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Rheological and textural properties of fish gelatine-microbial transglutaminase gel microparticles as thickener for texture-modified food

Siti Munirah, R. and *Syahariza, Z. A.

Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia

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Abstract

Gel microparticles are tiny, soft particles made from proteins or polysaccharides using particle size reduction. In creating texture-modified food and thickened liquids, gel microparticles are frequently employed to customise the rheological and textural features of foods or thin liquids. Gel microparticles aid by lowering flow behaviour, and modifying the taste perception of the texture-modified food. By cross-linking fish gelatine with the microbial transglutaminase enzyme (mTGA) using the homogenisation process, fish gelatine has tremendous potential to be used to create gel microparticles. The size measurements, texture profile, viscoelastic characteristics, and impact of sugar content on the texture profile of gel microparticles were all examined in the present work for the developed gel microparticles. The results of a particle size investigation revealed that gel microparticle sizes, which ranged from 18 to 1445 µm, increased with higher enzyme concentrations. With increasing mTGA concentration, the texture profile analysis (TPA) also revealed increasing values for hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness parameters. The cross-linked gel microparticles had higher dominance on elastic behaviour than viscous behaviour from the rheological investigation, as evidenced by a higher G' compared to G". Additionally, larger TPA values were seen as the sugar concentration increased. These outcomes are anticipated because sugar will strengthen fish gelatine's hydrogen bond. Based on all completed analyses, fish gelatine cross-linked with 0.7% mTGA to create gel microparticles has shown encouraging results, which can be used as a thickener in texture-modified food to aid those who have trouble swallowing.

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Introduction

Starches and gums have been widely used as thickeners in preparing texture-modified food as part of diet management practices for individuals with dysphagia (Zargaraan *et al*., 2013). However, because starch-based thickeners add to the starchy flavour, consumers find them less appealing (Cichero, 2013). Furthermore, the viscosity of a product may vary depending on the temperature at which it is prepared (Vilardell *et al*., 2015). Based on a report, starch-based thickeners make products thicker over time, making them less suitable for consumption of people with trouble swallowing, known as dysphagia. To meet the special dietary requirements, an alternative thickener is required.

Gel microparticles are soft, stable, and tiny particles with variable sizes, shapes, and textural characteristics (Nicolai, 2016). Shewan and Stokes (2013) have reviewed gel microparticles production, structure, and characteristics. According to Leon *et al*. (2016), proteins, polysaccharides, or their combination are typically used to make gel microparticles. These particles are manufactured using various processes, such as emulsification, spray drying, and gelation. To create gel particles that meet the definition of gel microparticles, bulk gels can be mechanically broken down to produce small-sized gel microparticles (Leon *et al*., 2016). Gel microparticles are frequently utilised as thickeners in soups and sauces, as well as structural agents that reinforce the dispersed phase (Aguilera and Park, 2016). Gel microparticles show great potential as thickeners due to their ability to tailor the textural and rheological properties of food, increasing gel-like properties, and reducing texture inconsistency in food (Stokes,

*Corresponding author.
Email: syahariza@usm.my

2012). Reduction in the flow rate of food bolus is vital to prevent choking, hence preparing safe food bolus for consumption.

In the past, Leon *et al*. (2019) successfully created gel microparticles containing fibre by combining whey protein and alginate. As determined by rheological investigations, gel microparticles with added dietary fibre showed a predominantly elastic nature. A texture profile analysis shows that the generated gel microparticles are more adherent and cohesive than commercial thickeners. Since research has yet to be done on the direct addition of gel microparticles to food models, it is unknown what the result will be (Shewan and Stokes, 2013).

One of the most used thickeners in food applications is gelatine (Ninan *et al*., 2011). Gelatine is made from collagen, an animal protein found in bones and connective tissues, which is then subjected to acid or alkaline hydrolysis (Burey *et al*., 2008). Given its ability to gel when subjected to a cold-set gelling process and its thermo-reversible characteristics, gelatine has a significant potential to produce gel microparticles (Haug and Draget, 2009). In the pharmaceutical industry, gelatine microparticles are one of the most frequently utilised drug carriers due to their non-toxicity, storage stability, affordability, and ease of synthesis (Kong *et al*., 2011; Martins *et al*., 2018). Meanwhile, gelatine is frequently employed as a thickener and gelling agent in the food business. In addition, using protein-based thickeners like gelatine may add some nutritional value to the consumer's food intake. The search for mammalian gelatine substitutes has been sparked by diseases including bovine spongiform encephalopathy (BSE), foot and mouth disease (FMD), African swine fever epidemics, and religious concerns (*halal*) (Huang, *et al*., 2019a; 2019b). Fish gelatine has been investigated as an alternative (Mariod and Adam, 2013). However, due to its lower gelling and inferior rheological characteristics compared to mammalian gelatine, fish gelatine is seldom used in food industry applications (Karim and Bhat, 2008).

