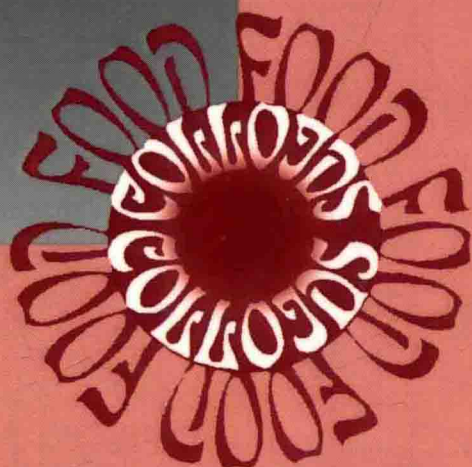


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Food Colloids

INTERACTIONS, MICROSTRUCTURE
and PROCESSING

edited by
ERIC DICKINSON

Food Colloids

Interactions, Microstructure and Processing

Edited by

Eric Dickinson

Procter Department of Food Science, University of Leeds, UK

RS•C

advancing the chemical sciences

The proceedings of Food Colloids 2004: Interactions, Microstructure and Processing held on 18–21 April 2004 in Harrogate, UK.

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Food Colloids
Interactions, Microstructure and Processing

Preface

There can surely never have been a time when the field of colloid and surface science was radiating more excitement or confidence than it is in the early 21st century. Under the fashionable—and as yet relatively untarnished—brand name of ‘nanoscience’ (and its sibling subject ‘nanotechnology’), a traditional discipline that a decade or so ago had become physical chemistry’s poor relation seems to have been relaunched into the scientific mainstream. As a consequence, food researchers working on the nanometre scale are deriving a technical payout from a dynamic growth in activity. They are gaining access to powerful modern instrumental and computational techniques. And they are deriving lively spin-off from the collaboration and recently discovered interest of other scientists whose previous ‘involvement’ with food was mainly limited to the kitchen or local supermarket.

This volume is based on a conference entitled ‘Food Colloids 2004’ held in Harrogate (UK) on 18–21 April 2004, the tenth in a series of European conferences on the subject of food colloids organized under the auspices of the Food (Chemistry) Group of the Royal Society of Chemistry. The programme was sub-titled ‘Interactions, Microstructure and Processing’ and it was arranged under six main themes: (i) interfacial characteristics of food emulsifiers and proteins; (ii) microstructure and image analysis; (iii) processing issues; (iv) phase transitions; (v) interactions of macromolecules, particles, droplets and bubbles; and (vi) perception of taste, texture and appearance.

The Harrogate conference was attended by 192 delegates from five continents. The technical programme consisted of 41 lectures and 97 posters. Most of the invited and contributed lectures are recorded in full in this book. In addition to being displayed at the formal poster sessions, over half of the poster contributions were displayed electronically on the conference web-site—before, during, and after the conference. Research papers based on selected poster presentations are to appear separately in a special issue of the journal *Food Hydrocolloids*.

The editor of this book acknowledges enthusiastic assistance from the other members of the International Organizing Committee in assembling the

scientific programme: Prof. Björn Bergenståhl (University of Lund), Prof. David Horne (Hannah Research Institute), Dr Reinhard Miller (Max-Planck-Institute, Golm), Prof. Juan Rodríguez Patino (University of Seville), Prof. Ton van Vliet (Wageningen University) and Prof. Pieter Walstra (Wageningen University). In addition, for their spirited commitment and hard work, he would like to express his personal and sincere thanks to the members of the Local Organizing Committee: Caroline Eliot-Laizé (Secretary), Jianshe Chen, Rammile Ettelaie, Jonathan James, Brent Murray, Malcolm Povey, and Luis Pugnaroni.

