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Effects of different types and concentration of salts on the rheological and thermal properties of sin croaker and shortfin scad skin gelatin

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Abstract

The aims of this study were to examine the effect of salts ($CaCl_2$, $CaSO_4$ and $MgSO_4$) on the rheological and thermal properties of gelatin extracted from the skins of tropical fishes, sin croaker (*Johnius dussumeiri*) and shortfin scad (*Decapterus macrosoma*). It was found that the melting temperatures of fish skin gelatins were increased by 1.5 times as compared to bovine gelatin which was only increased by 0.5 times after holding for 2 h at 5°C. The storage (G') and loss (G") modulus of fish skin gelatins were improved with the addition of calcium sulphate ($CaSO_4$) and magnesium sulphate ($MgSO_4$), respectively. However, the storage (G') and loss (G") modulus of gelatin solutions were decreased with the addition of calcium chloride ($CaCl_2$). Magnesium sulphate ($MgSO_4$) was found to be an effective salt to improve the bloom value, elastic and viscous moduli of the fish skin gelatin. This study showed that shortfin scad skin gelatin with salt addition possessed better thermal and rheological properties than sin croaker gelatin.

Introduction

Gelatin is a processed and refined animal protein, derived from bones, hides and collagenous connective tissues, but for many sociocultural reasons such as BSE (bovine spongiform encephalopathy) crisis, an alternative sources, like fish skins, are highly demanded (Gime´nez *et al*., 2005; Badii and Howell, 2006; Sarbon *et al.*, 2013). Therefore, new sources of gelatin have been explored, among which gelatins extracted from under-utilised fish sources such as fish skin, fish bone and fish frame. However, there are a number of studies have been conducted in developing fish gelatin such as horse mackerel skin (Badii and Howell, 2006); black and red tilapia skin (Jamilah and Harvinder, 2002) and hake skin (Gomez-Guillen *et al*., 2002).

Sin Croaker (*Johniecop sina*), gelama (local name) and Shortfin Scads skins (*Decapterus macrosoma bleeker*), selayang (local name) are the low value tropical fish that commonly found in Malaysia which normally used for salted fish production. Therefore, the development of fish skin gelatin from the skin of sin croaker (*Johniecop sina*) and shortfin scad (*Decapterus macrosoma bleeker*) resulting in value added to these underutilised fish species. Moreover, there has been much interest in investigating possible means of making more effective use of under-utilised resources and industrial waste (Nagai and Suzuki, 1999).

In food application, gelatin can be used as an ingredient to enhance the elasticity, consistency and stability of food products and therefore good rheological properties of gelatin are required. The good quality of food grade gelatin are depends largely on its thermal and rheological properties; especially the gel strength. Although mammalian gels are stronger, as this characteristic is directly related to the higher hydroxyproline content (Norland, 1990; Ledward, 1992), these could be attained by using gelatin modifying material (Sarabia *et al*., 2000) on the alternative fish gelatin. The rheological behaviour of gelatin is of special importance when they are used to modify textural attributes. Thermal properties of gelatin samples have been extensively studied by various workers. The melting temperature of gelatin derived from skin of tropical fish is significantly lower than that of gelatin from skin of warm blooded animals (Gilsenam and Ross-Murphy, 2000). Furthermore, the higher the denaturation temperature, the greater is thermal stability of gelatin (Bigi *et al*., 2001).

One possible means of manipulating the characteristics of a given gelatin is to trigger

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interactions by the addition of solute like salts, glycerol and enzymes (Elysee-Collen and Lencki, 1996; Ferna´ndez-Di´az *et al*., 2001). It has been stated that the effect of salt concentration on protein stability is very ion specific, with stabilising effects typically following the Hofmeister series (Von Hippel and Wong, 1962). Moreover, the effect of different salts on the rigidity and melting temperature of animal gelatin has been known for a long time (Harrington and Rao, 1970). Study by Sarabia *et al.* (2000) showed that, the addition of 0.5 M $MgSO_4$, $(NH_4)_2SO_4$ or NaH_2PO_4 to megrim gelatin was critical in raising the melting point, whereas chloride salt acted to reduce it. Whereas, Ferna´ndez-Di´az *et al*. (2001), stated that the addition of 0.1 and 0.5 M $MgSO_4$ to fish gelatins led to improve functional properties like melting temperature.

However, there were still little information available in relation to the effect of salts in food grade gelatin obtained from fish skins, which differ greatly from gelatins of animal origin. The aim of the present study was to take on a comparative study on the effects of calcium chloride ($CaCl_2$), calcium sulphate ($CaSO_4$) and magnesium sulphate ($MgSO_4$) salts at different concentration, on the thermal and rheological properties of gelatin extracted from skins of sin croaker (*Johnius dussumeiri*) and shortfin scad (*Decapterus macrosoma*) as compared to commercial bovine gelatin.

