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Thermal characterisation of gelatin extracted from yellowfin tuna skin and commercial mammalian gelatin

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Abstract

Glass transition and other thermal characteristics of gelatin from different sources were studied by differential scanning calorimetry (DSC) and modulated DSC (MDSC). The initial glass transition temperatures of equilibrated gelatin samples at 11.3% relative humidity, determined from reversible heat flow thermogram of MDSC, were 23, 75 and 59 °C, respectively, for tuna skin, bovine and porcine gelatin. When gelatin samples were equilibrated at higher relative humidity of 52.9%, glass transition temperature of fish skin and bovine gelatin decreased to −3 and 57 °C, respectively. Further increase of equilibration relative humidity to 75.3% showed increased value in the case of tuna skin, whereas bovine and porcine did not show any significant change. DSC and MDSC results indicated that tuna gelatin showed lower glass transition compared to mammalian source gelatin equilibrated at the same constant relative humidity. In general glass transition measured by DSC was found lower than the values measured by MDSC. The results in this study showed that the degree of plasticization varied with the source of gelatin as well as their extraction methods.

Keywords: Structure; Glass transition; Plasticization; DSC; State diagram

1. Introduction

Gelatin is a biopolymer that has very broad applications in the food, pharmaceutical and photographic industries. In the food industry gelatin is widely used as a gelling agent. Gelatin offers functionality, versatility, and health. Studies have shown gelatin to promote healthy joints and skin. The developed functional products based on gelatin are high-protein gelatin gum with green tea extract provides low glycemic index, high-protein milk based chocolate drink and water-based high-protein sports drink (Ohr, 2005). Skin and bone from bovine and porcine sources have usually been utilized commercially in gelatin production (Veis, 1964; Ward & Courts, 1977). In recent years however, fish gelatin has gained importance as the demand for non-bovine and non-porcine gelatin has increased due to the bovine spongiform encephalopathy (BSE) crisis and for religious and social reasons. In addition, fish skin is a major by-product of the fish-processing industry, causing wastage and pollution. It is estimated that fish-processing waste after filleting accounts for approximately 75% of the total fish weight (Shahidi, 1994) and 30% of the waste is in the form of bones and skins (Gomez-Guillen et al., 2002). This waste could be used to develop the value added by-products in addition to solve the problem of waste disposal.

The quality of a gelatin for a particular application depends largely on its rheological properties (Stainsby, 1987), as well as its physico-chemical properties that are greatly influenced not only by the species or tissue from which it is extracted, but also by the severity of the manufacturing method (Johnston-Banks, 1990). An optimization of the tissue extraction procedures and a better knowledge of the properties of fish-skin gelatin could be helpful in rationalizing the use of fish residues (Gomez-

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Guillen et al., 2002). So far fish gelatin has known limited application because the gels formed tend to be less stable and to have worse rheological properties than gelatins from land mammals (Leuenberger, 1991). This limitation has been attributed mainly to the low number of Proline rich regions of the collagen or gelatin molecule in cold water fish than in warm blooded animals (Ledward, 1986; Norland, 1990). In addition, the total glycine–proline–hydroxyproline sequence content is one of the main factors affecting collagen thermostability (Burjandze, 2000). Skins from tropical-fish species, such as tilapia, have been described as an optimal raw material for gelatin production (Grossman & Bergman, 1992; Holzer, 1996). However, gelatin extracted from cod skin shows poor rheological properties (Gudmundsson & Hafsteinsson, 1997).

Gomez-Guillen et al. (2002) studied the rheological characteristics (viscoelasticity and gel strength) and chemical/structural properties (amino acid composition, molecular weight distribution and triple helix formation) of gelatins extracted from skins of several marine species. Gelatins from flat-fish species (sole and megrim) showed the best gelling ability and the gels were more thermostable than those from cold-adapted fish (cod and hake). This different behavior was explained considering the amino acid composition, the α_1/α_2 collagen-chain ratio, and the molecular weight distribution. The squid gelatin showed the most significant changes regarding amino acid composition and molecular weight distribution, most of these differences arising from the low solubility of the squid connective tissue. However, the squid gelatin showed viscoelastic properties intermediate between those from flat-fish and cold-adapted fish species.

Yellowfin tuna (*Thunnus albacares*) are large pelagic fish in the tropics and subtropics; with landing accounting for about 22% of the world's tuna catch (Al-Abdessalaam, 1995). The annual landing of yellowfin tuna (*Thunnus albacares*) in the Oman was 7377 metric tonnes during 1999 of which 709 metric tonnes were exported (Ministry of Agriculture, 1999). Tuna is usually processed as canned food in factory or sliced raw meat in local markets, and by-products of tuna are affluent and can be collected at once. For this reason, if physical properties of gelatin from tuna skin resemble mammalian gelatin, tuna skin can possibly be a replacement resource of mammalian gelatin.

The stability of foods strongly depends on the state of water, which affects the characteristics of the products. Thermal analysis determines different phases and states of foods as a function of water content and temperature (Rahman, 2004; Rahman, 2006). Glass transition temperature is related to the structural characteristics of biological materials, such as crystallization, stickiness, collapse and molecular mobility (Rahman, 2006). The glass transition temperature values of abalone (Sablani, Kasapis, Rahman, Al-Jabri, & Al-Habsi, 2004), tuna (Rahman, Kasapis, Guizani, & Al-Amri, 2003), and king fish (Sablani et al., 2007) muscle were presented in the literature. The overall objective of this study was to extract and characterize the gelatin obtained from yellowfin tuna (*Thunnus albacares*) skin and to compare its thermal characteristics with other commercial gelatin from mammalian skins.

2. Materials and methods

2.1. Source of raw materials

In the month of August 2005, one batch of skins from Yellowfin tuna (*Thunnus albacares*) were collected from local super market in Muscat and stored at −20 °C until used for the experiments.

