Research Paper

日本冷凍空調学会論文集
Trans. of the JSRAE
Vol.25, No. 1 (2008)
pp.47～53
原稿受付:平成19年10月19日

What is the Actual Low-Temperature Glass Transition for Fish Flesh?

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Summary

We measured glass transition temperatures in the maximally freeze-concentrated phase (T_g') of tuna and cod tissue by differential scanning calorimetry (DSC) using an annealing technique. In our experiment, the T_g' (approximately −71°C) of tuna and cod tissue did not exhibit any significant change after isothermal holding for several weeks at a temperature higher than the T_g. Another T_g' appeared above −45°C when annealing was performed at a temperature higher than −60°C for one week. We also collected T_g' data for fish flesh from many published studies and discussed them in the light of our data. The T_g' values for fish flesh in most published reports are higher than −40°C, although there have been several reports that give values of around −70°C. The high glass transition temperature (i.e., −45°C) measured in our study agrees with most of the data in the literature. Furthermore, the results suggest that the glass transition behavior at the higher temperature may be correlated to the vitrification of protein itself.

Key words : Glass transition temperature, The maximally freeze-concentrated phase, Annealing technique, Tuna, Cod

1. Introduction

The use of commercial frozen fishery products has significantly increased in recent decades, and these products now play an important role in the world market. It is vital to gain a knowledge of the parameters that affect the quality and degradation of frozen fish in order to develop optimal freezing processes and frozen storage methods to economically maintain a high quality for frozen fish.

Recently, the preservation of food products in the glassy state has been developed as a new technology for freezing and frozen storage, particularly for high-moisture food materials such as fish flesh. The characteristic glass transition temperature, which is defined as the glass transition temperature in the maximally freeze-concentrated phase (T_g'), is often considered to be a criterion temperature for predicting frozen food stability [1-3].

Glass transition has been extensively studied in chemical compounds, including low-molecular-weight carbohydrates [1, 4-8] and proteins [9-11]. In addition, the glass transition of actual food products such as fish muscle, which consists of complex heterogeneous systems [12-27], has been investigated by many different methods,

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including differential scanning calorimetry (DSC) [14-19, 21,23-24, 27] and rheological analysis [20-25].

Consequently, numerous and diverse T_g' values for fish flesh have been reported. For example, some studies have reported T_g' values of around −70°C [13,15,17-19], although most of the reported data indicate that T_g' values for fish flesh are higher than −40°C [14,16,18]. In addition, until recently, there were disagreements concerning how to define and determine T_g', even for homogeneous aqueous solutions. That means, two transitional events used to be observed in the vicinity of T_g'. Thus, there is still no definitive answer for the question, "what is the true low-temperature glass transition of fish flesh?".

The objectives of this report are to determine the T_g' values for tuna and cod tissue from DSC measurements using a lengthy annealing process. Specifically, in the experiments conducted in this study, we employed a new annealing method, which was developed based on glass transition theory; it is described in the following section. Furthermore, we discuss reported T_g' values for fish flesh measured by many different methods and compare them with the results we obtained.

2. Theoretical on annealing for determining T_g'

When food such as fresh fish flesh is frozen slowly in a near-equilibrium freezing process, the freeze-concentrated solution may occur and it proceeds according to the freezing-point depression curve (the solid line in Fig. 1). As the temperature continuously decreases, the freeze-concentrated solute ideally reaches the maximum concentration and becomes a solid glass at T_g' [28]. Consequently, T_g' has been generally considered to be the intersection of the freezing point depression curve and the glass transition line in the phase diagram [1].

However, food is always frozen rapidly in conventional freezing processes, which results in incomplete ice formation, and thus the glass will be formed under dilute conditions at $T_g < T_g$' [1,12], as shown by the dashed line in Fig. 1. As a result, it is difficult to determine the T_g' value of any solution in conventional freezing, and thus an annealing procedure is necessary above T_g after the sample has been cooled to below T_g [6].

