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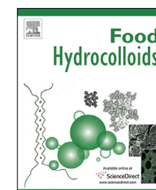

Insight into the mechanism of myofibrillar protein gel stability: Influencing texture and microstructure using a model hydrophilic filler

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In the present study we have characterized the influence of incorporating micron-sized glass particles into a comminuted protein matrix at increasing filler volume fractions (ϕ_f). Liquid expulsion during gelation was rapidly reduced in an approximately linear fashion from ~20 wt% in unfilled gels to no expulsion at $\phi_f = 0.03$. Similarly, large deformation mechanical attributes improved with increasing filler addition, reaching a plateau at when water expulsion was eliminated. Hardness increased from 4.3 N in unfilled gels to ~11.3 N at $\phi_f \geq 0.03$. Similarly, Resilience increased 2-fold from 0.18 in unfilled gels, reaching a plateau of ~0.34 at $\phi_f \geq 0.03$. SEM micrographs indicated the glass beads weakly interact with the protein network, leaving their hydrophilic surface exposed and available to interact with free/mobile water. Light micrographs showed that in the absence of filler particles the myofibrillar gels contain an integrated network of water channels which deteriorate the integrity of the gel matrix. By incorporating the ~4 μm glass beads, these channels decreased in size, and were no longer apparent when water expulsion was arrested. Low-field NMR T_2 relaxation measurements confirmed that incorporating the glass beads restricted the mobility of the aqueous phase prior to gelation. The dominant relaxation peak was centered around 130 ms prior to gelation, and shifted to ~70 ms after thermal treatment. Filled gels which exhibited no liquid expulsion during gelation had relaxation times <70 ms even prior to gelation. This work provides insight into the mechanism of myofibrillar protein gel stabilization during gelation and suggests that food-grade, hydrophilic colloidal particles may be of use in improving the textural properties of comminuted meat products.

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1. Introduction

Comminuted meat products such as frankfurters and bologna are made up of a dispersed fat phase embedded in a continuous, porous protein gel predominantly made up of salt-soluble myofibrillar proteins. They can therefore be viewed as a soft biopolymer composite containing an embedded particulate filler (Gordon & Barbut, 1991; Gravelle, Barbut, & Marangoni, 2015). Myofibrillar proteins are well known for their thermally-induced gelation abilities; myosin in particular is capable of forming a gel at low concentrations (~0.5 wt%; Hermansson & Langton, 1988). Prior to gelation, these salt soluble proteins must be extracted via chopping under saline conditions, and this process forms what is referred to as a finely comminuted meat batter (Gordon & Barbut, 1992b). Additional water is also commonly added during comminution, and it has been hypothesized that electrostatic repulsion within the myofilament lattice causes these proteins to swell (Offer & Trinick, 1983). During thermal treatment, the solubilized myofibrillar proteins undergo a non-reversible two-step rearrangement process (Tornberg, 2005). The first step occurs between 30 and 50 °C, and involves partial denaturation and protein aggregation, most notably in the heavy meromyosin head groups. Above 50 °C, denaturation of the helical light meromyosin tail groups leads to further protein/protein interactions, giving rise to the formation of a three-dimensional gel matrix.

Perhaps surprisingly, relatively little is still understood regarding the precise mechanism(s) responsible for stabilizing these types of products. This is likely due to the inherently complex

Abbreviations: CPMG, Carr-Purcell-Meiboom-Gill; FID, free induction decay; IPF, interfacial protein film; NMR, nuclear magnetic resonance; SEM, scanning electron microscope.

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and heterogeneous nature of comminuted meats, which contain a mixture of solubilized proteins (myofibrillar, sarcoplasmic, etc.), intact muscle fibers, connective tissue, fat particles, and minor components such as minerals and inorganic compounds (Tornberg, 2005). Additional factors such as pH and ionic strength during comminution, the addition of non-meat ingredients (i.e. binding agents and fillers), and processing conditions can also strongly influence the properties of the final product (Barbut, 2015). Regarding the aqueous phase, the bulk water holding capacity of myofibrillar systems has predominantly been attributed to the molecular interactions arising from the swelling of the myofibrils (Puolanne & Halonen, 2010). Three classical hypotheses have been put forth in an attempt to describe this influence; i) the net negative charge between myofilaments, which causes an electrostatic repulsion and swelling (Hamm, 1972), ii) an osmotic-like pressure within the filaments arising from preferential segregation of ions within the filament space (Offer & Knight, 1988), and iii) capillary forces arising from surface tension within the myofilaments and the three-dimensional gel network (Offer & Trinick, 1983). However, because the macroscopic bulk water holding properties of these materials can be sufficiently controlled by formulation and processing conditions, more fundamental work on characterizing water stability in myofibrillar protein gels has been lacking in recent decades (Puolanne & Halonen, 2010).