Eric Dickinson (Leeds)

July 2004

Contents

Gels and Gelation

INVITED LECTURE

- Rheology, Structure and Texture Perception in Food Protein Gels 3
E. A. Foegeding

- Mechanism of Acid Coagulation of Milk Studied by a Multi-Technique Approach 16
D. G. Dalgleish, M. Alexander, and M. Corredig

- Rheology of Acid Skim Milk Gels 26
C. M. M. Lakemond and T. van Vliet

- Linear and Non-linear Rheological Properties of β -Lactoglobulin Gels in Relation to their Microstructure 37
M. Pouzot, L. Benyahia, D. Durand, and T. Nicolai

- Gelation of Bovine Serum Albumin in the Presence of Low-methoxyl Pectin: Effects of Na^+ and Ca^{2+} on Rheology and Microstructure 48
L. Donato, C. Garnier, B. Novales, and J.-L. Doublier

Colloidal Interactions

INVITED LECTURE

- Stabilization of Food Colloids by Polymers 61
C. G. de Kruif and R. Tuinier

- Self-Consistent-Field Studies of Mediated Steric Interactions in Mixed Protein + Polysaccharide Solutions 74
R. Ettelaie, E. Dickinson, and B. S. Murray

Probing Emulsion Droplet Interactions at the Single Droplet Level	85
<i>A. P. Gunning, A. R. Mackie, P. J. Wilde, R. A. Penfold, and V. J. Morris</i>	

Adsorbed Layers

INVITED LECTURE	
Formation and Properties of Adsorbed Protein Films: Importance of Conformational Stability	99
<i>M. A. Cohen Stuart, W. Norde, M. Kleijn, and G. A. van Aken</i>	
Thermodynamic and Adsorption Kinetic Studies of Protein + Surfactant Mixtures	120
<i>R. Miller, D. O. Grigoriev, E. V. Aksenenko, S. A. Zholob, M. E. Leser, M. Michel, and V. B. Fainerman</i>	
Computer Simulation of Interfacial Structure and Large-Deformation Rheology during Competitive Adsorption of Proteins and Surfactants	131
<i>L. A. Pugnaloni, R. Ettelaie, and E. Dickinson</i>	
Molecular Interactions in Mixed Protein + Ionic Surfactant Interfaces	143
<i>P. A. Gunning, A. R. Mackie, A. P. Gunning, P. J. Wilde, and V. J. Morris</i>	
Conformational Changes of Ovalbumin Adsorbed at the Air–Water Interface and Properties of the Interfacial Film	152
<i>S. Pezennec, E. Terriac, B. Desbat, T. Croguennec, S. Beaufils, and A. Renault</i>	
Displacement of β -Casein from the Air–Water Interface by Phospholipids	160
<i>A. Lucero Caro, M. R. Rodríguez Niño, C. Carrera Sánchez, A. P. Gunning, A. R. Mackie, and J. M. Rodríguez Patino</i>	

Protein Functionality and Aggregation

INVITED LECTURE	
Milk Protein Functionality in Food Colloids	179
<i>H. Singh</i>	
Aggregation and Gelation of Casein Sub-Micelles	194
<i>M. Panouillé, T. Nicolai, L. Benyahia, and D. Durand</i>	

Caseinate Interactions in Solution and in Emulsions: Effects of Temperature, pH and Calcium Ions <i>M. G. Semenova, L. E. Belyakova, E. Dickinson, C. Eliot, and Y. N. Polikarpov</i>	209
Nanorheological Properties of Casein <i>K. M. Helstad, A. D. Bream, J. Trckova, M. Paulsson, and P. Dejmek</i>	218
Whey Protein Aggregation Studies by Ultrasonic Spectroscopy <i>M. Corredig and D. G. Dalgleish</i>	230
Critical Concentration for Fibrillar Aggregation of Bovine β -Lactoglobulin <i>L. N. Arnaudov and R. de Vries</i>	237
Properties of Fibrillar Food Protein Assemblies and their Percolating Networks <i>C. Veerman, L. M. C. Sagis, and E. van der Linden</i>	247