In contrast to mammalian gelatine, fish gelatine has lower rheological characteristics and inferior gel properties in texture and viscosity (Huang *et al*., 2019a). The lower rheological properties are because of the low amino acid content, particularly hydroxyproline and proline, which restricts its wide-scale usage. Therefore, various techniques have been studied and applied to overcome these limits, such as enzyme cross-linking, chemical modification, physical modification, and complex modification (Huang *et al*., 2019a). Among all the techniques, enzyme cross-linking, particularly microbial transglutaminase (mTGA) enzyme, has been proven to show excellent results in terms of lower price and non-toxicity (Shewan and Stokes, 2013; Huang *et al*., 2019b). This technique has been proven to increase the hardness and viscosity of fish gelatine (Huang *et al*., 2019b). Besides, enzyme cross-linking techniques have been successfully proven to increase gels and rheological properties (Huang *et al*., 2019a). This enzyme acts as a catalyst in the reaction between the carbonyl group of glutamines, and the amino group of lysine in proteins, which will generate inter- and intra-molecular covalent and hydrogen bonds between the groups, making the structure of fish gelatine gel stronger (Mostafa, 2020).

However, most of the earlier research on using gel microparticles concentrated on pharmaceutical uses, utilising various essential ingredients such as whey protein, whey protein isolate, and polysaccharides (Aguilera and Park, 2016; Leon *et al*., 2016; 2018). As far as we know, cross-linked fish gelatine gel microparticles are rarely used in food applications. Determining the rheological and textural characteristics of gel microparticles made from fish gelatine and cross-linked with different concentrations of microbial transglutaminase was the goal of the investigation. The need for a freeze-drying process is crucial, as this drying process could prevent the gels from collapsing by developing networks of layered structures and microcrystals. Thus, the original and reconstituted studies are included to know the effect of this drying process.

Furthermore, sugar has been proven to increase the properties of gelatine, such as textural parameters, due to the ability to stabilise hydrogen bonding (Koli *et al*., 2011; Sow and Yang, 2015). Nevertheless, there is also an opposite opinion that the addition of sucrose decreased gel strength by weakening gelatine-gelatine molecule interaction, and increasing distance between entangled points, hence reducing the amount of available junction zone, thus causing the textural properties of the gel to reduce (Sow and Yang, 2015). This may be owing to the differences in gelatine sources and sucrose concentrations. Therefore, it is essential to test how well the developed fish gelatine gel microparticles work in various sugar concentrations to determine their ability in developed gel microparticles.

Materials and methods

Materials

Gelatine from cold water fish skin was purchased from Sigma-Aldrich (Canada). Microbial transglutaminase enzymes were purchased from Activa (Canada). All other chemicals used were of analytical and food grade. Deionised water was used for all the experiments.

Sample preparation

The cross-linked fish gelatine gel microparticles were prepared according to the method of Leon *et al*. (2016) and Huang *et al*. (2019a) with slight modifications. The fish gelatine solution (6.67%, w/v) was prepared by dissolving fish gelatine in deionised water at 60°C with constant mild stirring using a magnetic stirrer. The pH of the fish gelatine solution was adjusted to 6.5 using 0.1 M sodium hydroxide (NaOH) and hydrochloride acid (HCl) solution. Cross-linked fish gelatine solution was prepared by adding the enzyme to fish gelatine solution at the concentrations of 0, 0.1, 0.3, 0.5, and 0.7% (w/v), and named T0-FG, T0.1-FG, T0.3-FG, T0.5-FG, and T0.7-FG, respectively. The mixture solution was incubated at 40°C for 40 min, followed by deactivation of the enzyme at 90°C for 15 min. The solution was cooled to room temperature, and kept in a refrigerator (4°C) for 12 h. The resultant gels were first subjected to crushing for 1 min in a speed hand blender, and then homogenised with a high-speed homogeniser (Ultra Turrax) at 10,000 rpm for 3 min until a uniform paste was obtained. The gels were kept in the refrigerator for another 12 h before being subjected to analysis. A portion of the samples was dried using a freeze-dryer (Labconco, United States), and the powder obtained after 3 d drying was kept for further analysis. Reconstituted samples were prepared by mixing the powder with the deionised water.

Particle size analysis

The particle sizes of wet samples were measured using a particle size analyser (Malvern 2000, Hydro 2000S, United Kingdom). The sampler settings for tray selection were chosen: sample tray (general purpose, < 200 g) and dispersion control (50% vibration feed rate and 2 bar dispersion air pressure). Distilled water was filled inside the Hydro 2000S tray. The auto-testing alignment was waited for until a successful alignment was achieved. Then, the wet sample was filled inside the Hydro 2000S tray. The result of the analysis was obtained. Each sample was tested in triplicate.

Texture profile analysis (TPA)

The textural properties of the modified fish gelatine were measured according to the method Huang *et al*. (2019a) with slight modification using TA-XT2 Texture Analyser (Stable Micro Systems, United Kingdom). The resultant gels were kept in a 50 mL beaker, with a sample height of 2 mm and diameter of 35 mm, as the samples were too soft to stand by themselves. Following this, gelatine gels were compressed at the following settings, test speed: 1.0 mm/s, pre speed: 5 mm/s, post speed: 10 mm/s, and strain: 60%. The compression was done using a cylindrical aluminium probe (P/1"). The results obtained from the gels were crushed as this imitates mastication. The results from the force-time curve of the TPA, including hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness were obtained. Each sample was tested in triplicate. The same procedure and settings were used to study the effect of sugar concentration on the texture profile of gel microparticles. The sugar concentration was prepared by adding 2% (w/v) into the prepared gel microparticles (reconstituted samples) after being homogenised, and before being kept in a refrigerator. The same procedure was repeated for 4, 6, 8, and 10% sugar concentrations.

