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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effect of Addition of Different Gums on The Technological and Rheological Properties of Fish Gelatin

Farklı Gam İlavesinin Balık Jelatinin Teknolojik ve Reolojik Özellikleri Üzerine Etkisi

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Abstract

Technological and rheological properties of fish gelatin (FG) with the addition of different gums (xanthan gum, gellan gum, agar-agar, locust bean gum, carrageenan, guar gum, gum arabic) were determined. Increase in the storage modulus (G′) and loss modulus (G″) was observed with the addition of gums to FG. The elastic structure of FG became stronger and showed a significantly higher gel property (G′>G″). The addition of gum arabic was seen to adversely affect the structure of FG, causing a decrease in gel strength and a more viscous structure. The highest gel strength was achieved with the addition of gellan gum (7.50%). The melting temperatures, gel strength, and consistency index of FG were increased with the addition of all gums, except gum arabic. Addition of 5.00% xanthan gum to FG resulted in an increase in the melting temperature to 15.93°C, which was the highest melting temperature obtained with FG. Similarly, an increase in the melting point was detected with the addition of gellan gum, agar-agar, carrageenan, and carob gum compared to the control. Different hydrocolloids enhanced Kgel, G,G, consistency index, gel strength, and melting temperature of FG. Bloom values for Bovine Gelatin (BG) and FG were 247.16 and 31.29 g, respectively. The bloom value increased to 409.363 with the addition of gellan gum and changed between 8.11-131.08 with the other gums. The water holding capacity (WHC) was found to be 784.36% in BG and 35.14% in FG. The highest WHC among all the mixtures was determined as 232.5% with the addition of 5.00% xanthan gum. The best overall results were obtained with the addition of gellan gum. Gellan gum added to FG could potentially make it suitable for usage in the food industry.

Keywords: Fish gelatin (FG), Gums, Gel strength, Melting point.

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Öz

Farklı gamlar (ksantan gam, gellan gam, agar-agar, keçiboynuzu gam, karagenan, guar gam, gam arabik) ilavesinin balık jelatininin (FG) teknolojik ve reolojik özellikleri üzerine etkisi belirlenmiştir. Balık jelatinine gamların eklenmesiyle birikim modülünde (G′) ve kayıp modülünde (G″) artış tespit edilmiştir. Gam ilavesi ile balık jelatinin elastik yapısı güçlenmiş ve önemli ölçüde daha yüksek bir jel özelliği kazanmıştır (G′>G″). Gam arabik ilavesinin balık jelatininin yapısını olumsuz etkilediği, hem jel mukavemetinde azalmaya hem de daha viskoz bir yapıya neden olduğu tespit edilmiştir. En yüksek jel kuvveti olan 11390.17 Pa değerine % 7.50 gellan gam ilavesiyle ulaşılmıştır. Balık jelatininin erime sıcaklıkları, jel kuvveti ve kıvam indeksi, gam arabik hariç tüm gamların eklenmesiyle artmıştır. Balık jelatinine %5.00 ksantan gam ilave edilmesi, balık jelatini ile elde edilen en yüksek erime sıcaklığı olan 15.93^{o}C'ye erime sıcaklığında bir artışa neden olmuştur. Benzer şekilde gellan gam, agar-agar, karagenan ve keçiboynuzu gam ilavesiyle de kontrole göre erime noktasında artış tespit edilmiştir. Sığır jelatin (BG) ve balık jelatini (FG) için bloom değerleri sırasıyla 247.16 ve 31.29 g olarak tespit edilmiştir. Farklı hidrokolloidler, balık jelatininin Kgel, G',G'', kıvam indeksi, jel kuvveti ve erime sıcaklığını arttırıcı etki göstermiştir. Bloom değeri gellan gam ilavesiyle 409.363 g 'ye yükselirken, diğer gamlarla 8.11 ile 131.08 g arasında değişiklik göstermiştir. Su tutma kapasitesi (WHC) sığır jelatininde %784.36, balık jelatininde ise %35.14 olarak tespit edilmiştir. Tüm karışımlar arasında en yüksek WHC, %5.00 ksantan gam ilavesiyle %232.5 olarak belirlenmiştir. Çalışma kapsamında en iyi sonuçlar gellan gam ilavesiyle elde edilmiştir. Balık jelatinine gellan gam ilavesi ile jelatin gıda endüstrisinde kullanıma uygun hale gelme potansiyeli kazanmaktadır.

Anahtar Kelimeler: Balık jelatini (FG), Gam, Jel kuvveti, Erime sıcaklığı

1. Introduction

The use of gelatin is wide ranging and extensive, giving it a high economic value. Of the approximately 400 thousand tons of gelatin produced worldwide today, 46% is generated from pork, 29.4% from bovine skin, 23.1% from bones and 1.5% from other sources (GME, 2008). According to the Grand View Research reports, the share of gelatin in the world market will exceed 4 billion dollars by 2024 (Karayannakidis and Zotos, 2016). The use of fish skin or bone materials in the production of gelatin has become popular in recent years due to factors such as religious preferences, safety concerns, and economic considerations of pork and bovine gelatin (BG) (Sow and Yang, 2015; Yang and Wang, 2009). For this reason, fish gelatin (FG) has been used as an alternative to bovine and pork gelatin in the food industry (Kaewruang et al., 2014). Since FG does not have the quality parameters of mammalian gelatin, it can't be used directly as an alternative; it needs to be modified with the addition of different processes or additives for use in the food industry. The main advantages of FG are that its source is not associated with the risk of bovine spongiform encephalopathy (BSE) and can be used by diverse religions such as Muslims, Hindus, and Jews. In addition, the main by-product of fish production, which causes waste and pollution, can be utilized to generate the gelatin (Binsi et al., 2009).

