Review

Functions of Food Polysaccharides to Control the Gelatinization and Retrogradation Behaviors of Starch in an Aqueous System in Relation to the Macromolecular Characteristics of Food Polysaccharides

Takahiro FUNAMI*

Texture Design Division, San-Ei Gen F.F.I., Inc., 1-1-11, Sanwa-cho, Toyonaka, Osaka 561-8588, Japan

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Functions of food polysaccharides to control the gelatinization and retrogradation behaviors of starch were reviewed, with a brief introduction to the physicochemical properties of starch. The conclusions of some reports cannot be easily compared to understand the molecular mechanisms for the functions of polysaccharides, partially due to insufficient purification or characterization of the polysaccharide samples. In this sense, a series of studies has recently used well-characterized samples to clarify the effects of molecular weight and other macromolecular characteristics of polysaccharides on starch behavior. Based on these recent reports, schematic figures were drawn to explain their results on a molecular level, allowing for determination of a structure-function correlation of polysaccharides. The understanding of the molecular mechanisms is necessary for the progress of the food industry from both a fundamental and an application point of view.

Keywords: food polysaccharide, starch, gelatinization, retrogradation, molecular association, phase separation

1. Introduction

Starch is one of the most important and abundant food hydrocolloids. It is used not only as a main ingredient in staple foods such as bread and noodles, but also as a thickener, gelling agent, stabilizer, and fat substitute in processed food products. Starch is insoluble in water but swells upon heating to enhance viscosity in an excess amount of water. This phenomenon is known as gelatinization. The gelatinization or viscosifying ability of native starch is decreased in acidic or high temperature conditions, as well as through a vigorous agitation process during cooking due to the rupture of the granules. Once gelatinized, starch dispersions lose their transparency and water-holding capacity on storage, causing syneresis. Storage also increases the rigidity of starch systems, resulting in textural hardening. This phenomenon is called retrogradation. These shortcomings of native starch decrease the palatability of food products and are the reason for its limited use in the food industry. It is important for

food manufacturers to establish methods to control or regulate the gelatinization and retrogradation behaviors of native starch in an aqueous system, as it may potentially lead to cost reduction as well as quality control of starch-based or starch-containing food products. Modified starch (in a chemical or physical manner) provides a solution to overcome the disadvantages of native starch. Actually, chemically cross-linked starch, in which phosphate or adipate is commonly used as a bifunctional reagent, is often found in fillings, puddings, soups, and sauces due to increased resistance to mechanical shearing, thus providing short and non-sticky texture or ‘mouthfeel’ (Wurzburg, 2006). Also, esterified or etherified starch (so-called stabilized starch), in which acetate or propylene oxide is commonly used as a monofunctional reagent, is often found in frozen and retorted foods due to increased stability in viscosity enhancement, thus preventing textural hardening and syneresis during storage (Wurzburg, 2006). A variety of modified starches have been developed commercially with both types of manipulations, spreading its usage in the food industry. An alternative method of regulating the behavior of native starch is to use food hydrocolloids, partic-

*To whom correspondence should be addressed.

E-mail: tfunami@saneigenffi.co.jp

ularly food polysaccharides. This type of approach has been utilized in the food industry for decades to control viscosity, improve water-holding capacity and freeze-thaw stability, and inhibit textural hardening. Here, food hydrocolloids are defined as a state of edible colloid particles (10–1,000 nm in diameter) dispersed in water as a continuous phase; alternatively, polysaccharides and proteins themselves that regulate such a dispersing state in food systems. Food hydrocolloids have served as a texture modifier due to their ability to alter mechanical and geometrical characteristics of foods through gelling, thickening, dispersing, emulsifying, water-holding, binding, foaming, and film forming (Table 1). Recently, a worldwide trend, particularly from the consumer side, has been developing for “clean level” food ingredients that do not utilize any chemical processing or reagents during production, which may accelerate the usage of food hydrocolloids instead of chemically modified ingredients. Food polysaccharides are essentially from “natural” sources, ensuring safety through a long-term human diet history.

The aim of this paper was to first review public information about the functions of food polysaccharides in starch systems. Most studies have used an aqueous starch/polysaccharide composite system as a model, in which starch from wheat or corn is frequently used as a starch source considering its commercial importance. Their focus is not beyond the level of “phenomena”, and detailed molecular mechanisms are insufficient for explaining the results. Experimental conditions, including starch concentration, mixing ratio of starch and polysaccharide, storage temperature,

and analytical method, vary among studies, and most reports lack macromolecular profiling of the polysaccharides used, preventing comparisons of conclusions. The functions of food polysaccharides in starch systems should depend on their macromolecular characteristics (e.g., molecular weight) even within the same polysaccharide source or on whether the polysaccharides used are charged or uncharged in relation to molecular interactions or phase arrangement (Annable *et al.*, 1994). In this context, we focused on a series of recent studies to understand the functionality of certain food polysaccharides, on the molecular level, in controlling the physical properties of starch. Non-ionic galactomannans, such as guar gum and fenugreek gum, have been investigated as model polysaccharides to elucidate the role of the mannose backbone or galactose side chains in determining the behaviors of starch (Funami *et al.*, 2005a, b; Funami *et al.*, 2008a, b). Furthermore, two types of anionic polysaccharides, gum arabic and soybean soluble polysaccharide, have been investigated for their novel functionality in starch systems in relation to the coating ability at the interface (Funami *et al.*, 2008c).

