Food Structure

Volume 12 | Number 4 Article 7

1993

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FOOD STRUCTURE, Vol. 12 (1993), pp. 469-474 1046-705X/93$5.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

STRUCTURE-COMPRESSIVE STRESS RELATIONSHIPS IN MIXED DAIRY GELS

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Abstract

Mixed dairy gels (including a control without fat) of skim milk powder (SMP) and whey protein isolate (WPI) containing fat globules were formed by heating protein emulsions to 90°C and by acid release from glucono-δ-lactone to provide a pH of 4.3-4.4. Fat globules with artificial protein membranes (FGAPM) were prepared by homogenization of a butter oil/water mixture in the presence of WPI while fat globules without membranes were stabilized with polyoxyethylene sorbitan monolaurate (Tween 20). Both emulsions were added at a 4% (w/w) level to solutions having 3% SMP and 8.3% WPI. The gel containing FGAPM had significantly higher compressive strength than the control without fat (2.4 versus 1.8 kPa, respectively) and microstructurally it was a mixed gel in which the FGAPM, casein and whey protein aggregates formed a copolymer network. Addition of fat globules without membranes led to a filled gel weaker than the control without fat (1.4 versus 1.8 kPa, respectively). Bonding of the protein membrane in FGAPM to the gel network and presence of individually dispersed fat globules without membranes was demonstrated by transmission electron microscopy. The difference in microstructure is proposed to be responsible for the mechanical properties of each gel.

Key Words: Whey protein, casein, protein gels, transmission electron microscopy, fat globules, compressive strength.

Paper received March 19, 1993
Manuscript received December 14,1993
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Introduction

Gels are important food materials exhibiting structural features of polymeric networks and physicochemical and transport properties of aqueous solutions. The limited number of gel-forming proteins that can be used in foods have prompted the study of mixed and filled gels, in which a much wider range of functionality is expected by combining different polymer networks and fillers. Both major protein fractions in milk form gels: casein, by acid or enzyme-induction; and, whey proteins by heating. Mixed dairy gels have been obtained from blends of skim milk powder (SMP) and whey protein concentrate solutions by using glucono-δ-lactone as source of acid and heating to above 80°C (Aguilera and Kinsella, 1991; Aguilera and Kessler, 1988).

Incorporation of a dispersed lipid phase is usually desirable in fabricated foods, since it modifies favorably textural attributes and can be used as a carrier of fat-soluble nutrients, flavors and colors. Recently, small fat globules with artificial protein membranes (FGAPM) have been incorporated into the protein network of mixed dairy gels of various compositions, pH values and total solids content (Aguilera and Kinsella, 1991). It has been postulated that these fat globules act as nuclei during gelation, and later become an integral part of the gel network (Jost *et al.*, 1989; Xiong *et al.*, 1991).

Scanning and transmission electron microscopy (SEM and TEM) have been important tools in elucidating the microstructure of dairy gels. Acid casein gels were characterized as an open network of branched casein micelles chains arranged in a string-of-bead sequence or as thick aggregates leaving large cavities filled with liquid (Harwalkar and Kalab, 1980; Heertje *et al.*, 1985; Roefs *et al.*, 1990). Whey protein gels were described as formed by denatured protein varying in structure from an evenly dispersed matrix of fine strands (< 0.1 μm in diameter) to a network of linked particle aggregates of approximately 1 μm thickness and large pores (Mulvihill and Kinsella, 1988; Langley and Green, 1989). Similar microstructural forms were reported for β-lactoglobulin gels by Stading and Hermansson (1991). Interesting structural features in mixed dairy gels result from interactions between denatured β-lactoglobulin proteins and

Table 1. Composition and properties of simple and mixed dairy gels.

Gel	WPI (%)[a]	SMP (%)[a]	Fat (%)[a]	Protein (%)[a]	pH	Compression stress (kPa)[b]
SMp[c]	-	16.0	-	5.7	4.6	1.0 ± 0.18
WPI[c]	6.0	-	-	5.6	4.6	3.2 ± 0.30
WPI/SMP	3.0	8.3	-	5.7	4.3	1.8 ± 0.12
WPI/SMP/cream WPI	3.0	8.3	4	5.7	4.4	2.4 ± 0.32
WPI/SMP/emulsion	3.0	8.3	4	5.7	4.4	1.4 ± 0.14

[a]percent weight basis; [b]mean ± standard deviation;

[c]presented as reference, from Aguilera and Kinsella, 1991

the surface of casein micelles and the spanning of casein micelles chains by finely flocculated whey protein (Modler and Kalab, 1983; Aguilera and Kinsella, 1991).

