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The Influence of Particle Size and Protein Content in Particle-Filled Myofibrillar Protein Gels

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Abstract: The addition of glass microspheres as a model insoluble, hydrophilic filler in comminuted meat gels was investigated. The influence of protein content (9, 11, and 13%), filler size (~4 μm, 7 to 10 μm, and 30 to 50 μm), and volume fraction filler (ϕ_f) on the microstructure, cooking losses, and large deformation/textural properties were evaluated. Microstructural analysis indicated the glass microspheres did not strongly interact with the gel matrix. For all protein levels investigated, cooking losses decreased with increasing ϕ_f, and this impact was more pronounced with smaller filler particles. The textural attributes of the 9 and 11% protein gels exhibited a similar dependence on filler size. When incorporating the 4 μm and 7 to 10 μm particles at increasing ϕ_f, the Hardness, Resilience, Cohesiveness, and Springiness all displayed a sharp increase to a plateau. The larger 30 to 50 μm particles exhibited no increase in any of the textural properties until higher ϕ_f were employed. In the 13% protein gels, the influence of the particles were diminished, and the effect of particle size was substantially reduced. The influence of these insoluble model filler particles was attributed to their ability to decrease the mobility of the aqueous phase, which explains their minor impact on more stable formulations. Through this work, it has been demonstrated that micrometer-sized hydrophilic particles have the potential to improve the stability and enhance the textural properties of comminuted meat gels. These findings suggest that micrometer-sized inert particles might function as an effective stabilizer in comminuted meat batters at low concentrations.

Keywords: composite gel, gelation, meat, microstructure, texture, water binding
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Introduction

Many foods are structured as a discrete particulate phase dispersed throughout a continuous matrix, such as a gel. Some examples include fat-containing custards, cheeses, and processed cheese products, as well as several processed meats, such as salamis, pâtés, and comminuted products such as bologna and frankfurters. The dispersed phase can have a dramatic impact on both the small and large deformation properties (i.e., rheological, textural, and mechanical attributes) of such composite materials (van Vliet, 1988; van Vliet and Walstra, 1995). From a consumer perspective, the large deformation properties are most relevant, as they will dominate during handling, preparation, and consumption (Rosa, Sala, van Vliet, and Van de Velde, 2006; van Vliet and Walstra, 1995).

The influence of the filler phase on the mechanical properties of overall product can be influenced by several factors. These include the size, quantity (volume fraction), spatial distribution, and physical state of the filler particles (Gravelle, Barbut, and Marangoni, 2015; Sala, van Vliet, Cohen Stuart, van de Velde, and van Aken, 2009), as well as the chemical nature of the discrete phase, and the mechanical strength relative to that of the gel (Gravelle et al., 2015; Kim, Renkema, and van Vliet, 2001; van Vliet, 1988). The impact of the filler on

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the mechanical properties of various particle-filled food gels have previously been analyzed using particulate-reinforcement theories, such as those originally proposed by van der Poel (1958) and Kerner (1956), with subsequent modifications by Smith (1974, 1975) and Lewis and Nielsen (1970), respectively. These theories characterize how the modulus of the composite is influenced by the particulate phase as a function of volume fraction filler (ϕ_f). Such theories have been used to describe several food systems, including cheddar (Yang, Rogers, Berry, and Foegeding, 2011) and mozzarella cheeses (Thionnet, Havea, Gillies, Lad, and Golding, 2016), emulsion-filled protein gels such as gelatin (Sala, Van Aken, Cohen Stuart, and Van de Velde, 2007), soy protein isolate (Kim et al., 2001), acidified milk (van Vliet, 1988), whey (Sala et al., 2007), and concentrated caseinate gels (Manski, Kretzers, van Brenk, van der Goot, and Boom, 2007), as well as comminuted meat systems (Gravelle et al., 2015).

Although the theoretical models currently available have been used with relative success in characterizing particle filled food gels, they are not without some limitations. These models generally assume the filler is spherical, homogeneously dispersed, non-interacting, and strongly bound to the continuous phase (Ahmed and Jones, 1990). Therefore, particle-particle interactions caused by flocculation during gelation, or particle clustering can skew the theoretical interpretation (Kim et al., 2001; van Vliet, 1988). Additionally, a weak interaction between the filler and matrix can affect the influence of the filler (Gravelle et al., 2015) and these theories do not incorporate particle size into the framework which is based solely on ϕ_f (Ahmed and Jones, 1990). Although some studies have found filler size has little, or no influence on the reinforcing behavior (Manski et al., 2007), in emulsion filled gels, the influence of filler size has been associated with the surface tension, and thus the modulus of the filler. In such systems, a higher filler modulus (i.e., smaller particle) should produce a greater reinforcement (Rosa et al., 2006; Sala et al., 2009; van Vliet, 1988; Wu, Xiong, Chen, Tang, and Zhou, 2009). However, our group recently demonstrated that in a comminuted meat system (~14% protein, 2.5% salt), particle size had a strong impact on both cooking losses and mechanical strength when incorporating rigid model fillers (i.e., a fixed modulus much greater than that of the matrix) at increasing ϕ_f (Gravelle et al., 2015). A follow-up study also demonstrated that hydrophilic, micrometer-sized rigid particles produced significant reinforcing effects at ϕf far lower than those predicted by theory ($\phi_f < 0.05$; Gravelle, Marangoni, and Barbut, 2016a). As these particles were shown to only weakly interact with the protein network, it was suggested that the mechanism responsible for the observed reinforcement in the latter study differed from that of a traditional particle-filled composite material. In particular, their influence was drastically different than that expected for fat-filled meat batters (Barbut, Wood, and Marangoni, 2016; Wu, Xiong, and Chen, 2011b; Zhang et al., 2013; Zhao et al., 2014), as these composite materials were fully stabilized at low ϕ_f, above which changes in textural properties were not observed. Additionally, due to the physical nature of the glass particles, they are unaffected by the cooking process and will not negatively impact the stability of the overall product. Therefore, the glass microspheres seem to behave as an inert additive which supports the structural integrity of the protein network through the cooking/gelation process. The focus of the present work is to build on the previous findings by varying protein content (9, 11, and 13%), filler size (4 μm, 7 to 10 μm, and 30 to 50 μm), and ϕ_f, to determine how these parameters modulate the influence of the filler on microstructure, batter stability (cooking losses), and textural attributes of a model particle-filled comminuted meat system. By extension, this fundamental approach should provide insight into a novel approach at improving product stability, for example, during reformulation.

Materials and Methods

The meat used in this research was purchased form a commercial vendor and therefore no Animal Care Protocol was required.

