How a Micro-Phase Separation Model Explains Gelation Properties of Egg White Protein Gels

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Student: Phanin Leksrisompong

Advisor: E. Allen Foegeding

Department of Food, Bioprocessing and Nutrition Sciences

North Carolina State University, Raleigh, NC 27695, USA

Executive summary

One of the main functionalities of food proteins is the ability to form heat-induced gels. The gelation process involves protein denaturation follow by aggregation. Altering the way molecules organize and interact in the system affects microstructure, mechanical properties and ultimately texture of food products. Texture is one of the most important sensory attributes determining quality and consumer acceptability of foods. Other than sensory properties, food texture has been shown to impact human health in areas such as satiation and satiety. It is well known that altering processing parameter (i.e., heating rate), solvent condition (i.e., pH), and biopolymer mixtures creates different food structures. The objective of this research was to understand the mechanisms responsible for structural formation and gelation properties of egg white protein (EWP) gels based on micro-phase separation considerations; which is a new perspective on the structure of protein gels based on solution conditions favoring either single phase (stranded) or micro-phase separated aggregates (particulate).

The first and second studies investigated how micro-phase separation alters the effect of heating rate on viscoelastic and fracture properties of EWP gels. Protein solutions (1 - 15% w/v protein, pH 3.0 - 8.5) were heated using a range of heating rates (0.1 – 35 °C/min) to achieve a final temperature of 80 °C. Single or micro-phase separated solution conditions were determined by confocal laser scanning microscopy. Under single phase conditions, gels formed by faster heating had the lowest rigidity (G′) at 80 °C; however, a common G′ was achieved after holding for 4 hr at 80 °C. Fracture properties of gels prepared by fast heating were also the weakest but extending holding time after fast heating for only 5 min produced gels as strong as gels prepared by slower heating. *This strongly suggested that the variations seen in viscoelastic and fracture properties were simply due to variations in time allowed for proteins to form a gel network.* In contrast, under micro-phase separation conditions, faster heating allowed phase separated particles to be frozen in the network prior to precipitation. Thus, gels produced by faster heating had higher G′ values. There was no effect of heating rate on held water; supporting previous investigations that showed the gel point sets the gel microstructure and that microstructure determines water-holding properties. Overall, the effect of heating rate appears to depend on phase stability of the protein dispersion and total thermal input. The second experiment used one heating condition (80 °C for 60 min) and probed in detail the pH range of 4.5 to 7.0 for EWP. Minimal differences in fracture properties and held water of EWP gels at pH 4.5 versus 7.0 were

observed. In contrast, whey protein isolate (WPI) gels, used as a control to compare gelation properties, were stronger and had higher held water at pH 7.0 as compared to 4.5. This was due to a mild degree of micro-phase separation of EWP gels across the pH range whereas WPI gels only showed an extreme micro-phase separation at pH values close to the isoelectric points of the two predominate proteins, β-lactoglobulin and α-lactalbumin. It was concluded that formation and physical properties of globular protein gels can be explained by degree of micro-phase separation. *Moreover, the ability of EWP to form strong, elastic gels across a wide range of pH is due to the unique mixture of proteins found in egg white.*

In the final series of experiments, microstructure and gelation properties of EWP/polysaccharide mixed gels were investigated. *Polysaccharides are often added to foods to increase water holding and freeze-thaw stability; so understanding their interactions with EWP is essential to proper food applications.* Altering polysaccharide type and concentration produced a wide range of microstructures and gelation properties of EWP/polysaccharide mixed gels. The effect of polysaccharides on the properties of the mixed gels can be grouped based on the charge density of polysaccharide. In addition to polysaccharide charge density, the degree of micro-phase separation was shown to be key in predicting the microstructure of mixed gels and in turn the gelation properties. The last chapter of this dissertation combined the knowledge acquired from previous chapters to understand the effect of biopolymer mixtures on freeze/thaw stability of the mixed gels. There was a general trend of ι-carrageenan increasing freeze/thaw stability but current methods for determining water holding limited development of a physical model to explain the effects.

