EFFECT OF DIFFERENT β-GLUCANS ON THE GELATINIZATION AND RETROGRADATION OF RICE STARCH

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ABSTRACT

Four β-glucan preparations, i.e. curdlan (CL), oat (OG), barley (BG), and yeast (YG) β-glucans, were compared for their effects on the gelatinization and retrogradation of rice starch (RS). The preparations all exhibited different β-glucan purities ranging from 58-100% and other minor chemical components. Rapid visco analysis (RVA) showed that addition of the different β-glucans increased the peak, breakdown, final, and setback viscosities, but tended to decrease the pasting temperature of RS to different degrees. Differential scanning calorimetry (DSC) demonstrated that the β-glucans all had a negligible effect on the onset (T_o), peak (T_p), and conclusion (T_c) temperatures, but slightly decreased the gelatinization enthalpy (ΔH_1) of RS. Storage of all the gels at 4°C resulted in a marked decrease in the T_o, T_p, T_c, and melting enthalpy (ΔH_2) values. The retrogradation ratio ($\Delta H_2/\Delta H_1$) and the phase transition temperature range (T_c - T_o) of all the gels increased with storage time, but addition of any of the β-glucans could reduce these effects. Dynamic viscoelastic measurements indicated that addition of any of the β-glucans resulted in an increase in the storage modulus (G'), loss modulus (G''), and loss tangent ($\tan\delta$) of the fresh RS gels. The G' values of all the gels increased whereas the tan δ values decreased during refrigerated storage. Steady flow tests illustrated the time-dependent shear-thinning (thixotropic) behavior of all the gels. The hysteresis loop area and the gel hardness of RS gels increased with storage time, but these effects were reduced by the addition of various β-glucans. The extent of the abovementioned effects differed among the different β-glucan preparations, generally in the order OG ≈ BG > CL ≈ YG. This difference could be explained in terms of molecular weight and structure as well as purity of the β-glucans and the impurities present in the various β-glucan preparations.

KEY WORDS: RICE STARCH/ β-GLUCAN/ GELATINIZATION/ RETROGRADATION/ RHEOLOGY

124 pp.

ผลของเบต้า-กลูแคนชนิดต่างๆ ต่อเจลาติไนเซชั่นและเรโทรเกรเดชั่นของแป้งข้าวจ้าว
(EFFECT OF DIFFERENT β-GLUCANS ON THE GELATINIZATION AND RETROGRADATION OF RICE STARCH)

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บทคัดย่อ

การศึกษาเพื่อเปรียบเทียบผลกระทบของเบต้ากลูแคน 4 ชนิด ได้แก่ เคริดแลน, โอ๊ต, บาร์เลย์ และยีสต์เบต้ากลูแคน ต่อการเกิดเจลาติไนเซชั่นและเรโทรเกรเดชั่นของแป้งข้าวจ้าว เบต้ากลูแคนที่เตรียมได้ทุกชนิดมีความบริสุทธิ์ในช่วง 58-100% และมีส่วนประกอบอื่นๆเล็กน้อย ผลการทดลองโดยวิธี Rapid visco analysis (RVA) แสดงให้เห็นว่า การเติมเบต้ากลูแคนแต่ละชนิดลงในแป้งข้าวจ้าวจะเพิ่มค่าความหนืดสูงสุด ค่าความหนืดที่ลดลงในช่วงการให้ความร้อน ค่าความหนืดสุดท้าย และค่าความหนืดที่เพิ่มขึ้นในช่วงการลดอุณหภูมิ แต่มีแนวโน้มจะลดอุณหภูมิของการเกิดเจลาติไนเซชั่นของแป้งข้าวในระดับที่แตกต่างกัน ข้อมูลที่ได้จาก Differential scanning calorimetry (DSC) แสดงให้เห็นว่า เบต้ากลูแคนมีผลต่ออุณหภูมิที่เริ่มเกิดเจลาติไนเซชั่น (T_o) อุณหภูมิสูงสุด (T_p) และอุณหภูมิเมื่อสิ้นสุดการเกิดเจลาติไนเซชั่น (T_c) น้อยมาก แต่จะลดค่าพลังงานความร้อน (enthalpy) ที่ใช้ในการเจลาติไนเซชั่น (ΔH_1) ของแป้งข้าว การเก็บรักษาเจลแป้งข้าวที่ 4 องศาเซลเซียส พบว่าค่า T_o, T_p, T_c และค่าพลังงานความร้อนที่ใช้ในการหลอมเหลว (ΔH_2) ของเจลแป้งลดลง อัตราการเกิดเรโทรเกรเดชั่น ($\Delta H_2/\Delta H_1$) และช่วงอุณหภูมิของการเปลี่ยนแปลงสถานะ (T_c - T_o) ของเจลเพิ่มขึ้นเมื่อเก็บเป็นระยะเวลานานขึ้น แสดงให้เห็นว่ามีการเกิดเรโทรเกรเดชั่นมากขึ้น แต่ผลกระทบดังกล่าวจะลดลงโดยการเติมเบต้ากลูแคนทุกชนิด การวัดรีโอโลยีแบบไดนามิคแสดงให้เห็นว่าการเติมเบต้ากลูแคนแต่ละชนิดมีผลทำให้ค่า storage modulus (G'), loss modulus (G'') และ loss tangent ($\tan\delta$) ของเจลแป้งข้าวที่เตรียมใหม่เพิ่มขึ้น ค่าG'ของเจลทุกชนิดจะเพิ่มขึ้นในขณะที่ค่า $\tan\delta$ ลดลงระหว่างการเก็บรักษา การศึกษาคุณสมบัติการไหลแสดงให้เห็นว่าเจลของของผสมทุกชนิดมีพฤติกรรมการไหลแบบ time-dependent shear-thinning (thixotropic) ค่าพื้นที่ของ hysteresis loop และความแข็งของเจลแป้งข้าวจะเพิ่มขึ้นระหว่างการเก็บรักษา แต่อิทธิพลดังกล่าวจะลดลงโดยการเติมเบต้ากลูแคนแต่ละชนิด ผลกระทบดังกล่าวข้างต้นจะแตกต่างกันตามชนิดของเบต้ากลูแคน ซึ่งโดยทั่วไปสามารถเรียงลำดับได้ดังนี้ โอ๊ต ≈ บาร์เลย์ > เคริดแลน ≈ ยีสต์เบต้ากลูแคน ความแตกต่างนี้สามารถอธิบายได้ด้วยความแตกต่างของน้ำหนักโมเลกุล, โครงสร้าง, ความบริสุทธิ์ และสิ่งเจือปนที่มีอยู่ในเบต้ากลูแคนแต่ละชนิด

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LIST OF ABBREVIATIONS

α	Alpha
&	And
AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of variance
β	Beta
BG	Barley β-glucan
BEPT	Birefringence end-point
CL	Curdlan
Con A	Concanavalin A
cP	Centipoise
db	Dry basis
DP	Degree of polymerization
DP3	Cellotriosyl residue
DP4	Cellotetraosyl residue
DSC	Differential scanning calorimeter
ed.	Edition
Ed. (Eds.)	Editor, editors, edited by
et al.	Et alii (Latin), and others
g	Gram
G'	Storage modulus, Pa
G''	Loss modulus, Pa
GT	Gelatinization temperature
h	Hour
Hz	Hertz
ΔH	Enthalpy, J/g
i.e.	Id est (Latin), that is

LIST OF ABBREVATIONS

(continued)

J	Joule
K	Consistency coefficient, Pa s^n
mg	Milligram
ml	Millimeter
min	Minute
M_W	Molecular weight
N	Normality
N	Newton
n	Flow behavior index, dimensionless
η_a	Apparent viscosity, Pa s
NMR	Nuclear Magnetic Resonance
OG	Oat β-glucan
p.(pp.)	Page (pages)
Pa	Pascal
rad	Radian
RD6	Kor Khor 6 (glutinous rice variety)
RS	Rice starch
RVA	Rapid Visco Analyzer
RVU	Rapid Viscosity Units
R^2	Coefficient of determination
s	Second
SD	Standard deviation
SPSS	Statistical Package for the Social Science
tanδ	Loss tangent, dimensionless
$T_c - T_o$	Phase transition temperature range, °C
T_o, T_p, T_c	Onset, peak, conclusion temperature, °C
TPA	Texture profile analysis
μm	Micrometer

LIST OF ABBREVATIONS

(continued)

w/v	Weight by volume
w/w	Weight by weight
YG	Spent brewer's yeast β-glucan
/	Per
%	Percent
°C	Degree Celsius
$\dot{\gamma}$	Shear rate, 1/s
σ	Shear stress, Pa
σ_0	Yield stress, Pa
ω	Angular frequency, rad
θ	Zeta

CHAPTER I
INTRODUCTION

Rice starch is the major component of grains and a common ingredient used in the food industry. Starches are mainly used in foods as an agent for thickening and gelling. Starch properties depend on the physical and chemical characteristics such as mean granule size, granule size distribution, amylose/amylopectin ratio and mineral content (Singh, Singh, Kaur, Sodhi, & Gill, 2003). Rice starch granule being very small in size provides a texture perception similar to that of fat (Champagne, 1996), and is non-allergic due to the hypoallergenicity of the associated proteins. Rice starch, in its gelatinized form, has a bland taste and is smooth, creamy and spreadable.

From a rheological point of view, the gelatinization and retrogradation of starch, which are important properties in food processing, cooking, handling, distribution, and storage, can all be affected by the presence of hydrocolloids. Starch/hydrocolloid mixtures are widely used in the food industry to modify and control the texture, improve moisture retention, control water mobility, and extend shelf life of food products (Appelqvist & Debet, 1997).

However, it, in common with other cereal starches, has also negative aspects such as gel retrogradation and tendency to produce undesirable weak-bodied, cohesive, rubbery pastes or gels under extended cooking, high shear or acidic conditions (BeMiller, 2007). To overcome these shortcomings, the blending of native rice starch with various polysaccharide hydrocolloids (gums) has been investigated by many workers (Alloncle, Lefebvre, Llamas, & Doublier, 1989; Biliaderis, Arvanitoyannis, Izydorczyk, & Prokopowich, 1997; Christianson, Hodge, Osborne, & Detroy, 1981; Ferrero, Martino, & Zaritzky, 1994; Kim & Yoo, 2006; Kulicke, Eidam, Kath, Kix, & Kull, 1996; Satrapai & Suphantharika, 2007; Shi & BeMiller, 2002; Techawipharat, Suphantharika, & BeMiller, 2008).

Starch-hydrocolloid mixed system is regarded as a suspension of starch granules dispersed in the solution of the hydrocolloid (Closs, Conde-Petit, Robert,

Tolstoguzov & Escher, 1999). Function of hydrocolloid to starch, including inhibition of retrogradation or the improvement of water-holding capacity for the starch system, depending on the macromolecule characteristics of hydrocolloid (Funami, Kataoka, Omoto, Goto, Asai, & Nishinari, 2005b).

In the aspects of a natural polysaccharide hydrocolloid, β-glucan is attracting the increasing attention of the pharmaceutical and functional food industry not only because of its thickening or gelling properties but also its multiple beneficial effects on human and animal health such as immune-stimulation, anti-inflammatory, antimicrobial, antitumoral, hepatoprotective, cholesterol-lowering as well as antifibrotic, antidiabetic, and hypoglycemic activity (Lazaridou & Biliaderis, 2007; Wood, 2007; Zeković, Kwiatkowski, Vrvić, Jakovljević, & Moran, 2005). Additionally, β-glucan is nutritionally non-functional in the human digestive tract, and hence contribute to no caloric value (Charalampopoulos, Wang, Pandiella, & Webb, 2002; Morgan, 2000). β-Glucan is a non-ionic homopolysaccharide comprised of D-glucopyranosyl units and can be found in a wide variety of microorganisms and plants.

Cereal β-glucans are water soluble linear (1→3),(1→4)-β-D-glucans, containing approximately 70% (1→ 4) and 30% (1→ 3) β-glucan (Dais and Perlin, 1982; Lazaridou & Biliaderis, 2007). Among all cereal grains, oat and barley contain the highest levels of β-glucan, with the latter at 2–11% of dry weight (Charalampopoulos et al., 2002; Jadhav, Lutz, Ghorpade, & Salunkhe, 1998; Lehtonen & Aikasalo, 1987). They are also reported to be an effective hypoglycemic and hypocholesterolemic agent (Gordon, 1989; Wood, 2007).

Curdlan is a bacterial polysaccharide produced by strains of *Alcaligenes faecalis* and *Agrobacterium radiobacter*. It is a linear (1→3)-β-D-glucan, with low molecular weight, that despite its insoluble in water at room temperature, but its aqueous suspension can form gel upon heating (Harada, Misaki, & Saito, 1968; Harada, 1992; Harada, Terasaki, & Harada, 1993). It is tasteless, has no evident toxicity (Spicer, Goldenthal, & Ikeda, 1999) and widely used as food additives. In addition, it can exhibit the strong anti-tumour properties due to its free-radical scavenging and antioxidative activities (Kishk & Al-Sayed, 2007).

Yeast β-glucan is water insoluble branched (1→3),(1→6)-β-D-glucan extracted from yeast cell walls (about 50–60%) (Lipke & Ovalle, 1998; Nguyen, Fleet, & Rogers, 1998; Thammakiti, Suphantharika, Phaesuwan, & Verduyn, 2004). The major component of yeast β-glucan (about 85%) is a backbone chain of β-1,3 glucan and the minor component (about 3%) is a branched β-1,6 glucan (Manners, Masson, & Patterson, 1973). The molecular and structural features of β-glucans are important determinants of their physical properties, such as water solubility and rheological behavior, and thereby affect the functionality of food systems. Among β-glucan from different sources, the spent brewer's yeast β-glucan has more advantage than the others because it can extract from yeast (a by-product from brewery industry) with low cost of production, simple extraction technology, and potential infinite supply will dominate the market for the foreseeable future (Zekovic et al, 2005).

Despite many studies on the effect of other hydrocolloids addition, there are very few reports on the effect of oat β-glucan (Biliaderis et al., 1997; Colleoni-Sirghie, Jannink, & White, 2004; Zhou, Robards, Glennie-Holmes, & Helliwell, 2000), barley β-glucan (Brennan & Cleary, 2007; Faraj, Vasanthan, & Hoover, 2006; Kim & Setser, 1992; Symons & Brennan, 2004), curdlan (Lee, Baek, Cha, Park, & Lim, 2002), and yeast β-glucan (Satrapai & Suphantharika, 2007) addition on the gelatinization and/or retrogradation of various starch. In particular, comparative studies among the effects of these various β-glucans on the gelatinization and retrogradation of rice starch have not been carried out.

The objective of this study was to compare the effects of different β-glucan preparations, i.e. curdlan, barley, oats, and spent brewer's yeast β-glucans, on the pasting, gelatinization, and retrogradation of rice starch by rapid visco-analysis (RVA), differential scanning calorimetry (DSC), dynamic and steady shear rheometry, and textural analysis.

CHAPTER II
LITERATURE REVIEW

1. Starch

Starch is the major carbohydrate reserve in plant tubers and seed endosperm. The largest source of starch is corn (maize) with other commonly used sources being wheat, potato, tapioca and rice. Starch is used in a wide range of products, either as a raw material or as a food additive; therefore, it plays many roles in food. Although the properties of starch are naturally inconsistent, being dependent on the vagaries of agriculture. There are several suppliers of consistently uniform starches as functional ingredients, such as a thickener, gelling agent, water retention agent, emulsion stabilizer, and bulking agent.

1.1 Starch structure and properties

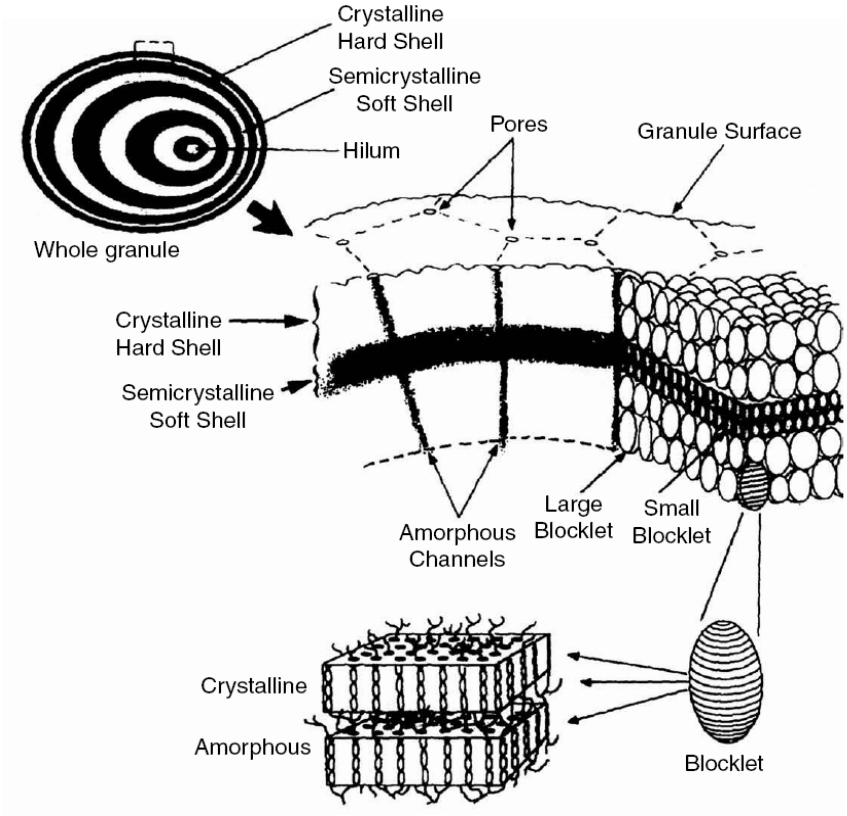
Starch molecules are aligned radially in the granules (Figure 2.1) that have a semi-crystalline characteristic. Starch granules consist of amorphous and crystalline regions. The crystalline regions are formed the short branch chains of amylopectin molecules arranged in clusters. The areas of branching points are believed to be amorphous, suggested that some amylose molecules are located in this region with some interaction with the branch chain of amylopectin (Qiang, 2005).