Various studies with FG have generally compared its characteristics with pork gelatin. The existing collagen in fish skin was shown to be of a wider variety than mammalian collagen, hydroxyproline and proline contents were lower than mammalian gelatin, and serine and threonine contents were higher in FG (Balian and Bowes, 1977). The higher the proline and hydroxyproline levels, the higher the melting point and gel strength (Karim and Bhat, 2009). It has been reported that the FG yield varies between 6-19%, which is lower than mammalian gelatin. The main quality parameters required for the use of gelatin as an additive in the food industry are high gel strength, as well as suitable melting and gelling temperatures. Various modifications have been reported to increase the weak gel strength of FG. These include mixing high gel strength gelatins with FG, treatment of gelatin with carbohydrates, proteins and different salts, addition of transglutaminase, addition of phenolic compounds, use of cross-linking agents, high pressure, radiation and UV radiation (Avena-Bustillos et al., 2006; Gilsenan and Ross-Murphy, 2000; Lin et al., 2017; Pranoto et al., 2007). The physicochemical properties of FG were reported to be modified by mixing it with food polysaccharides (Sow et al., 2018).

In the current study, gelatin was produced from waste fish skins in order to evaluate the use of this industrial waste material as a source of an economically important food additive. Additionally, changes in the rheological and technical characteristics of the produced FG was determined after the addition of different gums (xanthan gum, gellan gum, agar-agar, carob gum, carrageenan, guar gum, gum arabic).

2. Materials and Methods

2.1. Materials

The skin of rainbow trout (Oncorhynchus mykiss) was used as a source of gelatin. Trout skins were obtained from a trout farm in Çanakkale and stored at -18°C until use. The gums (gellan gum, gum arabic, xanthan gum, carob gum, guar gum, carrageenan, agar-agar) and BG were sourced commercially.

2.2. Methods

2.2.1. Gelatin extraction

Gelatin from fish skin was obtained with a previously reported method (Garcia and del Carmen Guillen, 2003). Frozen fish skins were cut into small pieces with the help of scissors and washed with tap water. The cut skins were immersed in 0.5 M NaCl at 5°C and mixed with a baguette for 5 minutes. The skins were then taken to a beaker containing NaOH (1:5 w/v) and incubated for 40 minutes in a shaking incubator (INFORS) at 20°C at a mixing speed of 180 rpm. Following this, the skins were washed three times with distilled water. Next, the swollen skins were extracted with 0.1 M acetic acid at 50°C at a mixing speed of 180 rpm for 18 hours. The extracted FG was passed through a filter paper and dried at 80°C in an incubator (ERTICK) (Işık, 2018).

2.2.2. Preparation of FG solutions

A total of 7 different gums, including xanthan gum (1), gellan gum (2), agar-agar (3), carob gum (4), carrageenan (5), guar gum (6), gum arabic (7), were evaluated as additives in preliminary experiments. Five

different amounts of each gum corresponding to 0.5, 2.50, 5.00, 7.50 and 10%, to 6.67% (w/v) were added to FG. Prior to the addition of the gums, the dried FG was ground into a more easily soluble form. The solutions were kept at room temperature for 1 hour to hydrate followed by mixing in a shaking incubator at 65°C for 20 minutes at 180 rpm until all the gelatin was dissolved. The prepared solutions were incubated at 4°C for 16-18 hours to form a gel (Cai et al., 2017).

2.2.3. Determination of physicochemical properties of FG

Ash, protein and pH values of the BG and FG samples were determined according to the AOAC (2000) method (AOAC, 2006).

2.2.4. Determination of rheological properties

Small-deformation oscillatory measurements were performed with FG containing 6.67%, w/v of the gum in a controlled-strain rotation rheometer with a peltier system (TA Instruments New Castle, USA). The plate and plate geometry (40 mm diameter of the upper plate), was used with a gap setting of 0.8 mm.

2.2.4.1. Gelation kinetics

To determine gel kinetic parameters, time sweep analysis was performed by using a previously published method (Kuan et al., 2016) with some modifications. Briefly, FG gels were placed on the rheometer plate at a temperature of 24°C. For the product to reach equilibrium, first, the temperature was reduced from 24°C to 4°C at a rate of 1°C/min and a temperature sweep was performed. Time sweep analysis at 4°C for 4000 seconds at a constant frequency of 1 Hz and strain of 1% was performed for samples in which linear viscoelastic regions (LVR) were determined. The data were analyzed using the software supplied with the device. Elasticity and viscosity values were expressed as storage modulus (G′) and the loss modulus (G″), respectively.

2.2.4.2. Determination of gel strength and steady shear experiments

Gel strength is a measure of the stability of the gel against angular frequency, and serves as an indicator of the suitability of the gel for storage. Following the time sweep analysis, the gel strength was determined in the range of 0.1-10 Hz (Anvari & Chung, 2016) at 1% strain with the frequency sweep test (Kuan et al., 2016). For this purpose, compatibility with storage conditions (J') was calculated with the following equation:

$$J' = \frac{G'}{G'^2 + G''^2} \qquad \text{(Eq. 1)}$$

G_N^0 is the indicated gel strength is related to J_N^0. J_N^0 also indicates the frequency with the lowest G″ value (Ferry, 1980).

$$G_N^0 = \frac{1}{J_N^0} \qquad \text{(Eq. 2)}$$

Rheological data were tested for compatibility with the Power-Law model. Flow behavior, consistency coefficient (K-Pa.s^n) and flow behavior index (n) values of FG+gum solutions were calculated by using Power-law (Equation 3). The model with the highest coefficient of determination (R^2) was established.

Power law (Ostwald-de Waele equation) model: $\sigma = K(\dot{\gamma})^n$ (Eq. 3)

2.2.4.3. Gelling and melting temperatures

Temperature sweep analysis was performed to determine the gelation and melting temperatures of gelatin solutions. The samples were heated to 10°C, followed by equilibration for about 300 seconds, heating to 50°C at the rate of 1°C/min and then cooling back to 10°C. The crossover point where the storage modulus (G′) was reduced and the loss modulus (G″) was increased was considered as the melting temperature. In the cooling process, the temperature at which G′ and G″ underwent a crossover was considered as the sol-gel transformation or gel formation point.