Material and Methods

Raw materials

Scianidae (*Johnius dussumeiri*) sin croaker and carangidae (*Decapterus macrosoma*) shortfin scads with average size of 25 - 26 cm and 20 - 21 cm in lengths, respectively, were obtained fresh from the fish wholesaler in Port Klang, Selangor, Malaysia, chilled in the ice and transported to the laboratory. The skins were removed manually after filleting and stored at -20°C until used. The high bloom strength bovine gelatin (Halagel), which was used for comparison, was imported from Pakistan (Ahmad, 1999). All reagent used were analytical grade.

Gelatin extraction

Gelatin was extracted following the procedure reported by Badii and Howell (2006). Thawed skin was thoroughly cleaned and rinsed with excessive water to remove superfluous material and treated with 0.2% (w/v) aqueous sodium hydroxide (NaOH) solution for 40 min. Then it was treated with 0.2% (w/v) aqueous sulphuric acid (H_2SO_4) for 40 min followed by 1.0% (w/v) aqueous citric acid ($C_6H_8O_7$). The ratio used was 1 kg skin (wet weight) to 7 L of acid or alkali solution for each treatment. Each treatment was repeated 3 times. The skins were then subjected to a final wash with distilled water to remove any residual matter. The final extraction was carried out in distilled water at controlled temperature within the range of 40 - 50°C for overnight. The clear extract obtained was filtered with a whatman filter paper (no. 4), followed by evaporation under vacuum. It was then freeze-dried and ground to obtain gelatin powder. In the entire test conducted, 6.67% gelatin powder by weight was dissolved in different salt solutions of $CaCl_2$, $CaSO_4$ and $MgSO_4$ at 0.1, 0.2 and 0.4 M concentration, respectively.

Small oscillatory measurement

Small deformations oscillatory were performed with a controlled strain oscillatory rheometer, Physica Model No. MCR 300, (Physica Messtechnik GmbH, Darmstadt, Germany). Stainless steel concentric cylinder cup geometry (CC27, cup internal diameter, 28.925 mm) with gap size 1 mm was used. Temperature sweep was carried out from 50°C to 5°C, holding at 5°C for a period of 2 h and heat up to 50°C at a scan rate of 1°C/min with frequency 1 Hz and controlled strain 2%. Gelatin powder by weight were dissolved in different salt solutions of $CaCl_2$, $CaSO_4$ and $MgSO_4$ at concentration 0.1, 0.2 and 0.4 M, respectively in warm distilled water (50°C) at 6.67% concentration using magnetic stirrer before the start of the test. The gelatin solutions were carefully poured into the rheometer cup and covered with a thin layer of silicone oil (Sigma cat. no. M-6884). The melting temperatures were taken as the point at which the phase angle peaked immediately after a sharp increase. The gelling temperatures were also taken as the point at which the first temperature of minimum phase angle (Sarabia *et al.*, 2000). In order to allow suitable gelling temperature in all studied samples, the reference for storage (G') and loss (G") modulus (Pa) values were taken at 5°C to compare the characteristics at a given standard temperature.

Differential scanning calorimetry (DSC)

Melting temperature of gelatin at different salts concentrations were investigated by using differential scanning calorimetry (DSC), Pyris Diamond DSC, (Perkin Elmer Instruments, Norwalk, Connecticut, USA). Sample was weighed approximately 10 mg (± 0.01 mg) in a precision balance (Sortorius, CP225D, Goettingen, Germany). Each sample was filled in an aluminium pan which was hermetically sealed using an encapsulation press. A scanning rate of 5°C/ min was used throughout the study. In general, triplicate

sample pans were heated from 5°C to 60°C and cooled back to 5°C.

Determination of gel strength

The gel strength (bloom value) was determined according to the method described by Wainewright (1977). Dried gelatin powder by weight was dissolved in different $CaCl_2$, $CaSO_4$ and $MgSO_4$ solutions at concentration 0.1, 0.2 and 0.4 M, respectively in warm distilled water at 60°C to form 6.67% (w/v) solution. Bloom jars (Schott Duran, 55122 Mainz, Germany) which were filled with the solution was covered and were kept at 7°C (maturation temperature) for 16 to 18 h. After allowed to cool for 15 min at room temperature, the gel strength of the samples were determined on TA.XT2 Texture Analyser (Stable Micro System, Godalming, Surrey, UK) at 8 to 9°C according to British Standard, BS 757 (BSI, 1975), with a load cell of 5 kg cross–head speed 1 mm/s and equipped with a 0.5 inch in diameter, flat bottomed plunger. The force (g) was determined when the probe proceeded to penetrate into the gel to a depth of 4 mm. The average of three determinations were obtained and translated as gel strength (g).

Statistical analysis

All data were stated as mean ± standard deviation. Data was subjected to two-ways ANOVA by using MINITAB Statistical Software (version 16.0). Level of significance was set at $p < 0.05$ and the significant difference between the means were determined by Tukey's Multiple comparison.