Egg white protein and WPI are used extensively as ingredients in the food industry. Despite their similarity (i.e., both are globular proteins), they were shown to respond differently to changes in pH and interactions with polysaccharides. These variations were explained based on the degree of protein micro-phase separation. *This study demonstrated how the micro-phase separation model can be used to explain the textural and water holding properties of egg white gels. This in turn can be used by product developers in designing foods containing egg white proteins and achieving desirable textural and water holding properties.*

Introduction

One of the main functionalities of food proteins is the ability to form heat-induced gels. The gelation process involves protein denaturation (exposure of sites for inter-molecular interactions) follow by aggregation to form a gel (Ferry 1948). This process depends on the balance between attractive and repulsive forces amongst the denatured protein molecules during aggregation. Varying solvent conditions (i.e., pH and ionic strength), protein type, concentration and processing parameters (i.e., heating temperature, time and rate) and addition of other polymers affect this process by altering the way molecules organize and interact in the system. Of these parameters, heating rate, pH and biopolymer interactions are of interest in this study. Protein aggregation/gelation is very pH dependent. It influences intermolecular interactions through changing the electrostatic properties as well as affecting chemical reactivity (i.e., thiol groups) of the proteins and in turn impacts the types of networks formed and their physical properties. Physical properties of gels can also be altered by heating rates due to difference in the kinetics of protein denaturation and aggregation (Ferry 1948; Arntfield and others 1989). In addition, using mixtures of biopolymers (e.g., proteins and polysaccharides) is a way to alter food structure and physical properties of gels (Nishinari and others 2000).

Heat-induced globular protein gels form two general types of networks: those formed from linear or rod-like aggregates (stranded) and those made with particulate aggregates (particulate). The two types of heat-induced protein gels have different microstructure, rheological properties, water holding properties and ultimately texture of food products. Texture is one of the most important sensory attributes determining quality and consumer acceptability of foods. Other than sensory properties, food texture has been shown to impact human health in areas such as satiation and satiety.

Recently, Ako and others (2009) provided a new perspective on the structure of protein gels based on solution conditions favoring either single phase or micro-phase separated aggregates, allowing for construction of a state diagram for gelation of β-lactoglobulin. This clearly showed how pH relative to protein isoelectric point (pI) and solvent ionic strength determine gel types (e.g., stranded vs. particulate). Protein solutions under micro-phase separation conditions (low electrostatic repulsion among molecules) form precipitates at concentrations below the critical concentration for gelation (C_o) and particulate gels above C_o. Particulate gels are the result of a competition between large aggregates settling due to Stokes'-

based considerations versus frozen in the network prior to precipitation. In contrast, protein solutions in the single phase region (high electrostatic repulsion among molecules) form soluble aggregates at concentrations below C_o and stranded gels above C_o.

Objectives

The initial objective of this research was to determine how heating rate can be used to alter gel structure. The overall hypothesis was that different heating rates would change gel structure, and thereby produce different gel textures and water holding properties associated with different heating rates. Initial experiments clearly showed that the effects of heating rate were highly dependent on solution conditions, and therefore explained by the micro-phase separation model. We then conducted a series of experiments to show that the micro-phase separation model not only explains heating rate and pH effects on egg white protein gelation, it also is a key element of how gelation will be altered by addition of polysaccharides. The final result was a comprehensive model for molecular mechanisms determining textural and water holding properties of egg white protein gels that is beneficial in guiding product developers in using egg white in various food applications.

Effects of Heating Rate and pH on Viscoelastic and Fracture Properties of Globular Protein Gels as Explained by Micro-Phase Separation.

Background

Previous investigations have shown that heating rate affects the physical properties as well as microstructure of protein gels; however, there is a disagreement as to the mechanism. Some researchers reported that faster heating produced more rigid and stronger gels (Stading and others 1992), while other researchers reported the opposite results (Foegeding and others 1986; Arntfield and Murray 1992; Stading and others 1993; Langton and Hermansson 1996; Li and others 2006). One complicating factor among studies is that they were conducted at different solution conditions (e.g., pH and ionic strength). In addition, some studies only compared gels that were heated to a common endpoint temperature, without considering the effect of integration of time and temperature.

Based on Ferry's two-step model, Foegeding and others (1986) proposed that fast heating rates to a common end point allowed less time for proteins to spend at temperatures above the denaturation temperature. This implies that less rigid gels produced by fast heating were due to insufficient time for aggregation rather than insufficient time for denaturation. This is based on denaturation being a very rapid process. Riemann and others (2004) investigated the effect of heating rate and holding time on gelation of Alaskan Pollock surimi and turkey breast. Their results confirmed that integration of time and temperature was the key thermal processing parameter. The findings of Riemann and others (2004) suggests that the differences in heating rate were simply due to not allowing for sufficient time to have the same final gel structure formed.

Accounting for sufficient time to allow for complete gel network formation does not explain why in some investigations faster heating rates produced more rigid gels. This requires additional considerations regarding the aggregation process. Ako and others (2009) showed that, at concentrations below the critical gelation concentration, proteins in solution conditions favoring micro-phase separation will precipitate; while those in single phase conditions form soluble aggregates. *Based on the time required for complete network formation and the micro-phase separation model, the effects of heating rate (or time) are predicted to depend on the solution's state: micro-phase separated or single phase regions.*

Objective

The objective of these studies were to determine how micro-phase separation alters the effect of heating rate on viscoelastic and gelation properties of egg white protein (EWP) and whey protein isolate (WPI) gels. Results were interpreted based on the kinetic model of Ferry (1948) and the micro-phase separation model of Ako and others (2009).