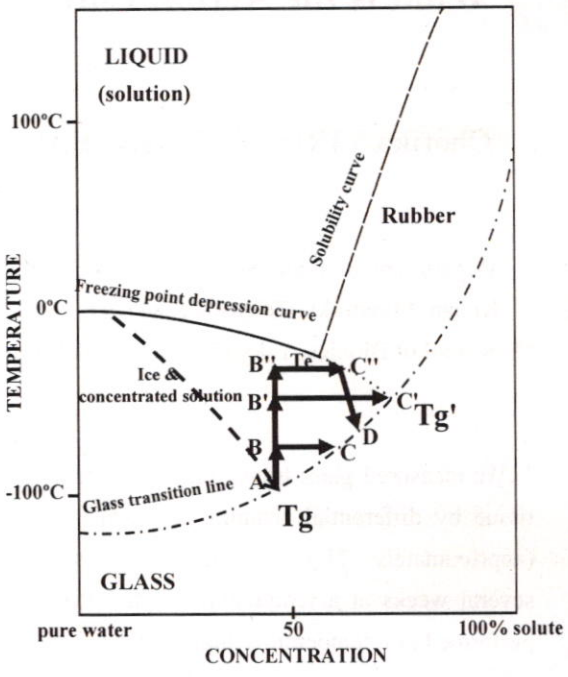

Fig. 1 Schematic state diagram for an aqueous solution.

Annealing is a process whereby the sample is isothermally held for a sufficiently long time above T_g but below the melting point of ice (T_m). The glass phase begins to soften and flow, that is, it enters the rubber phase, while it is surrounded by ice crystals. Thus, the concentration of the glass phase increases as more ice forms from the freezable water that is in the rubber phase. In practical terms, the sample is first cooled to below T_g, it is then annealed by warming it to the annealing temperature (T_{ann}), and it is held at this temperature for the allotted time period. After that, the annealed sample is recooled to below T_g, and then rewarmed to room temperature in order to measure the new T_g.

Even when the sample is annealed at $T_g < T_{ann} < T_g$' for a long time, the concentration of the glass phase increases but it does not attain its maximum possible value due to kinetic hindrance. This causes

the new T_g to approach the glass transition line, which is lower than T_g' (A → B → C in Fig. 1). If the glassy sample is annealed at $T_{ann} = T_g$' for a sufficiently long time, the freeze-concentrated solute can approach the maximum concentration at T_g' (A→ B' → C' in Fig. 1). Therefore, the optimum annealing temperature is T_g'. If a glassy sample is annealed above T_g', the concentration of its freeze-concentrated solution reaches the extrapolation line between the freezing-point depression curve and the glass transition line. As a result, some of the ice may melt, and liquid water molecules begin to interact with the glassy material, softening it into rubber (A →B"→ C" in Fig. 1). Thus, when the temperature is again reduced at a finite rate to measure T_g', glass may be formed under dilute conditions at new $T_g < T_g$' (C"→ D in Fig. 1). Since T_g' is unknown before the annealing process is performed, it is necessary to conduct a trial-and-error experiment involving annealing at several different temperatures.

3. Materials and methods

Fresh bigeye tuna (*Thunnas obesus*) and cod (*Gadus morhua*) fillets were purchased from a local retailer. Since both of tuna and cod meat was obtained from each a single portion of ordinary muscle, the effect of initial freshness on the transition temperatures was assumed to be negligible. The water content of the fresh tuna and cod samples were approximately 72 and 80%, respectively.

After purchasing, samples of 9–13 mg were immediately cut from both kinds of fish fillets, and hermetically sealed in aluminum DSC pans. The DSC pans containing the samples were frozen by quenching in liquid nitrogen for 10 min, and stored in a freezer that was maintained at several different temperatures (−90°C, −60°C, −40°C, −30°C, and −20°C) for 1 month. At intervals of one week, the DSC sample pans were removed from the freezer and inserted in the sample holder on a DSC (Perkin Elmer, Inc., Diamond DSC, Shelton, USA). The samples were kept in liquid nitrogen when they were transferred from the freezer to the DSC sample stage.

In the DSC measurement, the sample was held at −120°C for 10 min before being rewarmed to 20°C at a rate of 5°C/min. The temperature was calibrated with indium (melting point = 156.6°C) and cyclohexane (solid-solid transition temperature = −87.06°C). The heat flow was calibrated with indium (ΔH_m = 28.5 J/g). An empty pan was used as a reference. In this study, the transition temperatures were reported as the onset of the transition. Measurements were done in duplicate or in triplicate.