The starches are composed primarily of D-glucopyranose polymers linked together by α-1,4 and α-1,6 glycosidic bond. Glucose polymerization in starch results in two types of polymers, amylose and amylopectin (Figure 2.2). The amylose and amylopectin content and structure affect the architecture of the starch granule, gelatinization and pasting profiles, retrogradation, and textural attributes, as shown in Table 2.1 (Patindol & Wang, 2002; Thomas & Atwell, 1999). The ratio of these two polymers within a given type of starch is very important point to consider with respect to starch functionality in foods.

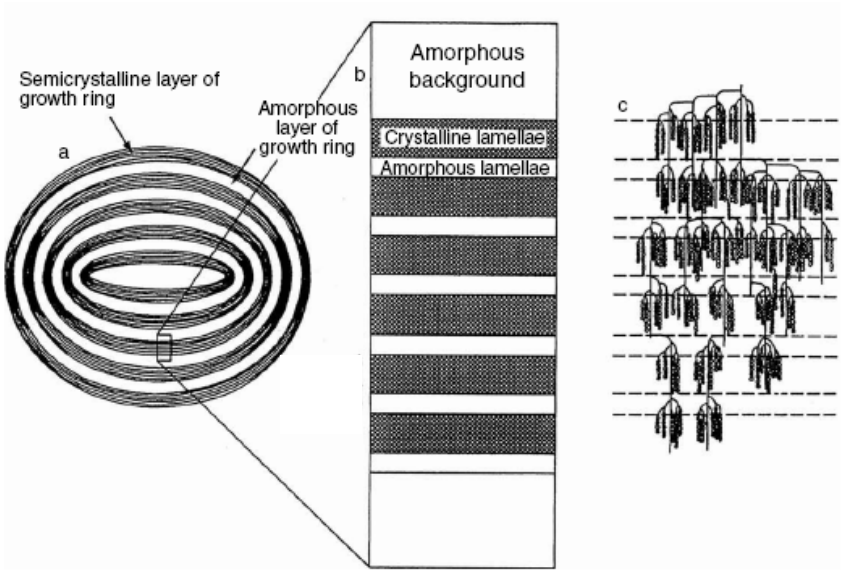
Amylose is a linear polymer consists of α-1,4-linked D-glucopyranose. The degree of polymerization (DP) is between 100 and 10,000. However, amylose from some starch sources contains about 2 to 8 branch points per molecule. The chain length of these branch chains varies from 4 to 100 DP (Hizukuri, Takeda, & Yasuda, 1981). Amylose chain gives the molecules a right-handed spiral or helical shape. The interior of the helix is lined with hydrogen atoms and is hydrophobic, resulting in amylose complex forming with free fatty acids, fatty acid components of glycerides, some alcohols and iodine (Fennema, 1985). Amylose gives a blue color with iodine. As the starch granules are heated, starch gels generally comprise a complex system of partly gelatinized granules in a matrix of amylose. Amylose, linear polymer, undergoes gelation (retrogradation) at a faster rate and, depending on concentration, at higher temperatures than amylopectin (Miles, Morris, Orford, & Ring, 1985a; Leloup, Colonna, Ring, Roberts, & Wells, 1992).

Amylopectin is much larger and highly branched molecule, is composed of α-1,4-linked D-glucopyranose connected by α-1,6-linked branch points. On average, amylopectin has one branch point every 20 to 25 residues. The branch points are not randomly located (Imberty, Buléon, Tran, & Pérez, 1991). The amylopectin chains can be classified into three different types of sub-chains, termed A, B and C, according to their length and branching points (Figure 2.1B and 2.3). The shortest A chains (DP 6-15) carry no branch points and are linked to the amylopectin molecule by a single α-1,6-linkage. The B chains are branched by A chain or other B chains. B chains are further classified, depending on their respective length and number of cluster they span, into B1 (DP ~15-25), B2 (DP ~ 40-50), with B3 and B4 chains being longer. There is only one C chain per amylopectin molecule and it is identified as having the only non-reducing end. It is now widely accepted that linear branched chains with DP ~15 in amylopectin are the crystalline regions present in the granules (Imberty et al., 1991). These short chains form double helical ordered structure; part of the double helices can pack together in organized arrays in cluster form**.** The large size and the branched nature of amylopectin reduce its mobility in solution and eliminate the possibility of significant levels of interchain hydrogen bonding. Amylopectin (Yuan, Thompson, & Boyer, 1993) has high water-binding capacity and undergoes less

retrogradation, thus forming clear gels that are soft and flow well. Amylopectin give a reddish-brown color with iodine.



(A)



(B)

Figure 2.1 Overview of starch granule structure. (A) At the lowest level of granule organization (upper left), the alternating crystalline (hard) and semi-crystalline (soft)

Figure 2.1 (Cont.) shells are shown. The shells are thinner towards the granule exterior and the hilum is shown off center. At a higher level of structure the blocklet structure is shown, in association with amorphous radial channels. Blocklet size is smaller in the semi-crystalline shells than in the crystalline shells. At the next highest level of structure one blocklet is shown containing several amorphous crystalline lamellae (Gallant, Bouchet, & Baldwin, 1997). (B) Schematic diagram of starch granule structure. (a) A single granule, comprising concentric rings of alternating amorphous and semi-crystalline composition. (b) Expanded view of the internal structure. The semi-crystalline growth ring contains stacks of amorphous and crystalline lamellae. (c) The currently accepted cluster structure for amylopectin within the semi-crystalline growth ring. A-chain sections of amylopectin form double helices, which are regularly packed into crystalline lamellae. B-chains of amylopectin provide intercluster connections. Branching points for both A and B chains are predominantly located within the amorphous lameilae (Jenkins & Donald, 1995).

Table 2.1 Starch granule characteristics (Murphy, 2000)

Starch	Type	Diameter microns (μm)	Morphology	Gelatinisation temp. °C	Pasting temp. °C (a)	Amylose content	Cooked properties
Maize (b)	Cereal	5–30	Round Polygonal	62–72	80	25	Opaque gel
Waxy Maize	Cereal	5–30	Round Polygonal	63–72	74	< 1	Clear cohesive
Tapioca	Root	4–35	Oval Truncated 'kettle drum'	62–73	63	17	Clear cohesive, tendency to gel
Potato	Tuber	5–100	Oval Spherical	59–68	64	20	Clear cohesive, tendency to gel
Wheat	Cereal	1–45	Round Lenticular	58–64	77	25	Opaque gel
Rice	Cereal	3–8	Polygonal Spherical Compound granules	68–78	81	19	Opaque gel
Sago	Pith	15–65	Oval Truncated	69–74	74	26	Opaque gel
High Amylose Maize	Cereal	5–30	Polygonal Irregular Elongated	63–92 (c)	> 90	50–90	Very opaque, very strong gel

(a) Measured for 5% starch suspension.
(b) Maize is also often referred to as 'corn', 'dent corn' or 'regular maize'.
(c) High amylose maize starches are not completely gelatinised in boiling water.

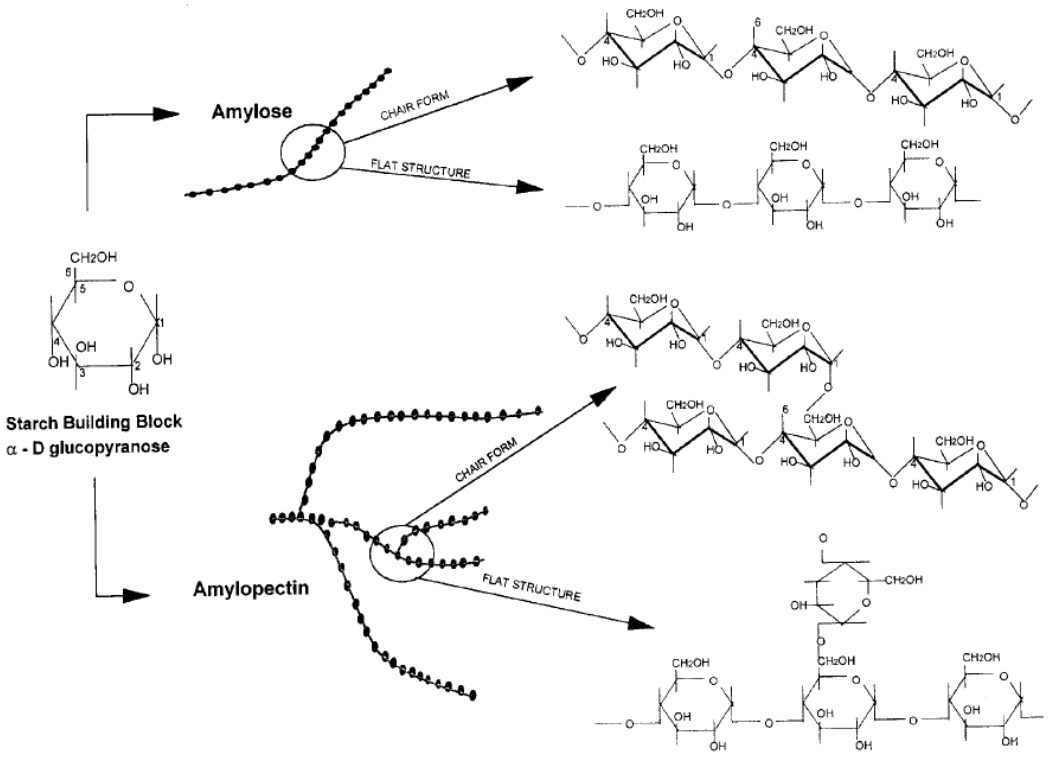


Figure 2.2 Linear and branched starch polymers (Taggart, 2004).

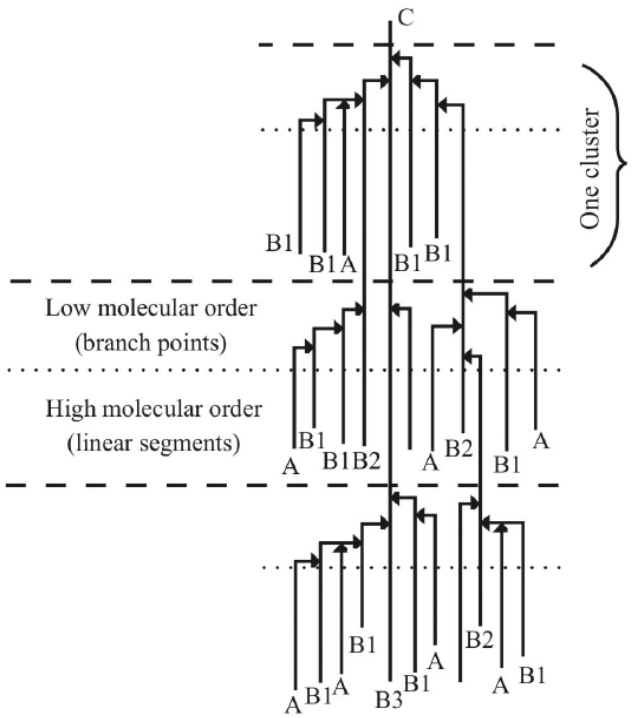


Figure 2.3 Schematic representation of the sub-chains within an amylopectin molecule. Where the branch points sit are regions of low order, and neighboring B chains can form double helices to make up the crystal structure (Donald, 2004).

1.2 Rice starch

Rice (*Oryza sativa* L.) is a short-lived plant, belongs to the grass family and has been one of the most commonly used grain products since ancient times. These plants are native to tropical and subtropical southern Asia, southeastern Asia and southeastern Africa (Crawford & Shen, 1998). Rice provides more than one fifth of the calories consumed worldwide by humans. Rice is a staple for a large part of the world's human population, about 90% of world rice crops is produced and consumed in East, South and Southeast Asia (Juliano, 1985; Whistler, BeMiller, & Paschall, 1984). It can be divided into two sub-species, *indica* and *japonica*, containing genotypes that vary greatly in terms of starch properties.

Rice starch is commonly isolated by alkaline and enzymatic processes, which have been removed the most of native proteins and lipids (Puchongkavarin, Varavinit, & Bergthaller, 2005). The protein content of isolated rice starch is generally 0.5% or less. In application aspect, the small granule size and soft gel formed from rice starch have made it desirable as a nutrient, texturing agent and also widely applied as several functions such as thickener, gelling agents and volatile flavor compounds binding agent.

1.2.1 Chemical compositions of rice starch

The physiochemical properties of non-waxy and waxy rice starch were shown in Table 2.2. Moreover, Noosuk, Hill, Pradipasena, and Mitchell (2003) displayed the composition and structural properties of different Thai rice starches in Table 2.3 The amylose content of rice is a significant factor that affects its eating quality (Takeda, Hizukuri, & Juliano, 1987), and the molecular structure of the amylopectin, as with starches generally, has an important influence on the physical properties such as viscosity, pasting behavior, and gelatinization (Patindol et al., 2002). The content of amylose in rice decreases with grain size, and is very low in waxy rice.

The nitrogenous substances include proteins, peptides, amides, amino acids, nucleic acids, and enzymes that may be present in the rice starch granule. The relatively high content of nitrogenous substances in these cereal starches may have undesirable effects, such as the formation of mealy flavor and odor in the

pregelatinized starches, the tendency of cooked starch to foam, and color formation in the starch hydrolyzate (Swinkels, 1985).

Besides proteins, other minor constituents including lipids, phosphorus, and trace elements, are commonly found in isolated rice starch (Champagne, 1996). Non-waxy rice contains 0.3-0.4% bound lipids; waxy rice starch reportedly contains less of this fraction (0.03%) (Morrison, Milligan, & Azudin, 1984; Morrison & Azudin 1987). The lipids also increase the pasting temperatures and reduce the water-binding ability of these starches. The presence or formation of insoluble amylose–lipid complexes causes turbidity and precipitation in starch pastes and starch solutions. Phosphorus plays an extremely important role in starch functional properties, such as, paste clarity, viscosity consistency, and paste stability. Phosphorus in starch is mainly present in two forms; phosphate-monoesters and phospholipids. Ash is the residue of the starch product after complete combustion at a specified temperature. Starch gives a relatively high ash residue because of the presence of phosphate groups in salt form. The ash residue of the cereal starches corresponds partly with the amount of phospholipids in the starch granules.

Table 2.2 Physicochemical properties of rice starch (Juliano, 1984)

Property	Non-waxy rice starch	Waxy rice starch
Final BEPT (^{o}C)	58-79	58-78.5
Granule size (μm)	1.6-8.7	1.9-8.1
Density (xylene displacement) (g cm^{-3})	1.49-1.51	1.48-1.50
Residual Kjehdahl N (% dry basis)	0.02-0.12	~0.02
Residual P (mg/g)	0.12-0.45	0.02-0.03
Bound lipids (% dry basis)	0.2-0.4	~0.03
6% gel viscosity (cP)	140-1200	64-1890
Iodine binding capacity (% dry basis)	2.36-6.96	0.15-0.86

Table 2.3 Comparison and structure of Thai native rice starches (Noosuk, Hill, Pradipasena, & Mitchell, 2003)

Starches derived from	Amylose content [2] [%]			Fat content[1]	Protein content[1]	Moisture content[1]	Granule size mean diameter[2]	Crystallinity[2]	Short-range molecular order from IR[3]
	Con A	Iodine method [% db]							
	[%]	Total	Soluble	[%]	[%]	[%db]	[μm]	[%]	
1. RD6 rice									
• 0 month	2.08 ± 0.22	8.40 ± 0.27	2.56 ± 0.03	0.66 ± 0.10	0.64 ± 0.00	8.20 ± 0.06	4.95 ± 0.01	34.62 ± 0.18	0.91 ± 0.04
• 5 month	1.70 ± 0.08	8.18 ± 0.33	2.21 ± 0.08	0.74 ± 0.07	0.77 ± 0.05	9.91 ± 0.08	5.29 ± 0.03	34.44 ± 0.06	0.89 ± 0.03
2. Commercial waxy rice	2.39 ± 0.25	10.09 ± 0.23	3.78 ± 0.07	0.35 ± 0.18	0.38 ± 0.01	9.76 ± 0.07	4.25 ± 0.04	33.58 ± 0.76	0.86 ± 0.03
3. Jasmine rice									
• 0 month	15.14 ± 0.21	25.62 ± 0.27	15.12 ± 0.13	0.67 ± 0.14	0.70 ± 0.01	6.85 ± 0.00	4.55 ± 0.05	25.70 ± 0.10	0.81 ± 0.03
• 5 month	14.80 ± 0.02	26.29 ± 0.13	15.52 ± 0.05	0.74 ± 0.12	0.78 ± 0.05	9.09 ± 0.16	5.80 ± 0.01	22.27 ± 0.45	0.75 ± 0.01
4. Commercial rice	21.21 ± 0.19	39.48 ± 0.20	21.97 ± 0.27	0.41 ± 0.07	0.56 ± 0.03	9.46 ± 0.07	4.94 ± 0.08	28.94 ± 0.60	0.74 ± 0.02
5. Supanburi1 rice									
• 0 month	22.43 ± 0.15	40.73 ± 0.33	23.08 ± 0.04	0.67 ± 0.07	0.79 ± 0.05	6.74 ± 0.28	5.85 ± 0.05	23.00 ± 0.10	0.72 ± 0.05
• 5 month	22.70 ± 0.24	40.91 ± 0.20	22.65 ± 0.04	0.55 ± 0.16	0.78 ± 0.02	8.76 ± 0.23	6.02 ± 0.06	24.50 ± 0.30	0.73 ± 0.04

[1] means of duplicate ± SD.
[2] means of triplicate ± SD.
[3] Ratio of absorbance at wave number 1047 to 1022 cm^{-1} (means of triplicate ± SD).

