

## Properties of gels from mixed agar and fish gelatin

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### Abstract

Agar (A) and fish gelatin (Fg) are acceptable gelling agents in halal food. Agar gel is brittle, has high gelling and melting temperatures and high syneresis, while fish gelatin gel is soft with low gelling and melting temperature, so neither is alone practical. The main objectives of this study were to characterize the properties of mixed gels between fish gelatin and agar, and to understand sol-gel-sol behavior of these mixed gels, in order to provide an alternative gelling material particularly suited for halal food. The agar concentration was fixed at 1% (w/v), while the fish gelatin concentration was varied (0, 5, 10, 15, 20 and 25%, w/v). The key properties of mixed gels were determined: sol-gel-sol transitions during cooling (60 - 0°C) and heating (0 - 100°C) at the gelling temperature ( $T_{gel}$ ) and melting temperature ( $T_m$ ) were determined by oscillatory rheometry. Gel strength, springiness, and syneresis were also measured. It was found that the thermal transitions (sol-gel-sol) of agar gel were at comparatively high temperatures (37°/87°C). For the mixed gels these temperatures decreased dramatically between fish gelatin contents 5% and 10%; at contents over 10% the transition temperatures again increased slightly. Individual transitions of each mixture component were detectable in the storage modulus ( $G'$ ) profile only for the A:Fg = 1:5 mixture, indicating a bicontinuous gel, with  $T_{gel}$  and  $T_m$  closest of all the blends to those of 1% agar. The mixtures with 1:15 to 1:25 A:Fg had storage modulus trace behavior similar to a single component gelatin during cooling and heating, but the storage modulus values synergistically exceeded those of both fish gelatin and agar. This is a synergistic effect of two biopolymers in this specific mixing range. The  $T_{gel}$  and  $T_m$  were shifted toward those of Fg, and this shift should be carefully tuned for each specific application. Compared to agar gel, springiness and syneresis of these co-gels were improved. In these cases with high content of Fg, the fish gelatin acts as the dominant continuous phase, while the 1% agar component is a dispersed phase in the co-gel. Therefore, two modes of behavior were observed depending on the mixing ratio: bicontinuous gels without disperse phase, and gels with continuous and disperse components. In conclusion, the key gelling and gel properties for food applications of fish gelatin and agar mixtures are mostly between the extremes of pure components. Therefore mixtures enable food applications not possible with either component alone, but mixing ratio should be tuned for the specific application. Particularly in applications to halal food products these mixed gels may prove very useful.

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### Introduction

Gelatin is a gelling agent from animal sources. Commercial gelatin is mainly produced from skins of livestock animals. In Europe, 80% of edible gelatin is produced from pig skin, 15% is from cattle hide split and 5% is from pig and cattle bones and fish (GME, 2013). It is a popular gelling agent with wide range applications, in industries such as food, pharmaceuticals, medical products, cosmetics, and paperboard or paper products. However, the food applications are limited among other reasons by religion-based regulations. For example, Hindus do not consume cow-derived products, while Muslims do not consume pork-derived products. In addition,

the bovine spongiform encephalopathy (BSE) has become an issue of concern on consuming cow-derived products (Gudmundsson, 2002). These limitations and concerns encourage the development of alternative non-mammalian gelatins, and fish gelatin seems an attractive option to use as a basis.

Fish gelatin is obtained mainly from fish skin. Low gelling and melting temperatures, poor stability and low gel strength (Gomez-Guillen *et al.*, 2002; Lui *et al.*, 2008) limit its applications. Other major properties for practical use in making gels are water solubility and gelling ability. Similar to mammalian gelatins, fish gelatin dissolves in water at temperatures of 40-60°C, and forms a gel when cooled down, due to a coil-helix transition on molecular level. Fish

