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Physical properties of gelatin extracted from skin of Thai panga fish (*Pangasius bocourti* Sauvage)

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Abstract

Gelatin from the skin of Thai panga fish (Pangasius bocourti Sauvage) was pretreated with a solution of 0.8 M sodium chloride and 0.1 M sodium hydroxide and extracted by acetic acid solution pH 4.55 at 55°C for 1 h. Physical properties of the obtained fish gelatin and the commercial bovine bone gelatin were compared. The gel strength (513.75 g), viscosity (3.88 cP), turbidity (73.21%), foaming properties (foam formation ability 1.13 and foam stability 0.71), emulsion stability (34.2 to 44.6%) and adhesiveness (-369.1 g.sec) of the fish skin gelatin were higher, but color (L* 43.62, C* 3.66 and h° 45.28), cohesiveness (0.838) and gel elasticity were lower than those of the bovine bone gelatin. Gelling and melting points of the fish skin gelatin (16.40°C and 26.87°C, respectively) were lower than those of the bovine bone gelatin (18.45°C and 29.90°C, respectively). Results obtained suggest that the gelatin extracted from the skin of Thai panga fish was a potential raw material for producing a gelatin film or use as foaming agent, emulsifying agent or thickener, but not suitable for use as gelling agent.

Keywords: Thai panga fish, *Pangasius bocourti* Sauvage, gelatin, extraction, physical properties

1. Introduction

Thai panga fish (Pangasius bocourti Sauvage) is a new economic fresh water fish that was promoted to be cultured in areas along the Mae Khong shore of Thailand. Only the flesh of this fish is frozen and exported to Europe and USA as fish fillet. The remaining part of the fish becomes waste (National Food Institute, 2006). About 30% of the wastes are skin and bone that are high in collagen and can be used for production of gelatin (Wasswa et al., 2007).

Gelatin is a class of protein fractions derived from collagen by destruction of intermolecular cross-links and partial breakage of peptide bond. Gelatin is widely used in food industries as gelling, foaming, binding and emulsifying agents (Schrieber and Gareis, 2007). Most of commercial gelatin obtains from mammals, mainly bovine and porcine. Until recently, foot-and-mouth disease as well as bovine spongiform encephalopathy in products from mammals have been concerned, gelatin production from other sources such as by-products of seafood have been searched. Moreover, religious sentiments of Judaism, Islam and Hindus

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forbid consumption of porcine or bovine products (Karim and Bhat, 2009). However, physical properties of fish gelatin differ considerably from bovine gelatin. The gelling and melting temperatures of fish gelatin, especially those produced from cold water fish, were lower than those of the bovine gelatin (Regenstein and Zhou, 2007). In contrast, fish gelatin was superior in film formation, emulsification property and stability of viscosity (Schrieber and Gareis, 2007).

The objective of this study is mainly to compare the physical properties of the gelatin extracted from the Thai panga fish skin with the commercial bovine bone gelatin. The knowledge gained from this work would direct the proper use of the obtained gelatin and would promote production of the fish gelatin commercially.

2. Materials and Methods

2.1 Raw material and reagents

The frozen Thai panga fish (Pangasius bocourti Sauvage) skin was packed in thermal insulated box and transported from a processing plant at Nakhonphanom province of Thailand within 24 h. The fish skin was kept at -20°C and the experiment was conducted within 1 year. Proximate composition of the skin was 60.86±0.65% moisture, 35.83±2.61% crude protein, 2.19±0.64% crude lipid and 0.18±0.08% crude ash. Extraction chemicals included sodium hydroxide (Merck, Germany), sodium chloride (Union Science, Thailand) and glacial acetic acid (Labscan, Thailand). The limed bovine bone gelatin (type B) with 250 bloom was purchased from Nitta Gelatin (Japan). Soybean oil purchased from Thai Vegetable Oil Co., Ltd. (Thailand).

