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The Effect of Thermal History on The Glass Transition of Dried Gelatin Gel

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Glass transition behavior of gelatin samples prepared by drying at various temperature (10-70℃) was investigated by using differential scanning calorimetry (DSC). Circular dichroism measurement (CD) and wide angle X-ray diffraction measurement (WAXD) were also performed to analyze the effect of the preparation temperature on the microstructure in gelatin. The DSC investigation showed that the heat capacity increment ΔC_p of the gelatin sample during glass transition depended on the drying temperature, namely the thermal history. In addition, from results of CD and WAXD measurements, it was suggested that the difference of the content of amorphous (or crystalline) region in gelatin sample, which caused by such the difference in drying temperature, affected on the value of ΔC_p during the glass transition.
Key words: gelatin gel, glass transition, differential scanning calorimetry, thermal history

1. INTRODUCTION

Gelatin is a fibrous protein polymer produced from collagen and an important industrial biopolymer, particularly as a food ingredient because of its gelling properties of hot solution; upon cooling, cross-linked zones, similar to collagen triple helix, are formed among random gelatin polymers, resulting in network structure [1]. Gelatin gel is regarded as the composition of cross-linked zones (crystalline regions) interconnected with random coil regions [2]. It has been reported that the amount of the collagen-like helix that contributes to the cross-linked zone in gelatin depends on temperature [3].

Recently, it is known that low moisture foods are often solid material with a glassy structure at room temperature. Because, at temperature above glass transition temperature, various physical properties are affected and so some deteriorative change may occur, glass transition is associated with the stability of food products [4]. Therefore, studying glass transition for food ingredient is useful to food storage.

As for gelatin, several researchers have reported the glass transition behavior and showed that gelatin at low-moisture content could take glassy state at room temperature [5]. The glass transition of gelatin originates from not the crystalline region of the cross-linked zones, but the amorphous region derived from randomly coiled chain segments [2]. Because the formation of the cross-linked zone in gelatin depends on the gelling temperature [3] as mention before, the thermal history in preparation of gelatin sample such as drying temperature may influence the thermal properties of gelatin including the glass transition. However, there are a few studies that investigated the effect of thermal history on the glass transition behavior of gelatin [2].

In this study, the thermal behavior, particularly the glass transition behavior, of dried gelatin prepared through several thermal history was investigated by differential scanning calorimetry (DSC). In addition, the structure of the gelatin sample prepared at various temperature was analyzed with the measurements of circular dichoism (CD) and wide angle X-ray diffraction (WAXD). Finally, the result of DSC was compared with that of CD and WAXD.

2. EXPERIMENTIAL

2.1 Differential Scanning Calorimetry Measurement

The granular gelatin (Wako Pure Chemical Ind., Lid., Osaka, Japan) that was first glade industrial product prepared from cattle bone and skin by the lime-process was used. The granular sample was swollen in distilled water for 30 minutes at room temperature and dissolved at 70 ℃ for 30 minutes with stirring. The final concentration of the sample solution was 10%(w/w). This aqueous gelatin solution was poured into stainless vat. Each stainless vat was sealed with the cover and placed at six temperature; 10℃, 20℃, 30℃, 40℃, 50℃ and 70℃. After 2days, the seals were opened and the gelatin solution was dried at the same temperature as before. Two different drying methods were used according to drying temperature. As for the sample at 30-70℃, the sample solutions were dried in oven for 24hours. The sample at 10℃ and 20℃ were dried in desiccators containing silica gel at drying temperature for 5days.

After the drying process, the dried samples were exposed to atmosphere in equilibrium with saturated sodium chloride solution at 10℃ for 2days to adjust the water content to about 20%(w/w).

Heat-flux DSC-50 (SHIMADZU Co., Kyoto, Japan) was used. The sample was shaped into pellet-like form and encapsulated in hermetically sealed aluminum DSC

pan. The sample weight was about 10mg. Alimina powder was used as a reference sample. The sample was set in DSC apparatus and heated at 5℃/min. The experimental data was analyzed with the software of TA60-WS thermal analysis (SHIMADZU Co., Kyoto, Japan).

2.2 Measurement of Circular Dichoism

0.05%(w/w) aqueous solution was prepared. Before CD measurement, aliquots of this solution were placed for 48 hours at six fixed temperatures; 10℃, 20℃, 30℃, 40℃, 50℃, and 70℃.

