

Gelation and thermal characteristics of microwave extracted fish gelatin–natural gum composite gels

P. K. Binsi¹ · Natasha Nayak¹ · P. C. Sarkar² · C. G. Joshy¹ · George Ninan¹ · C. N. Ravishankar¹

Revised: 23 December 2016 / Accepted: 13 January 2017 / Published online: 31 January 2017
© Association of Food Scientists & Technologists (India) 2017

Abstract In this study, the gelation and thermal characteristics of microwave extracted fish scale gelatin blended with natural gums such as gum arabic (AG), xanthan gum (XG), guar gum (GG), and tragacanth gum (TG) was evaluated. The nature of interaction and behavior of gelatin in presence of various gums was confirmed by particle size analysis, viscosity profile, FT-IR analysis and turbidity measurements. DSC data revealed that addition of AG, TG and GG remarkably improved the thermal stability of fish gelatin gel. The composite gels of TG, AG, and XG exhibited higher hardness and bloom strength values as compared to pure fish gelatin implying its textural synergy. Based on qualitative descriptive analysis, TG was found to be superior in improving the stability of fish gelatin gel, closely followed by AG. The results suggest that addition of these gums can reduce syneresis and retard melting of gelatin gels at ambient temperature, which are otherwise soft and thermally unstable.

Keywords Fish gelatin · Gum arabic · Xanthan gum · Gum tragacanth · Guar gum · Composite gel

Introduction

The use of fish gelatin is limited by several factors such as inferior gelation rate, thermal stability, and rheological properties as compared to its mammalian counterpart. Several

methods have been suggested to increase the thermal stability of gelatin gels, including enzymatic cross-linking, addition of co-gelators, hydrocolloids etc. (Somboon et al. 2014). Higher thermal stability of gelatin gels permits the use of gelatin for the development of stable materials with extensive applications in food, pharmaceutical and tissue engineering applications. Natural gums such as gum arabic, xanthan gum, tragacanth gum and guar gum are the most popularly used functional hydrocolloids for this purpose. Gum arabic is the most widely used industrial gum owing to its superior surface activity and solubility characteristics. Chemically, gum arabic is a weak polyelectrolyte that carries a net negative charge above pH 2.2 (Yang et al. 2012). Similarly, xanthan gum is a naturally occurring anionic gum that has considerable practical value due to its high shear thinning behavior despite having high viscosity at rest. The high viscosity of xanthan gum solutions at low shear rates provides long-term stability to colloidal systems (Sworn 2000). Tragacanth gum is another important industrial gum, which consists of two primary fractions, tragacanthic acid/bassorin and tragacanthin. Tragacanthin is the lesser component and is water soluble. The major component, bassorin, is polymethoxylated tragacanthin and hence insoluble in water, but has the capacity to swell and form a gel (Verbeken et al. 2003). The neutral gum, guar gum (GG) is a linear polysaccharide, extensively used in a range of applications because of its unique ability to alter rheological properties of other polymers, thereby acting as a co-gelator. The properties of guar gum are not affected by ionic strength or pH at moderate temperature, owing to the non-ionic character of galactomannan. In general, these hydrocolloid gums permits the use of gelatin at lower concentrations while exhibiting higher bloom strength. The reason for this phenomenon is that the electrostatic attractions between the oppositely charged biopolymers induce the formation of biopolymer complexes in the case of charged polymers such

✉ P. K. Binsi
binsipk@yahoo.com

¹ ICAR - Central Institute of Fisheries Technology,
Matsyapuri, Willingdon Island, Cochin 682 029, India

² ICAR - Indian Institute of Natural Resins and Gums,
Namkum, Ranchi 834 010, India

as gelatin, gum arabic, tragacanth gum and xanthan gum. On the other hand, neutral hydrocolloids such as guar gum impart a bulking effect to the gelling system by way of its structural complexity. Although relevant studies have been made in the past on the use of composite gels made of gelatin with various additives, the interaction between gelatin and different natural gums in modifying the properties of gelatin has not been adequately assessed.

Skins and bones from many fish species have been extensively investigated as raw material for collagen and gelatin production (Binsi et al. 2009; Chandra et al. 2013; Chandra and Shamasundar 2015; Pranoto et al. 2016). Lately, fish scale has been focused as an ideal source for collagen and gelatin extraction (Zhang et al. 2008; Fengxing et al. 2008; Yong-an and Jing-na 2010; Zhang et al. 2011; Mori et al. 2013; Tu et al. 2015). Akagunduz et al. (2014) reported higher yield and bloom strength values for gelatin extracted from scales compared to that from bones in sea bream. However, most of these studies followed heat extraction protocol, which requires long extraction period of 12–16 h. Recently, microwave assisted extraction method has been suggested as one of the effective methods for improving quality characteristics, including gel strength, melting point, and viscosity of gelatin, with considerably reduced extraction time in comparison to the conventional extraction processes (Park et al. 2013). Hence, in the present study, gelatin was isolated from fish scale using microwave assisted extraction protocol. Further, the interaction effect of anionic gums namely gum arabic, xanthan and tragacanth gum, and the neutral guar gum on fish gelatin in modifying the thermal stability, rheological properties and textural characteristics of gelatin gels at ambient temperature was evaluated.

Materials and methods

Raw material

The gelatin used in the present study was extracted from scales of rohu fish (*Labeo rohita*) by microwave assisted heating method. The gelatin was freeze-dried to a constant moisture content of 6% and then used for subsequent analysis. The molecular weight of the gelatin as determined by size exclusion chromatography using standard molecular weight markers was 282 kDa.

Chemicals

Analytical grade arabic, xanthan, tragacanth and guar gum were sourced from M/s HiMedia Pvt Ltd Mumbai (India).

Preparation of gel forming solutions (GFS)

A 3% (w/v) solution of gelatin was prepared by hydrating the freeze dried gelatin at 40 °C for 15 min with occasional stirring and the gums aforementioned were dispersed at concentrations of 0.5, 1 and 1.5% of GFS (w/v). The pH of GFS was adjusted to 8.0, prior to the addition of different gums. The concentrations of gelatin and various gums were established based on preliminary experiments, which were the critical concentrations required to form stable gels after aging at 4 °C for 24 h. Gel forming solutions (GFS) were further heated at 60 °C for 30 min in a temperature controlled water bath. The resulting GFS of fish gelatin are hereafter designated as FG, AG, XG, TG and GG for pure gelatin, gum arabic–gelatin, xanthan–gelatin, tragacanth–gelatin, and guar gum–gelatin GFS, respectively. The GFSs were further analyzed for color, particle size and zeta potential, turbidity and viscosity prior to the preparation of composite gels.

Preparation of composite gels (gelatin-gum gels)

Definite volumes of hot GFS solutions were poured into glass petri dishes and were aged at 4 °C for 24 h to obtain the composite gels of respective gums (hereafter designated as FG, AG, XG, TG and GG gels). The gels were further characterized by texture profile analysis (TPA), bloom strength analysis, Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC).

Analysis of the gel forming solutions (GFS)

Nitrogen solubility index of gelatin

In order to assess the optimum pH for interaction of the polymers, the nitrogen solubility index of gelatin was determined as described earlier by Binsi et al. (2009).

Determination of particle size and zeta potential

Particle size and zeta potential of GFS was determined by Nicomp 380 ZLS laser Diffraction Sizer (Santa Barbara, CA, USA). Particle size distribution was determined for a single concentration of respective gums (1%), from the number-weight distribution of particles suspended in a dispersing medium, using the principles of dynamic light scattering. The measurement was made in triplicates for each GFS sample.

Turbidity analysis

The turbidity of GFS were analyzed using a UV/visible spectrophotometer at 600 nm (Shimadzu UV-1601 UV/Vis

double beam spectrophotometer, Kyoto, Japan). Distilled water was used as a blank reference. All measurements were performed in triplicates.