Results and Discussion

Effect of $CaCl_2$, $CaSO_4$ and $MgSO_4$ on gelling and melting temperature

The effect of ionic strength on the gelling and melting temperatures of sin croaker, shortfin scad and bovine gelatin as studied by using rheometer were shown in Table 1 and 2, respectively.

The addition of $CaCl_2$ notably decreased the gelling temperatures of sin croaker, shortfin scad and bovine solutions (Table 1). At 0.4 M $CaCl_2$ addition to the gelatin solution showed that, there was no gelling ability of solution for sin croaker gelatin. Gelling ability of shortfin scad gelatin (6.3°C) was higher than sin croaker gelatin when 0.4 M $CaCl_2$ was added. However, bovine gelatin in $CaCl_2$ solution showed the highest (10.7°C) gelling temperatures as compared to fish gelatin.

However, the addition of $CaSO_4$ to the gelatin solutions showed a decreased in gelling temperature for bovine gelatin with the temperature was remained constant at 19.2°C for each level of concentration. The gelling temperatures of shortfin scad gelatin were presented constant at 9.9°C for each level of $CaSO_4$ addition. Conversely, the gelling temperatures for sin croaker gelatin were gradually increases with the increased of $CaSO_4$ at each level of concentration. The gelling temperature at 0.1, 0.2 and 0.4 M $CaSO_4$ added were 8.0, 8.9 and 10.9°C, respectively compared to only 7.1°C without addition of salt.

Furthermore, the addition of $MgSO_4$ did not affect much on the gelling temperatures of bovine gelatin. At all concentration of $MgSO_4$ added, the gelling temperature for bovine gelatin was 20°C. While, the addition of $MgSO_4$ increases the gelling temperature of both sin croaker and shortfin scad gelatin solutions. Although there were not much different between gelling temperatures for sin croaker and shortfin scad gelatin solutions, the gelling temperatures of shortfin scad was shown higher (13.0°C; 13.8, 13.9°C) than that of sin croaker (11.9, 12.0 and 15.0°C) as $MgSO_4$ was added at 0.1, 0.2 and 0.4 M, respectively. The addition of $MgSO_4$ was observed to be able to improve the gelling temperature of fish gelatin as compared to the $CaCl_2$ and $CaSO_4$. As expected, bovine gelatin presented the highest gelling temperature as compared to both fish gelatins. This results are agreed according to Sarabia *et al.* (2000), the chloride salts (NaCl and $MgCl_2$) lowered melting temperature of the gelatin solution. The small ion radius (chlorides) can approach more readily and hence can interact to the centre of the positively charged protein chain.

Table 1. Gelling temperatures of bovine, shortfin scad and sin croaker skin gelatin at different salt concentrations as measured by rheometer

Salts	Concentration (M)	Bovine	Shortfin scad	Sin croaker
		Gelling Temperatures(°C)		
	0	19.6±0.07[a]	9.9±0.28[b]	7.1±0.01[c]
$CaCl_2$	0.1	16.7±0.00[a]	9.0±0.04[b]	7.9±0.06[c]
	0.2	14.0±0.14[a]	8.1±0.01[b]	6.5±0.24[c]
	0.4	10.7±0.07[a]	6.3±0.28[b]	-
$CaSO_4$	0.1	19.2±0.00[a]	9.9±0.00[b]	8.0±0.01[c]
	0.2	19.2±0.00[a]	9.9±0.01[b]	8.9±0.01[c]
	0.4	19.2±0.00[a]	9.9±0.07[b]	10.9±0.03[c]
$MgSO_4$	0.1	20.0±0.71[a]	13.8±0.71[b]	11.9±0.71[c]
	0.2	20.0±0.71[a]	13.9±0.71[b]	12.0±0.71[c]
	0.4	20.0±0.71[a]	15.0±0.78[b]	13.0±0.71[c]

[a-c] Mean within a row with same superscript are not significantly different ($p < 0.05$). Values represent means of triplicate measurements.

Table 2. Melting temperatures of bovine, shortfin scad and sin croaker gelatin at different salt concentrations as determined by rheometer

Salts	Concentration (M)	Bovine	Shortfin scad	Sin croaker
		Melting Temperature (°C)		
	0	28.8±0.07[a]	23.8±0.00[b]	17.7±0.07[c]
$CaCl_2$	0.1	28.1±0.10[a]	20.7±0.07[b]	17.7±0.10[c]
	0.2	27.4±0.21[a]	18.7±0.07[b]	16.6±0.10[c]
	0.4	25.4±0.00[a]	17.6±0.10[b]	12.5±0.00[c]
$CaSO_4$	0.1	30.0±0.00[a]	24.2±0.00[b]	18.5±0.00[c]
	0.2	30.0±0.00[a]	25.2±0.07[b]	18.8±0.10[c]
	0.4	30.0±0.00[a]	25.7±0.00[b]	20.6±0.10[c]
$MgSO_4$	0.1	31.0±0.70[a]	25.9±0.80[b]	20.1±0.20[c]
	0.2	31.0±0.70[a]	25.9±0.80[b]	21.8±0.80[c]
	0.4	31.0±0.70[a]	25.9±0.80[b]	22.8±0.70[c]

[a-c] Mean within a row with same superscript are not significantly different ($p < 0.05$). Values represent means of triplicate measurements.