Methods

Protein solutions (1 - 15% w/v protein, pH 3.0 - 8.5) were heated using a range of heating rates (0.1 – 35 °C/min) to achieve a final temperature of 80 °C. Single or micro-phase separated solution conditions were determined by confocal laser scanning microscopy. The gelation process and viscoelastic properties of formed gels were evaluated using small strain rheology. Fracture properties (strength and deformability) of gels were measured by torsional deformation and held water (HW) was measured as the amount of fluid retained after a mild centrifugation. Gelation curves (G´ development vs. time) were normalized based on gel time to evaluate possible master curves across heating time. Data were analyzed using ANOVA using PROC GLM of SAS (version 9.1, SAS Institute Inc., Cary, NC) and comparisons of the means using Tukey's significant difference test ($p < 0.05$).

Results

Figure 1 shows the microstructures of heat-induced WPI and EWP gel across the pH. The bright areas represent protein (stained by Rhodamine B) and the dark areas represent zones devoid of proteins which are mainly comprised of water. Under conditions where most proteins were single phase (pH 3.0, 7.0 and 8.5), gels formed by faster heating had the lowest rigidity (G′) at 80 °C; however, a common G′ was achieved after holding for 4 hr at 80 °C and master curves normalizing based on gel time were observed (Figure 2). Fracture properties of gels prepared by fast heating were also the weakest, however, if given enough time (only 5 min) after reaching end-point temperature, gels prepared by fast heating are as strong as gels prepared by slow heating (Table 1). *This confirmed that the variations seen in viscoelastic and fracture properties were simply due to variations in time allowed for proteins to form a gel network.* In contrast, under micro-phase separation conditions (pH 4.5), faster heating allowed phase separated

particles to be frozen in the network prior to precipitation. Thus, gels produced by faster heating had higher G′ values and master curves based on normalized gel time were not detected for both proteins (Figure 2). There was no effect of heating rate on HW; supporting previous investigations (Verhuel and Roefs 1998) that showed the gel point sets the gel microstructure, and that microstructure determines water-holding properties (Table 2).

Conclusion

In summary, these results suggest a similar heating rate/solution phase state mechanism controlling gel firmness (G') and strength. The effect of heating rate on viscoelastic properties, fracture properties and HW of globular protein gels can be explained by phase stability of the protein dispersion and time allow for gel network formation. When proteins have a high net negative charge and form soluble aggregates (single phase conditions), there is no heating rate effect and gels with equal firmness and strength will be formed if given enough time. In contrast, when electrostatic repulsion is low (micro-phase separated conditions), there is a competition between protein precipitation and gel formation; thus a faster heating rate produces a firmer and stronger gel. *These findings have significant implications regarding applications of egg white proteins in food products. It shows that the heating conditions need to be adjusted to solution properties (e.g., pH) to obtain the desired texture.*

Gelation Properties of Egg White Protein/Polysaccharide Mixed Gels Based on Micro-Phase Separation Considerations

Background

Biopolymer mixtures can be use to alter food structure (Nishinari and others 2000). When protein and polysaccharide are mixed with water there are several possible outcomes. At low concentrations, both biopolymers are soluble and form a mixed solution. At higher concentrations, there is the possibility of associative (interactions between biopolymers) or segregative (no interactions between biopolymers) types of interactions (Tolstoguzov 1991). This means that when concentrations are high enough for gelation, three types of mixed gels can be formed depending on the biopolymers' molecular properties (i.e., shape, size and charge) and solvent qualities (i.e., pH and ionic type/strength): interpenetrating, associative phase separated and segregative phase separated networks (Tolstoguzov 1991; Piculell and Lindman 1992; Turgeon and others 2003).

Formation and properties of WP/polysaccharide mixed gels has been investigated (Sanchez and others 1997; Beaulieu and others 2001; van den Berg and others 2007; de Jong and van de Velde 2007; de Jong and others 2009; Çakır and Foegeding 2011). Altering polysaccharide type and concentration produces a wide range of microstructures in mixed gels. The microstructures of mixed gels have been characterized as homogenous, micro-phase separated and polysaccharide continuous (van den Berg and others 2007). Molecular properties of polysaccharides, mainly charge density and polysaccharide concentration, determined the microstructure, and in turn structure determines fracture and water holding properties of WPI/polysaccharide mixed gels (de Jong and van de Velde 2007). Polysaccharides can be divided into three categories; low, medium and high charge density, based on how they alter gel structure (de Jong and van de Velde 2007). Solvent quality (i.e., pH and ionic strength) also has significant effects on gel structure (Çakır and Foegeding 2011; Çakır 2011). Changes in microstructures have been associated with specific sensory properties of mixed gels (van den Berg and others 2007; de Jong and van de Velde 2007; Çakır and Foegeding 2011).