This work was aimed at describing the relationship between the main microstructural features of dairy gels containing milkfat globules and their texture measured as the compressive strength.

Materials and Methods

Materials

Whey protein isolate (WPI) prepared by ion-exchange chromatography was from Le Sueur Isolates (Le Sueur, MN); and low-heat spray-dried skim milk powder (SMP) was from Maryland and Virginia Milk Producers Association, Inc. (Laurel, MD). Protein contents of WPI and SMP were 92.7 and 35.7% weight basis, respectively. Glucono-δ-lactone (GDL) (Sigma Chemical Co., St. Louis, MO) was used as acid precursor. Polyoxyethylene sorbitan monolaurate, Tween 20, and sodium azide were also purchased from the Sigma Chemical Co. (St. Louis, MO).

Fat globules with artificial protein membranes (FGAPM) were prepared by dispersing a butter oil/water suspension (18%, w/w) and WPI (0.8%, w/w) with a Polytron PCU-2 mixer (Kinematica GmbH, Luzern, Switzerland) and homogenizing the mix at 55°C and 3,000 psig (20,670 kPa) for five times (total residence time 3.5 minutes) in a Rannie homogenizer model Mini-Lab, type 8.30H (Niro Atomizer Food and Dairy, Inc., Hudson, WI). Fat globules without membranes were formed similarly using Tween 20 (0.7% w/w) instead of WPI. Emulsions were used within 2 hours after being prepared. The butter oil/water emulsions with WPI and with Tween 20 are referred to as the cream and the emulsion, respectively.

Preparation of gels

Gels listed in Table 1 were prepared as follows: SMP, WPI, and/or cream or emulsion were blended in 80 ml beakers and adjusted to their final concentration with distilled water (3% WPI, 8.3% SMP, and 4% fat, w/w wet basis). Sodium azide was added to solutions at 0.02% as preservative. Blends were gently swirled to achieve a uniform dispersion and were degassed for 40 to 80 minutes under 85 kPa vacuum. Beakers were placed in a 90°C water bath until the temperature of the sample reached 60 ± 2°C. At this time, GDL (0.3 g) was added and the content of the beaker was divided into two cylindrical glass tubes (100 mm long x 12.3 mm diameter) precoated with Sigmacote (Sigma Chemical Co., St. Louis, MO), sealed with rubber stoppers, and held vertically in the water bath at 90 ± 1°C for 30 minutes. Glass tubes were removed from the water bath and cooled for 15 minutes in iced water and stored at 5°C overnight. Part of the gels was saved for the microscopy study. Gels are referred to as WPI/SMP/cream or WPI/SMP/emulsion depending on whether they contained an emulsion stabilized with WPI or Tween, respectively.

Mechanical testing

Gels were allowed to equilibrate at 25°C for 1 hour prior to compression testing. After cutting the gels, at least 4 samples (11 mm long sections) were uniaxially compressed to 20% deformation using an Instron Universal Testing Machine model 1122 (Instron Corp., Canton, MA). The cross-head speed of the compressing plate was set at 10 mm/min. The compressive stress was calculated as the peak force at 20% compression divided by the initial cross-section area. The slight change in the cross-section area during compression was neglected. After compression tests, gels were mixed with 1 vol distilled water, thoroughly crushed, and equilibrated for 30 minutes before the pH determination.

Samples for TEM

Creams and gels were studied with TEM using a sample preparation procedure similar to that described by Liboff *et al.* (1988). Cream was mixed with a 2% agarose solution (3 parts sample to 1 part agarose solution) made from an ultra-low-temperature gelling agarose (Sea-Prep Agarose, FMC Marine Colloids Div., Rockland, ME) prepared as follows: The agarose powder was added slowly to distilled deionized water at 22°C under constant stirring. The solution was heated to boiling while being stirred on a hot plate and then

cooled to 22°C. Cream was mixed into the agarose solution by gently stirring with a wooden applicator stick and allowed to gel overnight at 4°C.