Viscosity profile

The viscosity measurements were performed using LVDV-III ultra-programmable viscometer (Brook-field, USA). The viscosity was measured at a single concentration of various gums (1%) at 25 °C after maturing the GFSs at 25 °C for 1 h, at shear rate range of 25–250 s⁻¹. The spindle was kept to rotate for 1 min before measuring the viscosity, where the time lapse between every 25 units of shear rate increment was 10 s, and the measurements were made in triplicates. The best-fit model selected on the basis of standard error was found to be power law model and logistic model for different GFS.

The power-law model is explained by

$$\eta = k\gamma^n$$

where η is the viscosity in cP, n is the flow behaviour index (dimensionless) and γ is the shear rate (s⁻¹).

The logistic model is explained by the formula

$$\eta = \frac{\eta_{\infty}}{1 + \sigma_0 * \exp(-k * \gamma)}$$

where η_{∞} is the ultimate viscosity or the viscosity at near Newtonian region, σ_0 is the yield stress (Pa), k is the consistency coefficient (Pa sⁿ).

Colour

The color of GFS was determined by a Hunter Lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). The samples were filled in a 64 mm glass sample cup to a predetermined level and L*, a* and b* parameters were determined. ΔE values were calculated by the software keeping pure gelatin as the standard.

Analysis of composite gel

FT-IR analysis

Infrared study was carried out on freeze-dried gel samples for a single concentration of gum (1%), using a Shimadzu Prestige 21 FT-IR (Schimadzu Corporation, Tokyo, Japan), using KBr pellet method in the wavelength range of 4000–400 cm⁻¹.

Differential scanning calorimetry (DSC)

The gel setting point (T_s), gel melting point (T_m), glass transition (T_g) and melting enthalpy (ΔH_m) of FG and

gelatin gum composite gels at 1% gum concentration were measured by DSC (Mettler Toledo TC15). Known amount of samples were weighed and placed in aluminum pans, heated at the rate of 10 °C/min, from –50 to 250 °C, under inert atmosphere (100 ml/min of N₂) along with an empty pan as a reference. The freezing and melting curves of FG with different gums were generated separately; the maximum peak of temperature of the endothermic peak was taken as T_m , and the area under the endothermic peak as ΔH_m .

Texture profile and Bloom strength analysis

Texture profile analysis and bloom strength of the gels were determined on composite gels containing 3% gelatin and varying concentrations of gums (0.5–1.5% of gelatin) using Lloyds Texture Analyzer (Lloyd Instruments, Model LRX Plus, and UK). Prior to analysis, the gel samples were matured at 4 °C for 24 h. The testing was done immediately after the samples were removed from the refrigerator. The gels were compressed once to 40% of the original height at a compression rate of 0.5 mm/s at ambient temperature. Three measurements were made for each sample. The results of TPA were tabulated using Nexygen software and the results were expressed in terms of hardness (N). The samples for bloom strength analysis were prepared as described previously by Binsi et al. (2009).

Statistical analysis

All the measurements were taken in triplicates and the data was subjected to Analysis of Variance (ANOVA) by SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA) at 5% level of significance. Further, qualitative descriptive analysis (QDA) of selected parameters (Melting temperature, hardness and bloom strength) was performed to identify the best gum among the four gums tested in improving the stability of fish gelatin gel. As part of QDA, principal component analysis was performed using SAS 9.3 (SAS Institute Inc, NC, USA) to identify the most suitable gum to modify the properties of fish gelatin gels.

Results and discussion

Effect of pH on net charge of gelatin as determined by nitrogen solubility index (NSI)

The nature of interaction between two polymers is mostly decided by the net charge on the polymers. Hence, the solubility profile of gelatin at various pH was determined by the nitrogen solubility index method, which indirectly indicates the surface charge distribution of polymers. The

lowest protein solubility was achieved at pH 8.4, which may be the iso-electric point of the gelatin used in the present study (Fig. 1). This further suggests that the gelatin used in this study was Type A gelatin. At pH values near to the iso-electric point, the net surface charge on the polymer is zero. At pH above 8.4, both the polymers carry a net negative charge in the case of anionic gums. This generates a repulsive force between the polymers, thereby preventing network formation. Hence, GFS remained as a high viscous solution during gel maturation. On the contrary, as the pH moved away from iso-electric point to acidic range, phase separation was evident after ageing the gels at 4 °C. This may be as a result of the strong electrostatic interaction between the positively charged gelatin and negatively charged anionic gums, yielding visible coacervates (phase separation). At pH 8, GFS yielded a strong gel (as assessed by hardness values obtained by texture profile analysis, data not given) without any visible phase separation after ageing the GFS for 24 h at 4 °C. At pH 8, gelatin carries net positive charge which is just sufficient for its optimum electrostatic interaction with anionic polymer. Hence in the present study, pH 8 was opted for gel preparation and further analysis.

Analysis of gel forming solutions of gelatin and gelatin-gum blends

Determination of particle size and zeta potential of gelatin-gum GFS

The gelatin-gum composite GFS exhibited higher particle size as compared to that of pure gelatin indicating certain extent of interaction between gums and gelatin (Table 1). AG and TG exhibited almost similar values as that of FG, while XG and GG showed significantly higher values of mean diameter. The reason for this might be that xanthan and guar gums possess high proportion of polar groups that induce the formation of a hydration sphere around the

polymer chain, resulting in higher mean diameter. Zeta potential, which is the electro-kinetic potential at the slip plane of moving particles, was determined for better understanding of gelatin-gum interactions and complex formation. The more pronounced zeta potential values, whether positive or negative, tend to stabilize particle suspension in a better way (RaviKumar et al. 2004). It is the charge on the particle's mobile surface and is used to determine the degree of flocculation or de-flocculation in nanosystems. The pH at which zeta potential value is zero is often regarded as iso-electric point (IEP). The zeta potential of FG at pH 8 was found to be +0.22 mV, which almost coincided with the pH of lowest solubility of gelatin as determined by the NSI method. The mild electro-positive nature is attributed to the amino groups on gelatin that are positively charged below IEP. The most intensive interaction between the polymers occurs when the electrical charge of the mixtures is nearly neutralized. In the present study, the zeta potential of gelatin with all the anionic polymers registered higher positive value as compared to pure gelatin solution. This suggests a possible conformational change in gelatin molecules upon interacting with gum macromolecules, exposing more residues of positive charge to the bulk phase. On the other hand, the non-ionic guar gum registered a net negative zeta potential, suggesting that guar gum being highly hydrophilic in nature, might have altered the hydration sphere of gelatin molecules, by deriving more H⁺ ions from the solution.

Effect of gums on the turbidity of gelatin-gum GFS

The turbidity of a mixed biopolymer system is often a consequence of phase separation resulting in the formation of particles that scatter light (Schmitt et al. 2009). Turbidity measurements on gelatin-gum GFS can provide information about electrostatic complex formation between the two biopolymers. The pure gelatin GFS was almost transparent at pH 8 (0.09 OD) (Table 1). The turbidity value of gelatin-gum GFS, on the other hand, increased linearly with gum concentration, which is likely due to the formation of light scattering polymer network, upon cooling. The turbidity data does not reveal whether turbidity was caused by the formation of insoluble aggregates or by viscous sol. XG showed highest turbidity value compared to all other gelatin-gum blends which may be attributed to the higher molecular weight and higher charge density of XG along with the sufficient anionic charge to interact with FG. On the other hand, TG exhibited low turbidity, which may be due the lower molecular weight and the formation of soluble complexes which have lesser light scattering properties. The turbidity values indicate the nature of network formation and growth mechanism of electrostatic complex, which are primarily governed by electrostatic