1.2.2 Granular structure and crystallinity

Rice contains compound granules with diameters up to 150 μm form as clusters containing between 20 and 60 individual granules (Champagne, 1996) and fill most of the central space within the endosperm cells. However, in waxy rice the endosperm is opaque because of air spaces between the starch granules. The starch granules are accumulations of numerous starch molecules that can be fractionated into highly branched amylopectin and amylose which is less branched. The primary variations in rice starch composition are the relative amounts of amylose and amylopectin and their structure.

Rice starch granules are the smallest size known to exist in cereal grains, with the size reported to range from 3 to 8 μm (Table 2.1). There is some variation in starch granule size between different rice genotypes. The average starch size from some waxy rice ranged from 4.9 to 5.7 μm (Qi, Tester, Snape, & Ansell, 2003). Rice starch granules have a smooth surface but angular and polygonal shapes. The starch from some rice mutants are different in size and shape compared to that of starch from regular rice (Wong, Kubo, Jane, Harada, Satoh, & Nakamura, 2003).

Starch is partially crystalline, as typical cereal starches, rice starch has the A-type X-ray diffraction pattern. The degree of crystallinity of rice is reported to be low (Ong & Blanshard 1995; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003a). The estimated crystallinities of rice starches in the study of Ong and Blanshard (1995) ranged from 29.2% to 39.3%. Crystallinity is likely influenced by amylose content and amylopectin structure. However, it seems that there is no association between crystallinity and amylose: amylopectin ratio (Ong & Blanshard, 1995). Vandeputte et al. (2003a) reported that waxy rice starch was more crystalline than non-waxy starch, as was starch from non-waxy starch with a high gelatinization temperature compared to that with low gelatinization temperature starch.

1.2.3 Amylose and amylopectin

Amylose essentially consists of long chained α-1, 4 linked glucose molecules, but it also may contain a few α-1,6 branch points. The fine structure of rice amylose has not been fully elucidated. In the rice starch studied by Juliano (1998), 40-67% (wt) of the amylose was linear and 33-60% (wt) was branched. The amylose chain has a helical conformation with six anhydroglucose units per turn. Hydroxyl groups of glucosyl residues are located on the outer surface of the helix, while the internal cavity is a hydrophobic tube. Therefore, hydrophobic complexing agents can lie within the amylose helix stabilized by van der Waals forces between adjacent C-hydrogens of amylose. The ability to form helical inclusion compounds with iodine gives rise to the blue color observed when starch is placed in a solution of iodine. Other compounds reported to complex with rice amylose are detergents such as SDS and fatty acids. Amylose content of starch may also be estimated measuring the melting enthalpy of amylose-lipid complex using DSC (Kugimiya & Donovan, 1981). In this technique, lipid is added to starch during starch gelatinization. Starch-lipid complex is formed during cooling and storage. The complex is then melted by heating near or slightly above 100°C. Based on the value of enthalpy of melting complex, amylose content can be calculated.

Rice amylopectin is a highly branched molecule with the branch points being α-(1, 6) bonds. Multiangle laser light scattering with differential refractive index (MALLS-RI) detection has shown the molecular mass (M_w) of waxy rice amylopectin to be approximately 5.7×10^9 while non-waxy rice starch amylopectin is approximately 2.7×10^9 (Yoo & Jane, 2002). Amylopectin structural properties vary depending on rice cultivar. Hizukuri (1986) have shown that the structure of amylopectin can be generalized in terms of its types of chains (A, B and C) which differ in length. The A-chains (unbranched) are linked to B-chains and do not carry any other chains; the B-chains (B1-B4), carry one or more A-chains and/or B-chains; and the C-chain, has the reducing end of the molecule (Figure 2.3). Hizukuri (1993) reported that the average DP of waxy rice cultivars was 19,000 glucose units. Recently, there are several attempts focusing on the effects of genetics and growing environments on amylopectin structure.

1.2.4 Swelling power and solubility

When starch is heated in excess water, the crystalline structure is disrupted due to the breakage of hydrogen bonds, and water molecules become linked by hydrogen bonding to the exposed hydroxyl group of amylose and amylopectin. This causes an increase in granule swelling and solubility. Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. The extent of this interaction is thought to be influenced by a sample's amylose content, amylose and amylopectin structure, degree of granulation and other factors. For example, amylose-lipid complexes have been shown to restrict swelling and solubilization. The swelling behavior of cereal starch is primarily related to amylopectin structure (Tester & Morrison, 1990a). Lower solubility was observed by these authors to be related to the largest DP, this was also reported by Juliano and Villareal (1987). The large DP molecules might have a lower tendency to leach out of the granule during heating or may trap other molecules. Lii, Tsai and Tseng (1996a) observed that swelling power was inversely proportional to the rigidity of starch granules, thus higher swelling power suggests a less rigid granular structure exists.

1.2.5 Gelatinization, pasting and retrogradation

Gelatinization and retrogradation are extremely important in food processing operations, storage and also reflect starch-based food quality. Gelatinization and retrogradation effect on physical starch characteristics is shown in Figure 2.4. Especially, characteristic of native rice starch is very sensitive after retrogradation and easily loss its quality during storage time.

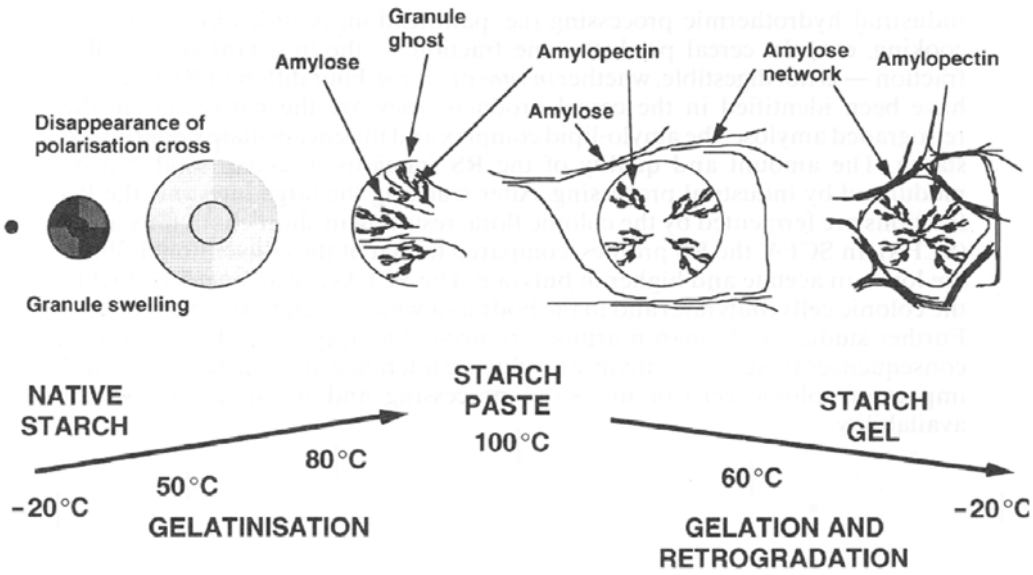


Figure 2.4 Gelatinization and retrogradation on physical starch characteristics. (Bornet, 1993)

1.2.5.1 Gelatinization

Gelatinization is a collapse (disruption) of molecular orders within the starch granule, when starch is heated in the presence of water, manifested by irreversible changes in properties such as granular swelling, native crystalline melting, loss of birefringence, and starch solubilization. The point of initial gelatinization and the range over which it occurs is governed by starch concentration, method of observation, granular type, and heterogeneities within the granule population under observation (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988).

For gelatinization to occur the regions of amorphous starch must first melt or undergo glass transition (Slade & Levine, 1988). The heat energy required to completely gelatinize starch in rice is critical to the rice processor, who must optimize heat input, cooking time, and temperature and, at the same time, minimize the cost of the entire process. Gelatinization temperature (GT) is measured by differential scanning calorimetry (DSC). DSC measures the range in transition temperature required for gelatinization to occur. Thermal properties typically reported using DSC include gelatinization onset (T_o), peak (T_p), conclusion (T_c) and enthalpy (ΔH) (Figure 2.5). Currently, there is no clear understanding of the relationship

between starch structure and thermal properties. Noda, Takahata, Sato, Ikoma, and Mochida (1996) postulated that DSC parameters (T_o, T_p, T_c, ΔH) are influenced by the molecular architecture of the crystalline region of starch, which corresponds to the distribution of amylopectin short chains (DP 6-11), and not by the proportion of crystalline region which corresponds to the amylose content. Cooke and Gidley (1992) have shown that the ΔH values of gelatinization primarily reflect the loss of double helical order rather than the loss of crystallinity. However, Tester and Morrison (1990a) reported that ΔH reflects the overall crystallinity (quality and amount of starch crystallites) of amylopectin. Tester (1997) suggested that the extent of crystalline perfection is reflected in the gelatinization temperature. The degree of crystalline perfection according to these authors is impacted by the molecular structure of amylopectin (unit chain length, extent of branching, molecular weight, and polydispersity), starch composition (amylose to amylopectin ratio and phosphorous content), and granule architecture (crystalline to amorphous ratio).

DSC values are impacted by many aspects of sample preparation and instrument operation. Consequently, it is often difficult to compare data obtained from various DSC studies. It has been reported that the frequency of temperature modulation and the underlying heating rate significantly influenced the gelatinization temperatures and enthalpy changes in total and non-reversing endotherms. Soaking time is a significant factor in achieving reproducible DSC data (Chiang & Yeh, 2002). Tester and Morrison (1990b) indicated that low and high GT starches had very similar amylopectin chain lengths after debranching and on debranching of insoluble residues after lintnerization. The low GT starches could be annealed to behave like high GT starches, but the latter also responded a little to annealing. It was concluded that low GT starches have less crystallinity and less perfect cystallites than the high GT starches due to minor amylopectin structural differences. Jane et al. (1999) also reported that amylopectin branch chain lengths and distributions determine starch GT, enthalpy change, and pasting properties. Starch GT increased with increasing branch chain length. After more than a decade, the most studies agree that variation in rice GT is a result of differences in the proportion of amylopectin that is short versus long chains, thus degree of crystallinity is what is being measured as GT (Umemoto, Nakamura, Satoh, & Terashima, 1999).

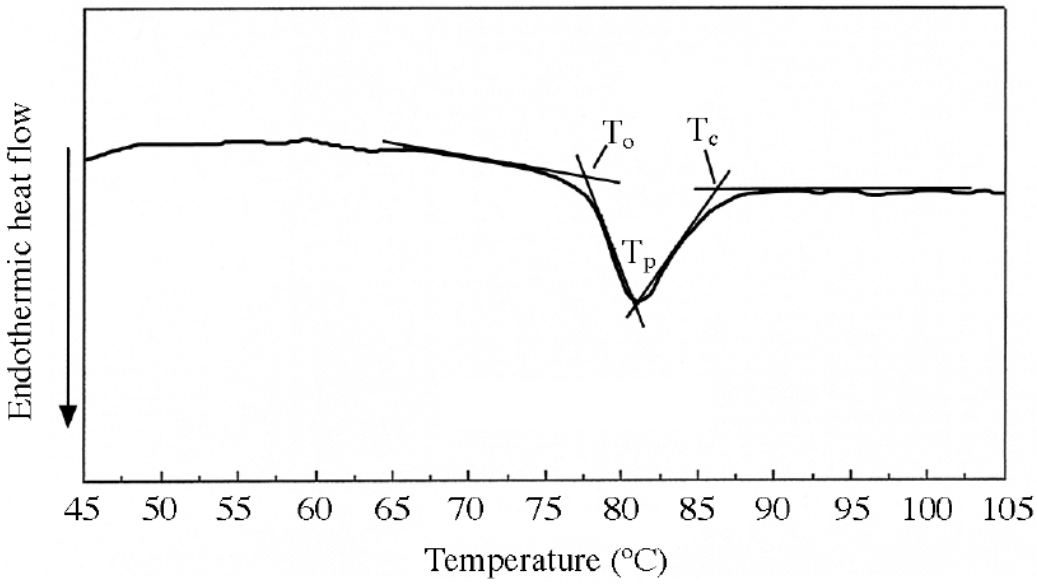


Figure 2.5 Thermal property of rice starch determined by differential scanning calorimetry (DSC). T_o: onset temperature, T_p: peak temperature, T_c: conclusion temperature (Bao & Bergman, 2004)

1.2.5.2 Pasting

Pasting is the phenomenon following gelatinization in the dissolution of starch. It involves granular swelling, exudation of molecular components from the granule, and eventually, total disruption of the granules (Atwell et al, 1988). When the starch granule is heated up to the GT in excess water, heat transfer and moisture transfer phenomena occur. The granule swells to several times its initial size as a result of the loss of the crystalline order and the absorption of water inside the granular structure. The pasting viscosity during swelling and gelatinization can be recorded using a Brabender Visco Amylograph, Rapid Visco Analyzer (RVA), or other viscometers, which record the viscosity continuously as the temperature is increased, held constant for a time, and then decreased (Figure 2.6). At the initial step, the viscosity increases rapidly with the increase of temperature as the granule swells. The peak viscosity is reached when granules swelling have been balanced with the granules broken by stirring. With continued stirring, more granules rupture and fragment, causing a further decrease in viscosity. On cooling, some starch molecules partially reassociate to form a precipitate or gel (retrogradation), in which amylose molecules aggregate into a network, embedding remnants of starch granules. Rice

starch pasting parameters have been reported to be correlated with amylose content (Bao, He, Xia, Chen, & Zhu, 1999; Vandeputte et al. 2003b; Noda, Nishiba, Sato, & Suda, 2003) but different in waxy rice compared to non-waxy rice.

The pasting viscosity of rice flour, compared to its starch counterpart is reportedly different due to the influence of proteins and lipids which are removed during the production of starch (Singh, Okadome, Toyoshima, Isobe, & Ohtsubo, 2000; Fitzgearld, Martin, Ward, Park, & Shead, 2003). Experiments that analyzed pasting viscosities before and after protein and/or lipid flour removal indicate that protein has a major influence on flour pasting viscosity (Martin & Fitzgerald, 2002; Fitzgerald et al., 2003). An increase of rice grain protein has been associated with decreased peak viscosity (Martin & Fitzgerald, 2002). They concluded that the decreases seen in peak viscosity during rice storage result from an increased number of disulfide bonds. Changes in protein content and disulfide bonds thus appear to be, at least in-part, controlling the differences seen in rice pasting viscosity during storage.

Rheological properties during rice starch gelatinization have been characterized by many researchers. Lii, Lai, and Tsai (1996b) reviewed their results and concluded that the starch granular properties are the major factors responsible for starch rheological behavior, followed by the degree of amylose leaching during the gelatinization process, especially in high concentration systems. The change in viscoelastic properties of rice starch suspensions during gelatinization can be placed into three or four transition stages: starch suspension into sol, sol transition to gel, network destruction and network strengthening. During the early heating of starch granules in water, the increase of storage modulus G' and $\tan\delta$ is relatively small, which indicates that amylose molecules are dissolved and the suspension has been transformed into a "sol"' (Sodhi & Singh, 2003). Then the G' and G'' increase to a maximum in which the temperature coincides with the onset temperature, which is attributed to the close-packed network of swollen starch granules. Tanδ decreases simultaneously, which indicates that a three-dimensional gel network has been constructed from amylose, reinforced by strong interactions among the swollen starch particles (Lii et al., 1996b; Sodhi & Singh, 2003). The G' increases during the gelatinization of rice starch are reported to be mainly governed by granule characteristics, which include swollen granule rigidity and the

interaction between the close-packed granules (Lii et al., 1996b). In the third stage, continued heating beyond T G', the G' decreases and tan δ increases, indicating that the gel structure has been destroyed during prolonged heating (Lii et al., 1996b; Sodhi & Singh, 2003). The destruction is likely due to the melting of the crystalline regions remaining in the swollen starch granule or results from the disentanglement of the amylopectin molecules in the swollen particles, which softens the particles. Another reason the network collapses may be due to the loss of interaction between particles and the network. The fourth stage, G' and G'' are reported to increase and tan δ increase even higher after an inflection point (Figure 2.7). These authors attributed the G' increase to the leached low molecular weight amylopectin, which interacted with the amylose matrix to strengthen the continuous phase (network). However, a larger tan δ indicated that the dispersed phase (particles) became softer due to continuing dissolution of amylopectin.