Materials

Fresh boneless, skinless chicken breast meat (~25 kg) was purchased from a national supermarket (Kirkland Signature, Costco Wholesale Canada Ltd., Ottawa, ON, Canada). Within 24 h of purchasing, all visible fat and connective tissue was removed and the meat was chopped in a bowl chopper (Model 15L, Feuma Gastromaschinen GmbH, Gößnitz, Germany) at the low speed setting for approximately 60 sec and mixed by hand to produce a homogeneous mixture. The meat was then portioned into ~800g batches in bags, vacuum packed, and stored at –20°C until use. Protein content was determined, in triplicate, to be 21.2 wt % using the Dumas method and a nitrogen conversion factor of 5.53 (Mariotti, Torné, and Mirand, 2008).

Three sizes of spherical glass beads were obtained with a mean diameter of ~4 μm (Cospheric LLC, Santa Barbara, CA), 7 to 10 μm, and 30 to 50 μm (Potters Industries, LLC, Malvern, PA), according to manufacturer specifications. The density of all particles was 2.5 g/ml.

Preparation of filled myofibrillar protein gels

All composites were prepared in a household food processor (Braun Multipractice 4259, Braun Household, Kronberg, Germany) and formulated to have a final protein content of either 9, 11, or 13 wt % in the batter (see Table 1). Glass beads were added on a volume fraction basis (volume fraction filler, ϕ_f), from $\phi_f = 0$ to 0.20, depending on the protein concentration and particle size employed. The ranges employed were determined based on preliminary screening trials to determine the ranges necessary to stabilize the composites. The 4 μm and 7 to 10 μm particles were first dispersed in a portion of the added water prior to being incorporated into the meat slurry, to avoid the formation of dry aggregates. The 30 to 50 μm particles were mixed in by hand immediately after the chopping procedure, as they incorporated into the meat more readily, without clumping. Prior to preparation, each portion of meat was completely defrosted overnight under refrigerated conditions (~4°C), and any remaining connective tissue was removed by hand. Meat batters were prepared using a previously described method (Gravelle et al., 2015). Briefly, 2 parts meat (70 to 120 g, depending on the formulation) were chopped for 60 sec, followed by the addition of 1 part distilled water (10 sec chopping) and 2.5% NaCl (10 sec chopping). The slurry was then put in an ice bath for 5 min to facilitate the extraction of salt-soluble myofibrillar proteins (ionic strength ~0.42 M). The remaining water was added to achieve the desired protein concentration, and the mixture was further chopped for a total of 80 sec. For batters prepared with the 4 μm and 7 to 10 μm particles, these particles were dispersed in this final portion of the water added prior to incorporation. To ensure the mixture was chopped homogeneously, the batter was scraped off the base and walls of the chopping unit at regular intervals throughout the preparation procedure. After chopping, the batter was refrigerated at ~4°C for a minimum of 1 h prior to thermal treatment. All formulations were independently prepared and each was repeated 3 times in a randomized block design.

Table 1. Formulations of comminuted meat batters used to prepare glass-filled composite meat gels[1]

Protein level, %	Trimmed chicken breast meat, %	Water	Fat content, %
9	42.45	57.55	1.87
11	51.89	48.11	2.28
13	61.32	38.68	2.70

[1]Note: The protein level indicates the protein content of the meat batter only, and was not adjusted when incorporating filler particles. All formulations were prepared with an additional 2.5% NaCl.

After chilling, for each composite batter, 40-g samples were stuffed into four 50 mL polypropylene centrifuge tubes (Fisher Scientific, Ottawa, ON, Canada) and centrifuged (model 225, Fisher Scientific) at a low speed for 30 sec to remove air pockets. To induce gelation, the composite batters were gradually heated to an internal temperature of 72°C in a water bath (Haake W-26, Haake, Berlin, Germany). The heating process took approximately 75 min and the internal temperature was monitored using thermocouple unit (Fluke Co. Inc., model #52 K/J, Everett, WA) fed through a rubber stopper. Once the target temperature was reached, the composites were briefly transferred to an ice bath to arrest the gelation process and subsequently refrigerated overnight prior to performing texture profile analysis.

Liquid loss

After the initial cooling, the composite gels were equilibrated to room temperature and the excess liquid which was expelled during thermal treatment was drained and weighed. Liquid loss was expressed as the mass of the total expelled liquid relative to the mass of the meat batter (i.e., excluding the filler) prior to thermal treatment. No fat loss was observed after overnight storage under refrigeration conditions.

Large deformation/textural properties

Evaluation of mechanical and textural properties of the gels was performed using a 2 cycle uniaxial compression test (Bourne, 1978). For each sample, a total of 12 cylindrical cores (height: 10 mm; diameter: 15 mm) were compressed twice between 2 parallel plates to 50% of their original height using a texture analyzer (model TA.XT2, Stable Micro Systems, Texture Technologies Corp., Scarsdale, NY) outfitted with a 30 kg load cell. The crosshead speed was fixed at 1.5 mm/s and all composites were tested at room temperature. From this test, a variety of parameters were obtained, including Hardness, Resilience, Springiness, and Cohesiveness (Bourne, 1978).

Scanning electron microscopy (SEM) analysis

Samples were prepared for SEM by sectioning small cubes (~3 mm^3) and cross-linking the protein gel network by fixing them overnight in a phosphate buffer (pH 6.5) containing 2% gluteraldehyde. The samples were then rinsed with phosphate buffer 3 times (10 min each) and washed with a 1% osmium tetroxide solution

in phosphate buffer for a minimum of 1 h. After osmium treatment, the samples were again rinsed in buffer 3 times and dehydrated in a series of alcohols (50, 70, 80, 90, and 100% ethanol) for a minimum of 10 min each. The 100% ethanol rinse was repeated 3 times, and the samples were then transferred in anhydrous ethanol to a CO_2 critical point drying chamber (built in-house) for solvent removal. The chamber was maintained at ~15°C and pressurized to ~1,200 lb/in^2 (~8.3 MPa) during the ethanol/CO_2 solvent exchange process. Once pressurized, the chamber was allowed to vent for 2 min while continuously being refilled with liquid CO_2. The purge valve was then closed and the samples were allowed to soak for 2 min. This cycle was repeated a minimum of 6 times to ensure full removal of the ethanol. The chamber was then heated to ~40°C to facilitate critical point evaporation. After 5 to 10 min the chamber was slowly purged so as not to damage the samples. Following this procedure, the fixed, dehydrated gels were mounted on a pin stub with double sided carbon tape or carbon paint and sputter coated with ~15 nm of gold-palladium (model K550, Emitech, Ashford, Kent, UK). For imaging, samples were placed in the SEM chamber (Hitachi S-570, Tokyo, Japan) under vacuum, and viewed under an accelerating voltage of 10 kV. Digital images were captured using Quartz PCI Imaging software, version 8 (Quartz Imaging Corp., Vancouver, BC, Canada).