2.2 Gelatin extraction

Gelatin extraction was performed by the method of Prommajak and Raviyan (2010). The flesh attached to the fish skin was manually scraped off. The skin was then cut into the square dimension with the size of 1-2 cm. The fish skin was pretreated for 4 h at ambient temperature in a mixture containing 0.8 M sodium chloride and 0.1 M sodium hydroxide at a skin-per-solution ratio of 1:20 (w/v) using SGM300 orbital shaker (Gallenkamp, England) at 120 rpm. The solution was changed after using for 2 h. The pretreated skin was next rinsed 3 times with water at room temperature, left the water to drain off and extracted at 55°C in a WB14 water bath (Memmert, Germany) for 1 h using acetic acid solution pH 4.55. The ratio of skin-per-extracted solution was 1:6 (w/v). After extraction, the mixture was filtered through a piece of double-layer cheese cloth. Activated charcoal (1% of initial fish skin weight) was put into the gelatin solution and the mixture was continuously stirred at 400 rpm by magnetic stirrer for 1 h at room temperature. After stirring, the mixture was centrifuged at 2,000 g by Rotina

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46R (Hettich, Germany) for 30 min to separate the activated charcoal from the gelatin solution. The filtrate was then dried out overnight at 50°C using hot air oven (Termaks, Norway). The dried gelatin sheets had 12±1.19% moisture content. Physical properties of the obtained fish gelatin and the commercial bovine bone gelatin were analyzed and the results were compared.

2.3 Gel strength determination

Gel strength was analyzed by the method of Zhou and Regenstein (2004). Gelatin solution of 6.67% (w/w) was prepared by dissolving dried gelatin with distilled water and heated at 60±1°C for 30 min in a water bath. The gelatin solution was then filled in a cup (30 mm diameter × 15 mm height) and kept at 2±0.4°C for 16-18 h. The gel strength was measured by the texture analyzer (TA.XT Plus, Stable Micro System, England), using a 12.7 mm diameter plunger (P/0.5R probe), 0.5 mm/s compression rate and 4 mm penetration depth. The gel strength is a maximum force required in penetration.

2.4 Viscosity determination

Gelatin solution (6.67% w/w) was adjusted to pH of 3 to 10 using 0.1 M HCl or 0.1 M NaOH. The solutions were then diluted to a final concentration of 2% using distilled water previously adjusted to the same pH of the gelatin solution. Viscosity of gelatin solutions was determined by Brookfield viscometer using S18 probe at 200 rpm. The viscosity was recorded at 10 s of the measurement.

2.5 Turbidity and color determination

Turbidity of gelatin solution was determined by measuring transmittance at 620 nm of 6.67% (w/w) gelatin solution using spectrophotometer model Lambda 35 (Perkin Elmer, UK) following the method of Schrieber and Gareis (2007).

Color of the 6.67% (w/w) gelatin gel sample was determined. The gel sample was prepared according to the method used in sample preparation for gel strength determination. The color was measured in L*, C* and h° color space system using Minolta Chroma Meter, CR300 model (Minolta, Japan).

2.6 Foaming properties determination

Foaming properties of gelatin were measured by the method modified from the method of Marinova et al. (2009). Sample preparation was followed the method used in sample preparing for viscosity determination. The 15 ml gelatin sample was filled into a 50 ml closed graduated cylinder. The cylinder was vigorous hand shakings for 20 times. The foam volumes

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generated immediately after stop shaking (0 min) and the foam volume left after stop shaking for 15 min were recorded. Foaming properties were calculated as follow:

$$\text{Foam formation ability} = \frac{\text{volume of foam at 0 s}}{\text{initial volume of solution}}$$

$$\text{Foam stability} = \frac{\text{volume of foam at 15 min}}{\text{volume of foam at 0 min}}$$