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gelatin from cold-water fish has lower values of gel strength, gelling and melting temperatures, and also lower gel modulus than gelatin from warm water fish and mammals (Haug *et al.*, 2004a; Muyonga *et al.*, 2004). Gelling and melting points for fish gelatins range within 8-25°C and 11-28°C, respectively, while those of mammalian gelatins range within 20-25°C and 28-31°C (Karim and Bhat, 2009). This is due to varying amino acid compositions and structure. The amino acids that play an important role for gelling ability are proline and hydroxyproline or imino acids. Fish gelatin has lower concentrations of proline and hydroxyproline or imino acids than mammalian gelatin (Haug *et al.*, 2004a; Muyonga *et al.*, 2004; Avena-Bustillos *et al.*, 2006). Gelatins from different types of fish also differ in amino acid compositions. Cold-water fish have lower content of proline and hydroxyl proline than warm water fish, causing lower gel strength (Norland, 1990; Gomez-Guille *et al.*, 2002; Muyonga *et al.*, 2004; Avena-Bustillos *et al.*, 2006). Gelatin is derived from collagen, which consists of three chain types ( $\alpha$ -chains,  $\beta$ -chains, and  $\gamma$ -chains) that form a triple helix with counter clockwise or left-handed orientation (Te Nijenhuis, 1997), and this is stabilized by inter chain hydrogen bonds. Treating collagen with dilute acid or alkaline destroys the hydrogen bonds, and hence releases the triple helix conformations to coiled chain forms (Schrieber and Gareis, 2007). It has been reported that the distribution of those chains also influences gelatin gel properties. Gelatin with more  $\alpha$ -chains has higher gel strength (Liu *et al.*, 2008), while with more  $\beta$  and  $\gamma$ -chains it has lower values of viscosity, gelling and melting points (Muyonga *et al.*, 2004). Currently, the food applications of fish gelatin are still limited due to poor properties, as mentioned above. Methods have been suggested to improve gel strength, or gelling and melting points, namely enzymic modification (Yi *et al.*, 2006), adding some solutes (Elysee-Collen and Lencki, 1996), or mixing fish gelatin with other suitable gelling agents (Haug *et al.*, 2004b; Pranoto *et al.*, 2007).

Combining different types of gelling agents is widely practiced to improve gel properties. Also the properties of fish gelatin can be modified by mixing with some polysaccharides, and in this study agar was selected to complement fish gelatin. The reasons for selecting agar were its ability to gel even at very low concentrations, wide availability, as well as compatibility with applications where mammalian gelatins face limitations or concerns. Agar gel is strong, with low elasticity and high syneresis, while fish gelatin is a weaker gelling agent that gives lower gel strength and needs higher concentration to be

able to form gel (Haug *et al.*, 2004a). So mixing agar with fish gelatin could provide an alternative gelling material, which could usefully compromise between the components mixed.

Agar consists of polysaccharides extracted from red (Rhodophyceae) seaweed, of *Gelidiaceae* and *Gracilariaceae* species. The polysaccharides are linear polymers based on a disaccharide repeat structure of 3-linked  $\beta$ -D-galactopyranosyl and 4-linked 3,6-anhydro- $\alpha$ -L-galactopyranosyl units (Araki, 1966; Norziah *et al.*, 2006). Agar contains mainly agarose but also agaropectin, and the former contributes to gelation while the latter weakens the gel. Agar can be solubilized at around 90-100°C and in solution the polymers take the form of random coils. With cooling this solution forms a gel. As the temperature decreases, right-handed double helices form. These double helices become linked and form bundles of right-handed double helices. At junction zones the bundles interact, further forming a three-dimensional network. This network has great ability to immobilize water molecules in its interstices (Norziah *et al.*, 2006).

There are prior reports on mixed gel studies of agar and other polysaccharides, for example; agar and k-carrageenan (Noziah *et al.*, 2006), agar and locust bean gum (Selby and Wynne, 1973), agarose and galactomannan (Rees, 1972) and agar and gellan (Banerjee and Bhattacharya, 2011). By mixing gelatin and agar one might be able to identify new and improved gelling systems. So far, studies of mixed gels mostly use porcine gelatin in the mixed gel model; for example, with agar (Clark *et al.*, 1983, Fujii *et al.*, 2000), maltodextrin (Kasapis *et al.*, 1993), k-carrageenan (Antonov, 1999); agarose (Shrinivas *et al.*, 2009), gellan (Lau *et al.*, 2000; Lee *et al.*, 2003; Wang *et al.*, 2008), alginate (Panouille and Larret-Garde, 2009) and starch (Firoozmand and Rousseau, 2013). There are only few reports on fish gelatin and polysaccharide mixtures, such as fish gelatin with gellan (Fonkwe *et al.*, 2003; Pranoto *et al.*, 2007), and fish gelatin with k-carrageenan (Haug *et al.*, 2004b; Pranoto *et al.*, 2007).