Rheological measurement

The viscoelastic properties of the modified fish gelatine gels were studied by dynamic oscillatory measurements according to Tan *et al*. (2020) using an AR 1000-N stress-controlled rheometer (TA Instruments, New Castle, DE, United States) equipped with a steel cone-plate geometry (diameter: 40 mm; cone angle: 2°; transition gap: 51 µm). Approximately, 1 mL of sample was employed in all measurements. Before the rheological test, a stress sweep was performed at 1 Hz to determine the linear viscoelastic region (LVR) of modified fish gelatine gels. The samples were equilibrated for 1 h at 0°C before frequency sweeps. Then, frequency sweeps were performed between 0.1 to 100 rad/s at 0°C. The values of the storage or elastic modulus (G'), loss or viscous modulus (G"), and loss tangent (Tan δ = G"/G') were calculated (in duplicate using fresh samples) using the TA Rheology Advantage Data Analysis software (version V5.7.0).

Statistical analysis

Statistical analysis for particle size and textural measurements was conducted using SPSS Statistics Desktop 22.0 (IBM Corporation, US). The results obtained were expressed as mean ± standard deviation of triplicates. Comparison of means was performed using independent *t*-test, one-way ANOVA, and two-way ANOVA using Tukey's test at a 5% probability level.

Result and discussion

Particle size analysis

Table 1 shows the particle sizes of cross-linked samples for original and reconstituted samples. It is apparent that the particle size of both original and reconstituted samples increased significantly with the increased concentration of the mTGA enzyme. It can be concluded that the size range of original samples that have been cross-linked with mTGA was from 10 to 1,258 µm, while that for reconstituted cross-linked samples was from 18 to 1,445 µm. This suggested that mTGA had appreciable impacts on the mean particle diameters of fish gelatine. mTGA catalysed fish gelatine to form a macromolecular polymer with high molecular weights, as mTGA will aid in forming additional bonding between the carbonyl group of glutamine in mTGA and the amino group of lysine in proteins, which will generate bigger molecules (Huang *et al*., 2019b).

Generally, a dispersion of gel-like particles with an equivalent diameter ranging from 0.1 to 100 mm is commonly referred to as microgels, microspheres, or microbeads (Loewen *et al*., 2017). In contrast, other studies claimed their gel microparticles to be up to 200 µm, and up to 1,000 µm (Leon *et al*., 2016; Mishra and Singh, 2020). Based on the results obtained in the present work, the resultant gels from T0.1-FG and T0.3-FG could be categorised as microparticles. Other samples can also be categorised as microparticles even though the sizes exceed 1,000 µm, as there is no fixed standard for the range value of gel microparticles.

Meanwhile, the sizes of the original samples were significantly different from those of reconstituted samples, with increasing particle sizes for all concentrations after freeze-drying. The freeze-drying process was reported to preserve most gel characteristics, and prevented the developed network from collapsing by forming microcrystals or layered structures during its unidirectional freezing that would change the original size of the gel (Cao and Mezzenga, 2020). Generally, traditional drying processes such as vacuum and oven drying cause the collapse of the developed network structure due to the cracking or shrinkage of the gel structure due to solid capillary forces caused by the evaporating water (Zhao *et al*., 2018).