2. Literature review

For the usage of food polysaccharides, synergistic interactions with starch components to increase viscosity of pastes or sols upon heating and to prevent the structural hardening of gels during storage have been the subject of extensive study.

2.1 Gelatinization A significant increase in the viscos-

Table 1. Functions of food polysaccharides.

Origin	Polysaccharide	Functions							
		Gelling	Thickening	Dispersing	Emulsifying	Binding	Water holding	Foaming	Film forming
Plants	Seeds	Guar gum	⊙	○			⊙	○	
		Tara gum	⊙	○			⊙	○	
		Locust bean gum	○ ¹⁾	⊙		○	⊙	○	
		Tamarind seed gum	○	⊙	○		○		
		Soybean-soluble polysaccharide			⊙			○	
		Psyllium seed gum		⊙		○	⊙		
	Tubers	Starch	⊙	⊙		⊙	⊙		
		Konjac glucomannan	⊙	○	○	○	○		
	Fruits	Pectin	⊙		⊙				
	Tree exudates	Gum arabic			⊙	⊙		○	⊙
		Gum karaya	○	⊙	○	⊙	⊙	⊙	○
		Gum ghatti			⊙	○		○	⊙
		Gum tragacanth		⊙	⊙	○	○	○	○
	Pulps	Cellulose		○	⊙	⊙			
	Seaweeds	Carrageenan	⊙	○	⊙	○	⊙		○
		Agar	⊙		○				○
		Alginate	⊙	○	○	○	○		○
Microorganism	Xanthan gum	Xanthan gum	○ ¹⁾	⊙	⊙	○	○		
		Gellan gum	⊙		⊙				○
		Curdlan	⊙				○		○
		Pullulan				⊙			⊙

⊙: Very frequently used in the food industry; ○: Frequently used in the food industry.

¹⁾ Does not form gels as a single ingredient but does when combined.

ity of wheat starch paste (5.64% starch), through the addition of certain polysaccharides (< 1%), has been demonstrated in terms of viscography during gelatinization using xanthan gum, guar gum, or carboxy methylcellulose as the polysaccharide source (Christianson *et al.*, 1981). A soluble complex was evidenced, as a result of the molecular associations between amylose and polysaccharide, as the main cause for viscosity increases during gelatinization. A similar result has been reported, in terms of viscography, using starch from wheat or corn pasted in the presence of guar gum or locust bean gum (Alloncle *et al.*, 1989). The addition of each galactomannan (0.35%) lowered the pasting temperature of starch (4%), with a dramatic increase in viscosity during gelatinization. It was suggested that the mutual exclusion between galactomannan and starch was the main cause, resulting in increased gum concentration in the continuous phase and increased volume fraction of the starch granules in the dispersed phase, although molecular associations between the two substances were not completely precluded. A variety of combinations have been investigated in terms of viscography, using starch from normal corn, waxycorn, regular rice, glutinous rice, tapioca, potato, or wheat pasted in the presence of guar gum, xanthan gum, gellan gum, alginate, ι-carrageenan, κ-carrageenan, carboxymethylcellulose, or hydroxypropylmethylcellulose (Shi and BeMiller, 2002). The addition of xanthan gum or carboxymethylcellulose (0.4%) decreased the viscosity of potato starch paste (3.6% starch), while increasing the viscosity of other starch pastes during gelatinization. Viscosity decrease of potato starch was related to the retardation of pasting, destruction of the starch granules, as well as inhibited amylose leaching, for which electrostatic repulsions between negatively charged batches may be responsible. Viscosity increase of starch pastes through the addition of food polysaccharides is currently utilized in the food industry for texture modification of soups, sauces, and fillings, providing a short and non-sticky texture or 'mouthfeel' based on the reduction in starch usage level.

