A Thesis for the Degree of Doctor of Philosophy

Microstructural, rheological and physicochemical properties of milk protein and rice starch during heat-induced gelation

우유단백질 및 쌀전분의 열 유도 젤화 과정 중의 미세구조,
유변학적 및 이화학 특성 규명

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by

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Abstract

Physicochemical and gelatinization properties of starches obtained from different rice cultivars are responsible for their processability in food production. In this study, the relationship between the structure evolvement of rice starches upon heating and their properties was investigated with the emphasis on rice cultivars. Starches separated from 10 different Korean rice cultivars were studied with respect to their morphological, viscoelastic, hydration, pasting, and thermal properties. The granular morphology of the 10 samples was found to be mostly polyhedral but was not significantly different among the rice cultivars. The rheological behavior during gelatinization upon heating brought out differences in onset in elastic modulus (G′) and degree of steepness. Upon gelatinization, the G′ values of the rice starch pastes ranged from 37.4 to 2,057 Pa at 25 °C, and remarkably, the magnitude depended on the starch varieties. R1 showed the lowest critical strain (γ_c), whereas W1 and W2 possessed the highest γ_c. The amylose content in starch affected the linear viscoelasticity of rice starch pastes and gels. In addition, the amylose content in rice starches also affected their pasting properties; the sample possessing the highest amylose content showed the highest final viscosity and setback value, whereas waxy starch

samples displayed low final viscosity and setback value. The onset gelatinization temperatures of the starches from 10 rice cultivars ranged between 57.9 and 64.4 °C, but the thermal properties did not correlate well with the amylose content. Furthermore, amylose content could be correlated to hydration (Pearson correlation coefficient (PC): -0.9269) and pasting properties (PC: 0.8514) of rice starches whereas it was insufficient in accounting for their viscoelastic and thermal characteristics (PC: -0.3745). The combined analysis of hydration, pasting, viscoelastic, and thermal data of the rice starches is useful in fully understanding their behavior and in addressing the processability for food applications.

Gelation characteristics were investigated by in situ rheological test during gelation of β-lactoglobulin (BLG) system heated from 25 to 100 °C. Some valuable parameters derived to represent gelation characteristics were maximal G′ (G'_{max}), and initial gelation temperature (T_i). Changes in rheological properties were investigated by using small-amplitude oscillatory shear (SAOS) test during gelation of BLG system heated from 25 to 100 °C. The effects of pH (3.5, 5.5, and 6.8), were studied on the viscoelastic properties during gelation. BLG solutions prepared at different conditions were poured directly on the measuring stage of the rheometer installing a 20-

mm parallel-plate geometry with a 1-mm gap. During heating, rheological parameters such as elastic modulus (G′), and loss modulus (G″) were measured as a function of temperature at 1 Hz and a maximum target strain of 0.01. G′ and G″ rapidly increased with increasing temperature and then tended to reach a plateau with G′ becoming higher than G″. Some valuable including G′ and initial gelation temperature (T_i) were derived to represent gelation characteristics. Various gelation conditions yielded systems showing different rheological properties. G'_{max} at pH 3.5 and 6.8 was much higher than that at pH 5.5. High electrostatic repulsion offered high G'_{max}. Different gel preparation conditions endowed different electrostatic conditions to BLG system which resulted in different gel properties.

Also changes in rheological properties were investigated with small-amplitude oscillatory shear (SAOS) test during shear stress sweep at a constant frequency of 1 Hz. The elastic moduli (G′) of the cured BLG gels prepared ubder different conditions were measured as a function of strain (γ) after cooling. The G′ and γ showed remarkable dependence on pH. The pH affected dominantly the critical strain (γ_c). At pH 3.5 or 6.8, the G′ was higher than that at pH 5.5. The texture of BLG gels at pH 6.8 was rubber-like while that at pH 3.5 was very brittle. The gels prepared at pH 5.5 were

slightly rubbery but overall weak. These experimental results would provide useful information for food industry using rice starch and BLG.

Key words: rice starch; viscoelastic property; thermal property; gelation temperature, β-lactoglobulin, rheological property

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Chapter 1. Introduction

1. Background

Historically, starchy foods that come from seeds, roots, and tubers, have always been eaten by humans. It is interesting that very early agricultural production of grain crops such as barley, rice, wheat and corn, is related to isolation of starch from grain crop (Schwartz & Whistler, 2009). In 1900s, the starch industry began to increase with establishment of starch production plant. In New York, USA, the first starch plant (wheat starch production facility) started in 1807 and changed to corn starch production facility in 1849. After that the number of starch plant in the USA increased to 140 by 1880 and the corn, wheat, potato and rice starches were produced. Rice starch manufacture began in the USA in 1815. However, production did not expand significantly (BeMiller, 2009).

Rice is a major world food crop and about 90% of the total rice is produced and consumed in Asia including East Asian region. Similar to other food crops, in order for industrial uses, it is more important to use as a starchy food source than usage as a whole grain. In Korea, rice is the staple food and the only self-sufficient crop. Also a number of research have been carried out to improve rice cultivar and expand the industrial usage of rice such as snack, cake, noodle, and candy.

In industrial aspect, not only the stable supply of substances for food production but also understanding the influence of microstructure on product quality such as texture and softness is very important. Many food products use starch as a stabilizer and a viscosity enhancer, and gelling property is a key to control the quality of starch product. There are a variety of factors representing gelation behavior of food ingredients. And it is important to understand the parameters related to gelation in order to expect structure evolution of protein and starch gels for various industrial applications.

2. Objectives of Research

The first objective was to investigate the morphological, viscoelastic, hydration, pasting, and thermal properties of starches from 10 Korean rice cultivars, and to understand the relationship between structure evolvement upon heating and their properties.

The other objective was to elucidate the gelation properties of BLG and to understand heat-induced gelling properties of typical food ingredients (rice starch and milk protein, BLG) in a Korean food industry.

3. Review of Literature

3.1. Starch

A carbohydrate is essential for human beings to maintain their lives and activities with protein and fat. Starch is the major source of carbohydrate and is produced from crops such as wheat, corn, and rice. Recently, FAO (Food and Agriculture Organization of the United Nations) has reported the crops are major source of carbohydrates, especially rice had 43.3% of total carbohydrate supply from crops followed by wheat (40.0%) and corn (10.3%) in 2007.

Starch is the main component of cereal grains and is used in the food industry as an agent for thickening and gelling (Singh, 2006). Starch, nature's most abundant reserve polysaccharide, consists mainly of amylose and amylopectin. Amylose is an essentially linear molecule consisting of α-(1→4)-linked D-glucopyranosyl units, whereas amylopectin has short α-(1→4)-linked D-glucosyl chains with 5-6% non-randomly distributed a-(1→6)-bonds. The amylopectin structure model maintains it to be a cluster (Robin et al., 1974). The starch granule consists of 70% of amorphous region, which is mainly filled with amylose, and 30% of crystalline region, which is composed of amylopectin chains (BeMiller, 2007). Many former studies

revealed that physicochemical characteristics like the amylose/amylopectin ratio and the granule size are responsible for the properties of starch (Fredriksson & Silverio, 1996; Madden & Christensen, 1996, Singh et al., 2003)

Rice is a major world food crop and has very wide ranges of cooking quality and rheological properties that are largely determined by swelling, gelatinization, and pasting of starch (Tester & Morrison, 1990). The swelling behavior of cereal starches is dependent on amylopectin content; amylose inhibits swelling of starch as a dilutent (Juliano, 1985). Traditionally, rice has been used as a hypoallergenic carbohydrate ingredient because it does not have gluten. And its digestibility, compared to other cereal grains, has led to use in infant and geriatric food. Rice starch is prepared from broken rice for many reasons. There are two methods of rice starch isolation. The traditional method involves alkali solubilzation of the glutelin constituting 80% of protein in rice, while the mechanical method releases starch via wet-milling process. Depending on the relative amylopectin : amylose ratio in rice, the starch may show a various gelatinized textures and strength. Also, there are several basic factors that affect starch properties such as rice variety, protein content, and modification

(BeMiller, 2007).

3.2. Swelling of starch

Starch swelling begins with water absorption in amorphous region of starch and then causes crystalline structures, which is composed of amylopectin, to melt and break due to breakage of hydrogen bonds. And water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin (BeMiller, 2007). The extent of granule swelling is determined by measuring the swelling factor (SF), which is reported as the ratio of volume of swollen granules to the volume of dry starch (Tester & Morrison, 1990; BeMiller, 2007).

As stated in many previous studies, swelling of starch is an important property not only for a process prior to gelatinization but also a factor to understand the changes in starch structure such as leaching of amylose, loss of birefringence, loss of crystallinity and expansion of starch granules (Fennema, 1996). Also, it is certain that swelling and gelatinization behavior of starch are contributed by various factors such as amylose, amylose-lipid complex and amylopectin in starch.

3.3. Starch gelatinization

Gelatinization is the thermal restructuring of crystalline region in starch granules. However in a broader concept, gelatinization includes swelling of starch molecules and leaching of soluble fraction of starch (Tester & Morrison, 1990a).

In gelatinization, water acts as a plasticizer. When heated in the presence of sufficient water and a proper temperature is reached, the plasticized amorphous regions of the granule undergo a phase transition to rubbery state. During gelatinization, leaching of amylose occurs and total gelatinization normally proceeds over a temperature range. Because gelatinization of starch is an endothermic process, the temperature range and the enthalpies are precisely measured by DSC (Fennema, 1996).

The crystallinity of the starch is measured by the enthalpy of gelatinization (ΔH). Onset temperature (To) and completion temperature (Tc) determine the boundaries of the different phases in a semicrystalline material like starch (Tester & Morrison, 1990b; Biliaderies et al., 1986)

3.4. Starch pasting

Pasting property of starch is an important factor in anticipating the behavior of starch granule during processing and measured by the Rapid Visco Analyzer (RVA). As heating continues, an increase of viscosity would be observed, which indicates the occurrence of pasting. The temperature at the onset of viscosity increase is pasting temperature. Continued heating makes viscosity reach the peak viscosity (PV), where the swelling rate equals the starch granule's collapse. Once PV is achieved, the breakdown (BD) appears as a result of dissolving granules (Adebowale & Lawal, 2003; Fennema, 1996).

In cooling stage, viscosity rises again and reaches setback (SB), which is caused by retrogradation of starch, typically due to amylose. SB is an indicator of final texture of starch gels (Batey, 2007).

3.5. Milk Protein (β-Lactoglobulin)

Many globular proteins can form gels by heating. Heating a globular protein solution results in aggregation of protein molecules (Clark & Lee-Tuffnell, 1986; Ko, 2005). The physical properties of protein gels are

influenced by microstructure of the gel. And various measuring methods have been used to correlate the physical properties to the microstructure.

The gelation of BLG involves many kinds of interactions such as disulfide bonds, hydrogen bonds, and hydrophobic interactions, which are dependent on pH, ionic strength, and protein concentration (Aguilera, 1995; Kinsella and Whitehead, 1989; Ziegler and Foegeding, 1990; Petit et al., 2012; Woo et al., 2013). The gelation process of globular proteins such as BLG is considered a three-step process, which is unfolding of protein molecules with exposure of buried SH and hydrophobic groups, the aggregation of denatured protein molecules, and the formation of a three dimensional network. The microstructural morphology of BLG molecules changed significantly by protein concentration, pH, and NaCl concentration (Woo et al., 2013).

Because gelation conditions affect conformation and interactions of BLG molecules, they influence rheological characteristics and texture. Gelation conditions cause dissimilarity in structure evolution. In other words, the gel structure and rheological properties of heat-induced BLG gels may be controlled by various physicochemical conditions which is necessary for food process applications such as viscosity enhancer and stabilizer (Ko,

2005).

Determination of parameters representing gelation characteristics is important in understanding the incipient gelation of globular proteins upon heating. When protein molecules start to form a network, the moment is recognized as gelation point. At the moment, the system changes from a viscous liquid (sol) to an elastic solid (gel). Rheological study would be helpful to understand sol-gel transition of aqueous globular proteins. Initially, intermolecular aggregation upon heating forms protein clusters at which G′ increases suddenly. At that point, protein clusters began merging and subsequently combined into a matrix. It can be assumed that gelation occurs at this point. The gelation temperature can be used as an important criterion for the gelation behavior. Some theory and methods have been proposed to determine the gelation temperature of the system to form a gel upon heating. Rheometry has become widely used to determine gelation temperature since it provides continuous rheological data for the entire gelation process (Lin et al., 1991). A simple method is to determine gelation parameters from a point at which the G′ rose above a threshold value, ~2-3 Pa (Tobitani and Ross-Murphy, 1997b; Tobitani and Ross-Murphy, 1997a). However, the threshold value sometimes can be confused with noise of signals of the measuring

system. When the signal fluctuates higher than the threshold value, it is hard to determine the gelation point. Winter and Chambon's criterion has been used to determine the gelation point (Winter and Chambon, 1986): tan δ is independent of frequency at the gel point for chemical gels. The congruent functions G′ (ω) = G″ (ω) are as much a rheological property at the gel point as are infinite viscosity and zero equilibrium modulus. It is known, however, that in gelation kinetic experiments, the crossover method is often very difficult to apply (Stading and Hermansson, 1990; Ross-Murphy, 1991). In some cases such as gelation of BLG, no crossover of the G′ and G″ was found during the gelation process for some protein-based gelation system (Tobitani and Ross-Murphy, 1997a) as well as G′ was already higher than G″ before the gelation temperature. Therefore, a different approach is needed to determine gelation point for the gels not showing crossover between G′ and G″.

3.6. Small amplitude oscillatory shear (SAOS) test

Small amplitude oscillatory shear test is commonly used to characterize dynamic viscoelastic properties. Among various measurements, SAOS measurement can distinguish the weak and hard gel (Rossmurphy &

Shatwell, 1993; Ko, 2005). During increasing shear stress, the inner structure of gels first becomes fully extended and then breaks under shear (Burche & Halpin, 1964; Foegeding, 1992; Ko, 2005).

Onset point of the structure collapse is termed the limit of linearity or critical strain (γ_c). Above this point, structure change is irreversible. The limit of linearity indicates the gel structure and strength of gel network (Shin, 1990; Eleya, 2004; Ko, 2005; Montejano, 1986).

Many kinds of food gels have been studied using the SAOS measurement. The gelatinization of starch has been investigated using an oscillatory rheometer; the dynamic measurement for viscoelastic ranges is established for the starch based food system (Navarro, 1997). A previous study (Baltsavias, 1997) investigated the rheological properties of dough with various ingredients using the SAOS. The linearity of dough was limited to a great extent and beyond it significant structure breakdown occurred. In order to control food gel's texture effectively, it is necessary to understand the relationship between the structure and the physical properties. The SAOS method is one of the useful measurements to produce information on microstructure and physical properties of starch and protein gels.

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Chapter 2. Physicochemical properties of starches separated from different Korean rice cultivars

1. Introduction

Gluten plays an important role in retaining gas that lends the desired volume and texture to dough, but it is allergenic and causes celiac disease in some populations (Demirkesen, Mert, Sumnu, & Sahin, 2010). The only effective treatment for celiac disease is strict adherence to a gluten-free diet (Gallagher, Gormley, & Arendt, 2004). Rice does not contain gluten; it is therefore desirable for the preparation of gluten-free products. Moreover, rice augments human health owing to low levels of sodium, protein, fat, and fiber, and is applied for a substantial measure of easily digestible carbohydrates (Ji, Zhu, Qian, & Zhou, 2007). These properties make rice flour highly desirable and the most suitable cereal source for gluten-free food products (Yoon et al., 2011). Although rice has been routinely consumed as a whole grain, its processed products have not been developed actively as compared with those from other crops such as wheat. Consequently, in order to expand its scope in food applications, an understanding of its physicochemical properties is especially required, since there is limited availability of rice products in the form of bread and noodles because of the absence of gluten (Gujral, Guardiola, Carbonell, & Rosell, 2003).