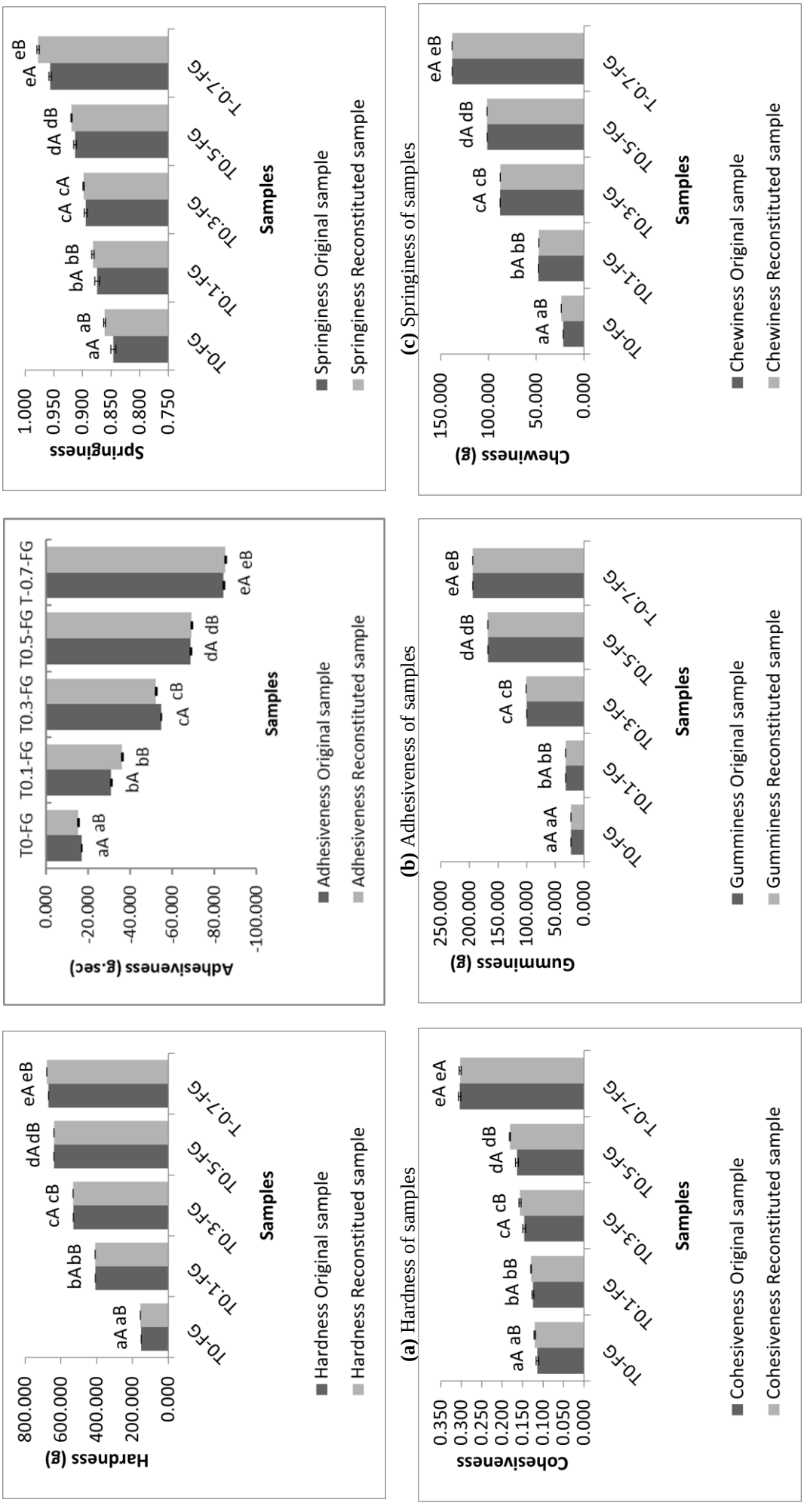
Table 1. Particle size of cross-linked fish gelatine gel microparticle and reconstituted cross-linked fish gelatine gel microparticle.

Sample	Particle size (µm)	
	Original sample	**Reconstituted sample**
T0-FG	5.01 ± 0.00^{aA}	6.61 ± 0.00^{aB}
T0.1-FG	10.05 ± 0.00^{bA}	18.21 ± 0.00^{bB}
T0.3-FG	1096.48 ± 0.00^{cA}	1118.92 ± 0.00^{cB}
T0.5-FG	1134.89 ± 0.00^{dA}	1201.13 ± 0.00^{dB}
T0.7-FG	1258.92 ± 0.00^{eA}	1445.44 ± 0.00^{eB}

Values are mean ± standard deviation (n = 3). Means in the same column with different lowercase superscripts are significantly different from each other at $p < 0.05$ level of significant by one-way ANOVA. Means in the same row with different uppercase superscripts are significantly different from each other at $p < 0.05$ level of significant by independent *t*-test.

Texture profile analysis (TPA)

Textural measurements using a texture analyser represent the process during mastication, as it imitates the actions between the tongue and teeth (Huang *et al*., 2019b). The results are presented in Figures 1a until 1f. Based on the figures, the textural parameters ((a) hardness, (b) adhesiveness, (c) springiness, (d) cohesiveness, (e) gumminess, and (f) chewiness profiles of cross-linked fish gelatines increased with increasing concentrations of mTGA.



(d) Cohesiveness of samples **(e)** Gumminess of samples **(f)** Chewiness of samples

Figure 1. Effects of increasing concentration of mTGA enzyme on original and reconstituted gel microparticles on textural parameters of **(a)** hardness, **(b)** adhesiveness, **(c)** springiness, **(d)** cohesiveness, **(e)** gumminess, and **(f)** chewiness. Bars in each graph between different concentrations for original and reconstituted samples with different lowercase superscripts are significantly different from each other at $p < 0.05$ level of significant by one-way ANOVA. Bars in each graph between original and reconstituted samples for each concentration with different uppercase superscripts are significantly different from each other at $p < 0.05$ level of significant by independent *t*-test.