2.2.4.4. Determination of gel strength (Bloom value)

Bloom value was determined according to a previously described method (Europe, 2000). The FG+gum mixture was dissolved in 105 mL of distilled water and taken into a bloom jar. Gel strength was measured with a

texture analyzer (TA HD plus) at 4°C. Gel force (g) was measured as the force required for 4 mm penetration into the samples at a penetration rate of 1 mm/s.

2.2.5. Texture profile analysis

The hardness properties of gelatin were determined using a 36 mm diameter (R/36) probe at 4°C using a texture analyzer (TA HD plus). Test parameters included a test speed of 2 mm/s; Target mode: Compression distance: 5 mm; Duration: 3.0 s; Trigger type: Automatic (force); Trigger force: set to 0.1g (Kuan et al., 2016).

2.2.6. Determination of water holding capacity (WHC) and oil binding capacity (OBC)

WHC and OBC were determined according to the method of (Lin et al., 1974). 1g sample was weighed into a centrifuge tube; 50mL of distilled water and 10mL of corn oil were added to it. After 1 hour of storage at room temperature, the tube was vortexed 4 times for 5 seconds at intervals of 15 minutes. At the end of the process, the tube was centrifuged for 20 minutes at 450 x g. The upper phase was removed and the phase remaining in the centrifuge tube was filtered through a filter paper at an angle of 45 degrees for 30 minutes. The tube and the pellet were weighed and the results were calculated with the following equations:

$$WHC\% = \frac{[Pellet\ weight\ (g) - Gelatin\ weight\ (g)]}{Gelatin\ weight\ (g)} x100 \quad \text{(Eq. 4)}$$

$$OBC\% = \frac{[Pellet\ weight\ (g) - Gelatin\ weight(g)]}{Gelatin\ weight\ (g)} x100 \quad \text{(Eq. 5)}$$

2.2.7. Microstructural properties

Images of gelatin samples were taken with a FEI-QUANTA FEG 250 Scanning Electron Microscope (SEM).

2.2.8. Statistical analysis

The data were analyzed with the JMP 5.0.1.a package program. One-way ANOVA and Tukey's comparison test was used to compare the differences statistically.

3. Results and Discussion

Seven different gums (xanthan gum, gellan gum, agar-agar, carob gum, carrageenan, guar gum, gum arabic) were added to FG in a range of 0.5-10% (w/v). The melting temperature and gel strength were evaluated with rheological analyses in a total of 35 samples (*Table 1*). The ideal gum amount was determined on the basis of the highest gel strength and melting temperature (*Table 2*). As seen in Table 1, the melting points of the samples varied between 14.50-29.74 ºC. While the highest melting point was observed in BG (29.74 ºC), the lowest melting point was found in FG with the addition of 2.50% gellan gum (14.50 ºC). The gel strength of the samples ranged from 749.77-11,390.17 Pa. The highest gel strength was 11390.17 Pa in FG with 7.50% gellan gum added, while the lowest value was 749.77Pa in FG with 5.00% gum arabic added. These findings are in agreement with the commonly accepted 8–25 °C range for fish gelatin to gel (Karim and Bhat, 2009). The incorporation of various hydrocolloids, such as gums, has been reported to enhance the rheological properties of fish gelatin. These hydrocolloids can augment the gel strength and melting degree of the gelatin. This finding is supported by prior research in the field (Haug et al., 2004; Huang et al., 2019).

The pH value of 5 in FG and BG increased to 5.08 only in FG samples with added locust bean gum; all other samples showed a decrease in the pH value. Examples of decreasing pH values were: FG+xanthan gum pH 4.95, FG+gum arabic pH 4.92, FG + carrageenan pH 4.87, FG + guar gum pH 4.86, FG + agar agar pH 4.82 and FG + gellan gum pH 4.78. FG with added gellan gum had the lowest pH value of 4.78 (Table 2). In the literature, it has been reported that the pH value of the produced gelatin changes depending on the extraction processes applied (Songchotikunpan et al., 2008). Depending on the pre-treatment applied to the collagen, different types and properties of gelatin can be obtained including type A and type B gelatin. Type A gelatin has an isoelectric point at pH 6.9, while type B has an isoelectric point at pH 5 (Karim and Bhat, 2009). In addition, pH is one of the important factors affecting the gelling properties of FG (Kaewruang et al., 2014). The gelatin produced in the current study is type B gelatin and may have the potential to be used particularly in acidic foods.

Table 1. Gel strength and melting point values of FG with the addition of gum at different concentrations

