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Study on the glass transition of *Katsuobushi* (boiled and dried bonito fish stick) by differential scanning calorimetry and dynamic mechanical analysis

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ABSTRACT: The glass transition state of *Katsuobushi* (boiled and smoke-dried bonito) was studied by differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). DSC and DMA data proved *Katsuobushi* as a glassy material. The glass transition temperature (*Tg*) measured by DSC was about 33°C in 14.8% moisture. In general, moisture content of *Katsuobushi* on the market is approximately 12–15% and the *Tg* value of *Katsuobushi* containing such moisture was approximately 10–30°C and this was within the room temperature range. Furthermore, the *Tg* of *Katsuobushi* showed strong dependence on moisture content, and the *Tg* value varied from 11 to 165°C with moisture levels from 18.04 to 0%.

KEY WORDS: **boiled and dried bonito, dynamic mechanical analysis, differential scanning calorimetry, glass transition temperature, *Katsuobushi*.**

INTRODUCTION

Japan is the world's leading consumer of bonito. About 25% of the total amount of bonito caught in the world is consumed in Japan, of which over 50% is used for manufacturing *Katsuobushi*. *Katsuobushi* is highly dried bonito meat. It is one of the well-known traditional Japanese foods that is used as a flavoring for various kinds of Japanese dishes. *Katsuobushi* is processed as follows. Slivered bonito are boiled, and bones and skins are removed, and the muscle parts are smoked, dried, and a fine mold is applied on the surface in the final stage. The final product of *Katsuobushi* looks like a stone or hard wood, and is said to be 'the hardest food in the world'. *Katsuobushi* is a very hard and brittle material, has good storage stability and its cross-section surface looks just like red broken glass. Additionally, it is empirically known that *Katsuobushi* changes from a stiff solid state to a soft state during cooking by moisture absorption or increasing temperature. These characteristics of *Katsuobushi* strongly suggest it is a glassy material.

Recently, it has been shown that many low-moisture foods, such as cereal snacks,[1,2] dried fruits and vegetables,[3–10] bread[11] and powdered milk,[12] are glassy materials. 'Glass' is defined as a solid, brittle material that has an amorphous, liquid-like structure without obvious fluidity. By elevating the temperature, a glass changes from the brittle to rubbery state through the so-called glass transition process, which is accompanied by a rapid increase of molecular mobility and a drastic drop of the elastic modulus, at its glass transition temperature (*Tg*). It is widely recognized that the glass transition temperature is a very useful parameter for the understanding and prediction of the shelf-life of many low-moisture foods, because several deterioration reactions of foods, such as texture loss, enzymatic spoilage, flavor release and the Maillard browning reaction,[13] are significantly reduced because molecular motions of glassy material are strictly prohibited at temperatures below the *Tg*. The effect of moisture upon the glass transition temperature has also been reported; addition of water decreases the *Tg* of many low-moisture foods. There is some research about glass transition of low-moisture fishery products, such as mackerel protein hydrolysates[14] and fish myofibrillar protein-based films.[15,16] Furthermore, in high-moisture fishery products, reports have been made about *Tg* of fresh tuna,[17,18] cod muscle,[19] and muscle tissue of cod and mackerel.[20] However, there is very little research about the glass transition of processed fishery products, which are used practically.

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We consider that several physical characteristics of *Katsuobushi* as mentioned above can be explained by using the glass transition concept. Furthermore, it can be considered that information on the glass transition behavior of *Katsuobushi* may give useful information for the optimum storage conditions for its long-term stability. The optimum storage condition of *Katsuobushi* is often determined based on experience only. However, it can be determined theoretically if the glass transition concept is used. Previously, we performed differential scanning calorimetry (DSC) analysis of *Katsuobushi* and reported that a stepwise change in heat capacity that is indicative of the glass transition was observed.[21] The objectives of the present study are to clarify and determine the state diagram of *Katsuobushi* on the point of the glass transition. In order to obtain information about the exact glass transition temperature of *Katsuobushi* as a function of moisture content, DSC and dynamic mechanical analysis (DMA) were used concomitantly. These techniques have often been used in studies of the glass transition of food materials.[15,16,22,23] It is expected that the experimental results obtained in the present study will provide very useful information to control and predict the shelf life of *Katsuobushi* and similar dried fish products manufactured from several fishes, such as tuna, mackerel, sardine and Japanese horse mackerel, which are also processed into popular preserved foods in Japan.