2.2 Retrogradation Acceleration of short-term retrogradation has been demonstrated in terms of rheology for corn starch paste (4%) through the addition of food polysaccharides (0.1-0.5%), including guar gum, locust bean gum, or xanthan gum, based on the time-course of elasticity growth (~15 h) by dynamic viscoelasticity measurements to determine the rate constant (Alloncle and Doublier, 1991). Increase in the effective concentration of amylose in the continuous phase was suggested as the main cause, through the thickening or concentrating effects of polysaccharides. A similar result has been reported in terms of differential scanning calorimetry (DSC) for the composite system of corn

starch and konjac-glucomannan (Yoshimura *et al.*, 1996). The addition of konjac-glucomannan accelerated retrogradation of the composite system (total concentration 33%) during storage at 5°C for 5 days, represented by increased retrogradation ratio calculated as a percentage of enthalpy change required to melt retrograded starch upon re-scanning to that to gelatinize starch upon the initial scanning. Conversely, the addition of the polysaccharide retarded retrogradation during storage for longer periods. Increase in water-holding capacity of the composite system (total concentration 3.5%) has been demonstrated in terms of syneresis through the addition of konjac-glucomannan (Yoshimura *et al.*, 1996). Some polysaccharides increase the effective concentration of starch in the continuous phase, leading to the acceleration of retrogradation during storage for shorter periods, while preventing molecular ordering or crystallization of starch, particularly the amylopectin fraction of relatively short-chain segments, leading to the retardation of retrogradation during storage for longer periods. It has been demonstrated that the effects of cellulose on starch retrogradation depend on its water-solubility (Kohyama and Nishinari, 1992). Water-insoluble cellulose (3.3%) accelerated the retrogradation of starch paste from sweet potato (29.7%) after storage at 5°C for 14 days, while water-soluble cellulose derivatives carboxymethylcellulose and methylcellulose retarded retrogradation. Water-insoluble cellulose may function as a crystalline core in the starch system, promoting retrogradation. Control of starch retrogradation through the addition of food polysaccharides is currently utilized in the food industry to modify the texture and storage stability of bakery goods, noodles, and *harusame* (noodle-shaped jellies of green-pea starch), leading to improved final product quality as well as reduced processing costs.

2.3 Freeze-thaw stability Starch in an aqueous system undergoes retrogradation during frozen storage, although the degree of retrogradation is lower than in refrigeration. Furthermore, syneresis occurs upon freeze thawing, causing texture deterioration (softening in most cases) in addition to loss of taste. These changes occasionally decrease the palatability of starch-based food products and are preventable by the addition of food polysaccharides. Freeze-thaw stability has been investigated in terms of syneresis and DSC characteristics for starch from sweet potato (6.7 or 6.4%) pasted in the presence of sodium alginate, carboxymethylcellulose, curdlan, gellan gum, guar gum, gum arabic, κ-carrageenan, locust bean gum, or xanthan gum (0.3 or 0.6%) (Lee *et al.*, 2002). Among the polysaccharides tested, the addition of guar gum or xanthan gum effectively decreased syneresis of starch gels upon 5 repeated freeze-thaw cycles; while sodium alginate was proven to prevent the crystallization of starch

during frozen storage. For starch gels from sago (6%), the addition of guar gum or locust bean gum (< 1%) effectively decreased syneresis and inhibited structural softening (i.e., sponge-like structure) upon 5 repeated freeze-thaw cycles (Ahmad and Williams, 2001). The effects of galactomannan pertaining to the prevention of amylose retrogradation showed molecular-weight dependence. The larger the molecular weight of galactomannan, the larger the effects. The addition of κ -carrageenan or locust bean gum (10%) increased the freeze-thaw stability of potato starch gels (5%) in terms of rheology, represented by a lower increasing degree of storage modulus G' , for which network coupling and mutual exclusion between starch and polysaccharide may be responsible (Liehr and Kulicke, 1996). The functions of polysaccharides to increase freeze-thaw stability of starch systems are well explained in relation to the glass transition (Ferrero *et al.*, 1994). In the rubbery state (above the glass transition temperature), the mobility of amylose and amylopectin molecules is greater than that in the glassy state (below the glass transition temperature), enhancing molecular associations for gelation and molecular arrangements for crystallization. The addition of polysaccharides shifts the glass transition to higher temperatures as a result of the decrease in the amount of water that acts as a plasticizer, leading to the prevention of retrogradation during frozen storage. Additionally, polysaccharides are expected to inhibit ice-crystalline growth in the glassy state and to withhold water required for starch crystallization by holding it within their molecular structures.

2.4 Functions of food polysaccharides and their macromolecular characteristics The effects of galactomannans on the gelatinization and retrogradation behaviors of starch from sago using guar gum or locust bean gum of various molecular weights have been reported (Ahmad and Williams, 2001). Gelatinization behavior of sago starch (10%) was almost independent of the molecular weight of each galactomannan (0.125–1%), in terms of the DSC pasting temperature and endothermic enthalpy change during gelatinization, while retrogradation behavior was dependent. The rate constant of amylose gelation and the elastic modulus of the starch system increased with molecular weight upon storage at 25°C for 6 h. This report certainly contributes to the progress in this research subject, but may be somewhat deficient in discussing the molecular mechanism behind the results.