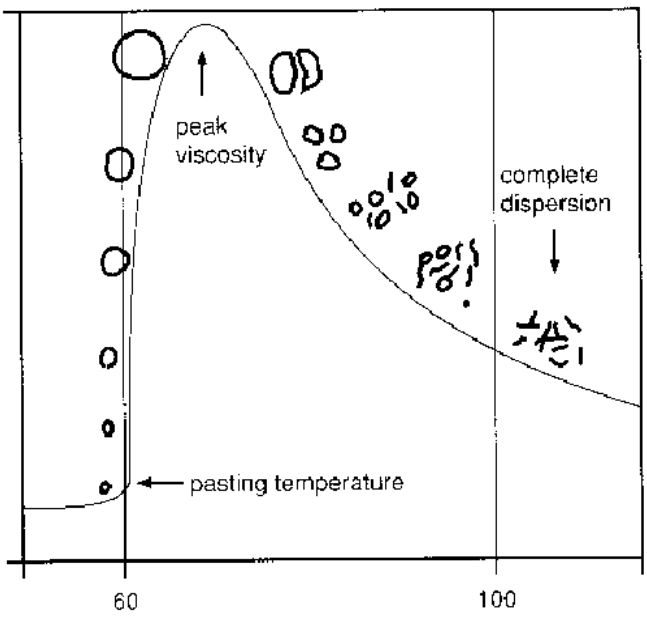


Figure 2.6 Swelling, disruption and dispersion of a starch granule during gelatinization (Sanders, 1996)

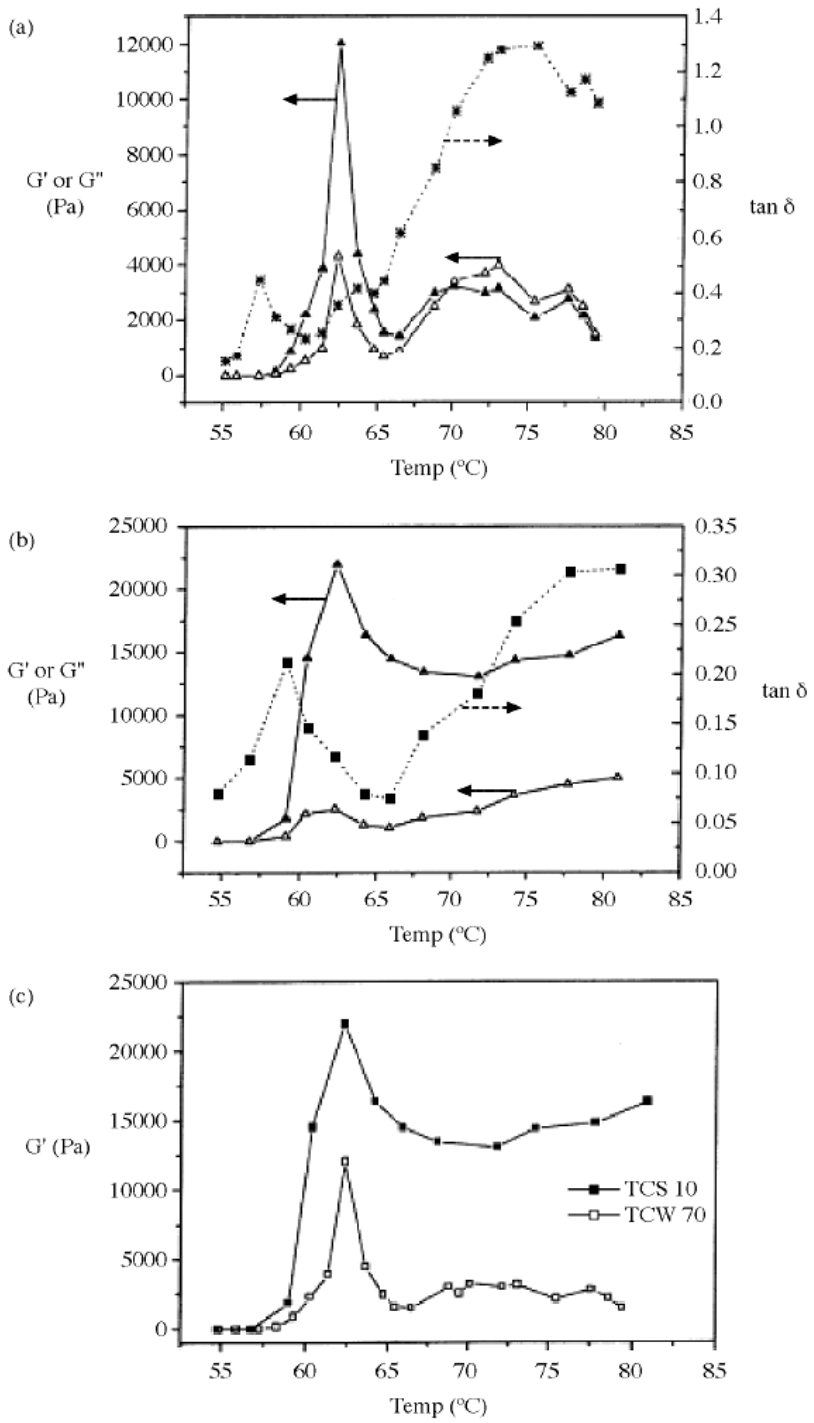


Figure 2.7 Temperature sweep data for gelatinization of 25% TCW70 (a), waxy rice and TCS10 (b), normal rice with amylose content of 17.1% rice starches suspension, and comparison G' of between TCW70 and TCS10 (c). Symbols: G' (-▲-), G'' (-□-) and $\tan\delta$ (-*-) (Bao & Bergman, 2004). Note: TCW70 and TCS10 are Taichung waxy 70 and Taichung Sen10, respectively.

1.2.5.3 Retrogradation

Retrogradation is a process which starch chains begin to reassociate in an ordered structure. Two or more starch chains initially form a simple junction point, which then may develop into more extensively ordered regions and ultimately, under favorable conditions, to a crystalline order (Atwell et al., 1988).

This phenomenon is generally regarded as a crystallization or recrystallization (i.e. formation and subsequent aggregation of double helices) process of amylose and amylopectin, after a heated starch paste cools to below the melting temperature of starch crystallites. This process results in viscosity increase, gel firming, syneresis and textural staling of predominantly starch-containing systems. Short-term retrogradation of amylose occurs within the continuous phase (a few hours) and subsequent long-term crystallization of amylopectin, which occurs at a much slower rate, (requiring several weeks) (Zhou, Robards, Helliwell, & Blanchard, 2002). The rapid initial rate of retrogradation relates to the loss of networked amylose, the development of amylose aggregates, and binding of granule remnants into assemblies by amylose and amylose aggregates. Amylopectin forms shorter double helices which can be attributed to restrictions imposed by the branching structure of the amylopectin molecules and the chain lengths of the branches. Because the amount of amylopectin in most starches is greater than amylose, most of the crystallites formed during starch retrogradation are related to the association of amylopectin chains and contributed the long term rheological and structural changes of starch systems (Lii et al., 1998; Zhou et al., 2002). The retrogradation properties can be measured by DSC, rheological properties, starch gel hardness, and NMR. Methods to study retrogradation of starch have been reviewed by Karim, Norziah and Seow (2000).

Tako and Hizukuri (2000) proposed some mechanisms for rice starch retrogradations which are based on the formation of hydrogen bonding at various molecular levels. It is assumed that intramolecular hydrogen bonding may take place between OH-6 and the adjacent hemiacetal oxygen atom of the D-glucosyl residues. Intermolecular hydrogen bonding may take place between OH-2 of the amylopectin and an adjacent O-6 of the amylose. Another intermolecular hydrogen bond may form between OH-2 of a D-glucose residue of the former molecule and O-6 of a D-glucose residue of a short side chain (A and B1) of the latter molecule. After

saturation of intermolecular hydrogen bonding between amylose and amylopectin molecules, an intermolecular association may also take between amylopectin molecules through hydrogen bonding. The mechanism of retrogradation is complicated because retrogradation rate may vary from one cultivar to another due to differences in the proportion and interaction of amylopectin and amylose, chain length distribution, and molecular size of branched molecules (Hizukuri, 1986; Eliasson & Gudmundsson, 1996).

2. Hydrocolloids (gums)

Hydrocolloids, or gums, are substances consisting of hydrophilic long-chain, high molecular weight molecules, usually with colloidal properties, that in water-based systems produce gels, i.e., highly viscous suspensions or solutions with low dry – substance content. The term 'hydrocolloid' refers to a contraction of hydrophilic colloid. Hydrocolloids are not really colloids, because they are truly water soluble, exhibiting certain of colloidal properties. This term also refers to a range of polysaccharides and proteins.

Hydrocolloids can have linear or branched molecules, as biopolymers. The linear type is the most abundant in nature and has sugar units that repeat over the entire length of the polymer. They usually have side chain units which greatly influence the properties of hydrocolloid. Most hydrocolloids occur naturally, but there are also several natural hydrocolloids that have been chemically modified. The commercially important hydrocolloids and their origin are given in Table 2.4.

When hydrocolloids are in solution, one can visualize a cylinder of organized water surrounding the molecule. The water molecules are oriented with respect to the hydroxyl groups found on the individual sugar units of the hydrocolloid molecule as shown in Figure 2.8. The main effects of hydrocolloids result from their ability to organize water and/or form networks. Visualize a hydrocolloid molecule as looking like a long, flexible piece of yarn. Now visualize a cylinder of water surrounding yarn, to some arbitrary distance, such that this layer of organized water of hydration actually moves around with the gum molecule. This water is organized in the sense of being associated with the long, thin gum molecule, particularly at hydroxyl group along the polysaccharide chain and at any of the anionic groups that present on some gums, and moves around with gum molecule to some extent. Increased associations generally lead to increases in volume and swelling. The chain length, or DP, influences a gum's viscosity and hydration rate. Long molecules tend to produce higher viscosities and take longer to hydrate than short ones. A highly branched molecule takes up less space than strength one with the same molecular weight and therefore provides less viscosity.

Nowadays, hydrocolloids are widely used in a variety of industrial sectors to perform a number of functions including thickening and gelling aqueous solutions,

stabilizing foams, emulsions and dispersions, inhibiting ice and sugar crystal formation and the controlled release of flavors, etc.

The functional properties of polysaccharides are generally considered with regard to the results that are expected (Table 2.5). In the simplest situations, functionality is the straight forward result of the properties of the biopolymer in an aqueous medium (i.e., its thickening or gelling properties). In many cases, however, when more complex properties have to be analyzed, the link between the behavior of the biopolymer and the expected property is less clear. This is the case, for example, for binding properties. Also, many times several mechanisms are involved. The functional properties of hydrocolloids lie mostly in the physicochemical mechanisms underlying their behavior in an aqueous medium. In other words, they are the result of macromolecule–water and macromolecule–macromolecule interactions, as well as interactions of macromolecular chains with the surface of the dispersed entities (solid particles, droplets, or gas cells) (Table 2.6).

All of these mechanisms are driven by the thermodynamics of the system. Water solubility is related to solvent quality-that is, the strength of interactions between the polysaccharide and water (the solvent) through hydrogen bonds created by means of hydrophilic (hydroxyl) groups along the macromolecular chain, dividing them into two categories (Figure 2.9): thickener molecules move about randomly and exhibit little interaction with each other, whereas gelling molecules form junction zones. Junction zones have to be created in order to yield the three-dimensional network that gives the solid-like character of the system despite its high water content. Thermodynamics is also at the basis of the peculiar properties of mixed biopolymer systems. Phase separation takes place if polymers are incompatible. The incompatibility should result in phase separation and should yield two separated phases at thermodynamic equilibrium; however, additional phenomena can take place, particularly when one or two components can form physical gels. This impedes the phase separation to be completed at the macroscopic level, and the final structure of the system is not at thermodynamic equilibrium. The result is a complex morphology yielding a specific texture, whether pleasant or unpleasant, for the consumer. Molecular binding between unlike polymers has been suggested as a possible mechanism underlying the properties of mixed polysaccharide systems resulting in

dramatic synergistic effects. These phenomena are not yet clearly understood, however, and the molecular mechanisms of binary biopolymer systems are still a matter of debate. In the case of dispersed systems, adsorption of the polymer onto the interface may be desired. This phenomenon can be the major mechanism responsible for the stabilizing effect. Water–solvent interactions are also generally involved. When part of the macromolecular chain cannot interact with water, due to the presence of numerous hydrophobic groups, adsorption onto the interface is favored. In contrast, if the overall polymer chain is hydrophilic, there will be no affinity between the polymer and the interface. In such a case, the polymer is excluded from the vicinity of the particle, and a depletion–flocculation phenomenon may occur (Gunning, Hibberd, Howe, & Robins, 1988; Cao, Dickinson, & Wedlock, 1990; Walstra, 1993). Particles are led to flocculate in order to organize themselves and hence to minimize the overall excluded volume.

Rheological properties of the every system are deeply changed when polysaccharides are present; therefore, rheological methods are the appropriate tools for studying the functional properties of hydrocolloids. The methods provide direct information on the molecular mechanisms underlying the properties. These mechanisms can be related to entanglement of macromolecular chains, the lifetime of interchain interactions, or the density of junction zones. Oscillatory shear measurements, relaxation tests, and creep-recovery tests have been used for understanding of the rheological behavior of polysaccharide systems.

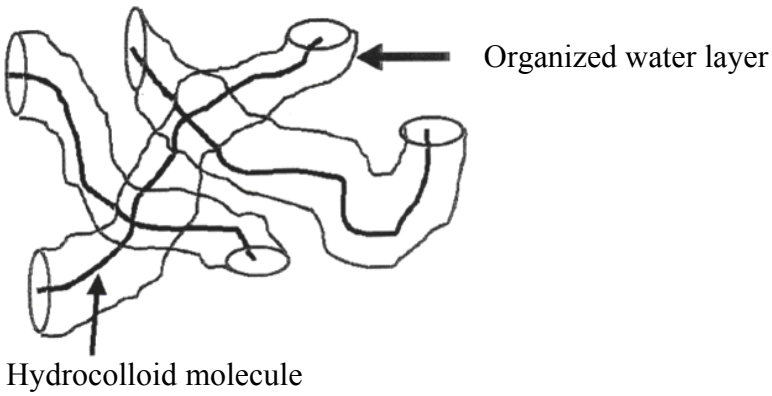


Figure 2.8 Hydrocolloid molecules surrounded by organized water (Hoefler, 2004).

Table 2.4 Source of commercially important hydrocolloids (Williams & Phillips, 2000)

Botanical	
trees	cellulose
tree gum exudates	gum arabic, gum karaya, gum ghatti, gum tragacanth
plants	starch, pectin, cellulose
seeds	guar gum, locust bean gum, tara gum, tamarind gum
tubers	konjac mannan
Algal	
red seaweeds	agar, carrageenan
brown seaweeds	alginate
Microbial	xanthan gum, curdlan, dextran, gellan gum, cellulose
Animal	Gelatin, caseinate, whey protein, chitosan

Table 2.5 Functional properties of hydrocolloids (Doublier & Cuvelier, 2006)

Function	Example
Thickening agent	Sauces, gravies, soups, salad dressings, creams, ice creams
Gelling agent	Desserts, confectionery, jams, jellies, restructured products, pet foods
Binding agent	Processed meat products
Emulsifier	Sauces, salad dressings, mayonnaise
Foaming or whipping agent	Whipped toppings, mousses
Stabilizer of emulsion, suspension, or foam	Salad dressings, mayonnaise, beverages, whipped toppings
Encapsulating agent	Powdered flavors
Film forming	Protective coatings
Flocculating or clarifying agent	Beverages
Crystallization inhibitor	Ice creams

Table 2.6 Main physicochemical mechanisms involved in the function of hydrocolloids (Doublier & Cuvelier, 2006)

Physicochemical Property	Functional Property
Macromolecule/solvent interactions	Solubility, swelling
	Viscosity increase, thickening
	Binding
	Stabilization
Macromolecule/macromolecule interactions	Gelling
	Binding
	Stabilization
Surfactant or macromolecule interactions with:	
Oil droplets	Adsorption, emulsification
Solid particles	Whipping, stabilization
Gas	—

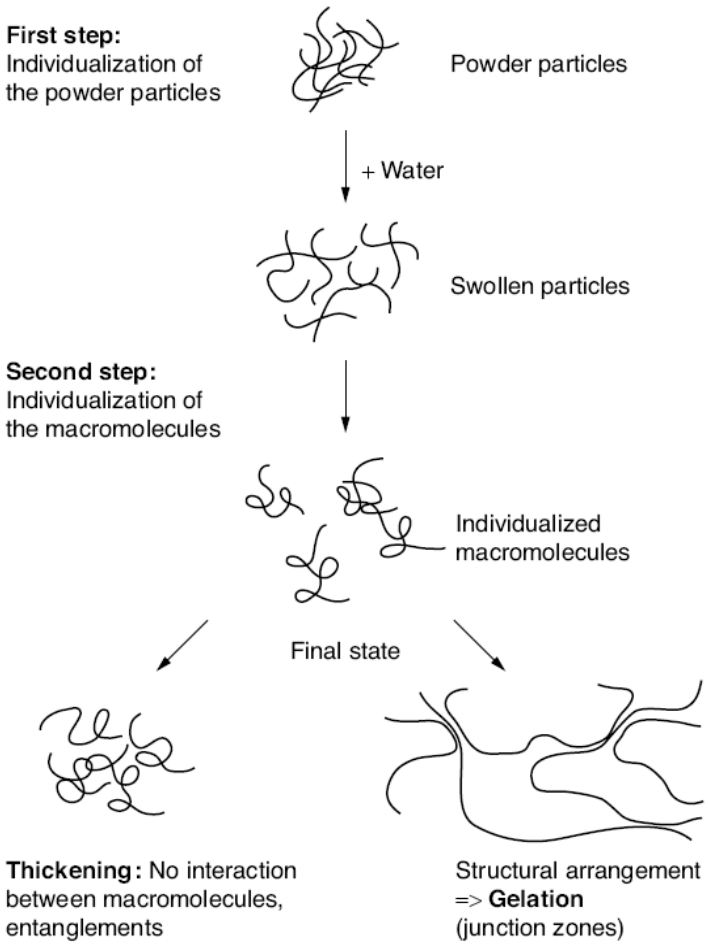


Figure 2.9 Schematic description of the different steps of the solubilization process of polysaccharides (Doublier & Cuvelier, 2006).