In order to facilitate the industrial application of rice flour, there is a

need to investigate not only the physicochemical and gelatinization properties but also the relationship between inner-structure and rheological properties of rice starch. A wide variety of rice cultivars need to be studied as each rice cultivar possesses unique properties (Lee et.al, 2012), including the size, shape, and ratio of amylose/amylopectin of the starches that are responsible for their processability, as they affect the physicochemical and gelatinization characteristics (Christensen & Madsen, 1996; Li & Yeh, 2001; Singh, Singh, Kaur, Sodhi, & Gill, 2003). Particularly, macroscopic properties of rice starches strictly correspond to microscopic changes, that is, association and disassociation of its molecular strands. A change of macroscopic state such as gelatinization upon heating is significantly reflected in the change of microstructural properties of materials. Furthermore, functional characteristics such as solubility, swelling, water absorption capacity, gelatinization, and pasting provide useful insights into the diverse potential applications of the starches for industrial purposes (Hoover, 2001).

Few researches, however, on the characterization of starches separated from Korean rice cultivars have been reported, though Korean food industries use rice or rice starch for many kinds of food products such

as Korean traditional cake, snack, noodle, bread, drink, and candy. The objectives of this partial study were to investigate the morphological and physiochemical properties of starches from 10 Korean rice cultivars, and to understand the relationship between structure evolvement and gelatinization.

2. Materials and Methods

2.1. Materials

Ten rice cultivars, Goami (R1), Dongjin (R2), Seolgaeng (R3), Ilmi (R4), Hanmauem (R5), Hopyeong (R6), Hiami (R7), Deuraechan (R8), Boseokchal (W1), and Shinseonchal (W2), grown at the National Institute of Crop Science, Rural Development Administration, Korea, in 2009, were tested for the morphological, structural, and hydration properties of their starches. The cultivars from R1 to R8 are japonica-type non-glutinous rice and ranked according to amylose content, whereas cultivars W1 and W2 are waxy rice.

2.2. Isolation of rice starches

Each starch of 10 rice cultivars was separated by the alkaline

steeping method (Yamamoto, Sawada, & Onogaki, 1973). Rice grains were washed twice with tap water. After draining the water out, the soaked rice was crushed in a blender (HMF-1100; Hanil Electric, Seoul, Korea). The ground rice was allowed to settle in 0.05 M sodium hydroxide solution at about 25°C for 24 h. The supernatant was decanted off, and the process was repeated twice. The rice slurry was suspended in deionized water and pH was adjusted to 5.5–6.0 using 1 M HCl at 25°C to isolate rice starch. Subsequently, rice starch slurry was passed through a 100 mesh-sieve and followed by a 270 mesh-sieve. The starch thus obtained was filtered to discard water and was subsequently dried in an air flow drying oven at 15°C for 24 h. Finally, the starch cake was crushed by the blender and passed through a 100 mesh-sieve to obtain rice starch powder.

2.3. Scanning electron microscopy (SEM)

The starch samples isolated from 10 different rice cultivars were dehydrated using anhydrous ethanol to minimize moisture in the specimen for SEM. Then, the specimen was sputtered with gold for 30 s. The rice starch specimens were examined in a low vacuum scanning electron microscope (S-3500N, Hitachi Science Systems, Ltd., Ibaraki, Japan) under

an accelerating voltage of 15 kV and 3.0 nm resolution. The monochrome images of the rice starches were obtained at 65,000 x magnification.

2.4. Particle size analysis

The particle size of the rice starch was measured with a commercial particle size analyzer (DelsaNano C, Beckman Coulter, Fullerton, CA, USA). The starch sample was dispersed in distilled water (0.2 g/mL), exposed to an ultrasonicator for 1 min (VCX 750; Sonics & Materials, Inc., Newtown, CT, USA) in an ice bath, and poured into a polystyrene cuvette. The particle size measurement was run in triplicate and carried out at 25°C.

2.5. Amylose content measurement

Amylose content of rice starches was measured by performing the iodine test (Juliano, 1971). Briefly, 20 mg (dry weight) of starch powder was added to 10 mL of 0.5N KOH with constant stirring at 700 rpm for 30 min to induce alkali gelatinization. Further, distilled water was added to the starch suspension under stirring to a total volume of 100 mL. Ten milliliters of the diluted starch suspension was mixed with 5 mL of 0.1N HCl and 0.2 mL of

iodine solution (0.2% I_2 and 2% KI). Finally, the total volume was made up to 50 mL with distilled water. The suspension was allowed to stand for 20 min at 25°C to bring about a chromogenic reaction, and its absorbance was measured at 680 nm on a UV-spectrophotometer (8452A Hewlett-Packard; Agilent, Santa Clara, CA, USA). The amylose content of rice starches was quantified by interpolation of the absorbance values in the standard curve.

2.6. Determination of swelling power

Among the water hydration properties, the swelling power was determined by suspending 0.5 g of starch (dry sample weight) in 30 mL of distilled water at 25°C with stirring using a magnetic stirrer at 700 rpm for 30 min. The starch suspension was poured into a Falcon tube (50 mL), heated to 80°C with shaking in a water bath (60 shakings per min, SWB-55; HYSC, Seoul, Korea) for 30 min and then cooled to 25°C. The heated starch suspensions were centrifuged at 7,200 × *g* for 30 min to separate the supernatant and the sediment. Subsequently, the supernatant was dried at 105°C for 5 h in a hot air oven (OF-22G; Jeio Tech, Seoul, Korea). The weights of the sediment left in the tube (wet sediment weight) and of the dry supernatant (dry supernatant weight) were measured, and the swelling power

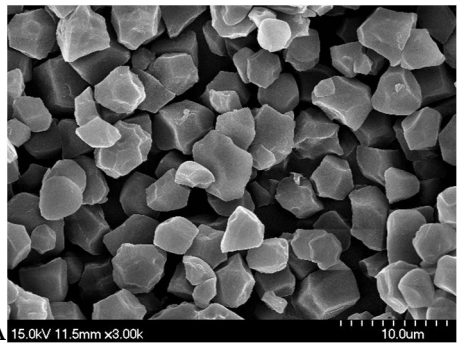
(SP) was calculated using the following equation:

$$Swelling\ Power\ (SP) = \frac{wet\ sediment\ weight}{dry\ sample\ weight \times \left(1 - \frac{dry\ supernatant\ weight}{dry\ sample\ weight}\right)}$$

3. Results and Discussion

3.1. Morphology of rice starch granules

Scanning electron micrographs showed the shape and size of the rice starch granules obtained from different rice cultivars; many polyhedral granules and several round granules were observed (Figure 2.1, Table 2.1). The morphology of all rice starches regardless of their cultivars was rather similar, indicating that all samples were congeneric. These observations were in agreement with earlier reports on the starch morphology of other rice cultivars (Lawal et al., 2011; Sodhi & Singh, 2003).

A 
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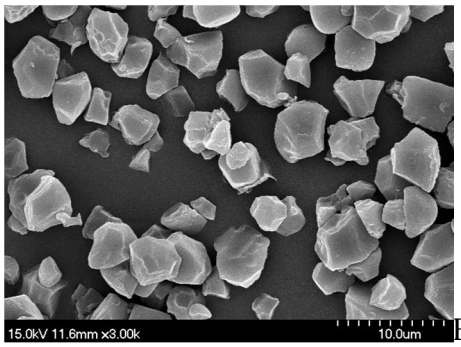
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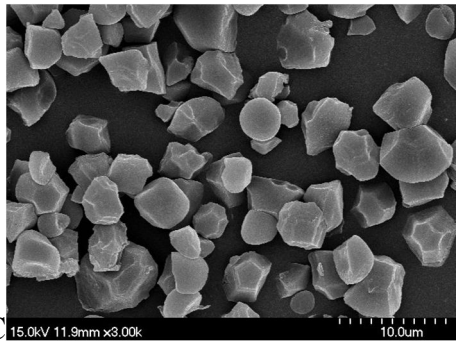
C 
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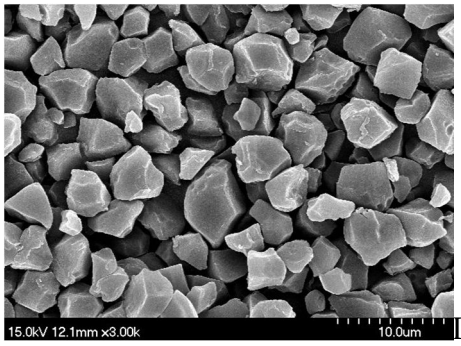
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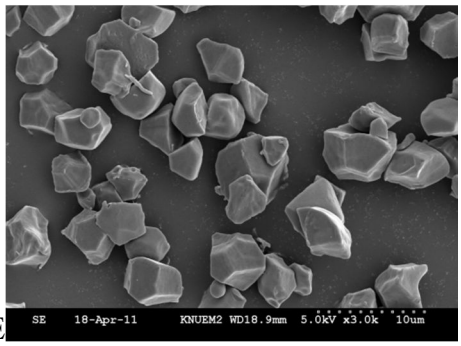
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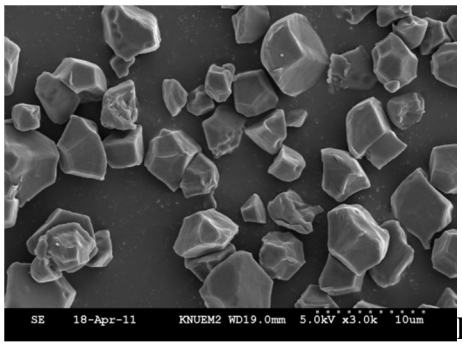
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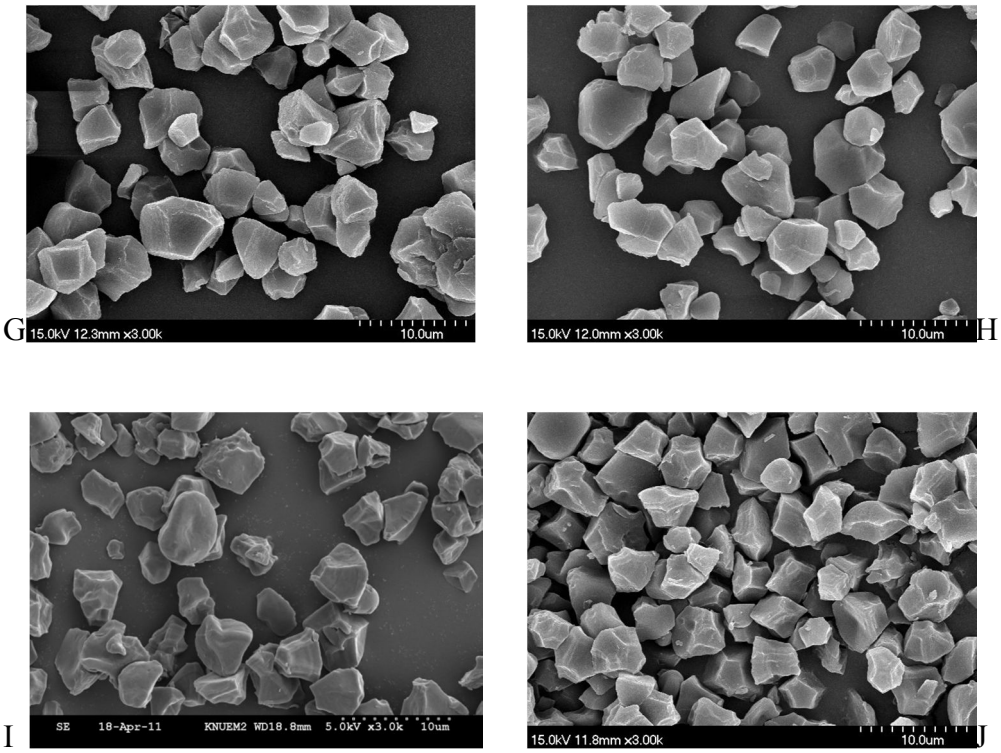

Figure 2.1 Scanning electron micrographs of the starches isolated from 10 different rice cultivars (A) R1-Goami, (B) R2-Dongjin, (C) R3-Seolgaeng, (D) R4-Ilmi, (E) R5-Hanmauem, (F) R6-Hopyeong, (G) R7-Hiami, (H) R8-Deuraechan, (I) W1-Boseokchal, and (J) W2-Shinseonchal.

Table 2.1 Amylose content, granule size and swelling power of the starches from different rice cultivars

Cultivars	Sample No.	Amylose content (%)	Granule size (μm)	Swelling power (%)
Goami	R1	29.4	2.45±0.10	7.70±0.50
Dongjin	R2	19.6	2.90±0.41	8.26±0.88
Seolgaeng	R3	19.3	3.78±1.69	9.20±0.80
Ilmi	R4	18.5	2.74±0.26	13.82±1.32
Hanmauem	R5	18.1	3.27±0.37	10.83±0.93
Hopyeong	R6	18.1	3.17±0.36	9.04±0.75
Hiami	R7	18.0	4.74±1.02	9.76±0.59
Deuraechan	R8	17.7	3.13±0.06	13.18±0.95
Boseokchal	W1	0	3.36±0.24	20.51±5.91
Shinseonchal	W2	0	2.87±0.46	22.76±5.92

3.2. Granule size

The particle size analysis revealed that the sizes of starch granules were considerably varied by the rice cultivars. R3, R7, and R8 showed relatively large granules. On the other hand, R1 and R4 consisted of a number of small polyhedral granules along with a few large granules. Waxy starches, W1 and W2, displayed a unique morphology in the micrographs: W2 starch showed granules of uniform size (2.87±0.46 μm), whereas W1 exhibited some large granules (3.36±0.24 μm) and many irregularly shaped small granules. It is difficult to correlate the amylose content to granule size of starches from 10 rice cultivars. The granule sizes ranged from 2.45 to 4.74 μm for all rice cultivars studied (Figure 2.2, Table 2.1). R7 showed the biggest granule size, and R1, which has the highest amylose content, showed the smallest granule size (2.45±0.10 μm). The granule sizes of R2–R8 were significantly different (2.74–4.74 μm) even though they had similar amylose contents (17.7–19.6%), which is one of the factors affecting processability of starches for food applications.

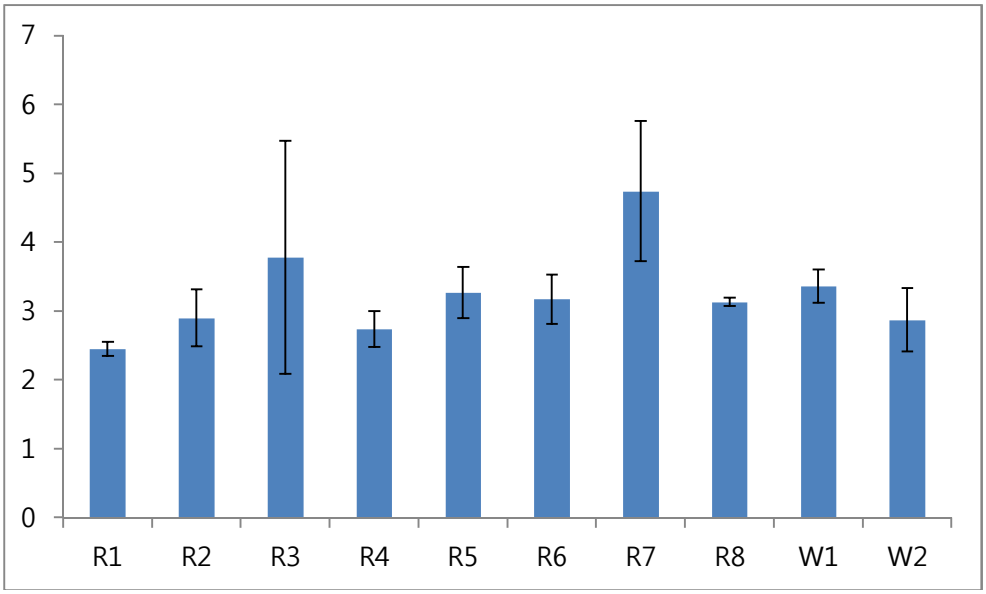

Figure 2.2 Granule size (μm) of the starches from 10 different rice cultivars

3.3. Amylose content

The amylose content of starches from different rice cultivars (Figure 2.3, Table 2.1) was found to be between 17.7% and 29.4% except in W1 and W2, which were derived from the waxy rice cultivars. Amongst the starch samples studied, R8 had the lowest amylose content (17.7%), whereas R1 had the highest starch content (29.4%). Cultivars R2–R8 had similar amylose contents that varied within a narrow range (19.6–17.7%).

