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ARTICLE

Effects of Pressure-shift Freezing on the Structural and Physical Properties of Gelatin Hydrogel Matrices

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Abstract

This study investigates the effects of the gelatin concentration (10-40%, w/v), freezing temperatures (from -20°C to -50°C) and freezing methods on the structural and physical properties of gelatin matrices. To freeze gelatin, the pressure-shift freezing (PSF) is being applied at 0.1 (under atmospheric control), 50 and 100 MPa, respectively. The freezing point of gelatin solutions decrease with increasing gelatin concentrations, from -0.2°C (10% gelatin) to -6.7°C (40% gelatin), while the extent of supercooling did not show any specific trends. The rheological properties of the gelatin indicate that both the storage (G') and loss (G'') moduli were steady in the strain amplitude range of 0.1-10%. To characterize gelatin matrices formed by the various freezing methods, the ice crystal sizes which were being determined by the scanning electron microscopy (SEM) are affected by the gelatin concentrations. The ice crystal sizes are affected by gelatin concentrations and freezing temperature, while the size distributions of ice crystals depend on the freezing methods. Smaller ice crystals are being formed with PSF rather than under the atmospheric control where the freezing temperature is above -40°C. Thus, the results of this study indicate that the PSF processing at a very low freezing temperature (-50°C) offers a potential advantage over commercial atmospheric freezing points for the formation of small ice crystals.

Key words: pressure-shift freezing, freeze-drying, freezing rate, gelatin, micro-pore

Introduction

Freezing has been the most effective food preservation technique, although drip loss during freezing contributes to the loss of food quality. Formation of ice crystals during freezing causes tissue damage by generating exudates. In addition to economic losses for food manufacturers, drip losses diminish food nutrition and quality for consumers. Rapid freezing is generally accepted as a favorable alternative for minimizing tissue damage due to the formation of small uniform ice crystals (Choi *et al*., 1998). Various technologies have been introduced as novel quick freezing technologies including cryogenic freezing, immersion freezing, and fluidized bed freezing (Khairullah and Singh, 1991; Pham, 2014; Xu *et al*., 2013). These novel quick freezing techniques were based on thermal conduction or convection because heat must be removed from the interior of food and through its surface to the exterior. Thus, the freezing rate is inevitably slower inside food than near its surface.

High pressure techniques have been applied in the food industry. The three categories of high pressure applications are food texturization, food preservation, and food control of the phase transition of water. Schlüter *et al*. (1998) classified high pressure freezing according to applied pressure and temperature conditions. In particular, pressure-shift freezing (PSF) shows potential advantages for minimizing the loss of quality caused by ice crystallization.

Water undergoes phase transitions under high pressure and forms various types of ice (Kalichevsky *et al*., 1995). The decrease in the latent heat of fusion with increasing pressure is an intriguing phenomenon (Bridgman, 1912; Otero and Sanz, 2006). Additionally, the freezing point of water decreases with increasing pressure up to 200 MPa, where the freezing point of pure water is reported to be approximately -20°C, providing the basic principle of PSF (Bridgman, 1912). Foods subjected to sub-zero tempera-

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tures under high pressures are not frozen but supercooled. During supercooling, freezing is very rapid and there is no difference in the freezing rate throughout food (Le Bail *et al*., 2002; Otero and Santz, 2006). The PSF technique should be the most rapid freezing technique among all current freezing methods. Studies are underway to investigate the effects of PSF on the physical attributes of protein-based foods such as tofu, meat, and model foods (Chevalier *et al*., 2000; Fuchigami *et al*., 2003; Kanda *et al*., 1992). In the study of Koch (1996), aPSF-treated potato maintain edits texture with higher bursting strength than a potato frozen in an air blast freezer. The color change was greater and the concentration of dissolved matter was higher in the drip for a potato that underwent air blast freezing than PSF. Fuchigami *et al*. (1997a, 1998) and Koch *et al*. (1996) also reported that the effects of PSF on textural properties and microstructure were better than traditional freezing methods. Additionally, Otero *et al*. (1998) showed that PSF reduced drip in eggplants relative to conventional freezing. In the study of Fernández *et al*. (2006), ice crystals were more numerous and smaller in gelatin samples formed by PSF than freezing under constant pressure (pressure assisted freezing, PAF). Ko *et al*. (2006) also recommended PSF over PAF at higher pressure (> 100 MPa) in the case of meat.