3. β-glucans

β-glucan is generally described as a polymer of glucose widely found in such sources as cereal grains, including oats and barley, as well as in yeast, bacteria, algae, and mushroom. It is attracting the increasing attention of the pharmaceutical and functional food industry because of its positive effects on human and animal health, and because it is a natural polysaccharide found in microorganisms and plants. Different source of β-glucan and fine structure are shown in the Table 2.7.

Table 2.7 Sources and fine structure of different β-glucans
(http://www.immunebody.com/zArchive/pg_md_whatis.html)

Beta Glucan Type	Structure	Description
Bacterial		Linear β1,3-glucan (Curdlan)
Fungal		Short β1,6 branched β1,3-glucan (*i.e.* Schizophyllan)
Yeast		Long β1,6 branched β1,3-glucan (WGP BetaGlucan, Betafectin™)
Cereal		Linear β1,3/ β1, 4-glucan (*i.e.* oats, barley, rye)

3.1 Cereal β-glucans

Polysaccharides serve the growing plant as a structural component that maintains the tissue integrity, as a conduit structure for the movement of water and low-molecular-weight solutes that help maintain osmotic pressure, and as a barrier against microbe and insect penetration. Pentosans and (1→3),(1→4)-β-D-glucans are the major components of cereals, and cellulose is a minor one; however, this study will primarily on the β-D-glucans. In foods, they control rheological properties, water binding, and the sensory perception of texture, and they are important sources of nutrients and dietary fiber (Fincher & Stone, 1986).

3.1.1 Origin of cereal β-glucans

β-glucans occur in grasses of the *Poaece* family. They are constituents of the cell wall and appear during cell expansion (Carpita & Gibeaut, 1993; Fincher,

1992; Fincher & Stone, 1986; Carpita, 1996). β-glucans are also present in the cell wall of certain cereal grains, particularly those of oats and barley (Fincher, 1992; Fincher & Stone, 1986; Wood, 1986).

In barley and oats, β-glucans are the main non-starch polysaccharide, typically forming anywhere from 2 to 7% by weight of the grain (McCleary & Glennie-Holmes, 1985; Fincher & Stone, 1986; Wood, 1986, 1994; Newman, Newman, & Graham, 1989). In oats, the bran contains from 5–10% β-glucan (Beer, Arrigoni, & Amado, 1996). In barley (*Hordeum vulgare*), the endosperm cell walls are composed of about 70% β-glucan (Fincher & Stone, 1986) whereas the aleurone cell walls are about 20% (Bacic & Stone, 1981). To be able economically to extract and purify β-glucan, cultivars having high β-glucan contents are desirable.

β-glucan was not used as a general food hydrocolloid until the 1990s, even though there has never been a shortage of suitable raw material for β-glucan production. The reason that β-glucan has not been promoted for use in foods has been due to the considerable cost of extracting and purifying the β-glucan.

3.1.2 Extraction and purification

Traditional methods for extracting β-glucans from oats or barley flours have involved three key steps (Wood, Weisz, Fedec, & Burrows, 1989). Firstly enzymes present in the flour are deactivated to decrease hydrolysis of the β-glucan to lower molecular weight products. Then warm or hot water is used to extract the β-glucan from the flour. Lastly, the spent flour is removed, and a β-glucan containing gum is recovered by precipitation on addition of a water-miscible organic solvent. The gum contains about 40–60% β-glucan and has an average molecular weight between 3×10^5 and 10^6. Variations exist on this basic process and include extraction under alkali conditions and hydrolysis or precipitation of solubilised starch and protein after the extraction step, which increases the quantity of β-glucan in the gum (Bhatty, 1993, 1995). Consequently, the presence of impurities (such as starch, protein and lipid) may affect the functionality of β-glucan solution when incorporated into real food systems, but the same will be true for high-purity β-glucan gums because real food systems are multicomponent and possible interactions or incompatibilities between β-glucan and other food components are not sufficiently known.

3.1.3 Molecular structure

β-glucans are unbranched polysaccharides formed of glucopyranosyl units joined by groups of contiguous (1→4)-β-linkages and isolated (1→3)-β-linkages (Figure 2.10). Isolated (1→4)-β-linkages never occur, instead most of the (1→4)-β-linkages are in groups of two or three. This forms the main structural motif: chains of cellotriosyl (DP3) and cellotetraosyl (DP4) residues, joined by single (1→3)-β-linkages. The β-glucan thus has a cellulose-like backbone but contains kinks at the position of the (1→3)-β-linkages. The kinks disrupt the strong hydrogen bonding network that is normally found in cellulose, thus unlike cellulose the cereal β-glucans can be dissolved in water (Morgan, 2000).

The structural sequence of β-glucan has been probed in some details (Yin & MacGregor, 1989; Woodward, Phillips, & Fincher, 1988; Izydorczyk, Macri, & MacGregor, 1998a, 1998b; Wood, Weisz, & Blackwell, 1991). The enzyme, lichenase, solely cleaves the (1→4)-β-linkage immediately following a (1→3)-β-linkage. Lichenase treatment of the β-glucan generates a series of oligomers that have the same number of glucopyranosyl residues as the cello-oligomer residues in the original β-glucan polymer (Figure 2.11), although they contain a (1→3)-β-linkage at the end of the chain instead of a (1→4)-β-linkage. The oligomers can be characterised by chromatography to obtain the distribution of the cello-oligomers in the original β-glucan (Figure 2.12). About 90% of the β-glucan consists of cellotriosyl and cellotetraosyl oligomers joined by (1→3)-β-linkages. The other 10% contains cello-oligomeric residues having a higher DP (Izydorczyk, Macri, & MacGregor, 1998a, 1998b). Cello-oligomers having DP in the range of five to nine are the most common. Thus β-glucan in the native state, that is within the cell wall, consists mainly of cellotriosyl and cellotetraosyl residues joined by single (1→3)-β-linkage, but incorporates longer cello-oligomers up to at least DP 19. Ratios of cellotriosyl (DP3) to cellotetraosyl (DP4) residues in β-glucan are in the ranges from 2.1 ± 0.1 for oats, 3.2 ± 0.3 for barley and 3.5 ± 0.4 for wheat and vary according to the temperature and conditions under which the β-glucan is extracted (Wood, Weisz, & Blackwell, 1991).

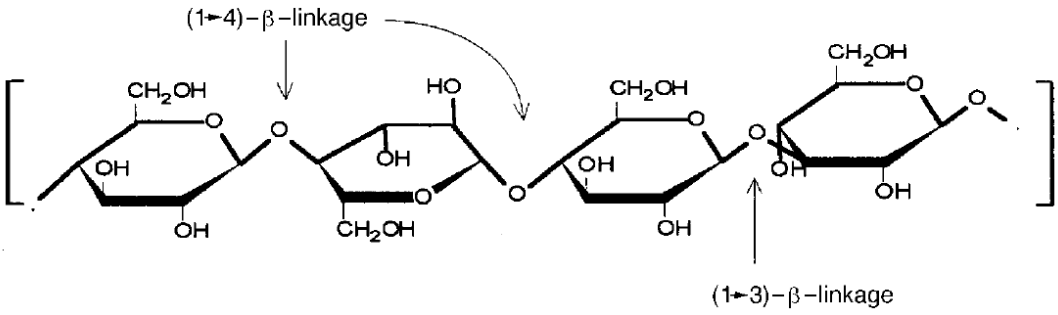


Figure 2.10 Molecular structure of a mixed linked (1→4),(1→3)-β-glucan (Morgan, 2000)

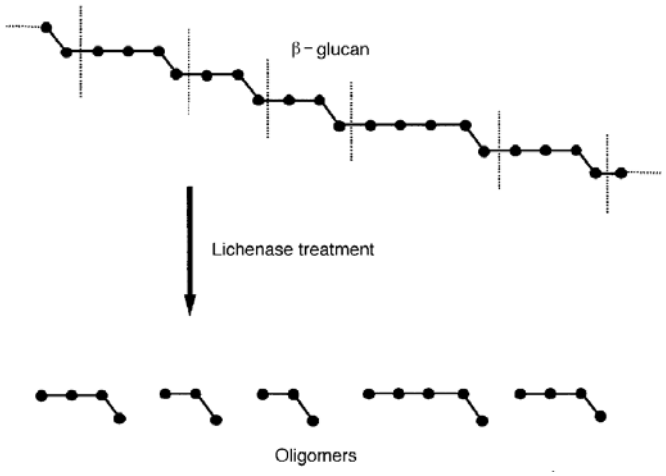


Figure 2.11 Lichenase treatment of a β-glucan (horizontal lines are (1→4)-β-linkages, angled lines (1→3)-β-linkages and vertical dashed lines are the site of lichenase hydrolysis) (Izydorczyk, Macri, & MacGregor, 1998).

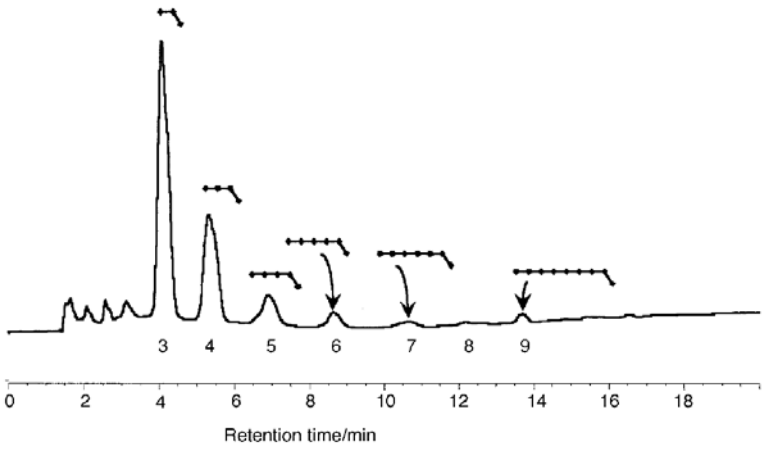


Figure 2.12 High-perfomance ion-chromatography of oligomers released from lichenase treatment of barley β-glucan (Izydorczyk, Macri, & MacGregor, 1998).

3.1.4 Solubility

The solubility of β-glucan is dependent on the fine structure. Most water soluble β-glucans contain approximately 30% (1→3) and 70% (1→4) linkages, which are organized into blocks of two or three (1→4)-linked residues separated by single (1→3)-linked residues (Clarke & Stone, 1963). The higher solubility of mixed-linkage β-glucans than cellulose is due to the presence of (1→3)-β-bonds, which introduce irregularity into the structure. (1→3)-β-D-glucan forms a helix. A few blocks of adjacent (1→4) linkages in the chain relative to water-soluble barley β-glucan, and the result is a regular shape, which permits more extensive aggregation and gives it poor solubility in water (Reese & Perlin, 1963).

Evidence for the occurrence of two or more adjacent (1→4) linkages has been reported for barley (Woodward, Fincher, & Stone, 1983; Luchsinger, Chen, & Richards, 1965) and oat (1→3),(1→4)-β-D-glucans (Wood, Weisz, & Blackwell, 1991). The (1→4) linkages render the polysaccharide more insoluble, and the differences in the number of consecutive (1→4) linkages may explain the differences in solubility of some β-glucan preparations (Woodward, Phillips, & Fincher, 1988; McCleary, 1988). Results describing the occurrence of consecutive (1→3)-linked units in barley and oat β-glucan are contradictory (Woodward, Fincher, & Stone, 1983).

While bound to protein, the mixed-linkage β-glucans are insoluble. An enzyme, acidic carboxypeptidase, which is present in raw barley, solubilizes the water-insoluble β-glucan in barley (Bamforth, Martin, & Wainwright, 1979). Comparison of the solubility of β-glucans in different cereals is hampered by variations in the experimental conditions and particle size. In general, the order of solubility of β-glucans is oat > barley > wheat (Wood, 1993). Solubility seems to correspond with the ratio of cellotriosyl to cellotetraosyl (DP3/DP4) units in the cereal β-glucan structure (approximately 4, 3, and 2 for wheat, barley, and oat β-glucan, respectively) and with molecular weight (Lazaridou et al., 2003). Drying of isolated barley cell walls and isolated β-glucan preparates reduces the solubility (Palmer & Bathgate, 1976). Nevertheless, Woodward, Phillips, and Fincher (1988) suggested that small differences in the fine structure determine the solubility; however, the fractions also differed in protein and uronic acid levels, so the contribution of different factors to the solubility remains unclear.

3.1.5 Molecular weight

The molecular weight is a fundamental parameter characterizing a polysaccharide and determining its rheological properties. Molecular weight distributions of polydisperse polymers are commonly estimated by gel filtration chromatography. There was the variation in molecular weights obtained for mixed-linkage β-glucans (Autio, 2006). This large variation is due to the diversity of the methodology used for the determination of molecular weight and the extraction protocol (solvents, conditions, and sample history). Low molecular weight β-glucans in solution exhibit fairly rapid aggregation, which can occur during extraction (Lazaridou et al., 2003). In general, the β-glucans of oat have the highest molecular weights, followed by those of barley, malt, and rye.

3.1.6 Rheological properties

The flow properties of oat β-glucans have been studied with the help of rotational law constants of a β-glucan solution at 25°C as a function of concentration. Oat β-glucans exhibit strong shear thinning behavior in the shear rate range of 20 to 1600/s but no thixotropy. The shear thinning is greater at higher concentrations (Doublier & Wood, 1993). The viscosity of β-glucans decreases strongly during heating but is recovered during cooling. With lower molecular weights, a decrease in viscosity and shear-thinning properties has been observed (Vaikousi, Biliaderis, & Izydorczyk, 2005).

For viscoelastic behavior, the mechanical spectra of β-glucan solutions are typical of concentrated solutions: At low frequencies, $G'' > G'$, and at high frequencies $G' > G''$; both moduli increase with frequency (Autio, 1988). As the polymer concentration is increased, the transition from solid- to liquid-like responses moves to lower frequencies, a characteristic property of solutions in which the rheology is mainly governed by the degree of entanglement of macromolecules (Doublier & Wood, 1993). Low-shear-rate viscosity and viscoelastic measurements have shown that oat β-glucan is rheologically behaves like a random-coil, nongelling polymer in aqueous solution at concentrations between 0.1 and 2%.

3.1.7 Gel formation

Gel formation results from hydrogen bonds forming between β-glucan chains during gelation. According to a popular model, a plausible cause for aggregation of (1→3)(1→4)-β-D-glucans would be the cellulose-like sequences of more than three contiguous β-(1→4)-linked glucosyl units which stick together and lead to gelation (Fincher & Stone, 1986). An alternative model for gelation has been proposed lately, according to which association of consecutive cellotriose units (linked by β-(1→3) bonds) may form extended junction zones and lead to the development of a gel network structure (Böhm & Kulicke, 1999). Gel formation can also occur at low temperatures and gel strength increased with increased concentration. Low-molecular-weight barley and oat β-glucans in solution aggregate rapidly, leading to a network structure. The gelling rate increased with decreased molecular weight. The lower molecular weight suggested the higher the G' and the faster the gelling. Doublier and Wood (2003) suggested that the lower molecular weight molecules are more mobile, and cellulose-like blocks in the molecules achieve the orientation necessary for aggregation. However, Lazaridou et al. (2003) suggested that molecular size rather than fine structure determines the gelling behavior. In their study, samples with the lowest proportion of long cellulosic-like chain segments showed the greatest tendency to gel.

3.1.8 Physiological properties and health benefits

Cereal β-glucan products have captured the attention of both industry and the scientific community because the soluble β-glucans abundant in certain cereals (especially oat and barley) have been shown to have physiological effects on human health and diet (Wood, 2007). Soluble mixed-linkage β-glucan has a high viscosity in water (Wood, 1986; Autio, Myllymäki, & Mälkki, 1987), and it has been suggested that the creation of viscous conditions within the small intestine is one of the mechanisms involved in the lowering of postprandial blood glucose and insulin levels (Wood et al., 1994) and in hypocholesterolemic, that is cholesterol-lowering, responses to oat and barley in animals and in humans (Bengtsson, Åman, & Graham, 1990; Lund, Gee, Brown, Wood, & Johnson, 1989; Anderson & Bridges, 1993).

However, only a small part of the daily diet is likely to include foods containing β-glucan. The major health benefit is therefore likely to be from replacing some or all of the fat in the diet with a low calorie alternative without compromising taste or texture. For hydrocolloid use these β-glucans products should be recognized primarily for the functionality they impart to foods, and secondly to the reduction in fat they engender in processed foods, rather than to any other perceived health benefit.

3.2 Bacterial β-glucan (Curdlan)

Curdlan is an extracellular microbial polysaccharide and was first discovered and investigated by Harada et al. in 1964. (Harada, Masada, Fujimori, & Meada, 1966; Meada, Saito, Masada, Misaki, & Harada, 1967). Curdlan is composed entirely of 1,3-β-D-glucosidic linkages, which occur widely in nature involved in cell structure and food storage in bacteria, fungi, algae and high plants (Deslandes, Marchessault, & Sarko, 1980).

Curdlan has utility as a food additive in its ability to form an unique elastic gel. It forms a heat-set gel at both relatively high and low temperatures or on neutralisation or dialysis of alkaline solution of curdlan (Konna & Harada, 1991; Konna et al., 1994). In addition, curdlan is believed to show strong bioactivities (Sasaki, Abiko, Sugino, & Nitta, 1978; Leao, Buchi, Lacomini, Gorin, & Oliverira, 1997; Gao et al., 1997). Because of its unique properties, curdlan has been the subject of remarkable investigation especially for use in the food industry.