Much of the literature on the stability of comminuted meat systems has been focused on determining the mechanism responsible for stabilizing the fat phase, as instability can lead to degradation of the textural and mechanical attributes of these products (Gordon & Barbut, 1992b; Youssef & Barbut, 2010). There has been significant evidence demonstrating that during the early stages of gelation, the exposed hydrophobic regions of the partially denaturation myofibrillar proteins associate with the dispersed fat globules, forming a layer of protein at the interface known as the interfacial protein film (IPF; Gordon & Barbut, 1990b, 1990c; Gordon & Barbut, 1992b; Lee, 1985). It has been suggested that the IPF is continuous with the surrounding three-dimensional gel matrix, thus physically bridging the fat phase and the protein gel matrix (Gordon & Barbut, 1990a; Hermansson, 1986). It was thus hypothesized that the IPF was intimately involved in stabilizing the fat phase during thermal treatment, preventing fat migration by one of two possible mechanisms; i) the proteins directly associated with the interface 'emulsify' the fat in a manner analogous to how the dispersed phase is stabilized in a true emulsion, or ii) the interfacial proteins become physically linked to the gel network, thus acting more as a physical barrier to fat migration (Gordon & Barbut, 1992b).

Recently, further evidence supporting the physical entrapment theory was reported (Liu, Lanier, & Osborne, 2016); however, these authors proposed that the capillary forces presumed to stabilize the water within the myofilament lattice are also responsible for physically restraining the fat globules from migrating through the gel network. In agreement with this hypothesis, it has been seen that water loss precludes fat loss (Schmidt, 1984), suggesting that stability of the aqueous phase has influence over that of the dispersed fat component. From this, it would follow that achieving stability of the aqueous phase within the myofibrillar protein gel dictates the stability of the entire composite system. Fillers such as starches are often used to stabilize the aqueous phase by sequestering water via gelatinization. A recent study characterized the influence of modified starches in surimi (comminuted fish meat) gels and demonstrated a correlation between initial swelling temperature and starch viscosity on improving gel strength (Kong et al., 2016). We have recently reported that the addition of hydrophilic model filler particles which do not absorb water can decrease liquid expulsion during protein gelation, and increased the mechanical strength of the composite (Gravelle et al., 2015). This influence was found to be dependent on filler size, where smaller particles were found to have a greater influence on reducing liquid expulsion and increasing mechanical and textural properties at lower incorporation levels. As an extension of this work, in the present study we have investigated the influence of incorporating near-colloidal glass beads (~4 μm) as a model hydrophilic filler for stabilizing the aqueous phase in comminuted myofibrillar protein gels.

2. Materials and methods

2.1. Materials

Approximately 25 kg of fresh boneless, skinless chicken breast meat 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 (Schneidmeister SMK 40, Berlin, Germany) at the low speed setting for approximately 60 s and incorporated by hand to produce a homogeneous mixture. The meat was then portioned into ~800 g batches in bags, vacuum packed, and stored at −20 °C until use. Protein content was determined to be 21.2 wt% using the Dumas method and a nitrogen conversion factor of 5.53 (Mariotti, Torné, & Mirand, 2008).

Spherical glass beads of ~4 μm diameter and $\rho = 2.5$ g/mL were obtained from Cospheric LLC (Santa Barbara, CA, USA). Particle size distribution was performed using an optical microscope, as described in Section 2.5. Greater than 95% of the particles were found to be in the range of 1.0–6.0 μm in diameter.

2.2. Preparation of composite gels

All composites were prepared in a household food processor (Braun Household, Germany) and formulated to have a final protein content of 11 wt% in the continuous phase. Glass beads were added on a volume fraction basis (volume fraction filler, ϕ_f), from $\phi_f = 0$ to 0.05. Filler particles were first dispersed in a portion of the aqueous phase prior to being incorporated into the meat slurry, to avoid the formation of dry aggregates. Prior to preparation, each portion of meat was completely defrosted overnight under refrigerated conditions (~4 °C), and subsequently inspected for any residual connective tissue, which was removed by hand. Briefly, the meat batters were prepared by chopping 100 g meat for 60 s, followed by the addition of 50 g distilled water (10 s chopping) and 3.75 g NaCl (2.5% salt; 10 s chopping). The slurry was then put on ice for 5 min to facilitate the extraction of salt-soluble myofibrillar proteins (ionic strength ~0.42 M). Finally, the remaining water containing the appropriate amount of dispersed filler particles was added and the mixture was further chopped for a total of 80 s. 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 in triplicate.

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 s to remove large 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 core temperature was monitored using thermocouple unit

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