2.2. Fish gelatin extraction

Frozen skin was thawed at room temperature for about 1 h and then the attached meat was removed by scratching with a knife. The gelatin extraction procedure was carried out similarly as described by Gomez-Guillen and Montero (2001). Thawed skin was washed with running tap water and dipped in 0.5 M sodium chloride for 5 min at 5 °C. Glass stirrer was used to stir the skin dipped in sodium chloride solution. Skin was then washed with tap water three times before treating with 0.1 N sodium hydroxide. The beaker containing the sodium hydroxide was placed on a magnetic stirrer for 40 min at room temperature of 20 °C. It was then washed three times with distilled water with gentle shake and placed in 0.1 N acetic acid solution for heating and stirring at 50 °C on a hot plat for 18 h. The solution-skin mass ratio for extraction was 5. Gelatin extracted in solution was separated by using two layer filter cloth. The solution was then dried in an oven initially at 80 °C and then 100 °C until reached to a solid state. The yield of solids extracted was estimated from the total solids in the fresh skin before extraction and the solids dissolved in the solution. It was expressed as kg/100 kg solids in fresh skin.

2.3. Commercial mammalian gelatins

Commercial gelatins were bought from BDH Laboratory (gelatin powder: catalogue number 44045 4B) and Sigma–Aldrich (porcine powder: catalogue number G 2500 and bovine powder: catalogue number: G 9382).

2.4. Chemical composition and pH

Moisture, crude protein, crude lipid and ash contents of the extracted gelatin derived from tuna skin were determined in triplicate as described in AOAC (1990). Moisture contents were determined by drying 2 g samples in a mechanical convection oven for at least 18 h at 105 °C. Gelatin (0.5 g) was dissolved in 20 g of distilled water and the pH of the solution was then measured with Mettler Delta pH meter. Protein content was calculated by converting the nitrogen content, determined by Kjeldahl's method ($6.25 \times N$) in a 2000 digestion system and 2300 Kjeldahl

analyzer unit. All values were calculated as a kg/100 kg sample. The ash content of the samples was determined by burning the sample in a furnace. The crucibles were heat dried at 550 °C in a muffle furnace for 30 min and cooled in desiccators. Samples of 2 g of gelatin were weighed and ashed at 530 °C in the muffle furnace for 18 h. It was then cooled and weighed; and the percentage of ash was determined. Fat was determined by extracting with light petroleum ether, and then subsequent removal of solvent by distillation. The residue was dried at 103 °C in an oven for at least 1 h.

2.5. Samples equilibration at different water activity

The equilibrium isotherms of tuna gelatin samples were determined by the isopiestic method at 25 °C. Samples (about 5 g) were placed in a glass jar with a beaker of saturated salt solutions (with a layer of crystal at the bottom) of lithium chloride, magnesium nitrate, and strontium chloride, and then equilibrated for six weeks at room temperature (20 °C). The equilibrated samples were placed in air tight glass bottles and stored at −20 °C until used for DSC and MDSC analysis.

2.6. Differential scanning calorimetry (DSC)

The melting point, glass transition and deterioration temperature of gelatin samples at different relative humidity values were measured by DSC (DSC Ql0, TA Instruments, New Castle, DE, USA). Mechanical refrigerated cooling system was used to cool the sample up to −90 °C. The instrument was calibrated for heat flow and temperature using distilled water (melting point (m.p.) = 0 °C; ΔH_m = 334 J/g) and indium (m.p. = 156.5 °C; ΔH_m = 28.5 J/g). Aluminum pans of 30 μL, which could be sealed with lid, were used in all experiments with an empty sealed pan as reference. Nitrogen at a flow rate of 50 mL/min was used as a carrier gas.

Samples of 5–10 mg of gelatin were placed in an aluminum pan and then sealed. The sealed pan with samples were cooled to −90 °C at 5 °C/min, and equilibrated for 10 min. After equilibration, it was scanned from −90 °C to 300 °C at a heating rate of 5 or 10 °C/min (with or without 30 min annealing at T_g −1 °C). Initial experiments showed that 10 °C heating rate provided good sensitivity in identifying the glass transition, thus experiments were conducted at a heating rate of 10 °C/min. Each thermogram was analyzed for the onset, mid, and end of glass transition, melting (or denatured) endotherm, and decomposition characteristics. At least five replicates were performed for each sample.

2.7. Modulated differential scanning calorimetry (MDSC)

The glass transition temperature of gelatin powder at different moisture contents was measured by modulated differential scanning calorimetry (MDSC Q1000, TA Instruments, New Castle, DE). The sample preparation and calibration procedures were same as described in the DSC. Samples of around 10 mg gelatin were cooled to −90 °C at 5 °C/min, and equilibrated for 10 min. Following equilibration, samples were scanned from −90 °C to 200 °C at a constant rate within 10 °C/min with a modulation of ±0.50 °C amplitude and 40 s period of modulation. Thermograms were analyzed from their total, reversible and non-reversible heat flow. The glass transition could be identified as a shift in thermogram line (vertical) in the reversible heat flow line. For all experimental measurements, the average value and standard deviation of at least five replicates were obtained in order to identify the variability of the experimental data.

3. Results and discussion

3.1. Chemical composition

The yield of extracted solids from whole skin was 57.8% based on total solids (62.2% based on ash free solids basis) in the skin. In terms of total skin mass, the yield was 18.0%. Similarly Muyonga, Cole, and Duodu (2004) reported yield of extraction 66.3% for adult Nile perch based on ash free dry basis. However, extraction was higher in the case of young Nile perch. In the case of tilapia the yield was much lower to around 8% (Jamilah & Harvinder, 2002). The lower yield could be due to the loss of extracted collagen through leaching during the series of washing steps or due to incomplete hydrolysis of the collagen (Jamilah & Harvinder, 2002). The chemical compositions of tuna skin gelatin and other commercial gelatins are presented in Table 1. Whole raw skin contained water 60.1 kg/100 kg of skin (60.1%) and extracted gelatin after drying, contained water 8.3 kg/100 kg sample (8.3%), respectively (Table 1). The protein and fat contents were 78.1 and 5.6%, respectively. As a comparison, protein, water, and fat contents in extracted gelatin from Nile perch were 88.0, 10.5 and 0.10%, respectively, when temperature of extraction was 50 °C (Muyonga et al., 2004).