The results for determination of amylose content in the starch samples showed that R1, R2–R8, and W1 and W2 were high-, medium-, and low-amylose rice cultivars, respectively. Based on the results, the different thermal and rheological properties from different rice cultivars with similar amylose content were examined. Furthermore, besides the amylose content, efforts were also taken to determine other properties that could be directly related to the processability for food applications. Although amylose content has been used to broadly define starch properties, sometimes it is hardly related to physicochemical properties of rice starch, flour, and processed products (Choi, 2002; Yoon, Koh, & Kang, 2009).

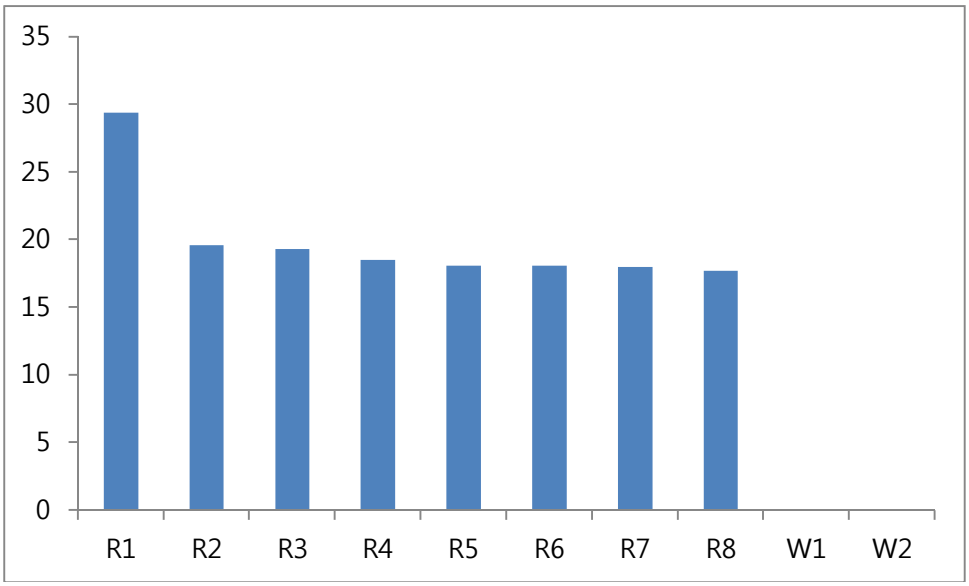

Figure 2.3 Amylose content (%) of the starches from 10 different rice cultivars

3.4. Swelling power

The swelling power of starches, which indicates its ability to hydrate and increase its weight due to wetting, was determined for different rice cultivars (Figure 2.4, Table 2.1). In general, the swelling power of rice starches is inversely proportional to its amylose content as amylopectin contributes to the swelling of starch granules, whereas amylose and lipids inhibit swelling (Morrison, Tester, Snape, Law, & Gidley, 1993; Tester & Morrison, 1990a, 1990b). In the present study, R1 contained the highest amylose content and showed the lowest swelling power (7.7 g/g starch), whereas starches having a low amylose content, like W1 (20.5 g/g starch) and W2 (22.8 g/g starch), displayed a relatively high swelling power. Amongst R2–R8, the higher the amylose content of the starch, the lower was the swelling power. The variation in swelling power may also be attributed to the difference in internal structure due to variable amylose content, lipid content, and distribution of branch chain-length of amylopectin of the starch granules (Sodhi & Singh, 2003)

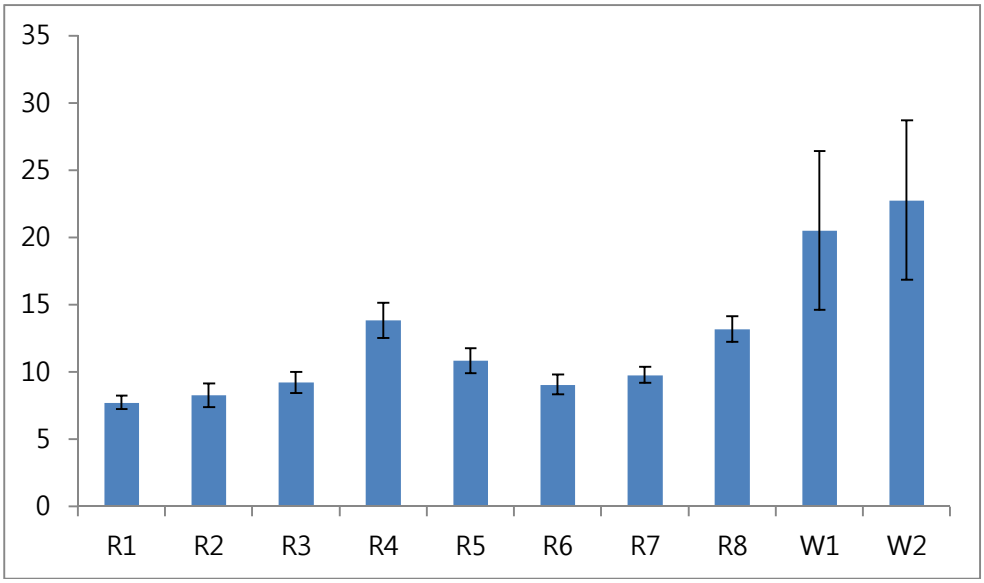

Figure 2.4 Swelling power (%) of the starches from 10 different rice cultivars

4. Conclusion

Rice flour, owing to the absence of gluten, is a popular candidate as a cereal source for gluten-free food products. However, replacing wheat flour with rice flour for a variety of food products has been difficult because the gluten plays an important role in forming desirable food matrix and texture. Thus, an understanding of the physicochemical properties and structure evolution upon heating of rice flour is necessary to expand its processability in food applications.

In this study, I investigated hydration and morphological properties of 10 different rice starches and compared the measure of amylose content to their processability for food applications. Amylose content could be fairly correlated to hydration properties of rice starches whereas it was insufficient in accounting for their morphological characteristics. The combined analysis of hydration and morphological data of starches is expected to be useful for gauging its processability in food applications.

A systematic analysis involving several parameters like hydration, pasting, viscoelastic, and thermal property data of rice starch will be helpful in fully understanding its behavior at ingredient level, dough and batter level, and product level.

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Chapter 3. Gelatinization properties of starches separated from different Korean rice cultivars

1. Introduction

Gelatinization of rice flour is the most important factor for its application in bakery and noodle industry, as gluten-free flour may form an improper or insufficient network for the products (Bhattacharya, Zee, & Corke, 1999; Hormdok & Noomhorm, 2007). Thus, the texture formation by interactions among starch molecules, which can be achieved only by gelatinization, is more significant for products made from rice flour compared to those made from wheat flour (Huang & Lai, 2010). Several reports have suggested that gelatinization is the compelling factor in the processing of starch-based food products and that involves the physicochemical and functional properties of starch (Ahmed, Ramaswamy, Ayad, & Alli, 2008; Copeland, Blazek, Salman, & Tang, 2009; Lee, Lee, Lee, & Ko, 2012; Lu, Duh, Lin, & Chang, 2008; Sasaki, Yasui, & Matsuki, 2000). The structure of the gel or paste is determined by the starch concentration and structure of swollen starch granules, the amount and type of amylose and amylopectin that leaches out from the granules, and heating conditions such as heating temperature, time, and rate (Genovese & Rao, 2003; Keetels, van Vliet, & Walstra, 1996a, 1996b; Lu et al., 2008).

In order to facilitate the industrial application of rice flour, there is a

need to investigate both physicochemical and gelatinization characteristics of rice starch. A wide variety of rice cultivars need to be studied as each rice cultivar possesses unique properties (Lee & We, 2012), including the size, shape, and ratio of amylose/amylopectin of the starches that are responsible for their processability, as they affect the physicochemical, thermal, and pasting characteristics (Christensen & Madsen, 1996; Li & Yeh, 2001; Singh, Singh, Kaur, Sodhi, & Gill, 2003). Particularly, macroscopic properties of rice starches strictly correspond to microscopic changes, that is, association and disassociation of its molecular strands. A change of macroscopic state such as gelatinization upon heating is significantly reflected in the change of microstructural properties of materials (Hoover, 2001). Furthermore, functional characteristics such as solubility, swelling, water absorption capacity, gelatinization, and pasting provide useful insights into the diverse potential applications of the starches for industrial purposes (Hoover, 2001). For investigating the relation of microstructure and macroscopic state of rice starch, the viscoelastic and pasting properties of rice starch could be analyzed with rapid visco analysis (RVA) and differential scanning calorimetry (DSC) (Hsu, Lu, & Huang, 2000; Lapasin, Pricl, & Tracanelli, 1992).

Few researches, however, on the characterization of starches separated from Korean rice cultivars have been reported though Korean food industries use rice or rice starch for many kinds of food products such as Korean traditional cake, snack, noodle, bread, drink, and candy. The objectives of this study were to investigate the viscoelastic, pasting, and thermal properties of starches from 10 Korean rice cultivars, and to understand the relationship between structure evolvement upon heating and physicochemical properties.

2. Materials and Methods

2.1. Materials

Ten rice cultivars, Goami (R1), Dongjin (R2), Seolgaeng (R3), Ilmi (R4), Hanmauem (R5), Hopyeong (R6), Hiami (R7), Deuraechan (R8), Boseokchal (W1), and Shinseonchal (W2), grown at the National Institute of Crop Science, Rural Development Administration, Korea, in 2009, were tested for the rheological, pasting and thermal properties of their starches. The cultivars from R1 to R8 are japonica-type non-glutinous rice and ranked according to amylose content, whereas cultivars W1 and W2 are waxy rice.

2.2. Isolation of rice starches

Each starch of 10 rice cultivars was separated by the alkaline steeping method (Yamamoto, Sawada, & Onogaki, 1973). Rice grains were washed twice with tap water. After draining the water out, the soaked rice was crushed in a blender (HMF-1100; Hanil Electric, Seoul, Korea). The ground rice was allowed to settle in 0.05 M sodium hydroxide solution at about 25°C for 24 h. The supernatant was decanted off, and the process was repeated twice. The rice slurry was suspended in deionized water and pH was adjusted to 5.5–6.0 using 1 M HCl at 25°C to isolate rice starch. Subsequently, rice starch slurry was passed through a 100 mesh-sieve followed by a 270 mesh-sieve. The starch thus obtained was filtered to discard water and was subsequently dried in an air flow drying oven at 15°C for 24 h. Finally, the starch cake was crushed using the blender and passed through a 100 mesh-sieve to obtain rice starch powder.

2.3. Measurement of viscoelastic characteristics

The viscoelastic properties of heat-treated rice starch suspensions

were measured using a small amplitude oscillatory shear measurement during heating and cooling treatments on a rheometer (AR 1500; TA Instruments Inc., New Castle, DE, USA). A parallel plate (PP) with a 20-mm diameter was attached to the rheometer and separated with a 1000-μm gap. Rice starch powder (0.2 g dry weight) was added to 1.4 mL of distilled water (starch:water = 1:7). A 0.335-mL aliquot of the rice suspension was loaded on the rheometer after which the PP geometry was moved downward and silicon oil was spread on the edge of the rice suspension sample to prevent loss of moisture during the rheological measurement. The starch suspension loaded on the rheometer was heated from 20°C to 90°C at the rate of 10°C/min and maintained at 90°C for 30 min. Elastic modulus (G′, or storage modulus), viscous modulus (G″, or loss modulus), and tan δ were measured at 1.0% strain and 1 Hz frequency.

2.4. Measurement of linear viscoelastic behavior

Upon curing the rice starch suspensions for the *in situ* measurement of their viscoelastic characteristics (as described in the earlier section), the temperature was decreased to 20°C at the rate of 10°C/min and maintained for 30 min to cool down the gel completely. The samples were further tested

on the rheometer under strain sweep to determine the linear elastic region. The samples were also subjected to stress sweep experiments at a constant frequency of 1 Hz. The strain applied for the strain sweep test ranged from 10^{-5} to 10^{2} Pa measured at logarithmic scale increments. During stress sweep, *in situ* elastic modulus (G′) was measured as a function of strain (γ). The limit of linearity or critical strain (γ_c), the end-point of the linear region, was determined when G′ deviates considerably from the original G′ value. The strain value was taken as a measure of the γ_c where G′ was 95% of the cooled G′ in the strain sweep experiment. All viscoelastic measurements were carried out in triplicate (TA Instruments Inc., New Castle, DE, USA).

2.5. Measurement of pasting properties

The effects of heating on the pasting properties of rice starch suspensions were measured using a rheometer equipped with a starch pasting cell (AR 1500ex; TA instrument, New Castle, DE, USA), which can be operated like a rapid visco-analyzer. Rice starch powder (3 g) was mixed with 25 mL of distilled water and equilibrated at 50°C for 1 min. The starch suspension loaded on the starch pasting cell of the rheometer was heated from 50°C to 95°C at the rate of 12°C/min, maintained at 95°C for 2 min,

and then cooled to 50°C at 12°C/min. During the measurement, pasting parameters such as peak (PV), trough (TV), final (FV), breakdown (BD, PV-TV), and setback (SB, FV-TV) viscosities, peak time, and pasting temperature (PT) were determined. All the measurements were carried out in triplicate.

2.6. Measurement of thermal properties

The thermal properties of rice starch suspensions were measured on a differential scanning calorimeter (DSC 200; Netzsch-Gerätebau GmbH, Selb, Germany). Rice starch powder (2.5 g) was added to distilled water (7.5 g), an aliquot (10 mg) of the suspension was placed in an aluminum pan for DSC measurement, and the pan was sealed tightly with a lid and allowed to stand for 1 h before the measurement. An empty pan with a lid was used as a reference during the measurement. Each starch suspension sample was heated from 30°C to 130°C at the rate of 10°C/min. Onset (To), peak (Tp), and completion (Tc) temperatures of the thermal transition were determined during temperature sweep in the DSC. Further, enthalpy was also calculated for the samples tested.

2.7. Statistical analysis

Linear viscoelastic behavior of the starches was statistically analyzed using the Duncan's multiple range tests (SAS, v. 9.1, SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Viscoelastic characteristics

Viscoelastic properties of the starch samples obtained from 10 rice cultivars are listed in Table 3.1. The rice starch suspensions were heated from 20 °C to 90 °C, which changed their form from a liquid-like suspension to a solid paste-like or gel during the SAOS measurement. The starches obtained from 10 rice cultivars showed different pasting behavior and consequently displayed different macroscopic textures. The kinetics of the heat-induced gelatinization of rice starch suspensions during *in situ* viscoelastic measurement showed that G′ values at or below 70°C were very close to zero (Figure 3.1) after which G′ increased rapidly, peaked, and then tended to decrease a little. This indicates that swollen starch molecules heated above

their gelatinization temperature lose crystallinity, but become gelatinized with an amorphous structure that can absorb water well. During gelatinization, G′ starts rising because of the formation of the amorphous network and reaches a maximum value on complete development of the network. The increase in G′ is attributed to the degree of granular swelling to accommodate the entire available volume of the system (Keetels & Van Vliet, 1994), and further inter-granule contact may also form a network of swollen granules (Evans & Haisman, 1980; Wong & Lelievre, 1982). Upon heating after the peak, the G′ decreases, which is known as the trough G′. The decrease in G′ is due to the melting of the remaining crystallites or loosening of hydrogen bonds at a high temperature, which leads to softening of swollen granules (Singh, Kaur, Sandhu, Kaur, & Nishinari, 2006). The shape of the G′ curve obtained from the SAOS measurement depended upon the rice cultivar. Rice starches from different cultivars presented different viscoelastic curves; their differing shapes are indicative of the onset of G′ increase and the degree of steepness during gelatinization.