4. Results

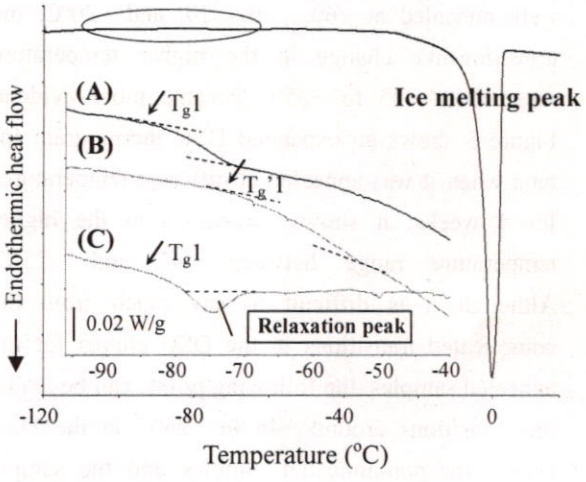

Fig. 2 Typical DSC thermograms for tuna which was not annealed (A), annealed at -60°C for 1 month (B), and annealed at -90°C for 1 month (C). Arrows indicate the glass transition temperatures.

Figure 2 shows DSC warming thermograms for tuna. It shows a typical curve from −120°C to 20°C. Three other curves, labeled A, B and, C are enlarged on the same figure; they represent a non-annealed sample, a sample annealed at –60°C for 1 month, and a sample annealed at –90°C for 1 month, respectively. Although DSC thermograms for cod are not shown, they were similar to those of tuna. The non-annealed samples for tuna (Fig. 2A) and cod had identical glass transition temperatures (denoted by T_g1) at around –85°C, and subsequently

the ice started to melt at approximately −26°C for tuna and −32°C for cod. All tuna and cod flesh samples that had been annealed at −60, −40, −30, and −20°C for periods of a few hours up to 1 month exhibited an increase in the glass transition temperature T_g1 to around −71°C. In particular, the results for annealing at −90°C, which is below the glass transition temperature of fish flesh (T_g1 = −85°C), exhibited an enthalpy relaxation peak near the glass transition at −85°C (Fig. 2C). These results imply that the transition around −71°C (denoted by $T_g'1$) should be taken as the true glass transition for fish flesh; this is also in agreement with the data of Nesvadba [13], Inoue and Ishikawa [15], Agustini *et al.* [17], and Orlien *et al.* [19].

On the other hand, when both of tuna and cod were annealed at −60, −40, −30, and −20°C, the transition-like change in the higher temperature range from −45 to −25°C became more evident. Figure 3 shows an expanded DSC thermogram for tuna when it was annealed at different temperatures for 4 weeks; it shows transitions in the higher temperature range between −45 and −25°C. Although it is difficult to say much from the complicated transitions in the DSC curves for the annealed samples, the following points can be made: the transitions around −45 to −25°C in the DSC curves for non-annealed samples and the sample annealed at −90°C were almost identical, and, with the exception of the sample annealed at −90°C, the transitions temperatures of the annealed samples shifted to lower temperatures as the annealing temperature decreased.

The above relation, namely, the effect of the annealing temperature on the transition temperatures for both types of fish, is shown in Fig. 4. It clearly shows that the transition temperatures for samples (both tuna and cod) annealed at higher than −60°C decreased with a decrease in the annealing temperature. Furthermore, it shows that the transition temperatures for cod were considerably lower than those for tuna at all annealing temperatures. It should be stressed that even when the annealing time was extended from 1 week to 4 weeks the transition temperatures for both fish types did not change.

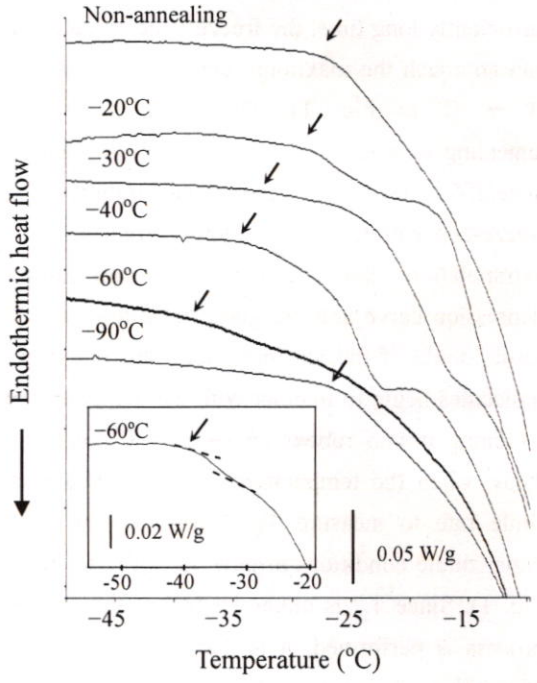

Fig. 3 DSC thermograms showing the transition temperatures for tuna at different storage temperatures (storage time 4 weeks). Arrows indicate the transition temperatures at different storage temperatures. The inset shows the enlarged DSC thermogram of tuna stored at −60°C for 4 weeks.