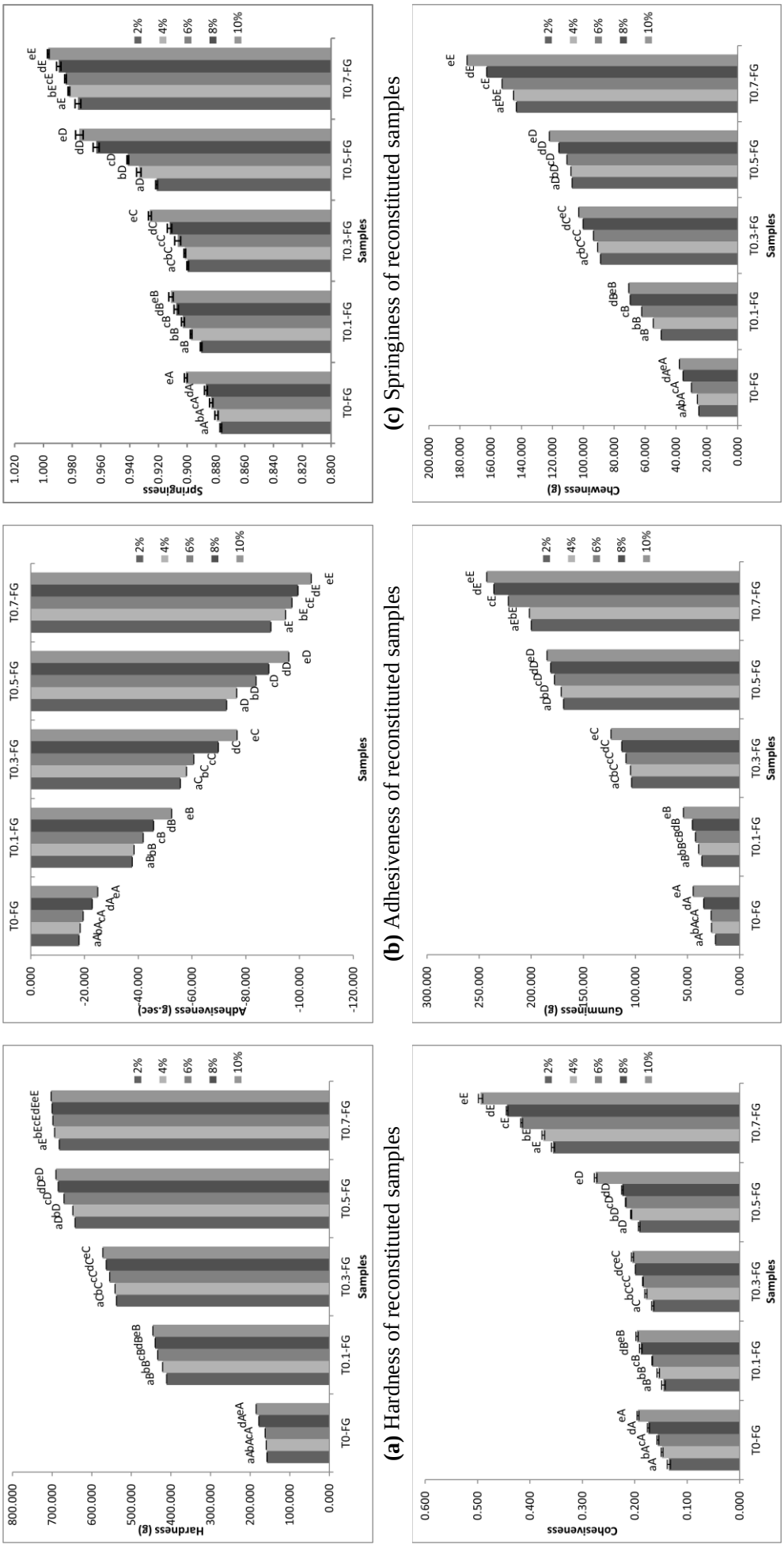
This could have been due to the increased covalent cross-linked between the carbonyl group of glutamines in enzymes and the amino group of lysine in fish gelatine. The higher the amount of mTGA used for the cross-linking, the higher the number of cross-linking sites between the carbonyl and amino groups in the proteins (Huang *et al*., 2017). As a result, gels with improved properties in terms of rheological and textural properties were produced. These properties will help the resultant gels to act as thickeners, behaving better than single-fish gelatine or uncross-linked fish gelatine (Huang *et al*., 2019a). Nevertheless, excessive amounts of mTGA might produce non-thermo reversible gels with lower viscosity and textural parameters, such as hardness due to impeding intermolecular aggregation that reduces the gel network formation as a high concentration of mTGA will form excessive intramolecular covalent bonds (Wangtueai *et al*., 2010). For each variety of fish gelatine, excessive amounts of mTGA have been observed to vary; nonetheless, most fish gelatine will exhibit adverse properties when cross-linked with mTGA at levels greater than 0.7% (Wangtueai *et al*., 2010; Mariod and Adam, 2013; Huang *et al*., 2017).

The three primary textural characteristics assessed for texture-modified foods are cohesiveness, adhesiveness, and hardness (Aguilera and Park, 2016). Figure 1a shows the hardness values of the original and reconstituted gel microparticles with increasing concentrations of mTGA enzyme. Hardness refers to the strength of the gel structure under compression (Huang *et al*., 2019b). From Figure 1a, the hardness of the original gel microparticles increases with a higher concentration of mTGA. Higher hardness values indicate a higher maximum force required to compress the food between molar teeth (Huang *et al*., 2019b). The higher the mTGA used to cross-link, the more complex the gel produced due to the addition of peptide bonds in the fish gelatine molecules, causing the resultant gels to have more gel network formation (Wangtueai *et al*., 2010).

Adhesiveness is the work necessary to overcome the attractive forces between the product and a specific surface (Huang *et al*., 2019b). The lowest adhesiveness value was found in T0.7-FG as exhibited in Figure 2, for both original and reconstituted samples. Lower adhesiveness is needed as lower force is crucial for the gel to adhere to the mouth (Leon *et al*., 2019). Meanwhile, springiness measures how much the gel structure recovers after the initial compression (Huang *et al*., 2019b). Higher springiness indicates that the gelatine gels require more mastication energy in the mouth (Huang *et al*., 2019b). Figure 1c shows that the concentration of mTGA, ranging from 0.1 to 0.7%, could increase the springiness of the gel microparticles, with the highest springiness value for a sample cross-linked with 0.7% of mTGA. This could have been due to the different types of fish gelatine used and other factors such as types of mTGA and incubation times. It has been reported that various concentrations of mTGA could not alter the springiness of lizardfish scales gelatine gels (Wangtueai *et al*., 2010).