To our knowledge, heat-induced gelation of EWP/polysaccharide mixed gels had not been studied thus far. We hypothesize that the combined models of protein-protein micro-phase separation (Ako and others 2009) and polysaccharide charge density (de Jong and van de Velde

2007) can be used understand how different structures are formed with EWP/polysaccharide mixtures.

Objective

The objective of this study is to investigate the effect of polysaccharide type and concentration on the microstructure and gelation properties of EWP/polysaccharide mixed gels.

Methods

Protein (10 % w/v protein; pH 7.0) and polysaccharides (0 to 0.6% w/w; guar gum, locust bean gum, high methoxyl pectin, κ-carrageenan, low methoxyl pectin, and ι-carrageenan) solutions were used to prepare mixed gels. Single or micro-phase separated solution conditions were determined by confocal laser scanning microscopy. Large deformation behavior and fracture properties of the gels were determined by uniaxial compression. Held water was measured as the amount of fluid retained after a mild centrifugation. Data were analyzed using ANOVA using PROC GLM of SAS (version 9.2, SAS Institute Inc., Cary, NC) and comparisons of the means using Tukey's significant difference test ($p < 0.05$).

Results

Overall, EWP/polysaccharide mixed gels showed phase separated structures except for EWP/low methoxyl pectin mixed gels (Figure 3). The dark areas represent the area devoid of protein which is known as the "serum phase" and comprised of mostly water and polysaccharide. Based on the polysaccharide charge density model of de Jong and van de Velde (2007), the effect of polysaccharides concentration on microstructure and gelation properties of EWP/polysaccharide mixed gels can be grouped into 4 categories: neutral (guar gum and locust bean gum), low charge density (high methoxyl pectin), medium (κ-carrageenan) and high (low methoxyl pectin and ι-carrageenan) charge density polysaccharides.

Altering polysaccharide type and concentration produced a wide range of microstructures of the mixed gels (Figure 3). At low polysaccharide concentrations, addition of lower charge density polysaccharides resulted in a higher degree of micro-phase separation than when higher charge density polysaccharides were added with exception of ι-carrageenan (Figure 3; the second row). Concentration of neutral, low, and medium charge density polysaccharides (categories 1

to 3) also affected the degree of micro-phase separation and dictated whether the protein or polysaccharide would constitute the continuous phase (Figure 3; the first to the fourth column). Whereas, increasing concentration of high charge density polysaccharides resulted in no change in the microstructures of mixed gels (Figure 3; the fifth and the sixth column).

Interactions of EWP and galactomannans are assumed to result in segregative phase separation because galactomannans have no charge (Grinberg and Tolstoguzov 1997). Mixture of negatively charged polysaccharides (categories 2 to 4) and EWP results in more complex structures. Since some egg white proteins are positively charged at pH 7.0, one would expect both associative and segregative phase separation (Grinberg and Tolstoguzov 1997) to occur when negatively charged polysaccharides are added. This resulted in more complex microstructures than when only segregative phase separation dictates the microstructure.

Mechanical and water holding properties of EWP gels were greatly altered by the polysaccharides. Figure 4 shows changes in post-fracture force-deformation (normalized force and deformation after sample fracture; indicates a texture pattern), held water, recoverable energy (indicates structural damage under mild stress) and fracture stress (gel strength) of EWP gels containing different amounts of polysaccharides. Galactomannans (e.g., guar gum) disrupted the structure such that addition of 0.2% or more made the gels so weak they could not be tested (Figures 3 and 4). High methoxyl pectin also disrupted the gel network. This was seen by the post-fracture force-deformation initially decreasing then, as polysaccharide concentration increased, increasing. The decrease in recoverable energy also signifies a weakening of the structure. Kappa-carrageenan and low methoxyl pectin both increased held water, but only kappa-carrageenan increased gel strength (i.e., fracture stress). This clearly shows that polysaccharides vary widely in their effect on EWP gel properties and that selection should be made on the desired outcome.

Conclusion

In a complex system where there are more than just two biopolymers, a combination of polysaccharide charge density model (de Jong and van de Velde 2007) together with micro-phase separation model (Ako and others 2009) (i.e., protein charge density) can predict the microstructure of the mixed gels. Based on these microstructures, the gelation properties of EWP/polysaccharide mixed gels can be predicted to a certain extent.