The G′ peak values differed among the rice cultivars. R1 had the highest amylose content but showed the lowest G′ peak (849.2 Pa) at 90°C. The G′ peak values for R2 and R4 were 895.9 Pa at 90°C and 830.5 Pa at

88°C, respectively. Interestingly, as the amylose molecules hardly developed an amorphous structure, the G′ lowered. A decrease in the amylose content (indicating a corresponding increase in amylopectin content) may cause an increase in cross-linkable sites that could correspond to a higher G′ value. The highest G′ peak was obtained for R6, which was 1,493.0 Pa at 90°C. W1 and W2, being waxy starch, showed significantly different G′ peaks; there were adequate cross-linkable sites for gelatinization of W1 and W2 compared to non-glutinous starches. Particularly, R4, R5, R6, R7, and R8, having similar amylose contents at approximate 18%, showed different G′ peaks, which ranged from 830.5 Pa to 1,493.0 Pa. Interestingly, the onset of increase in G′ in R4, R5, R6, R7, and R8 showed an inverse correlation with their swelling power. The onset of increase in G′ reflects the onset of gelatinization at which internal networking commences. The starches isolated from different rice cultivars show a difference in the melting of the amylopectin strands inside the swollen starch granules, which may result in variable onset of increase in G′ and the G′ peak among the starches (Keetels & Van Vliet, 1994).

The G′ breakdown, the difference between G′ peak and G′ trough, was inversely well correlated with the amylose content of rice cultivars; thus,

the lower the amylose content, the higher the G′ breakdown value. R1 displayed the lowest G′ breakdown, whereas R2 and R3 showed the next lowest G′ breakdown among the rice cultivars. The similar amylose content in R4, R5, R7, and R8 was also reflected in the similar G′ breakdown ranging from 544.3 to 714.5. As anticipated, W1 and W2 displayed significant differences in their G′ breakdown compared to other non-glutinous starches. R6 showed the highest G′ breakdown, which was significantly higher than those from R4, R5, R7, and R8. The amylose content of the starches seems to be a major factor in the G′ breakdown, which agrees well with the fact that the G′ breakdown differs significantly in starches from various rice cultivars (Sodhi & Singh, 2003). However, like in the case of R6, the amylose content cannot fully explain the observed breakdown G′. The viscoelastic behavior of the rice starches could not be explained merely by consideration of individual factors such as amylose content, and other tests are required for further understanding, as combined factors in starch depending on its cultivar could influence these properties (Lee & We, 2012).

The rice starch pastes hardened upon cooling to 20°C, consequently raising their G′ values (Table 2). Further, the extent of this increase was

dependent on the origin of starches. G′ values upon cooling varied depending upon rice cultivars. R1 showed the highest G′ (2,176.0 Pa) when cooled, which has the highest amylose content among 10 rice starch samples. Remarkably, the G′ values for cooled R2 ~ R8 ranged 446.7 to 1335.3 Pa, which have lower amylose content (17.7 ~ 19.6%) than R1. This increase in G′ values upon cooling may probably be due to strengthening of the intra- and inter-molecular hydrogen bonds with the decrease in temperature (Aguilera, 1995; Eleya & Turgeon, 2000). Moreover, the increase in G′ upon cooling could be attributed to retrogradation of starch paste, related to the development of crystallinity in polymer-rich regions for the formation of starch paste network (Miles, Morris, & Ring, 1984).

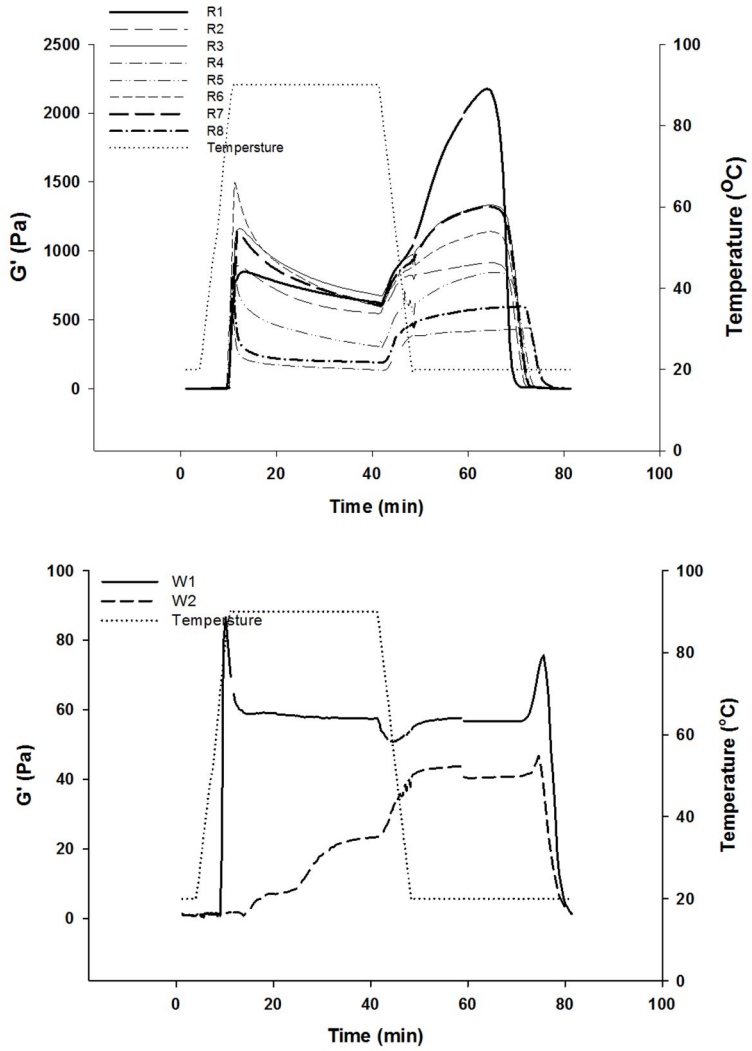

Figure 3.1 Heat-induced viscoelastic properties of the starches isolated from 10 different rice cultivars (R1-Goami, R2-Donjin, R3-Seolgaeng, R4-Ilmi, R5-Hanmauem, R6-Hopyeong, R7-Hiami, R8-Deuraechan, W1-Boseokchal, and W2-Shinseonchal). The starch suspensions were heated from 20 to 90℃ at 10℃/min and maintained at 90℃ for 30 min.

Table 3.1 Viscoelastic characteristics of the starches from different rice cultivars

	Gelatinization peak G' (Pa)	Gelatinization trough G' (Pa)	Breakdown G' (G'_P-G'_T)	G'_F-G'_P (Pa)	Retrogradation peak G' (Pa)
R1	849.2±19.9	615.9±10.9	233.3	1,326.8	2176±161.4
R2	895.9±72.3	545.5±33.6	350.4	21.0	916.9±73.6
R3	1164±64.0	671.9±22.0	492.1	171.3	1335.3±21.0
R4	830.5±74.4	132.1±7.8	698.4	-383.8	446.7±24.6
R5	916.0±30.9	300.1±46.8	615.8	-69.6	846.4±53.8
R6	1493±103.6	592.4±78.7	900.6	-351.3	1141.7±126.0
R7	1144±55.4	599.7±11.2	544.3	178.7	1322.7±35.6
R8	902.9±166.2	188.4±19.7	714.5	-303.1	599.7±57.9
W1	86.5±1.7	50.7±13.7	35.8	-9.3	77.2±11.1
W2	43.8±5.1	N/A	N/A	3.6	47.4±1.0

3.2. Linear viscoelastic behavior

The *in situ* SAOS measurements made during increasing shear stress to study the paste properties of their starches furnished the values pertaining to 95% G′ and γ_c for the starch pastes from different rice cultivars (Table 3.2). The G′ obtained from the rice starch pastes ranged from 37.4 to 2057 Pa at 25°C, and the magnitude remarkably depended on the starch varieties. The cooling greatly assisted in the increase of G′ as the bond strength of the hydrogen bonds among starch molecules increased with decreasing temperature.

Under small shear stress, G′ value remained almost constant and unchanged, while on increasing the shear stress G′ decreased dramatically at higher strain. The structure of the rice starch paste broke beyond γ_c with increasing shear stress, as the stress between the structural bonds at break point is higher than the adhesion or cohesion stress~~es~~ (van Vliet & Walstra, 1995); this lowered the strain energy in the starch paste and resulted in the collapse of the structure. The onset of decrease in the G′ value indicates the breaking of weak bonds within the starch paste network and a transition from a linear to a non-linear behavior (Shih, Shih, Kim, Liu, & Aksay, 1990). Under the small strain region, the structure of starch is practically unaltered

and returns to its original shape once the stress applied is removed. This range of stress-strain corresponds to the linear viscoelastic region. Above the limit of linearity, the shear stress is strong enough to bend, break, and reform the structural elements in the starch pastes. On applying larger shear stress to the starch pastes, the G′-strain relationship deviates from the linear viscoelastic region. Out of the linear viscoelastic region, the change in structure is irreversible and cross-links among molecules may collapse (Demirkesen, Mert, Sumnu, & Sahin, 2010).

R1 exhibited the lowest γ_c (brittle), whereas W1 and W2, the highest γ_c (rubber-like). The W1 and W2 pastes resisted relatively large strain changes, whereas R1 dropped suddenly upon resisting small amount of strain change. The γ_c for R2, R3, R5, R6, and R7 was found to be intermediate. It is therefore believed that amylose content in starch is the most valuable factor in controlling γ_c and G′ of the rice starch paste. The γ_c is a measure of the brittleness of the paste and gel as it indicates the strain above which the gel responds in a nonlinear fashion. Thus, gels showing high γ_c values can be considered rubbery, whereas they are brittle if γ_c is low. These G′-γ response correlations provide an indirect means to reflect upon the microstructure of rice starch paste. The γ under applying the shear stress brought out the

properties of the rice starch pastes due to the difference in the rice starch microstructure originating from different cultivars. The structure of the gelatinized starch pastes responds to small stresses like a viscoelastic solid. During increased shear stress, the network structure of the starch pastes becomes fully extended before breaking under the shear (Bueche & Halpin, 1964; Foegeding, 1992). Above the γ_c, the structure change is irreversible and cross-links in the gel structure may collapse or fracture. The limit of linearity is known to affect the structure (Eleya, Ko, & Gunasekaran, 2004; Shih et al., 1990).

Table 3.2 Linear viscoelastic behavior of the starches from different rice cultivars

	95% G' (Pa)	Critical strain
R1	2057±169.8[a]	0.035±0.005[c]
R2	872±77.3[d]	0.094±0.012[c]
R3	1274±30.1[b]	0.101±0.000[c]
R4	415±25.2[f]	1.109±0.200[c]
R5	807±51.2[d]	0.180±0.065[c]
R6	1087±121.7[c]	0.087±0.012[c]
R7	1249±35.2[b]	0.094±0.012[c]
R8	568±69.4[e]	0.879±0.137[c]
W1	60.4±14.6[g]	5.746±1.980[a]
W2	37.4±7.3[g]	2.661±1.173[b]

3.3. Pasting properties

Pasting properties of starches from 10 rice cultivars (Table 3.3) demonstrated that W1 starch had the highest PV (4.55 Pa) and BD (2.83 Pa) but the lowest PT (64.65°C). Although W1 and W2 starches' PV and BD values differed, PV and BD of non-waxy starches (R1 - R8) showed lower PV and BD values than waxy starches (W1 and W2). The PV (2.19 Pa) and BD (0.75 Pa) of R1, which has the highest amylose content, was the lowest among 10 rice cultivars. These results agreed with those of previous studies that had reported that larger amylose content in rice starch correlated with lower PV and BD (Reddy, Subramanian, Zakuiddin Ali, & Bhattacharya, 1994; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003).

And W1 and W2 starches had relatively lower FV and SB than those of R1–R8 starches. The highest FV (3.85 Pa) and SB (2.38 Pa) were observed in R1 starch among 10 rice cultivars. The increase of SB and FV values may cause the formation of amylose junction zones (Jane et al., 1999). Especially, FV increase of the starch paste is resulted from cooling, which enhances the intermolecular hydrogen bonds and the aggregation of the amylose molecules. From the previous studies, amylose content and the distribution of amylopectin branch chain are major factors in determining the

pasting properties of starch. In addition, high SB of the starch may be due to the amount and the molecular weight of amylose leached from the granules and the remnants of the gelatinized starch (Karim, Norziah, & Seow, 2000; Mervyn J Miles, Morris, Orford, & Ring, 1985; Loh, 1992). In this study, the highest amylose content was related to the highest FV and SB, whereas the higher amylopectin (waxy starch) was linked to the lowest FV and SB.

In R2–R8 starches, which contained similar amylose contents (17.7–19.6%), the pasting parameters were not proportional to amylose content but differed from one another. The PV values of R4 (4.25 Pa) and R8 (4.13 Pa) starches were similar even though their amylose contents (18.5% and 17.7%, respectively) were significantly different. On the contrary, R5, R6, and R7 starches having approximately the same amylose content (18.0 to 18.1%) showed different PV values; the PV value of R7 (3.25 Pa) was relatively lower than that of R5 (3.39 Pa) and R6 (3.39 Pa). Moreover, the PV values of R5, R6, and R7 starches were much lower than those of R4 and R8. In starches with similar amylose content, PV may be more dependent on the amylose density (the ratio of amylose content to granule size) or granule size of starch. Starch having a high amylose density showed high BD and SB values which may occur due to leaching of amylose fragments (soluble

amylose) from the starch granule upon heating by the breakdown of α-1,4 linkages in amylose during gelatinization.

The results showed that a combination of amylose content, amylose density, and pasting properties may be useful in explaining the processing properties of starches as compared to the amylose content alone. I propose that amylose content is insufficient in completely explaining the pasting properties of starches and an understanding of amylose density instead may be more effective in evaluating pasting properties of starch molecules for industrial applications.

Table 3.3 Pasting properties of the starches from different rice cultivars

	PT(°C)	PV(Pa)	TV(Pa)	BD(Pa)	FV(Pa)	SB(Pa)
R1	70.60±0.28	2.19±0.06	1.45±0.01	0.75±0.05	3.85±0.01	2.38±0.02
R2	70.30±0.28	3.17±0.00	1.56±0.05	1.60±0.05	3.19±0.17	1.67±0.12
R3	69.50±0.28	3.23±0.05	1.31±0.02	1.92±0.03	2.41±0.04	1.13±0.02
R4	69.35±0.07	4.25±0.00	1.52±0.00	2.74±0.00	3.25±0.04	1.66±0.03
R5	68.90±0.00	3.39±0.06	1.12±0.06	2.27±0.00	2.43±0.06	1.26±0.01
R6	69.50±0.28	3.39±0.02	1.37±0.02	2.03±0.05	2.49±0.02	1.12±0.00
R7	68.85±0.64	3.25±0.02	1.30±0.02	1.95±0.07	2.41±0.08	1.17±0.00
R8	69.40±0.00	4.13±0.04	1.36±0.04	2.78±0.13	2.83±0.10	1.37±0.14
W1	64.65±0.35	4.55±0.04	1.73±0.04	2.83±0.07	2.66±0.08	0.84±0.05
W2	68.00±0.28	3.61±0.01	1.52±0.01	2.09±0.09	2.24±0.05	0.75±0.04

PT, pasting temperature (°C); PV, peak viscosity (Pa); TV, trough viscosity (Pa); BD, break down (PV-TV)(Pa); FV, final viscosity(Pa); SB, setback(Pa)

3.4. Thermal properties

The results of thermal analysis of the starches isolated from the rice cultivars are summarized in Table 3.4. The thermal parameters, To, Tc, Tp and ΔH of the starches depended significantly on the rice cultivars. R1 sample with highest amylose content showed the lowest To and W2 (waxy starch) displayed the highest To. Similar to previous study, waxy starch (W1 and W2) showed the highest △H, whereas △H was the lowest in R1 sample. Meanwhile, the R5 and R6 starches having approximately the same amylose content showed considerably different Tc (77.1°C and 74.2°C, respectively) and ΔH values (10.9 and 7.1 J/g, respectively). Whereas, also, R1 and R6 starch showed similar ΔH, the amylose content of R1 (29.4%) and R6 (18.1%) starch varied to a fair extent. In conclusion, amylose content did not correlate with any thermal parameter obtained by DSC analysis. The result concurred with earlier reports that the correlation between amylose content and thermal characteristics of starches was very weak (Noda, Tohnooka, Taya, & Suda, 2001; Singh et al., 2006).