Despite numerous investigations of PSF, little information was available regarding the effects of PSF at relatively moderate pressure levels (<100 MPa) on the quality attributes of foods. Additionally, relatively high pressure (>150 MPa) has been reported to cause protein denaturation and discoloration of meat (Ko *et al*., 2006). In the present study, the effects of PSF under mild pressure conditions (50 and 100 MPa) on the physical and structural properties of gelatin matrices were explored at various freezing temperatures and gelatin concentrations and compared with conventional atmospheric freezing.

Materials and Methods

Sample preparation

The extra gelatin (Duksan Pure Chemicals, Korea) from cowskin was used for PSF. Gelatin solutions were prepared by dissolving 10-40% (w/v) gelatin in distilled water and heating in an autoclave at 121°C for 10 min (Choi *et al*., 1998). After cooling under ambient conditions for 2 h, the gelatin solutions were poured into disposable cylindrical containers (Micro-weighing dish, Wheaton, USA) with a 20 mm diameter and 8 mm height. These containers were sealed with para-film.

Differential scanning calorimetry (DSC)

The thermal properties of gelatin solutions were determined by DSC analysis (DSC200F3, NETZSCH, Germany). Each sample (approximately 1-3 mg) was sealed hermetically in an aluminum pan and equilibrated at 10°C for 1 min. The temperature was decreased from 10°C to -70°C and then increased to 10°C at a rate of 5°C/min. The extent of supercooling and the freezing point were obtained from each DSC curve.

Oscillatory shear rheometry

For oscillatory rheometry (Anton-Paar, USA), gelatin samples were loaded on the sample plate and covered with paraffin oil to prevent evaporative loss. Each sample was equilibrated at 25°C for 3 min and sheared at a fixed vibrational angular frequency of 10 rad/s with a strain amplitude of 0.01-100%.The storage (G') and loss (G'') moduli were measured using a parallel plate (20 mm diameter and 3 mm thickness), and the normal force was approximately 5 N.

PSF treatment

The PSF treatment of each gelatin sample was conducted using a high pressure system composed of a pressure generator (S40-40-4.5HP, a Seowon compressor, Korea), a pressure intensifier, multiple pressure vessels (300 mL working volume) and a coolant circulator (FP80-MS/P, JULABO, Germany). Ethanol was used as the pressure-transmitting medium. A thermocouple (k-type) was inserted into the geometric center of a sample and connected to a data logger (MV104-2-2-1F, Japan) to monitor the sample temperature during freezing. The PSF of gelatin solutions was carried out at three different pressure levels: 0.1 (control), 50 and 100 MPa. Samples were pressurized at the targeted pressure levels, and the vessels were cooled to selected freezing temperatures (-20, -30, -40 and -50 °C). When the sample temperature reached its target, pressure was released to initiate PSF and was maintained for 10 min to complete the freezing process. Finally, samples were transferred to a freezer at -70°C and stored for 24 h. Then, the para-film and frozen sample were removed from each container. To prevent surface melting during handling, liquid nitrogen was sprayed on the sample surface. The samples were moved to a freeze-drier and lyophilized at 0.5 mbar for 12 h at -80°C.

Size distribution of ice crystals

Each cylindrical freeze-dried sample was cut in sections parallel to the bottom of the cylinder. Samples were obser-

ved with a scanning electron microscope (SEM) at an acceleration voltage of 14 kV. Each sample was photographed at least 20 times. The pores had globular shapes, and pore sizes were measured using an image analysis program (Uthsca Image Tool for Windows version 3.0, the University of Texas Health Science Center, USA). The pore size was reported as the average of measured values.

Puncture test

Dried samples were compresse dusing a texture analyzer (CT3, Brookfield, USA) equipped with a conical probe with a 24 mm diameter and a 30° angle. Samples were prepared witha 10 mm diameter and 5 mm thickness. The test speed was 0.5 mm/s, and the trigger load was 3 g.

Statistical analysis

A randomized complete block design was used to analyze the main effects (gelatin concentration, freezing temperature and freezing method). Means from three replicate experiments were analyzed by one-way analysis of variance (ANOVA) using the SAS statistical program (ver. 9.3), and Duncan's Multiple range test was conducted when main effects were significant ($p<0.05$).