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Table 1. Effect of heating rate and holding time on fracture stress (σ_f, gel strength) and fracture strain (γ_f, gel deformability) of 15% (w/v) egg white protein (EWP) and whey protein isolate (WPI) gels at pH 4.5 and 7.0.

Heating Rate + Holding Time	**EWP**				**WPI**			
	pH 4.5		**pH 7.0**		**pH 4.5**		**pH 7.0**	
	σ_f (kPa)	**γ_f**	**σ_f (kPa)**	**γ_f**	**σ_f (kPa)**	**γ_f**	**σ_f (kPa)**	**γ_f**
Slow*** + 0 min hold	-	-	23^{b}	1.5^{a}	-	-	36^{d}	1.1^{c}
Medium** + 0 min hold	21^{e}	1.1^{b}	21^{bc}	1.6^{a}	-	-	46^{c}	1.2^{abc}
Fast* + 0 min hold	20^{e}	1.1^{b}	16^{c}	1.5^{a}	-	-	26^{e}	1.4^{a}
Fast* + 5 min hold	31^{d}	1.4^{a}	22^{bc}	1.5^{a}	-	-	53^{b}	1.3^{ab}
Fast* + 10 min hold	32^{d}	1.4^{a}	23^{b}	1.5^{a}	-	-	55^{b}	1.3^{abc}
Fast* + 15 min hold	36^{cd}	1.5^{a}	25^{ab}	1.7^{a}	-	-	59^{b}	1.2^{bc}
Fast* + 20 min hold	38^{bc}	1.4^{a}	23^{b}	1.6^{a}	-	-	57^{b}	1.1^{bc}
Fast* + 2 hr hold	42^{b}	1.4^{a}	25^{ab}	1.6^{a}	13^{a}	0.7^{a}	81^{a}	1.1^{c}
Fast* + 4 hr hold	49^{a}	1.5^{a}	30^{a}	1.5^{a}	13^{a}	0.7^{a}	88^{a}	1.1^{c}

[a-d]Numbers in columns with a different letter superscript are significantly different ($p < 0.05$)

*20˚C/min for EWP; 17˚C/min and 35˚C/min WPI pH 4.5 and 7.0, respectively

** 1˚C/min

*** 0.1˚C/min

Table 2. Effect of heating rate and holding time on held water of 15% (w/v) egg white protein (EWP) and whey protein isolate (WPI) gels at pH 4.5 and 7.0.

	EWP		**WPI**	
Heating Rate + Holding Time	pH 4.5	pH 7.0	pH 4.5	pH 7.0
Slow*** + 0 min hold	-	77 [ab]	-	92 [b]
Medium** + 0 min hold	74 [a]	76 [ab]	-	93 [b]
Fast* + 0 min hold	-	74 [b]	-	92 [b]
Fast* + 5 min hold	76 [a]	77 [ab]	66 [b]	94 [ab]
Fast* + 10 min hold	77 [a]	78 [a]	67 [ab]	94 [ab]
Fast* + 15 min hold	77 [a]	79 [a]	72 [ab]	94 [ab]
Fast* + 20 min hold	77 [a]	79 [a]	75 [a]	94 [ab]
Fast* + 2 hr hold	78 [a]	78 [ab]	73 [ab]	96 [a]
Fast* + 4 hr hold	79 [a]	78 [a]	74 [ab]	96 [a]

[a-b]Numbers in columns with a different letter superscript are significantly different ($p < 0.05$)
*20˚C/min for EWP; 17˚C/min and 35˚C/min WPI pH 4.5 and 7.0, respectively
** 1˚C/min
*** 0.1˚C/min
" – " indicates missing data points were due to:

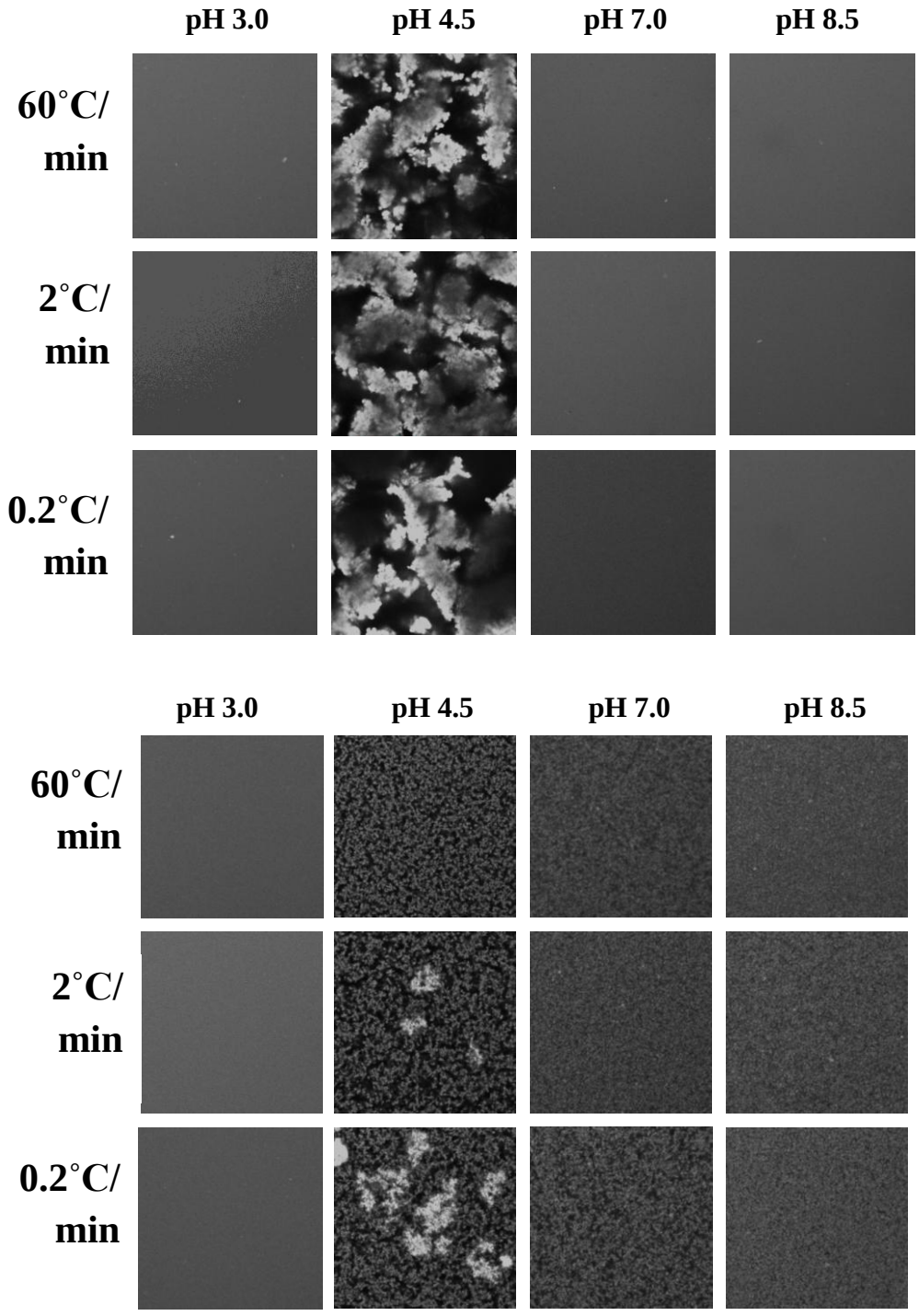

Figure 1. Cross-sections of the networks of 7% (w/v) whey protein isolate (top) and egg white protein (bottom) gels formed at three different heating rates (60 °C/min, 2 °C/min and 0.2 °C/min) and four different pHs (3.0, 4.5, 7.0 and 8.5). The width of the images represents 70.7 μm.