Curdlan is tasteless, odorless and colorless. There are several applications of curdlan used as a food additive or an ingredient in procedures for food production. For example, it is applied as moisture and shape retention in cake and ice cream, gelling and freeze-thaw stabilizer in jellies and fabricated foods, water holding, texturing and binding agent in meat product and processed cooked foods (Miwa, Nakao, & Nara, 1994).

3.2.1 Production

Curdlan is produced in a fermentation process from the mutant strain of the bacteria *Alcaligenes faecalis* var. *myxogenes* 10C3 which can be isolated from soil (Harada, Masada, Fujimori, & Meada, 1966; Meada et al., 1967). Commercial curdlan may contain cellular debris, proteins and nucleic acids and other organic acids.

3.2.2 Molecular structure and granule

The chemical structure of curdlan is shown in Figure 2.13 (a). Curdlan is one of the biopolymeric molecules known as 1,3-β-D-glucans with low molecular weight ($M_w \approx 4\text{-}7\times10^4$) (Harada et al., 1968). Such a polysaccharide is characterised by repeating glucose subunits joined by a β-linkage between the first and third carbons of the glucose ring, which differs only in the linkage manner of repeating units from cellulose. Curdlan is a neutral polysaccharide without acidic components. The number-average degree of polymerization (DP) of curdlan is about 450 (Konna & Harada, 1991; Konna et al., 1994).

In the solid state, curdlan may exist in a triple helical structure. Curdlan has been shown to have a triple helical structure. In its natural state, curdlan is poorly crystalline and is found as a granule in the form of doughnut shaped structure (Kanzawa, Harada, Koreeda, & Harada, 1987) as shown in Figure 2.13 (b). The granule is insoluble in distilled water due to the ionisation of hydrogen bonds. There is extensive hydrogen bonding that is holding the granule together, most likely by strongly binding the helices to form microfibrils and then binding together the microfibrils. When these bonds are broken through swelling, the granule loses its structure, i.e., the microfibrils have dissociated from each other during hydrolysis.

a **b**

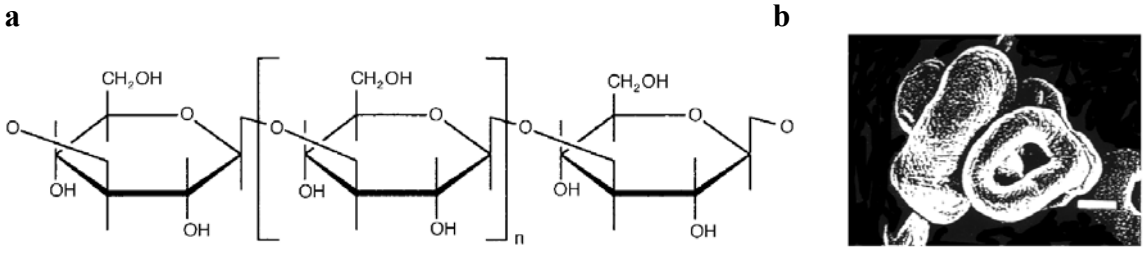


Figure 2.13 Chemical structure of curdlan (a) (Nishinari & Zhang, 2000); Electron micrograph of curdlan granule (b) (Kanzawa, Harada, Koreeda, & Harada, 1987)

3.2.3 Functional properties

3.2.3.1 Solution properties and conformations

While the primary structure is a long linear chain, curdlan forms more complex tertiary structures due to intra- and intermolecular hydrogen bonding. Curdlan is not soluble in water at room temperature but dissolves in an alkaline aqueous solution, cadoxen [Tri (ethylene diamine) hydroxide] aqueous solution and DMSO (dimethyl sulfoxide). The water insolubility of curdlan may be attributed to the existence of extensive intra- and intermolecular hydrogen-bonded crystalline domains like that of cellulose (Imeson, 1997). It has been reported that 1,3-β-D-glucans with a very low degree of polymerisation of below 25 DP is soluble in water (Ogawa & Tsurugi, 1973) and it was suggested that curdlan is soluble in water at elevated temperatures (Kanzawa, Harada, Koreeda, & Harada, 1987) At present, however, there is no direct evidence to clarify the water solubility of curdlan though an aqueous suspension of curdlan becomes clear when heated at above 55ºC.

3.2.3.2 Gel formation and properties

Curdlan can form a gel through the heating process alone, rather than relying on accompanying conditions such as pH, sugar concentration, or the presence of cations. Although curdlan aqueous gels can be formed by various methods, heat treatment is generally used. A curdlan aqueous suspension is capable of forming two types of gels depending on heating temperature, one of which is a thermo-reversible gel termed as a low-set gel formed by heating up to about 55ºC then cooling, and the other thermo-irreversible gel termed as a high-set gel formed by heating at above 80ºC. This change is explained by the hypothesis that microfibrils dissociate at 60ºC as the hydrogen bonds are broken, but then reassociate at higher temperatures as hydrophobic interactions between the curdlan molecules occur.

The physical properties of aqueous curdlan gels were studied extensively. The gel strength increases with concentration of curdlan (Meada et al., 1967). The gel strength is strongly dependent on heating temperature and it is found that the strength of a gel formed by heating for 3 min. at 90ºC is much greater than that obtained by heating for 4h at 70ºC. The high-set gel (Konno et al., 1994) has the

properties of being much stronger and more resilient and syneresis (i.e. exude water because of the shrinkage of the gel) than the low-set gel and neutralised gel. It has been reported that tannin, sugar and starch are capable of reducing the syneresis of curdlan gel (Harada, 1992; Nishinari, Hirashima, Miyoshi, & Takaya, 1998).

3.2.3.3 Molecular conformations

The molecular crystal structure of the anhydrous form of curdlan is composed of a triple stranded helix. The three strands of the glucan helix are parallel, right-handed and in phase along the helix axis, and the crystal structure is extensively hydrogen-bonded (Chuah, Sarko, Deslandes, & Marchessault, 1983).

Fulton and Atkins (1980) investigated the molecular structure and gelling mechanism of curdlan using X-ray diffraction and infra-red spectroscopy. They proposed that triple helices are dominant for most curdlan molecular chains. The triple stranded molecules are bound by hydrogen bonding to the interstitial water of crystallisation to form a micellar domain, in other words, interstitial water forms a hydrogen bonded network with the triple helices, binding them into a micellar structure (Figure 2.14a). The gelling mechanism of curdlan involves the interactions between these micelles and not the untwining and retwining of single helices into triple stranded junction zones. It is the association of these micelles which forms the junction zones of the gel network (Figure 2.14b).

Harada and co-workers (Konno et al., 1994; Okuyama et al., 1991; Kasai & Harada, 1980) indicated that the helix structure was transformed from single strand to triple strands at higher temperatures. An elaborate interpretation of structural change was proposed as shown in Figure 2.15. The gelation mechanism of the low-set gel is different from that of the high-set gel. For a low-set gel, curdlan micelle interior is packed mostly by 7/1 single helical molecules which are hydrogen-bonded to one another by water molecules and some parts of the micelle are occupied by triple helical molecules which are also hydrated, whereas for a high-set gel, curdlan molecules change their conformation to 6/1 triple helices and curdlan micelle is occupied by molecules of triple-stranded helix in which hydrophobic interactions between curdlan molecules take a predominant contribution to the formation of the gel.

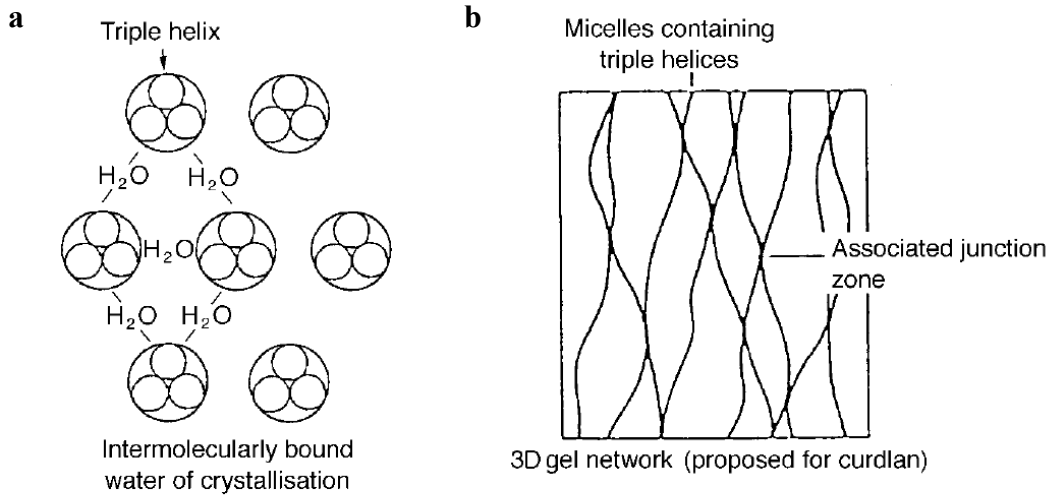


Figure 2.14 Schematic gel network of curdlan (Fulton & Atkins, 1980)

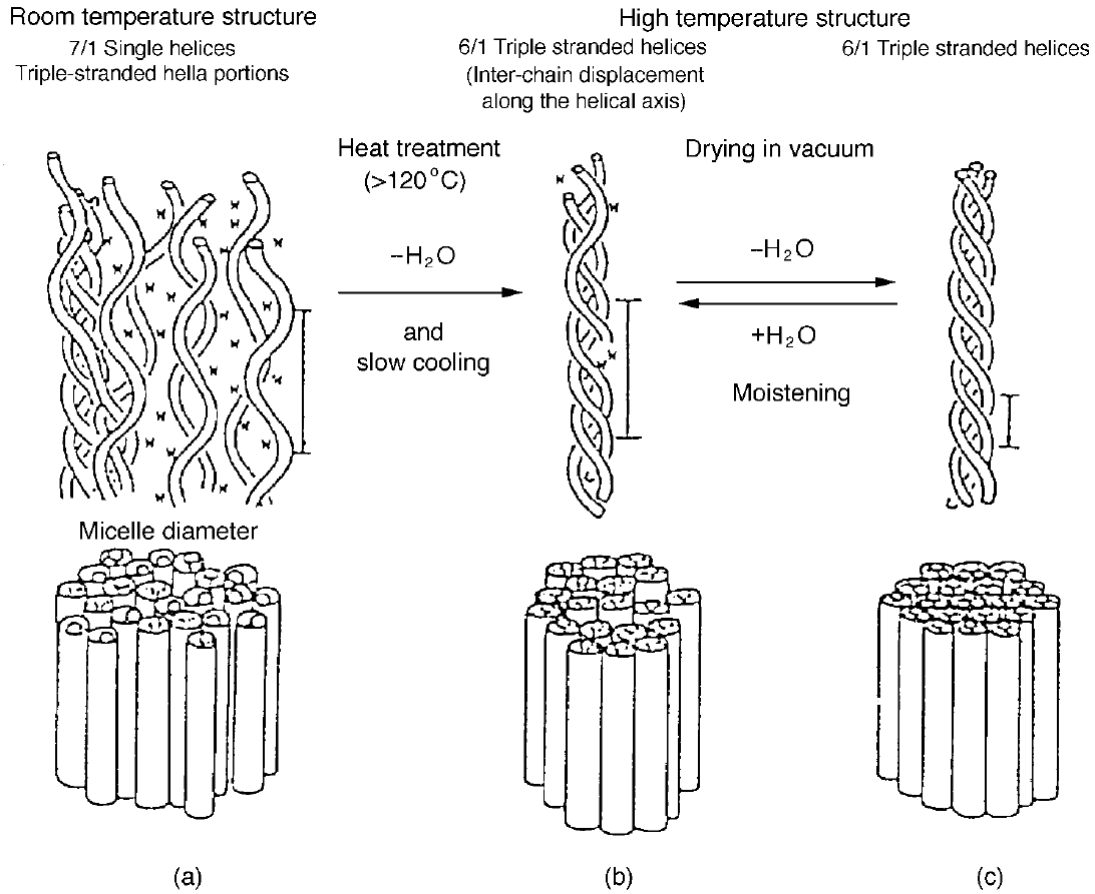


Figure 2.15 Schematic representation of structural change between three forms of curdlan (a) room temperature structure; (b) high temperature structure at high humidity; (c) high temperature structure at low humidity (Kasai & Harada, 1980).

3.2.3.4 Thermal and morphological analysis

According to thermal analysis, the DSC curves of original curdlan in aqueous suspension show a sharp endothermic peak at 50–64ºC, a shallow endothermic peak at 70–100ºC and another endothermic peak at ca. 150ºC (Figure 2.16) (Watase & Nishinari, 1994). The first endothermic peak is ascribed to swelling of curdlan due to the breakup of some hydrogen bonds and the second the occurrence of hydrophobic interaction between curdlan molecules. As for the endothermic peak at ca. 150ºC, it might be caused by the structural change of curdlan gels by further heating, corresponding to some molecular conformation transformation mentioned above.

Though, curdlan may be considered as the best polymer to clarify the mechanism of gel formation due to its neutral characteristics. It has been suggested that curdlan can exist as a triple helix, single helix, single chain, or a random coil depending mainly on crystallinity of curdlan, heating temperature and type and concentration of solvents used.

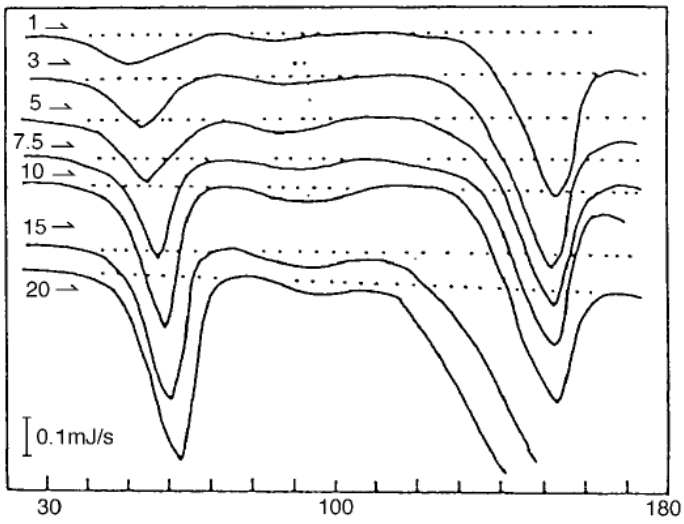


Figure 2.16 DSC heating curves of curdlan aqueous dispersions at various concentrations. Figures beside each curve represent the curdlan concentration in wt% (Watase & Nishinari, 1994).

3.3 Yeast β-glucan

Among β-glucan sources, mushrooms and yeasts are also known to contain 1,3-β-D-glucosidic linkages (Harada, 1992). β-glucan from yeast cell wall (*Saccharomyces cerevisiae*) is an important source of which contains about 55 – 65% of β-glucan (Klis, Mol, Hellingwerf, & Brul, 2002).

3.3.1 Molecular structure

The major component of yeast β-glucan (about 85%) is a backbone chain of β-1,3 glucan and the minor component (about 3%) is a branched β-1,6 glucan (Manners et al., 1973). Yeast β-glucan is sometime designed as β-(1,3/1,6) glucan. Molecular structure of yeast β-glucan are shown in Figure 2.17, respectively.

3.3.2 Physicochemical properties

β-glucans has demonstrated advantages in improving the physical properties of foods as a thickening and water holding agent, also it is a good emulsifying stabilizer and fat replacer, it has a good fat-like mouthfeel (Reed & Nagodawithana, 1991 ; Temelli, 1997; Temelli & Burkbus, 2000; Thammakiti et al., 2004; Worrasinchai, Suphantharika, Pinjai & Jamnong, 2006). In addition, β-glucan is nutritionally non-functional in the human digestive tract and, therefore, functions as a non caloric food. (Temelli & Burkus, 2000). Recently, there is a report which clarifies that the gelatinization and retrogradation characteristics of the rice starch are largely modified by the spent brewer's yeast β-glucan addition and the extent of this effect depended upon the β-glucan content (Satrapai & Suphantharika, 2007).

3.3.3 Functional properties

β-glucan has many reports about their bioactive and medical properties. The mechanism of the effect of β-glucan is not yet fully understood and probably depends on the specific molecular structure. In previous studies, the β-glucan showed very interested affects, such as immune-stimulating, anti-inflammatory, anti-microbial, anti-infective, anti-viral, anti-tumoral, cholesterol-lowering, radioprotective and wound healing (Kogan, 2000; Stone & Clarke, 1992).