The main objectives of this study were to characterize the properties of mixed gels between fish gelatin and agar, and also understand sol-gel behavior of these mixed gels, and provide an alternative gelling material for applications, with emphasis on halal food.

## Materials and Methods

### *Agar and fish gelatin samples*

A commercial food grade powdered agar was

obtained from Sac Sci Eng Co., Ltd, Thailand. The supplier provided following information on the agar sample. It contained  $\leq 6\%$  ash, had gel strength  $\geq 300$  g/cm<sup>2</sup>, and gelling temperature  $\sim 35^\circ\text{C}$  for 1.5% agar (w/v) gel. Fish gelatin was supplied by Cartino gelatin Co., Ltd, Thailand. According to the supplier; it contained 12% moisture and 2% ash. The viscosity of a 6.67% solution at  $60^\circ\text{C}$  was 20 MPa.s, and the bloom strength of gel was 150 g.

#### *Preparation of solutions and gels*

Agar (A) and fish gelatin (Fg) samples were individually solubilized in deionized water at  $95^\circ\text{C}$  and  $60^\circ\text{C}$ , respectively. The concentration of agar was 1% (w/v), while those for Fg were 5, 10, 15, 20 and 25% (w/v). For the binary mixtures, the agar concentration was fixed at 1% and that of Fg was varied across the same concentrations as for Fg alone. The solutions were kept warm, at least  $60^\circ\text{C}$ , before further use. The 1% agar is the minimum concentration, from preliminary testing of 0.25-2.5% concentrations, with gelling point above  $35^\circ\text{C}$ , which is in the range of normal room temperature in Thailand.

#### *Rheological properties measurement*

Rheological measurements with a controlled stress rheometer (Haake RheoStress 1, England) had 35 mm diameter parallel plate-plate geometry and 1 mm gap. Temperature sweeps with oscillatory stress were used to determine the sol-gel transitions of gels. The temperature sweep rate on both cooling (from  $60$  to  $0^\circ\text{C}$ ) and heating (from  $0$  to  $100^\circ\text{C}$ ) was set at  $2^\circ\text{C}/\text{min}$ . The angular velocity was 1 rad/s, with constant strain amplitudes set at 1% for agar alone, and 0.5% for Fg alone as well as mixed gels: these amplitudes were well within the linear viscoelastic regions. The warm solutions were loaded onto the pre-heated peltier plate ( $60^\circ\text{C}$ ) of the rheometer. To prevent evaporation of water, the air-exposed circular periphery of samples was coated with olive oil. Both storage ( $G'$ ) and loss ( $G''$ ) modulus were recorded throughout the temperature sweeps. The gel point temperature ( $T_{\text{gel}}$ ) determined during cooling sweep was defined by  $G' = G''$  (or equivalently  $\tan\delta = 1$ ), and the melting temperature ( $T_m$ ) determined during heating was defined by the same identities (Gudmundsson, 2002).

#### *Compression measurement*

A plastic cup, 20 mm in diameter and 40 mm in height, was filled with warm sample solution, with care to avoid air bubbles, and then cooled at room temperature (about  $29^\circ\text{C}$ ) for around 20 min. Then

samples were then put into a refrigerator and kept there at  $4^\circ\text{C}$  for 12 hrs, before measurements. Texture profile analysis (TPA) was performed with Texture Analyzer (TA-X Tplus, England) using a P/50 HS hemispherical plastic probe. Gel in a cup was centrally positioned below the probe. The compression at 0.5 mm/s was continued to a depth of 30% of original gel height in cup. Hardness and springiness were obtained from the TPA curve (Bourne, 2002). At least 5 replicate tests were done for each type of sample.