Models	Gums	Melting point (ºC)	Gel strength (Pa)	Models	Gums	Melting point (ºC)	Gel strength (Pa)
1	Bovine Gelatin (BG)	29.74±0.005a	5600.87±0.07b	20	FG+5.00% Carob gum	15.51±0.005cde	1455.83±3.18 ghi
2	Fish Gelatin (FG)	15.27±0.011efg	1649.14±0.04ghi	21	FG+7.50% Carob gum	15.52±0.023bcd	2581.93±1.8c
3	FG+0.5% Xanthan Gum	15.22±0.011 efg	1723.57±1.57efg	22	FG+10% Carob gum	15.58±0.021 bcd	1206.43 0.95hij
4	FG+2.50% Xanthan Gum	15.61±0.005bcd	1327.60±2.9 ghi	23	FG+0.5% Carrageenan	15.43±0.017cde	2236.56±22.08def
5	FG+5.00% Xanthan Gum	15.93±0.011b	2658.29±5.64cde	24	FG+2.50% Carrageenan	15.80±0.017bc	1399.635±4.36 ghi
6	FG+7.50% Xanthan Gum	15.50±0.001cde	1684.17±4.25fgh	25	FG+5.00% Carrageenan	15.22±0.017 efg	1046.77±1.28 hij
7	FG+10% Xanthan Gum	15.80±0.046bc	1931.85±4.79efg	26	FG+7.50% Carrageenan	15.71±0.005bcd	1543.205±1.67 ghi
8	FG+0.5% Gellan Gum	14.80±0.017klm	951.89±21.94ij	27	BJ+10% Carrageenan	15.69±0.0175 bcd	3196.742±1.38c
9	FG+2.50% Gellan Gum	14.50±0.003m	2896.58±14.13cd	28	FG+0.5% Guar Gum	14.79±0.005lm	928.82±1.48 ij
10	FG+5.00% Gellan Gum	14.90±0.046ijk	1532.51±2.19 ghi	29	BJ+2.50% Guar Gum	15.00±0.323def	1377.61±2.79 gh
11	FG+7.50% Gellan Gum	15.60±0.017bcd	11390.17±10.17a	30	FG+5.00% Guar Gum	15.22±0.017 efg	1814.04±0.04efg
12	FG+10% Gellan Gum	--		31	FG+7.50% Guar Gum	15.11±0.017fgh	1026.42±0.56hij
13	FG+0.5% Agar Agar	15.10±0.017ghi	1109.88±5.66 hij	32	FG+10% Guar Gum	15.21±0.005 efg	1000.51±14.19 hij
14	FG+2.50% Agar Agar	15.10±0.001 ghi	1681.85±1.37 hij	33	BJ+0.5% Gum Arabic	14.79±0.011lm	1136.54±0.04 hij
15	FG+5.00% Agar Agar	15.20±0.028 efg	2593.06±10.17cde	34	FG+2.50% Gum Arabic	14.89±0.005jkl	934.45±4.04 ij
16	FG+7.50% Agar Agar	15.31±0.005def	3384.56±62.91c	35	FG+5.00% Gum Arabic	15.06±0.028hij	749.77±2.16j
17	FG+10% Agar Agar	15.60±0.017 bcd	5656.14±56.14b	36	FG+7.50% Gum Arabic	14.79±0.005lm	777.44±1.82j
18	FG+0.5% Carob gum	15.60±0.075 bcd	1466.10±66.1 ghi	37	FG+10% Gum Arabic	15.11±0.260def	1438.67±0.07 ghi
19	FG+2.50% Carob gum	15.80±0.011bc	3506.20±6.2c				

*Values in a column followed by different superscript letters are significantly ($p < 0.05$) different (Duncan's test). -: It could not be measured due to conversion to a solid form

Table.2. Physicochemical and technical properties of FG-gum mixtures showing the best rheological properties.

Samples Code	Gelatin-gum mixing ratio (%)	Conc.(%)	pH value	Ash (%)	Water Holding Capacity (%)	Oil Binding Capacity (%)	Degree of Bloom (g)
Control 1	6.67%FG	100%	5±0.02b	6.80±0.02c	35.14±5.105d	146.43±11.975bc	31.29±0.02^{d}
Control 2	6.67% BG	100%	5±0.01b	3.75±0.01$_{1}$	784.36±13.87a	190.87±6.155a	247.16±0.03^{b}
5	6.67% FG+0.033 Xanthan	5.00%	4.95±0.02bc	6.41±0.01d	232.59±16.68b	156.77±13.77bc	9.94±0.02^{h}
11	6.67% FG +0.050 Gellan	7.50%	4.78±0.03f	7.02±0.02b	104.17±1.72c	166.33±4.51b	409.33±0.03^{a}
17	6.67% FG +0.667 Agar Agar	10%	4.82±0.03ef	5.89±0.05f	50.50±14.995d	149.34±7.125bc	131.08±0.03^{c}
19	6.67% FG +0.166 Carob	2.50%	5.08±0.03a	5.79±0.04g	106.82±15.17c	138.83±4.935c	11.52±0.02^{g}
27	6.67% FG +0.667 Carrageenan	10%	4.87±0.01de	8.21±0.01a	99.22±2.92c	140.01±3.83c	19.89±0.01^{e}
30	6.67% FG +0.333 Guar Gum	5.00%	4.86±0.02de	5.69±0.1h	124.36± 4.485c	153.75±0.25bc	8.11±0.01^{i}
37	6.67% FG +0.667 Gum Arabic	10%	4.92±0.02cd	5.99±0.01e	102.19±0.375c	142.89±4.425c	18.76±0.01^{f}

*Values in a column followed by different superscript letters are significantly ($p < 0.05$) different (Duncan's test)

The % ash amounts of FG alone and after the addition of different amounts of gum are shown in *Table 2*. The amount of ash was 6.80% in FG and 3.75% in BG; BG had the lowest ash content of all the samples examined. Among the FG and gum mixtures, FG+carrageenan had the highest ash content with 8.21% while the ash content of the FG+gellan gum mixture was 7.02%. FG+guar gum had the lowest ash content of 5.69% (Muyonga et al., 2004) reported that ash content increased with increasing age in fish; the ash content of older fish skin was higher than that of young fish skins. The ash content of FG was seen to be higher than mammalian gelatin; higher quality FG have a low ash content (Muyonga et al., 2004) reported that a demineralization process applied at the extraction stage can affect the ash content of gelatin. Higher ash content may be seen if the demineralization process is insufficient. It is recommended that high quality gelatin should have an ash content of less than 0.5%, and in food applications, the ash content should be less than 3% (Yearbook, 2019). The FG extracted in the current study as well as the FG-gum mixtures generated had an ash content of more than 3%. This may be decreased further by applying different demineralization processes.

3.1. Rheological properties of FG-gum mixtures

The viscoelastic properties of BG, FG and FG with different gum additions were determined with the frequency sweep test under 1% strain. The frequency-dependent change in G′ and G″ values are shown in *Figure. 1*. The data were adapted to the Power- Law model and the consistency indices (K) and flow indices (n) are given in *Table 3*.