Gels and agarose-gelled creams were cut into 1 mm cubes and fixed in 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) overnight. After several rinses in phosphate buffer, the samples were post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.0) for 3 hours, dehydrated in a graded series of ethanol, and embedded in Spurr's resin by polymerization in a vacuum oven overnight at 70°C. Sectioning was done on a Sorvall Porter-Blum Ultramicrotome. The sections, 70 to 90 nm thick, were picked up on carbon coated Formvar grids, stained with uranyl acetate followed by Reynolds' lead citrate (Reynolds, 1963), and examined with a Philips 300 TEM operated at 80 kV.

Results and Discussion

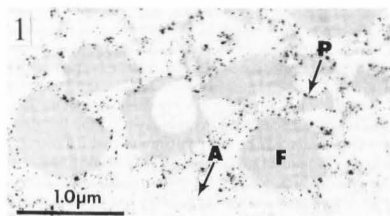
Both the cream and the emulsion were similar in composition and appearance (white and opaque, viscous liquids). Figure 1 shows well-preserved FGAPM in the cream surrounded by an uneven layer of WPI protein (P). Most fat globules (F) observed under the microscope had diameters smaller than 1 μm. This was in agreement with the particle size distribution determined by Coulter counter, which showed that 97.4% of the fat globules were smaller than 1.26 μm (Aguilera and Kinsella, 1991). The microstructure of the cream is similar to that of peanut oil/BSA emulsions presented by Liboff *et al.* (1988). Electron-dense granules in the outer layer of fat globules are probably fixation artifacts consisting of a complex of glutaraldehyde and osmium tetroxide as discussed by Parnell-Clunies *et al.* (1986) and present also in micrographs of other emulsions (Jost *et al.*, 1988; Liboff *et al.*, 1988). Granules were generally located at the outer edge of fat globules but never in the interior nor in the agarose phase (A), so it is conceivable that they are associated with the protein surrounding the fat globules.

Table 1 presents relevant chemical and mechanical data for mixed gels of similar protein content and pH, as well as for pure SMP and WPI gels, so they can be used to compare with the mixed gels. The protein added to the gel with the cream was negligible (ca. 3% of total protein in the gel). Large differences in compression stress were detected among mixed gels and between pure gels of similar protein content, in agreement with results of Xiong *et al.* (1991) and Aguilera and Kinsella (1991). Addition of cream to the WPI/SMP solution resulted in a gel having a compressive strength 1.33 times larger than the WPI/SMP gel, consistent with previous results reporting increased strength of gels upon addition of protein-emulsified fat (Aguilera and Kessler, 1989; Aguilera and Kinsella, 1991; Yost, 1992). This reinforcing effect cannot be accounted for by the addition of fat since the WPI/SMP/emulsion gel having similar fat and protein content as the WPI/SMP/cream gel presented a compressive stress of 0.77 times that of the WPI/SMP gel. Recent work by McClements *et al.* (1993) confirms that fat droplets stabilized with WPI increase the gel strength while those stabilized by non-ionic surfactants, like Tween 20, decrease it. These authors also found that the compressive stress of gels was relatively insensitive to the mean droplet diameter within the range of 0.8-3.5 μm, when surfactants were used. It can be concluded that presence of the protein layer on the fat globules was decisive for reinforcement and that its absence resulted in weakening of the gel. This finding may explain why the presence of fat in dairy products affects texture either positively or negatively. It also reassures that some dairy products derived from homogenized milk have different rheological properties than those made from non-homogenized milk. In effect, fat globules in milk or cream lose part of their membrane during homogenization; the newly formed surfaces of the smaller fat globules adsorb casein particles or plasma proteins and the fat globules can then participate in acid or enzymic coagulation processes (Buchheim, 1986).

The mechanical behavior of mixed gels can be understood by examining the general microstructure of the three types of mixed gels in Figure 2. FGAPM in the WPI/SMP/cream gel were always found attached to the protein network (N) as shown in Figure 2A, contrary to the case when Tween 20 was used as emulsifier (Fig. 2B). It appears that fat globules having a preformed membrane (M) become an integral part of the proteinaceous strands of a WPI/SMP gel, shown in Figure 2C. Thus, gels containing FGAPM, classified in the past as "filled" gels, may be called "copolymer" gels, where the monomeric units are casein micelles, whey protein aggregates, and protein-coated fat globules. Reinforcing in compression would then be the result of a larger number of gelling units forming the network.