Foams and Emulsions

Disproportionation Kinetics of Air Bubbles Stabilized by Food Proteins and Nanoparticles <i>B. S. Murray, E. Dickinson, Z. Du, R. Ettelaie, T. Kostakis, and J. Vallet</i>	259
Coarsening and Rheology of Casein and Surfactant Foams <i>A. Saint-Jalmes, S. Marze, and D. Langevin</i>	273
Interfacial and Foam Stabilization Properties of β -Lactoglobulin–Acacia Gum Electrostatic Complexes <i>C. Schmitt, E. Kolodziejczyk, and M. E. Leser</i>	284
Interactions between β -Lactoglobulin and Polysaccharides at the Air–Water Interface and the Influence on Foam Properties <i>R. I. Baeza, C. Carrera Sánchez, J. M. Rodríguez Patino, and A. M. R. Pilosof</i>	301
Proposing a Relationship between the Spreading Coefficient and the Whipping Time of Cream <i>N. E. Hotrum, M. A. Cohen Stuart, T. van Vliet, and G. A. van Aken</i>	317
Utilization of a Layer-by-Layer Electrostatic Deposition Technique to Improve Food Emulsion Properties <i>D. J. McClements, T. Aoki, E. A. Decker, Y.-S. Gu, D. Guzey, H.-J. Kim, U. Klinkesorn, L. Moreau, S. Ogawa, and P. Tanasukam</i>	326

Sensory Perception

INVITED LECTURE

- Perceiving the Texture of a Food: Biomechanical and Cognitive Mechanisms and their Measurement 339

D. A. Booth

- Colloidal Behaviour of Food Emulsions under Oral Conditions 356

G. A. van Aken, M. H. Vingerhoeds, and E. H. A. de Hoog

- Sensory Perception of Salad Dressings with Varying Fat Content, Oil Droplet Size, and Degree of Aggregation 367

E. Tornberg, N. Carlier, E. P. Willers, and P. Muhrbeck

- Acoustic Emission from Crispy/Crunchy Foods to Link Mechanical Properties and Sensory Perception 380

H. Luyten, W. Lichtendonk, E. M. Castro, J. Visser, and T. van Vliet

Structure Control and Processing

INVITED LECTURE

- Solubilization and Bioavailability of Nutraceuticals by New Self-Assembled Nanosized Liquid Structures in Food Systems 395

N. Garti, I. Amar-Yuli, A. Spornath, and R. E. Hoffman

- Particle Dynamics in a Transient Gel Network—Ageing of a Monodisperse Emulsion 420

A. D. Watson, G. C. Barker, D. J. Hibberd, B. P. Hills,

A. R. Mackie, G. K. Moates, R. A. Penfold, and M. M. Robins

- Gels, Particle Mobility, and Diffusing Wave Spectroscopy—A Cautionary Tale 432

D. S. Horne, E. Dickinson, C. Eliot, and Y. Hemar

- Small-Angle Static Light-Scattering Study of Associative Phase Separation Kinetics in β -Lactoglobulin + Xanthan Gum Mixtures under Shear 443

S. I. Laneville, C. Sanchez, S. L. Turgeon, J. Hardy, and P. Paquin

- Break-up and Coalescence of Bubbles in Agitated Protein Solutions at High Air Volume Fraction 466

B. J. Hu, A. W. Pacek, and A. W. Nienow

Subject Index

482

Gels and Gelation

Rheology, Structure and Texture Perception in Food Protein Gels

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1 Introduction

One could propose that mankind's original encounter with a protein gel occurred when the first human accidentally dropped an egg into a fire or hot water and discovered the hard cooked egg. If we accept this humble origin of a culinary art, then the presence of current research¹ dealing with the gelation of ovalbumin demonstrates that making protein gels is a simple process while understanding the molecular mechanisms continues to present scientific challenges.

The seminal review by Ferry² defines some general properties of protein gels as "...systems in which small proportions of solid are dispersed in relatively large proportions of liquid by the property of mechanical rigidity ... the characteristic property common to all gels." The rheological characteristics of gels were further refined³ to include viscoelastic properties and to distinguish between 'solid' and 'solid-like' gels. The key elements of a solid-like gel are a storage modulus, $G'(\omega)$, which has a plateau extending to times at least of the order of seconds, and a loss modulus, $G''(\omega)$, that is much smaller than the storage modulus in the plateau region. For gels formed by denatured proteins, Ferry² went on to propose: (1) they are the result of forming a three-dimensional network of solute; (2) they arise from interactions that act along the entire solute molecule, and therefore require a proper balance of attractive and repulsive forces regulated by pH and ionic strength; and (3) their macroscopic appearance and water-holding properties reflect microscopic networks that have extremes of coarse or fine structures. The following mechanism for gelation of denatured proteins was suggested: native protein (corpuscular) \rightarrow denatured protein (long chains) \rightarrow association network.