3. New insights from recent studies into the molecular mechanisms

The addition of food polysaccharides modifies the gelatinization and retrogradation behaviors of starch as a result of thermodynamic incompatibility and/or molecular interactions between the two substances, arranging the phase

behavior. It has been reported that phase behavior of the composite system is determined not only by the mixing ratio but also by the molecular weight of each component (Annable *et al.*, 1994; Closs *et al.*, 1999). Thus the functions of food polysaccharides in starch systems should depend on the macromolecular characteristics (e.g., molecular weight and conformation) of polysaccharides and also on whether polysaccharides used are charged or uncharged (Annable *et al.*, 1994). Also, the functions of food polysaccharides in the systems should depend on starch concentration. Starch systems can be regarded as a suspension of swollen or gelatinized granules dispersed in a macromolecular (e.g., amylose) medium (Alloncle and Doublier, 1991), and the physical properties of starch systems are governed by the characteristics of either the dispersed or the continuous phase. The relative importance of both phases essentially depends on starch concentration (Alloncle and Doublier, 1991; Doublier, 1987; Doublier *et al.*, 1987; Ellis *et al.*, 1989; Miles *et al.*, 1985a; Ring, 1985), and role of the dispersed phase is greater with increasing starch concentration. In this context, for the studies on the starch/polysaccharide composite systems, it is important to use well-characterized polysaccharide samples, with their effects being investigated at multiple starch concentrations.

3.1 Non-ionic polysaccharide galactomannans

As a source of non-ionic food polysaccharide, galactomannans, including gums from guar (*Cyamopsis tetragonoloba*) and fenugreek (*Trigonella foenum-graecum*), have been highlighted (Funami *et al.*, 2005a, b; Funami *et al.*, 2008a, b). These gums have a common molecular structure of β -(1-4) linked mannan (M) backbone substituted by a single galactose (G) side chain through α -(1-6) linkages with theoretical average G/M ratio of 1/2 for guar gum and 1/1 for fenugreek gum on a mol basis. Functions of these gums have been investigated using native normal corn as a starch source.

3.1.1 Molecular characterizations Weight-average molecular weight M_w ranged from 2.0×10^3 to 23.4×10^5 g/mol for guar gum and from 7.5×10^4 to 20.7×10^5 g/mol for fenugreek gum. Care should be taken of the difference in the data for guar gum from those in the previous paper (Funami *et al.*, 2005a) as a result of recalculation. Also, z-average root-mean-square radius of gyration R_g ranged between 76.0 and 135.8 nm for guar gum, whereas for fenugreek gum it was between 16.3 and 122.3 nm.

3.1.2 Gelatinization The addition of each galactomannan (0.5%) modified the gelatinization behavior of starch (15%) in terms of the rapid visco analyzer (RVA) profiles. RVA pasting temperature of the composite system shifted to higher temperatures with increasing M_w of each galactoman-

nan (Fig. 1a), opposite to the effect of starch concentration. When M_w is standardized by the molecular weight of the mannan backbone, to eliminate the difference in the substitution pattern of the galactose side chains, the effect of each galactomannan on the pasting temperature overlaps each other better than when M_w is standardized by the molecular weight of the galactose side chains (Figs. 1b & c). A similar standardization is applicable to the results at a lower starch concentration (5%) in the presence of the same addition level of galactomannan, although the pasting temperature shifted oppositely to lower temperatures with increasing M_w of the

polysaccharide (Fig. 2). Also at 5% starch, the pasting temperature showed M_w -independence at boundary of a critical value. RVA peak viscosity of the composite system (15% starch) increased with M_w of each galactomannan (Fig. 3a), analogous to the effect of starch concentration. When M_w is standardized by the molecular weight of the mannan backbone, the effect of each galactomannan on the peak viscosity overlaps each other better than when M_w is standardized by the molecular weight of the galactose side chains (Figs. 3b & c). A similar standardization is applicable to the results at the lower starch concentration in the presence of the same

Fig. 1. RVA pasting temperature of the starch/galactomannan composite system as a function of molecular weight of galactomannans. Starch concentration was 15 w/w%. (a): non-standardized M_w ; (b): M_w standardized by mannose; (c): M_w standardized by galactose. Closed: Guar gum; Open: Fenugreek gum.

Fig. 2. RVA pasting temperature of the starch/galactomannan composite system as a function of molecular weight of galactomannans. Starch concentration was 5 w/w%. (a): non-standardized M_w ; (b): M_w standardized by mannose; (c): M_w standardized by galactose. Closed: Guar gum; Open: Fenugreek gum.

Fig. 3. RVA peak viscosity of the starch/galactomannan composite system as a function of molecular weight of galactomannans. Starch concentration was 15 w/w%. (a): non-standardized M_w ; (b): M_w standardized by mannose; (c): M_w standardized by galactose. Closed: Guar gum; Open: Fenugreek gum.

addition level of galactomannan (Fig. 4).