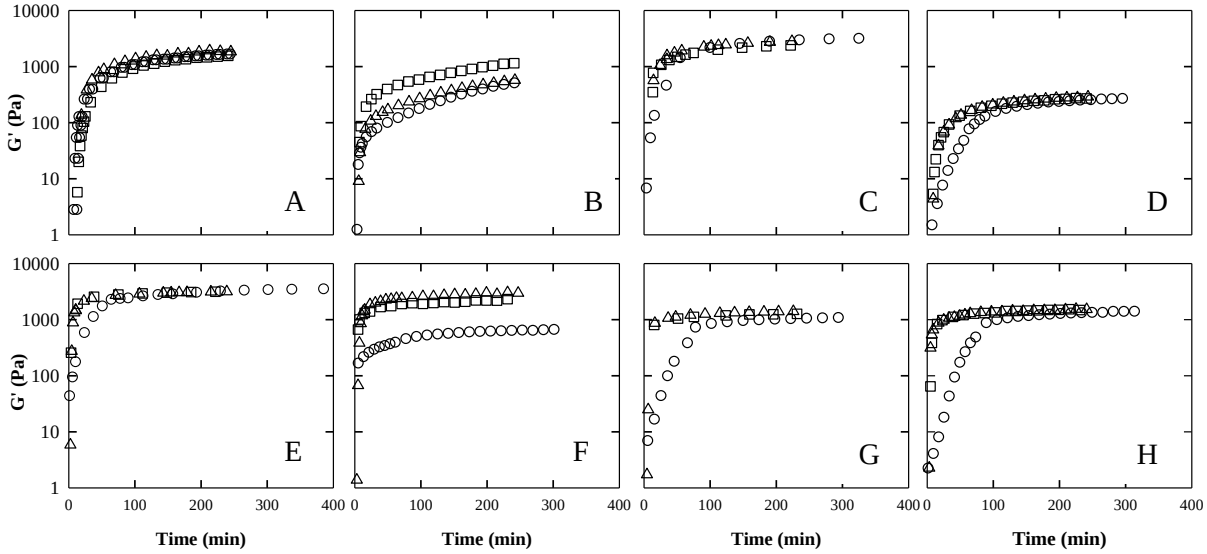

Figure 2. Normalized development of storage modulus (G´, gel firmness) over time for three different heating rates of 7% (w/v) whey protein isolate (WPI) or egg white protein (EWP) at various pHs: A. WPI pH 3.0; B. WPI pH 4.5; C. WPI pH 7.0; D. WPI pH 8.5, E. EWP pH 3.0; F. EWP pH 4.5; G. EWP pH 7.0; and H. EWP pH 8.5. Heating rates were 20°C/min, □; 2°C/min, Δ; 0.2°C/min, ○.

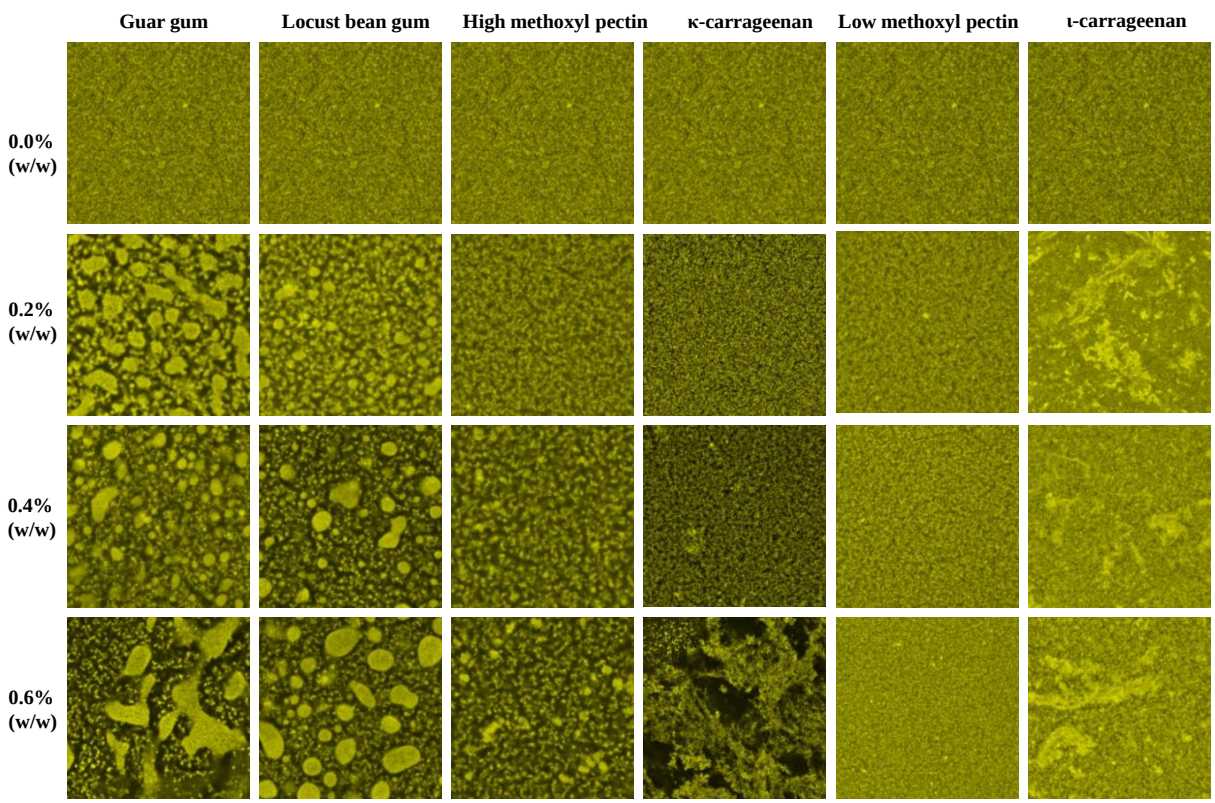

Figure 3. Microstructure of EWP/polysaccharide mixed gels (from left to right column: guar gum, locust bean gum, high methoxyl pectin, κ-carrageenan, low methoxyl pectin, ι-carrageenan) at four different concentrations (from top to bottom row: 0%, 0.2%, 0.4%, 0.6% (w/w) polysaccharides). Image size: 70.7 x 70.7 μm.