#### *Syneresis*

The sample solutions were prepared as described above, and gelled in 50 ml plastic cups. The syneresis of gels was determined as in Banerjee and Bhattacharya (2011). Syneresis in percent was calculated as  $100(m_1 - m_2)/m_1$ , where  $m_1$  and  $m_2$  are gel weights before and after discarding water by centrifugation.

#### *Statistical analysis*

The test data of  $T_{\text{gel}}$ ,  $T_m$ , gel hardness and springiness, were statistically analyzed by two-way Analysis of Variance (ANOVA). The comparison of means between treatments at 5% significance level was carried out using Duncan's Multiple Range Test.

## **Results and Discussion**

#### *Sol-gel transition of fish gelatin gel*

At  $60^\circ\text{C}$  the Fg was in a solution form, dispersed in water as random coils, and on cooling to  $0^\circ\text{C}$  gelation was observed from the dynamic moduli  $G'$  and  $G''$ , as shown in Figure 1a. A steep increase in  $G'$  indicates that the Fg molecules start to rearrange and gel, by linking at junction zones, so that a three-dimensional network is formed (Gilsenan and Ross-Murphy, 2000). The gel formation is very much dependent on concentration, as seen in Figure 1. A high density of Fg molecules in solutions makes crosslinking, aggregate formation, and network formation easier than at a lower density.

Similar to mammalian gelatins, fish gelatin is a thermoreversible gel. During heating the gel melted, indicated by a sharp decrease in  $G'$ . At low Fg concentrations the melting started earlier than at higher concentrations, and concentration affected also gelling temperature and time. This is expected typical behavior across various gelling agents.

#### *Sol-gel transition of mixed gels*

The mixtures of 1% agar and 0-25% Fg were tested for sol-gel transition by temperature sweep,

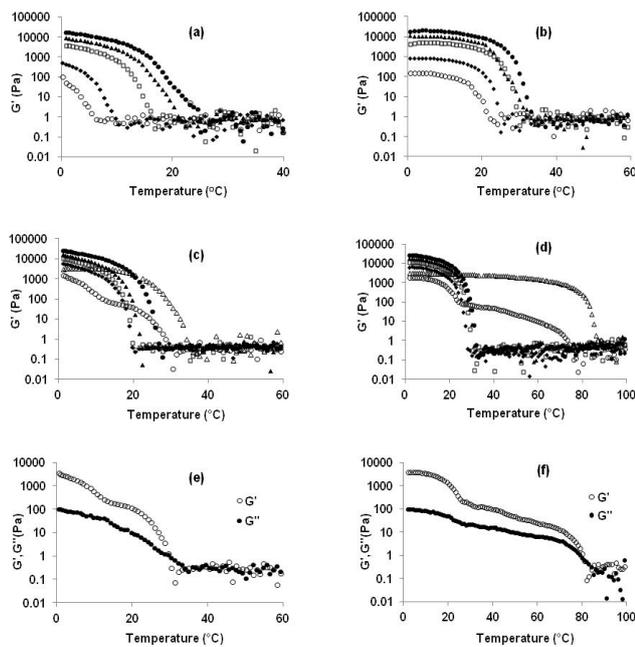


Figure 1. Temperature dependence of storage modulus ( $G'$ ) during cooling ( $60\text{-}0^{\circ}\text{C}$  in left column) and heating ( $0\text{-}100^{\circ}\text{C}$  in right column): at various concentrations of fish gelatin only (a, b), mixed gels between 1% agar and 0-25% Fg (c, d), and a 1:5 ratio of agar and Fg in mixed gel (e, f). The Fg concentrations are 0% ( $\Delta$ ), 5% ( $\circ$ ), 10% ( $\blacklozenge$ ), 15% ( $\square$ ), 20% ( $\blacktriangle$ ), and 25% ( $\bullet$ ).