Results and Discussion

Thermal properties of gelatin samples

The DSC profiles for samples with various gelatin concentrations are given in Fig. 1. The freezing point of the gelatin samples decreased from -0.2°C (10% gelatin) to -6.7°C (40% gelatin) when the gelatin concentration was increased. Fullerton *et al*. (1994) reported that the freezing points of their solutions were related to their water content. In the present study, the same trend was observed, where the initial freezing point decreased when the water content was lower. The freezing point depression (ΔT_f) is expressed as $\Delta T_f=K_f \cdot M$, where K_f is the cryoscopic constant (1.86 for water) and M is the solute molality (Toledo, 2007). Meanwhile, the apparent extent of supercooling did not follow a trend with respect to the gelatin concentration. For example, the supercooling extent was lowest (-25.5°C) for a 20% gelatin sample and highest for 30% gelatin. These results are not consistent with the results reported by Otero and Sanz (2006), who postulated that the extent of supercooling during PSF increased with increasing pressure level. However, Fernández *et al*. (2006) noted that the supercooling of pure water was not statistically correlated with the pressure level because of the stochastic nature of ice nucleation, and that result was consistent with the absence of a correlation between the gelatin concentration and supercooling extent in the present study.

Rheological properties

The storage modulus (G') and loss modulus (G'') were observed at different gelatin concentrations (Fig. 2). At a fixed oscillatory angular frequency (10 rad/s), both moduli

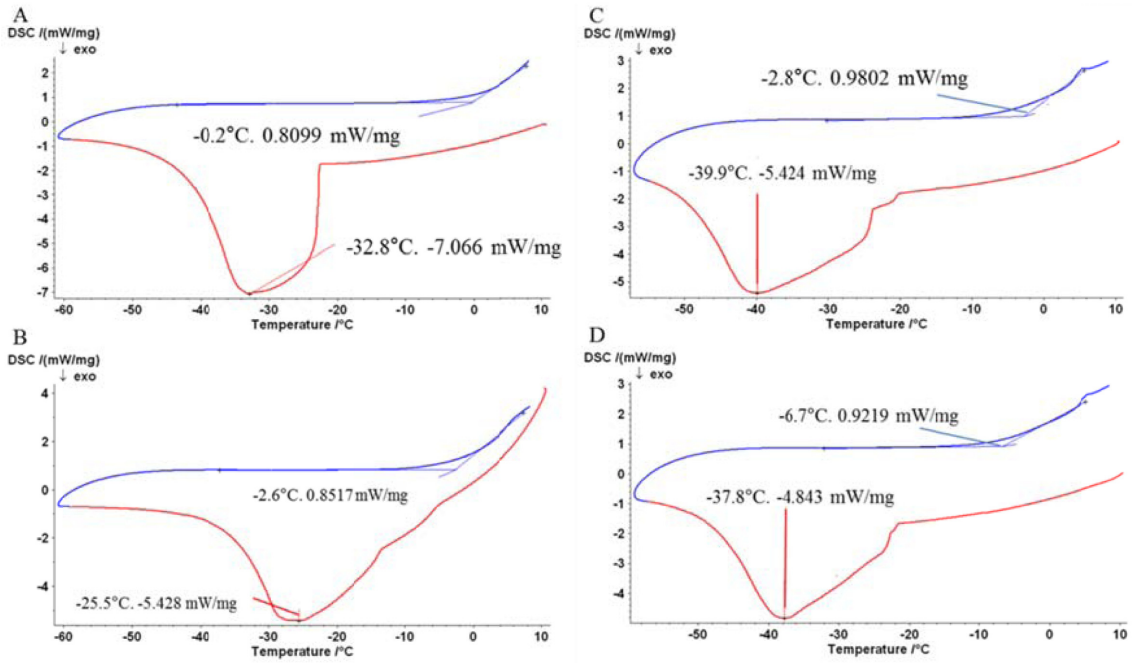

Fig. 1. DSC profiles of (A) 10%, (B) 20%, (C) 30% and (D) 40% gelatin solutions during cooling and heating procedures.