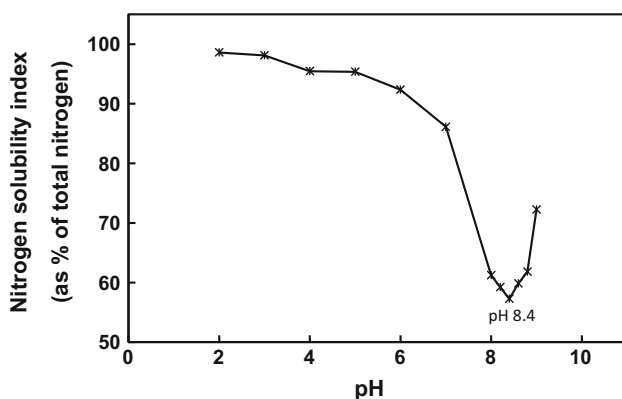


Fig. 1 Nitrogen solubility index of fish gelatin in distilled water

Table 1 Physico-chemical properties of composite gel forming solutions

Particle size analysis (Based on Nicomp number-weight distribution)					
Sample	Zeta potential (mw)	Particle size (nm)		Mean distribution (%)	
FG*	0.22	12.7		100.0	
AG 1%	20.84	11.2		90.3	
		31.8		9.70	
XG 1%	44.48	52.1		89.3	
		122.9		10.7	
		657.7		0.10	
TG 1%	9.55	10.7		98.9	
		42.5		1.10	
GG 1%	-6.63	114.6		99.9	
		930.2		0.10	
Turbidity and color parameters of gel forming solutions					
	Turbidity (A _{660 nm})	L*	a*	b*	ΔE
FG	0.09	4.34	-0.23	-1.11	
AG					
0.5%	0.18	7.55	-0.21	-3.41	3.94
1.0%	0.25	7.59	-0.24	-2.44	3.51
1.5%	0.36	6.65	0.04	-0.58	2.38
XG					
0.5%	0.29	24.51	-0.77	-4.49	20.45
1%	0.50	40.1	-1.07	-3.14	35.82
1.5%	0.81	48.31	-1.45	-1.61	43.98
TG					
0.5%	0.15	7.17	-0.22	-3.71	3.84
1%	0.23	8.01	-0.18	-2.94	4.10
1.5%	0.29	8.47	-0.14	-1.8	4.18
GG					
0.5%	0.25	12.3	-0.65	-2.91	8.17
1%	0.33	15.15	-0.75	-3.27	11.03
1.5%	0.67	24.81	-1.27	-2.55	20.54

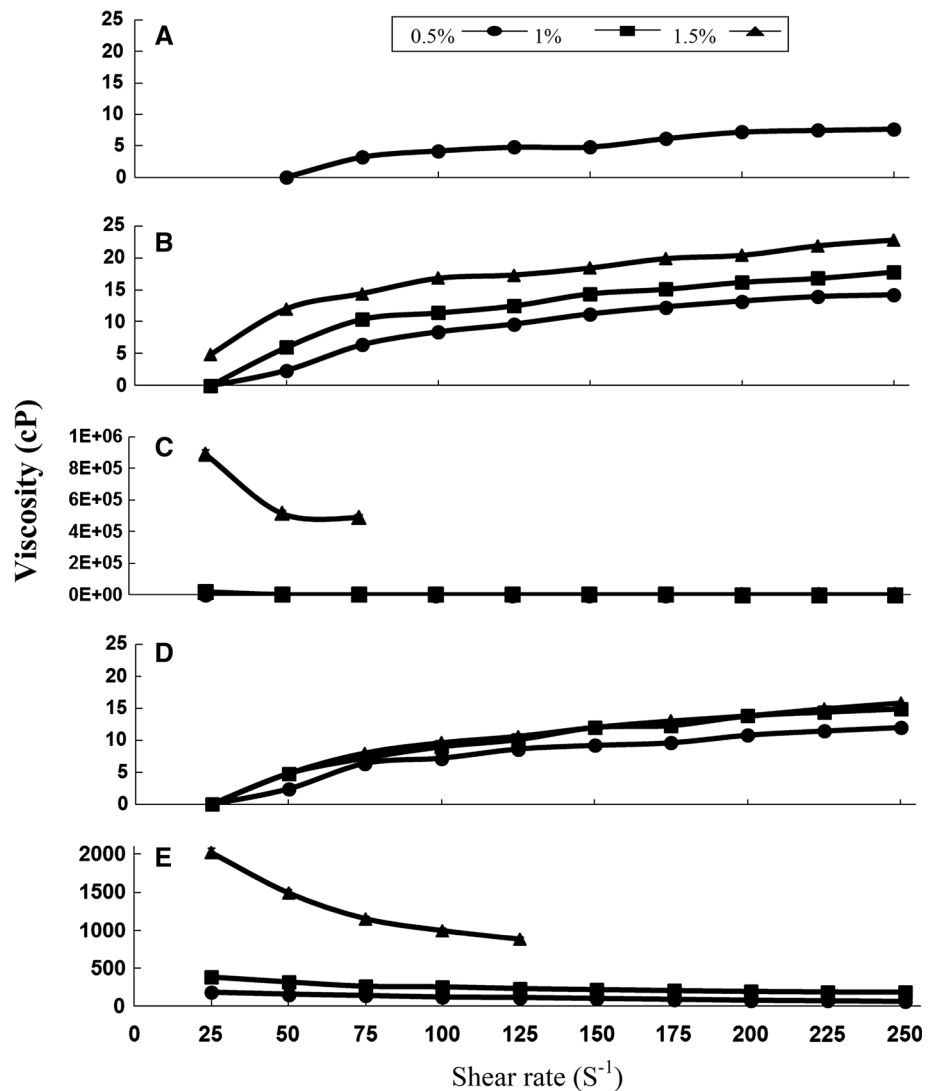
attraction between the polymers involved. Paulsson et al. (1990) reported that transparent protein gels consists of a well-ordered homogeneous network, whereas non-transparent gels consists of randomly ordered colloidal networks or aggregates. The results of turbidity measurements were in agreement with that of particle size analysis.

Apparent viscosity measurement of gelatin-gum GFS

Changes in the viscosity of gelatin-gum GFS as a function of concentration and speed of rotation indicated non-Newtonian behavior induced by structural deformation during rotation (Fig. 2). As the viscosity values were found to be strongly dependent on speed of rotation, the viscosity determined is described as 'apparent viscosity'. Generally, viscosity of gelatin is determined at a concentration of

6.67% (w/v). However, in the present study, concentration of gelatin was maintained at 3% (w/v), as both interaction between gelatin and various gums, as well as bulking effect of different gums were expected. The apparent viscosity profile of FG solution indicated non-Newtonian behavior with shear thickening effect and power law was found to be suitable to predict the flow behavior. A shear thinning and Newtonian-behavior for fish gelatin extracted by conventional hot water extraction method has been reported in earlier studies (Marcotte et al. 2001; Binsi et al. 2009); however, shear thickening behavior observed in the present study is an unusual behavior. The probable reason is that, the gelatin used in the present study was extracted by microwave assisted heating method, where certain extent of re-organization and cross-linking of polymer chains are expected. On further shearing, these polymers might have