MATERIALS AND METHODS

Sample preparation

Katsuobushi manufactured within 1–2 months was purchased at a supermarket in Tokyo. *Katsuobushi* manufactured by company A (Ninben Co., Ltd, Tokyo, Japan) was mainly used in the present study. In the DSC experiment, two specimens of *Katsuobushi* manufactured by two different companies, B (Yanagiya-Honten Co., Ltd, Shizuoka, Japan) and C (Akimoto-Suisan Co., Ltd, Shizuoka, Japan) were also examined. All of the *Katsuobushi* was of the so-called '*Honkarebushi*' type, which was *Katsuobushi* treated by mold fungus to add flavor and improve stability by reducing moisture. Before process, these were stored in a refrigerator at 0–2°C. *Katsuobushi* was sliced into 2–3 mm-thick sections by a power band saw and the red meat region, which has a shiny red, glass-like appearance, was selected for analysis after removal of dark muscle and the surface moldy region. For DSC measurement, sliced *Katsuobushi* was ground into powder and compressed into a pellet of approximately 5 mm in diameter and 1 mm in thickness. For DMA measurement, it was shaped into a bar ($5 \times 20 \times 1.5$ mm) by using a cutter knife and a file. To examine the plasticizing effect of moisture, samples with different moisture contents were also prepared by equilibrating them in separate chambers with different relative humidity (RH) for 10 days for powder samples (DSC) and 20 days for bar samples (DMA). Saturated salt solutions of LiBr, LiCl, CH_3COOK, $MgCl_2$, NaBr and NaCl, giving RH of 6.6, 11.3, 23, 33, 58 and 75.5%, respectively, were used in the present study. Low moisture samples (0–3%) were prepared by drying in an oven at 110°C for a given length of time.

Thermal analysis

Differential scanning calorimetry

A Shimadzu DSC-50 differential scanning calorimeter (Shimadzu Co., Ltd, Kyoto, Japan) fitted with an LTC-50 cooling system (Shimadzu Co., Ltd) was used. The temperature calibration was performed with indium (melting point, 156.6°C; ΔH_m, 28.5 J/g) and distilled water (melting point, 0.0°C; ΔH_m, 333 J/g). α-Alumina powder was used as a reference. N_2 at a flow rate of 20 mL/min was used as carrier gas. Approximately 20 mg of the sample was weighed and hermetically sealed into an aluminum pan by using a sealer. Samples were cooled with liquid nitrogen as a cooling medium and scanned from –70 to 200°C at a heating rate of 5°C/min. To determine the moisture content of the sample, the top cover of the DSC pan was pierced by a drill and dried at 110°C until it reached a constant weight after DSC measurement. Thermal analysis software TA-60WS (Shimadzu Co., Ltd) was used to analyze the experimental data. The glass transition temperature was determined from the onset, midpoint and end-point temperatures of the stepwise change in heat capacity.

The obtained glass transition temperatures of *Katsuobushi* as a function of moisture content were fitted by using the Gordon-Taylor equation:[24]

$$Tg = \frac{X_s(T_{gs}) + kX_w(T_{gw})}{X_s + kX_w} \tag{1}$$

where T_g, T_{gs} and T_{gw} are the glass transition temperatures (°C) of the mixture, solid and water, respectively, X_s and X_w are the percentages of solid and water contents, respectively, and k is a fitting parameter that is expressed by the ratio between the change in heat capacity of water at its glass transition temperature (ΔCp_w) to that of the dry solids (ΔCp_s).[25]

$$k = \frac{\Delta Cp_w}{\Delta Cp_s} \qquad (2)$$

The glass transition temperature and the change in heat capacity of pure water were taken as $Tg_w = -135$°C and $\Delta Cp_w = 1.39$ J/g per K.[26,27]