It is amazing that the description for gelation of denatured proteins proposed by Ferry in 1948, based on primarily 4 research articles, is generally

valid today. While it has since been proven that proteins do not have to unfold into long chains to form gel networks,^{4,5} there have been, and continue to be, numerous investigations into how factors (pH, salts) regulate the association of denatured molecules, the resulting microstructure, and the links between microstructure and macroscopic properties (texture and water-holding).

Research on protein gelation can be generally categorized into two areas, the first being the nature of the gelation process. This involves the various factors that transform proteins from non-aggregating to aggregating structures; the definition and determination of the gel point; the factors responsible for development of gel elasticity; and theories capable of explaining all aspects of the process. The second area concerns the physical and chemical properties of gels. To that end, the microstructure of gels along with the rheological and fracture properties have received the greatest attention, but appearance, intra-gel diffusion, and water-holding attributes have also been investigated.^{6,7} While the nature of the gelation process can be considered a general scientific curiosity, the appearance, water-holding and textural properties of gels have direct applications to food quality. Some of the foods that rely on the gelation of denatured proteins to produce desirable characteristics are cooked eggs, processed meats, and some cheeses. In theory, if one understands the physical and chemical basis for the quality characteristics of a food protein gel, such as a cooked egg, then the potential exists to form the same structure with another protein. Indeed, this was pointed out⁸ in 1930 as a motivation for developing whey protein ingredients to replace egg proteins in foods. The many soy protein-based imitation meat products on the market today serve as examples of this application. It is also possible to augment a product by adding proteins as gelling agents. An example is the addition of milk proteins to water-added hams as a means of increasing water-holding and rheological properties.⁹

The fundamental science of globular protein gelation took a step forward with the publication of three articles in the early 1980s.¹⁰⁻¹² These articles were the first to focus on molecular and microstructural changes associated with heat-induced gelation of a variety of globular proteins. The protein gelation process was reviewed by Clark and Lee-Tuffnell⁵ and by Clark.¹³ In these reviews, the theoretical foundation of the gelation process is discussed, along with comprehensive coverage of protein aggregation, changes in protein secondary structure, and microstructure. These areas will be addressed in the present article only from the standpoint of how they help to explain differences in gel structure type and textural properties. This review will focus on gelation from the perspective of producing food structure and textural properties.

2 Gel Structure Type

In a recent article¹⁴ entitled 'From food structure to texture', Wilkinson and coworkers state that advances in the understanding of texture perception will depend on advances in sensory analysis of texture, physiological perception, and food structure. Included under the heading of food structure are