As a general trend, the G′ and G″ values of FG were seen to increase with the addition of gum, suggesting that the elastic structure of FG was strengthened with the addition of gums. The elastic property of the samples was more dominant than the fluidity property and formed a gel-like structure. Among all the FG+gum mixtures, FG+gellan gum showed a significantly higher gel property (G′>G″). The addition of gum arabic negatively affected the structure of FG, causing a decrease in gel strength and a more viscous structure. The results obtained with oscillation tests were compatible with the shear steady flow data. For all samples, G′ appeared to be at least 100 times larger than G″. This is typical of a strong gel system (Yang and Wang, 2009).

Frequency sweep test was performed at 10°C to compare the mechanical strength of FG and FG-gum mixtures (Anvari and Chung, 2016). Frequency scanning under dynamic conditions (in the linear viscoelastic region) showed that both G′ and G″ values increased slightly with increasing frequency in all gum added and control samples (Fig. 1). This property is typical for a concentrated polymer solution (Mei et al., 2012). In addition, G′ was seen to be weakly frequency dependent compared to G″ (Fig. 2), typical of systems with solid-like rheological properties (Zhong and Ikeda, 2012). FG+gellan gum, FG+agar agar and FG+carrageenan showed slower characteristic slopes for the frequency dependence of G′. This suggests the presence of a strong gel structure and shows that gellan gum, agar-agar and carrageenan can strengthen the gel structure of FG. All samples were found to exhibit true gel behavior, regardless of the gum type. The G′ curves for each sample were almost parallel to each other regardless of the oscillation frequency. The G″ was found to be higher than the G″ modulus practically over the entire frequency range. This feature is considered to be typical of systems with a rigid and brittle structure (Lin and Huang, 2003). There was a significant increase in both G′ and G″ with the addition of all gum samples to FG except gum arabic. Addition of hydrocolloids such as gums can increase of G′ and G″ due to intramolecular interactions (Sow et al., 2018).

The flow behavior consistency coefficient (*K*-Pa.s^n) and flow behavior index (n) values of FG+gum solutions are shown in *Table 3*. The K coefficient of the samples varied between 767.54 -12,051 Pa.s^n. The lowest K value was determined as 767.54 Pa.s^n in FG with 5.00% gum arabic added while the highest K value of 12051 Pa.s^n was seen in FG with 7.50% gellan gum added.

The samples with the best K (consistency index) values were determined as FG with addition of 2.50% carob gum, 5.00% xanthan gum, 5.00% guar gum, 7.50% gellan gum, 10% carrageenan and 10% agar-agar, respectively (*Table 3*). The best performing FG+gum mixture was found to be FG+gellan gum; addition of 7.50% gellan gum increased the consistency index and therefore strength of FG to values greater than that obtained with BG. This may have resulted from the contribution of increasing gellan gum concentration and stronger intermolecular interactions to the G′ and G″ values. Similar results were also reported with hydrocolloids such as carrageenan (Haug et al., 2004; Sow et al., 2018). However, the addition of gum arabic negatively affected the rheological properties of FG at all concentrations and decreased the consistency index.

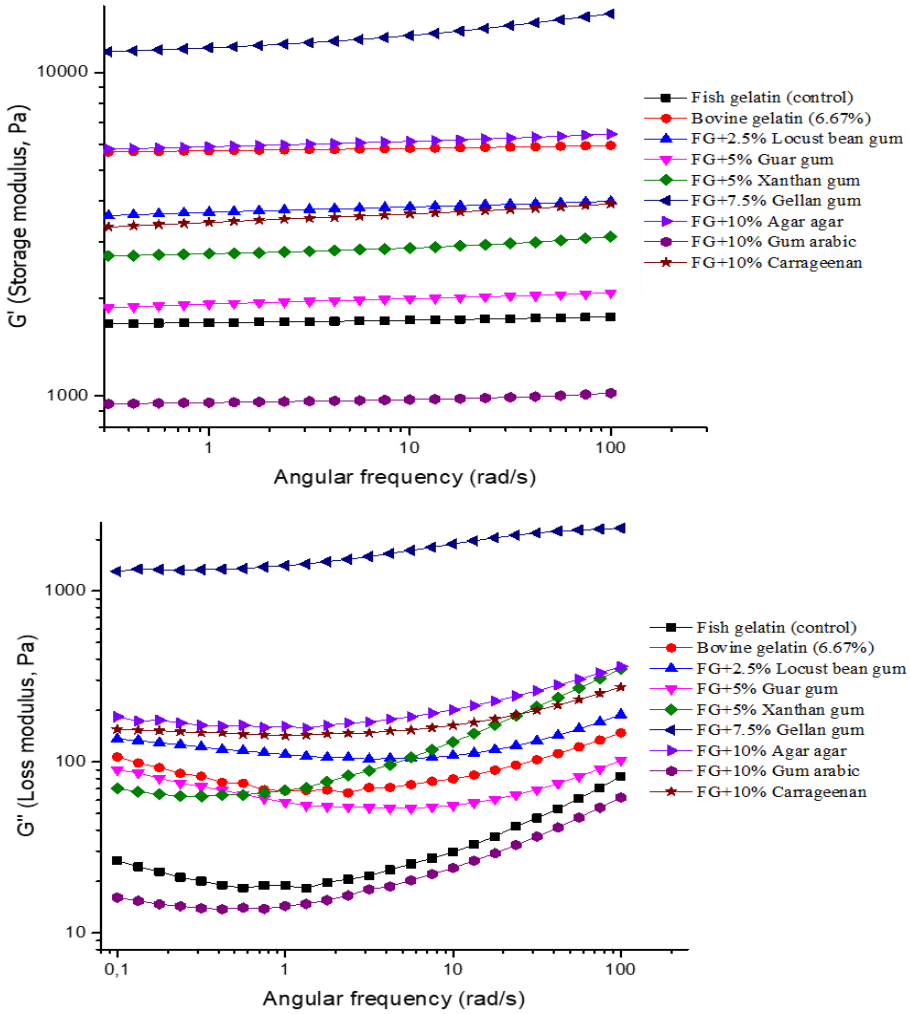

Figure. 1. Variation in G' and G'' values of bovine gelatin (BG), fish gelatin (FG) and FG-gum mixtures as a function of angular velocity.