Dynamic mechanical analysis

Perkin-Elmer DMA-7 (Perkin-Elmer Corp., Wilton, CT, USA) was used in a three-point bending mode. The system calibration was performed using Perkin-Elmer calibration software, with indium (melting point, 156.6°C). Samples were cooled with liquid nitrogen and scanned at 3°C/min from –100 to –200°C. As mentioned above, the sample size used in DMA was larger than that of DSC, so the scanning rate was reduced to avoid a heating time lag and to ensure a correct rise in temperature during scanning. The frequency of dynamic force was 1 Hz. The sample bars were coated with silicone grease to limit moisture evaporation during measurements. The moisture content of the sample was determined by drying the same sample at 110°C to constant weight. In these analyses, values of the storage modulus (E′) and the tangent of the phase angle (tan δ) were obtained as a function of temperature. In the present study, the glass transition temperature was determined from the peak top temperature of tan δ as reported in the study of Johari *et al.*[28]

RESULTS AND DISCUSSION

Differential scanning calorimetry measurement

Typical DSC data of *Katsuobushi* (company A, water content: 14.8%) are shown in Fig. 1. The DSC scan was performed three times for the same sample in an aluminum pan, namely first run, second run and third run, after cooling the sample with liquid nitrogen to confirm the reversibility of the heat capacity change. In the first-run curve, an endothermic peak overlapping stepwise change in endothermic direction was observed at a temperature of around 60°C. The peak observed here was most likely not due to protein denaturation because the protein in *Katsuobushi* was considered to be already denatured by boiling and smoke drying during the manufacturing process. The disappearance of this endothermic peak in the second-run curve suggested that it was not caused by some first-order phase transitions (e.g. melting). For many glassy materials, such an irreversible endothermic peak, which is attributed to the phenomenon generally called 'enthalpy relaxation', has often been observed in DSC data.[29] As glassy materials are far from the thermodynamic equilibrium, molecular re-arrangement takes place, leading to a lower state of enthalpy during long-term storage. 'Enthalpy relaxation' is a recovery phenomenon of enthalpy, and it is observed as an endothermic peak in the DSC heating curve and disappears on reheating scanning. Furthermore, this endothermic peak appeared several times at the same temperature range after storage and this peak magnitude was dependent on storage time and temperature (data not shown). Such dependency was a characteristic of enthalpy relaxation behavior of amorphous materials.[30] From these results, the same phenomenon of enthalpy relaxation must have occurred in the case of *Katsuobushi*. In many cases, the existence of the endothermic peak caused by enthalpy relaxation made it difficult to determine the exact Tg value. Therefore, in the present study, Tg values were determined from the second-run curve after eliminating this endothermic peak by the first heating.[14,22,31,32] In the second-run curve, a clear stepwise change in heat capacity indicated the glass transition was observed at a temperature of around 30°C (shown by an arrow in Fig. 1). The third-run curve of the same sample gave a similar stepwise change at almost the same temperature range as the second-run curve. This result implies that this stepwise change is reversible. As the glass transition is generally a reversible phenomenon,[33] it can be considered that the observed change in DSC is caused by the glass transition. These stepwise changes in heat capacity occurred over a relatively broad temperature range ($\Delta Tg = Tg^{\text{end-point}} - Tg^{\text{onset}} = 67.56$°C) as compared to synthetic polymers, and this is probably attributed

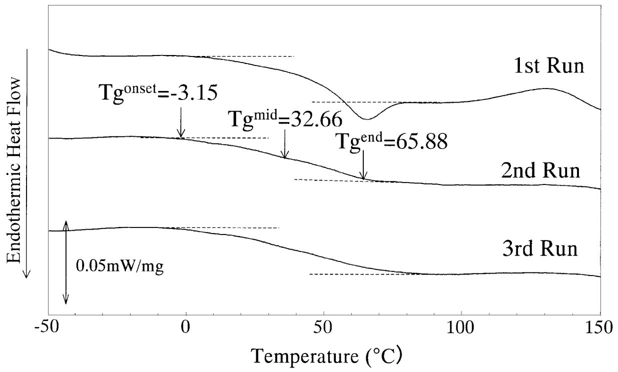

Fig. 1 Typical differential scanning calorimetry thermograms of *Katsuobushi* (A) at moisture content 14.8%. The sample was cooled with liquid nitrogen and heated at 5°C/min. The glass transition temperature (Tg) was determined from the midpoint temperature of stepwise change in heat capacity.