Fig. 2 Viscosity profile of
a Fish Gelatin GFS 1%.
b Gelatin-Arabic gum GFS 1%.
c Gelatin-Xanthan gum GFS.
d Gelatin-Tragacanth gum GFS
 1%. **e** Gelatin-Guar gum GFS
 1%



formed a transient weak three dimensional network with interpolymer junction zones imparting a pre-gelling effect to GFS. However, addition of gums resulted in distinctly different behaviors depending on the nature of the gums and concentration used, with a linear increase in apparent viscosity with increase in the concentration of gums in the GFS. Addition of AG and TG resulted in higher viscosity values without altering the shear thickening behavior of pure gelatin solution with characteristic flow behavior index (n) values of >1 (Table 2). On the other hand, GG and XG blends were characterized by an initial high yield stress value and thereafter showing shear thinning/pseudoplastic behavior followed by a plateau region with near-Newtonian behaviour referred as ‘ultimate viscosity’. Hence a logistic model was derived to explain the flow behavior of XG and GG blends. The ultimate viscosity of GFS were found to be increasing with increase in the concentration of gums. Similarly, the consistency

coefficient (k) of fish gelatin increased with the addition of various gums. However, higher values were shown by power-law fluids. The yield stress values exhibited at the beginning of the rotation in XG and GG was attributed to the first stage of unbranching and extensive hydration. Rheologically, XG and GG are categorized as non-gelling gums, as they form weak junction zones (Saha and Bhattacharya 2010). Hence, the shear thinning behavior observed in XG and GG might be related to the higher rate of disruption of weak entanglements, compared to the formation of new entanglements during shearing. Xanthan gum is characterized by a highly branched skeleton stabilized primarily by weak Van der Waal’s forces, which undergoes unwinding and structural reorganization during shearing or rotation. This permits the formation of a temporarily hydrated cluster of static nature, which is primarily caused by the intermolecular association due to acetate residues. Previously, Szczesniak (1986) indicated the

Table 2 Rheological model parameters for different gel forming solutions

Sample	Concentration of gum (%)	η_{∞} (cP)	Yield stress (σ_0) (Pa)	Consistency Coefficient (k)	Flow behaviour index (n)	Regression coefficient (R^2)
Power-law model						
FG ^a	0.0	–	–	0.03	1.98	0.96
AG	0.5	–	–	0.47	1.14	0.95
	1.0	–	–	1.78	1.67	0.97
	1.5	–	–	2.58	1.47	0.99
TG	0.5	–	–	0.19	1.76	0.96
	1.0	–	–	0.28	1.73	0.97
	1.5	–	–	0.30	1.73	0.97
Logistic model						
XG	0.5	995.90	16,753.60	0.05	–	0.99
	1.0	1138.80	119,677.0	0.09	–	0.98
	1.5	1432.10	292,744.0	0.11	–	0.99
GG	0.5	9.81	188.0	0.005	–	0.99
	1.0	153.00	284.60	0.01	–	0.98
	1.5	176.20	2292.40	0.09	–	0.98

^a FG Fish Gelatin GFS, AG Gelatin-Arabic gum GFS, XG Gelatin-Xanthan gum GFS, TG Gelatin-Tragacanth gum GFS, GG Gelatin-Guar gum GFS

appearance of a yield stress followed by shear-thinning as a typical behavior of xanthan gum. Unlike the branched structure of xanthan gum, guar gum has a basic straight chain structure of galactomannan molecule with regularly arranged single membered galactose branches. It is highly hydrophilic in nature, with 137 substantially polar surface hydroxyl groups (Vijayendran and Bone 1984), and hence can confer an unusual effect on other hydrated colloidal systems through hydrogen bonding. A distinct loss of network rigidity during higher shear rates was evident in GG, as indicated by a shear thinning behavior. As the speed of rotation increases, the static structure formed at rest will be destroyed and the molecules will be orientated parallel to each other, permitting more inter-molecular sliding resulting in lowering of viscosity. Previously, Sahin and Ozdemir (2004) observed a similar shear thinning behavior of guar gum and xanthan gum when added to ketchup, with a higher yield stress value for xanthan gum.

Color measurement of gelatin-gum GFS

Distinct differences were observed in the color parameters of FG gel and the different gelatin-gum composite gels (Table 1). Color of dilute solutions can be attributed to hues resulting from colloidal substances or completely dissolved solutes. This is particularly important as the gums used in the present study exhibited wide variations in modifying the transparency of GFS, as indicated by the results of turbidity measurement. Among the various gums,

AG and TG yielded clear and transparent GFS, whereas xanthan and guar gums gave viscous translucent-opaque sols having a marked effect on the light scattering property of the solution due to larger particle size. FG gel gave L^* value very close to that of distilled water indicating that the gel was almost colorless and transparent. This was apparent from turbidity measurements where low absorbance values were observed for FG gel. Similarly, AG and TG also showed low lightness values close to FG, and was least affected by increase in gum concentration. On the other hand, XG showed 6–12 fold increase in lightness values compared to that of pure gelatin GFS, when the concentration was increased from 0.5 to 1.5% in GFS, which might be due to the high molecular weight and greater light scattering property of XG blends. AG showed moderate increase in L^* value in the range of three to sixfold at similar concentrations, which may be attributed to its ability to swell and confer high viscosity to aqueous solutions.

Addition of gums resulted in marked difference in the chroma parameters such as a^* and b^* values of the GFS. FG yielded a GFS with— a^* value indicating the green chroma, which showed a gradual shift towards the red chroma on the addition of AG and TG. On the other hand, XG and GG further increased the greenness of GFS as the concentration increased. All the GFS showed— b^* values indicating a shift towards blue chroma. The b^* value of FG increased drastically with the addition of gums at 0.5% level, however showed a reduction in blueness with

increase in concentration. This may be due the light scattering effect of colloidal particles in composite GFS as indicated by the highest value shown by XG.

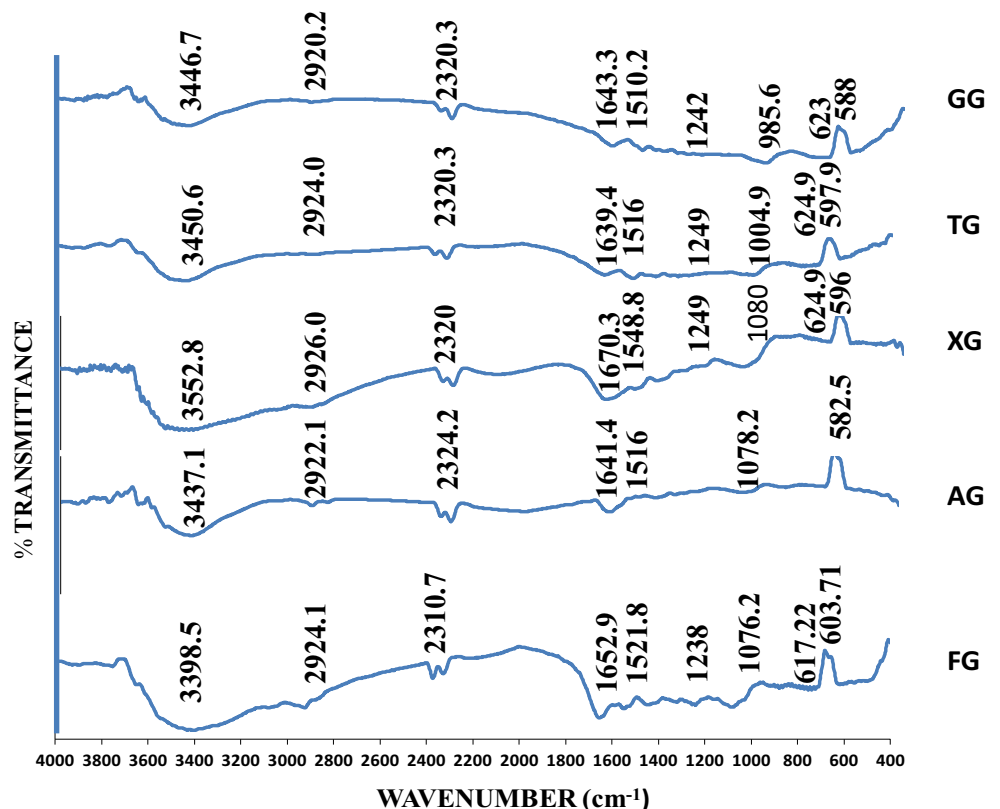
Analysis of gelatin-gum composite gels

FT-IR spectra of gelatin-gum composite gels

In order to understand the nature of interaction between gelatin and various gums, FT-IR spectroscopic analysis of gelatin and composite gels were carried out (Fig. 3). A typical FT-IR spectrum of fish gelatin showed three major peak regions marked as 1 (3600–2700 cm^{-1}), 2 (1900–900 cm^{-1}), and 3 (400–900 cm^{-1}). The regions assigned to the bonds are Amide A and B; Amide I, II and III; and Amide IV, V and VI, respectively (Kong and Yu 2007). Among these absorption bands, the Amide-I band between 1600 and 1700 cm^{-1} and Amide-II between (1565 and 1520 cm^{-1}), are the most useful peaks for infrared analysis of the secondary structure of proteins like gelatin (Muyonga et al. 2004). In the present study, the gelatin gum composite gels were freeze-dried before analysis in order to avoid the interference from water molecules. FG exhibited Amide-I band at 1652.9 cm^{-1} and Amide-II band at 1521 cm^{-1} . Amide-I represents C=O stretching/hydrogen bonding coupled with COO^- and Amide-II arises from bending vibration of N–H groups and stretching vibrations