3.1.3 Long-term retrogradation The addition of each galactomannan (0.5%) modified long-term retrogradation behavior of starch (15%) in terms of the rate constant k , determined by creep tests, reducing the time-dependence of the creep compliance during storage (at 4°C for 14 days) to the first-order kinetic equation. The resultant rate constant decreased with increasing M_w of each galactomannan, opposite to the effect of starch concentration (Fig. 5a), representing slower development of structural hardening during long-term retrogradation. When M_w is standardized by the molecular weight of the galactose side chain, the effect of each galactomannan on the k overlaps each other better than when M_w is standardized by the molecular weight of the mannan backbone (Figs. 5b & c).

3.1.4 Molecular mechanism Functions of galactomannan are related to the molecular associations with starch components during gelatinization and retrogradation. Earlier onset of viscosity increases at the lower starch concentration is attributable to enhanced molecular associations between each galactomannan and leached amylose (Christianson *et*

al., 1981; Shi and BeMiller, 2002), and the M_w -independence can be explained by a fixed chain-length of amylose that participates with the molecular associations. Amylose leaching occurs as a trigger of starch swelling. Retarded onset at the higher starch concentration, on the other hand, is attributable to the masking of these molecular associations due to increased volume fraction of the starch granules in the continuous phase, and each galactomannan may instead lubricate the system by preventing granule abrasions. Another possibility lies with the decreased amount of leached amylose that participates in these molecular associations. The amount of leached amylose decreased as the function of M_w of galactomannan, along with the viscosity increase of the system (Fig. 6).

The increase in viscosity at the lower starch concentration is attributable to the molecular associations between each galactomannan and the swollen starch particles or amylopectin, particularly the amylopectin fraction with long-chain segments, which leaches out of the starch granules during gelatinization in a manner similar to amylose (Tester and Morrison, 1990). The increase in viscosity at the higher

Fig. 4. RVA peak viscosity of the starch/galactomannan composite system as a function of molecular weight of galactomannans. Starch concentration was 5 w/w%. (a): non-standardized M_w ; (b): M_w standardized by mannose; (c): M_w standardized by galactose. Closed: Guar gum; Open: Fenugreek gum.

Fig. 5. Rate constant k representing long-term retrogradation of the starch/galactomannan composite system as a function of molecular weight of galactomannans. Starch concentration was 15 w/w%. Time-dependence of the creep compliance J was reduced to the first-order kinetic equation to determine the k :

$$J = J_0 \exp(-kt)$$

where t and J_0 stand for storage time and the initial creep compliance ($t = 0$), respectively. (a): non-standardized M_w ; (b): M_w standardized by mannose; (c): M_w standardized by galactose. Closed: Guar gum; Open: Fenugreek gum.

Fig. 6. The amount of leached amylose as a function of the RVA peak viscosity. Closed: Guar gum; Open: Fenugreek gum.

starch concentration, on the other hand, is attributable to enhanced granular abrasions.

Long-term retrogradation of starch is described as the crystallization of amylose (Miles *et al.*, 1985b) and amylopectin (Kalichevsky *et al.*, 1990; Orford *et al.*, 1987; Ring *et al.*, 1987; Zobel and Stephen, 2006), as well as the co-crystallization between these starch components (Miles, *et al.*, 1985a; Russell, 1987). The crystallization of amylopectin is predominant over other factors in long-term retrogradation, particularly the fraction with short-chain segments of approximately 15 DP (Kalichevsky *et al.*, 1990; Ring *et al.*, 1987). Retarded long-term retrogradation is attributable to the molecular associations between each galactomannan and the corresponding amylopectin fraction, preventing the formation of crystalline structures. Functions of each galactomannan to stabilize water molecules should be considered as another factor to retard long-term retrogradation of starch; the polysaccharide may act as a water binder, effectively depriving amylose or amylopectin of usable water for crystallization and thus preventing starch retrogradation (Lee *et al.*, 2002).

Results suggest an important role of the mannan backbone in explaining the effects of galactomannans during starch gelatinization. Viscosity profiles of starch systems relate to the molecular associations between the mannan backbone and amylose or amylopectin leached out of the starch granules, although the effects depend on starch concentration. The mannan backbone should reasonably interact with these amylosic molecules more easily than the galactose side chains when the molecular similarity (e.g., linearity and smoothness) between these substances is considered. Results also suggest an important role of the galactose side chains in explaining the effects of galactomannans during starch retrogradation. The galactose side chains impart the flexibility

with galactomannan molecules, increasing the accessibility of the polysaccharide to starch components. The galactose side chains should reasonably act on the crystallizing sites, particularly the amylopectin fraction with short-chain segments, more easily than the mannan backbone when the complicated and heterogeneous molecular structures of amylopectin are considered. It is unclear at the present stage, however, which effect of the galactose side chains is more molecularly important, the galactose side chains themselves as a direct active site or the flexible mannan backbone as a result of an indirect contribution of the galactose side chains.