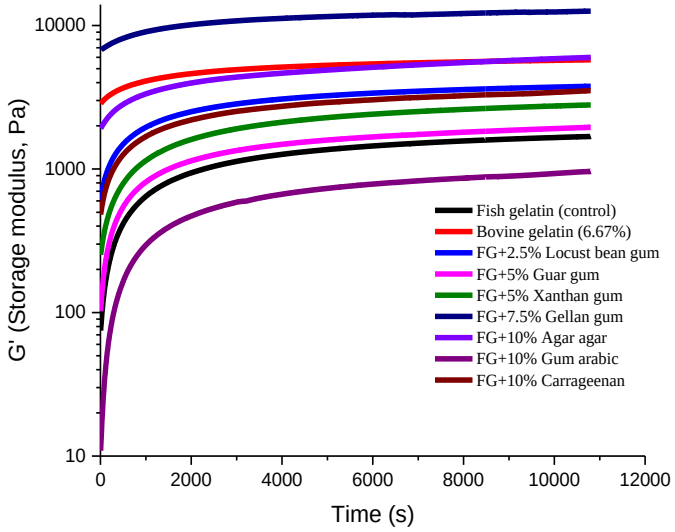

Figure. 2. Time-dependent variation of G' in FG and FG-gum mixtures.

Table.3. Power-Law (PL) model parameters of gum added FG solutions

Samples Code	Samples Name	k (Pa.s^n)	n	R^2	Gelation Kinetics		
					Kgel	C (-)	R^2
Control 1	Fish Gelatin (FG)	1685.1±5.5b	0.0085±0.002a	0.99	378.63±5.29b	1863.9±10.41g	0.96
Control 2	Bovine Gelatin (BG)	5720.9±22.3g	0.0081±0.009a	0.99	630.99±3.93e	112.54±1.09a	0.98
5	FG+Xanthan Gum	2761.3±18.2d	0.0206±0.09b	0.95	604.16±0.92d	1784.7±3.14f	0.96
11	FG+Gellan Gum	12051±1ı	0.0411±0.01e	0.94	1307.1±0.4ı	346.1±0.57b	0.97
17	FG+Agar Agar	5892.7±2.6h	0.018±0.003b	0.99	913.95±2.03h	2786.3±0.28ı	0.97
19	FG+Carob Gum	3683.3±3.9f	0.018±0.004b	0.99	702.08±1.08g	1419.6.7±23.20c	0.98
27	FG+Carrageenan	3429.6±5.4e	0.0282±0.003c	0.99	672.03±0.62f	1614.8±8.44d	0.98
30	FG+Guar Gum	1914.5±4.9c	0.0188±0.002b	0.98	416.2±1.93c	1958.7±3.15h	0.97
37	FG+Gum Arabic	955.87±3.22a	0.0112±0.003a	0.95	330.02±2.81a	1650.6±4.60e	0.96

*Values in a column followed by different superscript letters are significantly ($p < 0.05$) different (Duncan's test)

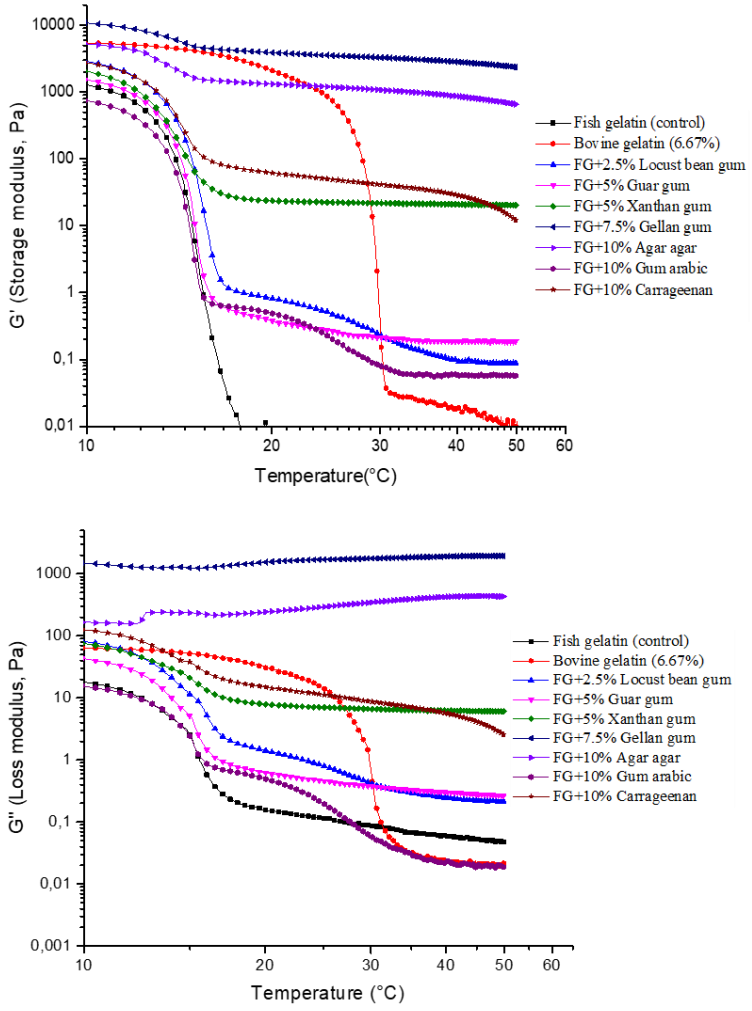

Figure. 3. Variation of G' and G'' values of FG and gum mixtures depending on temperature

The flow behavior index (n) was found to be below 1 in all samples. Elastic properties of all the samples tested were more dominant than viscous properties. The rheological behavior of FG with the addition of 10% agar-agar was found to be similar to that of BG. With the addition of 7.50% gellan gum, the gel structure of FG became approximately 2 times stronger than BG. Evaluating the effects of addition of carrageenan on the structure and rheological properties of FG (Sow et al., 2018). Reported that carrageenan strengthened the rheological properties of FG, corroborating the data from the current study.