2.7 Emulsion stability determination

Emulsion property of gelatin was measured by the method that modified from Kittiphattanabawon (2004). Gelatin solutions at a concentration of 1, 2 and 3% were mixed with soybean oil at a ratio of 3:1 (v/v) using blender for 30 s. The 5 g of the obtained emulsion was centrifuged at 2500g for 15 min by Rotina 46R (Hettich, Germany). The separated water layer was weighed. Emulsion stability was calculated as follows:

$$\text{Emulsion stability (\%)} = \frac{\text{initial emulsion weight} - \text{separated water weight}}{\text{initial emulsion weight}} \times 100$$

2.8 Texture profile analysis (TPA)

TPA was analysed by the method that modified from Wangtueai and Noomhorm (2009). Gelatin gel was prepared according to the method used in gel strength determination. TPA was analysed using the Texture analyzer (TA.XT Plus, Stable Micro System, England). The gel was pressed with a 36 mm diameter aluminum probe at a compression rate of 1 mm/s until 30% deformation was reached. The pause between the first and second compression was 3 s. Parameters obtained from the analysis included fracturability, hardness, adhesiveness, cohesiveness, chewiness, gumminess, springiness and resilience.

2.9 Viscoelastic properties determination

Viscoelastic properties were analysed by a Rheometer (AR2000, TA Instruments, USA) using the modified method of Liu et al. (2008). The probe used in this study was a cone-plate geometry with 1° cone angle. The 1 ml of 6.67% (w/w) gelatin solution was cooled from 40 to 5°C and kept at 5°C for 5 min. Then, the gel was heated from 5 to 40°C. Conditions used in the study were 1°C/min scan rate, 1 Hz frequency, 3.0 Pa oscillation stress and 1 mm gap. The obtained elastic modulus (G'), viscous modulus (G'') and phage angle were plotted as a function of temperature.

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2.10 Statistical analysis

The experiments were conducted in triplicate. Differences between the physical properties of Thai panga fish gelatin and bovine bone gelatin were determined by analysis of variance (ANOVA) and Duncan's multiple range test using SPSS software (SPSS Inc., Chicago, Illinois).

3. Results and Discussion

3.1 Gel strength

The gel strength of Thai panga fish gelatin was 513.75±10.06 g, higher than that of the bovine bone gelatin (P<0.05) which had the gel strength of 465.76±15.38 g. This result was in agreement with the previous published data of Cho et al. (2005) who discovered that the gel strength of gelatin from the yellowfin tuna skin was considerably higher than those of the bovine hide and pig skin gelatins.

Type of fish itself would affect the gel strength of gelatin. Theoretically, the gelatin obtained from the tropical fish trended to have higher gel strength than that from the cold water fish because collagen produced from tropical fish was generally more stable when compared to the collagen from cold water fish (Gilsenan and Ross-Murphy, 2000). The gelatins obtained from Thai panga fish and yellowfin tuna which were the tropical fish thus showed high value of gel strength. In addition, there were other factors such as extraction condition that extensively affected the gel strength of gelatin. In production of bovine bone gelatin, the bone usually has to be exposed to hydrochloric acid solution for 5-7 days to get rid of minerals and then treated with alkaline solution for 35-70 days (Wasswa et al., 2007). These severe extraction conditions caused hydrolysis of collagen molecules that leading to decreasing of gel strength (Eysturskarð et al., 2009). Extraction of gelatin in this study required total soaking time in the pretreatment and extraction solution for 5 h which was not long enough to significantly hydrolyze α-chain collagen that was a component contributes to strength of gelatin. For food production, the gelatin of high gel strength required shorter gelling time and lower amount of gelatin to obtain the desired firmness (Schrieber and Gareis, 2007).

3.2 Viscosity

The viscosities of gelatins obtained from the Thai panga fish at all studied pH were higher than those of the bovine bone gelatins, excepted at pH 5 and 8 (Fig. 1). The average viscosity of the fish gelatin was 3.88±0.66 cP and was higher than that of the bovine gelatin which found 3.16±0.33 cP (P<0.05). Viscosity of gelatin could be varied from 3.0 to 6.0 cP, depended on extraction temperature and pH (Schrieber and Gareis, 2007). High viscosity of

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the fish gelatin was demonstrated to be a feasible property for production of gelatin film and stabilization of food emulsion (Baziwane and He, 2003).