Statistical analysis

Sample preparation was performed using a complete randomized block, and all samples were prepared in three independent replicates from a single 25 kg batch of skinless chicken breast meat. Statistical analysis was performed using the GraphPad Prism software package (Prism 5.0, GraphPad Software Inc., San Diego, CA). Statistical significance was evaluated at each protein level using a 1-way ANOVA test with a post-hoc Tukey's multiple comparison test and a 95% confidence interval. Error bars depicted in figures indicate the standard error of the mean.

Results and Discussion

Liquid loss during thermal gelation

A certain level of cooking loss is regularly observed in comminuted meat products, and the extent of such losses are commonly used as an indicator of product stability (Wu et al., 2011a; Barbut, 2015). In the present study, lean chicken breast meat was used with no added fat, resulting in no observed fat loss in any formulations; i.e., only liquid losses (water exuded during thermal gelation) were observed. Figure 1 depicts the liquid losses as a function of ϕ_f for the three protein concentrations investigated. As would be expected, in the unfilled gels ($\phi_f = 0$), liquid loss decreased with increasing protein content. At all protein levels, liquid losses decreased with increasing filler content; however, this effect was strongly dependent of the filler size employed. This general trend is consistent with that previously reported when a single protein concentration (~14%) was used (Gravelle et al., 2015). It should also be noted that the particles used in the present study were 1 to 2 orders of magnitude smaller than those previously used (~50 μm to > 1 mm), and were also incorporated at lower ϕ_f. The influence of each filler size was also dependent on the protein content of the gel, where a greater ϕ_f was required for the less stable gels (i.e., lower protein content). For example, when using the 7 to 10 μm particles, in the 9% protein gels liquid loss was arrested at $\phi_f > 0.08$, while in the 11% protein gels, no losses were observed at $\phi_f > 0.04$.

In general, the gels prepared with 9 and 11% protein (Fig. 1a and 1b) exhibited similar trends across the 3 particle sizes. For both these protein levels, the 4 μm particles produced a rapid decrease in liquid loss, while the 7 to 10 μm beads produced a slightly more gradual reduction. The 30 to 50 μm particles resulted in a much more gradual decrease, and required a substantially higher ϕ_f to arrest water expulsion; $\phi_f > 0.20$ and $\phi_f = 0.10$ to 0.12 for the gels prepared with 9 and 11% protein, respectively. The gels formulated with 13% protein (Fig. 1c) exhibited smaller differences between particle sizes with increasing ϕf, albeit the same general trend was apparent. In these gels, liquid losses decreased with increasing filler content and this reduction occurred more gradually as particle size increased. The difference in the trends observed for the 9 and 11% protein gels as compared to the 13% gels may be due, at least in part, to the greater stability of the latter system. It has previously been proposed that the ability of glass microspheres to decrease liquid loss in a comminuted meat system is due to the hydrophilic nature of the glass, which reduces the mobility of the aqueous phase during thermal gelation (Gravelle et al., 2016b; Gravelle, Marangoni, and Barbut, 2016a). This effect was attributed to the high surface tension of the glass beads distributed throughout the gel matrix, coupled with the capillary pressure arising from the porous protein network (Stevenson, Liu, and Lanier, 2012; Liu, Lanier, and Osborne, 2016). By this hypothesis, the presence of the glass beads reinforces the network of capillaries, reducing water migration within the gel network, thus decreasing the occurrence of microfractures

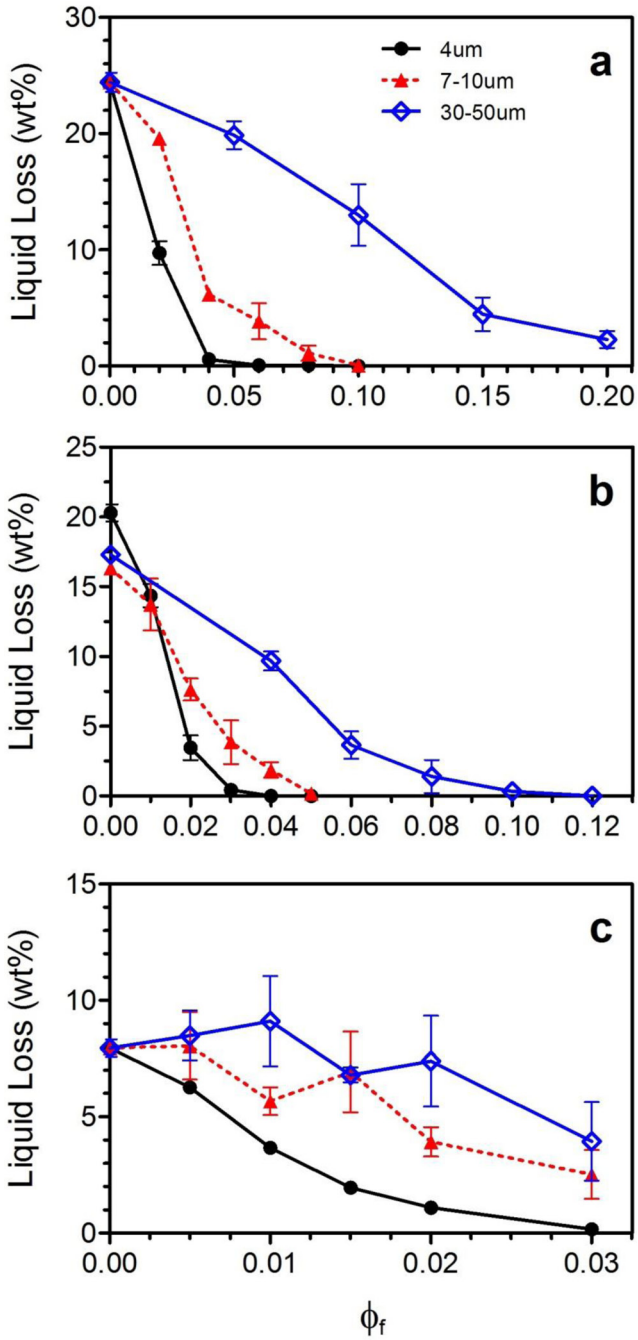

Figure 1. Liquid loss as a function of volume fraction filler particles (ϕ_f) for comminuted meat gels prepared with varying sizes of glass beads as the filler component. Filler sizes were 4 μm (filled circles), 7 to 10 μm (filled triangles), or 30 to 50 μm (open diamonds). Meat gels were prepared with 9 (a), 11 (b), and 13% protein (c).

and water channels which may facilitate further fluid losses (Gravelle et al., 2016b; Liu, Lanier, and Osborne, 2016; Wu et al., 2011b). As this was previously shown in batters having relatively high cooking losses (~15 to 20 wt % in 11% protein products), in the present work, the 13% gels would have a more dense, interconnected protein network. The increased number of protein-protein interactions could be expected to decrease the mobility of the water phase, thus decreasing migration through the gel matrix. This would, in turn, isolate the influence of the glass particles, producing a more local effect. Consequently, the influence of filler size is diminished, despite the greater total glass surface area present in the gels prepared with smaller particles.