Table 1
Chemical compositions of whole skin and gelatin extracted from yellowfin tuna

Sample	$X_w \times 100$	$X_p \times 100$	$X_f \times 100$	$X_a \times 100$	$X_c \times 100$
Whole skin	60.1 (1.2)	28.8 (0.3)	9.2 (0.3)	1.1 (0.1)	0.6 (0.2)
Tuna gelatin	8.3 (0.6)	78.1 (0.2)	5.6 (0.1)	7.8 (0.2)	0.1 (0.1)
Porcine gelatin	12.3 (0.1)	85.6 (0.1)	1.3 (0.2)	0.4 (0.5)	0.1 (0.2)
Bovine gelatin	9.7 (0.3)	87.6 (0.3)	1.2 (0.1)	0.9 (0.2)	0.1 (0.2)
BDH gelatin	9.3 (0.2)	88.2 (0.4)	1.3 (0.1)	0.7 (0.1)	0.1 (0.1)

Note: Values in parentheses are standard deviations. X_w: water (g/g sample), X_p: protein (g/g sample), X_f: fat (g/g sample), X_a: ash (g/g sample), X_c: carbohydrate (g/g sample).

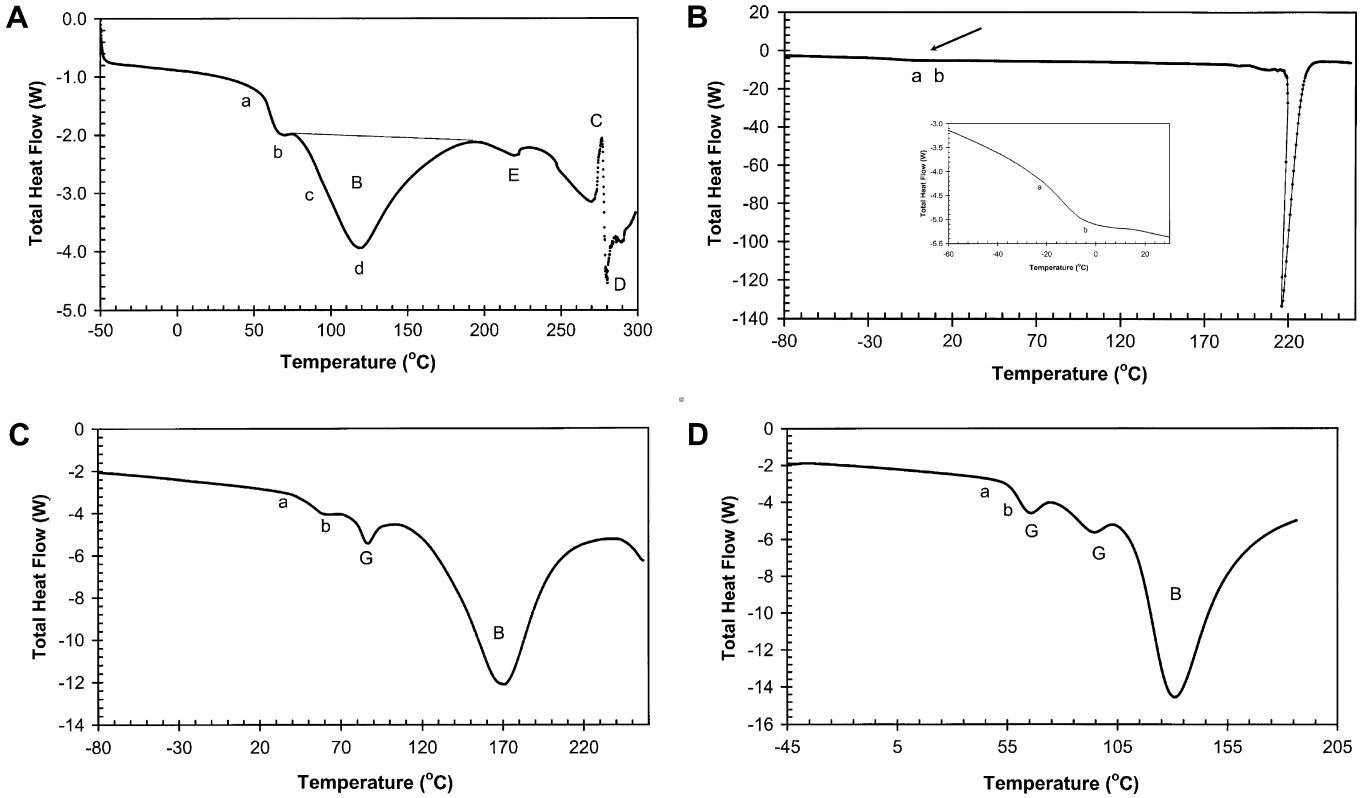

Fig. 1. DSC thermograms of: (A) Commercial gelatin containing 9.3 kg water/100 kg sample showing glass transition is merged with melting endotherm (heating rate of 5 °C/min); (B) tuna gelatin (A complete thermogram, and a Glass transition region by zooming the location are shown); (C) bovine gelatin and (D) porcine gelatin. Samples (B), (C), and (D) were equilibrated at 52.9% relative humidity (heating rate: 10 °C/min, annealing: 30 min).

3.2. Thermal transition by DSC (un-equilibrated samples)

Fig. 1A presents a typical curve for a commercial gelatin showing a glass transition (ab: shift in the thermogram line), melting (B: an endothermic peak) and deterioration (C: exothermic, D: endothermic). In addition there is a relatively small endothermic peak at E. Similar transitions were also found in case of commercial bovine and porcine gelatin. It was difficult to compare the thermal transitions of the gelatin of different sources with varied moisture content or water activity. For this reason, gelatins from different sources were equilibrated at relative humidity of 11.3%, 52.9% and 75.3% and then thermal transitions were measured.