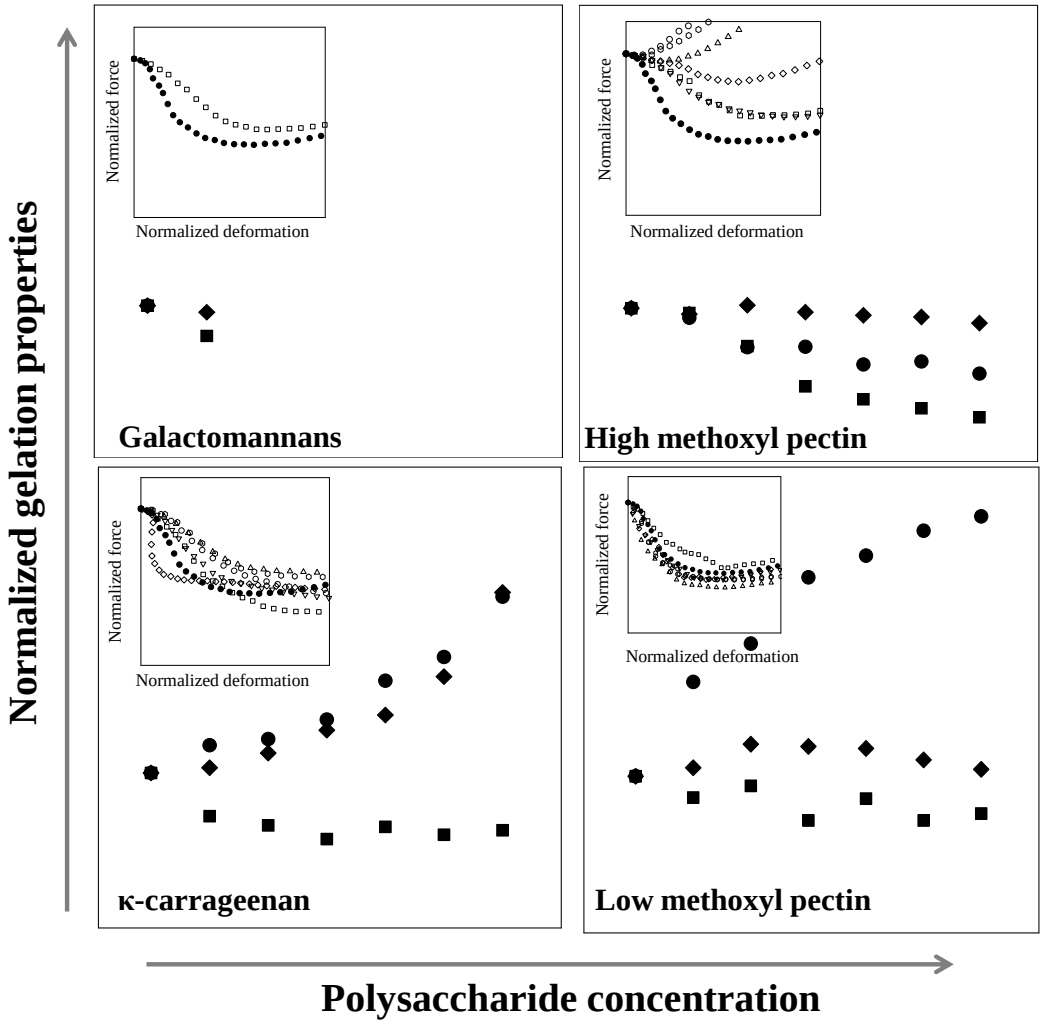

Figure 4. A summary of the effects of polysaccharide type and concentration on gelation properties. Egg white protein solutions (10 % w/v protein; pH 7.0) containing 0 to 0.6% w/w polysaccharide were formed into gels by heating at 80 ºC water bath for 30 min. Properties of: ● held water, ■ recoverable energy and ◆ fracture stress (gel strength) were measured on the formed gels. Insert shows post-fracture force-deformation of gels at various concentrations of added polysaccharide: ● 0%, □ 0.1 %, ▽ 0.2%, ◇ 0.3%, Δ 0.4%, ⬡ 0.5%, ○ 0.6% (w/w).