Large deformation properties of composite gels

Hardness and resilience. The mechanical/textural properties of the filled comminuted meat gels were evaluated by texture profile analysis (TPA; Bourne, 1978) which can be used to extract a variety of attributes. During the TPA test, the sample undergoes to a 2-cycle uniaxial compression to 50% of its original height with a 2 sec recovery period between compressions. The Hardness and Resilience are parameters which are extracted during the first compression cycle, and are depicted in Fig. 2. Hardness is defined as the peak resistive force during the first compression cycle, and gives a measure of gel strength. Resilience is given by the ratio of work done during the decompression of the first cycle relative to that measured during the initial compression. The latter parameter essentially describes how much the material 'fights' to regain its original shape, and thus contains information about both the immediate elastic and cohesive properties of the material.

Overall, for both Hardness and Resilience, a similar trend is observed at each protein level. When incorporating either the 4 μm or 7 to 10 μm particles, the Hardness and Resilience of both the 9 and 11% protein gels exhibited a rapid increase, roughly reaching a plateau at $\phi_f \geq$ 0.05 and 0.03, respectively. In the 9% protein gels, the 7 to 10 μm particles showed a short lag period, where no increase was observed until ϕ_f exceeded 0.02. Similarly, the 30 to 50 μm particles showed a large lag period, where no increase in Hardness or Resilience was seen until $\phi_f >$ 0.10. Additionally, although there was a statistically significant increase in Resilience at $\phi_f = 0.15$, this parameter again reduced when the ϕ_f was further increased. This may be attributed to the large number of rigid particles present in the system, which begin to experience particle-particle contacts at such high ϕ_f. Such contacts could still provide an increase in the Hardness (i.e., providing resistance during the first compression); however, the clustering of particles would also be expected to cause local microfractures, which would diminish the ability of the gel to recover during the decompression.

Similarly, the 11% protein gels containing the 30 to 50 μm particles also exhibited an initial lag phase, where no improvement in Hardness was seen until $\phi_f > 0.06$,

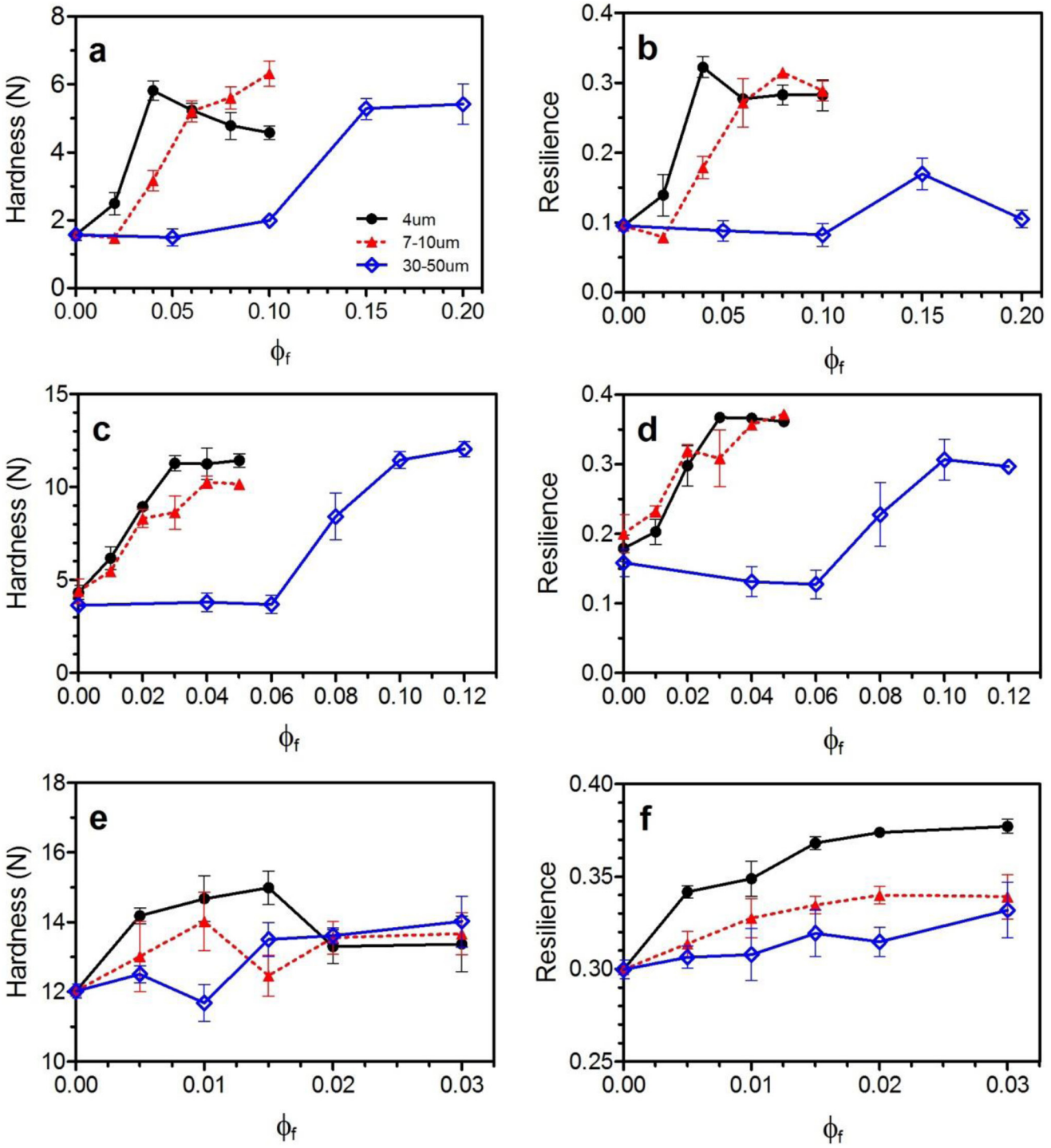

Figure 2. Texture profile analysis (TPA) Hardness (a,c,e) and Resilience (b,d,f) parameters for 4 μm (filled circles) 7 to 10 μm (filled triangles) and 30 to 50 μm (open diamonds) glass beads. Protein content of comminuted meat gels were 9 (a,b), 11 (c,d), and 13% (e,f).

and the Resilience decreased marginally within region. Beyond this region ($\phi_f > 0.06$), both the Hardness and Resilience increased to a plateau at $\phi_f \geq 0.10$. Interestingly, in both the 9% and 11% protein composites, this plateau was roughly equivalent for all three particle sizes, with the exception of the Resilience of the 9% gels prepared with the 30 to 50 μm particles (as discussed above). T2 relaxometry has previously been used to investigate the relative mobility of water in comminuted meat products (Bertram et al., 2001) and has been utilized in various systems (Bertram, Kristensen, and Andersen, 2004; Diao, Guan, Zhao, and Kong, 2016; Stevenson, Liu, and Lanier, 2012; Shao et al., 2016). Our group has recently shown that the glass particles improved stability by re-

ducing water mobility within the gel, decreasing water migration and the occurrence of microfractures within the gel matrix, and thus cooking losses (Gravelle et al., 2016a). By this hypothesis, once the aqueous phase is sufficiently stabilized and microfractures are minimized, no further reinforcing effects would be expected by this mechanism. Therefore, the results presented in Fig. 2a–d are consistent with this explanation.