3.3. Thermal transition by DSC (equilibrated samples)

Table 2 shows that tuna gelatin is more hygroscopic compared to mammalian source gelatin (bovine and porcine) since tuna gelatin adsorbed more moisture content compared to the bovine and porcine gelatins when equilibrated at different relative humidity. This also indicated

Table 2
DSC thermogram of different gelatins with annealing for 30 min

Sample	RH (%)	X_w	pH	T_{gi} (°C)	T_{gp} (°C)	T_{ge} (°C)	T_{mi} (°C)	T_{mm} (°C)	T_{mp} (°C)	ΔH (kJ/kg)
Tuna	11.3	0.089	5.46	30^{bc} (15)	43 (17)	54 (11)	139^{ba} (32)	149 (24)	164 (17)	134^{e} (27)
	52.9	0.181	5.70	-19^{d} (6)	−11 (3)	−3 (4)	196^{a} (23)	204 (21)	207 (14)	365^{a} (33)
	75.3	0.240	5.69	-8^{d} (9)	0 (12)	7 (12)	154^{ba} (58)	173 (55)	179 (47)	366^{a} (112)
Bovine	11.3	0.070	4.37	61^{a} (18)	76 (13)	78 (14)	118^{ba} (40)	135 (46)	153 (40)	168^{ed} (31)
	52.9	0.129	4.64	48^{ba} (6)	54 (4)	58 (3)	136^{ba} (40)	149 (37)	164 (37)	235^{cb} (52)
	75.3	0.154	4.65	34^{bc} (3)	45 (7)	48 (7)	114^{b} (60)	122 (57)	141 (45)	257^{b} (41)
Porcine	11.3	0.085	4.33	57^{a} (6)	65 (9)	69 (9)	139^{ba} (48)	151 (43)	163 (40)	197^{cd} (16)
	52.9	0.127	4.37	44^{bac} (4)	52 (4)	56 (1)	141^{ba} (18)	156 (8)	193 (22)	222^{cbd} (33)
	75.3	0.161	4.38	35^{c} (13)	39 (11)	50 (7)	178^{ba} (6)	184 (7)	190 (7)	281^{b} (162)

Note: X_w: moisture content (g/g sample), RH: Relative humidity, T_{gi}, T_{gp}, T_{ge}: initial, mid and end of glass transition temperature, T_{mi}, T_{mm}, T_{mp}: initial, maximum slope, and peak temperature of endothermic peak of melting, ΔH: enthalpy of melting. Values in parentheses are standard deviations. Values in the same column with different superscripts are significantly different ($p < 0.05$).

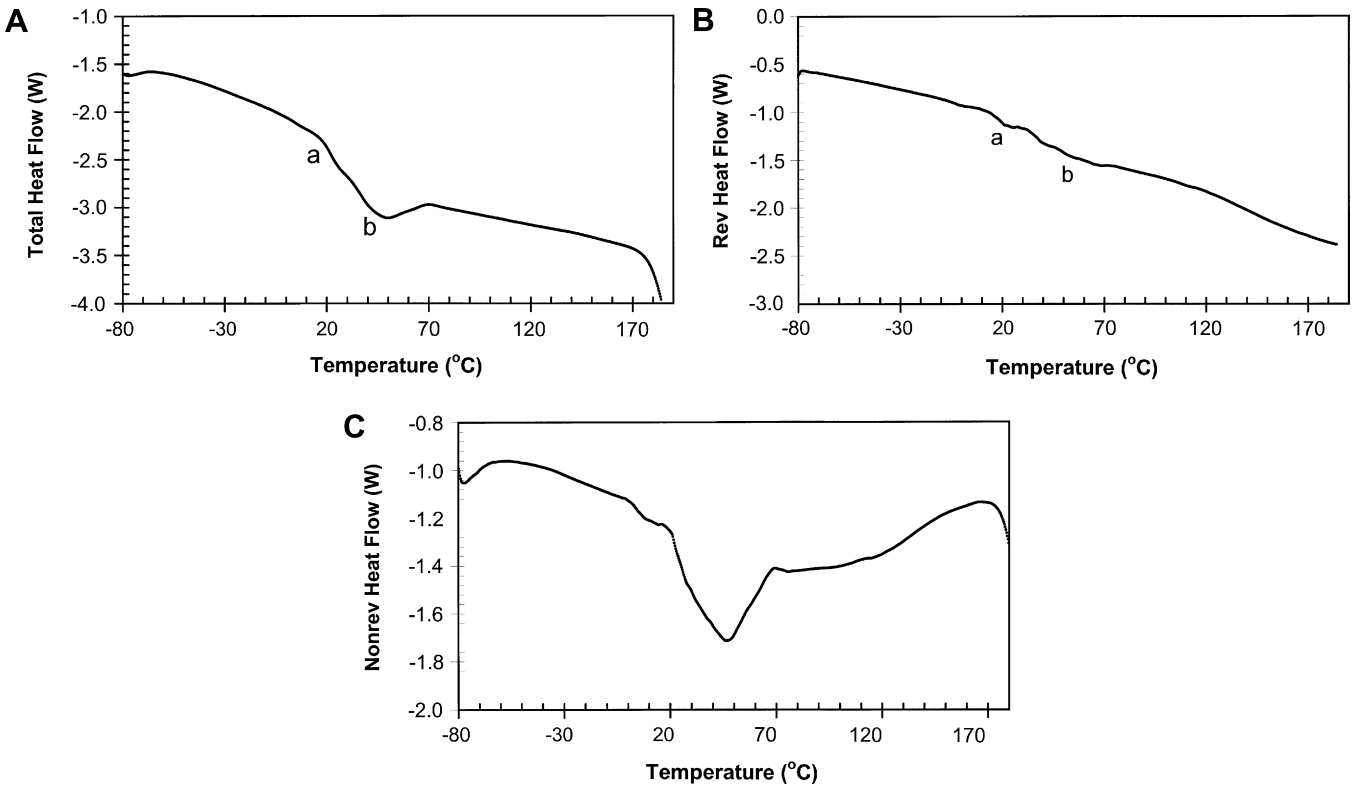

Fig. 2. MDSC thermogram of tuna gelatin equilibrated at 11.3% relative humidity (heating rate: 10 °C/min, annealing: 30 min); A: Total heat flow, B: Reversible heat flow, C: Non-reversible heat flow.

that tuna gelatin contained more polar sites to bind water molecules.