Moreover, saline ion can interact with the charge polar groups of the protein, or else they may remain free and mobile in the aqueous phase depending on the pH of the medium and the nature of the ions or the protein (Fennema, 1976).

Similar to the gelling temperature, the addition of $CaCl_2$ at 0.1, 0.2 and 0.4 M to the gelatin solution were decreased the melting temperatures of bovine, shortfin scad and sin croaker gelatin, respectively (Table 2). Instead, the melting temperatures were almost unchanged at 0.1, 0.2 and 0.4 M addition of $CaSO_4$ and $MgSO_4$ salts for bovine and shortfin scad gelatin solutions, respectively but slightly increases with the increased of $MgSO_4$ concentration in sin croaker gelatin solution. The melting temperatures for shortfin scad and bovine gelatin increased at 0.1 M of the addition of $CaSO_4$ and $MgSO_4$ saline concentration then maintained constant at 0.2 and 0.4 M. Results showed that 0.1 M $MgSO_4$ was the optimum ionic strength for all gelatin solutions. The melting and gelling temperature of bovine and shortfin scad gelatin solutions seem to be less dependent upon concentration of the addition of $CaSO_4$ and $MgSO_4$ saline concentration as compared to the sin croaker gelatin. Similar as previously discussed, the highest melting temperature was observed in bovine, shortfin scad and sin croaker gelatin solution by the addition of $MgSO_4$. The addition of $MgSO_4$ to the fish gelatin were increased 2°C and 5°C of the melting temperature for shortfin scad and sin croaker gelatin, respectively. According to Haugh *et al.* (2004), the difference between the gelling and melting temperature is most likely caused by some kinetic effects. An increase in concentration of fish gelatin will inevitably lead to a shorter distance between the gelatin α-chain in the solution and formation of junction zones and gel network will be favoured.

Effect of $CaCl_2$, $CaSO_4$ and $MgSO_4$ on thermal properties by DSC

Subsequently, the values of melting temperatures of bovine, shortfin scad and sin croaker gelatin with the addition of $CaCl_2$, $CaSO_4$ and $MgSO_4$ which were obtained by using rheometer were proved to be similar to that the melting temperature determined by using DSC as presented in Table 3, 4 and 5, respectively.

The addition of the $CaCl_2$ to the gelatin solution resulted in the decreases of the melting temperature of the gelatins. Table 3 showed that, the addition of $CaCl_2$ to the bovine gelatin did not alter the melting temperature. There were no significant differences ($p > 0.05$) in bovine gelatin at every concentration of $CaCl_2$ added. There was only 0.1°C decreased in melting temperature with every 0.1M $CaCl_2$ addition.

Table 3. Melting points of bovine, shortfin scad and sin croaker gelatin gel at different $CaCl_2$ concentration using DSC

Gelatins	Melting Temperature (°C)			
$CaCl_2$ Conc.(M)	0.0	0.1	0.2	0.4
Bovine	28.90 ± 2.61^{aA}	28.78 ± 1.44^{aA}	28.65 ± 0.18^{aA}	28.52 ± 0.49^{aA}
Shortfin scad	24.57 ± 0.50^{bA}	21.80 ± 0.02^{bB}	20.80 ± 1.11^{bB}	18.10 ± 0.13^{bC}
Sin croaker	18.51 ± 0.06^{cA}	17.38 ± 0.11^{cA}	15.71 ± 0.60^{cB}	11.29 ± 0.86^{cC}

[a-c] Mean within a column with same superscript are not significantly different ($p < 0.05$).
[A-C] Mean within a row with same superscript are not significantly different ($p < 0.05$).
[1]Values represent means of triplicate measurements.

Table 4. Melting points of bovine, shortfin scad and sin croaker gelatin gel at different $CaSO_4$ concentration using DSC

Gelatins	Melting Temperature (°C)			
$CaSO_4$ Conc.(M)	0.0	0.1	0.2	0.4
Bovine	28.90 ± 2.61^{aA}	28.92 ± 0.43^{aA}	30.47 ± 0.79^{aA}	32.76 ± 0.06^{aA}
Shortfin scad	24.57 ± 0.5^{bA}	24.81 ± 0.99^{bA}	25.34 ± 0.59^{bA}	26.16 ± 0.71^{bA}
Sin croaker	18.51 ± 0.06^{cA}	19.8 ± 0.13^{cB}	21.89 ± 0.00^{cC}	23.54 ± 0.41^{cD}

[a-c] Mean within a column with same superscript are not significantly different ($p < 0.05$).
[A-C] Mean within a row with same superscript are not significantly different ($p < 0.05$).
[1]Values represent means of triplicate measurements.