Figure 3 shows further detail of the presence of fat globules and gel network in both types of fat-containing mixed gels. Chains in the gel network consisted mostly of protein aggregates 0.1 to 0.3 μm in size, thus constituting a particle gel. Although there was an equal proportion of both sources of protein (approx. 2.8% w/w), it was difficult to distinguish clearly between whey protein aggregates and casein micelles in the network. Heating at high temperature (90°C) and low pH altered the original protein structures beyond recognition, inducing denaturation, aggregation, fusion and polymerization of components. Gels of whey protein or, β-lactoglobulin prepared under similar conditions are known to present a highly distorted network formed by fused whey protein aggregates (Beveridge *et al.*, 1983; Harwalkar and Kalab, 1985; Stading and Hermansson, 1991; Yost and Kinsella, 1992). Similarly, casein gels induced by GDL are formed by highly distorted, opaque casein particles ranging in size from approximately 0.2 to 0.4 mm, many times fused in groups (Harwalkar and Kalab, 1985; Aguilera and Kinsella, 1991).

In the past, gels containing fat were usually regarded as filled gels. Isolation and lack of interaction among fat globules stabilized with Tween 20 as well as



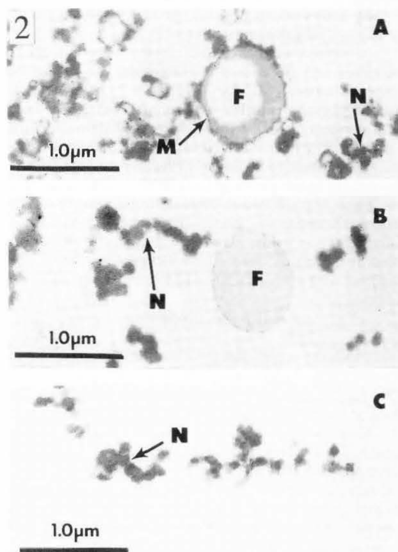
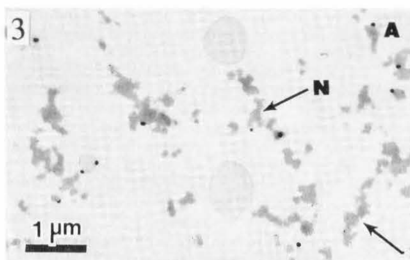
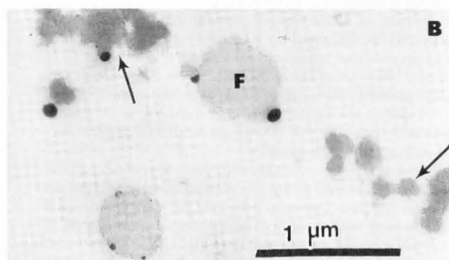
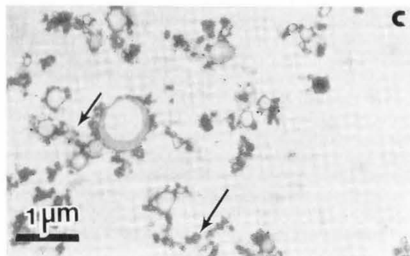


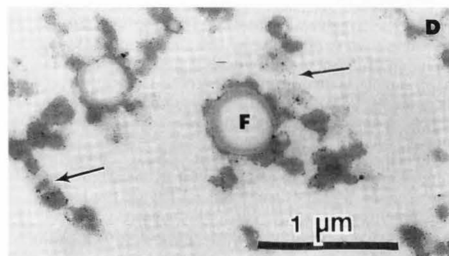
Figure 1. Transmission electron micrograph of cream stabilized with whey protein isolate. F = fat globule; P = adsorbed whey protein; A = agarose network.

Figure 2. Transmission electron micrographs of mixed WPI/SMP gels. **A.** Gel containing fat globules with an artificial protein membrane. **B.** Gel containing fat globules from an emulsion stabilized with Tween 20. **C.** Gel with no fat added. F = fat globule; M = artificial protein membrane; N = gel network.









with the extensive gel network is evident in Figures 3A and 3B. On the other hand, Figures 3C and 3D show several FGAPM bonded together by protein particles and incorporated as extra units into the gel network. An important feature of this gel is the continuity of the protein domain through the membrane of fat globules and the bonding between the membrane and the protein network. WPI/SMP gels containing FGAPM should be considered mixed gels, produced by "copolymerization" of three monomers: casein micelles, denatured whey protein particles and fat globules.