similarly as pure Fg that was discussed earlier. The moduli  $G'$  and  $G''$  were again recorded (Figures 1c-d) during both cooling and heating. Only one transition was observed with high Fg contents, in the (1:10-1:25) mixtures. This indicates that Fg gel acted as a continuous phase, while agar gel was a dispersed phase. The gelling and melting temperatures became more similar to pure fish gelatin as its content was increased, but the  $G'$  values were higher than for pure Fg at the same content. At 1:5 ratio of A:Fg, the mixture seemed to have qualitatively different viscoelastic behavior from the other ratios, as shown in Figure 1 (e and f). During both cooling and heating, two steps of phase change appear in the response curves. This suggests that both components in the mixture had their own phase transitions dramatically affecting the gelatin moduli, suggesting that neither of the components was dispersed in the other. During cooling (from  $60\text{-}0^{\circ}\text{C}$ ) the sol-gel transition of agar occurred first, and was in the range of  $30\text{-}13^{\circ}\text{C}$ , followed by that of Fg around  $13^{\circ}\text{C}$  or lower. During heating from  $0^{\circ}\text{C}$  to  $100^{\circ}\text{C}$ ,  $G'$  decreased and the fish gelatin in the mixture melted first to completion around  $26^{\circ}\text{C}$ , followed by agar that was completely solubilized at around  $80^{\circ}\text{C}$ . Despite the same 1% agar concentration, the  $G'$  values were much lower than with 1% agar alone, especially at  $30^{\circ}\text{C}$  and higher temperatures during heating process (Figure 1d).

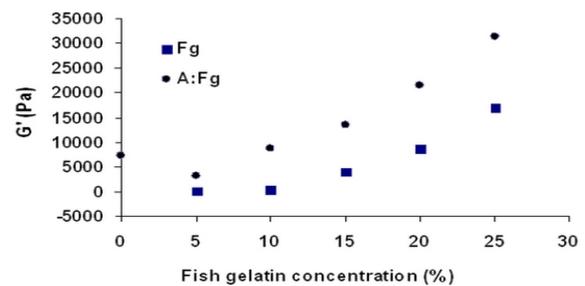


Figure 2. Storage modulus ( $G'$ ) at  $0^{\circ}\text{C}$  during cooling sweep of gels from fish gelatin alone (Fg) and from 1% agar with Fg at various concentrations (A:Fg).

At this stage of temperature sweep, the continuous phase Fg gel had melted while the dispersed agar was still a gel, but could not form a continuous network connecting the shear surfaces. The reduction of  $G'$  of such composite suspension might be due to the agar gel that remains intact until higher temperatures needed to melt it (Shrinivas *et al.*, 2009). Qualitatively similar results have been reported for 1% agarose and 5% pig gelatin (Shrinivas *et al.*, 2009).

Mixed gels of agar and kappa carrageenan also show two steps of temperature transition during heating (Norziah *et al.*, 2006). Mixed gels of agar and kappa carrageenan at different mixing ratios (100/0, 80/20, 60/40, 40/60 and 20/80) with a total concentration of 1.5% w/w have been studied. For all the mixtures and pure agar,  $G'$  increases during cooling. This might be due to similar gelation mechanisms of the mixed polysaccharides. The kappa carrageenan concentration affects the gelling temperature. During heating these mixtures have a typical  $G'$  profile indicating two melting steps. Kappa carrageenan gel melts first at  $15\text{-}38^{\circ}\text{C}$ , followed by melting of the stronger network of agar helices at  $72\text{-}84^{\circ}\text{C}$ .

The  $G'$  values at  $0^{\circ}\text{C}$ , from cooling sweeps, were extracted from storage modulus traces and are shown in Figure 2. The  $G'$  value of 1:5 mixture was lower than for 1% agar, or for the other mixture ratios. This could be due to the total concentration, accounting for both polymers, being lower than in the other mixture cases. However the  $G'$  being lower than 1% agar alone might be due to the bicontinuous gel structure observed in stepwise gelling and melting.

For higher Fg content mixtures (1:10-1:25), the  $G'$  increased and was higher than Fg or 1% agar alone, indicating partially synergistic effects (Banerjee and Bhattacharya, 2011). Clearly adding 1% agar in at least 10% Fg could improve the gel viscoelastic properties for food applications, in comparison to Fg or agar alone. However, also other properties need to be considered.