Table 5. Melting points of bovine, shortfin scad and sin croaker gelatin solution at different $MgSO_4$ concentration using DSC

Gelatins	Melting Temperature (°C)			
$MgSO_4$ Conc.(M)	0.0	0.1	0.2	0.4
Bovine	28.90 ± 2.61^{aC}	32.72 ± 0.28^{aCB}	36.23 ± 0.91^{bAB}	38.66 ± 0.54^{bA}
Shortfin scad	24.57 ± 0.50^{bD}	29.48 ± 0.03^{bC}	38.98 ± 0.01^{aB}	48.93 ± 0.42^{aA}
Sin croaker	18.51 ± 0.06^{cD}	28.22 ± 0.01^{cC}	32.99 ± 1.39^{cB}	36.54 ± 0.33^{cA}

[a-c] Mean within a column with same superscript are not significantly different ($p < 0.05$).
[A-C] Mean within a row with same superscript are not significantly different ($p < 0.05$).
[1]Values represent means of triplicate measurements.

However, the addition of $CaCl_2$ to the shortfin scads and sin croaker gelatin solution decreases the melting temperatures and there were significant difference ($p < 0.05$) in shortfin scads and sin croaker gelatin with the increased of $CaCl_2$ concentration. In addition, the melting temperatures for shortfin scads gelatin solution were gradually decreased with the increased of CaCl2 concentration. There was only 1°C difference in melting temperature of shortfin scads gelatin solution when 0.1 and 0.2 M of $CaCl_2$ were added. Sin croaker gelatin solution showed the lowest melting temperature among three gelatin studied.

However, the addition of gelatins with the difference $CaSO_4$ concentrations resulted in the increased of gelatins melting temperatures (Table 4). Bovine gelatin presents the highest values as compared to the shortfin scads and sin croaker gelatin. At 0.0, 0.1, 0.2 and 0.4 M $CaSO_4$ addition to the bovine gelatin, the melting temperature obtained were 28.90 ± 2.61, 28.92 ± 0.43, 30.47 ± 0.79 and 32.76 ± 0.06°C, respectively. These showed that there was a small effect to bovine gelatin when $CaSO_4$ was added between control and treated bovine gelatin at 0.1 M. In the same way, the addition of 0.1, 0.2 and 0.4 M $CaSO_4$ to the shortfin scads gelatin resulted in the increases of the melting temperature to 24.81 ± 0.99, 25.34 ± 0.59 and 26.16 ± 0.71°C,

Table 6. Viscoelastic values of bovine, shortfin scad and sin croaker gelatin at different salt concentrations after holding for 2 h at 5°C during gelling

Salts	Conc. (M)	Bovine		Shortfin scad		Sin croaker	
		G' (Pa)	G" (Pa)	G' (Pa)	G" (Pa)	G' (Pa)	G" (Pa)
	0	2160±0.00[a]	15.2±0.28	118±0.00[a]	3.08±0.32	44.0±0.00[a]	3.88±0.53
$CaCl_2$	0.1	1510±0.00[a]	12.8±0.21	78.7±0.00[b]	2.73±0.32	32.1±0.00[c]	3.59±0.00
	0.2	760±0.00[a]	12.6±0.87	42.1±0.00[b]	2.16±0.25	8.82±0.00[c]	2.40±0.00
	0.4	145±0.00[a]	4.61±0.36	5.90±0.00[b]	1.89±0.63	-	-
$CaSO_4$	0.1	2170±0.00[a]	16.0±0.21	169±0.00[b]	3.51±0.34	115±0.00[c]	4.68±0.54
	0.2	2180±0.00[a]	16.8±1.20	183±0.00[b]	4.00±0.00	175±0.00[c]	5.86±0.70
	0.4	2190±0.00[a]	17.8±0.71	203±0.00[b]	4.11±0.42	189±0.00[c]	6.02±0.73
$MgSO_4$	0.1	2210±0.00[a]	105±3.54	533±0.00[b]	6.00±0.45	610±0.00[c]	11.2±0.00
	0.2	2310±0.00[a]	120±0.00	704±0.00[b]	7.87±0.52	671±0.00[c]	12.4±1.20
	0.4	2450±0.00[a]	130±0.00	965±0.00[b]	10.1±0.72	849±0.00	14.6±1.13

[a-c] Mean within a column with same superscript are not significantly different ($p < 0.05$).
[1]Values represent means of triplicate measurements.