Initial thermogram showed exothermic or endothermic relaxation after glass transition. For this reason DSC run was performed with annealing for 30 min close to its glass transition and data are presented in Table 2. The DSC thermogram of tuna gelatin equilibrated at 52.9% relative humidity shown in Fig. 1B indicates a very narrow melting endothermic peak. This shows that the gelatin molecules were transformed into a more ordered structure with the adsorption of water. In this case water molecule acted as a bridging of the macromolecular network rather than breaking the network bonds. The DSC thermogram of sample equilibrated at 75.3% relative humidity indicated higher amorphous nature showing wider melting peak. In this case, water breaks the macromolecular networks. Typical thermogram for bovine gelatin shown in Fig. 1C indicates glass transition (marked as ab), melting peak (marked B) and another small endothermic peak (marked G). Porcine gelatin showed also similar thermal characteristics with glass transition and two endothermic peaks (Fig. 1D). These endothermic peaks before melting may be due to other structural changes in protein molecules.

The glass transition of tuna gelatin equilibrated at 11.3% relative humidity (8.9 g water/100 g sample) was 30 °C (initial glass transition), which decreased to −19 °C when equilibrated at 52.9% relative humidity (18.1 g water/100 g sample) ($p < 0.05$). The decrease in glass transition indicates plasticization of the polymer molecules, and increase in glass transition indicates anti-plasticization by ordering of polymer molecules. The decrease of glass transition in case of tuna gelatin shows plasticization with water molecules as expected and structural changes occurred in gelatin with hydration. Tuna gelatin equilibrated at 75.3% relative humidity (24.0 g water/100 g sample) showed insignificant change in glass transition compared to the sample equilibrated at 52.9% relative humidity ($p > 0.05$) (Table 2). This indicated no further plasticization with equilibration in higher relative humidity. The effect of adsorbed water on the gelatin of different sources varied due to their molecular structure. In addition, the degree of plasticization or anti-plasticization varied with the molecular nature of gelatin from different sources. The complexity of glass transition and plasticization in the case of biological macromolecules are evident in literature. The following section presents selected analysis of the results from the literature. Ong, Whitehouse, Abeysekera, Al-Ruqair, and Kasapis (1998) studied the glass–rubber transition and structural properties of composite containing acid pigskin gelatin, oxidized starch, and glucose syrup at 30% water content. They found that concentrated oxidized starch with glucose syrup systems were capable of forming intermolecular amylase-like structures at high temperature (e.g. 80 °C), which remained solid-like (absence of glass–rubber transition) throughout the cooling run down to −20 °C. They suggested the development of crystal

structural domain (water/polymeric aggregates) in starch dominated systems prevents them from exhibiting glass-related viscoelasticity. Fringant, Rinaudo, Foray, and Bardet (1998) also experienced complexity in plasticization in esters of starch. They observed internal plasticization (when relaxation was not observed), and external plasticization (when an impressive β relaxation was observed). In the case of glucose and lactose, at 10% mass ratio of anhydrous casein showed no effect on the glass transition of casein as a function of water content, however 50% mass ratio showed some increase in glass transition temperature, i.e. an anti-plasticizing effect with only glucose (Kalichevsky, Blanshard, & Tokarczuk, 1993). Mitsuiki, Yamamoto, Mizuno, and Motoki (1998) studied the glass transitions of low moisture galactans (agars and carrageenans) and starch. The glass transition temperatures of agars leveled off at higher water contents above 20% water content, whereas transition temperatures of carrageenans and wheat starch continued to decrease with increasing water content due to plasticization. The NMR and sorption isotherm indicated that strength of binding of water with agars was weaker, suggesting that most of the water molecules sorbed by agar were close to free and could not plasticize the molecules. In addition, pH of whey protein films affected its glass transition temperature (Anker, Stading, & Hermansson, 1999). Marzec and Lewicki (2006) measured the effect of water activity on the plasticization of extruded flat bread using mechanical compression. They found plasticization by water adsorption from water activity from 0.05 up to 0.55, and then at higher water activity anti-plasticization was observed. However, they did not conduct DSC to find the effect of water on thermal characteristics.

Bovine and porcine samples equilibrated at 11.3% and 75.3% relative humidity showed significant difference in glass transition ($p < 0.05$). However bovine and porcine samples equilibrated at 52.9% relative humidity showed insignificant difference with the samples equilibrated at 11.3% and 75.3% relative humidity ($p > 0.05$). However, it was noticed that the standard deviations were higher compared to the other samples. Thus these samples were replicated 10 times, but further reduction in the variability (i.e. standard deviation) were not observed. However, the reasons of this variability need to be studied further. In general, tuna gelatin equilibrated at a constant relative humidity of 11.3%, 52.9% or 75.3% showed lower glass transition compared to the mammalian source (bovine and porcine) gelatin ($p < 0.05$). Equilibration of tuna, bovine, and porcine gelatin with different water activities (11.3%, 52.9% and 75.3% relative humidity) showed insignificant effect on the melting points ($p > 0.05$). Sobral and Habitante (2001) measured the thermal transitions of pigskin gelatin by DSC. They found the initial glass transitions were 90, 40 and 20 °C, respectively for the pigskin gelatin conditioned by desorption at 11.3%, 52.9%, and 75.3% relative humidity. Their results are comparable to the samples in this study equilibrated at 52.9% and 75.3% relative humidity, while 11.3% equilibrated sample showed much lower value compared to 90 °C as found by Sobral and Habitante (2001). Sablani, Kasapis, Al-Rahbi, and Al-Mugheiry (2002) used a rheological method for determining T_g and found for young pigskin gelatin much lower values as 7, 15, and 35 °C for moisture contents of 25, 20 and 3 g/100 g sample, respectively. However, gelatin containing 10.6 g water/100 g sample showed an average onset glass transition of 60 °C (Bell & Touma, 1996). Similarly, Slade and Levine (1987) found the onset glass transition containing 10.2 g water/100 g sample at 73 °C. These wide variations in gelatin molecules from different sources indicated the complexity of the hydration and plasticization causing difference glass transition. Bell and Touma (1996) also explained that the wide variation of the glass transition values in literature may be due to different types of gelatin transformed during different extraction method and characteristics of animal skins based on species, age, and sex.