of C–N groups. The exact location of Amide-I band depends on the hydrogen bonding in the polypeptide chain and the protein conformation (Uriarte-Montoya et al. 2011). Yakimets et al. (2005) stated that the absorption peak at 1633 cm^{-1} is characteristic of the random coiled structure of gelatin. The frequency range of 1660–1650 cm^{-1} is characteristic of α -helical and 1640–1620 cm^{-1} of β -sheet structures (Hashim et al. 2010). In the present study, Amide-I band was evident at this region, suggesting that FG retained the triple helical structure to a certain extent during microwave extraction. It is also possible that the microwave-extracted gelatin contained a significant amount of intermolecular cross-links, either present in the native collagen or introduced as a result of exposure to microwave radiation. In conventional hot water extraction method, the conversion of collagen to gelatin is generally achieved by the hydrolysis of intermolecular cross-links in the triple helical structure of collagen. However, in microwave extraction method, solubilisation of collagen might have occurred by intra-chain cleavage of peptide bonds, as a result of vibrational force of water molecules coupled with heat energy supplemented during extraction. It is also logical to infer that gelatin short peptides or collagen like peptides might have undergone self-assembly during freeze-drying process which were responsible for the high helical content of pure gelatin observed in the present study. Interestingly, in

Fig. 3 FT-IR spectra of gelatin and gelatin-gum composite gels where (FG) Fish Gelatin GFS 1% (AG) Gelatin-Arabic gum GFS 1% (XG) Gelatin-Xanthan gum GFS 1% (TG) Gelatin-Tragacanth gum GFS 1% (XG) Gelatin-Guar gum GFS 1%



composite gels, this band had undergone a prominent shift. This might be because the gums prevented the self-assembly of gelatin during drying either by interacting with gelatin through hydrogen bonding or by way of occupying the voids between the polypeptide chains. The most pronounced shift in Amide-I band was evident in TG and XG composites compared to AG and GG gels. Except XG, all the other gums showed a shift towards lower wavelength region whereas XG showed a peak at 1670.3 cm^{-1} . This suggests that, apart from the well-known electrostatic interaction between amino group of gelatin and carboxylic group of anionic gums, gelatin could have undergone structural re-arrangements in the presence of various gums. Similar pattern was observed for Amide-II band also, where XG showed a major shift towards higher wavelength of 1548.8 cm^{-1} , whereas the other gums showed minor shift towards lower wavelength region. It was inferred that xanthan gum, owing to its structural complexity and high hydrophilicity, might have occupied the interstitial spaces between the gel networks, facilitating the formation of weak hydrogen bridges. Additionally, FG exhibited a minor peak of Amide-III band at 1238.29 cm^{-1} , which was almost unresolved in composite gels. The low intensity peak observed for Amide-III band in composite gels indicates some loss in helicity of native gelatin. The Amide-III represents the combination peaks between C–N stretching vibrations and N–H deformation from amide linkages as well as absorptions arising from wagging vibrations from CH_2 groups from the glycine backbone and proline side-chains (Jackson et al. 1995). The ratio between the Amide-III band ($1245\text{--}1240\text{ cm}^{-1}$) and Amide-I band ($1660\text{--}1650\text{ cm}^{-1}$) was lower in TG, AG and GG as compared to pure gelatin, indicating conformational rearrangement of pure gelatin in the presence of these gums. On the other hand, XG showed considerably higher values compared to FG, suggesting that the native structure of gelatin was unaltered in the presence of xanthan gum. In general, the addition of different gums to fish gelatin introduced structural changes in the gel network to various degrees, depending on the type of gum added. Shift of Amide-I, II and III bands to lower wave numbers, increase in intensity of Amide-III and broadening of Amide-I are therefore associated with increased intermolecular interactions (by hydrogen bonding) in collagen (Muyonga et al. 2004). Accordingly in the present study, interaction between the gelatin and the gums were evident in TG, AG and GG composite gels.

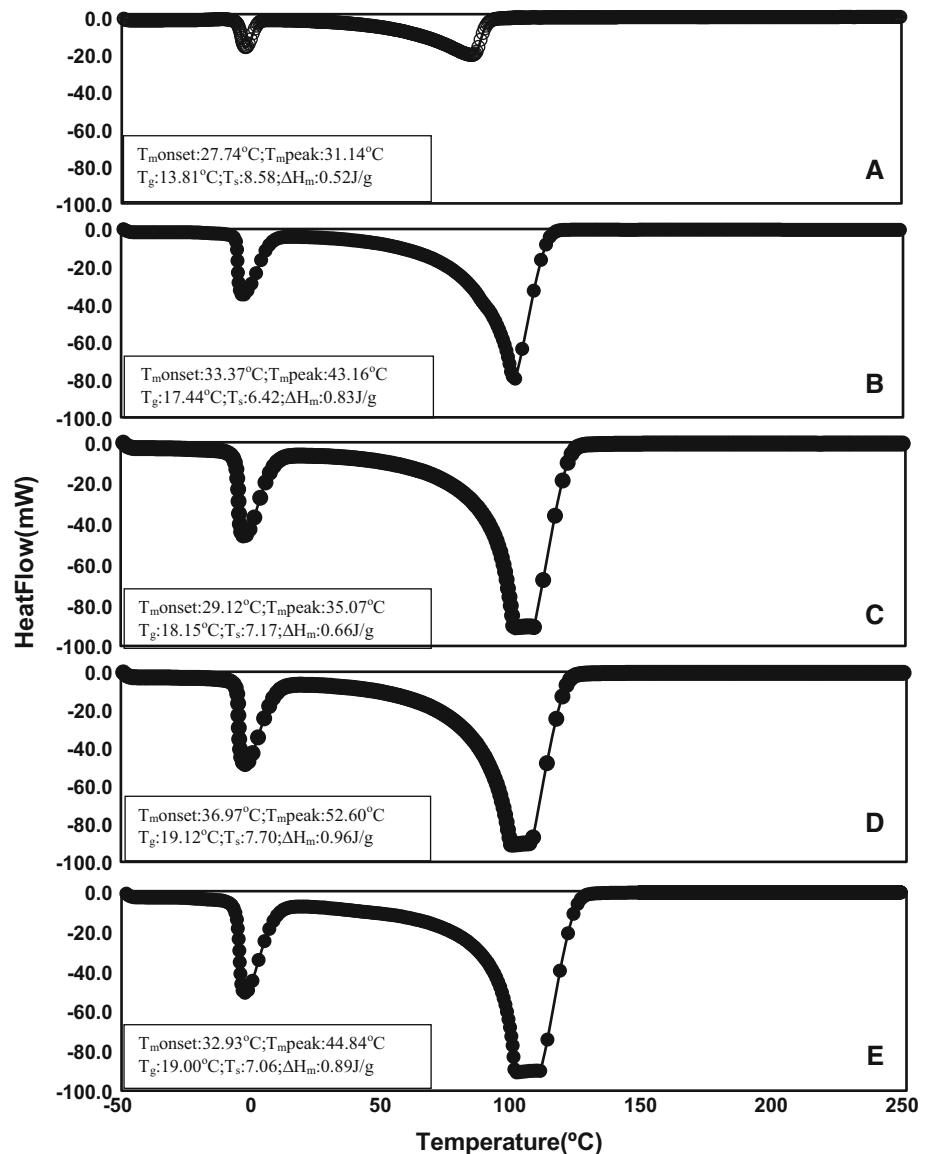
The Amide-A and Amide-B bands were also distinct in the FT-IR pattern of gelatin and composite gels. The pure gelatin exhibited a much broader Amide-A band compared to composite gels. The Amide-A band of pure gelatin, which appeared at 3398 cm^{-1} , was shifted to the higher wavelength in all the composite gels. The maximum shift