to the fact that *Katsuobushi* is a multicomponent system consisting of several types of protein, sugars and minerals. In a multicomponent system, multiple glass transitions are often observed in a DSC curve.[23] If these glass transition temperatures are within a similar temperature range, not each baseline shift in a DSC curve can be isolated, and may be detected in the form of an overlap. As a result, a DSC curve of this multicomponent system shows only one baseline shift that has a relatively broad temperature range. Therefore, it can be considered that inseparable transitions associated with different components may produce the broader *Tg* range in the case of *Katsuobushi* also. In Fig. 2, the DSC results of *Katsuobushi* manufactured by two different companies (B, C) are also presented. Both samples revealed similar results to the *Katsuobushi* of company A, indicating that the glass transition behavior is a general characteristic of *Katsuobushi*.

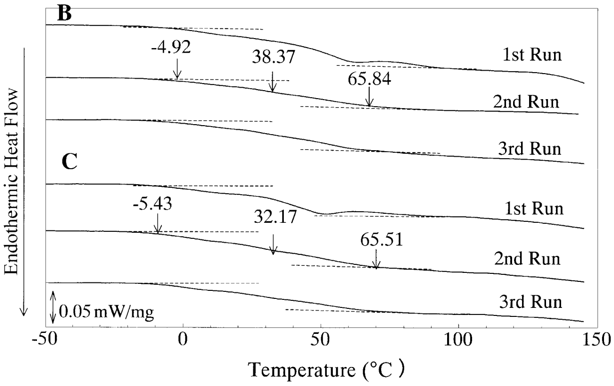

Fig. 2 Typical differential scanning calorimetry thermograms of *Katsuobushi* (B, C). Moisture contents of B and C are 14.4% and 13.7%, respectively.

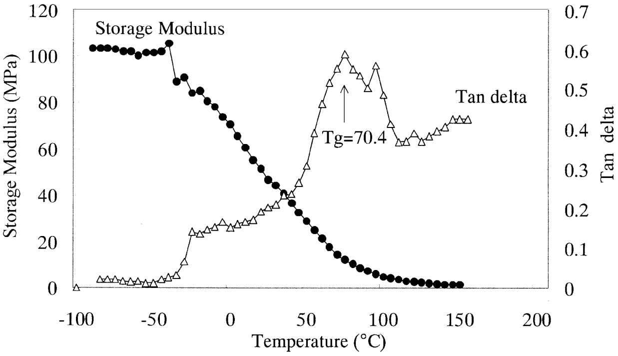

Fig. 3 Typical dynamic mechanical analysis plot of *Katsuobushi* (A) at moisture content 14.8%, showing tan delta (tan δ) and storage modulus (E′) as a function of temperature. Glass transition temperature (*Tg*) is a peak top temperature of the tan delta curve.