As another measure of the rheological properties of FG, BG and FG+gum mixtures, time dependent gelation was determined (*Figure 2*). The G′ value was seen to increase as a function of time when the samples were kept at a constant temperature at 4°C. This indicates that as the residence time increased, the helical structure was increased, reflecting on an increased gel strength. This also indicated that gelation had taken place. The G' value of all samples were seen to increase rapidly for about 1,000 seconds, followed by a gradual plateauing. These data suggest that existing junctions of the fortified gels were constantly reorganized but had the tendency to stabilize over time (Choi and Regenstein, 2000; Kuan et al., 2016).

Gelation kinetic parameters were determined by fitting the experimental data to the logarithmic function model (*Table 3*). The gel strength of FG was increased with the addition of each of the gum samples except gum arabic. This increase was approximately 7 times with the addition of 7.5% gellan gum, giving this sample the highest gel strength. This was followed by agar-agar (5,656.14 Pa), carob gum (3506.20 Pa), carrageenan (3,196.742 Pa), xanthan gum (2,658.29 Pa) and guar gum (1,814.04 Pa). The kgel values of the control FG and BG samples were 378.63 and 630.99 respective; upon addition of the gum samples, the kgel values of FG varied between 007-307.1. Considering the effect of the gums on gelation kinetics and strength of FG, gellan gum was seen to improve Kgel, G″ and gel strength of FG more than the other gums. Similarly, the addition of agar-agar, carrageenan and carob gum improved the structure compared to control FG and BG. The kgel of FG increased approximately 2 times with the addition of carrageenan and locust bean gum, approximately 2.5 times with the addition of agar agar, and approximately 3.5 times with the addition of gellan gum. As a general trend, G′, gel strength and kgel values increased with the increase in the concentration of gum added. These results are in line with those of previously reported studies (Cai et al., 2017; Kuan et al., 2016).

Changes in G′ and G″ values during the heating of gelatin solutions are given in *Figure* 3. The melting temperatures of the samples varied between 14.79°C and 15.93°C. The melting temperature of the control FG and BG samples were 15.11°C and 29.74°C, respectively. A decrease in G' and G" values was observed in all of the samples with the heating process. The melting temperature of FG increased with the addition of xanthan gum, agar agar, gellan gum, carrageenan, locust bean and guar gum, compared to the control FG sample due to the high solute concentration in the gums. An increase in the melting point of FG is important for its use as an alternative to mammalian gelatin in food products. Nonetheless, the 29.74°C melting point of BG could not be reached even at the best highest concentrations of the gums used.

3.2. Gel strength (Bloom Degree)

The bloom degree of the FG samples with different added gums are shown in *Table 2*. The bloom degrees of the control BG and FG samples were 247.16g and 31.29g, respectively. The bloom degree of the FG+gellan gum sample at 409.33g was higher than the control BG sample, followed by FG+agar agar with a bloom value of 131.08g. The lowest bloom value of 9.94g was obtained with the FG+xanthan gum mixture. The gel-forming property of gelatin depends on many factors such as extraction method, temperature, pH, type of raw material used for extraction and additives used during extraction. The cooling temperature and ionic strength of the gelatin sample ban also affect the bloom values (Europe, 2000). The quality of gelatin is measured by gel strength or bloom value and are classified as low (<150), medium (150-220) and high bloom (220-2300) values (Johnston-Banks, 1984). While the gelling strength of commercial gelatins is in the range of 100-300, it is desirable to produce gelatin at 250-260 blooms (Johnston-Banks, 1984). In the current study, samples with low bloom values were obtained, except for the FG+gellan gum mixture and the control BG sample.

3.3. Texture Profile Analysis (TPA)

TPA results of the FG samples with the addition different gums are shown in *Table 4*. The hardness levels of the controls BG and FG were 298.17g and 17.98g, respectively. With the addition of gums to FG, the level of

hardness approached that of BG with hardness of values as high as 231.76g observed in the FG+gellan gum sample. This was followed by FG+agar agar with a hardness value of 104.15g. The hardness values of the other FG+gum samples varied between 16.18 and 42.86g. Considering the degree of elasticity, FG+gellan gum showed the best result with 0.44, followed by BG with 0.399 and then FG+carob gum and FG+gum arabic samples. The degree of elasticity was 0.357 in the control FG samples and the lowest elasticity value was determined as 0.33 in the FG+carrageenan sample.

Evaluation of the parameter gumminess indicated that BG had the highest value of 0.821 while FG+agar agar had the lowest gumminess value of 0.392. The control FG sample had a gumminess value of 0.462. The gumminess value closest to BG was determined in the FG+xanthan gum sample. Evaluation of the parameter chewiness indicated that the best outcome was determined as 97.64 in BG, while the lowest value was measured as 2.81 in FG+guar gum. The control FG sample had a chewiness value of 2.96 suggesting that this parameter was lowered even further with the addition of guar gum. Addition of the other gum samples had an overall positive effect on the chewiness of FG, although the effect was somewhat modest. Among the FG samples with gum addition, the highest chewiness was observed with FG+gellan gum with a value of 48.84. Determination of resilience recovery suggested similar values in FG (0.606) and BG (0.626). The ability of elastic recovery, or resilience, might vary based on the imino acid content of the gel, its molecular weight distribution, and the extraction technique used (Chandra and Shamasundar, 2015).