3.4. Thermal transition by MDSC without annealing (equilibrated samples)

More sensitive MDSC was used to measure the thermal transitions of gelatins from different sources. Typical MDSC thermogram of tuna gelatin equilibrated at 11.3% relative humidity shows a shift in thermogram line indicating the glass transition and another small endothermic peak before melting peak. Similarly typical thermogram of porcine equilibrated at 52.9% relative humidity shows two endothermic peaks after glass transition and a melting endothermic peak. Similar thermograms were also observed for bovine samples. Experiments were then conducted with annealing due to complexity showing the numbers of endothermic peaks after glass transition and before melting. In addition, the effects of annealing on gelatin molecule were also explored. The endothermic peaks after glass transition indicate the structural relaxation or other structural changes of macromolecules.

3.5. Thermal transition by MDSC with 30 min annealing (equilibrated samples)

Table 3 shows the glass transition temperature determined from total and reversible heat flow thermograms. Typical MDSC thermogram for tuna gelatin equilibrated at 11.3% relative humidity showing total, reversible and non-reversible heat flow is shown in Fig. 2. Fig. 2A shows total heat flow with a shift in the thermogram line with a merged endothermic peak. The reversible heat flow (shift due to specific heat change) shows a clear glass transition by a shift in the thermogram line (Fig. 2B). Non-reversible thermogram shows endothermic peak indicating a kinetic process existed during the transformation of glassy to rubbery state (Fig. 2C). In addition, Fig. 2C shows an increasing trend in the thermogram line after the endothermic peak indicated ordering of the macromolecule before the start of melting process. In this case, MDSC method could

Table 3
Glass transition from total heat flow thermograms and specific heats changed from total heat flow and reversible heat flow (with annealing 30 min)

Sample	RH (%)	Total heat flow				Reversible heat flow			
		T_{gi} (°C)	T_{gp} (°C)	T_{ge} (°C)	ΔC_p (kJ/kg K)	T_{gi} (°C)	T_{gp} (°C)	T_{ge} (°C)	ΔC_p (kJ/kg K)
Tuna[A]	11.3	18^{c} (5)	23 (3)	35 (3)	700^{bc} (94)	23^{c} (4)	30 (6)	38 (7)	570^{a} (39)
	52.9	-19^{e} (3)	−9 (1)	−1 (1)	695^{bc} (346)	-3^{e} (3)	2 (3)	8 (2)	327^{bc} (143)
	75.3	-7^{d} (9)	1 (9)	8 (9)	646^{c} (335)	12^{d} (11)	24 (15)	32 (10)	457^{ba} (170)
Bovine[B]	11.3	70^{a} (9)	76 (9)	79 (12)	874^{bac} (536)	75^{a} (9)	77 (10)	82 (6)	246^{c} (92)
	52.9	49^{b} (8)	54 (7)	58 (6)	798^{bac} (76)	57^{b} (7)	61 (6)	64 (5)	456^{ba} (38)
	75.3	45^{b} (19)	51 (18)	60 (12)	1105^{a} (318)	49^{b} (17)	53 (14)	57 (12)	414^{ba} (134)
Porcine[B]	11.3	49^{b} (1)	58 (1)	61 (1)	604^{c} (68)	59^{b} (6)	63 (6)	70 (3)	305^{bc} (147)
	52.9	51^{b} (7)	59 (5)	61 (5)	895^{bac} (167)	60^{b} (3)	64 (4)	67 (3)	380^{bc} (75)
	75.3	48^{b} (18)	56 (13)	61 (12)	1019^{ba} (175)	60^{b} (9)	62 (9)	65 (9)	384^{bc} (170)

Note:
Values in parentheses are standard deviations.
Values in the same column with different superscripts are significantly different ($p < 0.05$).
ΔC_p Change in specific heat at the transition (kJ/kg K).
Other symbols are defined in Table 2.
[A] Average of five replicates.
[B] Average of ten replicates.

be used to explore other structural changes during the glass–rubber transition. However, a difficulty of using DSC is usually observed when other structural changes occur at a similar location of glass–rubber transition. It is clearly evident that MDSC can easily separate the glass transition in the reversible heat flow thermogram line.

MDSC thermograms for bovine gelatins are also shown in Fig. 3. Total heat flow shows that glass transition and endothermic process are occurring at a similar location (Fig. 3A). Reversible thermogram clearly shows glass transition (when separated from the total heat flow thermogram) at ab indicating a shift in the thermogram line (Fig. 3B). The non-reversible heat flow thermogram in Fig. 3C shows a kinetic process. The kinetic events could be due to extensive structural rearrangements influenced by whether the system exist a glassy or rubbery state (Slade, Levine, & Finley, 1989).

In the case of porcine gelatin two endothermic peaks were found close to the glass transition (Fig. 4A). The reversible heat flow thermogram shows glass transition at ab and an unusual exothermic peak after glass transition (marked J) (Fig. 4B). This may be due to other structural changes occurring after glass transition. The non-reversible thermogram in Fig. 4C showing endothermic peak above glass transition probably represented hydrogen bond disruption within the protein (Bell & Touma, 1996), which would be similar to the endothermic peak due to the unfolding of globular bovine somatotropin (Bell, Hageman, & Bauer, 1995a). However, these endothermic peaks were also evident in DSC thermogram.

Table 3 presents the glass transitions and specific heat changes at glass transition observed from total and reversible heat flow. The glass transition determined from the reversible heat flow is considered most accurate since other factors are separated from the glass transition, thus only glass transition determined from reversible heat flow with annealing is discussed. Tuna gelatin samples equilibrated at 11.3% relative humidity showed glass transition (initial) at 23 °C, which decreased to −3 °C when equilibrated at 52.9% relative humidity ($p < 0.05$). This indicated the plasticization of the gelatin molecules with adsorbed water. Further increase of water activity to 75.3% showed increase in glass transition to 12 °C ($p < 0.05$). This indicated further adsorbed water molecule transformed gelatin molecules into more order in their molecular structure. Crystallize material, if present, may increase glass transition of a system (Sperling, 1986). Bell, Hageman, and Muraoka (1995b) identified that in the dry state, the additives were acting as plasticizers, enhancing the mobility and thus the unfolding of the globular proteins, while hydrated state showed anti-plasticization stage and more stable.