Dynamic mechanical analysis measurement

Typical DMA data of *Katsuobushi* (company A, water content: 14.8%) are depicted in Fig. 3. In DMA data, the drop in storage modulus (E′) and the peak in tangent delta (tan δ), which are characteristics of the glass transition,[34] were observed in the temperature ranges of –40 to 80°C and 30 to 110°C, respectively. These changes in E′ and tan δ occurred over a relatively broad temperature range, like the heat capacity change in the DSC curve. This might also be caused by the fact that the sample was a multicomponent system. Furthermore, a small change in tan δ was observed around –30°C. In the case of soy protein sheets, changes in tan δ attributed to β-transition were observed at a temperature range of –72 to –33°C with moisture change from 26.0 to 2.8%.[24] Therefore, change in tan δ at –30°C may indicate β-transition of *Katsuobushi*. However, this change was not always observed in the experiments and this temperature range was relatively higher than the reported value for β-transition in the case of soy protein sheets. Therefore, it is more likely that a small change in tan δ observed in the present study was an experimental noise. The *Tg* of *Katsuobushi* obtained from the peak top temperature in tan δ was approximately 70°C and this *Tg* value was relatively higher than that measured by DSC at a similar moisture content. This difference was probably attributed to the difference in measurement method and the loss of moisture by evaporation during DMA scanning. In polymer literature, it is often said that the glass transition temperatures determined by DMA are higher than the corresponding glass transition temperatures measured by DSC.[35] Similar experimental results were reported for the glass transition of gluten[22] and soy protein.[24] For DSC measurement, the moisture content of the sample does not change at any temperature because all samples are sealed hermetically in aluminum pans. In contrast, DMA measurements are always performed in an open system, so evaporation of the sample moisture cannot be avoided. Actually, the moisture content of the sample dropped by about 6% during this scanning in spite of the silicone grease coating, which may bring about an increase in temperature of tan δ peak. Kalichevsky *et al.*[22] reported that loss of 1% moisture content during DMA scanning resulted in an increase of 5°C in the tan δ peak temperature in the case of gluten. Therefore, in case the sample contains some amount of moisture, the *Tg* value obtained by DSC is more accurate and reliable than that by DMA.

Effect of moisture content on *Tg* of *Katsuobushi*

Figure 4 presents the DSC curves of *Katsuobushi* with different moisture contents. In each curve, a clear stepwise change was observed. With the increase in moisture content, the glass transition temperature changed to a significantly lower temperature, reflecting the plasticizing effect of water. In the present study, the Tg^{midpoint} value of *Katsuobushi* varied from 11 to 165°C with moisture content from 18.04 to 0%. For two different *Katsuobushi* (B, C), similar moisture dependence behavior was observed in their DSC curves (data not shown). *Tg* values obtained from onset, midpoint and end-point temperature of the stepwise change in heat capacity are given in Table 1. Each glass transition temperature (onset, midpoint, end-point) showed clear moisture dependence. The onset *Tg* values suggested that the glass transition of *Katsuobushi* that had higher moisture content (over 15%) could be started even below 0°C. In the present study, the effect of moisture content on the ΔTg value was not unclear. Figure 5 shows the DMA curves of *Katsuobushi* with different moisture contents. In each curve, the drops in storage modulus E′ and the peaks in tan δ were observed. The onset temperatures of the drops in E′ and the peak top temperatures in tan δ shifted to a lower temperature with increasing moisture content. This tendency was the same as the result of DSC measurement. The peak top temperatures in tan δ of the samples with below 10% moisture were quite

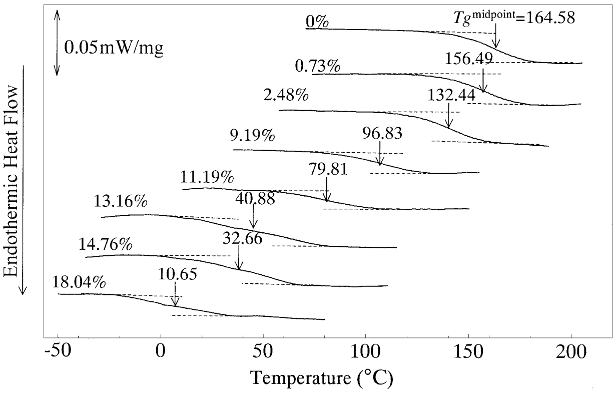

Fig. 4 Differential scanning calorimetry thermograms of *Katsuobushi* (A) at different moisture contents. These are second-run curves to eliminate relaxation hysteresis effects and glass transition temperature (*Tg*) values shown are midpoint temperatures of stepwise changes in heat capacity.