Table 7. Viscoelastic values of bovine, shortfin scad and sin croaker gelatin at different salt concentrations after holding 2 h at 5°C during melting

Salts	Conc. (M)	Bovine		Shortfin scad		Sin croaker	
		G' (Pa)	G" (Pa)	G' (Pa)	G" (Pa)	G' (Pa)	G" (Pa)
	0	4200±0.00[a]	20.0±1.07	1690±0.00[b]	17.9±0.21	1270±0.00[c]	24.0±0.42
$CaCl_2$	0.1	3990±0.00[a]	16.7±0.28	1470±0.00[b]	16.0±0.14	1260±0.00[c]	21.8±0.35
	0.2	2890±0.00[a]	12.6±0.28	1100±7.07[b]	12.4±0.14	1010±0.00[c]	18.7±0.28
	0.4	2210±0.00[a]	10.4±0.28	914±1.41[b]	14.9±0.14	448±0.71[c]	11.8±0.14
$CaSO_4$	0.1	4420±0.00[a]	21.0±0.00	1700±0.00[b]	18.1±0.28	1450±0.00[c]	24.4±0.42
	0.2	4870±7.01[a]	23.0±0.57	1800±0.00[b]	19.3±0.14	1730±0.00[c]	30.1±0.49
	0.4	4970±7.07[a]	26.9±0.64	1950±0.00[b]	21.8±0.21	1810±0.00[c]	31.6±0.42
$MgSO_4$	0.1	4500±0.00[a]	85.0±0.28	2000±0.00[b]	50.0±1.73	1730±0.00[c]	50.0±0.00
	0.2	5000±0.00[a]	185.0±1.41	2250±0.00[b]	85.8±2.62	1900±0.71[c]	61.9±1.32
	0.4	5100±0.00[a]	230.0±0.00	2910±7.07[b]	100.0±0.35	2810±7.07[c]	86.0±3.85

[a-c] Mean within a column with same superscript are not significantly different ($p < 0.05$).
[1]Values represent means of triplicate measurements.

respectively. Tsereteli and Smirnova (1991) reported that the melting temperature of the gelatin gels were corresponds to the maximum of heat absorption by the gelatins and heat required by fish gelatins for melting are small and the energy needed to melt the gel is related to the number of junction zones and to their thermal stability. Similar to the sin croaker gelatin, the addition of $CaSO_4$ at 0.1, 0.2 and 0.4 M resulted in the increased of melting temperatures which were 19.80 ± 0.13, 21.89 ± 0.00 and 23.54 ± 0.41°C, respectively. There were significant difference ($p < 0.05$) in melting temperature between bovine, shortfin scads and sin croaker gelatin for every concentration of $CaSO_4$ added. However, there were no significant difference ($p > 0.05$) in melting temperature at every concentration for bovine and shortfin scads gelatin. The incorporation of $CaSO_4$ only affects the melting temperature of bovine, shortfin scads and sin croaker gelatin at 0.2 M. By the addition of 0.2 M $CaSO_4$, the melting temperature of gelatin were increased to 5%, 3% and 18% respectively to the bovine, shortfin scads and sin croaker gelatin. Sin croaker gelatin represents the highest improvement in gelling temperature as compared to bovine and shortfin scads gelatin as $CaSO_4$ was added. According to Hofmeister series, SO_4^{-2} anion was structure stabiliser and these anions not only stabilized the gelatin structure but also enhance the hydrogen-bonded structure of water. In addition, the amino acid composition also affects thermal stability of proteins (Damodaran, 1996).

Furthermore, the addition of $MgSO_4$ to the bovine, shortfin scad and sin croaker gelatins were presented the highest value of melting temperatures as compared to the addition by $CaCl_2$ and $CaSO_4$, respectively. The melting temperature of each gelatin was increased with the increased of $MgSO_4$ concentration. Table 5 showed that the melting temperatures of sin croaker gelatin were increased by 52%, 78% and 97% respectively to the 0.1, 0.2 and 0.4 M $MgSO_4$ added. The melting temperatures of shortfin scads gelatin greatly increased when $MgSO_4$ was added at 0.1, 0.2 and 0.4 M by 20%, 58% and 99%, respectively. While the addition of $MgSO_4$ to the bovine gelatin solution showed less improvement than that of both shortfin scads and sin croaker gelatin. $MgSO_4$ salt was able to increase the melting temperature of fish gelatin effectively more than in bovine gelatin. Therefore, the properties of fish gelatin can be improved by the addition of $MgSO_4$ salt. The heat flow variation detected by DSC corresponds to the energy is necessary to melt the junction zones and to achieve the helix to coil transconformation (Michon *et al*., 1997).

Effect of $CaCl_2$, $CaSO_4$ and $MgSO_4$ on viscoelastic properties

The change of viscoelastic properties, i.e. storage (G') and loss (G") modulus during both gelling (from 50 to 5°C) and subsequent heating (from 5 to 50°C) of the gels at 6.67% gelatin in the different concentrations (0.1, 0.2 and 0.4 M) of $CaCl_2$, $CaSO_4$ and $MgSO_4$ solution were shown as in Table 6 and 7, respectively.