Conclusion

This work confirms that mixed WPI/SMP gels containing fat globules with artificial or reformed protein membranes prepared by homogenization are actually copolymer gels of whey protein aggregates, casein micelles, and FGAPM. Microstructural features of the gel network, as demonstrated by electron microscopy, were similar to a composite network of pure WPI or SMP gels, with or without incorporated FGAPM. The presence of FGAPM in the network adds extra polymer units and reinforce the structure, leading to higher compression strengths. Fat globules stabilized only with a surfactant (Tween 20) do not interact with the proteinaceous gel network leading to weaker gels filled with isolated fat globules. The artificial protein membrane over the fat globules is an essential microstructural feature for formation of strong mixed gels.

Acknowledgments

This work was performed while the senior author was visiting professor at the Department of Food Science, Cornell University as a Fulbright scholar. Financial assistance from the NE Dairy Foods Research Foundation and a grant (513-5600-G-00-1062-00; PSTC No. 11.373) from the Agency for International Development (AID) is appreciated.

References

Aguilera JM, Kessler HG. (1988). Physicochemical and rheological properties of milk fat globules with reconstituted membranes. Milchwissenschaft **43**, 411-415.

Aguilera JM, Kessler HG. (1989). Properties of mixed and filled dairy gels. J. Food Sci. **54**, 1213-1217 and 1221.

Aguilera JM, Kinsella JE. (1991). Compression strength of dairy gels and microstructural interpretation. J. Food Sci. **56**, 1224-1228.

Figure 3 (facing page). Transmission electron micrographs of mixed WPI/SMP gels containing fat droplets (F). **A** and **B**: emulsion stabilized with Tween 20; **C** and **D**: cream stabilized with whey protein isolate. Arrows point at gel network (N).

Beveridge T, Jones L, Tung MA. (1983). Stranded structure development in thermally produced whey protein concentrate gel. Food Microstruc. **2**, 161-163.

Buchheim W. (1986). Membranes of milk fat globules: Ultrastructural, biochemical and technological aspects. Kieler Milchwirt. Forsch. **38**, 227-246.

Harwalkar VR, Kalab M. (1980). Milk gel structure. XI. Electron microscopy of glucono-δ-lactone-induced skim milk gels. J. Texture Stud. **11**, 35-49.

Harwalkar VR, Kalab M. (1985). Thermal denaturation and aggregation of β-lactoglobulin in solution. Electron microscopic study. Milchwissenschaft **40**, 65-68.

Heertje I, Visser J, Smits P. (1985) Structure formation in acid milk gels. Food Microstruc. **4**, 267-278.

Jost R, Dannenberg F, Rosset J. (1989). Heat-set gels based on oil/water emulsions: an application of whey protein functionality. Food Microstruc. **8**, 23-28.

Langley KR, Green M. (1989). Compression strength and fracture properties of model particulate foods composites in relation to their microstructure and particle matrix interaction. J. Texture Stud. **20**, 191-200.

Liboff M, Goff HD, Haque Z, Jordan W, Kinsella JE. (1988). Changes in the ultrastructure of emulsions as a result of electron microscopy preparation procedures. Food Microstruc. **7**, 67-74.

McClements DJ, Monahan FJ, Kinsella JE. (1993). Effect of emulsion droplets on the rheology of whey protein isolate gels. J. Texture Stud. **24**, 411-422.

Modler HW, Kalab M. (1983). Microstructure of yoghurt stabilized with milk proteins. J. Dairy Sci. **66**, 430-437

Mulvihill DM, Kinsella JE. (1988). Gelation of β-lactoglobulin: Effects of sodium chloride and calcium chloride on the rheological and structural properties of gels. J. Food Sci. **53**, 231-236.

Parnell-Clunies EM, Kakuda Y, Humphrey R. (1986). Electron dense granules in yoghurt: Characterization by x-ray microanalysis. Food Microstruc. **5**, 295-302.

Reynolds ES. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**, 208-212.

Roefs SPFM, van Vliet T, van den Bijgaart HJCM, de Groot-Mostert, Walstra P (1990). Sstructure of casein gels made by combined acidification and rennet action. Neth. Milk Dairy J. **44**, 159-188.

Stading M, Hermansson A-M. (1991). Large deformation properties of β-lactoglobulin gel structures. Food Hydrocoll. **5**, 339-352.

Xiong YL, Aguilera JM, Kinsella JE. (1991). Emulsified milkfat effects on rheology of acid-induced milk gels. J. Food Sci. **56**, 920-925.