CD was measured with a Jusco Model J-720 spectropolarimeter (JASCO Co., Tokyo, Japan) from 190 to 260nm. Cell with 1mm path length was used.

2.3 Wide Angle X-ray Diffraction

The method of sample preparation was the same as that of DSC. The four kinds of samples (dried at 10℃, 30℃, 40℃, and 70℃, respectively) were used.

X-ray diffraction measurement was carried out using X'Pert-Pro (Philips Japan, Lid., Tokyo, Japan). The X-ray souse was Cu-K α (wave length 0.154184nm). The sample was cut into plate shape (the sample was about 0.5mm thick) and set in WAXD apparatus and then diffraction intensity was measurement at room temperature. The scanning region of the diffraction angle was from 3 to 60° at angular interval of 0.08° .

3. RESULTS AND DISCUTTION

3.1 DSC measurement

Figure 1 shows the DSC heating curves for the gelatin samples prepared at various temperatures. We performed the DSC rescan, namely second scan after cooling the sample in liquid nitrogen.

As for the first scan curves of the sample prepared below 30℃, a step-wise change in heat capacity, indicative of a glass transition was observed as indicated by an arrow. In addition to it, the endothermic peak was shown. The more the drying temperature increase from 10 to 30℃, the smaller the peak was. In the 2nd scan, only the glass transition behavior was shown. For the sample prepared at above 40℃, the typical glass transition behavior was occurred both in 1st scan and 2nd scan. However, the endothermic peak located apart from the step-wise change which was shown in the 1st scan curve for the sample prepared below 30℃ was not shown.

Cooling hot gelatin solution, cross-linked zones, similar to collagen triple helix, are formed among random coil of gelatin polymers [1,2]; that is to say, gelatin can have both crystalline structure (cross-linked zone) and amorphous structure (random gelatin coil).

This kind of polymer is called a semicrystalline polymer [6]. Example of semicrystalline polymer is starch; in native starches amylopectin exhibits partial crystallinity and amylose exists in the amorphous noncrystalline state. Starch is partially

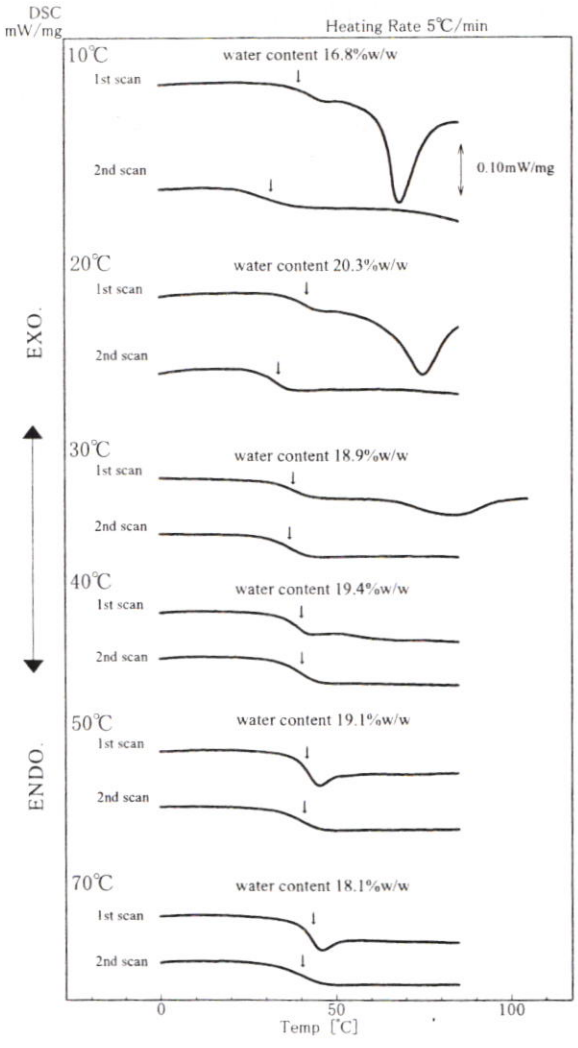

Fig.1 DSC Heating Curves for The Dried Gelatin Prepared at Several Temperatures The sample was prepared by drying 10%(w/w) gelatin solution.