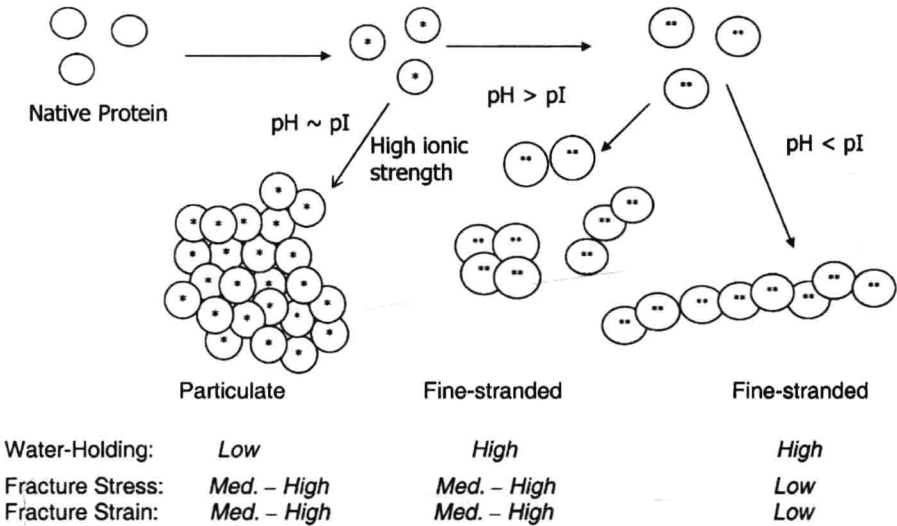


Figure 1 Model of globular protein gelation depicting changes from native protein to slightly unfolded (*) and more extensively unfolded (**) states, and resultant gel types. Characteristic physical properties are listed below the gel types.

“rheological parameters, microstructure and other relevant food characteristics and their relation to sensory perception.” This begs the question: are there specific structures in food protein gels? If so, what are the distinguishing characteristics that separate one structure from another? In many ways this issue has already been addressed. Heat-induced globular protein gels can be transparent, turbid or opaque depending on the gelation conditions (see Figure 1).^{2,12,15,16} Gels that are transparent or translucent are generally classified as *fine-stranded* (also called fibrillar, ‘string of beads’, or ‘true’ gels). They are formed under conditions of pH greater or less than the pI, and at low ionic strength. The name “fine-stranded” derives from transmission electron micrographs showing microstructures consisting of strands of various lengths, but with diameters corresponding to the length of one to several molecules.^{4,12,16} Fine-stranded gels formed at low and high pH have different textural properties. The gels formed at low pH are weak and brittle, and those formed at high pH are strong and elastic.^{17–19} Gels that are opaque are called *particulate* (also called aggregated, random aggregated, or coagulated).^{12,16,17} Particulate gels are formed under conditions where there is minimal charge repulsion, such as at a pH close to pI or at high ionic strength. The microstructure of particulate gels is composed of spherical particles with diameters in the micrometre range that are associated into a gel network.^{20,21} The large particles and low water-holding properties of particulate gels have led to the proposal^{12,22} that they are formed by phase separation. Needless to say, there are conditions that lie between the two limiting cases that produce gels with structures that can be considered either (i) a combination of stranded and particulate or (ii) distinct and intermediate between these extremes.

3 Gelation of Fine-Stranded and Particulate Gels

The networks formed in fine-stranded gels at low pH (generally ≤ 3.5) are different from those formed at high pH (generally ≥ 6.5). β -Lactoglobulin gel networks formed at pH 7.0 are composed of strands that are slightly thicker, longer and more curled than those formed at pH 3.5.²¹ Heating a β -lactoglobulin solution at pH 2 at a concentration below the critical gelation concentration ($< C_0$) produces fibrils rather than a gel network. Fibrils of β -lactoglobulin of length of 1–7 μm are observed.^{23,24} The fibril length is independent of ionic strength in the range 0.01–0.05 M.²⁴ Static and dynamic light scattering measurements support²⁵ the formation of β -lactoglobulin fibrils at pH 2.0. Recent atomic force microscopy images confirm²⁶ the formation of β -lactoglobulin strands composed of dimers or partially unfolded monomers. For β -lactoglobulin, an increase in pH (to 2.5 or 3.0) or ionic strength (> 50 –90 mM) causes the fibrils to shorten.^{23,24} The formation of fibrils and their association into a gel network seems to be a general property of gels formed at low pH and low ionic strength. This type of gelation can be explained²⁴ by an adjusted random contact model that takes into account the charge and semi-flexibility of the fibrils. This model has been shown²⁷ to apply to fibril network gels formed by bovine serum albumin, β -lactoglobulin and ovalbumin.