The minimum viscosity of gelatin basically reveals at the isoelectric point of the gelatin. Gelatin extracted with alkaline process generally has isoelectric point between 4 and 6 because the alkaline treatment leading to hydrolysis of amino acid asparagine and glutamine that results in aspartic acid and glutamic acid (Baziwane and He, 2003; Cole, 2000). Viscosity of gelatin solution was lowest at the isoelectric point and increased when pH was far away from this point (Linden, 1999). According to the minimum viscosity demonstrated in this study, the isoelectric point of the fish gelatin would be at pH 5, while that of the bovine gelatin would be at pH 6 to 7.

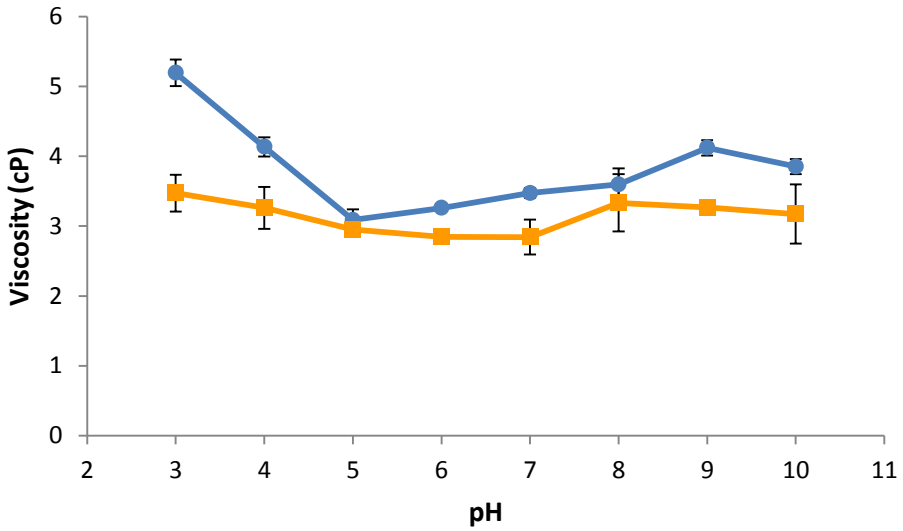

Figure 1. Viscosities of Thai panga fish skin gelatin (●) and bovine bone gelatin (■) at various pH.

3.3 Turbidity and color

Thai panga fish gelatin was visually more turbid than the bovine gelatin. Transmission of light at 620 nm through the Thai panga fish gelatin solution was 73.21±0.63%. While that of bovine bone gelatin was 95.24±0.44%, confirming the higher turbidity of Thai panga fish skin gelatin (P<0.05). However, the difference in gelatin turbidity was not due to the difference between fish and mammalian that used as raw materials, but due to inorganic, protein and mucosubstance contaminants (Avena-Bustillos et al., 2006).

Due to different turbidity, color of gelatin gel was measured by light reflection method using chroma meter. Lightness of bovine gelatin was higher than that of the fish gelatin (P<0.05). The chroma and hue angle values of fish and bovine gelatins were different (P<0.05)

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(Table 1). Fish gelatin had a color in an orange region, while the bovine gelatin had yellow color. However, both gelatins had very low chroma values (chroma value had a range between 0 and 100), indicated that both gelatins had pale color which made color difference was difficult to visually observe. High color intensity of gelatin was a result from Maillard reaction between protein and carbohydrate in raw material, which increased with reaction time (Schrieber and Gareis, 2007).

Table 1. Color of Thai panga fish skin gelatin and bovine bone gelatin.