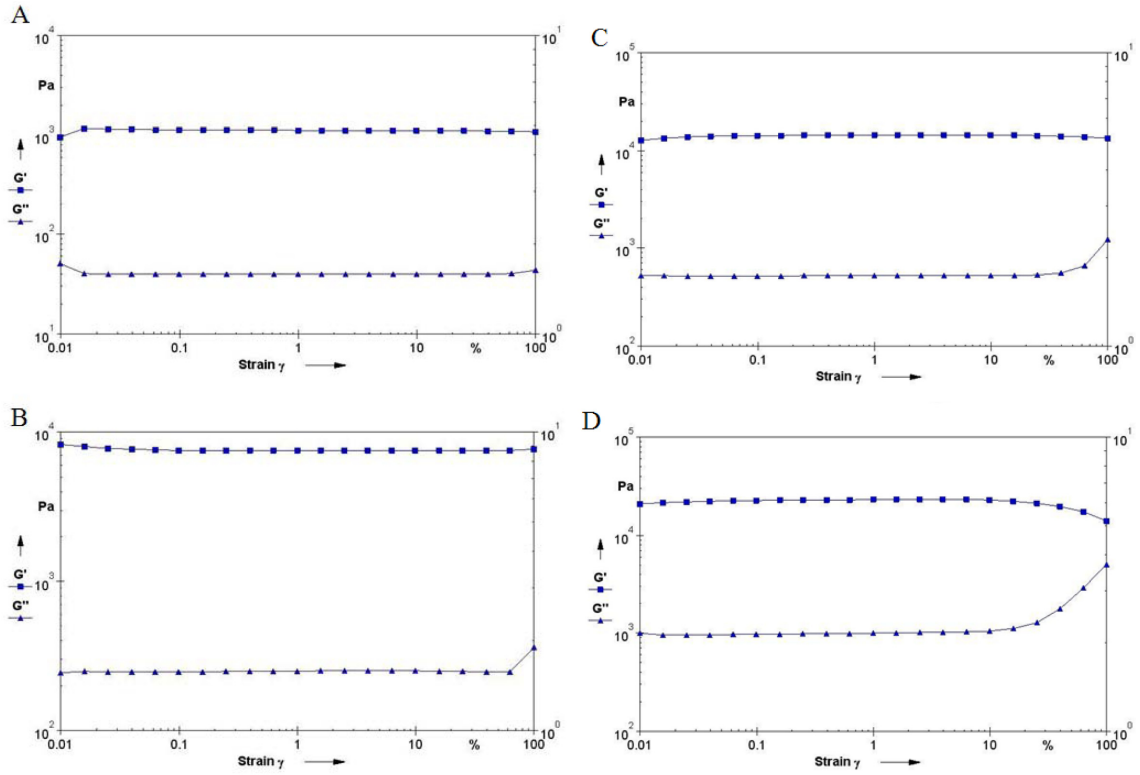

Fig. 2. Rheological properties of (A) 10%, (B) 20%, (C) 30% and (D) 40% gelatin solutions at a fixed vibrational frequency of 10 rad/s.

showed linear changes with strain in the range from 0.1% to 10%. In this range, higher G' values were observed with increasing gelatin concentration. The G' value for 10% gelatin samples was approximately 10.2 kPa, and G' increased to 20.1 kPa when the gelatin concentration was 40%. The G'' of the 10% gelatin samples wasapproximately 42 Pa. As was the case for G', G'' steadily increased with increasing gelatin concentration and reached 994 Pa at 40% gelatin. Thus, changes in the rheological properties of gelatin are expected to contribute to the formation of ice dendrites, thereby affecting the overall freezing rates of gelatin solutions.

Pore size distribution

The pore size indicates the dimensions of ice crystals formed during freezing, which correspond to the freezing rate. Rapid freezing causes the formation of small uniform ice crystals that minimize tissue damage in frozen foods that contain protein muscle. As depicted in Fig. 3 to Fig. 5, the pore size distributions were inversely proportional to gelatin concentrations, although some gelatin samples showed an irregular trend. Similar results were reported by Fuchigami *et al*. (2003), where PSF was applied togellan gum containing 0-20% sucrose. They concluded that ice crystal size decreased slightly as the concentration of sucrose in gels increased. The ice crystal size is expected to relate closely to the total solid/ice content. In the current study, gelatin would have raised the solution viscosity and possibly have led to the slow formation of ice dendrites. As a result, high concentrations of small ice crystals would have been generated in the gelatin matrix.