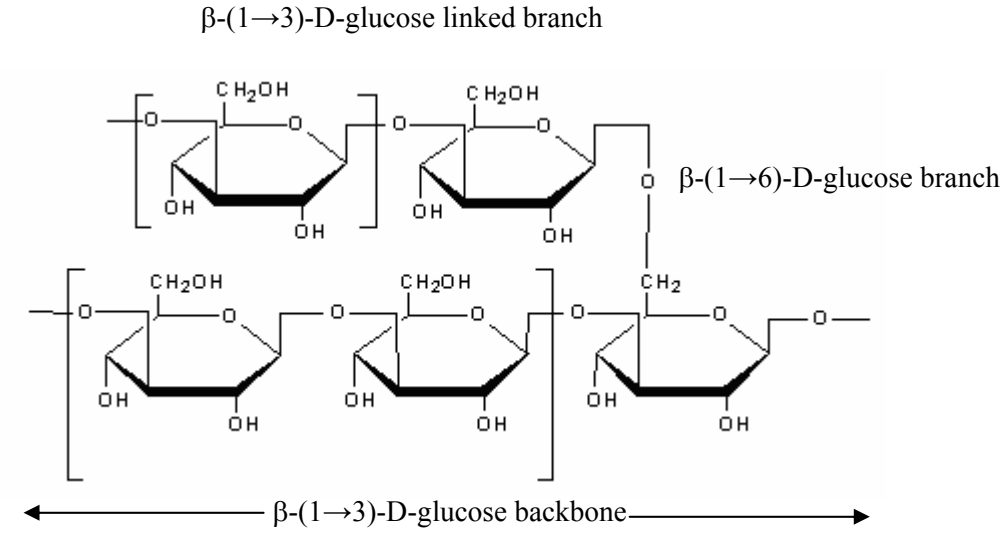


Figure 2.17 Molecular structure of yeast β-glucan (http://www.beta13dglucan.org/)

4. Interaction between starch and hydrocolloids

The gelatinization and retrogradation properties of starches can be modified by addition of a small amount of hydrocolloids (Alloncle et al., 1989; Biliaderis et al., 1997; Christianson et al., 1981 Ferrero et al., 1994; Yoshimura, Takaya, & Nishinari, 1999), resulting in an increase of peak viscosity, influences the retrogradation rate (Kohyama & Nishinari, 1992) improvement of syneresis and freeze thaw stability (Yoshimura et al., 1998, 1999), a change of phase transition temperature range and melting enthalpy of starch crystallites (Biliaderis et al., 1997), and an increase of dynamic modulus (G') (Liu & Lelievre, 1992).

A mechanism of the interaction of starches with hydrocolloids has been suggested (Christianson et al., 1981; Sajjan & Rao, 1987) involves the formation of soluble starch-gum associations, contributing to the increase viscosity. Hydrocolloids interact with amylose outside the starch granule to produce a more complex matrix of amylose and hydrocolloid surrounding the gelatinized granules. An increase in starch peak viscosity in the presence of hydrocolloids has been reported previously (Alloncle et al., 1989; Sasaki, Yasui, & Matsuki, 2000b; Satrapai & Suphantharika, 2006). Christianson et al. (1981) attributed the increase in viscosity to interaction between exudates from the starch granule (solubilized amylose and low-molecular weight amylopectin) and gums. A second explanation given was that addition of thickening gums enhanced the forces being exerted on the starch-water suspension with equal starch concentrations. Alloncle et al. (1989) proposed a model to interpret these effects in which the gums were located within the continuous phase of the starch pastes. In this model, the volume of the continuous phase accessible to the gum was reduced due to swelling of the starch granules during pasting, yielding an increase in gum concentration within the continuous phase, which was accompanied by a dramatic increase in overall viscosity.

The possible molecular interactions between starch and non-starch polysaccharides include the exclusion mechanism (Annable, Fitton, Harris, Phillips & Williams, 1994; Biliaderis et al., 1997; Morris, 1990) involves phase separation of biopolymer mixtures in aqueous solutions. It is a common phenomena due to incompatibility between unlike polymers, amylose and hydrocolloids, coexisting in the matrices. This process greatly enhances the concentration of each component in its

respective microdomain and thereby brings about a substantial enhancement in the viscosity of the mixed system with enhanced firmness of the mixed gel. The latter has often been described as reflecting a synergistic interaction between two polymers, but in reality it is rather than the thermodynamic incompatibility between two polymers that forces them to partition in two separate microphases and alters the rheological of the composite network.

Christianson et al. (1981) suggested that the presence of hydrocolloid in the starch media also influences the physical properties of starch granules such as shape, granule integrity, and exudates from starch granules, resulting in an earlier onset viscosity in amylograms compared with starch control. Shi and BeMiller (2002) stated that interactions between certain leached molecules, primarily amyloses, and certain hydrocolloids, were responsible for the viscosity increase occurring before starch and hydrocolloids were previously observed by Bahnassey and Breene (1994), and these were dependent on the hydrocolloid structure so structural and rheological properties of the hydrocolloid are very relevant.

When a starch/hydrocolloid mixture is used as a texture modifier, understanding of its rheological and thermal properties is important to improve the formulation of starch-based food. Thus, various studies on rheological and thermal properties of mixtures between starches and hydrocolloids have been reported (Shi & BeMiller, 2002; Sudhakar, Singhal, & Kulkarni, 1995). In general, the viscosity of a mixed system is greatly higher than starch alone since most biopolymers are strongly hydrophilic and compete with starch for water (Christainson et al., 1981; Sudhakar, Singhal, & Kulkarni, 1996). The extent of starch granule swelling or melting of crystalline parts during gelatinization is influenced by the presence of hydrocolloids and synergistic interaction between hydrocolloids and starch may be anticipated (Shi & BeMiller, 2002).

However, retrogradation of starch/ hydrocolloid mixed gel can be promoted at very early stage of storage results from the immobilizing water property of hydrocolloid. The promotion of retrogradation is attributable to increase in the effective concentration of starch or more specifically amylose in the continuous phase rather than the formation of some gelled or ordered structures between starch and hydrocolloid, leading to acceleration of amylose or short chain amylopectin gelation.

Hydrocolloid subsequently inhibits the progress of long-term retrogradation, which involves the crystallization of amylose or amylopectin as well as the co-crystallization between these two fractions during storage (Yoshimura et al., 1996; Funami et al., 2005).

To characterize thermal transition in starch systems (gelatinization, retrogradation, and glass transition), differential scanning calorimetry (DSC) was used to determine. Khanna & Tester (2006) reported that gelatinization temperature of starch-konjac glucomannan tended to be shifted to higher temperatures but decrease in enthalpy when compared with starch alone has been explained in terms of incomplete starch gelatinization as a result of limited water availability. Water is reduced in the mixed system because the non-starch polysaccharide readily hydrates and consequently reduces the amount of water available for gelatinization. Chaisawang and Suphantharika (2005) reported that gelatinization enthalpy of starch decreased with gum addition. The limitation in the amounts of water due to gum addition might contribute to a decrease in endothermic size reducing the energy difference between the granular starches with and without gum. When stored starch was regelatinized, the endothermic transition temperatures (T_o, T_p, and T_c) and starch retrogradation enthalpies associated with melting of retrograded starch lower than those for gelatinization of starch. Biliaderis et al. (1997) found that when β-glucan was incorporated in maize starch gelation was retarded possibly as a result of interference in the intermolecular associations among amylopectin molecules by the β-glucan. Substitution of 2.5 and 5% Glucagel® (83% db β-glucan) also affected starch granule swelling, pasting and gelatinisation temperatures in the β-glucan enrichment of breads (Brennan & Cleary, 2007). Satrapai and Suphantharika (2006) also suggested an increase in T_o, T_p, T_c and decrease in gelatinization enthalpy (ΔH_1) of rice starch/β-glucan gum mixtures with increasing β-glucan concentration. Storage and regelatinized of the mixed gels resulted in a decrease in T_o, T_p, T_c, and melting enthalpy (ΔH_2). The retrogradation ratio ($\Delta H_2/\Delta H_1$) and the phase transition temperature range (T_c - T_o) of the mixed gels increased with storage time, but this effect was reduced by the addition of β-glucan gum. Therefore, these results suggested retrogradation results in reassociation of gelatinized starch molecules, but in less

ordered and hence less perfect or stable forms than those present in the native starch granules (Karim et al., 2000).

From a rheological stand point, starch suspensions are viscoelastic systems (Ellis, Ring & Whittam, 1989) and their overall behavior depends on both, the matrix of dissolved macromolecules and the presence of swollen granules. Maximum viscosity is attained in systems where the granules are not completely broken and still keep their identity. Therefore, granule size influences the rheological behavior of gelatinized starch paste (Evans & Lips, 1992; Okechukwu & Rao, 1995). Alloncle and Doublier (1991) described such starch-gum dispersions as composites whose viscoelastic properties in the pasted and gelled states are governed primarily by the volume occupied by the swollen particles. Thus, increasing concentrations of hydrocolloids within the continuous phase will increase the viscoelastic behavior of the paste. It has also been proposed that diffusion of media water from the continuous phase into the starch granules increase the gum concentration surrounding them. Hence, viscosity increases because of competition for water rather than to any physical interaction between starch and gum. It is possible that both of these mechanisms are involved.

5. Rapid Visco Analyzer (RVA)

The RVA is employed to determine the pasting properties of the starch. It is used to produce viscogram profiles which applied a heating program (heating, holding and cooling) to a starch/water system. It can provide a complete pasting profile in 20 min or less (Perez, Breene, & Bahnassey, 1998). The resulting viscogram profile or pasting curve shows the changes in the viscosity of sample as the cooking temperature changes. Viscogram profiles consist of pasting temperature, peak viscosity, breakdown, holding strength, final viscosity, and set back as shown in Figure 2.18.

Heated starch granules in the presence of excess water undergo a process called gelatinization. Below the onset temperature of gelatinization (usually below 50°C), starch granules are insoluble in water and the viscosity of dispersion remains low. When starch granules are heated above the gelatinization temperature in a sufficient amount of water, the granules absorb a large amount of water and swell to many times their original size and the viscosity increases. The temperature at the onset

of the rise in viscosity can be considered as the starting point of the gelatinization and is defined as the pasting temperature in a RVA test (Newport Scientific, 1995). When most of the granules became swollen, a rapid increase in viscosity occurred. As the temperature increased further, the starch granules began to rupture and the amylose molecules leached out into the continuous phase until reaching a viscosity called the peak viscosity. The peak viscosity is considered to represent the equilibrium point between swelling and rupture of starch granules (Newport Scientific, 1995). Swelling of granules, accompanied by leaching of starch biopolymer, increased the viscosity and during further heating, granules would rupture further which resulted in a decrease in the viscosity. When the system was at the holding temperature (95°C), the sample was subjected to mechanical shear stress, which led to further disruption of starch granules and amylose leaching, followed at a slower rate by leaching of the amylopectin fraction. The leached-out polymer molecules were more or less aligned in the direction of flow, which contributes to shear-thinning behavior or breakdown in viscosity at a constant temperature. Therefore, the reduction in the viscosity after appearance of the peak was likely to be caused by mechanical rupture of starch granules. As the sample was subsequently cooled down to 50°C, the viscosity increased to a final viscosity at the end of RVA experiment, which was attributed to reassociation of amylose molecules or short-term retrogradation. The extent of short–term retrogradation evaluated from the RVA pasting curves is called setback.

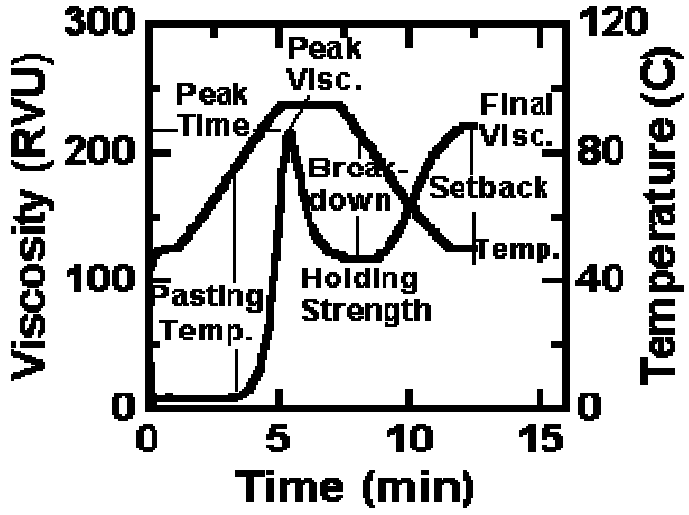


Figure 2.18 Typical RVA pasting profile of starch for viscosity (—) and temperature (---) as a function of time. (Newport Scientific, 1995)

- Peak viscosity = maximum viscosity developed during or soon after the heating portion of the test, in RVU.
- Peak time = time at which the peak viscosity occurred, in minutes.
- Pasting temperature = temperature where viscosity first increases by at least 2 RVU over a 20 s. period, in °C.
- Peak temperature = temperature at which the peak, normally occurring around the commencement of sample cooling, in RVU.
- Breakdown = peak viscosity minus trough viscosity, in RVU.
- Final viscosity = viscosity at the end of the test, in RVU.
- Setback from peak = final viscosity minus peak viscosity, in RVU.
- Setback from trough = final viscosity minus trough viscosity, in RVU.

6. Thermal analysis by differential scanning calorimetry (DSC)

DSC is a thermoanalytical technique for monitoring changes in physical or chemical properties of materials as a function of temperature by detecting the heat changes associated with such processes. In DSC curve (Figure 2.5), when a thermal transition occurs, the energy absorbed by the sample is replenished by increased energy input to the sample to maintain the temperature balance. Because the energy input is precisely equivalent in magnitude to the energy absorbed in the transition, a recording of this balancing energy yields a direct calorimetric measurement of energy transition which is then recorded as a peak. The area under the peak is directly proportion to the enthalpic change (ΔH) and its direction indicates whether the thermal event is endothermic or exothermic. DSC is commonly used for both measuring gelatinization and retrogradation of starch. In the case of retrograded starch, value of ΔH provides a quantitative measure of the energy transformation that occur during the melting of recrystallized amylopectin as well as precise measurement of the transition temperatures (i.e. onset, T_o; peak, T_p; and conclusion, T_c) of this endothermic event.

Recrystallized amylopectin melts in the temperature range 40-100°C, while amylose crystallites melts at much higher temperature (120-170°C) (Eerlingen, Jacobs & Delcour, 1994; Sievert & Pomeranz, 1989). Because retrogradation of amylopectin involves a crystallization process of the outer branches (DP14-18), the limited dimensions of the chains make amylopectin recrystallization a slow process. In

contrast to what is observed with amylose, therefore, the stability of these crystallites is lower than that of amylose crystallites.

Starch retrogradation enthalpies are usually 60-80% smaller (< 8 J/g) compared with gelatinization enthalpies (9-15 J/g). However, the retrogradation temperature range (T_c - T_o) is usually broader than the gelatinization range for a given sample. Furthermore, the endothermic transition temperatures (T_o, T_p, T_c) associated with melting of retrograded starch occur at temperature 10-26°C lower than those for gelatinization of starch granules (Baker & Rayas-Duarte, 1998; White, Abbas, & Johnson, 1989; Yuan et al., 1993), suggesting that retrogradation results in crystalline forms that are different in nature from those present in the native starch granules.

7. Rheology

The rheology has been defined as "a science devoted to the study of deformation and flow of materials". Rheology encompasses the area of fluid flow, which is concerned with two physical quantities: stress and strain (solid) or shear rate (fluid)

$$\text{Shear stress } (\sigma) = \frac{\text{shear (force)}}{\text{area (shear)}} \qquad (\text{Pa or N/m}^2)$$

$$\text{Shear rate } (\dot{\gamma}) = \frac{\text{velocity}}{\text{distance}} \qquad (1/\text{s})$$

$$\text{Strain} = \frac{\text{defection}}{\text{distance}} \qquad (-)$$

The rheology allows two different stress forms: the dynamic oscillation measurement and the stationary or in stationary shear flow.

The general instrument commonly used for measuring viscosity and the rheological properties is a rotational viscometer. It operates by rotating a spindle of define geometry within the fluid. The rotation rate of the spindle is rated to the shear stress within the fluid. Cone and plate rheometer was used in this study. It composes of a rotational cone and a fixed flat plate. The types of cone are determined by using the cone radius (R) and the cone angle (α). The ISO standard recommends using $\alpha = 1^o$ and excludes $\alpha > 4^o$ (Mezger, 2002). Moreover, temperature is environmental parameter, which influences viscosity or any rheological properties.

7.1 Steady flow measurement

Fluid may be described as Newtonian or non-Newtonian depending on their rheology (flow) characteristics.

7.1.1 Newtonian fluid behavior

Newtonian fluid obeys Newton's law of viscous flow. Newtonian fluid has a constant viscosity regardless of shear, therefore the viscosity of a Newtonian fluid will not vary with agitation rate (Figure 2.19).

7.1.2 Non-Newtonian fluid behavior

Non-Newtonian fluid dose not have a constant viscosity. It is composed of dilatant, pseudoplastic, bingham-plastic and Herschel-bulkley plastic fluids. This measured viscosity is called apparent viscosity. The apparent viscosity varies with the shear rate, it can be distinguished into 2 types of behavior, shear thinning (pseudoplasticity) behavior, where the viscosity decreases with increasing shear rate such as starch paste; and shear thickening (dilatancy) behavior, where the viscosity increases with increasing shear rate (Figure 2.19) (Chen & Ramaswamy, 1999).

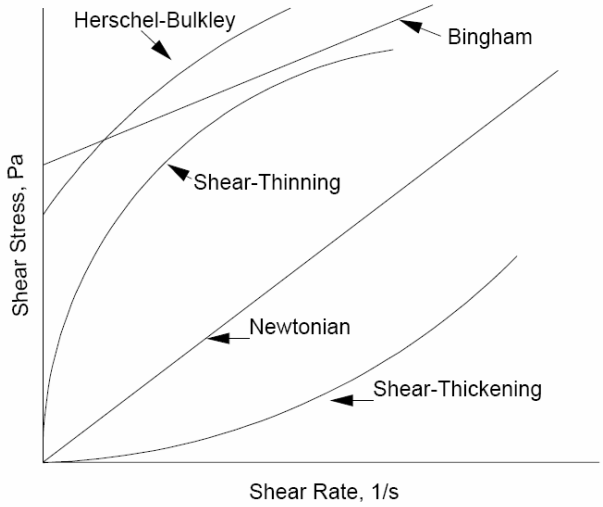


Figure 2.19 Comparison shear rate of the shear stress-rate of shear relationships for non-newtonian fluids and Newtonian fluids (Steffe, 1996)

Non-Newtonian fluids are usually considered to be those for which the relation connecting shear stress and shear rate is not linear, that is, the "viscosity" of a non-Newtonian fluid is not constant at a given temperature and pressure but depends on the rate of shear or, more generally, on the previous kinematic history of the fluid. Non-linear fluids in shear flow may be classified into three broad types:

(a) fluids for which the rate of shear at any point is some function of the shear stress at that point and depends on nothing else; (time-independent fluids);

(b) more complex systems for which the relation between shear stress and shear rate depends on the time the fluid has been sheared; (time-dependent fluids)

(c) systems which have characteristics of both solids and fluids and exhibit partial elastic recovery after deformation; these are called viscoelastic fluids.