Colors	Thai panga fish skin gelatin	Bovine bone gelatin
Lightness (L*)	$43.62^{b} \pm 1.53$	$45.78^{a} \pm 1.52$
Chroma (C*)	$3.66^{b} \pm 0.38$	$4.25^{a} \pm 0.63$
Hue angle (h°)	$45.28^{b} \pm 5.21$	$70.89^{a} \pm 9.62$

* Mean ± SD of triplicate determinations. Different letters within the same row indicated significant difference (P<0.05).

3.4 Foaming properties

Fig. 2 shows foam formation ability of gelatin at various pH. Average foam formation ability of Thai panga fish skin gelatin was 1.13±0.24, higher than that of the bovine bone gelatin which was 1.03±0.32 (P<0.05). The fish gelatin showed better foam formation ability at pH 9 and 10, while the bovine gelatin had better foam formation ability at pH 4 (P<0.05). Maximum foam formation ability of the fish gelatin was 1.3 times of initial gelatin solution at pH 5 to 9, while that of bovine gelatin was 1.47 times at pH 5. The higher foam formation ability of fish gelatin, when compared with bovine gelatin, was also found in red snapper and grouper bone gelatin (Jeya Shakila et al., 2012). However, foam formation ability of cuttlefish skin gelatin was lower than bovine gelatin (Aewsiri et al., 2011).

Average foam stability of the fish gelatin was 0.71±0.16, higher than that of the bovine gelatin which was 0.64±0.13 (P<0.05). Foam of fish gelatin had maximum stability at pH 3-6 and then slowly decreased with the increasing pH. The bovine gelatin foam had maximum stability at pH 7 and largely decreased at pH lower than 5 (Fig. 2). This result suggested that selection of an appropriated gelatin should depend on the pH used in food production.

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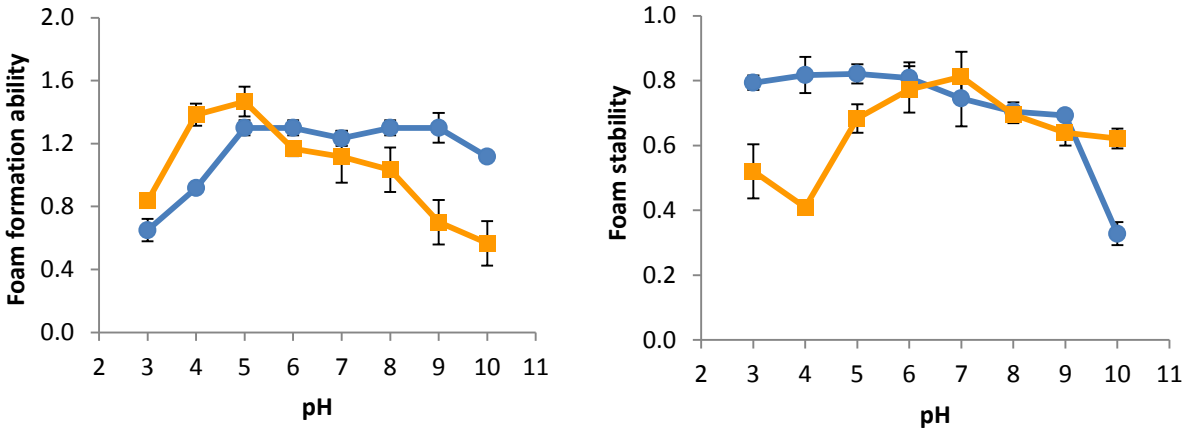

Figure 2. Foam formation ability and foam stability of Thai panga fish skin gelatin (●) and bovine bone gelatin (■) at various pH.

3.5 Emulsion stability

Emulsion stability depended on type and concentration of gelatin (Table 2). Compared with bovine gelatin, using Thai panga fish gelatin as emulsifier resulted in higher emulsion stability (P<0.05). Stability of emulsion containing 3% of fish gelatin was higher than those of emulsion containing 2 and 1% gelatin, respectively. However, no interaction between type and concentration of gelatin for emulsion stability was illustrated.