Finally, the influence of particle size and ϕf on the Hardness and Resilience parameters of the 13% protein gels were greatly reduced (see Fig. 2e,f). This mirrors the impact on cooking losses, where it was suggested that the higher protein content increased the stability of these gels, thus decreasing water mobility and reducing the impact of incorporating glass beads, as their effects would be more localized within the gel. While particle size had little impact on the TPA Hardness with increasing ϕf, there was still a minor improvement in Resilience as particle size decreased (Fig. 2f). These differences may have been associated with the minor differences observed in the cooking losses (Fig. 1c). Although it was suggested that the impact of the glass beads may be more localized within the gel, the far greater surface area provided by the smaller particles should provide a greater enhancement in local stability of the water phase. This would still be expected to decrease microfractures with in the gel, and thus provide an increase in the Resilience of the composite. This impact would be more apparent in the Resilience measurement, as it incorporates the post-deformation decompression. During compression, preexisting microfractures would be susceptible to further propagating, which would diminish the ability of the gel to recover, even if the large deformation resistance was comparable during the initial compression. This would account for the minor impact of particle size on the TPA Hardness parameter.

Cohesiveness and springiness. The second set of large deformation parameters evaluated by TPA were Cohesiveness and Springiness. Cohesiveness is defined as the total work done during the second compression relative to that of the first, while the Springiness characterizes the relative height reduction between the 2 compressions. Therefore, these 2 parameters incorporate a recovery period (2 sec in the present work) which allows the sample to relax. The Cohesiveness and Springiness as a function of ϕ_f for the 3 different protein concentrations under investigation are presented in Fig. 3.

The influence of filler size and protein content on the Cohesiveness of the composites was comparable to that of the Resilience; however, as noted above, the Resilience describes the immediate response, and is also strongly dependent on the elastic properties of the material. Despite this, the overall trends at each protein level are quite similar. In the low protein composites (9%), the addition of both the 4 μm and 7 to 10 μm particles produced an initial minor decrease in Cohesiveness (although not statistically significant) to $\phi_f = 0.02$, followed by a sharp increase to a plateau at a Cohesiveness value of ~0.6. Consistent with the influence on Resilience, the smaller particles required a lower ϕ_f to reach the plateau. The 30 to 50 μm particles also exhibited a gradual, minor decrease in Cohesiveness, up to $\phi_f = 0.10$, followed by slight increase, and subsequent decrease at $\phi_f = 0.15$ and 0.20, respectively. However, as these changes were not statistically significant, the larger particles effectively had no influence on the Cohesiveness of the composite gel.

The composites formulated with 11% protein displayed a similar effect to that of the 9% gels. At the lowest ϕ_f tested ($\phi_f = 0.01$), the Cohesiveness of the gels containing the 4 μm and 7 to 10 μm particles did not deviate from that of the unfilled gel, likely due to the stronger gel network formed, as compared to the 9% gels. After this initial 'lag phase', the Cohesiveness again abruptly increased to a plateau at $\phi_f \geq 0.03$, which corresponds to those formulations which exhibited little-to-no liquid expulsion (see Fig. 1b). Finally, the composites containing the 30 to 50 μm particles again showed a gradual decrease up to $\phi_f = 0.06$, beyond which the Cohesiveness also increased to approximately the same plateau value as that of the gels containing the smaller particles.

The Cohesiveness of the 13% protein gels appeared to experience a minor, gradual increase with ϕ_f for all filler sizes; however, these increases were only statistically significant in the samples filled with 4 μm particles, where all the filled gels were significantly higher than the control.

The effect of filler size on the Springiness of the composite gels was analogous to that seen in the Cohesiveness, although no lag was observed for the 9% protein gels containing the 4 μm particles. There was a brief lag period with the 7 to 10 μm particles, but as with the other large deformation parameters, there was a rapid increase in Springiness above $\phi_f = 0.02$. For both particle sizes, although there was some variation in the Springiness values after the initial increase, these differences were not statistically significant. We thus conclude that the influence of both the 4 μm and 7 to 10 μm particles result in a plateau in the Springiness of the composites at $\phi_f \geq 0.02$ and 0.04, respectively. Similarly, the variation seen in the Springiness when incorporating the 30 to 50 μm particles at increasing ϕ_f was not statistically