was shown by XG, and the minimum by AG composite gels. Amide-A represents NH-stretching coupled with hydrogen bonding. Normally, a free N–H stretching vibration is found in the range of $3400\text{--}3440\text{ cm}^{-1}$ (Muyonga et al. 2004). When the N–H group of a peptide is involved in a hydrogen bond, the position shifts to lower frequencies (Doyle et al. 1975). The shift towards higher wavelength range observed in the present study may be indicative of the presence of more exposed amino group in the composite gels which were involved in hydrogen bond in the gel matrix. The Amide-B band was observed at 3078.39 cm^{-1} in pure gelatin. A minimum shift in this wavelength range was observed in all the composite gels. An asymmetric peak in the wavelength region of $2922\text{--}2926\text{ cm}^{-1}$ was observed for FG and composite gels, which represents C–H stretching vibrations of the $-\text{CH}_2$ groups (D'Souza et al. 2008). A sizeable peak observed at 1076.2 cm^{-1} in FG underwent minimum band shift in AG and XG, while notably shifted to lower wavelength region in TG and GG. Absorption in this region is attributed to C–O vibration due to carbohydrates (Jackson et al. 1995). In pure gelatin, this effect may be due to pentosidine cross-links (Kent et al. 1985).

Differential scanning calorimetric analysis of gelatin-gum composite gels

In brief, the DSC thermograms were characterized by three endothermic peaks corresponding to gel setting/freezing, gel melting and polymer degradation zones (Fig. 4). Additionally, all gels showed a vague glass transition point (T_g), which is the temperature at which the polymer softens (changes from the glassy state to the rubbery state, at a given heating rate) because of the onset of long-range coordinated molecular motion of disordered (amorphous) structure (Sperling 2006). T_g of all the composite gels were significantly higher than FG gels ($p < 0.05$), suggesting a considerable change in melting properties of gelatin by the addition of different gums. This may be explained in terms of intermolecular cross-links formed between gelatin and gums by way of electrostatic interactions or by modifying the viscosity of gelling system which limits the fluidity of the gel. The former mechanism may be applicable to anionic gums such as xanthan, tragacanth and gum arabic, whereas the latter may be more appropriate for non-ionic guar gum. There was a distinct difference in the onset of melting as well as the peak melting temperature of FG and the composite gels, which was more likely due to the difference in the extent of helix-coil transition of gelatin molecule and intermolecular polymer dissociation. The melting curve of all the gels were characterized by two prominent transitions, representing the onset of melting and the peak of melting of the gels. The onset temperature of

Fig. 4 DSC thermograms of gelatin and gelatin-gum composite gels where **a** Fish Gelatin GFS 1%. **b** Gelatin-Arabic gum GFS 1%. **c** Gelatin-Xanthan gum GFS. **d** Gelatin-Tragacanth gum GFS 1%. **e** Gelatin-Guar gum GFS 1%



melting (T_{onset}), is defined by the extrapolated origin of the curve, being defined by the point of intersection of the tangent with the point of maximum slope, on the principal side of the peak with the base line extrapolated. The T_{onset} values of all the composite gels showed distinctly higher values for TG which was almost 10 °C higher than that of FG, and closely followed by AG and GG. In this study, the maximum of the endothermic peak in the thermogram was considered as the melting temperature (T_m) of the gels. Accordingly, the T_m exhibited by the various gel was in the order of TG > AG > GG > XG > FG. In the present study, the T_m of FG (31.14 °C) was higher than the values reported previously for fish gelatin and bovine gelatin (Cheow et al. 2007) even though the concentration of fish gelatin used was less than half of bovine gelatin. This might be related to the microwave assisted extraction

protocol used in the present study, wherein certain degree of gelatin cross-linking may be expected. Incorporation of gums further extended the melting point, which might be associated with the formation of a more compact protein network and generation of more junction zones.

The difference between T_m and T_{onset} , also gives an indication of the nature of structure built-up in the gelling system and indirectly the stability of the gel during slow heating. Among the four gums, maximum difference was exhibited by TG (15.63 °C), closely followed by GG (11.91 °C) and AG (9.79 °C), whereas XG registered a value (5.95 °C) close to that of FG (3.40 °C). This may be explained on the basis of polymer interaction effect and viscosity modifying effect of various gums. TG and AG being anionic in nature interacted with cationic groups of gelatin and formed a stable polymer network. On the

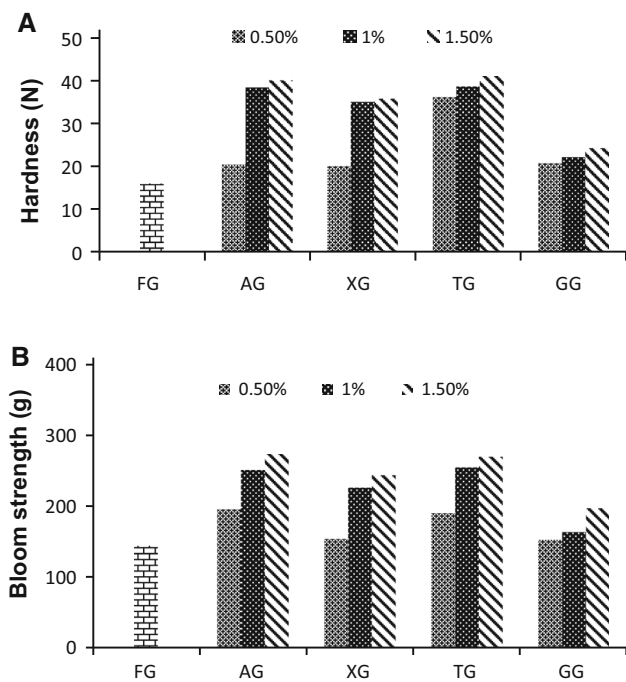


Fig. 5 **a** Texture profile of fish gelatin and gelatin-gum composite gels as a function of gum concentration. **b** Bloom strength values of fish gelatin and gelatin-gum composite gels as a function of gum concentration, where *FG* Fish gelatin gel, *AG* Gelatin-Arabic gum gel, *XG* Gelatin-Xanthan gel, *TG* Gelatin-Tragacanth gel, *GG* Gelatin-Guar gum gel

contrary, the possible mechanism for GG in enhancing the thermal characteristics of gelatin gel is the destabilization of the helical structure of gelatin molecules by reorienting the structural water in the gelatin molecule. This in turn results in the intermolecular entanglement between gelatin and gum polymers imparting ‘pseudo-junction zones’. On the other hand, the high molecular weight XG imparted void filling and viscosity modifying effect rather than contributing to polymer cross-linking. This was further reflected in the setting behavior of fish gelatin, which showed lower T_s values for composite gels, in the order of $FG > TG > XG > GG > AG$. It appears that, both viscosity modifying and cross-linking mechanism delays the setting phenomenon of gelatin gels at lower temperatures, probably due to the easy rearrangement of water molecules in pure gelatin gels owing to the vacant voids. In composite gels, the crystallization of water molecule is limited by the presence of hydrophilic gums, which retain the hydration layer around the polymer chains and limit crystallization.