Freezing temperatures also affected the pore size distribution in gelatin. In general, pore sizes in gelatin samples were smaller at lower freezing temperatures. During the freezing procedure, the freezing temperature is crucial to reduce the overall freezing rate (rapid freezing velocity). Based on Plank's equation, the difference between the freezing point of water and the freezing temperature of a solution acts as a driving force for freezing and has a greater effect than the latent heat on the freezing rate (Yanniotis, 2008).

The size distributions of ice crystals followed principles related to solute concentration and freezing temperature, whereas the freezing methods did not produce a trend in the size distribution. At relatively high freezing temperatures (>-40°C), ice crystals were smaller in gelatin after PSF at 100 MPa than freezing under atmospheric control. Although some gelatin samples formed by PSF at 50 MPa

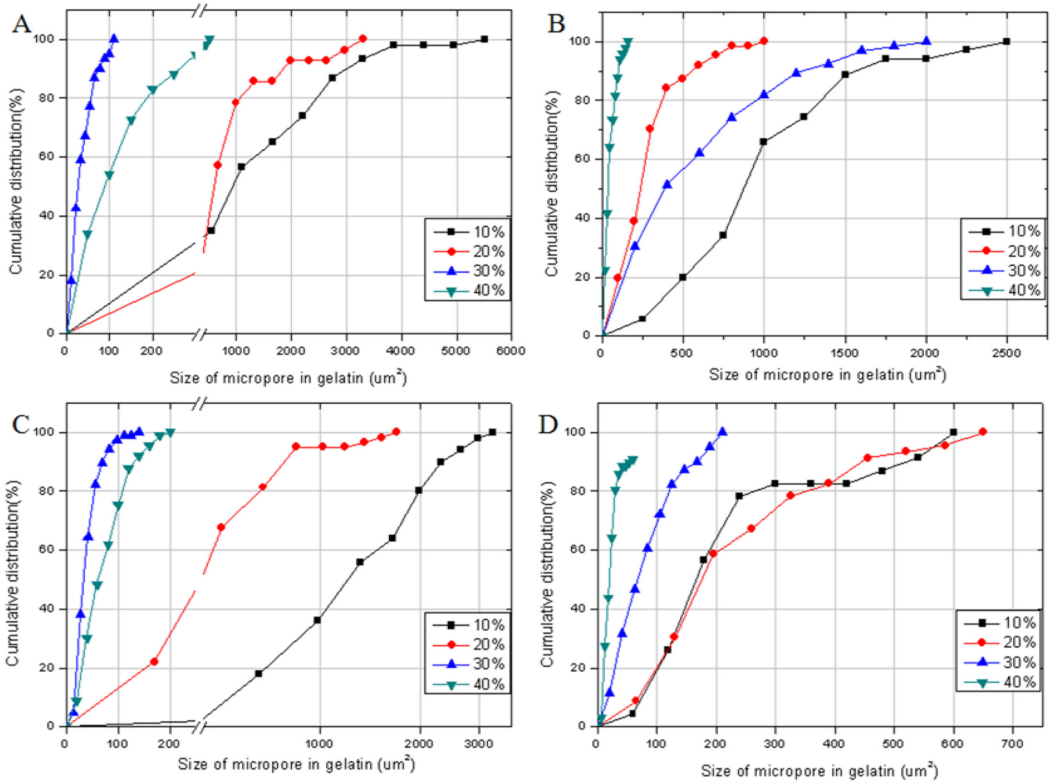

Fig. 3. Cumulative pore size distributions of gelatin frozen at (A) -20°C, (B) -30°C, (C) -40°C and (D) -50°C under atmospheric freezing conditions.