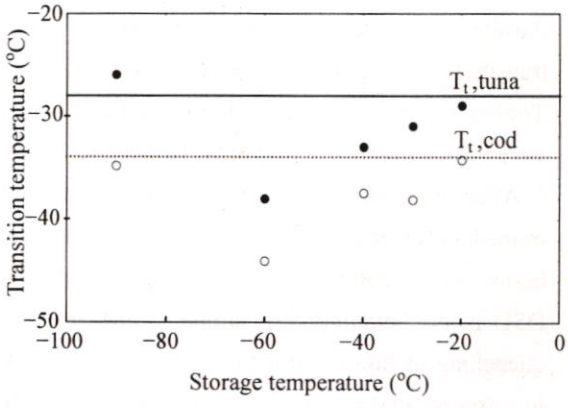

Fig. 4 Effect of annealing temperature on the onset of transition temperatures observed at around −25°C ~ −45°C for both tuna and cod. Closed and opened circles represented transition temperatures for tuna and cod flesh, respectively. $T_{t,tuna}$ and $T_{t,cod}$ are the observed transition temperatures for non-annealed

tuna and cod flesh, respectively.

Interestingly, the results of our preliminary study, which attempted to anneal cod flesh at −60°C for 7 weeks, showed an enthalpy relaxation peak around −45°C (onset temperature) as shown in Fig. 5. This appearance of an enthalpy relaxation peak supports the conjecture that these transitions are glass transitions. Therefore, it is appropriate to say that more than one glass transition may occur in frozen fish depending on the temperature range; one occurs at around −71°C, as described above, and another lies in the range between −45 and −25°C.

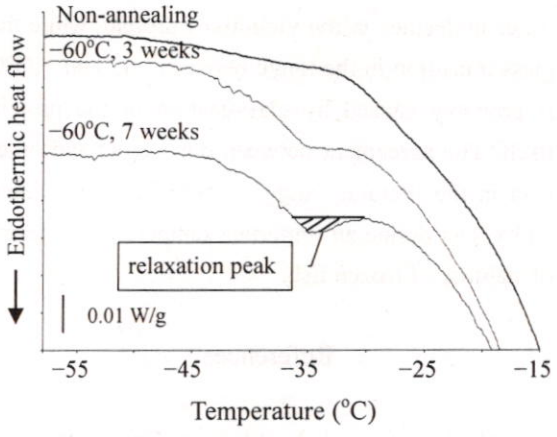

Fig. 5 DSC thermograms for cod that was not annealed, and annealed at −60°C for 3 and 7 weeks.

5. Discussion

The reported T_g' values and their measurement procedures for muscle tissue from various types of fish are listed in Table 1. The two glass transition temperatures determined in our study was in agreement with the results of previous studies, which seem to fall into two different groups, as Table 1 shows.

One possible explanation for these results is that the glass transition at T_g = −71°C for fish muscle is caused by rapid freezing, that is, the freeze-concentrated phase was not so concentrated as to depress T_g by the plasticizing effect. On the other hand, many reports have proposed that the low temperature glass transition of hydrated proteins that occurs at around −113 to −70°C is due to the freezing of the motion of water molecules in the vicinity of proteins [11,15,29]. Thus, T_g values in this range for fish flesh might also be considered to be transitions having a similar origin.

Table 1 T_g' data and their experimental conditions for various types of fish flesh.