Cohesiveness is the amount of compression needed or to what extent a sample deforms before it breaks (Cichero, 2015). The value for cohesiveness from Figure 1d is significantly different, but there was a sharp increase at T0.7-FG, giving it the highest value among all other samples. A higher value of this parameter is needed to form a safe bolus as it shows a higher degree to which the particles can be deformed before they break (Aguilera and Park, 2016). On the other hand, gumminess is described as the effort needed to break down a tender sample to form a swallow-ready-state bolus (Cichero, 2015). The gumminess values were significantly different from one another. Those values increased with increasing the concentration of enzymes. Besides, chewiness measures the length of time or number of chews so that the condition of the solid or semi-solid sample is swallow-ready (Cichero, 2015). According to Huang *et al*. (2019b), chewiness is also related to gumminess, in which the high value of chewiness will exhibit a high value of gumminess, as shown in sample T0.7-FG.

After the gel has been freeze-dried, the powder samples were reconstituted with deionised water to measure the effect of the drying method on the original gel. The results are visualised in Figures 1a to 1f, and labelled as reconstituted samples. The freeze-drying process can avoid capillary forces mainly caused by traditional drying methods, such as vacuum drying and oven drying, as these methods (vacuum and oven drying) cannot preserve the gel structure. However, freeze-drying could cause some drawbacks, which might slightly alter some of the characteristics of the original gels (Cao and Mezzenga, 2020). Based on Figures 1a to 1f, all



(a) Hardness of reconstituted samples **(b)** Adhesiveness of reconstituted samples **(c)** Springiness of reconstituted samples

(d) Cohesiveness of reconstituted samples **(e)** Gumminess of reconstituted samples **(f)** Chewiness of reconstituted samples

Figure 2. Effects of increasing sugar concentration on reconstituted gel microparticles on textural parameters of **(a)** hardness, **(b)** adhesiveness, **(c)** springiness, **(d)** cohesiveness, **(e)** gumminess, and **(f)** chewiness. Bars in each graph between different concentrations of sugar (%) in each same sample with different lowercase superscripts are significantly different from each other at $p < 0.05$ level of significant by two-way ANOVA. Bars in each graph between samples and the exact concentration of sugar (%) with different uppercase superscripts are significantly different from each other at $p < 0.05$ level of significant by two-way ANOVA.

parameters were significantly different from the original and reconstituted samples with increasing concentrations of mTGA. In Figure 1a, the hardness of reconstituted samples was slightly higher than original samples at every concentration. The chewiness also followed the same trend as hardness, except for adhesiveness. The springiness values were also significantly different at every concentration except at 0.3% mTGA. Higher cohesiveness was shown on every concentration after the freeze-drying process. However, there was no significant difference in cohesiveness value at T0.7-FG. In addition, there was no significant difference in gumminess value between one another at concentration T0-FG, while other concentrations significantly differed from the original and reconstituted sample. According to Ishihara *et al*. (2011), gel microparticles' low adhesiveness and high cohesiveness are particularly encouraging for creating food matrices for texture-modified food.

Effect of different sugar concentrations on texture profile analysis (TPA) of gel microparticles

To date, no report on the effect of sugar addition on the developed gel microparticles, particularly those made from cross-linked fish gelatine, is being documented. Hence, the present work aimed to find the effect of different sugar concentrations on the developed gel microparticles before being incorporated into food models, particularly those high in sugar. The effect of the added sugar in the gel microparticles was being analysed. The hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness results are presented in Figures 2a until 2f. The added sugars ranged from 2 to 10%, and were only being analysed on reconstituted samples as from the TPA and viscoelastic results; the reconstituted samples showed more promising resultant gels with more elasticity than the original samples.

Significant differences existed in all textural parameters between different sugar concentrations added to each sample. According to Harikedua (2018), other ingredients such as sucrose used in gelatine gel formulation (gelatine, water) might affect the gel strength of gelatine-based desserts, particularly the textural parameters. In each sample, the highest value for all textural parameters was obtained in the present work with the addition of 10% sugar for each sample. It has been reported that the gel strength and hardness of gelatine gel increased due to the addition of sucrose (Harikedua, 2018). Sugar will enhance the gel strength by stabilising the hydrogen bond between polypeptide bonds in the fish gelatine, which involves water, and forms ternary complexes that stabilise the gel (Koli *et al*., 2011). The hardness depends on the gel strength, increasing the hardness of the gel when a higher concentration of sugar was added. Higher cohesiveness shows that the gel needs a higher force to break down its internal structure, and that the samples have a higher degree of elasticity (Ninan *et al*., 2011). The higher chewiness values and lower adhesiveness values for samples at a sugar concentration of 10%, particularly in sample T0.7-FG, suggested that gel with firmer properties could be obtained. The chewiness is the force needed to masticate the gel into a ready-to-swallow state (Sow and Yang, 2015). Chewiness values followed the same trend as the hardness of the gel. Thus, fish gelatine's increased hardness and chewiness suggested a firmer texture of resultant gels.