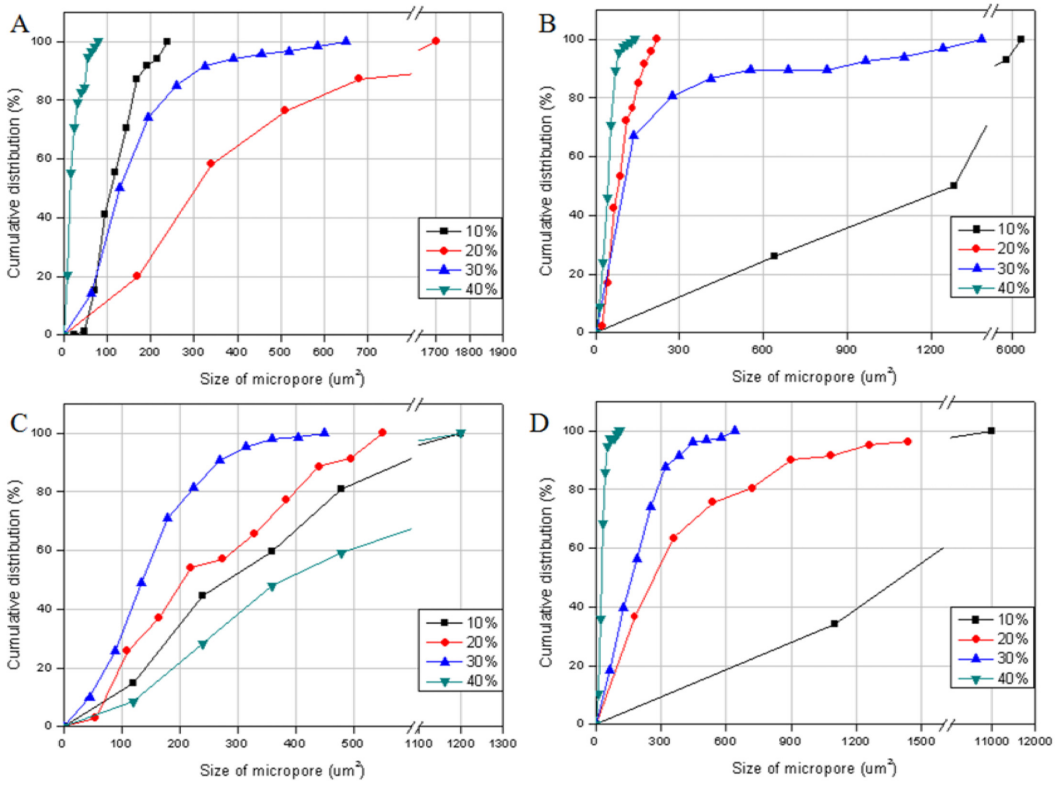

Fig. 4. Cumulative pore size distributions of gelatin frozen at (A) -20°C, (B) -30°C, (C) -40°C and (D) -50°C under PSF under 50 MPa.

