degradation of soy bean curds during storage were investigated. Ice recrystallization under -5 °C storage did not generally proceed in the samples prepared by SCF, whereas the samples prepared by slow freeing proceeded dramatically. Furthermore, increasing of breaking strength in the samples prepared by SCF were slightly prevented than by conventional rapid freezing.

On the other hand, distinguished changes in ice crystals and texture under -30 °C storage for 28 days could not be observed because the storage temperature was lower than the Tg' of the soy bean curds used as the sample.

The results indicated that the homogenous ice structure determined by SCF, which can attain a higher ice nucleation rate during the freezing process, is one of the effective factors to prevent ice recrystallization and quality degradation during the storage period, even under high storage temperature.

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