Over all, the viscoelastic properties of sin croaker, shortfin scad and bovine gelatin were notably decreased after the addition of $CaCl_2$. The storage modulus (G') of shortfin scad gelatin solutions during gelling (Table 6) were decreased with the increased of $CaCl_2$ concentrations. The addition of $CaCl_2$ to the sin croaker gelatin solution at 0.4 M resulted in no gelling ability. However, after cooling at 5°C for 2 h, the moduli of sin croaker gelatin at 0.4 M $CaCl_2$ solution were increased (Table 7). This increased was related to a quick cold maturation. The higher the $CaCl_2$ concentration, the lower the storage (G') modulus of shortfin scad, sin croaker and bovine gelatin were observed.

In contrast, the addition of $CaSO_4$ and $MgSO_4$ at all concentrations were increased the G' and G" values of all gelatins solution. After holding for 2 h at 5°C the moduli of fish gelatin solution increased drastically by more than 10 times. $MgSO_4$ was more effective salt to be used to increase the viscoelastic properties of gelatins. The G' values of fish gelatin during melting were higher than that during gelling

(Table 7). It has been shown that aging for 2 h has increased G' and G" values by many folds. Of all the salts assayed, $MgSO_4$ at the right ionic strength was the one that most influenced viscoelastic properties of gelatin solutions. Some properties such as melting point have been clearly attributed to the sulphate ion, while others, like setting times, have been associated in some way with the Mg^{2+} ion. However, the fact that both ions were located on the right extreme or at the end of the Hofmeister or lyotropic series. According to Steinberg *et al.* (1960), it is precisely the ions located on the left extreme that most influenced a disordered triple helical structure with diminished rheological properties. Although the addition of $MgSO_4$ slightly increased the elastic modulus (G'); in general, no evident improving effect in gel development by salts was observed. Salts have been reported to destabilize gelatin structure (Slade and Levine, 1987), probably as a direct consequence of both protein and salt competing for water to hydrate (ElyseÂe-Collen and Lencki, 1996). As noted by Stainsby (1987), promotion of helix formation by screening of ionic interactions by salts does not necessarily result in stronger gels and, therefore, there must be another factor involved. A weak gel may have being formed when the initial nucleation sites have not been able to anneal themselves into their most stable conformation to encourage further growth of these zones on subsequent cooling (Ledward, 1992), and this is directly related to the effect of the injected saline ions.

Effect of $CaCl_2$, $CaSO_4$ and $MgSO_4$ on gel strength of gelatins

Large deformations (gel strength) of the gelatin gels which were treated with different salt concentrations were carried out as presented in Table 8. The higher the penetration force on the gelatin gels showed that the higher the gel strength was obtained. This is due to the formation of a gelatin network with salt acting as elastic masses within the gelatin. Furthermore, by keeping the gelatin gel overnight would caused an increased in penetration force. As expected, the addition of $CaSO_4$ and $MgSO_4$ salt increased the gel strength (penetration force) of the gelatin gel while the addition of $CaCl_2$ decreased the gel strength of the gelatins. However, the effect on gel strength value was higher for a gelatin with high $MgSO_4$ concentration as compared to $CaSO_4$ concentration.

Similar to the viscoelastic properties discussed in 3.3, the addition of $CaCl_2$ at 0.1, 0.2 and 0.4 M resulted in a considerable decrease in gel strength, although the behavior was different, depending on the species. There were significant differences ($p < 0.05$) between bovine, shortfin scads and sin croaker gelatin at every $CaCl_2$ concentration. At 0.4 M $CaCl_2$, all gelatins shows the lowest value of gel strength which was decreased for about 33.8%, 14.2% and 27.4% for bovine, shortfin scads and sin croaker gelatin, respectively. However, there were great differences in gel strength values between every concentration for all gelatins. Study by Cheow *et al.* (2007), showed that bovine gelatin had considerably higher imino acid content than that of both shortfin scads and sin croaker gelatin which resulted in higher gel strength value. The pyrolidine rings of amino acids (proline and hydroxyproline) were considered to be determinant for the stability of the collagen helix although the hydrogen bonds through the hydroxyl groups also contributed to the intermolecular stability of the helix (Gilsenam and Ross-Murphy, 2000). In addition, studies by Choi and Regenstein (2000) observed that sodium chloride decrease the gel strength of several commercial gelatins from different sources and attributed this effect to the fact that sodium chloride is capable of breaking of hydrophobic and hydrogen bonds, which prevents stabilisation of the gel junction zones, either directly by preventing hydrogen-bond formation and/or by modifying the structure of the liquid water in the vicinity of these sites.