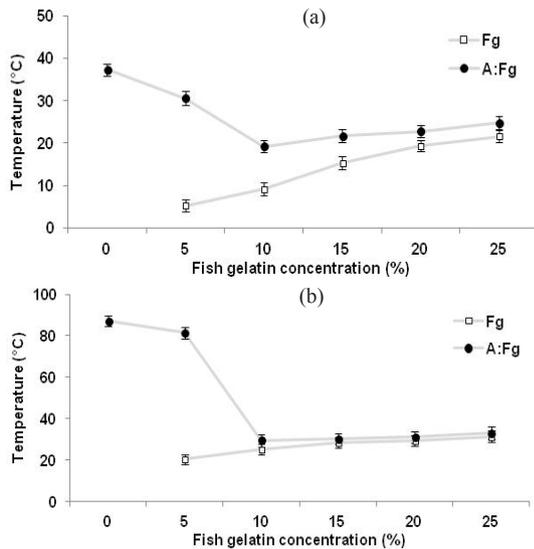


Figure 3. Gelling and melting temperatures ( $T_{gel}$  and  $T_m$ ) of gels from fish gelatin alone (Fg) and from mixtures of 1% agar and 0-25% fish gelatin (A:Fg). These were determined by the cross-over points of  $G'$  and  $G''$  (equivalently  $\tan \delta = 1$ ). Panel (a)  $T_{gel}$  (during cooling, 60-0°C), and panel (b)  $T_m$  (during heating, 0-100°C). Error bars represent mean  $\pm$  standard deviation of triplicates.

$T_{gel}$  and  $T_m$

Gelling temperature is interpreted as the temperature at which gelling occurs during cooling. In this study it was defined based on rheometry, as the temperature at which  $G' = G''$  or  $\tan \delta = 1$  in the cooling trace, while the melting temperature is similarly defined for the heating sweep.

The gelling and melting temperatures ( $T_{gel}$  and  $T_m$ ) were thus determined by the intersections of  $G'$  and  $G''$ , during the cooling and heating sweeps, respectively (Gudmundsson, 2002). The results are given in Figures 3a and 3b.  $T_{gel}$  of pure Fg (5-25%w/v) was in the range from  $5.3 \pm 0.2$  to  $21.57 \pm 0.5^\circ\text{C}$ , while  $T_m$  was in the range from  $20.4 \pm 0.3$  to  $31.5 \pm 0.3^\circ\text{C}$ , and these values increased with Fg concentration. Mixing in 1% agar, which had the high values  $T_{gel} = 37.25^\circ\text{C}$  and  $T_m = 87.12^\circ\text{C}$ , affected the mixtures but the values stayed lower than those of agar alone.

For the 1:5 ratio, although there were two phase transitions, one for each component at different temperatures, there was only one point in each temperature sweep at which  $G' = G''$  (see Figures 1e and 1f). The  $T_{gel}$  and  $T_m$  of this gel mixture were fairly close to those of agar at  $30.6^\circ\text{C}$  and  $81.5^\circ\text{C}$ , respectively.

The 1:10 mixed gel had the lowest  $T_{gel} = 19.23^\circ\text{C}$  as well as  $T_m = 29.6^\circ\text{C}$ , of all the mixture cases. These transition temperatures increased with higher Fg concentrations and seemed to approach those of Fg alone at the same concentration (see Fig. 3). Haug