crystalline carbohydrate polymer [6]. From the concept of semicrystalline polymer, the glass transition behavior observed in DSC curves of gelatin would originate from the amorphous structure in the gelatin [7]. On the other hand it was suggested that the endothermic peak corresponded to the melting reaction of the crystalline structure as was reported by Levine *et al.*[5,7]. As for the sample prepared below 30℃, the endothermic peak disappeared in 2nd scan. The reason for this disappearance may be that the cooling after the crystalline melting was so rapid that reforming of the crystalline was prevented. In case of the sample dried at above 40℃, the endothermic reaction same as those of the sample prepared below 30 ℃ was not observed, indicating that the crystalline structure was not formed. It was suggested that the temperature was so high that the drying process proceeded without formation of the crystalline structure

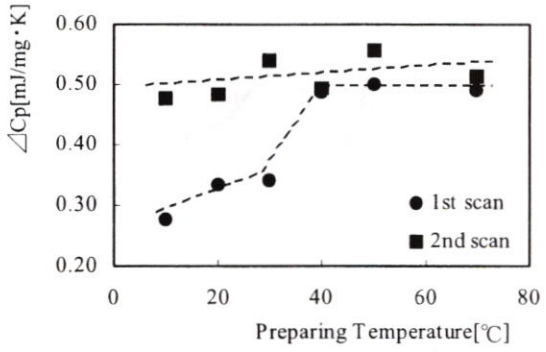

Fig.2 The Heat Capacity Increment ⊿Cp of The Dried Gelatin Prepared at Several Temperatures

(cross-linked zone).

Figure 2 shows the relation between the heat capacity increment at the glass transition ΔC_p and the drying temperatures. As for the 1st scan, the heat capacity increment ΔC_p for the sample dried at the temperature below 30℃ tended to increase with increasing the preparing temperature. The rapid increase of ΔC_p from 30℃ to 40℃ was observed, and then at higher than 40℃, the increment was almost constant. These facts suggested that increasing the drying temperature from 10 to 40℃, the content of amorphous structure increased, while the content of crystalline structure decreased. In the 2nd scan, the ΔC_p at all of the drying temperature examined seems to be approximately equal; through the heating process in 1st scan which brought about the glass transition and the melting reaction of the crystalline, effect of the drying temperature would be eliminated.

3.2 CD measurement

Figure 3 shows the CD value at 221nm of the aqueous gelatin solution thermostated for 48h at 10℃, 20℃, 30℃, 40℃, 50℃, and 70℃, respectively. In CD spectra of gelatin solution, the positive peak at about 220nm is associated with the collagen-like helix [3,8]. The CD value at the top of this peak indicates quantitative information of the structural order as helix in gelatin molecule. With increasing the temperature from 10 to 30℃, the CD value decreased. At higher temperature than 40℃, the CD value was almost constant, followed by the rapid decreasing between 20 and 30℃. It would be shown that the amount of helix content in this solution was dependent on the thermal history; the content of the collagen-like helix increased with decreasing of preparation temperature.

The thermal history dependence of CD value in Fig. 3 seems to be just opposite to that of the heat capacity increment ΔC_p, though the temperature region where the CD value decreased rapidly (between 20 and 30℃) was slightly different from that where the value of ΔC_p increased rapidly

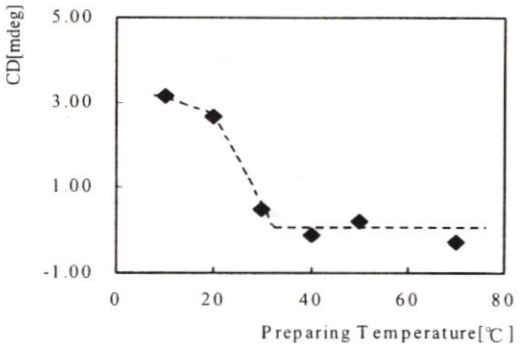

Fig.3 CD Value at 221nm of The Aqueous Gelatin Solution Prepared at Several Temperatures
Gelatin Concentration:0.05%(w/w)

(between 30 and 40℃); this difference would be caused by the difference of the gelatin concentration of the samples.

3.3 WAXD measurement

Several WAXD studies on gelatin have been reported that two characteristic peak derived from the crystalline structure of gelatin were observed [9,10,11] ; one is the peak at $2\theta = 7^\circ$ which is from the repeat of the collagen-like helical protofibril in the direction perpendicular to the fiber axis of a junction zone among gelatin molecules the crystalline structure formed by aggregated collagen-like helix. The other is the peak at $2\theta = 31^\circ$ from the amino-acid residues in junction zone and corresponds to the height of the amino-helical protofibril[9].