Although amylose content cannot be correlated to the thermal properties of the rice starches, waxy W1 and W2 showed unique thermal

characteristics, like relatively higher ΔH values, compared to non-glutinous rice starches. ΔH showed a strong correlation with the transition temperature (To, Tc, Tp), implying that ΔH was influenced by the crystallinity of the starch (Singh et al., 2006). Furthermore, W2 showed the highest To, Tc, and Tp values. In conclusion, amylopectin may contribute to structural stability upon heating. High To, Tc and Tp values result from a high degree of crystallinity in starch which provides the thermal stability thus rendering the granule more resistant to gelatinization (Barichello, Yada, Coffin, & Stanley, 1990). Therefore, the rice starches from different cultivars have different crystalline structure, which is influenced by the chain length of amylopectin, molecular order (double-helical structure), and crystallinity. Notably, the crystalline structures of W1 and W2 starches were relatively heat-resistant during thermal analysis.

Table 3.4 Thermal properties of the starches from different rice cultivars

	To (°C)	Tc (°C)	ΔT(Tc-To) (°C)	Tp (°C)	ΔH (J/g)
R1	58.8±0.3	71.8±0.7	13.3	64.8±0.2	6.5±0.5
R2	61.5±0.1	75.6±0.3	14.3	68.8±0.6	8.3±0.3
R3	62.5±0.1	74.4±0.5	11.4	67.9±0.2	10.0±1.3
R4	62.9±0.3	75.6±0.8	13.3	69.3±0.4	10.4±1.5
R5	63.8±0.3	77.1±0.2	13.3	69.9±0.4	10.9±1.1
R6	61.5±0.2	74.2±0.9	13.5	68.1±0.8	7.1±0.5
R7	61.2±0.1	74.6±0.5	13.3	68.5±0.2	7.6±0.5
R8	62.3±0.4	75.6±0.8	13.8	68.8±0.5	11.9±2.3
W1	60.4±0.3	76.3±0.3	15.6	67.2±0.2	12.6±0.9
W2	64.2±0.1	82.4±2.3	18.6	70.9±0.2	13.7±1.7

To, onset temperature (°C); Tc, conclusion temperature (°C); Tp, peak temperature (°C); ΔH, enthalpy of gelatinization (J/g)

4. Conclusion

Rice flour, owing to the absence of gluten, is a popular candidate as a cereal source for gluten-free food products. Replacing wheat flour with rice flour for a variety of food products has been difficult because desirable food matrix and texture is strongly related to gluten. Thus, an understanding of the physicochemical properties and structure evolution upon heating of rice flour is necessary to expand its processability in food applications. Further, such gelatinization of rice flour is important, as it is the only way to evolve structure.

In the current studies (Chapter II and Chapter III), I investigated morphological, hydration, viscoelastic, pasting, and thermal properties of 10 different rice starches and compared the amylose content to their processability for food applications. Amylose content could be correlated to hydration (Pearson correlation coefficient (PC): -0.9269) and pasting properties (PC: 0.8514) of rice starches, whereas it was insufficient in accounting for their viscoelastic and thermal characteristics (PC: -0.3745) (Table 3.5). The combined analysis of hydration, viscoelastic, pasting, and thermal data of starches is expected to be useful for gauging its processability in food applications (Figure 3.2-3.9).

Through the systematic analysis, the deeper understanding of rice starch behavior at ingredient level, dough and batter level, and product level may be provided and expand its practical use in industry. In addition to these integrated analyses, these properties could also serve in selecting the best cultivar fit for a particular food type such as bakery and noodle products.

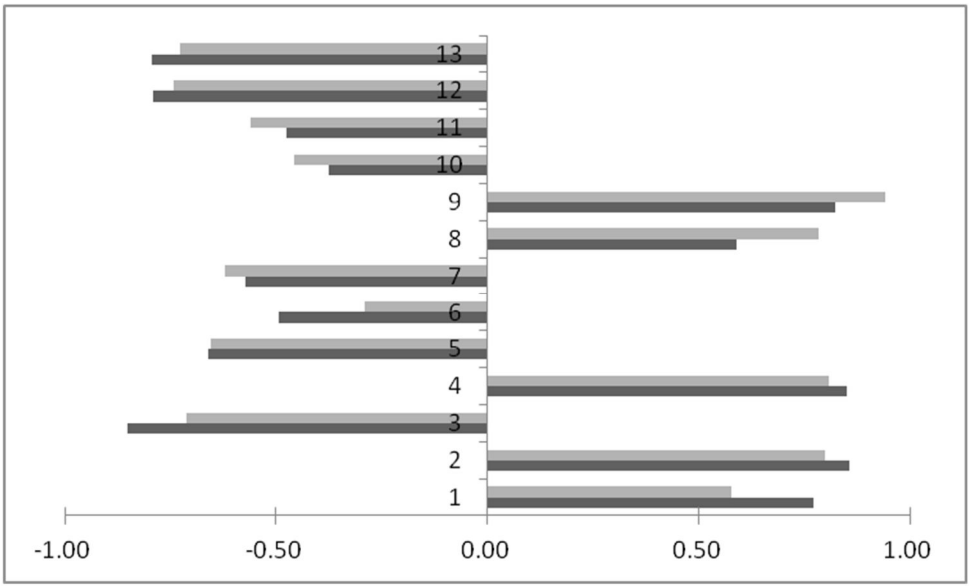

Figure 3.2 Comparison between amylose content and the ratio of amylose content to granule size by Pearson correlation coefficient

Dark grey bar : Pearson correlation coefficient with amylose content
Light grey bar : Pearson correlation coefficient with the ratio of amylose content to granule size
1. Gelatinization peak G', 2. Retrogradation peak G', 3. Critical strain, 4. Peak temperature, 5. Peak viscosity, 6. Though viscosity, 7. Breakdown, 8. Final viscosity, 9. Setback, 10. Onset temperature, 11. Close temperature, 12. Peak temperature, 13. Gelatinization enthalpy

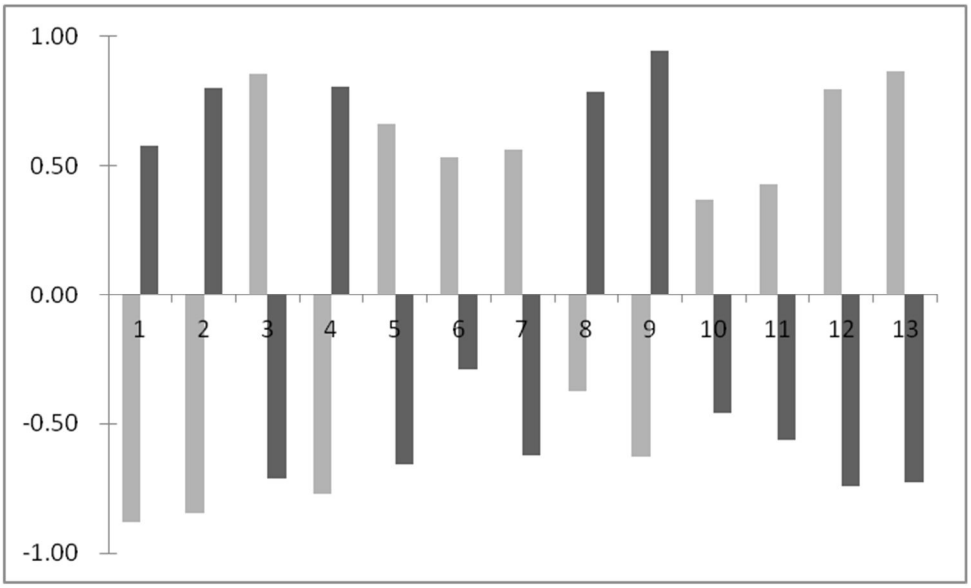

Figure 3.3 Comparison between swelling power and the ratio of amylose content to granule size by Pearson correlation coefficient

Dark grey bar : Pearson correlation coefficient with the ratio of amylose content to granule size

Light grey bar : Pearson correlation coefficient with swelling power

1. Gelatinization peak G', 2. Retrogradation peak G', 3. Critical strain, 4. Peak temperature, 5. Peak viscosity, 6. Though viscosity, 7. Breakdown, 8. Final viscosity, 9. Setback, 10. Onset temperature, 11. Close temperature, 12. Peak temperature, 13. Gelatinization enthalpy

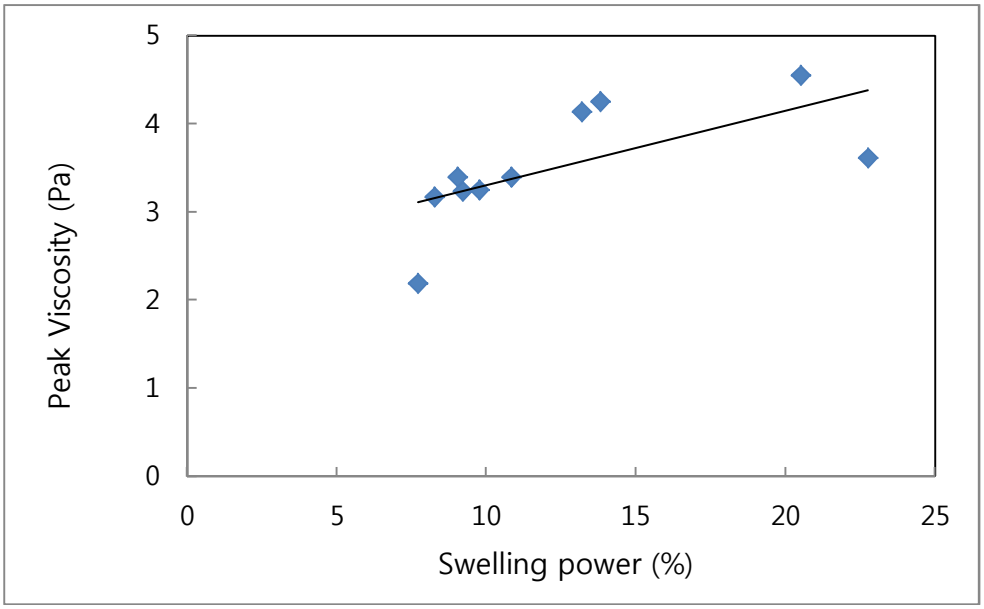

Figure 3.4 Relationship between swelling power and peak viscosity

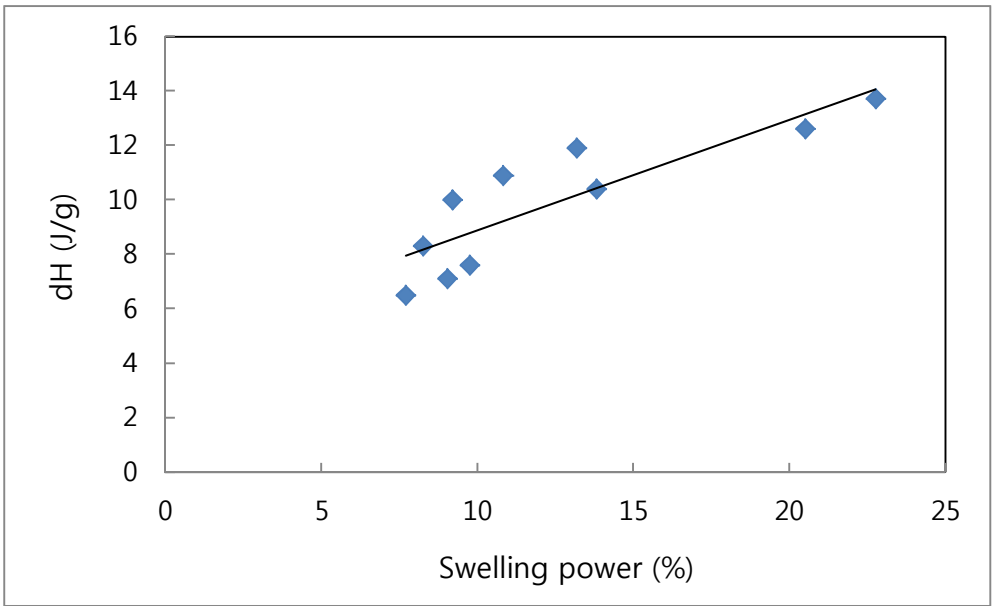

Figure 3.5 Relationship between swelling power and ΔH

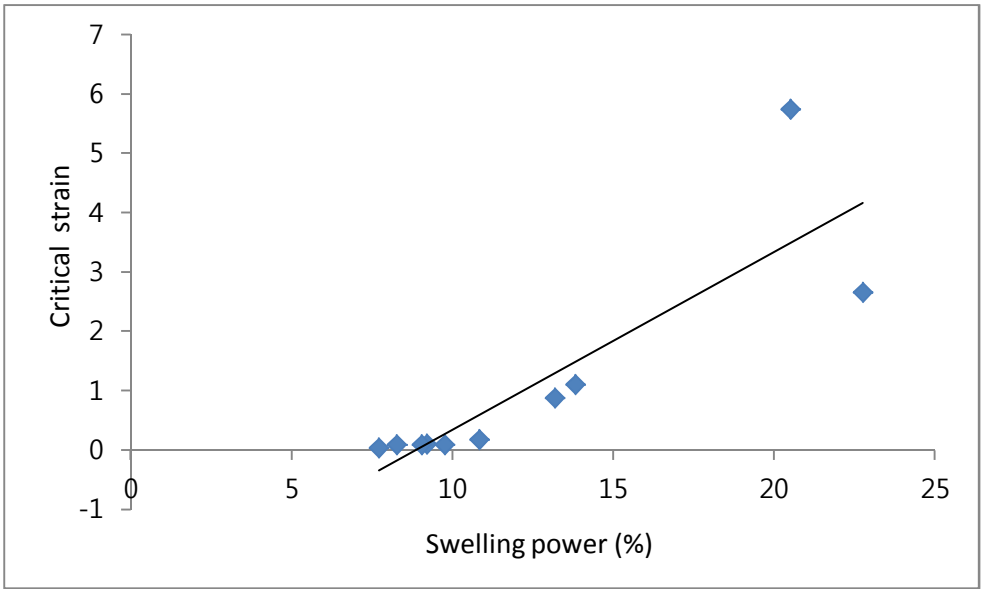

Figure 3.6 Relationship between swelling power and critical strain

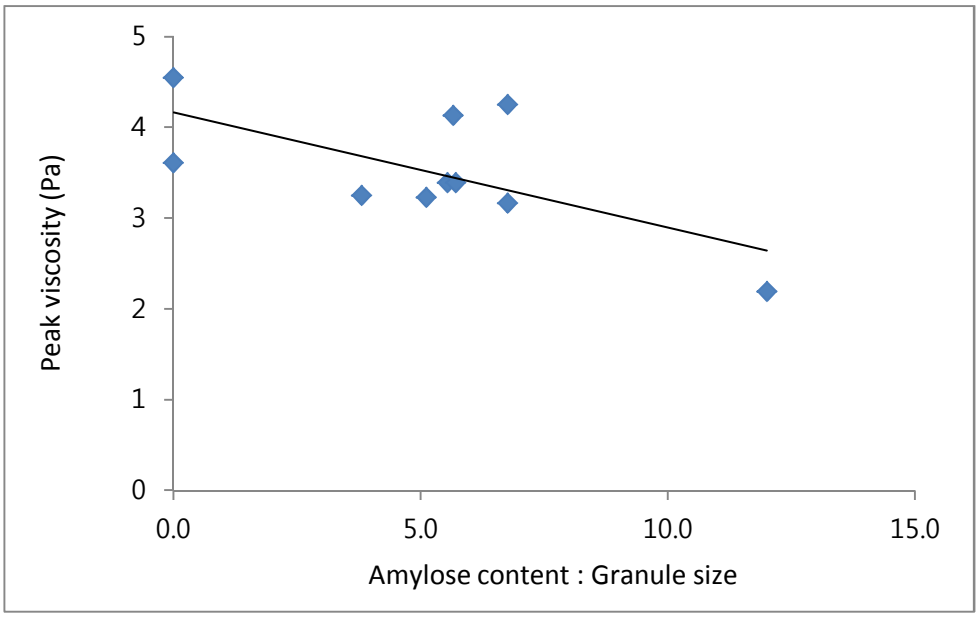

Figure 3.7 Relationship between the ratio of amylose content to granule size and peak viscosity