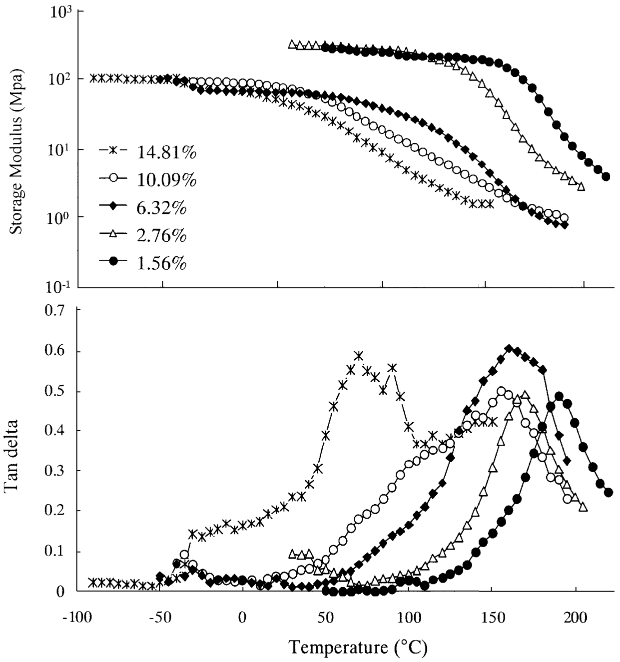

Fig. 5 Variation of storage modulus (E′) and tangent delta (tan δ) of *Katsuobushi* (A) with temperature at different moisture contents.

Table 1 Glass transition temperatures of *Katsuobushi* (A) determined by differential scanning calorimetry with different moisture contents

Moisture content (% dry basis)	Glass transition temperature (°C)			
	Tg_1 (onset)	Tg_2 (midpoint)	Tg_3 (end-point)	ΔTg (Tg_3–Tg_1)
0	140.13	164.58	180.77	40.64
0.73	132.02	156.49	179.09	47.07
2.48	113.77	132.44	154.96	41.19
9.19	79.41	96.83	124.01	44.60
9.49	59.05	82.90	101.06	42.01
11.19	48.93	79.81	94.24	45.31
13.16	14.92	40.88	74.71	59.79
14.76	–3.15	32.66	65.88	69.03
18.04	–23.68	10.65	31.87	55.55

close to each other, and this was probably attributed to the moisture loss of the samples. In other words, it seemed that moisture content of each sample became almost the same by heating above 100°C regardless of initial moisture contents. In tan δ curves, small changes at a low temperature range (around –40°C) were also observed in 14.81, 10.09 and 6.32% moisture samples. The fact that these changes had no moisture dependence and were not always observed suggested these were probably caused by an experimental noise.

These glass transition temperatures of *Katsuobushi* measured by DSC and DMA were plotted against the moisture content as shown in Fig. 6. The differences in *Tg* values obtained by DSC and DMA are attributed to a difference in the measuring method and moisture loss of the samples during DMA scanning as mentioned above. Figure 6 implies that *Tg* of *Katsuobushi* strongly depends on moisture content as already reported for other glassy foods.[1–12] The dotted line in Fig. 6 was drawn using the Gordon-Taylor equation 1. The *Tg* values of *Katsuobushi* (A) as a function of moisture content could be successfully fitted to the Gordon-Taylor equation. The experimental values of Tg_s and ΔCp_s obtained from DSC results were 164.6°C and 0.28 J/g per K, respectively. The values of the Gordon-Taylor parameter *k* obtained experimentally and predicted by using equation 2 were 4.75 and 4.94, respectively, and each value showed a very good agreement. In Fig. 6, the *Tg* values obtained in our previous study[21] were also plotted. The *Tg* values obtained in the previous study tended to be higher than those in the present study, especially for high-moisture content samples. It is difficult to explain such a large difference by the individual difference of the samples. It may be attributed to a misinterpretation of the DSC results in our previous study. The experimental results in the previous study have two unclear points. First, the existence of the reproducibility of the glass transition was not checked. Second, the moisture dependence of the *Tg* for high moisture content samples especially (6.0, 16.7%) was small, which was inconsistent with the general glass transition behavior of foods or food components.[1–12,14,16,22,24,31] From these aspects, the *Tg* value obtained in the present study is more reliable than that in previous study. The heat capacity changes detected as the glass transitions for high moisture content samples in our previous study were probably caused by other conformational changes of protein occurring at a high temperature range, such as disulfide or isopeptide bond formation.[36] The reason why the *Tg* in the present study could not be detected in the previous study may be attributed to the difference in the initial scanning temperature. In the previous study, DSC scanning always started at a higher temperature (20–25°C) than in the present study. In such scanning conditions, only a thermal event occurring above 50–60°C could be detected, considering the time required to achieve thermal equilibrium of the sample. Therefore, it is reasonable that the exact glass transition for high-moisture content samples occurring at a lower temperature range could not be detected in the previous study.