Stability of emulsion prepared from 3% bovine gelatin solution was comparable to the emulsion prepared from 2% fish gelatin solution. The emulsion with 2% bovine gelatin solution was not significant different from the emulsion with 1% fish gelatin solution (P>0.05). Emulsion prepared from 1% bovine gelatin solution had the lowest stability. High emulsion stability of fish gelatin was contributed to its high viscosity (Schrieber and Gareis, 2007). The good emulsion property of the fish gelatin can be used in production of ice cream, yoghurt or other dairy products.

Table 2. Stability of emulsion that emulsified by Thai panga fish gelatin or bovine gelatin.

Concentration of gelatin solution (%)	Emulsion stability (%)	
	Thai panga fish gelatin	Bovine bone gelatin
1	$34.19^{c} \pm 1.56$	$30.00^{d} \pm 0.99$
2	$37.60^{b} \pm 1.05$	$32.96^{c} \pm 1.02$
3	$44.57^{a} \pm 1.97$	$37.36^{b} \pm 0.89$

* Mean ± SD of triplicate determinations. Different letters indicated significant difference (P<0.05).

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3.6 Texture profile analysis (TPA)

Texture profiles of Thai panga fish gelatin and bovine bone gelatin were shown in Table 3. Fracturability, chewiness and gumminess of both gelatins were not significant different (P>0.05).

Hardness or firmness of the fish gelatin was higher than that of the bovine gelatin (P<0.05). The results agreed with the result of gel strength which applied the similar method but using different probe, distance and speed. Adhesiveness of the fish gelatin was also higher than that of the bovine gelatin (P<0.05). Thus, the fish gelatin could be used as a binding agent in cereal bars.

Cohesiveness, springiness and resilience of the bovine bone gelatin were higher than those of the Thai panga fish gelatin (P<0.05). Springiness related to the height that food recovered during timing between the first and the second bites. Resilience showed how the gelatin recovered from deformation in term of speed and force. These 3 parameters indicated that the bovine gelatin had higher elasticity than the fish gelatin. Although high in gel strength, the fish gelatin may not suitable for production of jelly that required high elasticity.

Table 3. Texture profile of Thai panga fish and bovine bone gelatin.

Texture profile	Thai panga fish gelatin	Bovine bone gelatin
Fracturability (g)	181.18 ± 49.96	150.68 ± 31.00
Hardness (g)	750.47^{a} ± 24.85	642.28^{b} ± 27.49
Adhesiveness (g.sec)	-369.09^{a} ± 49.33	-75.48^{b} ± 24.15
Cohesiveness	0.838^{b} ± 0.039	0.950^{a} ± 0.029
Chewiness	533.59 ± 56.45	562.60 ± 41.54
Gumminess	629.68 ± 48.13	610.41 ± 44.45
Springiness	0.847^{b} ± 0.046	0.922^{a} ± 0.018
Resilience	0.618^{b} ± 0.026	0.683^{a} ± 0.012

* Mean ± SD of 4 replications. Different letters within the same row indicated significant difference (P<0.05).

Wangtueai and Noomhorm (2009) studied texture profile of gelatin from lizardfish scale and found that its hardness, gumminess and chewiness were lower than those of bovine gelatin (P<0.05) but the springiness and cohesiveness were not different. The differences of this study to the previous study obviously contributed to differences of fish species and gel maturation temperature.

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3.7 Viscoelastic properties

During cooling from 40 to 5°C, elastic modulus (G′) and viscous modulus (G″) of the bovine gelatin were higher than that of the fish gelatin (Fig. 3A, C). However, after incubation at 5°C for 5 min, the elastic modulus and viscous modulus of the fish gelatin increased and eventually higher than those of the bovine gelatin (Fig. 3B, D). The result was in accordance with the results of gel strength and hardness in texture profile analysis that gelatin was stored at 2°C for 16-18 h. However, when the temperature increased, the elastic modulus and viscous modulus of the fish gelatin decreased at a higher rate than those of the bovine gelatin.