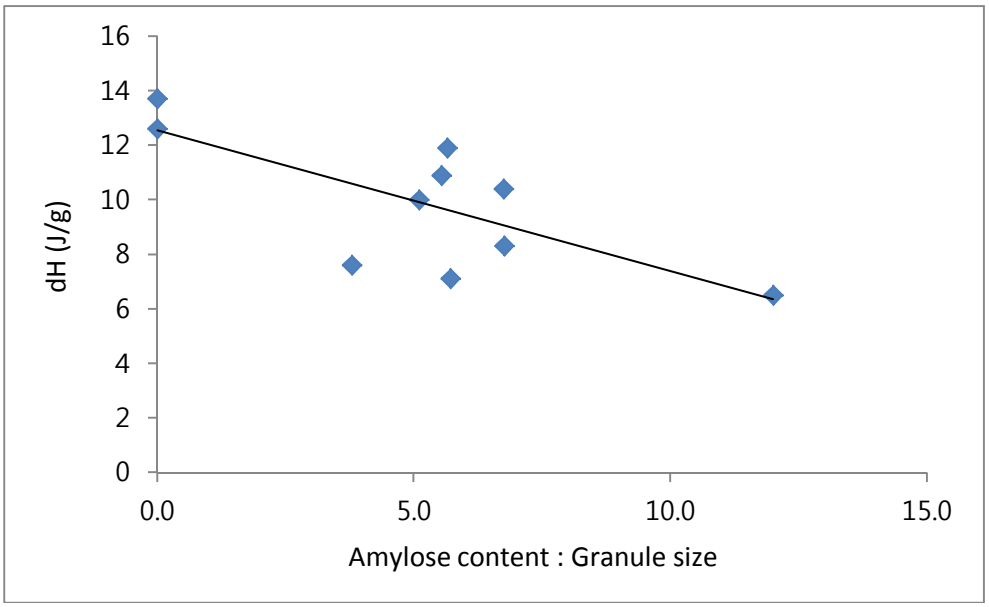

Figure 3.8 Relationship between the ratio of amylose content to granule size and dH

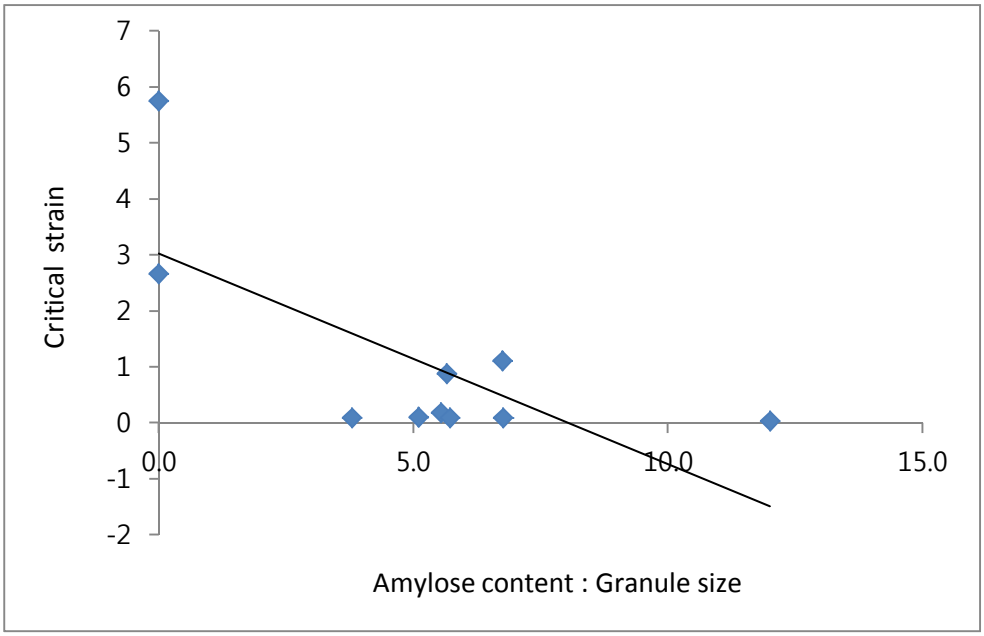

Figure 3.9 Relationship between the ratio of amylose content to granule size and critical strain

Table 3.5 Pearson correlation coefficients for the physicochemical, rheological and pasting and thermal properties of starches separated from 10 different rice cultivars

	AC(%)	GS(μm)	AC:GS	SP(%)	G-P G' (Pa)	R-P G' (Pa)	CS	PT(°C)	PV(Pa)	TV(Pa)	BD(Pa)	FV(Pa)	SB(Pa)	To(°C)	Tc(°C)	Tp(°C)
GS	-0.0871															
SP	-0.9269	-0.1338	-0.7995													
G-P G'	0.7716	0.2927	0.5771	-0.8818												
R-P G'	0.8549	0.0840	0.7976	-0.8450	0.6544											
CS	-0.8521	-0.0779	-0.7113	0.8552	-0.8315	-0.7075										
PT	0.8514	-0.2156	0.8068	-0.7715	0.6817	0.6709	-0.9363									
PV	-0.6618	0.0701	-0.6561	0.6582	-0.4099	-0.8727	0.6678	-0.6781								
TV	-0.4949	-0.3671	-0.2891	0.5299	-0.6335	-0.4270	0.7145	-0.4866	0.3763							
BD	-0.5723	0.1709	-0.6203	0.5612	-0.2667	-0.8157	0.5229	-0.5946	0.9684	0.1337						
FV	0.5878	-0.6088	0.7832	-0.3724	0.0499	0.4517	-0.1916	0.4271	-0.3502	0.3185	-0.4566					
SB	0.8228	-0.4734	0.9413	-0.6263	0.3133	0.6827	-0.5144	0.6763	-0.5833	-0.0383	-0.6104	0.9285				
To	-0.3745	0.0399	-0.4566	0.3703	-0.1337	-0.5900	-0.0376	-0.0301	0.3843	-0.3436	0.4989	-0.6233	-0.5421			
Tc	-0.4742	0.1149	-0.5615	0.4262	-0.1931	-0.6882	0.0370	-0.1052	0.4526	-0.1993	0.5319	-0.6300	-0.5918	0.9361		
Tp	-0.7907	-0.0909	-0.7436	0.7965	-0.6692	-0.7891	0.4470	-0.4163	0.4228	0.1550	0.4058	-0.5684	-0.6589	0.7531	0.8126	
dH	-0.7937	-0.1053	-0.7276	0.8637	-0.7490	-0.8626	0.6781	-0.6312	0.7157	0.2470	0.6972	-0.4507	-0.6239	0.6177	0.5822	0.7970

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Chapter 4. Heat-induced gelation properties of β-lactoglobulin

1. Introduction

Globular proteins play a major role as viscosity enhancer and stabilizer in food applications. Formula of ingredients and processing conditions are designed to obtain desirable gel characteristics upon heating for every specific process. Therefore, the understanding and control of the gelation properties is a key for a variety of utilization of globular proteins.

The gelation of globular proteins is an irreversible thermal process starting from the unfolding of protein molecules to the development of a three-dimensional intermolecular matrix structure. The intermolecular aggregation and matrix development influence gel properties including gelation temperature. Appearance, texture, and gelation temperature of heat-induced protein gels are strongly influenced by the gel microstructure. Microscopic study is necessary to understand macroscopic properties during sol-gel transition of aqueous globular proteins (Emmons et al., 1980; Holcomb, 1991).

The gelation of β-lactoglobulin (BLG) involves different types of interactions such as disulfide bonds, hydrogen bonds, and hydrophobic interactions, which depend on pH, ionic strength, and protein concentration (Aguilera, 1995; Kinsella and Whitehead, 1989; Ziegler and Foegeding,

1990; Petit et al., 2012). The gelation mechanism of globular proteins such as BLG is generally considered a three-step process characterized by 1) the unfolding of protein molecules with a concomitant exposure of buried –SH and hydrophobic groups, 2) the aggregation of denatured protein molecules, and 3) the formation of a three-dimensional network. The microstructural morphology of BLG molecules shows significant changes upon heating depending on BLG content, pH, and NaCl concentration (Ziegler and Foegeding, 1990; Petit et al., 2012).

Determining parameters to represent the gelation behavior is important in understanding structure evolution of protein gels for various food applications. Rheological techniques such as dynamic oscillatory shear test can probe *in situ* changes in rheological properties of globular protein systems upon heating. The physical and functional properties strictly correspond to microscopic changes, that is, association and disassociation of molecular strands. Rheological measurements provide indirect information on the structure of protein clusters upon heating. A state change such as sol-gel transition is significantly reflected in the change of rheological properties. The changes of rheological properties can be measured without interrupting structure development of protein gels upon heating. Dynamic rheological

properties, such as elastic modulus (G′) and viscous modulus (G″), obtained from the rheological measurement are used to characterize the gel properties. The evolution of G′ and G″ moduli as a function of gelation time or temperature can be used to probe structure development (Stading and Hermansson, 1991).

In this study, BLG, one of globular proteins and primary gelling agent in milk, was selected as a model protein. BLG gelation is related to both non-covalent and covalent protein-protein interactions such as hydrogen bonds, hydrophobic and electrostatic interactions, and disulfide (-SS-) bonds (Bezrukov, 1979; Schmidt, 1981). Thus, the BLG gelation is affected mainly by pH, ionic strength, and protein concentration. Since gelation conditions affect conformation and interactions of BLG molecules, they influence rheological characteristics and concomitant texture. Different gelation conditions induce dissimilarity in structure evolution. Alternatively, the gel structures and rheological properties of heat-induced BLG gels can be controlled by varying gelation conditions which is helpful for food applications such as viscosity enhancer and stabilizer. It is important to understand the dependence of protein concentration and temperature as well as other factors such as pH and ionic strength on developing gel structure.

Little attention has been focused on the combined effect of factors in the preparation conditions.

The objectives of this study were to investigate the gelation characteristics and the affecting factors using the BLG system at various gelation conditions: pH (3.5, 5.5 and 6.8), protein concentration (10%), and salt concentration (NaCl 0.2 M). Gelation temperatures of BLG were determined using the Hill function and their correspondences to various gelation conditions were studied.

2. Materials and Methods

2.1. Preparation of BLG solutions

Bovine BLG powder (BioPURE, Davisco Foods International Inc., Eden Prairie, MN, USA) composed of 97.4% protein, 0.1% fat, and 2.4% ash on dry basis was used in this study. Purity of BLG was 95.0% in protein content. BLG powder was added up to 10% (w/v) in distilled water containing 0.1 M NaCl, and then stirred for two hours. The BLG aqueous solutions were stored at 4ºC overnight for the complete hydration of the BLG molecules. Prior to experiments, the BLG solutions were warmed up at room temperature. The pH was adjusted to 3.5, 5.5, and 6.8 using 1 M HCl or NaOH.

2.2. Rheological test

The rheological test was applied to probe gelation process of BLG systems prepared at various conditions using a dynamic controlled-stress rheometer (Bohlin C-VOR, Bohlin Instruments Inc., East Brunswick, NJ, USA) which includes a heating/cooling stage. BLG solutions prepared at different conditions were poured directly on the measuring stage of the rheometer. The solution was covered with a thin film of paraffin oil to avoid

evaporation during the measurement. A 20-mm parallel-plate geometry with a 1-mm gap between the plates was used. Gels were formed *in situ* by allowing samples to cure from 25 to 100 °C at 1 ºC/min heating rate. Upon heating, *in situ* rheological parameters such as G′ and G″ were measured as a function of temperature at 1 Hz and a maximum target strain of 0.01. The strain value was checked over time and variations were less than 5% of the target strain. The tests were triplicated for each set of gel preparation conditions.

2.3. Curve fitting

Non-linear curve fitting was carried out using Sigma Plot (version 8.0, Systat Software Inc., San Jose, CA, USA). The non-linear regression was performed based on the Hill model.

3. Results and Discussion

3.1. Gel formation

Rheological properties of BLG systems heated from 25 to 100 °C changed from a liquid-like to solid-like status during the SAOS measurement. Different formulations of the gel system led to different types of gels with different macroscopic appearances and textures. Figure 4.1 shows usual gel formation kinetics during BLG gelation. The typical G′ and G″ response of the BLG with temperature during gelation was observed. The G′ and G″ values at below 80 °C were very close to zero. Thereafter, G′ rapidly increased and then reached a plateau. It wai explained that BLG solution was already structured well before the gelation temperature (Tobitani and Ross-Murphy, 1997a). As the temperature increased, G′ became much higher than G″, and tan δ was about 0.1 meaning that the system is very elastic. The linkage of disulfide bonds may be involved (Cheftel *et al.*, 1985).

When globular protein molecules are heated over the denaturation temperature, the molecules become unfolded, substantially exposing internal -SH and hydrophobic groups. The molecules then aggregate to form -SS- bonds and to reduce the exposure of hydrophobic groups to the outside

aqueous environment. During heating, G′ starts to increase because of the development of the protein network. As the protein molecules are heated enough, G′ value of protein solution becomes stable indicating the network is saturated. The status of aggregation and network depends on the conditions such as pH, and hydrophobic interaction of the protein molecules.

Figure 4.2 shows *in situ* G′ vs. temperature profiles of the gels with different pH conditions. The rheological curve shows different G'_{max}. The rheological parameters indicate that there are different microstructures in BLG system. These results explain that the gels behaved as elastic materials with the G′ higher than G″.