There were also significant differences in all parameters between different samples with the same sugar concentration, as depicted in Figures 2a to 2f. With increasing concentration of mTGA and sugar, the textural parameters increased except for adhesiveness. mTGA is used to catalyse lysine and glutamine in gelatine to form peptide bonds, which leads to a dense and homogenous gel network (Wangtueai *et al*., 2010). Therefore, it can be concluded that higher use of mTGA enzymes will cause higher values of textural parameters, as explained in the earlier section. In addition, adding sugar could stabilise the network structure of the gel, which enhances the rigidity of the samples by strengthening the hydrogen bonds (Li *et al*., 2020). A similar finding was reported by Choi and Regenstein (2000) that adding sucrose of approximately 2 to 14% in seven pork and fish gelatine gels increased the gel strength by 22%. The hardness is mainly related to gel strength (Ninan *et al*., 2011). Besides, the study by Altan Kamer *et al*. (2019) showed that adding sugar in fish gelatine gels exhibited a firmer texture based on TPA compared to gel without adding sugar. From the results, it can be concluded that adding sugar significantly affected the gel's textural properties.

Viscoelastic behaviour

The viscoelastic behaviour of developed gel microparticles was analysed using a rheometer, particularly a frequency sweep test based on storage modulus (G'), loss modulus (G"), and Tan δ. The

results are visualised in Figures 3a to 3f for original and reconstituted samples. The sample T0-FG was a control sample in which the fish gelatine was not cross-linked with mTGA, while other samples were cross-linked with a range of mTGA from 0.1 to 0.7%. Ideally, the gel will show elastic behaviour, which can be seen from the higher values of G' than values of G" at a frequency between 0.1 - 100 rad/s (Leon *et al*., 2018).

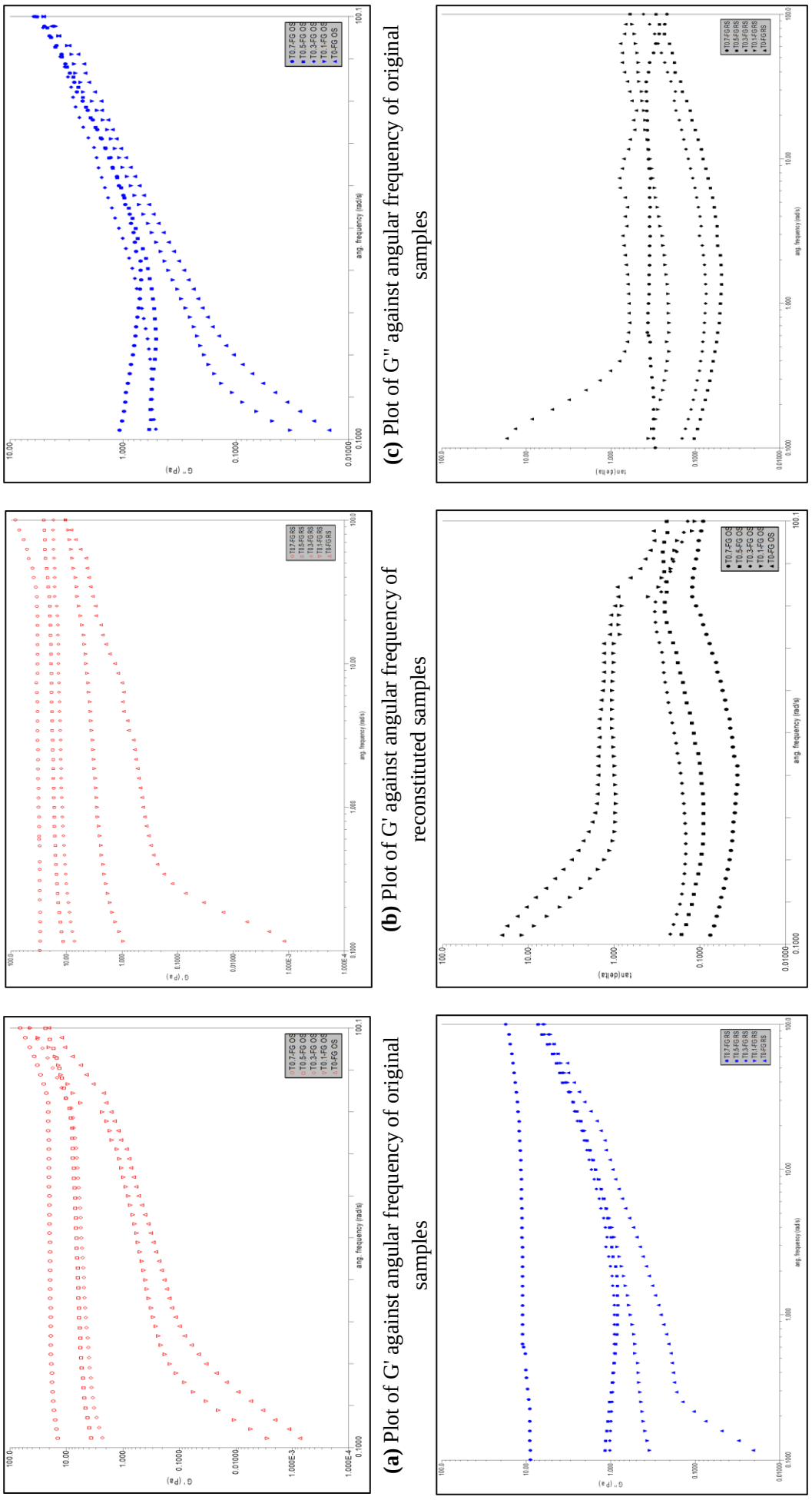
Generally, the values of G' were higher than G" for all original samples except T0-FG and T0.1-FG. This indicated that all original samples except T0-FG and T0.1-FG showed predominant elastic behaviour over viscous behaviour, as is typically observed for gelled structures (Leon *et al*., 2019). The same trend (G' > G") was also observed in reconstituted samples, except for T0-FG, which did not show G' values higher than G" values. The sample (T0-FG) might have more viscous behaviour than elastic. From Figures 3a till 3f, both G' and G" increased with increasing concentration of mTGA used for cross-linking. It can be concluded that cross-linking with mTGA influenced the rheological properties of the modified fish gelatine in the present work. This might have been due to the more vital bond formed by increasing the concentration of mTGA in the fish gelatine gel network, causing the gel to increase elastic behaviour (Huang *et al*., 2019b). This finding was in accordance with the TPA results, particularly cohesiveness, as the higher the concentration of mTGA used, the higher the cohesiveness obtained by the modified gel.