A rapid decreasing or increasing of phase angle indicated the gelling and melting points of gelatin (Liu et al., 2008). According to the gelling and melting points of gelatin samples in Table 4, the bovine gelatin showed better thermal stability as compared to the fish gelatin. The rate of gelling and melting of gelatin can be considered from slope at the point that phase angle decrease or increase rapidly. Accordingly, the gelling and melting rates of bovine and fish gelatin were similar (Fig. 3E, F).

Gelling and melting points of other fish gelatins were shown in Table 5. Melting point of Thai panga fish gelatin was higher than those of several cold water fish gelatins, such as megrim, herring, hake and cod gelatin, while lower than those of the terrestrial animal gelatins such as bovine and porcine gelatin. In general, physical properties of gelatin depended on amino acid composition of gelatin. Proline and hydroxyproline contents of warm-water fish gelatin were commonly higher than those of the cold-water fish gelatin, but lower than those of the terrestrial animal gelatin. Proline and hydroxyproline provided an important role in the triple helix structure of collagen, which apparently designated the stability of collagen and derived gelatin (Regenstein and Zhou, 2007).

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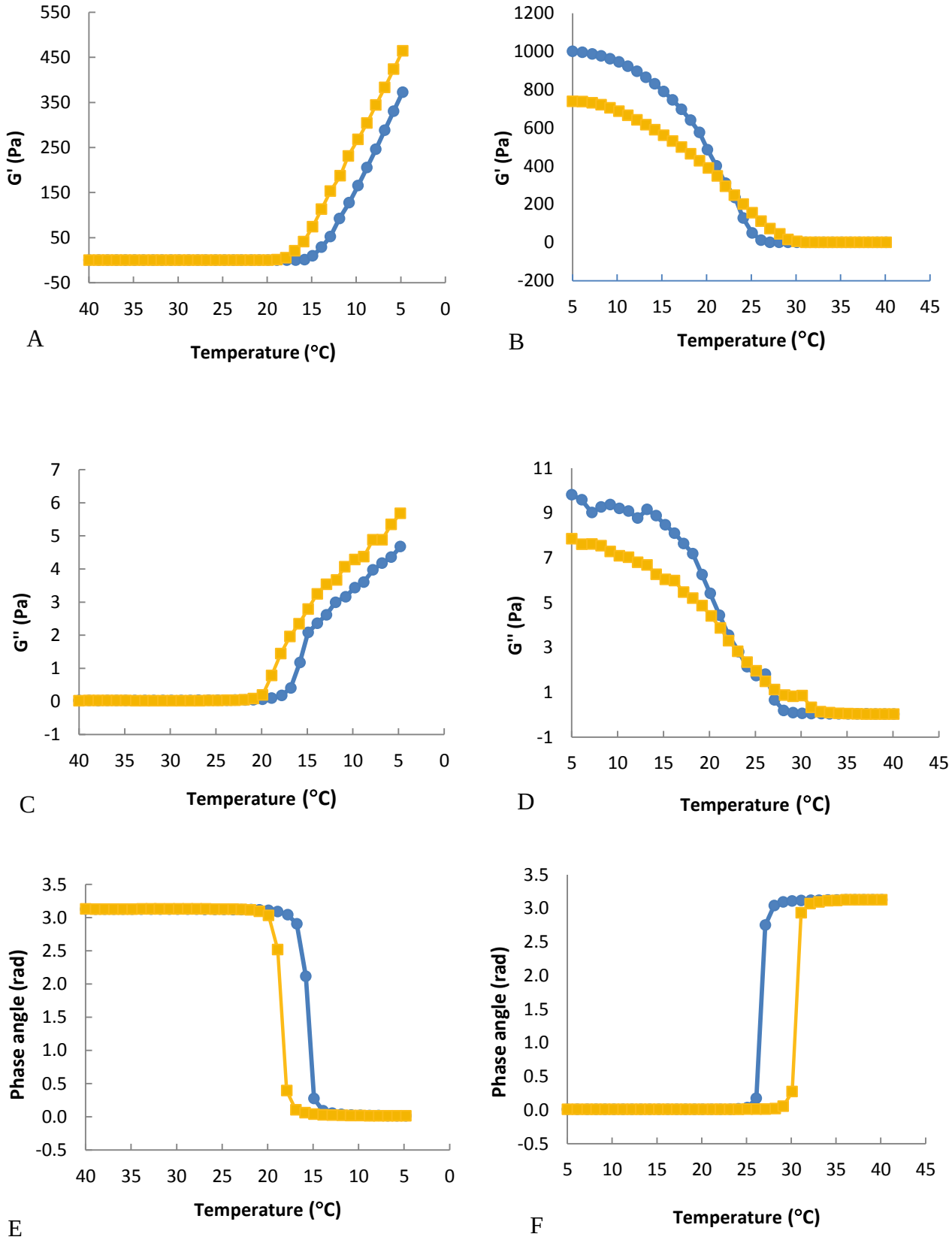