Yost RA. (1992). Rheological Properties and Microstructure of Filled Whey Protein Gels. M.S. Thesis, Department of Food Science, Cornell Univ., Ithaca, NY.

Yost RA, Kinsella, JE. (1992). Microstructure of

whey protein isolate gels containing, emulsifies butterfat droplets. J. Food Sci. **57**, 892-897

Discussion with Reviewers

E. Dickinson: It is known that the effect of filler particles on the rheology of polymer network gels is influenced to some extent by the size of the particles as well as by the nature of the interactions between the polymer network and the surface of the filler particles. Unless one takes particular care to control homogenization conditions, it seems likely that the droplet-size distributions produced with whey protein isolate will be rather different from those produced with Tween 20. How confident can the authors be that a significant part of the difference in gel strengths produced with the two different emulsifiers is not due to particle size effects?
Authors: Similar sizes of fat globules (generally < 1 μm) were observed inside the gels by inspection during the microscopy sessions. Other members of the group (McClements, Monahan and Kinsella, 1993) working with similar materials and procedures have recently reported that "...gel strength increased as droplet size decreased for droplets stabilized with WPI but was relatively insensitive to the size of droplets stabilized by the small molecule surfactants".

E. Dickinson: Depending on the surfactant/protein ratio in the gels containing the droplets emulsified with Tween 20, it is possible that some milk protein could become adsorbed at the emulsion droplet surface whilst some of the non-ionic surfactant becomes bound to adsorbed and unadsorbed milk protein. It is known that Tween 20 forms a 1:1 complex with β-lactoglobulin, and the implications of this interaction for the competitive adsorption of these two species in model oil-in-water emulsions has recently been discussed [Courthaudon *et al.* (1991) Colloids Surf. **56**, 293-300]. Have the authors considered the effects of surfactant-protein interactions on the rheology of the gels in the absence of emulsion droplets? What would be the effect of adsorption of caseins and/or whey proteins on the gel rheology?
Authors: In our gels, the presence of a thick layer of protein was observed in fat globules stabilized by WPI but not in globules stabilized with Tween. However, we agree that this is a subject that we need to be aware of in future work. In the present work, we ignored the effect of adsorption of surfactant to casein or whey proteins on the rheology of gels.

C.V. Morr: What is a "mixed gel"? Why not describe it as an oil/water emulsion?
Authors: "Mixed", as well as "filled", gels is a terminology now being widely used in the food materials literature, e.g., **1**. Brownsey GJ, Morris VJ (1988) Mixed and filled gels - models for foods. In: Food Structure: Its Creation and Evaluation. Blanshard JMV, Mitchell JR (eds.), Butterworths. **2**. Williams PA *et al* (1991) Mixed gels formed with konjac mannan...; and **3**. Doublier JL, Llamas G (1991) Flow and viscoelastic rop of mixed Xanthan...; **both** in: Food Polymers, Gels and Colloids. Dickinson E (ed.). The Royal Society of Chemistry, London; and **4**. Morris ER (1990) Mixed polymer gels. In: Food Gels. Harris P (ed.). Elsevier.

X.L. Xiong: A 20% compression seems too large to be within the linear viscoelastic range. Did the authors actually verify the linearity?
Authors: Indeed, 20% compression is outside the linear viscoelastic range (which is <10% deformation). However, compressive stress is only used as an index of texture and not to calculate Young modulus or to infer any basic mechanical property.

X.L. Xiong: Could the authors elaborate the basis for distinguishing WPI particles from casein micelles in a composite where both types of protein particles appear similar? In Figure 2, the microstructure of the composite gels shows the presence of both whey protein and casein-like micelles on the fat globule membrane. Would the membrane composition change during mixing of cream with WPI/SMP and would it be affected by the mixing time? Was β-lactoglobulin or α-lactalbumin preferentially adsorbed at the surface of fat globules in the acidic pH environment used in this study?
Authors: As mentioned in the text, it is difficult to distinguish between WPI aggregates and casein micelles. However, once the WPI membrane is built on the surface of globules, it could react with casein micelles giving the impression that both are present at the surface. We do not know whether the membrane composition varies during mixing previous to gelation, although it is an extremely mild mechanical process (gentle agitation to avoid air in the mix leading to the presence of bubbles in gels) compared to homogenization where new surfaces are formed. Preferential adsorption of individual protein moieties was not studied.