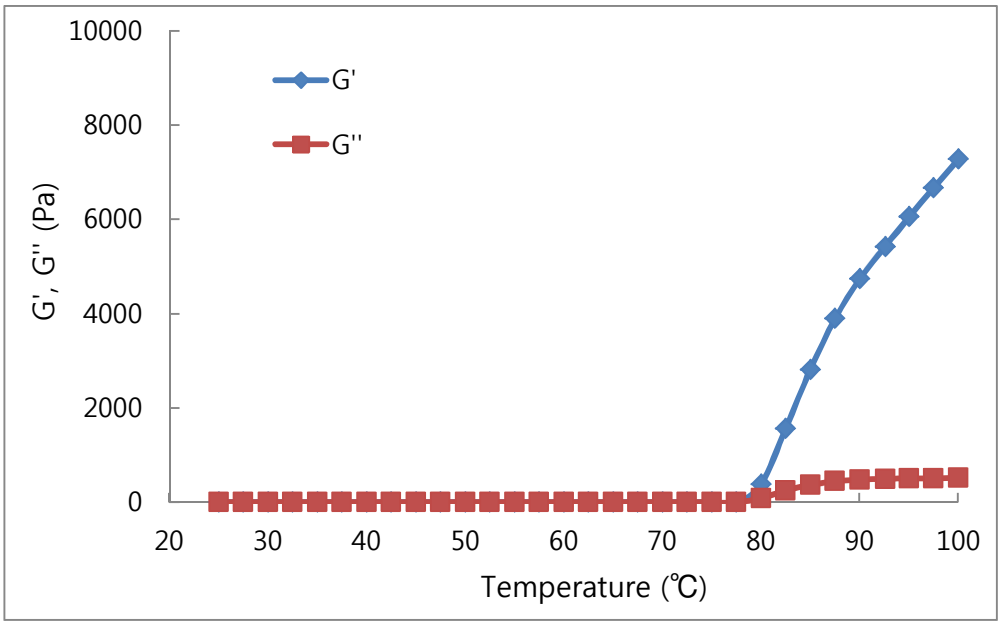

Figure 4.1 Typical elastic (G′) and viscous (G″) modulus response during gelation of BLG heated at 2.5 ℃/min from 25 to 100 ℃ (BLG 10%, NaCl 0.1 M, pH 6.8)

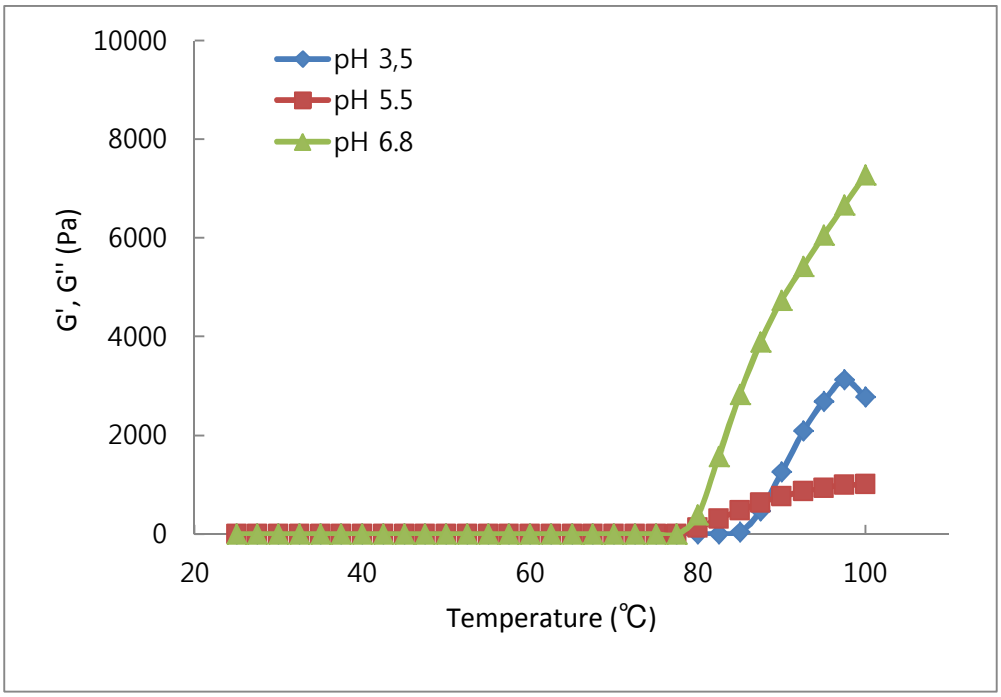

Figure 4.2 Typical elastic (G′) modulus response during gelation of BLG heated at 2.5 ℃/min from 25 to 100 ℃ (pH 3.5, pH 5.5, pH 6.8)

3.2. Effect of pH

The *in situ* SAOS measurements with increasing shear stress were applied to investigate the effect of pH and its contribution to the gel properties. It showed that the G′ and γ of BLG gels depended considerably upon pH of BLG solution. The fine-stranded or particulate gels formed at pH far from pI or at near pI, respectively, and their rheological characteristics were very distinctive. The G′ and γ under applying the shear stress reflected the properties of the BLG gels, which indicate the difference of the gel's microstructure resulting from the different gelation conditions.

In general, the G′ at pH 3.5 or 6.8 was higher than that at pH 5.5 (Figure 4.3-4.7, Table 4.1). High electrostatic repulsion may lead to high G′ values on the gels. The pH which decides the degree of electrostatic repulsion during the gelation is one of the most critical factors for the heat-induced gelation of BLG so that gel's rheological properties and microstructure are influenced by it. Among the gels established at pH 3.5 and 6.8 far from pI, G′ at pH 6.8 was higher than that at pH 3.5. This is an evidence that the characteristics of the gels prepared at pH 3.5 differed from those at pH 6.8 even though both gels were established at the high electrostatic repulsion. High electrostatic repulsion (pH 3.5 or 6.8) seemed to

form a high elastic gel whose microstructure differed from that made under less electrostatic repulsive condition. It is known that when pH is far from pI, aggregation of proteins is reduced because of high repulsion of equally charged molecules, leading to an ordered and translucent gel matrix (Stading and Hermansson, 1991; Foegeding et al., 1995). On the contrary, the pH close to pI results in high density network of protein aggregates due to differently charged protein molecules.

The ratio of $G'_{cooling}$ to G'_{curing} at pH 3.5 and 6.8 was smaller than that at pH 5.5 (Figure 4.3-4.7, Table 4.1). At pH 3.5 and 6.8, the developed gel matrix had sufficient G′ value relatively less affected during cooling. At high electrostatic repulsions, the cooling effect was weak since the gel already formed through heat-induced gelation process because of cross-links formed via sulfhydryl-disulfide (SH-SS) exchange interactions. However, at pH 5.5 the G′ values increased dramatically after cooling. At pH 5.5, BLG gels are usually formed via hydrogen bonds and hydrophobic protein-protein interactions. The cooling seems to affect the bonds, especially hydrogen bonds among protein molecules (Aguilera, 1995; McClements and Keogh, 1995; Eleya and Turgeon, 2000; Yang et al., 2004). During the cooling, the effect of hydrogen bonds in the gel matrix gradually increased, especially at

low temperature, so G′ increased. The G′ increased on cooling, which indicates that non-hydrophobic forces such as hydrogen bonds are important in developing the final gel texture at less electrostatic repulsions via hydrophobic interactions.

The γ_c at pH 6.8 was much higher than that at pH 3.5 and pH 5.5 (Figure 4.3-4.7, Table 4.1). The BLG gels at pH 6.8, which had high γ_c, were more rubbery than those at pH 3.5 and pH 5.5. The γ_c at pH 3.5 was much lower, indicating that the gels were very brittle. According to the large deformation measurements, slightly turbid gels were prepared with a high elasticity (rubbery) at pH 6.8, whereas at pH 3.5 almost translucent gels were obtained with a high brittleness. The critical strain at fracture of whey protein and BLG gels have been studied (Stading and Hermansson, 1991; Foegeding et al., 1995). They were fragile at low pH and elastic at high pH.

The texture profiles of G′ and γ_c at critical point of BLG gels are shown in Figure 3.4 to 3.10, which show the relationship between G′ and γ_c at critical point of BLG gels. From the figures, it can be explained that pH affected dominantly γ_c. The BLG gels formed at pH 3.5 had the lowest γ_c while gels at pH 6.8 had the highest γ_c. The γ_c of BLG gels developed at pH 5.5 was intermediate between that of the gels at pH 3.5 and pH 6.8. The

property of BLG gels at pH 6.8 was very rubber-like, while that at pH 3.5 was very brittle. The gels prepared at pH 5.5 were slightly rubbery but overall weaker than those at pH 3.5 or pH 6.8. It is therefore explained that the pH is the most critical factor to control the strain of gels. The effect of pH on γ_c was consistent. The cooling after gel formation affected the increase in G' and γ_c much, while the gelation condition affected only a little due to lack of time for protein denaturation.

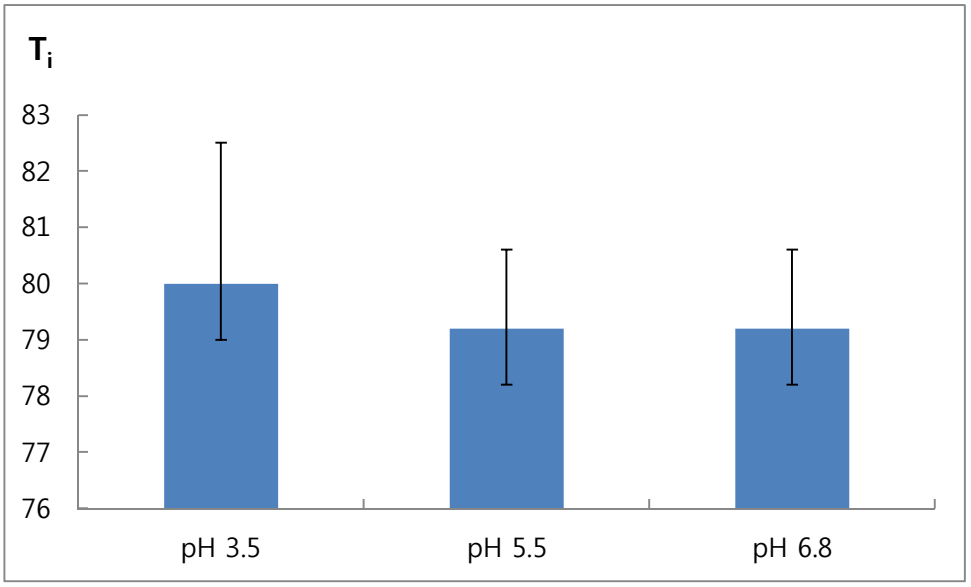

Figure 4.3 T_i (Initial gelation temperature) of BLG gels with different pH (pH 3.5, pH 5.5, pH 6.8)

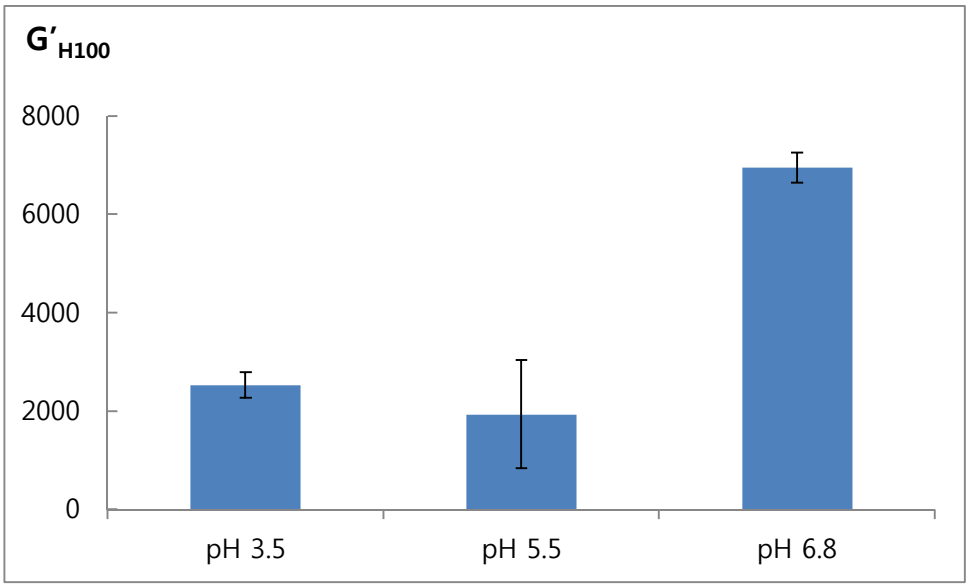

Figure 4.4 G'_{H100} (G′ at the end of heating step) of BLG gels with different pH (pH 3.5, pH 5.5, pH 6.8)

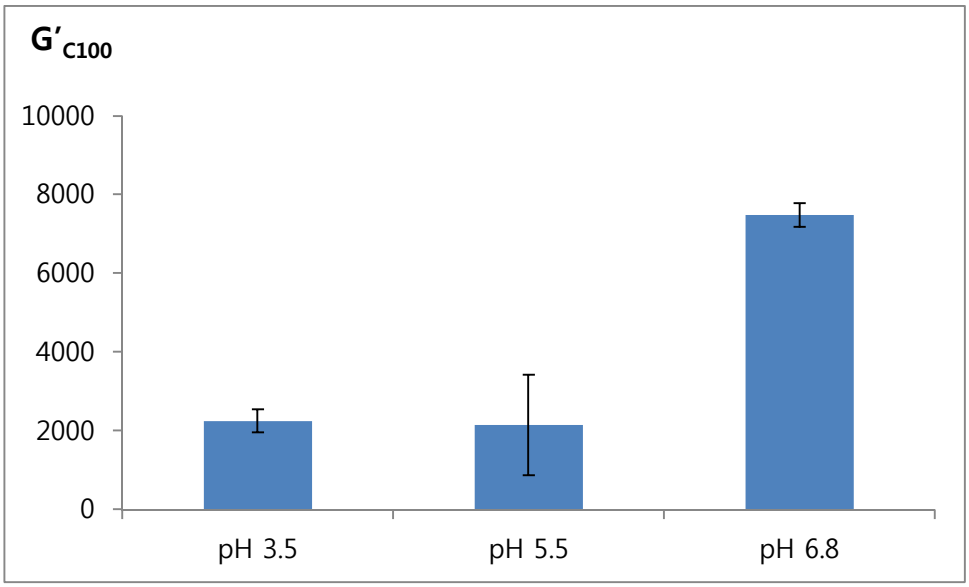

Figure 4.5 G'_{C100} (G′ at the beginning of cooling step) of BLG gels with different pH (pH 3.5, pH 5.5, pH 6.8)

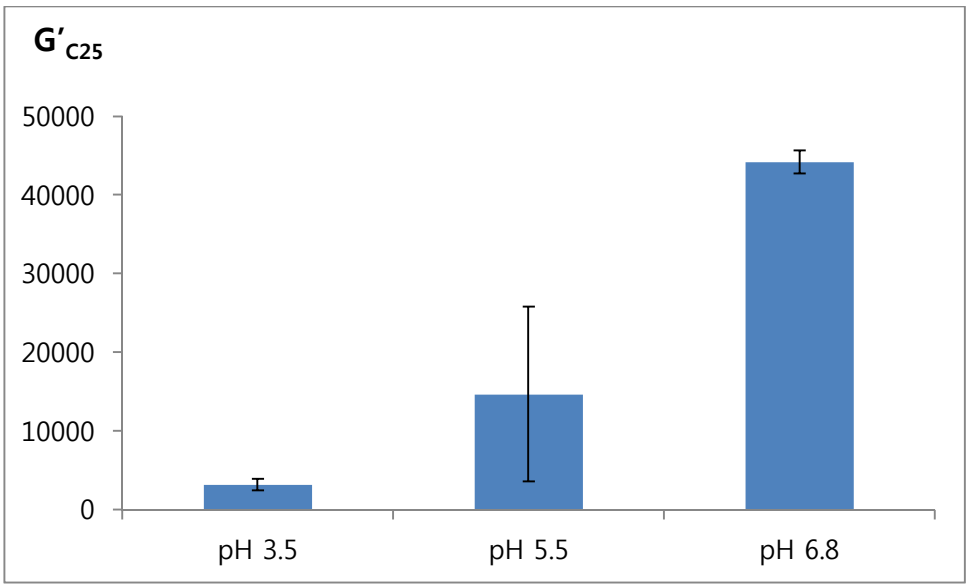

Figure 4.6 G'_{C25} (G′ at the end of cooling step) of BLG gels with different pH (pH 3.5, pH 5.5, pH 6.8)

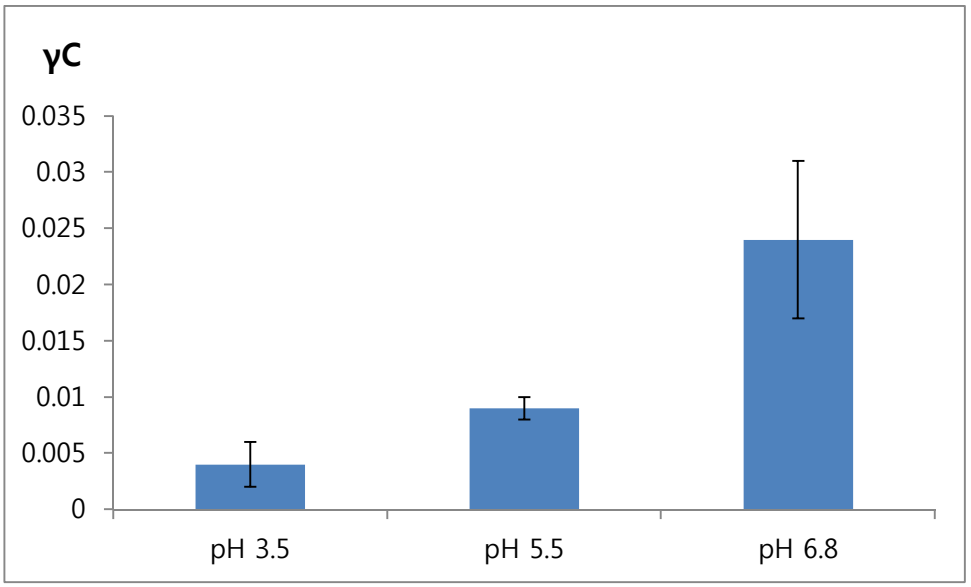

Figure 4.7 γ_C (95% strain of maximum G′ at strain sweep) of BLG gels with different pH (pH 3.5, pH 5.5, pH 6.8)

Table 4.1 T_i, G'_{H100}, G'_{C100}, G'_{C25}, and γ_C during BLG gelation at different pHs (3.5, 5.5, and 6.8)

	pH 3.5	pH 5.5	pH 6.8
Ti	80.0±2.5	79.2±1.4	79.2±1.4
G'_{H100}	2534±260	1931±1098	6946±306
G'_{C100}	2247±292	2144±1283	7480±298
G'_{C25}	3219±739	14686±11115	44160±1463
γ_C	0.004±0.002	0.009±0.001	0.024±0.007

T_i : initial gelation temperature

G'_{H100} : G′ at the end of heating step

G'_{C100} : G′ at the beginning of cooling step

G'_{C25} : G′ at the end of cooling step

γ_C : 95% strain of maximum G' at strain sweep

3.3. Determination of critical strain

Critical strain (γ_c) indicating the limit of linearity can be defined as the end point of the linear region. Because we cannot obtain direct information about the linearity of protein gels with increasing shear stress, the point of the limit of linearity should be inferred from the experiments. Experimentally, the critical point can be detected when G′ changes considerably from the original G′ value. And it is difficult to determine γ_c accurately.