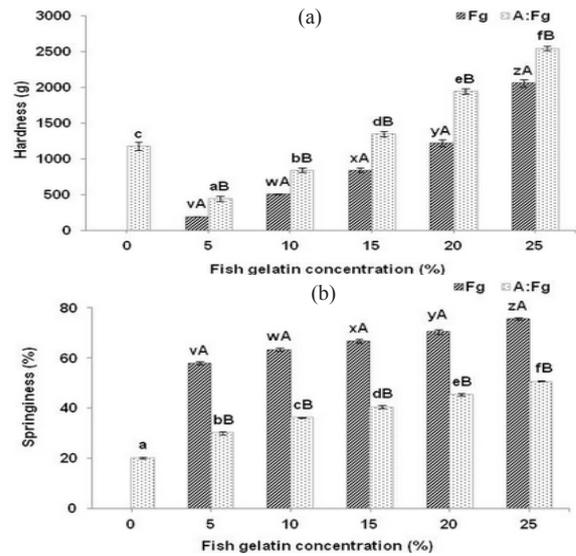


Figure 4. Hardness (a) and springiness (b) of fish gelatin (Fg) and mixed gels (A:Fg) with 1% agar and 0-25% fish gelatin. Different superscript letters indicate significant differences ( $p \leq 0.05$ ). Superscripts (a-f) compare within mixed gels, while (v-z) compare within fish gelatin only. Superscript capital letters compare between Fg and A:Fg mixtures, at the same Fg concentration. Error bars represent mean  $\pm$  standard deviation of at least 5 measurements.

*et al.* (2004a) reported the  $T_{gel}$  and  $T_m$  of 10-30% fish gelatin being  $4-10^\circ\text{C}$  and  $13-16^\circ\text{C}$ , respectively, which are much lower than the values determined in the current study. This could be due to different Fg source species (Eastoe and Leach, 1977) or Fg extraction process (Müller and Heidemann, 1993; Muyonga *et al.*, 2004) so the current study results must be considered specific to the type of Fg used, although we expect qualitatively similar behavior with other types.

Gelling and melting temperatures of 1% agar gel were very different ( $T_m - T_{gel} = 50^\circ\text{C}$ ), in other words agar had a high thermal hysteresis. For the mixed gel with A:Fg = 1:5 thermal hysteresis was very similar to that of agar, while for the mixtures with A:Fg = 1:10-1:25 hysteresis decreased with Fg content and at 8-10°C was much lower than of agar, being close to Fg alone at the same concentration. So, for agar and the 1:5 mixture  $T_m$  is far from  $T_{gel}$ , while this difference is much less for the other mixtures. This indicates that in the former cases the strength of molecular association and amount of junction zones in gel network are higher than in the latter cases and it takes more thermal activation to break the gel network (Rao, 2003).

$T_m$  was higher than  $T_{gel}$  for all mixtures, as expected due to thermal hysteresis. Hysteresis is a common physical phenomenon, and an indication of reluctance to switch state. In the case of gels, once molten by temperature increase they are reluctant to

solidify to a gel, and bringing the temperature down to melting temperature is not enough. Instead the temperature has to be lowered further, to the gelling temperature. Thermal hysteresis has its origins in stabilization of the order form by further aggregation (Morris *et al.*, 1980): when the conformational ordering on cooling is accompanied by aggregation of the ordered structure, the melting of aggregate usually occurs over a high temperature range (Rao, 2003).

Thermal hysteresis can be also explained by the Zipper model of Nishinari *et al.* (1990). It involves following mechanisms. During cooling the random coil forms of molecules are transformed to helix forms, and junction zones that fix the molecules into a rigid ordered molecular structure form when molecular zippers are sufficiently aligned and in contact. The sol-gel transition on heating begins by opening the molecular zippers, and this starts as soon as the temperature suffices to release segments of the zipper. On cooling, in contrast, the pairwise coupling cannot start easily because of difficulty for a long molecule to find its partner in appropriate positions for zipper construction. Hence further cooling is needed for gelling.

Thermal hysteresis also applies to the formation and disintegration of molecule aggregates (Rao, 2003), as opposed to only comprehensive phase transition of bulk material. These qualitative models do not provide a basis for quantitative prediction or parametric modelling of thermal hysteresis, such that would be useful in the current study. They do confirm that our experimental results qualitatively agree with generally accepted phenomena.

#### Textural properties

Gel hardness and springiness were measured using Texture profile analysis (TPA), with results shown in Figures 4a and b, respectively. As Fg concentration increased, hardness and springiness of both Fg alone and the mixed gels increased significantly, but the 1:5 ratio gave lower hardness than agar alone. This is qualitatively similar to observations of  $G'$  discussed earlier. At over 15% fish gelatin, the mixed gel strength synergistically exceeded those of individual fish gelatin and agar gel, similar to  $G'$ . The textural synergy has also been reported for gellan-carrageenan mixed gel system (Banerjee and Bhattacharya, 2011). The springiness of mixed gels shifted towards that of Fg. Overall, addition of Fg increased springiness, while reducing brittleness and hardness, in comparison to agar gel. Mixing Fg with gellan also improves its gel strength (Pranoto *et al.*, 2007).