3.2 Anionic polysaccharides with interfacial activity

As a source of anionic polysaccharides, soybean-soluble polysaccharide (SSPS) and gum arabic (GA) have been highlighted (Funami *et al.*, 2008c). These two anionic polysaccharides are widely used in the food industry as effective emulsifiers and stabilizers in oil-in-water emulsions rather than gelling or thickening agents. The emulsifying properties of these polysaccharides are regarded as a function of the proteinaceous moiety (Nakamura *et al.*, 2004a, b; Nakamura *et al.*, 2006 for SSPS and Dickinson *et al.*, 1989; Dickinson *et al.*, 1991; Randall *et al.*, 1988 for GA) because the character of the polysaccharide itself is predominantly hydrophilic. The carbohydrate fraction is effective in stabilizing the emulsions predominantly through steric effects. A common structural feature found in each polysaccharide is that it is highly branched and spherical, occupying a relatively compact space in an aqueous system in comparison with an ordinary linear polysaccharide of equivalent molecular weight. This realizes extremely low viscosity, even over 10% concentrations (Stephen and Churms, 1995), leading to novel functions in controlling rheological properties and in modifying textural attributes of starch systems. Functions of these polysaccharides have been investigated using native wheat as a starch source.

3.2.1 Molecular characterizations Molecular structures have been investigated in detail for SSPS (Nakamura *et al.*, 2001, 2002) and for GA (Al-Assaf *et al.*, 2003; Islam *et al.*, 1997; Randall *et al.*, 1988, 1989; Williams *et al.*, 1990). As an experimental material, SSPS consisted primarily of galactose, galacturonic acid, and arabinose, in this order; whereas GA was abundant in galactose, arabinose, and glucuronic acid, in this order. The M_w was almost equivalent between SSPS (65.3×10^4 g/mol) and GA (63.7×10^4 g/mol), while the R_g of GA (51.4 nm) was approximately twice as large as that of SSPS (26.4 nm), indicating more spherical or compact molecular structures for SSPS, consistent with atomic force microscopy images (Ikeda *et al.*, 2005).

3.2.2 Gelatinization The addition of each anionic polysaccharide (0.1–0.5%) hardly altered the RVA pasting tem-

Table 2. Gelatinization behavior of the starch/polysaccharide composite system by rapid visco analyzer.

Treatments	5 % starch		13 % starch	
	Pasting temperature (°C)	Peak viscosity (RVAU)	Pasting temperature (°C)	Peak viscosity (RVAU)
Starch alone	92.8 ^a	15.5 ^a	69.5 ^a	333.5 ^a
Starch+0.1% SSPS	93.5 ^a	13.7 ^{bc}	68.3 ^{ab}	310.2 ^{bc}
Starch+0.5% SSPS	93.2 ^a	12.2 ^{bc}	68.1 ^b	263.8 ^c
Starch+1.0% SSPS	94.0 ^a	10.1 ^{df}	67.6 ^b	218.7 ^d
Starch+0.1% GA	92.1 ^a	13.8 ^b	67.6 ^b	318.4 ^b
Starch+0.5% GA	92.6 ^a	12.4 ^{bc}	67.8 ^b	304.1 ^e
Starch+1.0% GA	92.0 ^a	11.7 ^{cef}	67.8 ^b	300.3 ^e

^{a-f} Values in the same column with different superscripts are significantly different ($P < 0.05$).

Table 3. Leached amylose and average particle diameter of the starch granules.

Treatments	Leached amylose (%)	Average diameter (μm)
5% Starch	51.2 ^a	43.2 ^a
5% Starch+0.1% SSPS	44.8 ^b	43.5 ^a
5% Starch+0.5% SSPS	43.4 ^{bc}	42.9 ^a
5% Starch+1.0% SSPS	41.4 ^c	43.4 ^a
5% Starch+0.1% GA	47.9 ^d	42.2 ^a
5% Starch+0.5% GA	45.2 ^b	41.9 ^a
5% Starch+1.0% GA	43.3 ^{bc}	42.8 ^a

^{a-d} Values in the same column with different superscripts are significantly different ($P < 0.05$).

perature of the composite system when the starch concentration was 5% (Table 2). Results were different at a higher starch concentration (13%), where the addition of each polysaccharide shifted the pasting temperature to lower temperatures. No differences were seen in this effect between SSPS and GA when compared at the same addition level. The addition of each anionic polysaccharide tended to decrease the RVA peak viscosity of the composite system with increasing addition level at each starch concentration. At the higher starch concentration, the effect of SSPS to decrease the peak viscosity was greater than that of GA when compared at the same addition level (except at 0.1%).

It was generally recognized that the higher the addition level of each polysaccharide, the lower the amount of leached amylose during gelatinization (Table 3). It should be noted that “amylose” here involves not only true amylose but also amylopectin with long-chain segments. The effect of SSPS in decreasing leached amylose was greater than that of GA when compared at the same addition level, although statistical difference was seen only at 0.1% addition. A linear positive relationship was found between the amount of leached amylose and the RVA peak viscosity (Fig. 7). The addition of each polysaccharide hardly altered the average particle diameter of the starch granules upon gelatinization

(Table 3), and thus no specific relationship was found between particle diameter and the RVA peak viscosity (Fig. 7).