Figure 3. Elastic modulus (G'), viscous modulus (G'') and phase angle of Thai panga fish skin gelatin (●) and bovine bone gelatin (■) during cooling (left) and heating (right).

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Table 4. Gelling and melting points of Thai panga fish and bovine gelatin.

Thermal properties	Thai panga fish gelatin	Bovine bone gelatin
Gelling point (°C)	$16.40^{b} \pm 0.17$	$18.45^{a} \pm 0.07$
Melting point (°C)	$26.87^{b} \pm 0.31$	$29.90^{a} \pm 0.14$

* Mean ± SD of triplicate determinations. Different letters within the same row indicate significant difference (P<0.05).

Table 5. Gelling and melting points of various gelatins.

Raw meterials	Gelling point (°C)	Melting point (°C)	Reference
Thai panga fish skin	16.4	26.87	-
channel catfish skin	22	25	Liu et al. (2008)
yellowfin tuna skin	18.7	24.3	Cho et al. (2005)
channel catfish head bone	18.4	25.1	Liu et al. (2009)
grass carp skin	19.5	26.8	Kasankala et al. (2007)
tilapia	18.2	25.8	Gudmundsson (2002)
red tilapia skin	-	22.45	Jamilah and Harvinder
black tilapia skin	-	28.90	(2002)
skate skin	16.12	19.30	Cho et al. (2006)
Chinese herring	5.1	16.7	Norziah et al. (2009)
megrim	11.8	20.0	Gudmundsson (2002)
	17	21	Gómez-Guillén et al.
dover sole	19	21	(2002)
hake	11	15	
squid	13	19	
cod	12	13	
	3.6	10.3	Gudmundsson (2002)
bovine	23.8	33.8	Cho et al. (2005)
	19.33	28.7	Norziah et al. (2009)
	22.6	29.7	Gudmundsson (2002)
bovine bone	24.5	31.2	
pigskin	27	29	Liu et al. (2008)
	25.6	36.5	Cho et al. (2005)
	24.7	32.3	Gudmundsson (2002)
porcine bone	26.0	33.2	
	21.9	29.7	

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4. Conclusion

Overall, this study demonstrated that the gelatin produced from the Thai panga fish skin had higher gel strength, viscosity, turbidity, foaming properties, emulsion stability and adhesiveness as compared to those of the bovine bone gelatin. While the bovine bone gelatin exhibited yellow color and had higher values of cohesiveness, gel elasticity, gelling temperature and melting temperature. These data provided the functional fact that leading to the application of different technological processes. It was evident that the Thai panga fish skin gelatin could be used for production of gelatin film, mallows products, dairy products such as ice cream and yoghurt, and cereal bar. However, Thai panga fish skin gelatin was not suitable for use as gelling agent due to its low gelling and melting temperature.

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