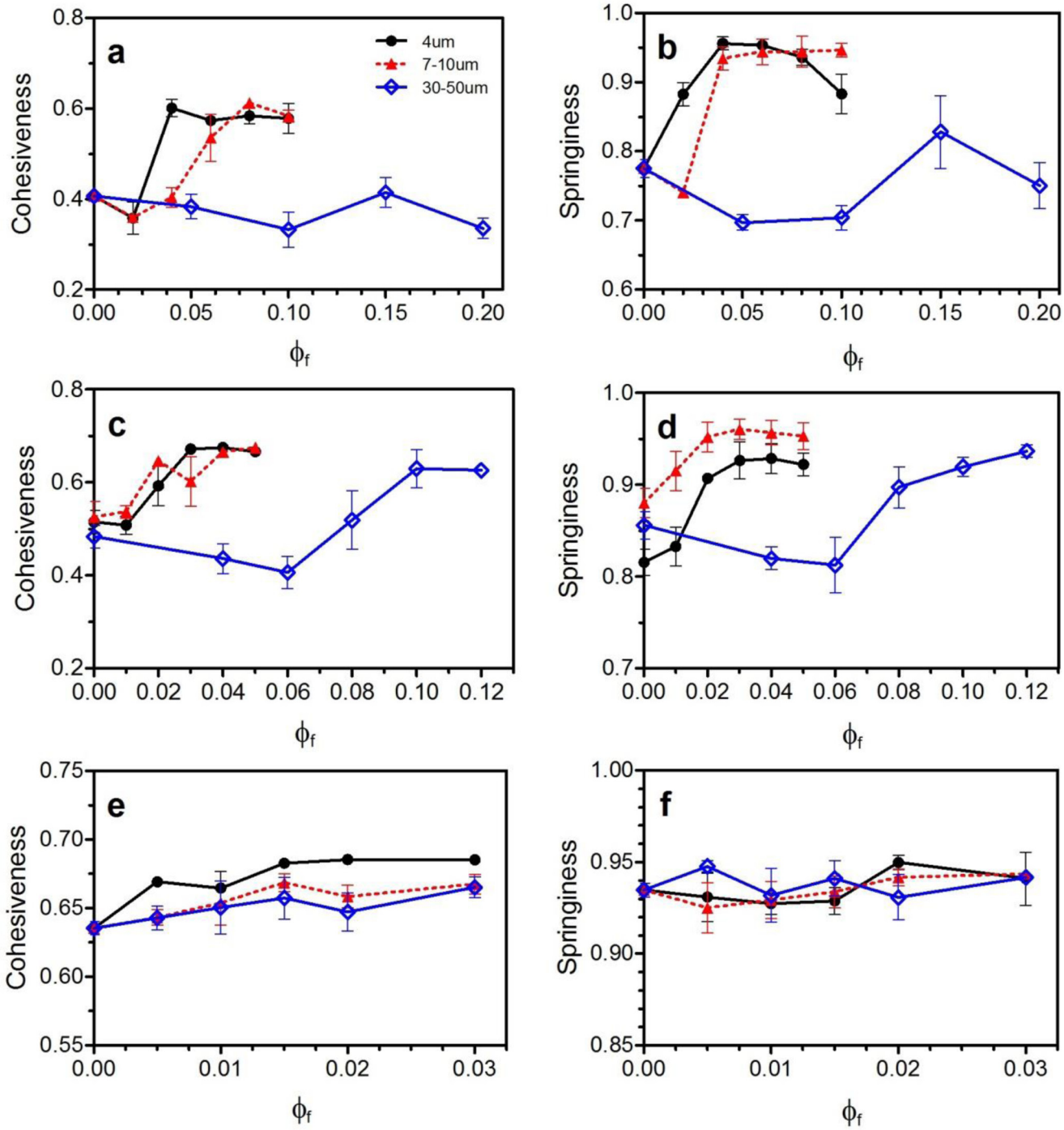

Figure 3. Texture profile analysis (TPA) Cohesiveness (a,c,e) and Springiness (b,d,f) parameters for 4 µm (filled circles) 7 to 10 µm (filled triangles) and 30 to 50 µm (open diamonds) glass beads. Protein content of comminuted meat gels were 9 (a,b), 11 (c,d), and 13% (e,f).

significant. However, this trend mirrored that seen in the Cohesiveness and Resilience parameters, and is likely associated with the mobility of the water phase. With insufficient particle surface area available for interaction, the water is still relatively free to migrate throughout the gel network during thermal treatment. This has been shown to irreversibly damage some of the newly formed protein-protein contacts (Gravelle et al., 2016b). Above a certain threshold (i.e., minimum available surface area), the water mobility is reduced sufficiently so that textural properties of the gel network are enhanced. However, because of the much lower surface area of the 30 to 50 µm beads, much higher ϕ_f were required to produce an effect, resulting in particle-particle contacts. As described above, although contacts between the rigid particles would enhance the initial Hardness of the composite, it has also

been suggested that adjacent particles being forced to move past one another would result in microfractures to the surrounding gel matrix. This would decrease the ability of the composite to recover, thus counteracting the influence of stabilizing the water phase. Such an effect can be seen at the highest incorporation level ($\phi_f = 0.20$), where the Resilience, Cohesiveness, and Springiness all decrease (Fig. 2b, 3a, and 3b, respectively).

The Springiness of the 11% protein composite gels exhibited a similar trend to that seen in the 9% gels. Both the 4 μm and 7 to 10 μm particles produce an immediate increase to a plateau, while the addition of the 30 to 50 μm particles results in a gradual minor decrease in Springiness up to $\phi_f = 0.06$, while above this level, Springiness increased. Due to the higher protein content, less particles were needed to stabilize the water phase, and thus the decrease in Springiness was not observed at the higher ϕ_f tested.

Finally, in the composites prepared with 13% protein, Springiness was not significantly influenced by the addition of glass beads of any size, for all compositions tested. This can again be attributed to the stronger, more interconnected gel matrix, which already produced higher textural values in the unfilled gels and increased the stability of the water phase. Therefore, the reduced water mobility in these more stable gels localizes the effect of the filler particles, which seems to diminish their ability to increase the large deformation/textural properties of the composite material.

Microstructure

Representative scanning electron micrographs of the composite gels prepared at different protein content and low ϕ_f are displayed in Fig. 4 (Note that the star-shaped objects apparent in Fig. 4a are phosphate crystals which were an artifact resulting from the fixation procedure prior to imaging). At all protein levels, the glass beads appear to be embedded in the protein matrix and retain some residual protein on the surface; however, the majority of the visible glass particles have exposed surfaces, indicating a weak affinity for the protein. This has also been reported previously (Gravelle et al., 2015; Gravelle et al., 2016a), and suggests the glass particles interact weakly with the gel matrix, and are thus loosely associated with the protein network. It is also noteworthy that despite the differences in protein content, the overall structure of the protein network appears to be quite similar. This can be attributed to the fact that all the formulation presented in Fig. 4 were quite stable, exhibiting little to no liquid losses.