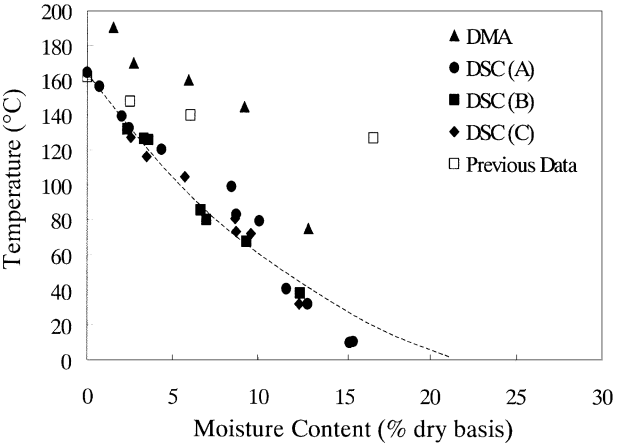

Fig. 6 Plots of dynamic mechanical analysis tan δ peak temperatures (▲) and differential scanning calorimetry midpoint temperatures of *Katsuobushi* as a function of moisture content. Differential scanning calorimetry data were obtained from three different *Katsuobushi* manufactured by company A (●), company B (■) and company C (◆). The dotted line was drawn using the Gordon-Taylor equation, as described in the text. The Gordon-Taylor parameter *k* obtained experimentally was 4.75. Furthermore, the data that were reported in our previous report (□),[21] were also plotted. The values of moisture content reported in our previous study were wet basis values, which were converted into dry basis values in the present figure.

Moisture content of *Katsuobushi* is normally 12–15% (14–18% dry basis). The *Tg* values (determined from the midpoint temperature in DSC heat capacity change) of *Katsuobushi* at this level of moisture content are 10–30°C, and these values are within the room temperature range. This implies that *Katsuobushi* changes easily from a stable glassy state to an unstable rubbery state by a slight moisture absorption or slight temperature increase during storage. Therefore, *Katsuobushi* must be stored at lower than room temperature, or the moisture content of the finished product must be lower than the current value to maintain quality of *Katsuobushi* over the long term. However, excessive drying of *Katsuobushi* is not desirable, because low moisture *Katsuobushi* makes it very rigid and hard to slice. To obtain optimum moisture content, therefore, further study based on its glass transition behavior will be required.

From the results of DSC and DMA measurements, it is clear that *Katsuobushi* is a glassy material. The moisture content of *Katsuobushi* is normally approximately 14–18% (dry basis) and the *Tg* value of *Katsuobushi* containing such moisture is 10–30°C. Furthermore, the *Tg* values of *Katsuobushi* are strongly affected by its moisture content and these values could be successfully fitted to the Gordon-Taylor equation. We can conclude that several characteristics of *Katsuobushi*, such as glass-like appearance, high storage stability and change of state with increased temperature or moisture, are a reflection of its glassy nature. It is expected that the experimental results obtained in the present study will provide useful information to predict and control the shelf life of *Katsuobushi* theoretically. In Japan, there are many dried marine products and it is expected that these preserved foods are also glassy materials like *Katsuobushi*. Therefore, research based on the glass transition concept will be increasingly required in the field of the Japanese fisheries industry in the future.

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