Table 8. Comparison of gel strength (g) (bloom value) of bovine, shortfin scad and sin croaker gelatins

	Concentration (M)	Bovine	Shortfin scad	Sin croaker
		Gel Strength (g) (Bloom Value)		
Salts	0.0	234.0 ± 0.4[a]	176.9 ± 0.1[b]	124.9 ± 0.6[c]
	0.1	233.8 ± 2.8[a]	168.2 ± 0.2[b]	122.1 ± 1.0[c]
	0.2	180.6 ± 0.9[a]	167.2 ± 0.9[b]	118.8 ± 0.5[c]
$CaCl_2$	0.4	158.0 ± 1.0[a]	151.3 ± 0.1[b]	90.5 ± 0.1[c]
	0.1	246.1 ± 0.5[a]	181.0 ± 0.5[b]	131.6 ± 0.6[c]
	0.2	249.2 ± 0.2[a]	184.5 ± 0.1[b]	143.7 ± 0.1[c]
$CaSO_4$	0.4	258.7 ± 0.4[a]	188.2 ± 0.4[b]	148.9 ± 0.9[c]
	0.1	265.0 ± 0.5[a]	189.3 ± 0.7[b]	150.8 ± 0.8[c]
	0.2	280.1 ± 0.8[a]	192.5 ± 0.2[b]	154.0 ± 0.9[c]
$MgSO_4$	0.4	297.6 ± 0.2[a]	197.1 ± 0.3[b]	156.3 ± 0.4[c]

[a-c] Mean within a column with same superscript are not significantly different ($p < 0.05$).
[1]Values represent means of triplicate measurements.

Conversely, the gel strength of bovine gelatin was significantly higher ($p < 0.05$) than that of shortfin scads and sin croaker gelatin at all concentration of $CaSO_4$ added. The gel strengths values of bovine gelatin with $CaSO_4$ added at 0.1, 0.2 and 0.4 M were 246.1 ± 0.50, 249.2 ± 0.20 and 258.7 ± 0.40 g, respectively. These values showed that only about 2.8%, 4.0% and 7.4% increment on the gel strength of bovine gelatin were obtained as 0.1 M, 0.2 M and 0.4 M $CaSO_4$ were added, respectively. Shortfin scads gelatin had similar effect as bovine gelatin which showed that the increases in $CaSO_4$ concentration were increased the gel strength of the gelatin. The gel strengths of shortfin scads gelatin at 0.1, 0.2 and 0.4

M of $CaSO_4$ added were 181.0 ± 0.50, 184.5 ± 0.10 and 188.2 ± 0.40 g, respectively. There were only 2.2%, 4.3% and 6.4% improvement in gel strength compared to no salt added. The gel strength values of sin croaker gelatin with 0.1, 0.2 and 0.4 M $CaSO_4$ added were 131.6 ± 0.60, 143.7 ± 0.10 and 148.9 ± 0.90 g, respectively. However, sin croaker gelatin gel with the addition of $CaSO_4$ salt represents the higher improvement in bloom value as compared to bovine and shortfin scads gelatin. There were 5.6%, 15% and 19.4% increment of sin croaker gelatin bloom value with the increased of $CaSO_4$ concentration.

Similarly, the gel strength of shortfin scad gelatin gels were increased with the increases of $MgSO_4$ concentration at 0.1, 0.2 and 0.4 M which were about 7, 9 and 11% increment, respectively. While, the addition of 0.1, 0.2 and 0.4 M $MgSO_4$ to the sin croaker gelatin solutions slightly increased the gel strength by about 20, 23 and 25%, respectively. The values of gel strengths treated with $MgSO_4$ were higher than that of $CaCl_2$ and $CaSO_4$. The increase in gel strength with addition of salt was due to the formation of a gelatin network with salt acting as elastic masses within the gelatin. The effect on gel strength value was higher for gelatin with high $MgSO_4$ concentration as compared to $CaSO_4$ concentration. This study showed that, $MgSO_4$ was most effective salt to increase gel strength of gelatins. According to Gudmundsson and Hafsteinsson (1997) the gel strength may also be dependent on the isoelectric point which may also be controlled to a certain extent, by pH. More compact and stiffer gels can be formed by adjusting the pH of the gelatin close to its isoelectric point where the proteins will be more neutral and thus the gelatin polymers are close to each other. A bivalent cation, such as Mg^{2+}, can form coordinate links involving this –OH group (Gustavson, 1956). This would explain the special ability of $MgSO_4$ to increase the gel strengths when the salt was added.

Conclusions

In conclusion, the addition of $CaCl_2$ to the gelatin solution reduced the value of storage (G') and loss (G") moduli with the concentration increases. The addition of CaSO4 and $MgSO_4$ at all concentration were increased G' and G" values of all gelatin solution. After holding for 2 h at 5°C the moduli of fish gelatin solution increased drastically by more than 10 times. However, $MgSO_4$ was the most effective salt to be used to increase the viscoelastic and thermal properties of gelatin solution. Fish gelatin gels only start forming network after keeping for 2 hour at 5°C. Therefore, aging process is very important for fish gelatin. Results obtained showed that shortfin scad gelatin has the potential as an alternative to the mammalian gelatin. It is therefore possible to improve the functional properties of shortfin scad gelatin to achieve characteristics similar to those of gelatins from mammalian.

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