The type of β -lactoglobulin aggregates formed at pH 7.0 varies among investigations. Kavanagh *et al.* reported²³ the formation of short ($< 0.05 \mu\text{m}$) linear aggregates based on transmission electron microscopy. Using size-exclusion chromatography and light scattering, Le Bon *et al.* found²⁸ that, when β -lactoglobulin was heated at 60 °C at pH 7.0 in a 0.1 M salt solution, self-similar aggregates are formed composed of 85 protein molecules and having a radius of 15 nm. A similarly sized aggregate ($18 \pm 4 \text{ nm}$) was observed²⁶ by atomic force microscopy for β -lactoglobulin solutions that were heated at 80 °C in pH 7.0 solutions containing 0.1 M salt. Although differences in aggregates have been observed among studies conducted at neutral pH, possibly due to variations in heating conditions and methods of analysis, the types of aggregates formed at high pH appear to be consistently different from those formed at low pH. This suggests that, at some level, the fundamental structural element is different for low pH and high pH fine-stranded gels.

Particulate gels can be formed in solutions where the pH is close to the pI of the protein (or proteins), or where the pH is not close to the pI but there is a relatively high ionic strength (usually $> 0.1 \text{ M}$).^{12,21} Once the pH and ionic strength are sufficient to form a particulate gel network, increasing ionic strength causes a decrease in rheological parameters (G' or G^*) and water-holding properties, accompanied by changes in the microstructure described as a coarsening of the network or an increase in voids.^{6,29,30}

Molecular changes associated with fine-stranded and particulate gels have been investigated using several spectroscopic techniques. Using Fourier transform infrared spectroscopy or Raman scattering spectroscopy, a more extensive unfolding was seen^{31,32} when β -lactoglobulin formed fine-stranded gels as compared to particulate gels. While there were apparent differences between

fine-stranded and particulate gels, no major differences were seen when these types of gels were formed under different gelation conditions. Therefore, differences in rheological properties associated with fine-stranded gels formed at low and high pH, as well as particulate gels formed close to the pI or at high ionic strength, cannot be explained based on protein structural transitions alone.

A series of studies has established^{30,33,34} how the incorporation of protein into the gel network at and after the gel point determines whey protein gel microstructure, permeability and rheology. These experiments were conducted under conditions where gelation was relatively slow (20–24 h at 68.5 °C), and mainly particulate gels were formed. The amount of aggregated protein forming the incipient gel network determines the gel network type (microstructure) and the permeability (a function of microstructure and other properties). Gel permeability decreases only slightly upon further heating after the gel point, suggesting that the network type is ‘fixed’ at the gel point. In contrast, the elasticity (G') is close to zero at the gel point, and it increases with the total amount of protein incorporated into the established gel network.^{3,33,34}

4 Fracture Properties

Fracture behaviour can be investigated by compressing, extending or twisting a sample to the point of fracture.³⁵ While compression is most often used, due to the simplicity of sample preparation, it is limited to conditions where there is a uniform expansion of the sample when compressed (*i.e.*, where friction between sample and testing apparatus is very low), and for fracture strains occurring at compression levels above $\sim 80\%$ of the original height h (*i.e.*, $\Delta h/h \leq 0.8$). Materials that fracture at high strains must be deformed in tension or torsion. Torsional deformation is pure shear, and there are minimal shape changing forces. So samples that exude fluids in compression and tension will not do so in torsion. Also, since the tensile and shear forces are of equal magnitude, the sample fracture pattern in torsion indicates the weakest mode. The angle of the fracture plane relative to the long axis is 45° for tension fracture and 90° for shear fracture.³⁵

While the rheological properties determined in the linear viscoelastic region are associated with small deformations of the gel network, the fracture properties are related to weaknesses in the structure due to defects and/or inhomogeneous elements in the network.^{36–38} The rheological and fracture properties of heat-induced protein gels can be altered by adjusting four general parameters: the protein concentration, the heating temperature/time, the solvent conditions/quality, and the addition of other macromolecules or filler particles.

The development of fracture stress and strain in heat-induced whey protein and egg white gels has been investigated by several approaches. When a 10% w/v solution (pH 7.0, 0.1 M NaCl) of whey protein isolate is heated at 80 °C, the development of fracture stress and strain can be observed by sampling over various heating times.³⁹ After 10 minutes of heating, a self-supporting gel is formed with a low fracture stress (13 kPa) and high fracture strain (1.58).