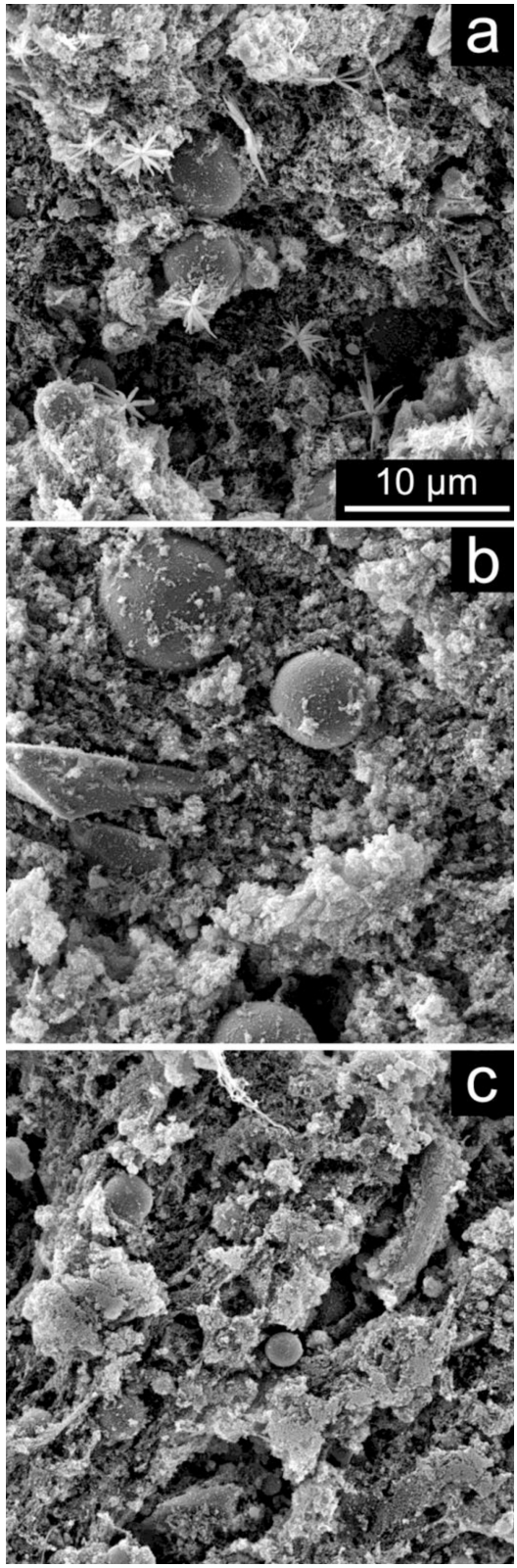

Figure 4. Influence of protein content on the microstructure of myofibrillar protein gels containing glass beads at low filler content 9% protein, 4 μm glass beads (a); 11% protein, 7 to 10 μm glass beads (b); 13% protein, 4 μm glass beads (c). The 9 and 11% protein gels were prepared with a $\phi_f = 0.04$, while the 13% was prepared with a $\phi_f = 0.03$. Note: the star-shaped objects are phosphate crystals formed after the fixation and drying procedures, for microscopy, and are not expected to influence the microstructure of the meat matrix.

Representative images of the different glass bead sizes embedded within the myofibrillar gel network (9% meat protein) are presented in Fig. 5. All the samples containing particles were formulated with a relatively low filler content ($\phi_f = 0.04$). For all 3 particle size ranges, the filler was incorporated homogeneously throughout the sample and all appear to exhibit a weak interaction with the protein network, as discussed above. The protein network of the formulation containing the 4 μm and 7 to 10 μm particles presented in Fig. 5b,c appears to be more dense and less porous than the other formulations. These samples were also more stable, having significantly lower cooking losses and higher TPA values than the unfilled gel (Fig. 5a) and that containing the 30 to 50 μm particles (Fig. 5d).

Figure 6 depicts the comminuted meat gels containing 30 to 50 μm glass beads at increasing filler content ($\phi_f = 0.04$, 0.08, and 0.15). Again, it can be seen that the density of the protein network increases as ϕ_f increases. As filler content increased, cooking losses decreased (see

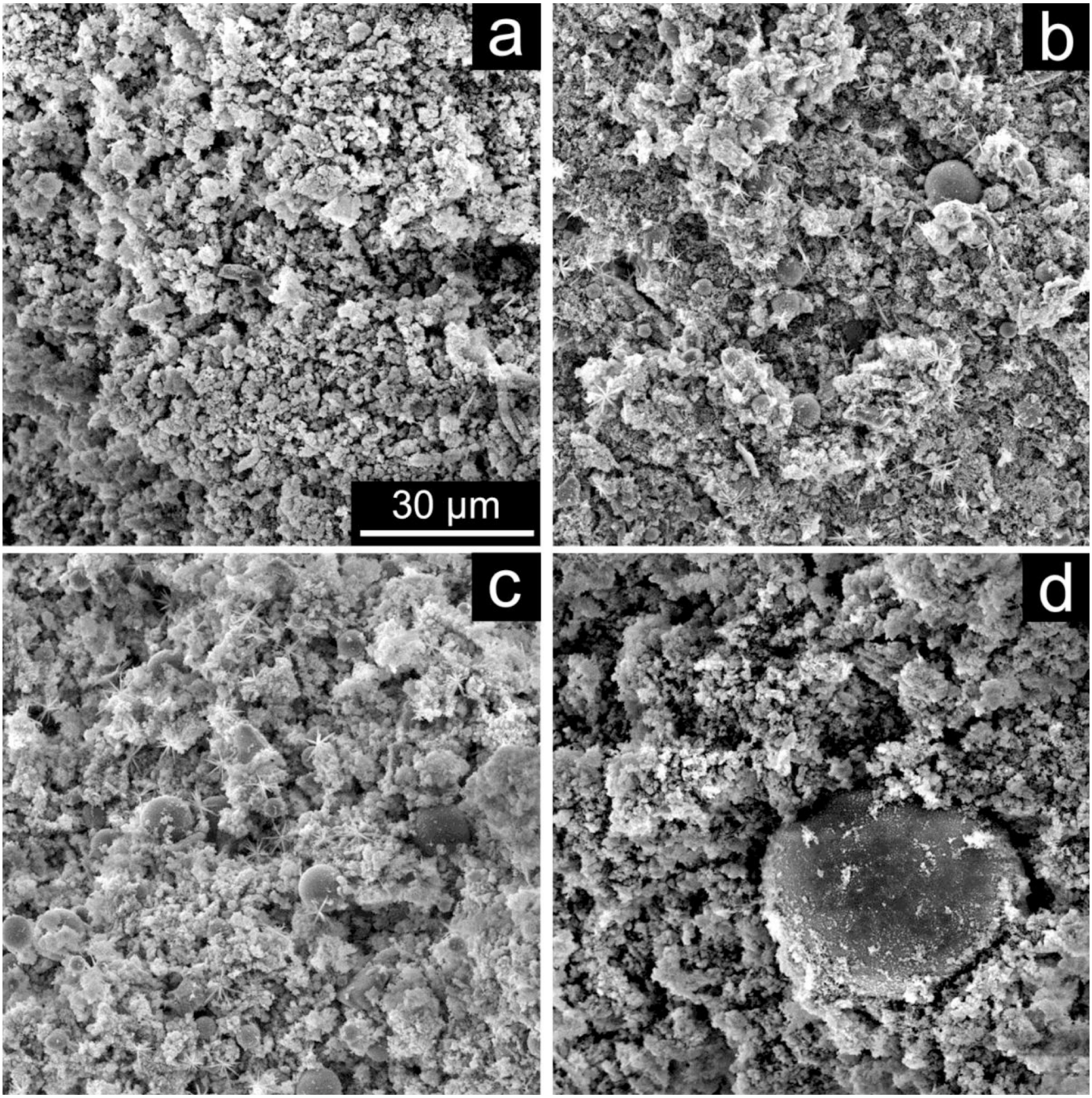

Figure 5. Scanning electron microscopy (SEM) micrographs of myofibrillar protein gels containing glass particles of distinct size ranges. No filler (a); ~4 μm (b); 7 to 10 μm (c); 30 to 50 μm (d). All gels were formulated to have 9% meat protein and 0.04 volume fraction filler ($\phi_f = 0.04$).