Table 1. Syneresis of gels from fish gelatine only and from mixtures of 1% agar (A) and 0-25% fish gelatine (Fg)

Fish gelatin (%)	Syneresis (%)*	
	Fg	Mixed gel (A:Fg)
0	-	1.13±0.07 <sup>dB</sup>
5	0.15±0.01 <sup>aA</sup>	0.57±0.04 <sup>cB</sup>
10	0.07±0.01 <sup>bA</sup>	0.22±0.03 <sup>bB</sup>
15	0.01±0.00 <sup>cA</sup>	0.06±0.01 <sup>aB</sup>
20	0.003±0.00 <sup>cA</sup>	0.04±0.00 <sup>aB</sup>
25	0.003±0.00 <sup>cA</sup>	0.02±0.00 <sup>aB</sup>

\*Mean ± standard derivation of triplicates.

Different small letters in the same column indicate significant differences ( $p \leq 0.05$ ).

Different capital letters in the same row indicate significant differences ( $p \leq 0.05$ ).

#### Syneresis

Syneresis is an undesirable phenomenon. During gel forming, unbound excess water is released from the gel matrix and causes the gel surface to wet, and the wetness promotes spoilage. Adding appropriate hydrocolloids at proper amounts can reduce syneresis significantly. In this study, agar gel alone had much higher syneresis than Fg gel (Table1). In comparison to agar, the syneresis of mixed gels was decreased (Table1) with larger decrease at higher Fg concentrations.

The observations from scanning electron microscope images in previous work (Shrinivas *et al.*, 2009) suggest that the bicontinuous mixed gel with 1% agarose and 5% fish gelatin may have large pores in the gel structure, and the water in these pores would contribute to high syneresis. When the concentration of fish gelatin is increased to the range 10-30%, the agar gel forms disperse encapsulated phase, shielded by the continuous fish gelatin phase that has low syneresis. Thus, the syneresis of the mixture should be dramatically less than in the bicontinuous case, or than with pure agar.

#### Conclusions

We have experimentally shown that the gel properties of 1% agar (A) can be significantly modified by addition of fish gelatin (Fg, 0-25%). When mixed in A:Fg = 1:5 ratio, bicontinuous gels formed with separate phase transitions for each mixture component, both during cooling and heating sweeps. The bicontinuous gel had  $G'$  and gel strength lower than pure 1% agar, while  $T_{gel}$  and  $T_m$  were affected only slightly. Further, during heating before agar was completely molten, molten fish gelatin was dispersed in unmolten agar gel. Fish gelatin (5-25%) can improve springiness and syneresis properties of 1% agar. These properties improve with increasing fish gelatin content within this range of mixtures. With at least 15% Fg, improved gel strength and storage modulus were observed. During cooling and heating sweeps the phase transitions were similar to Fg alone,

but the storage modulus was larger than those of Fg and agar, indicating synergistic effects in this range of compositions. However, the 21-25°C gelling and 30-33°C melting temperatures were closely similar to Fg alone, which may limit applications. In these mixtures, Fg acted as the dominant continuous phase, while the 1% agar was the dispersed phase. In conclusion, the mixing ratio of agar with fish gelatin affects obtained mixed gel properties, which need to match the application. Improved gelling and melting temperatures with reduced brittleness, compared to 1% agar, were obtained from a bicontinuous mixed gel containing 5% fish gelatin. This could be applied in a high temperature environment stability food gel, such as a gummy jelly product. The 1:15-1: 25 mixed gels had improved springiness and syneresis relative to 1% agar, but the low gelling and melting temperatures were similar to fish gelatin, over which the major improvement was in gel strength. These may be applied particularly in cold consumption food products. Over all, these mixtures may be particularly useful as alternative gelling agents in halal food products.

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