In case of bovine, glass transition decreased for the samples equilibrated at 52.9% relative humidity ($p < 0.05$). Further increase in equilibrium humidity had insignificant effect on glass transition ($p > 0.05$). However, porcine gelatin samples equilibrated at different relative humidity showed insignificant effect on the glass transition ($p > 0.05$). This trend was also evident form the ΔC_p at the glass transition temperature. Gelatin is a very complex molecule and its characteristics depend on many factors, such as the collagen type, tissue, animal species, and age. The type and concentration of acid strongly influence swelling properties and solubilization of collagen, leading to variations in molecular weight distribution in the resultant gelatins, depending on the persistence of some of the cross-links between collagen-chains (Gimenez, Turnay, Lizarbe, Montero, & Gomez-Guillen, 2005). According to Asghar and Henrickson (1982), the lyotropic effect of carboxylic acids on collagen seems to dominate the swelling capacity, rather than a specific ion effect, since it is the non-ionized acid that acts as the swelling agent by competing with the peptide group involved in intermolecular linking

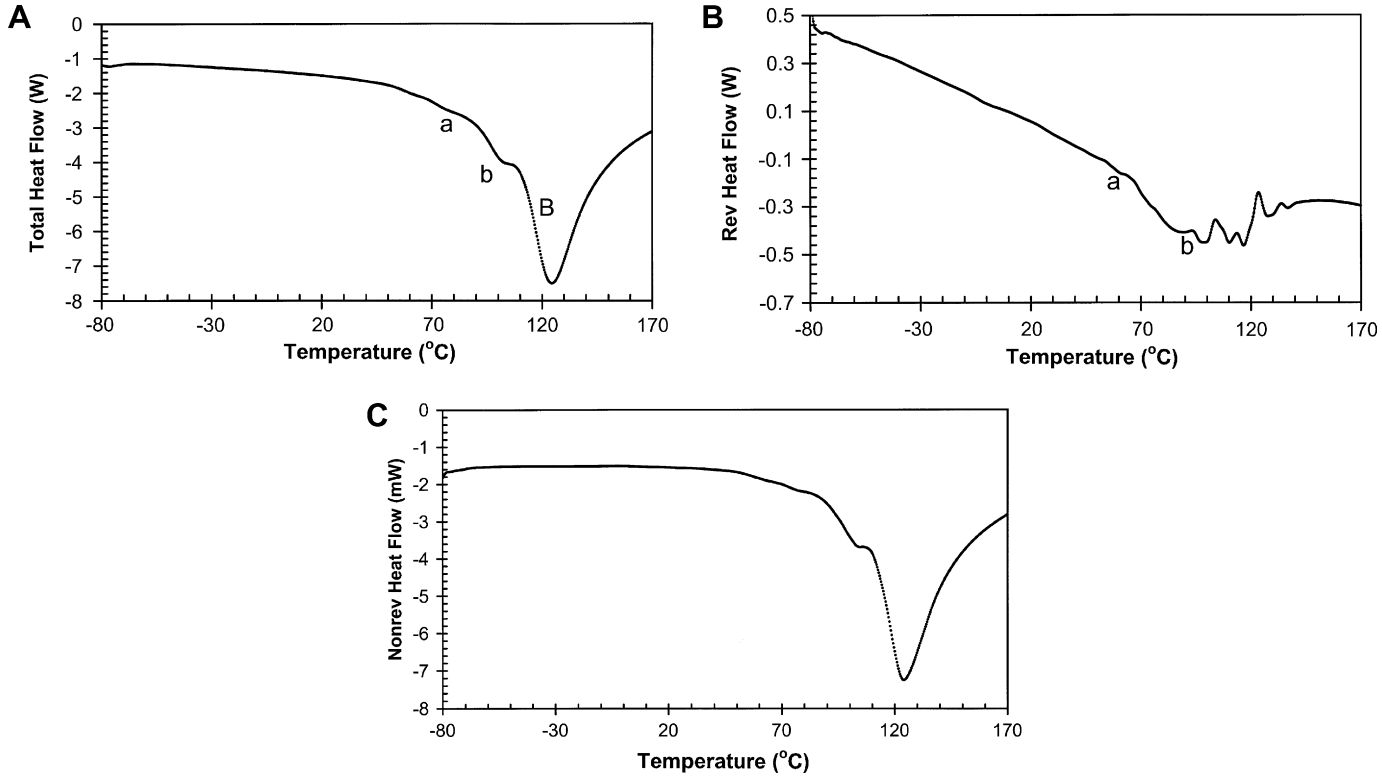

Fig. 3. MDSC thermogram of bovine gelatin equilibrated at 11.3% relative humidity (heating rate: 10 °C/min, annealing: 30 min); A: Total heat flow, B: Reversible heat flow, C: Non-reversible heat flow.