Melting of gelatin and composite gels are mainly associated with the helix-coil transition of native gelatin molecule and the rupture of intra- and inter-polymer hydrogen bonds. Hence, the values of the denaturation enthalpy associated with the endothermic peaks may be related to the relative amount of inter polymer cross-linking and the extent of conformational changes undergone by

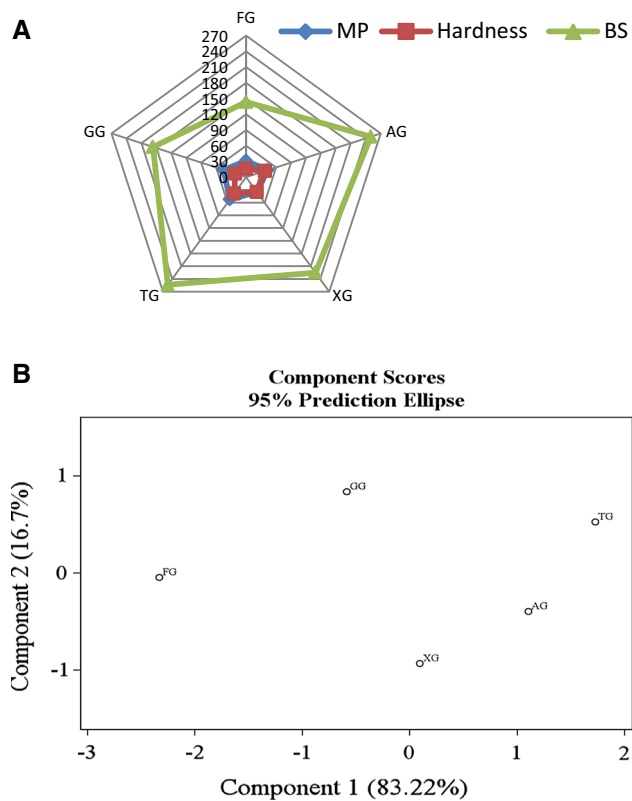


Fig. 6 **a** Qualitative descriptive analysis plot of different gelatin-gum composite gels. **b** Principal component analysis plot of different gelatin-gum composite gels

native gelatin molecule in the presence of various gums. The DSC plots of pure gelatin gel and composite gels indicated distinctly higher ΔH values for TG gels, indicating the higher thermal stability of gelatin-tragacanth network. AG and GG showed almost similar values, while XG showed significantly lower value ($p < 0.05$).

Strength of gelatin-gum composite gels

Gelatin gels are inherently soft and flexible and the strength of gelatin gels is dependent on the gelatin concentration, with little effect of ionic strength and pH (Papageorgiou et al. 1994). The FG gel showed a hardness value of 15.94 N (Fig. 5a) and a bloom strength value of 123.5 g at 3% gelatin concentration (Fig. 5b). Previously, a hardness value of 41 N was reported for bovine gelatin, 30 N for porcine and 5 N for fish skin gelatin gels at 10% gelatin concentration (Shafiqur Rahman and Al-Mahrouqi 2009). Similarly, a bloom strength value of value of 227.2 g for bovine skin gelatin and 181.3 g for common carp skin gelatin was reported at 6.67% gelatin concentration (Ninan et al. 2014).

The synergistic effect of gums on the gelation characteristics of FG was evaluated as a function of concentration

of various gums. The results indicated a sharp increase in the hardness values for all the composite gels, except for GG, when the concentration of gums increased from 0.5 to 1%. The highest score for hardness was obtained for TG, closely followed by AG, which were almost twice as that as obtained for FG gel (Fig. 5a). XG exhibited moderately high hardness values, whereas GG registered remarkably lower hardness value. However, the increase was minimum for further increase in concentration to 1.5%. Almost similar trend was observed for bloom strength values, as well (Fig. 5b). Hence, the structural and thermal characteristics of the composite gels were analysed only for 1% gum concentration. However, in the case of GG, the texturisation effect was more prominent at 1.5%. This may be because, at higher concentration, the gelatin network may be trapped within the increasingly viscous GG sol, making the gelatin gum blend more solid-like. There have been previous studies reporting the use of polysaccharides to improve the gelation and functional characteristics of fish gelatin, e.g. gellan (Fonkwe et al. 2003), and *k*-Carrageenan (Pranoto et al. 2007), wherein addition of hydrocolloids improved the texture of the fish gelatin gels. The texture stabilization of composite gels observed in the present study may be by way of introducing of more polymer junction zones, thereby minimizing syneresis of the gel. Anionic hydrocolloids interact with the positive charges on the surface of gelatin to strengthen the gelatin network. They also bind the water molecules in the interstitial spaces, and thereby reduce syneresis. Similar findings were reported by Hansen (1993) wherein anionic hydrocolloids (e.g. CMC, pectin, λ -Carrageenan) reduce syneresis in dairy foods by interacting with the positive charges on the surface of casein micelles to strengthen the casein network. The moderately high value exhibited by XG might be related to the high viscosity at rest, as indicated by high yield stress value in viscosity profile data. The strength of GG gel was the least, which might be attributed to its non-ionic nature that helps only in increasing the viscosity of the blend and introducing pseudo-junction zones in the gelatin matrix without bond formation. These junction zones can be easily slipped-off under compressive force, as applicable during texture measurement.

Based on qualitative descriptive analysis (QDA), gum TG was found to be superior in improving the stability of fish gelatin gel, compared to other three gums evaluated, even though AG exhibited values very close to that of TG (Fig. 6). Principal component analysis was also performed as part of QDA. It was evident from the plot of principal component 1 and 2, which explained almost 99% of total variability, that TG was superior to other gums followed by AG and XG (Fig. 6B).

Conclusion

Among the various gums analyzed as modifiers of gelation characteristics of microwave extracted fish gelatin, TG, AG, and XG were found to be effective in enhancing the hardness of the gelatin gel, whereas TG, AG and GG improved the thermal stability of fish gelatin gel. The results of the present study suggest that the behavior of composite gels under compression and thermal stress vary depending on the nature of interaction between gelatin and individual gums. In conclusion, natural gums are ideal for modifying the gelation and thermal characteristics of fish gelatin and hence can find application in a wide variety of gelatin based food products for modification of textural and functional performance of gelatin at ambient temperature.

Acknowledgements The authors would like to thank Director, ICAR-CIFT, Cochin for providing the facilities to undertake this work. This work was carried out under a research project funded by Ministry of Food Processing (Grant No. SERB/MOFPI/0038/2013), Govt. of India through Science and Engineering Research Board (SERB) of Department of Science and Technology (India).