Table 4. Texture analysis profile of gum added FG solutions

Samples Code	Samples Name	Hardness (g)	Elasticity	Gumminess	Chewiness	Resilience
Control 1	Fish Gelatin (FG)	17.989 ±0.002h	0.357±0.001d	0.462±0.002h	2.969±0.002h	0.606 ± 0.001c
Control 2	Bovine Gelatin (BG)	298.174±0.004a	0.399±0.002b	0.821±0.001a	97.641±0.001a	0.626 ± 0.001b
5	FG+Xanthan Gum	27.123±0.001g	0.349±0.002e	0.656±0.001b	6.207±0.002g	0.537 ± 0.003e
11	FG+Gellan Gum	231.765±0.003b	0.440±0.003a	0.471±0.001g	48.842±0.002b	0.089 ± 0.001ı
17	FG+Agar Agar	104.156±0.001c	0.350±0.002d	0.392±0.002ı	14.563±0.003c	0.128 ± 0.003h
19	FG+Carob Gum	30.758±0.002e	0.390±0.001c	0.601±0.001d	7.206±0.001e	0.586 ± 0.003d
27	FG+Carrageenan	42.863±0.004d	0.330±0.002f	0.521±0.001e	7.374±0.004d	0.276 ± 0.001g
30	FG+Guar Gum	16.183±0.003ı	0.350±0.003d	0.489±0.003f	2.812±0.002ı	0.405 ± 0.001f
37	FG+Gum Arabic	29.103±0.002f	0.390±0.001c	0.615±0.002c	6.979±0.001f	0.634 ± 0.004a

*Values in a column followed by different superscript letters are significantly ($p < 0.05$) different (Duncan's test)

3.4. Water Holding (WHC) and Oil Binding Capacity (OBC)

The water holding and oil binding capacities of control FG and the FG samples with different gum additions are shown in *Table 2*. The sample with the highest WHC was BG with 784.36% followed by FG+xanthan gum with a WHC of 232.59%. The control FG sample had the lowest WHC of 35.14%. Addition of each of the gum samples increased the WHC of FG.

BG had the highest OBC of 190.87% while the lowest OBC was determined in the FG+locust bean gum as 138.83%. Following BG, the highest OBC was observed in the FG+gellan gum mixture of 166.33% (Nurul and Sarbon, 2015). Emphasized that the WHC was related to the amount of hydrophobic amino acids present in the gelatin samples as well as the presence of high amounts of pores and voids in the gel network.

3.5. Microstructural characteristics (SEM)

Microstructures of the control FG and BG samples, as well as FG samples with 7 different gums added were examined by scanning electron microscopy (SEM) (*Figure 4*). SEM images indicated the presence of filamentous-

fibrous structure in the control BG sample (*Figure 4b*) while the control FG sample was more homogeneous and had smaller pores (*Figure 4a*). The FG+carob bean and FG+carrageenan samples were similar in structure to BG, while FG+agar-agar, FG+xanthan gum and FG+gum arabic were similar to FG. FG+guar gum and FG+gellan gum showed a homogeneous and almost smooth structure. These differences in the microstructures could be due to the differences in the chemical composition of the gums. Gums used in this study were obtained from different raw materials, with differences in production methods such as extraction and purification.

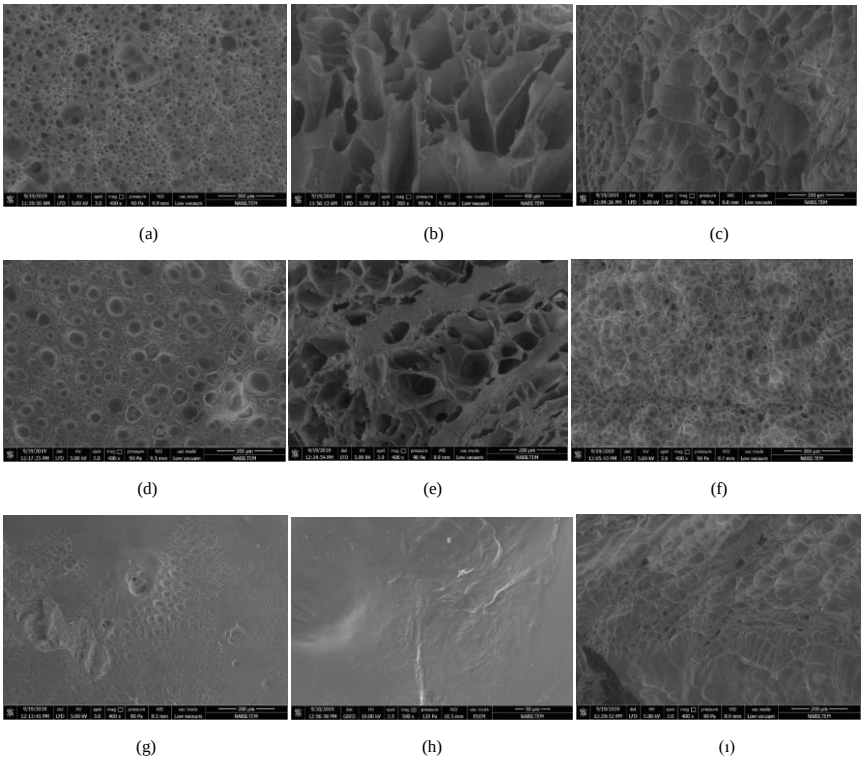

Figure 4. SEM images of bovine gelatin (BG), fish gelatin (FG) and FG with different gums added. (a: FG, b:BG, c:FG+Carob gum, d:FG+Agar agar gum, e: FG+Carrageenan, f: FG+Xanthan gum, g: FG+Guar gum, ı: FG+Gum arabic)

4. Conclusions

Kgel, G′,G″, consistency index, gel strength and melting temperature of FG were increased upon the addition of different hydrocolloids (gellan gum, carrageenan, agar agar, locust bean gum, and xanthan gum). The weaker rheological properties of the control FG samples compared to mammalian gelatin were improved upon addition of the gums. Thus, with the addition of gum, the elastic structure of FG was strengthened and the samples showed higher gel properties. Among the hydrocolloids used in the study, the sample that resembled the characteristics of BG the most was FG+gellan gum. Gum arabic, agar agar and carrageenan, also improved the characteristics of FG; however, the they still fell short of optimal outcomes. FG modified by the addition of gellan gum may have the potential for use in the food industry.

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