Fig. 1a), which is in contrast to the more dense appearance of the protein network. As was previously suggested (Gravelle et al., 2016a), the glass beads may assist in stabilizing the water during gelation by reducing migration through the protein network. Such mobility can cause microfractures, and lead to the formation of interconnected water channels and increased cooking losses. Therefore, the more dense appearance of the more stable samples (e.g., Fig. 5b,c, and Fig. 6c) may represent a decrease in such fractures, which has previously also been demonstrated using light micrographs (Gravelle et al., 2016a). Figure 6c also depicts an example of a high filler content ($\phi_f = 0.15$), in which particle-particle contacts are quite apparent. As noted earlier, such contacts would likely influence the large deformation properties of the composite material. These images are consistent with the interpretation discussed above; i) the observed increase in TPA Hardness at $\phi_f > 0.10$ which may have been caused by contacts between these rigid fillers, and ii) the lack of a significant increase in the Resilience, Cohesiveness, and Springiness, which incorporate the recovery of the material. The latter was attributed to the damage these particles would cause to the surrounding gel network as they slid past one another during the first compression. Therefore, it appears that the negative impact of particle crowding negated the improvement in TPA parameters associated with stabilizing the water phase.

Conclusion

The influence of glass beads as a model inert filler in a comminuted meat system was explored, based on several factors; protein content (9, 11, and 13%), filler size (4 μm, 7 to 10 μm, and 30 to 50 μm), and ϕ_f employed. Consistent with previous studies, at all protein levels, the glass particles were uniformly dispersed throughout the gel, and appeared to weakly interact with the protein network. This interaction was evident by the appearance of both exposed glass surfaces and regions with adhered protein, when observed in scanning electron micrographs. Liquid loss during thermal treatment was strongly influenced by both protein content and filler size. Particle size determines the filler surface area available for interaction with the aqueous phase, and thus a greater ϕ_f was required to decrease liquid losses as particle size increased. Increasing protein content also increased gel stability, resulting in reduced liquid losses observed in the unfilled gels, and lower ϕf needed to stabilize the composite system. The impact of the glass beads was also diminished in the more stable gels because water mobility was already restricted by the gel matrix, thus localizing the influence of the filler particles and decreasing the impact

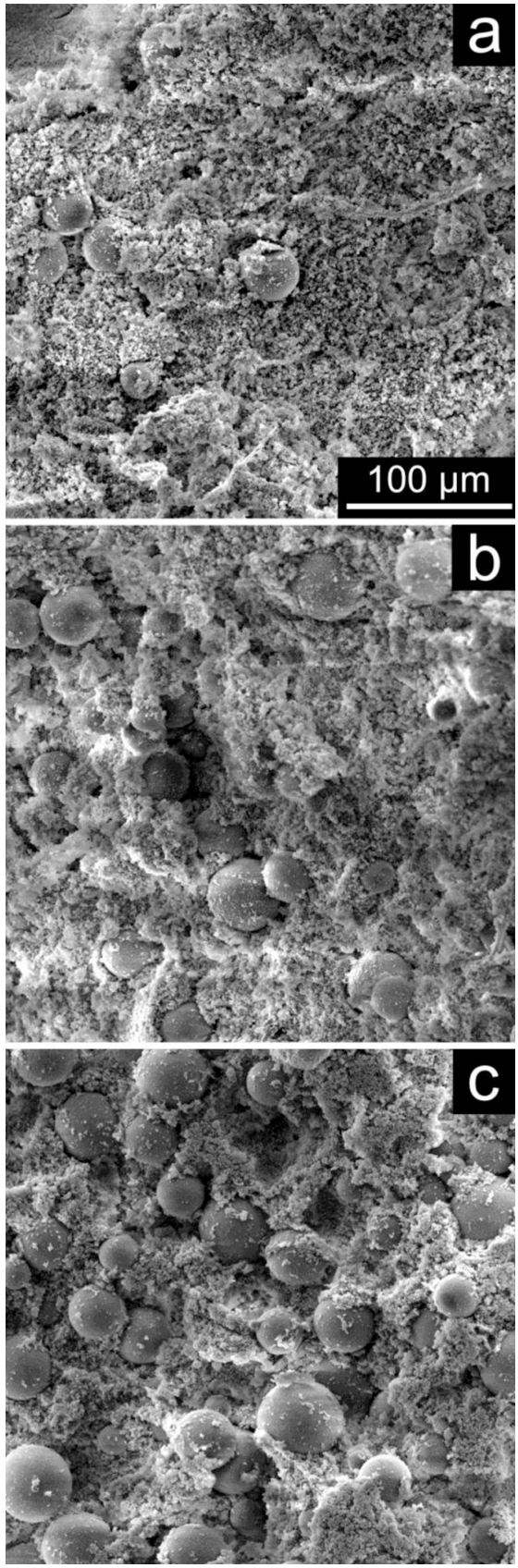

Figure 6. Scanning electron microscopy (SEM) micrographs of myofibrillar protein gels containing 30–50 μm glass particles at increasing volume fractions (ϕ_f). (a) 0.04; (b) 0.08; (c) 0.15. All gels were formulated to have 9% meat protein.

of filler size. Similarly, the textural properties of the composite gels were also affected by both protein content and filler size. When incorporating the 4 μm and 7 to 10 μm particles at increasing ϕ_f, all textural parameters reported (Hardness, Resilience, Cohesiveness, and Springiness) increased from the control value (i.e., the unfilled gel) to a similar plateau in both the 9 and 11% protein gels. In the 9% gels, there was a brief lag phase at lower ϕ_f prior to observing reinforcement; i.e., no statistical variation from the control was observed below $\phi_f = 0.04$. A much greater lag phase was observed when incorporating the 30 to 50 μm particles in both the 9 and 11% protein gels. Hardness values still increased to a plateau at $\phi_f > 0.10$ and 0.06, respectively. In the 9% gels, Resilience, Cohesiveness, and Springiness were unaffected by the addition of the 30 to 50 μm particles (which was attributed to particle crowding), while the 11% gels saw an increase in these parameters at higher ϕ_f. Finally, consistent with the liquid loss data, the addition of glass beads in the more stable 13% gels had much less of an impact on reinforcing the textural properties of the composites. The 4 μm particles produced a minor reinforcement, where a moderate increase in Hardness was observed at lower ϕ_f, and a gradual increase in Resilience and Cohesiveness was also seen with increasing ϕ_f. There was also a statistically significant increase in the Resilience of the composited containing 7 to 10 μm particles at the highest incorporation levels tested ($\phi_f = 0.02, 0.03$). Through this work it has been shown that the ability of insoluble, hydrophilic filler particles to reduce cooking losses and reinforce the textural attributes of comminuted meat products is strongly influenced by the protein content, filler size, and ϕ_f employed. Furthermore, this work has also established the relevant ranges of protein and filler content which can effectively impact the stability of such a comminuted meat system. These findings should be relevant to the food industry, as they suggest that an inert, food-grade particle with comparable properties to the glass beads employed here (hydrophilic, insoluble, and micrometer-sized) may function as an effective stabilizer at low concentrations. Such a filler could foreseeably decrease cooking losses and product shrinkage, thus improving overall product yield. This type of approach could also be useful in improving stability when reformulating products to meet evolving consumer demands.

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