References

- Akagunduz Y, Mosquera M, Giménez B, Aleman A, Montero P, Gomez-Guillen MC (2014) Sea bream bones and scales as a source of gelatin and ACE inhibitory peptides. *LWT Food Sci Technol* 55:579–585
- Binsi PK, Shamasundar BA, Dileep AO, Badii F, Howell NK (2009) Rheological and functional properties of gelatin from the skin of Bigeye snapper (*Priacanthus hamrur*) fish: influence of gelatin on the gel-forming ability of fish mince. *Food Hydrocoll* 23(1):132–145
- Chandra MV, Shamasundar BA (2015) Rheological properties of gelatin prepared from the swim bladders of freshwater fish *Catla catla*. *Food Hydrocoll* 48:47–54
- Chandra MV, Shamasundar BA, Kumar PR (2013) Visco-elastic and flow properties of gelatin from the bone of freshwater fish (*Cirrhinus mrigala*). *J Food Sci* 78(7):E1009–E1016
- Cheow CS, Norizah MS, Kyaw ZY, Howell NK (2007) Preparation and characterisation of gelatins from the skins of sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*). *Food Chem* 101(1):386–391
- D'Souza L, Devi P, Shridhar MPD, Naik CG (2008) Use of Fourier transform infrared (FTIR) spectroscopy to study cadmium-induced changes in *Padina tetrastratica* (Hauck). *Anal Chem Insights* 3:135
- Doyle BB, Bendit EG, Blout ER (1975) Infrared spectroscopy of collagen and collagen-like polypeptides. *Biopol* 14(5):937–957
- Fengxing Z, Zhang W, Shiyong X (2008) Study on the fish scale gelatin extracting technology. *Food Ferment Ind* 9:030
- Fonkwe LG, Narsimhan G, Cha AS (2003) Characterization of gelation time and texture of gelatin and gelatin–polysaccharide mixed gels. *Food Hydrocoll* 17(6):871–883
- Haihua C, Ju L (2009) Application of response surface methodology for microwave-assisted extraction optimization of Alaska Pollock fishskin gelatin. *Food Ferment Ind* 8:066

- Hansen PMT (1993) Food hydrocolloids in the dairy industry. In: Nishinari K, Doi E (eds) Food hydrocolloids: structures, properties and functions. Plenum Press, New York, pp 211–224
- Hashim DM, Man YC, Norakasha R, Shuhaimi M, Salmah Y, Syahariza ZA (2010) Potential use of Fourier transform infrared spectroscopy for differentiation of bovine and porcine gelatins. *Food Chem* 118(3):856–860
- Jackson M, Choo L, Watson PH, Halliday WC, Mantsch HH (1995) Beware of connective tissue proteins: assignment and implications of collagen absorptions in infrared spectra of human tissues. *Biochem Biophys Acta* 1270:1–6
- Kent MJC, Light ND, Bailey AJ (1985) Evidence for glucose-mediated covalent cross-linking of collagen after glycosylation in vitro. *Biochem J* 225:745–752
- Kong J, Yu S (2007) Fourier transform infrared spectroscopic analysis of protein secondary structures. *Acta Biochem Biophys* 39(8):549–559
- Marcotte M, Hoshahili ART, Ramaswamy HS (2001) Rheological properties of selected hydrocolloids as a function of concentration and temperature. *Food Res Int* 34(8):695–703
- Mori H, Tone Y, Shimizu K, Zikihara K, Tokutomi S, Ida T, Ihara H, Hara M (2013) Studies on fish scale collagen of Pacific saury (*Cololabissaira*). *Mater Sci Eng, C* 33(1):174–181
- Muyonga JH, Cole CGB, Duodu KG (2004) Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (*Lates niloticus*). *Food Chem* 86(3):325–332
- Ninan G, Joseph J, Aliyamveetil ZA (2014) A comparative study on the physical, chemical and functional properties of carp skin and mammalian gelatins. *J Food Sci Technol* 51(9):2085–2091
- Papageorgiou M, Kasapis S, Richardson RK (1994) Steric exclusion phenomena in gellan/gelatin systems I: physical properties of single and binary gels. *Food Hydrocoll* 8(2):97–112
- Park JH, Choe JH, Kim HW, Hwang KE, Song DH, Yeo EJ, Kim HY, Choi YS, Lee SH, Kim CJ (2013) Effects of various extraction methods on quality characteristics of duck feet gelatin. *Korean J Food Sci Animal Res* 33(2):162–169
- Paulsson M, Dejmeck P, Van Vliet T (1990) Rheological properties of heat-induced β -lactoglobulin gels. *J Dairy Sci* 73(1):45–53
- Pranoto Y, Lee CM, Park HJ (2007) Characterizations of fish gelatin films added with gellan and κ -carrageenan. *LWT-Food Sci Technol* 40(5):766–774
- Pranoto Y, Istigani M, Santoso U, Lestari LA, Erwanto Y, Rohman A (2016) Physicochemical properties of gelatin extracted from filvelined threadfin bream (*Nemipterus tambuloides*) skins. *KnE Life Sci* 3(3):92–97
- RaviKumar MNV, Mohapatra SS, Kong X, Jena PK, BakowskyU Lehd CM (2004) Cationic poly (lactide-co-glycolide) nanoparticles as efficient in vivo gene transfection agents. *J Nanosci Nanotechnol* 4(8):990–994
- Saha D, Bhattacharya S (2010) Hydrocolloids as thickening and gelling agents in food: a critical review. *Food Sci Technol* 47(6):587–597
- Sahin H, Ozdemir F (2004) Effect of some hydrocolloids on the rheological properties of different formulated ketchups. *Food Hydrocoll* 18:1015–1022
- Schmitt C, Bovay C, Vuilliomenet AM, Rouvet M, Bovetto L, Barbar R, Sanchez C (2009) Multiscale characterization of individualized β -lactoglobulin microgels formed upon heat treatment under narrow pH range conditions. *Langmuir* 25(14):7899–7909
- Shafiqur Rahman M, Al-Mahrouqi AI (2009) Instrumental texture profile analysis of gelatin gel extracted from grouper skin and commercial (bovine and porcine) gelatin gels. *Intern J Food Sci Nutr* 60(7):229–242
- Somboon N, Karrila TT, Kaewmanee T, Karrila SJ (2014) Properties of gels from mixed agar and fish gelatin. *Int Food Res J* 21(2):485–492
- Sperling LH (2006) Introduction to physical polymer science, 4th edn. Wiley, New York
- Sworn G (2000) Xanthan gum. In: Phillips GO, Williams PA (eds) Handbook of hydrocolloids. Elsevier, Amsterdam, pp 103–115
- Szczesniak AS (1986) Rheological basis for selecting hydrocolloids for specific applications. In: Phillips GO, Wedlock DJ, Williams PA (eds) Gums and stabilizers for the food industry. Elsevier Applied Science, London, pp 311–323
- Tu Z, Huang T, Wang H, Sha X, Shi Y, Huang X, Man Z, Li D (2015) Physico-chemical properties of gelatin from bighead carp (*Hypophthalmichthys nobilis*) scales by ultrasound-assisted extraction. *J Food Sci Technol* 52(4):2166–2174
- Uriarte-Montoya MH, Santacruz-Ortega H, Cinco-Moroyoqui FJ, Rouzaud-Sández O, Plascencia-Jatomea M, Ezquerro-Brauer JM (2011) Giant squid skin gelatin: chemical composition and biophysical characterization. *Food Res Int* 44(10):3243–3249
- Verbeke D, Dierckx S, Dewettinck K (2003) Exudate gums: occurrence, production, and applications. *Appl Microbiol Biotechnol* 63(1):10–21
- Vijayendran BR, Bone T (1984) Absolute molecular weight and molecular weight distribution of guar by size exclusion chromatography and low-angle laser light scattering. *Carbohydr Polym* 4(4):299–313
- Yakimets I, Wellner N, Smith AC, Wilson RH, Farhat I, Mitchell J (2005) Mechanical properties with respect to water content of gelatin films in glassy state. *Polymer* 46(26):12577–12585
- Yang Y, Anvari M, Pan CH, Chung D (2012) Characterisation of interactions between fish gelatin and gum arabic in aqueous solutions. *Food Chem* 135(2):555–561
- Yong-an WSHX, Jing-na WU (2010) Study on the extraction process of gelatin from tilapia fish scale. *Progress Fish Sci* 3:012
- Zhang FX, Xu SY, Wang Z (2008) Study on processing technology of demineralization of fish scale gelatin production. *Sci Technol Food Ind* 3:75
- Zhang F, Xu S, Wang Z (2011) Pre-treatment optimization and properties of gelatin from freshwater fish scales. *Food Bioprocess Technol* 89(3):185–193