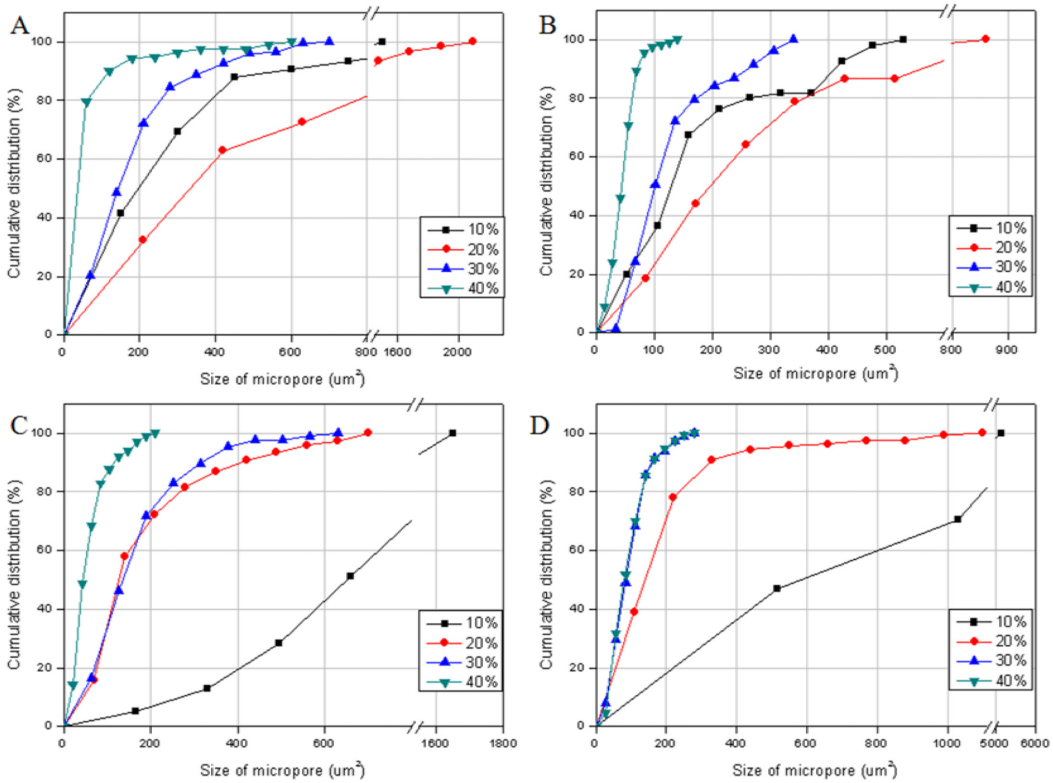

Fig. 5. Cumulative pore size distributions of gelatin frozen at (A) -20°C, (B) -30°C, (C) -40°C and (D) -50°C under PSF under 100 MPa.

also contained smaller ice crystals than the atmospheric control, the results were not consistent for every sample. It is possible that as mall extent of supercooling caused by PSF at 50 MPa produced inconsistent size distributions of ice crystals. Ko *et al*. (2006) indicated that PSF processing at relatively higher pressures (>100 MPa) was advantageous for the formation of small ice crystals. In contrast, PSF-treated gelatin at -50°C contained larger ice crystals than the atmospheric control. At -50°C, gelatin samples might have frozen below the pressure applied in this study (pressure assisted freezing) at a slower freezing rate than the atmospheric control.

Shear strength

Hardness data were obtained for 10% gelatin samples (Fig. 6), while the shear strength was too great to be measured for higher gelatin concentrations. The shear strength of gelatin tended to increase with decreasing freezing temperature, and this relationship was particularly clear for the PSF treatment. Because the freezing temperature was closely relatedto the freezing rate, higher shear strength values were found for gelatin frozen at lower temperatures. The shear strength of gelatin was higher for atmospheric freezing than PSF with the exception of gelatin frozen at -50°C (p<0.05). According to the DSC analysis, the freezing point and the extent of supercooling of 10% gelatin samples were estimated to be -0.2°C and -32.8°C, respectively. Although it was impossible to calculate the actual extent of supercooling of gelatin during atmospheric freezing, the supercooling results for pure water at 50 and 100 MPa were reported to be -4.7°C and -9.3°C, res-

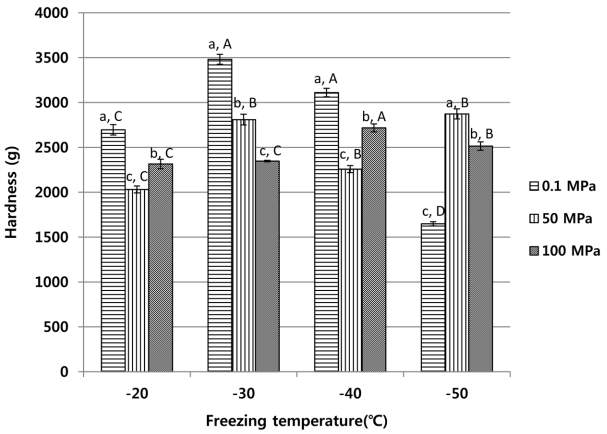

Fig. 6. Hardness of 10% gelatin samples frozen at various temperature and methods. [a-c]Means significantly differ at the same temperature (p<0.05). [A-D]Means significantly differ at the same pressure (p<0.05).

pectively (Chourot *et al*., 1997). The number of ice nuclei is proportional to the extent of supercooling, and smaller ice crystals correspond to more numerous ice nuclei (Fernández *et al*., 2006). Consequently, PSF-treated gelatin contained larger ice crystals than gelatin frozen under atmospheric conditions,which was consistent with the ice crystal size distribution and resulted in relatively low shear strength. Meanwhile, the shear strength of gelatin was greater following PSF treatment at -50°C than atmospheric conditions (p<0.05). This result implied that freezing was faster during PSF treatment at -50°C than under atmospheric control. Under higher pressures, the latent heat is lower, and the gelatin would freeze faster during PSF than normal freezing (Otero and Sanz, 2006). Thus, these results indicated that lower freezing temperatureswere necessary to achieve rapid freezing in PSF processing.

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