Referring to Figures 3a and 3b, the values of G' of both original and reconstituted samples were further compared to determine the effect of the freeze-drying process on the samples. The values of G' of reconstituted samples were higher than those of the original samples. This observation indicated that the reconstituted samples were more elastic than the original samples. It can be concluded that the freeze-drying process might influence the samples' viscoelastic behaviour by increasing their elastic properties. Based on Figures 3c and 3d, the trend of G" also shows the same as G' when comparing original samples and reconstituted samples. In summary, all reconstituted samples except T0-FG can be described as weak gel-like structure as G' > G", and both parameters varied with angular frequency (Leon *et al*., 2019). These behaviours described that the cross-linked gel microparticles produced and dried by using freeze-drying method could be used as a thickener for texture-modified food as it could contribute to weak gel consistency bolus. Weak gel bolus helps for easy mastication and safe swallowing as it provides bolus with soft properties and enough consistency to prevent choking hazards (Ishihara *et al*., 2011).

Tan δ values for all samples, except for T0-FG (original and reconstituted samples) and T0.1-FG (reconstituted sample), were < 1 in the studied range (Figures 3e and 3f), showing that they had higher elastic properties (Funami, 2016). As shown in Figures 3e and 3f, Tan δ of both original and reconstituted samples were approximately the same except for a few samples. In the original sample, only T0.3-FG, T0.5-FG, and T0.7-FG showed Tan δ < 1; in reconstituted samples, only T0-FG showed Tan δ > 1. These explained that cross-linking with mTGA could, to some extent, give the gel microparticles more elastic than viscous behaviour. According to Ishihara *et al*. (2011), 0.1 < Tan δ < 1 has been suggested as one of the rheological characteristics for safe swallow foods, which can be used to design a dysphagia diet. The rheological analysis results observed in the present work were in accordance with Funami (2011), in which gels have been suggested to be a good material for dysphagia diets owing to their viscoelastic properties. It can be concluded that sample T0.7-FG can be chosen as the ideal concentration for gel microparticles, even after freeze-drying. It is suggested that gel microparticles, especially T0.7-FG, can be used as thickeners for texture-modified foods, as Ellis and Jacquier (2009) suggested.

Conclusion

Fish gelatine cross-linked with mTGA gel microparticles was successfully prepared by the mechanical breakdown of bulk gels. The size of the gels was found to increase with increasing concentration of mTGA. Both original and reconstituted samples were subjected to textural and rheological analysis. The results showed that increasing the mTGA concentration could increase the textural and rheological properties. In addition, the reconstituted gel microparticles were analysed for their textural properties in various sugar concentrations using TPA. The developed gel microparticles appeared effective as thickeners,



(a) Plot of G' against angular frequency of original samples **(b)** Plot of G' against angular frequency of reconstituted samples **(c)** Plot of G'' against angular frequency of original samples **(d)** Plot of G'' against angular frequency of reconstituted samples **(e)** Plot of Tan δ against angular frequency of original samples **(f)** Plot of Tan δ against angular frequency of reconstituted samples

Figure 3. Plot of **(a)** G' against angular frequency of original samples, **(b)** G' against angular frequency of reconstituted samples, **(c)** G'' against angular frequency of original samples, **(d)** G'' against angular frequency of reconstituted samples, **(e)** Tan δ against angular frequency of original samples, and **(f)** Tan δ against angular frequency of reconstituted samples.

shown by elastic properties of gel microparticles with higher G' than G" for fish gelatine cross-linked with 0.7% of mTGA. Besides, the lowest value of adhesiveness and highest value of cohesiveness given by the sample T0.7-FG showed safe bolus properties for consumption by individuals with dysphagia. Therefore, the developed gel microparticles could be used as thickeners, as they could tailor the textural and rheological properties of texture-modified food, especially for individuals with dysphagia. Further research should be carried out to determine the effect of gel microparticles in a wide range of food types, and the interaction effect between food ingredients.

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