Remnants of the starch granules, so called “starch ghosts”, were frequently observed on the confocal laser scanning microscopy (CLSM) images for the control treatment without any food polysaccharide added (Fig. 8). Starch ghost is a synonym for the envelope of the starch granules after the starch components have been leached out upon heating in an appropriate amount of water. Starch ghosts were observed less frequently on the images in the presence of each anionic polysaccharide. Continuous and glutinous layer structures forming around the surface of the granules were lessened in the presence of each anionic polysaccharide. The distribution pattern of polysaccharide was different between SSPS and GA, although the majority of each polysaccharide seemed to be outside the starch granules, particularly for SSPS. SSPS existed continuously around the surface of the granules, whereas GA existed sparsely, forming “aggregated” structures.

3.2.3 Short-term retrogradation To investigate short-

Fig. 7. The amount of leached amylose and average particle diameter of the starch granules as a function of the RVA peak viscosity. Closed: Leached amylose; Open: Average particle diameter.

Fig. 8. CLSM images of the starch/polysaccharide composite system gelatinized. (a), (a'): starch alone; (b), (b'): starch/SSPS composite system; (c), (c'): starch/GA composite system.

term retrogradation behavior of the starch/polysaccharide composite system, time-dependence of G' was approximated by the first-order kinetic equation composed of two reactions with different rate constants, k_1 and k_2 (Yoshimura *et al.* 1998):

$$G'(t) = G'_0 + G'_{1s}\{1 - \exp(-k_1 t)\} + G'_{2s}\{1 - \exp(-k_2 t)\} \quad (k_1 > k_2),$$

where G'_{1s} and G'_{2s} represent the equilibrium G' for faster rate component and for slower rate component, respectively. Also, G'_0 represents the initial G' , and t is storage time. In this equation, a faster rate component should relate to the gelation of amylose as a trigger of retrogradation, whereas a slower rate component to the gelation of amylopectin with long-chain segments, the former of which dominates short-term retrogradation behavior of starch (Orford *et al.*, 1987). It is known that the gelation of amylose proceeds faster at higher concentrations (Miles *et al.*, 1985a, b) and that the chain length of amylopectin has an important effect on the rate of retrogradation: the longer, the faster (Kalichevsky

et al., 1990). The addition of each anionic polysaccharide (0.5%) increased the k_1 (i.e., faster rate constant) of the composite system (5% starch) upon storage (at 4°C for 24 h), but no marked differences were seen in the effect between SSPS and GA on the k_1 (Table 4). The k_2 (i.e., slower rate constant) was not sensitive to each polysaccharide. Also, the addition of each polysaccharide increased G'_0 , and this effect of GA was greater than that of SSPS. It decreased G'_{1s} and G'_{2s} , particularly G'_{1s} , on the other hand, and this effect of SSPS was greater than that of GA.

The addition of each anionic polysaccharide (0.5%) increased syneresis of the composite system (5% starch) upon storage at 4°C for 24 h, and this effect of SSPS was greater than that of GA (Table 4).

3.2.4 Phase behavior of the composite system Phase behaviors of the starch/polysaccharide composite system (2.0–5.0% starch and 0.25–2.0% polysaccharide) were investigated after incubation at 70°C for 48 h by visual inspection. Phase arrangement of the composite system depended on the

Table 4. Short-term retrogradation behavior of the starch/polysaccharide composite system.

Treatments	G'_0	G'_{1s}	k_1	G'_{2s}	k_2	Syneresis (%)
	(Pa)	(Pa)	($\times 10^{-3}$ Pa/min)	(Pa)	($\times 10^{-3}$ Pa/min)	
5% Starch	11.3	75.1	8.5	20.2	0.6	9.6 ^a
5% Starch+0.5% SSPS	17.0	28.8	18.8	15.3	0.6	27.1 ^b
5% Starch+0.5% GA	25.8	31.1	17.3	16.1	0.6	19.2 ^c

^{a-c} Values in the same column with different superscripts are significantly different ($P < 0.05$). Time-dependence of storage modulus G' was reduced to the first-order kinetic equation composed of two reactions with different rate constants, k_1 and k_2 :

$$G' = G'_0 + G'_{1s}\{1 - \exp(-k_1 t)\} + G'_{2s}\{1 - \exp(-k_2 t)\} \quad (k_1 > k_2)$$

where G'_{1s} and G'_{2s} represent the equilibrium G' for a faster reaction component and for a slower reaction component, respectively. Also, G'_0 represents the initial G' , and t is storage time.

total polymer concentrations, exhibiting two-phase separation at a lower concentration regime and no macroscopic separation at a higher concentration regime (Fig. 9).