Fish sample	T_{ann} (°C)	t_{ann} (min)	T_g' (°C)	References
Tuna	a		−71 to −68	Inoue and Ishikawa (1997)
Tuna			−63	Agustini *et al*. (2001)
Cod			−86 to −77	Nesvadba (1993)
Tuna	−40	420	−74	Orlien *et al*. (2003)
Tuna	−30	30	−20.5	Jensen *et al*. (2003)
Cod	−30	30	−21.3	Jensen *et al*. (2003)
Cod mince	−30	10	−21.4	Jensen *et al*. (2003)
Cod	−20	10	−15.4	Jensen *et al*. (2003)
Cod	−15	30	−10.6	Jensen *et al*. (2003)
Cod	−15	60	−11.7	Brake and Fennema (1999)
Mackerel	−15	60	−11.4	Brake and Fennema (1999)
Mackerel	−15b	60	−13.3	Brake and Fennema (1999)
Cod	c		−34	Reid *et al*. (1995)
Salmon			−37	Reid *et al*. (1995)
Shrimp			−33	Reid *et al*. (1995)
Tuna	d		−54.2	Rahman *et al*. (2003)
Abalone			−44.3	Sablani *et al*. (2004)
Tuna	e		−18 to −11.5	Levine and Slade (1989)
Cod			−15	Levine and Slade (1989)
Cod			−40	Simatos and Blond (1993)

T_{ann} = annealing temperature;

t_{ann} = annealing time;

[a]Samples which were not annealed;

[b]Sample which was annealed outside DSC;

[c]Samples which were partially lyophilized;

[d]Vertical extrapolation from the end point of freezing point depression curve to glass transition line;

[e]Estimation from the beginning of the melting peak

Secondly, the T_g in the range between −45 and −25°C, was also in the same range as the data of Reid *et al.* [14], Brake and Fennema [16], and Jensen *et al.* [18]. However, when compared with the T_g for fish annealed at the same temperature in our experiment, the transition temperatures of our results seem to be lower than those reported in the literature, as shown in Table 1. Most of earlier experiments were conducted with annealing times of 30 or 60 mins, which may have been too brief for fish muscle to attain equilibrium since it is a complex system with cell barriers. In contrast, the results of this study

were obtained after annealing between 1 week and 1 month and they exhibited a sufficiently clear stepwise change to determine the transition temperatures. Therefore, it can be concluded that an annealing time of 30 or 60 min is too short to anneal fish flesh.

According to recent reports about glass transition in protein-water systems, the glass transition commonly observed at temperatures higher than −70°C in these systems is considered to correspond to the primary chain of the hydrated protein [9,11]. In addition, Hansen *et al.* [22] reported that a transition temperature in frozen pork at around −55°C is associated with the glass transition in the structural proteins. Based on these observations, the T_g in the range between −45 and −25°C in our experimental results may be caused by glassification of the protein itself.

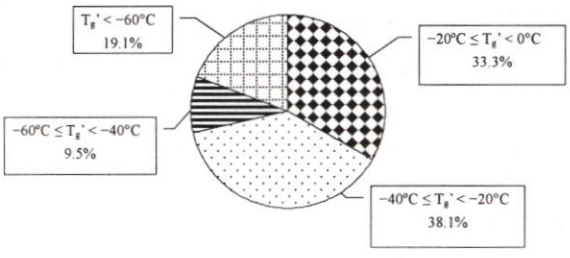

Fig. 6 Temperature distributions of T_g' data for fish flesh reported in the literature.

When considering the temperature distribution of these T_g' data reported for fish flesh (as shown in Fig. 6), it was found that more than 70% of the reported T_g' values are higher than –40°C. Interestingly, this temperature is the same as the optimum temperature for frozen storage of fish flesh over long time periods as reported by Bito [30] and Fukuda [31]. Bito [30] suggested that frozen tuna can be kept at –35°C for more than 1 year with no change in its metmyoglobin content, and Fukuda [31] showed that there was no significant change in myofibrillar Ca-ATPase activity in minced mackerel meat stored at –40°C for 3 months. This agreement between the T_g' data and the optimum temperature for frozen storage of fish flesh suggests that the T_g' of fish flesh is higher than –45°C, and this temperature can be considered to be an important temperature for the stability of frozen fish.

6. Conclusion

In our experiments, two glass transition temperatures were observed in frozen tuna and cod; one was −71°C and the other lay in the range between −45 and −25°C. Over 70% of all T_g' values for fish flesh reported in other studies in the literature, which were measured by many different methods, were higher than –40°C. The glass transition at −71°C is caused by the vitrification of water molecules in the vicinity of protein, while the glass transition in the range between −45 and −25°C is probably caused by glassfication of the protein itself. The agreement between our results and other data in the literature suggests that T_g values being –45°C can define an important temperature in terms of stability of frozen fish.

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