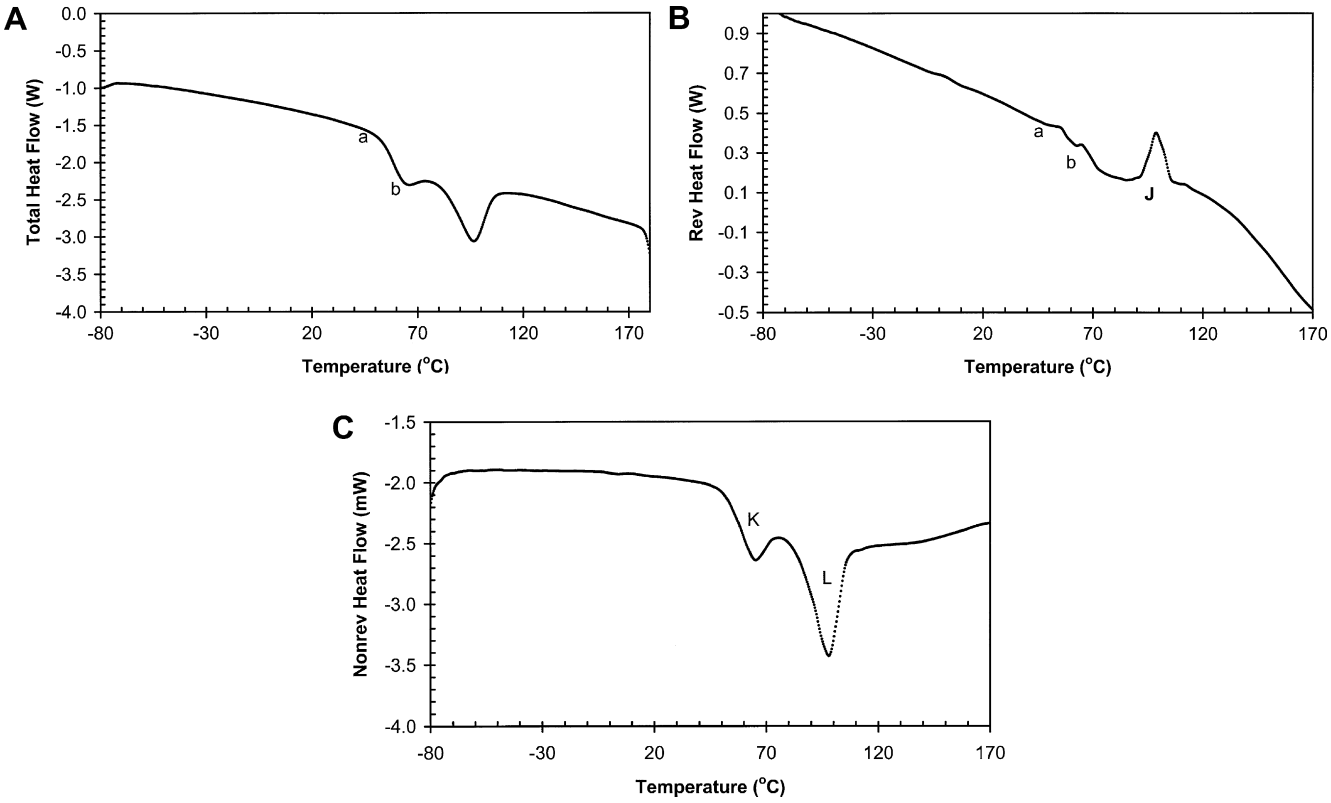

Fig. 4. MDSC thermogram of porcine gelatin equilibrated at 11.3% relative humidity (heating rate: 10 °C/min, annealing: 30 min); A: Total heat flow, B: Reversible heat flow, C: Non-reversible heat flow.

of protein chain, mainly because of the hydrogen bonding power of the acid.

Two cycles heating process was also applied to see the structural reversibility of the gelatin if it is heated up to 170 °C. Application of two cycles heating to tuna gelatin equilibrated at 11.3% caused a shift of the glass transition at a higher level (data not shown). This shift indicated that structural changes occurred during heating process. This may also indicate that larger molecules were formed by the heating process.

Gelatin from tuna skin showed lower glass transition compared to the gelatin from mammalian skins (bovine and porcine) ($p < 0.05$) (Table 3). Generally, fish gelatin showed lower gel strength than mammalian gelatin (Norland, 1987). In addition, tropical-fish, such as tilapia, was a superior material for gelatin processing (Grossman & Bergman, 1992), while cold-water fish, cod gelatin has poorer physical properties (Gudmundsson & Hafsteinsson, 1997). Cho, Gu, and Kim (2005) observed higher gel strength of gelatin from the dorsal skin of yellowfin compared with two mammalian gelatins (bovine and porcine). The variation is mainly due to the characteristics of collagen, which influence properties of gelatin. The amino acid content and sequence varies from one source to another but mammalian gelatin contained considerable large amounts of proline, hydroxyproline and glycine (Gilsenan & Ross-Murphy, 2000; Haug, Draget, & Smidsrod, 2004). The gelling point and gel melting of yellowfin tuna skin gelatin were much lower than bovine and porcine skin gelatins.

3.6. Comparison of DSC and MDSC

In general, glass transition temperature measured by DSC showed lower values compared to the MDSC reversible heat flow with 30 min annealing (Tables 2 and 3). The early transition by DSC could be due to the formation of stiff sample during heating scan while MDSC samples could experience relaxation due to heating/cooling oscillation cycle by modulation. In the cases of PVP and polydextrose, Bell and Touma (1996) reported lower MDSC values compared to DSC values. However, they pointed that in some instances this variation may not be significant. Rahman, Al-Marhubi, and Al-Mahrouqi (2007) studied the glass transition temperature of spaghetti by five different methods. At lower heating rate, MDSC values showed much higher glass transition values compared to the DSC values, since initial glass transition measured by DSC increased exponentially and reached a constant value of 55 °C at heating rate of 30 °C/min (or higher). Transition temperature, measured by MDSC, remained constant up to heating rate 15 °C/min and then decreased. The glass transition values determined from reversible heat flow was 60 °C. This suggests that more detailed studies need to be conducted to compare the glass transition measurement by DSC and MDSC methods. In addition physicochemical explanation should also be explored.

4. Conclusions

Gelatin was extracted from yellowfin tuna skin by alkali washing followed by heating in acetic acid solution, air-drying and grinding into powder. The yield of extracted soluble solids was 57.8% based on the total solids in the skin. Thermal characteristics were measured by differential scanning calorimetry (DSC) and modulated DSC (MDSC). Thermograms of tuna gelatin showed glass transition (shift in thermogram line), melting (endothermic peak) and deterioration (exothermic followed by endothermic). Water adsorption isotherm showed that tuna gelatin was more hygroscopic than commercial mammalian (bovine and porcine) gelatins equilibrated at the same relative humidity of 11.3%, 52.9% and 75.3%. In general, tuna gelatin showed lower glass transition compared to mammalian source. The results obtained in this study showed that the gelatins from different sources exhibited different degrees of plasticization by water molecules.

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