3.2.5 Molecular mechanism Functions of each anionic polysaccharide are related to the phase behavior of the composite system during gelatinization and retrogradation. As a cause for the earlier onset of viscosity increases, phase separation should be emphasized more than the molecular associations, as reported previously for the composite system of potato maltodextrin and gum arabic (Annable *et al.*, 1994) and for that of potato starch and xanthan gum (Conde-Petit *et al.*, 1997). The effective concentration of starch increases in the continuous phase (Alloncle *et al.*, 1989; Yoshimura *et al.*, 1998) as a result of mutual exclusion between each polysaccharide and starch or starch components. Viscosity decrease through the addition of each polysaccharide agrees with previous reports, in which starch from potato or rice (8 or 12%) was pasted in the presence of SSPS (1-5%) (Furuta *et al.*, 2003), and in which starch from sweet potato (6.4%) was pasted in the presence of GA (0.6%) (Lee *et al.*, 2002). Each polysaccharide added accelerates amylose gelation, as represented by increased k_t , which is also attributed to phase separation as a result of mutual exclusion among unlike polymers, increasing the effective concentration of amylose in the continuous phase. Molecular associations between amylosic chains and each polysaccharide are unlikely to occur when structural dissimilarity of the molecular conformation is considered. Increase in G'_0 also indicates that each polysaccharide added expedites amylose gelation. In this context, the effect of GA is greater than that of SSPS in accelerating amylose gelation, presumably due to the larger thermodynamic incompatibility with amylose. Each polysaccharide added lowers the rigidity of gelled structures of amylose, as represented by decreased G'_{1S} , as well as that of amylopectin with long-chain segments, as represented by decreased G'_{2S} . Depression of gel properties is related to the phase arrangement between each polysaccharide and these starch components, decreasing consistency and increasing inhomogeneity

of the whole system. Results are in agreement with a previous report (Alloncle and Doublier, 1991) showing that the incorporation of some polysaccharides accelerates short-term retrogradation of the starch/polysaccharide composite system but simultaneously depresses the final gel characters after retrogradation.

The lack of macroscopic separation at higher starch concentrations is believed to be an artifact due to the high viscosity of the medium, hindering total separation of the phases as in the case of amylose/guar gum composite system (Alloncle and Doublier, 1991). Thermodynamic incompatibility between starch components and polysaccharides has been indicated (Alloncle and Doublier, 1991; Conde-Petit *et al.*, 1997), and it is reasonable to think that phase separation occurs for any composite system tested, particularly at lower temperatures (e.g., 4°C), because temperature decreases generally shift the bimodal to lower total polysaccharide concentrations (Closs *et al.*, 1999).

Functions of each anionic polysaccharide are also related to the surface control by adsorbing onto and coating around the starch granules, preventing the swelling of starch and abrasions between granules. These are physical effects through the formation of a polysaccharide barrier or film. Inherent low viscosity with Newtonian flow behavior for each polysaccharide may additionally contribute to lubricating the system without generating adhesiveness. Increased syneresis, which is equivalent to decreased water-holding capacity and is ascribed to the incomplete hydration of the starch granules (i.e., swelling), is attributable to the formation of a polysaccharide barrier or film partly or entirely surrounding the surface of the starch granules to prevent moisture uptake occurring as a result of amylose leaching (and some amylopectin fraction) into the continuous phase, followed by melting of amylopectin crystalline structures (Keetels *et al.*, 1996). A linear negative relationship observed between the amount of leached amylose and the RVA peak viscosity for the starch/galactomannan composite system, in the previous section, illustrates that amylose diffusion should be prevent-

Fig. 9. Phase diagram of the starch/polysaccharide composite system gelatinized after incubation at 70°C for 48 h. Open: no separation (but this may be an artifact due to high viscosity); Closed: two-phase separation. (a): SSPS; (b): GA.

ed due to increased viscosity of the continuous phase in the presence of each galactomannan. This discrepancy suggests that a factor other than viscosity is involved in preventing amylose leaching in the presence of each anionic polysaccharide, emphasizing the coating effect. CLSM images indicate that the leaching of starch components, the majority of which is amylose, is prevented in the presence of each anionic polysaccharide. The difference in the functions of SSPS and GA can be explained by the distribution pattern of each polysaccharide outside the starch granules, and continuous and uniform layer for SSPS contributes more to the decreases in the RVA peak viscosity and in the amount of leached amylose than aggregated and sparse particles for GA.

4. Conclusion

The series of recent studies provides clues to elucidate the molecular mechanisms for the functions of some food polysaccharides in starch systems, where the structure-function correlation has been scarcely reported for polysaccharides. It is extremely important, from both a fundamental and an application point of view, to comprehend the functions of polysaccharides in food systems on a molecular level, for quality control of the final products and development of new products with increased palatability. In this context, these kinds of studies will greatly contribute to the progress in the food industry. Further investigations should accumulate more in-depth information on the interactions between starch components and polysaccharides using fractionated amylose and amylopectin of different unit chain-lengths.

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