Simple empirical methods have been used for determining the γ_c objectively in many colloidal gels including the globular protein gels (Shih et al., 1990; Hagiwara et al., 1997; Rueb and Zukoski, 1997; Eleya et al., 2004). The strain value was determined where the deviation was 5% from its maximum value. The value was taken as a measure of the γ_c. In this study, the same method was used. As shown in Figure 4.8-4.10, the G′ values of the BLG gels decreased at a certain point during shear stress sweep. The G′ of most BLG gels decreased of a sudden, whereas the rests (especially, the gels at pH 5.5) decreased slowly. In order to determine γ_c, 95% of initial G′ was estimated and its corresponding strain value was chosen (Figure 4.8 ~ 4.10). The γ_c is determined here as the strain the gel can maintain at 1 Hz when G′

was 95% of initial G′ in the strain sweep test. The γ_c is a measure of the brittleness of the gel network as it indicates the strain above which the gel responds in a nonlinear property.

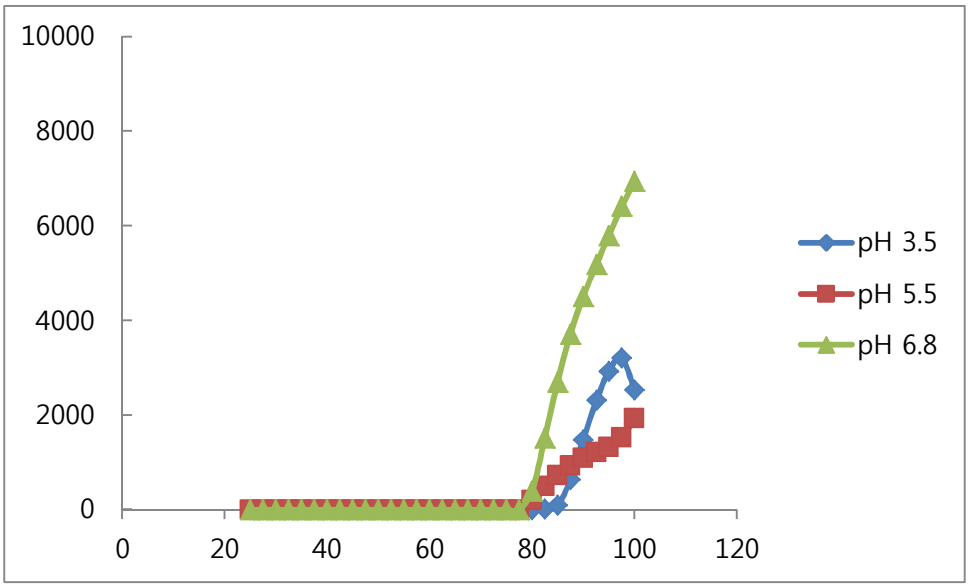

Figure 4.8 G′ at heating step during gelation of BLG heated at 2.5 ℃/min from 25 to 100 ℃ (pH 3.5, pH 5.5, pH 6.8)

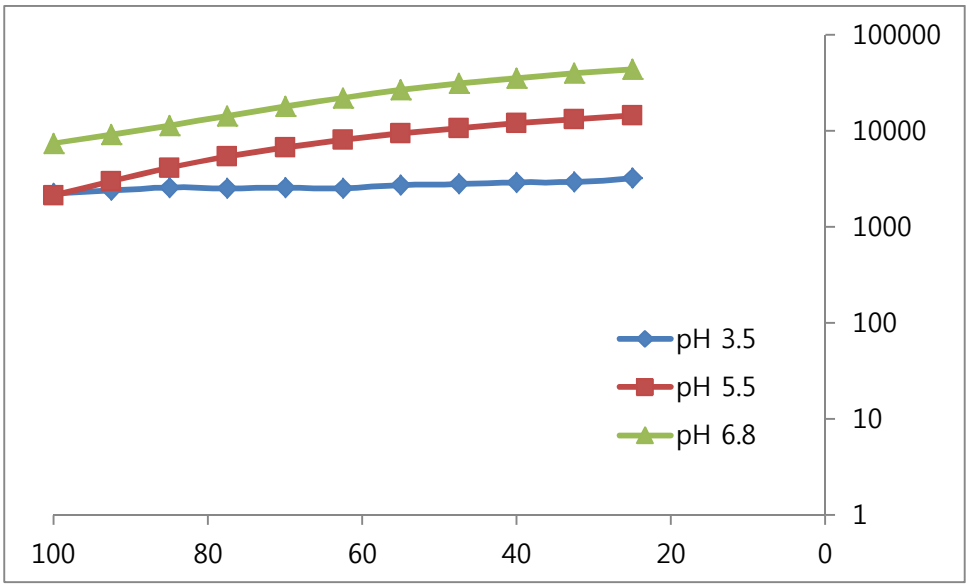

Figure 4.9 G′ at cooling step during gelation of BLG heated at 7.5 ℃/min from 100 to 25 ℃ (pH 3.5, pH 5.5, pH 6.8)

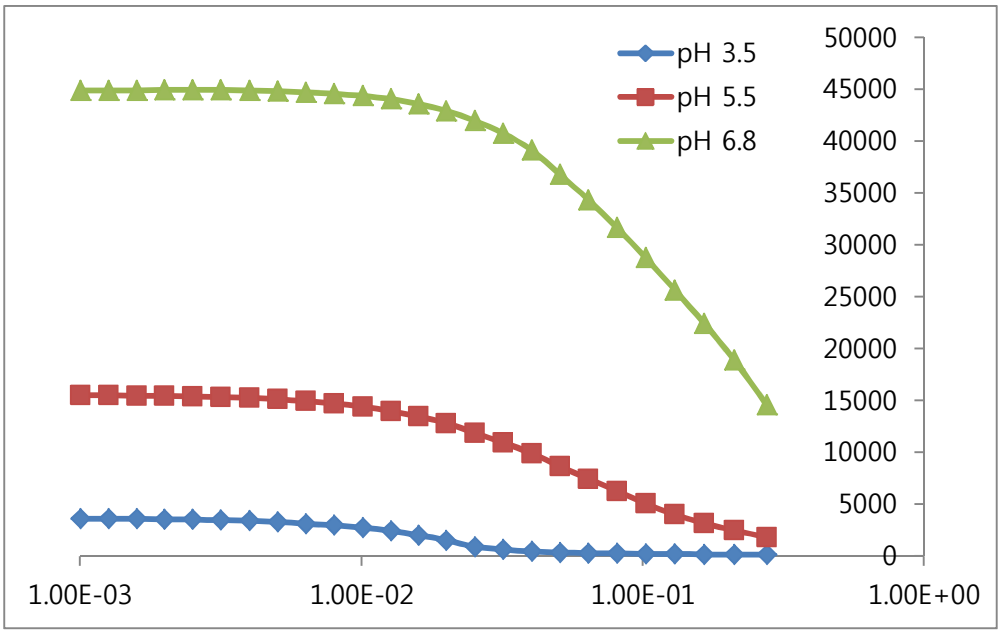

Figure 4.10 G′ at strain sweep of BLG gels (pH 3.5, pH 5.5, pH 6.8)

4. Conclusion

The gelation process of BLG and the gelation factors were investigated at various pH conditions. On heating BLG aqueous system, the developing rheological properties of BLG gels were determined with the dynamic rheological properties (G′) by the SAOS dynamic rheometry. And With the SAOS measurement, useful parameters such as T_i and G′ of each step during gelation and critical strain (γ_c) were calculated. These parameters could be used to represent gel characteristics as well as to describe the dependence of gel properties on gelation conditions.

The effect of pH on the rheological properties of the BLG gels was investigated during heat-induced gelation. G'_{max} at pH 3.5 and 6.8 was much higher than that at pH 5.5. High electrostatic repulsions led to high G′ values on the cured BLG gels.

The rheological properties of the BLG gels during shear stress sweep were studied at different pH conditions. The cured BLG gels hardened during cooling. The increase in G′ upon cooling is caused by the hydrogen bonds which allow enhanced bond formation in and between the protein molecules.

The texture of BLG gels at pH 6.8 was very rubber-like, while that at pH 3.5 was very brittle. The gels prepared at pH 5.5 were slightly rubbery but overall weak. High electrostatic repulsion produced stronger gels (high G′). The pH affected dominantly γ_c. The information of BLG gel structure evolution on heating would be useful for food industry application such as viscosity enhancer and stabilizer.

5. References

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Chapter 5. Overall discussion

Rice flour, owing to the absence of gluten, is a popular candidate as a cereal source for gluten-free food products. However, replacing wheat flour with rice flour for a variety of food products has been difficult because the gluten plays an important role in forming desirable food matrix and texture. Thus, an understanding of the physicochemical properties and structure evolution upon heating of rice flour is necessary to expand its processability in food applications.

In these studies (Part II & Part III), I investigated morphological, hydration, viscoelastic, pasting, and thermal properties of 10 different rice starches and compared the amylose content to their processability for food applications. Amylose content could be correlated to hydration (Pearson coefficient (PC): -0.9269) and pasting properties (PC: 0.8514) of rice starches whereas it was insufficient in accounting for their viscoelastic and thermal characteristics (PC: -0.3745). The combined analysis of hydration, viscoelastic, pasting, and thermal data of starches is expected to be useful for gauging its processability in food applications.

Through the systematic analysis, the deeper understanding of rice starch behavior at ingredient level, dough and batter level, and product level may be provided and expand its practical use in industry. In addition to these

integrated analyses, these properties could also serve in selecting the best cultivar fit for a particular food type such as bakery and noodle products.

The gelation process of BLG and the gelation factors were investigated at various pH conditions. On heating BLG aqueous system, the developing rheological properties of BLG gels were determined with the dynamic rheological properties (G′) by the SAOS dynamic rheometry. And its graphical parameters were used to calculate T_i, G′ of each step during gelation and critical strain (γ_c). These parameters could be used to represent gel characteristics as well as to describe the dependence of gel properties on gelation conditions. The effect of pH on the rheological properties of the BLG gels was investigated during heat-induced gelation. G'_{max} at pH 3.5 and 6.8 was much higher than that at pH 5.5. High electrostatic repulsions led to high G′ values on the cured BLG gels.

The rheological properties of the BLG gels during shear stress sweep were studied at different pH conditions. The cured BLG gels hardened during cooling. The increase in G′ upon cooling is caused by the hydrogen bonds which allow enhanced bond formation in and between the protein molecules. The texture of BLG gels at pH 6.8 very rubber-like while that at pH 3.5 was very brittle. The gels prepared at pH 5.5 were slightly rubbery

but overall weak. High electrostatic repulsion produced stronger gels (high G′). The pH affected dominantly γ_c.

국문 초록

10종의 국산 쌀 품종에 대하여 가공적성과 관련된 이화학적 특성 및 호화 특성을 조사하였고, 가열에 의한 전분의 구조 형성을 조사 후 품종별 차이를 비교하였다. 10종의 쌀 전분 모두 구형의 입자 상태로 조사되었고 품종간 차이는 크지 않았다. 쌀 전분의 호화 과정에서 G′ 값은 37.4 ~ 2,057 Pa로 품종별 큰 차이를 보였다. 아밀로오스 함량이 가장 높은 쌀 전분(R1)은 가장 낮은 γ_c (critical strain)을 보인 반면, 아밀로오스 함량이 가장 낮은 찹쌀 품종(W1, W2)은 가장 높은 γ_c을 보였다. 아밀로오스 함량은 전분 반죽의 물성적 특성에 많은 영향을 주는 것으로 조사되었고, 최종점도 (final viscosity) 및 setback 수치와 높은 상관관계를 보였다. 반면 찹쌀 품종은 낮은 최종점도 및 setback 값을 나타냈다.

쌀 품종별 호화 개시 온도는 57.9 ~ 64.4 ℃로 조사되었으며, 열특성(호화 개시 온도, 호화 종결 온도 및 엔탈피 등)은 아밀로오스 함량과 상관관계를 보이지 않았다. 또한 쌀 전분의 특성을 나타내는 지표간 피어슨 상관계수(Pearson coefficient)를 조사하였다.

아밀로오스 함량과 수화정도는 −0.9269로 높은 반비례의 상관 관계를 보였으며, 페이스팅 온도와는 0.8514의 높은 정비례 관계를 보였다. 하지만 호화개시온도와는 −0.3745의 낮은 피어슨 상관계수를 보였다. 쌀 전분의 가공적성을 보다 정확히 이해하기 위해서는 수화 특성, 물성적 특성, 열 특성 및 이화학적 특성과의 복합지표를 이용하여 종합적으로 비교하는 것이 필요하겠다.

베타락토글로블린 수용액을 25 ~ 100 ℃로 가열하면서 겔화를 유도하였고(pH 3.5, 5.5 및 6.8) G′ 및 G″ 값을 측정하였다. 그리고, 베타락토글로블린의 겔화 특성을 확인할 수 있는 지표로서 초기 겔화 온도 및 가열 과정에서의 최고 G′ 값, 냉각 과정에서의 최고 G′ 값 및 γ_c (critical strain)을 조사하였다. pH 3.5 및 6.8에서 측정된 G′ 값이 pH 5.5에서 측정된 값보다 높았으며, 이는 정전기적 반발력에 기인하는 것으로 분석되었다. pH 환경에 따라 정전기적 반발력이 다르며, 베타락토글로블린의 겔 특성에 영향을 주는 요인으로 작용한다. 또한 물성 특성을 알아보기 위해 small−amplitude oscillatory shear (SAOS) 테스트를 실시하였다(shear stress sweep, 1Hz). 스트레인에 따른 G′ 값의 변화를 측정하였으며, pH

에 따라 다른 관계를 보였다. pH 3.5 또는 6.8에서의 G′ 값이 pH 5.5에서 측정된 G′ 값보다 더 높았으며, pH 6.8에서 형성된 겔은 고무 형태(rubber-like)였으며, pH 3.5에서 형성된 겔은 딱딱하였고, pH 5.5에서 형성된 겔은 다소 고무와 같은 형태로 다른 겔보다 약한 겔을 형성하였다.

주제어: 쌀전분, 점탄성, 열특성, 물성, 베타락토글로블린, 겔화온도

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