Figure 4 shows the WAXD patterns obtained in this study. For the sample prepared at 10℃, at diffraction angle $2\theta = 7$℃, the sharp peak appeared. In the sample dried at 30℃, the peak at same diffraction angle was also shown, however, the peak shape became to be slightly broader. The sample prepared at 40 ℃ had only incomplete peak like shoulder at $2\theta = 7$℃. Furthermore, in the sample prepared at 70℃, no peak was observed at $2\theta = 7^\circ$.

The peak at $2\theta = 7^\circ$ reflects the crystalline formed by aggregation of collagen-like helix associated with cross-linked zone in gel network and the intensity of this peak is almost likely related to the content of the crystalline region in gelatin sample [10]. Though the peak intensity at $2\theta = 7^\circ$ could not be compared because it was difficult to prepare the sample of same thick at different drying temperature, it was clear that with increase in drying temperature from 10 to 70℃, the peak shape tended to be broader. This suggested the content of crystalline structure decreased, which was agreed with the increase in the content for the amorphous structure indicated by the behavior of ΔC_p observed in DSC measurement.

Ito et al. have observed the peak at 31° of the

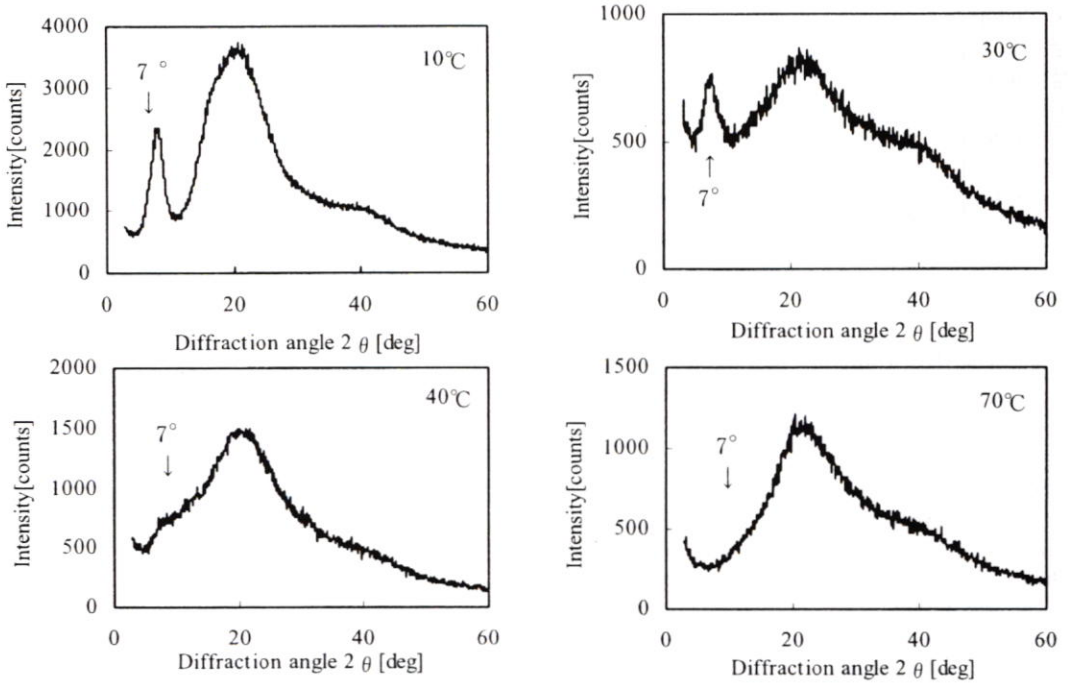

Fig.4 WAXD Patterns of The Dried Gelatin Prepared at Several Temperatures
The sample was prepared by drying 10%(w/w) gelatin solution.

deionized lime-processed bone gelatin (NP-S-744) and reported that this peak originates from amino acid residues in junction zones and corresponds to the height of the amino acid residues along the fiber axis in minor helices of the triple helical protfibril [9]. However, in this study, in all the gelatin sample examined, the peak at $2\theta = 31^\circ$ was not observed clearly. The reason why this peak is obtained at $2\theta = 31^\circ$ is not clearly.

4. CONCLUSION

The DSC investigation showed that the heat capacity increment ΔC_p of the gelatin sample during the glass transition depended on the drying temperature, namely the thermal history. In addition, from results of CD and WAXD measurements, it was suggested that the difference of the content of amorphous (or crystalline) region in gelatin sample, which was caused by such the different thermal history, affected on the value of the ΔC_p during the glass transition.

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