7.1.3 Time-dependent non-Newtonian fluids

The viscosity of more complex fluids depends not only on the rate of shear but also on the time the (constant) shearing has been applied. These fluids may be subdivided into two classes (Figure 2.20):

(a) thixotropic fluids – breakdown of structure by shear. If a thixotropic material is sheared at a constant rate after a period of rest, the structure will be progressively broken down and the viscosity will decrease with time.

(b) rheopectic fluids – formation of structure by shear. This is a case of gradual formation of structure by shear.

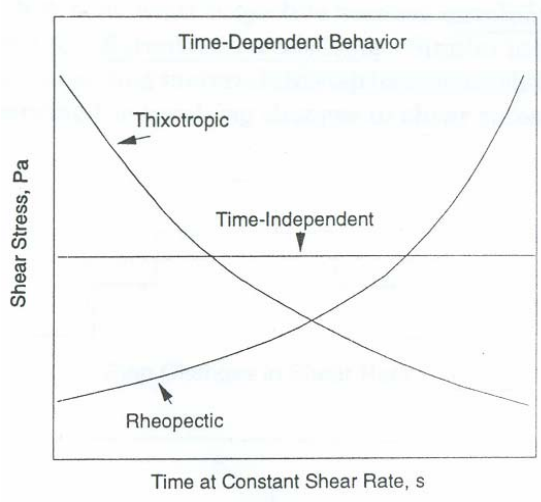


Figure 2.20 Time-dependent behavior of fluids (Steffe, 1996)

7.2 Dynamic oscillatory measurement

Starch pastes or gels can have both viscous (liquid-like) and elastic (solid-like) properties; i.e. they are viscoelastic. Dynamic test (small deformation test or destruction free method) was used to characterize property of viscoelastic behavior. These small-amplitude oscillatory tests are commonly performed in order of 1 to 3 or 5%. Basically, the gel specimen is subjected to a periodic, small amplitude sinusoidal torque (stress), the applied stress being altered at a given frequency (cycles s^{-1} or ω, radians s^{-1}). If the behavior of a viscoelastic material is linear, the strain will also vary sinusoidally with the stress, but will be out of phase with it. This behavior is intermediate between an ideally elastic material and a true Newtonian liquid where the stress is in phase ($\delta = 0$) and 90° out of phase, respectively, with the strain. Just as modulus is defined as the stress/strain ratio in any constant deformation experiment, then, for a dynamic sinusoidal experiment it follows that two moduli can be defined: (a) stress in-phase/strain or storage modulus (G') and (b) stress out-of-phase/strain or loss modulus (G'').

$$G' = \frac{\sigma_0 \cos\delta}{\gamma_0}$$

$$G'' = \frac{\sigma_0 \sin\delta}{\gamma_0}$$

Storage modulus (G') is a measure of the energy stored in the material and recovered from it per cycle. On a molecular basis, the magnitude of G' is dependent upon what rearrangements can take place within the period of oscillation (Ferry, 1980), and is taken as an indication of the solid or elastic character of the material.

Loss modulus (G'') is defined as the stress 90° out-of-phase with the strain divided by the strain and is a measure of the energy dissipated or lost (as heat) per cycle of sinusoidal deformation. It is, therefore, taken as an indication of liquid or viscous behavior.

Another parameter which is often useful in indicating the physical behavior of a system is the loss tangent ($\tan\delta = G''/G'$). It is the ratio of the energy lost to the energy stored for each cycle of the deformation. It is a useful indicator of the relative contributions of the viscous (G'') and elastic (G') components to the viscoelastic properties of a material. The logarithmic plot of the loss tangent gives rise to several characteristics.

Dynamic oscillatory rheometry has proved useful in monitoring structure development during aging of starch gels (Biliaderis & Zawistowski, 1990; Clark, Gidley, Richardson & Ross-Murphy, 1989; Miles et al., 1985a; Miles, Morris & Ring, 1985b). It allows continuous assessment of dynamic moduli without breaking structural elements formed in the sample upon aging. There are often three selected applications such as:

(a) deformation sweep at constant frequency (G' and G'' vs. strain) to determine the maximum deformation attainable by a sample in the linear viscoelastic region.

(b) frequency sweep (G' and G'' vs. ω) at constant deformation within the linear viscoelastic range to determine the elastic character of the gel and

(c) temperature sweep at constant frequency and deformation within the linear viscoelastic range (G' and G'' vs. Temperature) to evaluate thermal characteristics.

8. Texture profile analysis

Starch gel firmness or rigidity increases markedly with retrogradation. These changes have traditionally been followed using large deformation fundamental tests such as compression or empirical tests such as penetration which provide data on mechanical properties known to show good correlations with sensory textural attributes. Texture profile analysis (TPA) test, a sample of specific dimensions is compressed uniaxially; the compressive force is then removed and the sample is re-compressed. Such a compressive sequence represents two “bites” (Figure 2.23). During the test, compressive force is recorded as a function of the amount of compression (distance). Texture profile parameters may be derived from the TPA curves: the maximum force, which occurs at the end of the first compression, equates to “hardness”; the force of the first maximum is called “fracturability” (term as rupture strength of gel); the work done to compress the sample on the “first and second bites” is given as the area under the respective curves (Area1 and Area2), and the ratio of positive force area during the second compression portion to that during first compression, is related to “coadhesiveness”; the negative force area for first bite, representing the work necessary to pull the plunger away from the food sample is

called “adhesiveness”; the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite is called “springiness”. For food gel systems, the recommended deformation level ranged from 20 to 50% (Pons & Fiszman, 1996).

Inaba, Hatanaka, Adaci, Matsumura and Mori (1994) examined the changes in properties of gels prepared from various starches at different concentration with storage time. They demonstrated that compression work and rupture force of both potato and cassava starches increased with storage time and concentrations. Jankowski (1992) reported that the starch retrogradation markedly influence texture of cooked potatoes during post-cooking conditioning. Decrease of adhesiveness was the most distinctive feature and was attributed to the association of free amylose leached from starch granules in the cooking. Increase of coadhesiveness and hardness and decrease of fracturability of cooked tubers were slower than changes in adhesiveness; such effects were attributed to the development of a polymeric network within gelled starch in potato cells.

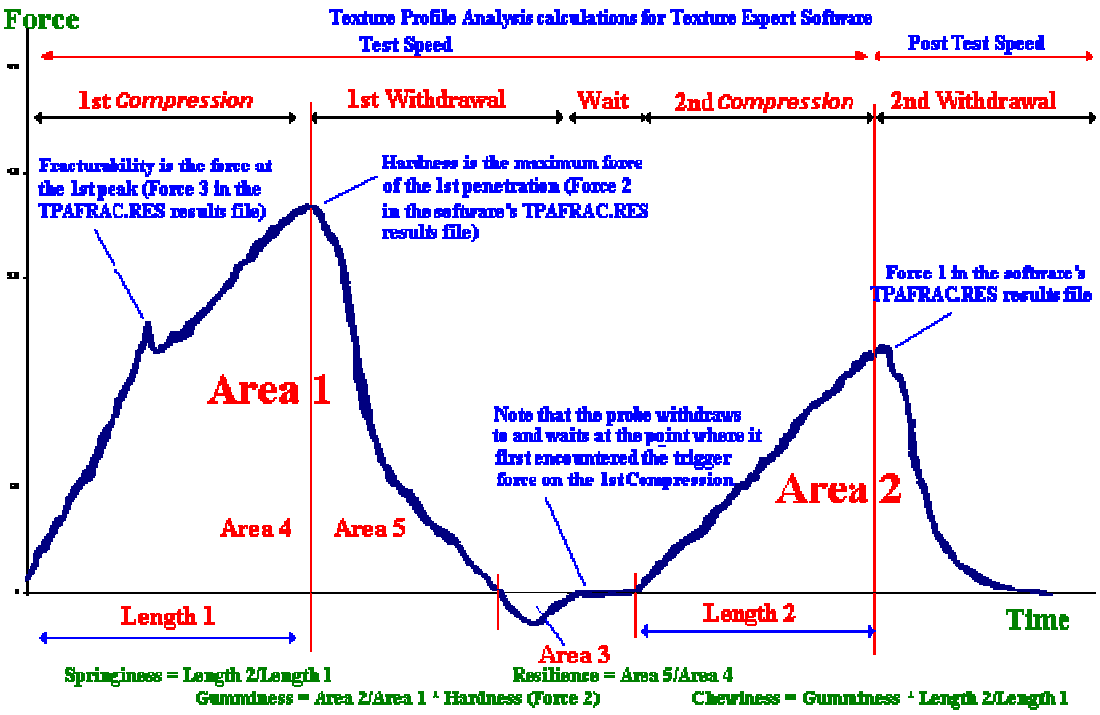


Figure 2.23 Generalized texture profile analysis curve
(http://www.texturetechnologies.com/texture_profile_analysis.html)

CHAPTER III
MATERIALS AND METHODS

1. Materials

Rice starch (RS) was kindly supplied by Cho Heng Rice Vermicelli Factory Co. Ltd., (Nakornpathom, Thailand) had moisture (AOAC, 2000) and amylose (AACC, 2000, Method 61-03) contents of 11.23% and 29.02%, respectively. β-glucans were purchased from commercial companies, i.e. oat β-glucan (OG) and barley β-glucan (BG) (Viscofiber®, Cevena Bioproducts Inc., Canada), curdlan (CL) (Takeda-Kirin Foods Co. Ltd., Japan). Spent brewer's yeast slurry (a strain of *Saccharomyces uvarum*), a by-product from brewery was provided by Boonrawd Brewery Co. Ltd., Thailand.

2. Methods

2.1 Preparation of β-glucan from spent brewer's yeast

Spent brewer's yeast β-glucan was prepared according to the procedure described previously (Thammakiti, Suphantharika, Phaesuwan, & Verduyn, 2004). Briefly, spent brewer's yeast slurry was autolysed at 50°C for 24 h. The autolysate was then heated at 80°C for 15 min, cooled to room temperature. Yeast cell walls were collected by centrifugation at 3565 × g for 10 min at room temperature. Yeast cell walls were resuspended in distilled water to obtain a suspension containing 15% solids content. The suspension was homogenized by using a high pressure homogenizer at 600 bar for six passes. Alkaline extraction with five volumes of 1.0 N NaOH at 80 ± 5°C for 2 h followed by an acid extraction with five volumes of 0.5 N acetic acid at 75 ± 5°C for 1 h. The extracted cell walls from each step were centrifuged and then washed three times with distilled water. The obtained β-glucan preparation should be a light-tan colored paste.

2.2 Chemical analysis

Chemical analyses were performed in triplicate for each β-glucan preparation, with the exception of insoluble dietary fiber determination which was done in duplicate. Total nitrogen, fat, moisture, and ash contents were determined using AOAC Official Methods (AOAC, 2000). Total nitrogen content was measured by a Kjeldahl analyser (Foss Tecator AB, Höganäs, Sweden) and multiplied by a factor of 6.25 to determine the crude protein content. Crude fat was determined by the Soxhlet extraction method using petroleum ether as the organic solvent. Moisture content was measured by using a direct heating method at 105°C to a constant weight. Ash was determined by incinerating dried samples at 600°C in a furnace. Carbohydrates were determined by subtracting the sum of protein, fat, and ash percentages from 100%. Insoluble dietary fiber content was determined using the enzymatic-gravimetric method as outlined in AOAC Method 991.42 (AOAC, 2000). Briefly, 1 g β-glucan preparation was weighed and wetted with 50 ml phosphate buffer (pH 6.0). Sample was gelatinized with Termamyl (α-amylase) for 15 min at 100°C. The enzymatically digested by using 5 mg protease (pH 7.5) for 30 min at 60°C, followed by 0.3 ml amyloglucosidase (pH 4.0-4.6) for 30 min at 60°C to remove protein and starch, respectively. Soluble dietary fiber was removed by filtering and washing residue with water. Remaining residue, insoluble dietary fiber, was washed with 95% ethanol and acetone, dried, and weight. One duplicate was analyzed for protein, and the other was incinerated at 525°C to determine ash. Insoluble fiber is weight of residue of protein and ash obtained from this analysis.

For the determination of β-glucan content, total glucose was determined by a sulfuric hydrolysis method (Dallies, François, & Paquet, 1998). This hydrolysis method is described as follows: 1 mg β-glucan preparation was wetted with 72 μl of 72% (w/w) H_2SO_4 and left at room temperature for 3 h. The slurry was diluted to 1 ml to a final concentration of 2N H_2SO_4 and heated for 4 h at 100°C and cooled. The hydrolysate was diluted to 9 ml with MilliQ water. Sulfate ions were precipitated by drop-wise addition of saturated $Ba(OH)_2$ until neutral pH was reached. The volume was adjusted to 25 ml and the $BaSO_4$ precipitate was pelleted at 3800 × g for 5 min. The supernatant was removed very carefully and left 4°C overnight to allow

precipitation of the remaining sulfate ions which were removed by second centrifugation. The monosaccharide was determined on 50 µl of supernatant by addition of 2 ml of glucose oxidase kit (GOD-PAP method, Human Gesellschaft für Biochemica und Diagnostica mbH, Germany), incubated for 10 min at room temperature. The absorbance of solution was determined at 500 nm with spectrophotometer.

Glycogen or starch content was determined by using amyloglucosidase enzyme from *Aspergillus niger* (Sigma Chemical Co., St. Louis, MO, USA) as explained by Parrou and François (1997). Briefly, 10 mg β-glucan preparation was wetted with 0.5 ml distilled water, then gelatinized for 15 min at 100°C and cooled to room temperature. The suspension was brought to pH 5.2 by addition of Sodium acetate buffer. Amyloglucosidase enzyme (1.2 Unit/ml) was then added and incubated at 57°C for 24 h. The suspension was centrifuged for 3 min at 5000 × g. The supernatant was ready to be used for glucose with a glucose oxidase kit (GOD-PAP method, Human Gesellschaft für Biochemica und Diagnostica mbH, Germany) as described above.

β-Glucan content was calculated by subtraction of the glucose obtained from glycogen or starch from the total glucose. This calculated β-glucan content was then multiply by 0.9, which was the molecular weight of anhydrous glucose divided by the molecular weight of glucose. The β-glucan contents were consequently reported as anhydrous glucose. Results are reported on a dry matter basis.

2.3 Determination of pasting properties

Pasting properties of rice starch (RS; 6%, w/w, dry basis) alone, RS/β-glucan (5.5% RS and 0.5% β-glucan) mixtures, and β-glucan (0.5%) alone suspended in distilled water were determined using a Rapid Visco-Analyzer (Model RVA-4C, Newport Scientific Pty. Ltd., Warriewood, Australia). Distilled water used in all experiments was added with 0.02%, w/w, sodium azide to prevent microbial spoilage of the stored samples. In the case of mixtures, the β-glucan solutions were firstly prepared by dispersing the calculated amounts of each β-glucan powders, i.e. curdlan (CL), oats (OG), or barley (BG) β-glucans, in distilled water with mild stirring for 15 min followed by heating at 80°C for 10 min and then quickly cooled to room

temperature (25°C). For the yeast β-glucan (YG) solution, the YG paste was dispersed in distilled water and stirred for 15 min. Subsequently, RS was slurried in the β-glucan solutions and stirred for 15 min at room temperature to avoid lump formation. The slurries (28 g) were then poured into aluminum canisters and stirred manually using plastic paddles for 20–30 s before insertion into the RVA instrument. The heating and cooling cycles were programmed in the following manner. The slurry was held at 50°C for 1 min, heated to 95°C within 3 min 42 s and then held at 95°C for 2 min 30 s. It was subsequently cooled to 50°C within 3 min 48 s and held at 50°C for 2 min, while maintaining a rotation speed of 160 rpm. Time measured in minutes and viscosity expressed in Rapid Visco Units (RVUs), 1 RVU = 12 cP, was collected by computer control program.

2.4 Differential scanning calorimetry measurement

Gelatinization temperatures and enthalpy of the RS in the presence or absence of various β-glucan preparations was measured using a differential scanning calorimeter (DSC 822^e, Mettler Toledo GmbH, Schwerzenbach, Switzerland). The total solids content of samples was selected to be 12%, w/w (dry basis), due to the sensitivity of the instrument, while keeping the RS/β-glucan ratio constant at 5.5/0.5. The samples were prepared by the procedure described above. After hydration for 1 h at room temperature, 10–15 mg of the well-stirred samples were exactly weighed into 40 µl aluminum crucibles and immediately hermetically sealed to prevent moisture loss. Scans were performed from 25 to 100°C at a controlled constant rate of 10°C/min. A sealed empty crucible was used as a reference and the DSC was calibrated using indium. The gelatinization enthalpy (ΔH_1) and transition temperatures, namely the onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c), were determined, based on the first-run DSC heating curves. The ΔH_1 was evaluated, based on the area of the main endothermic peak, and expressed in terms of J/g of dry starch using the equipment software. After the first-run heating, the gelatinized samples were cooled down and kept at 4°C for 0, 1, 3, 7, 14, 21, 35, 49, and 63 days. The stored samples were heated again to study the effect of β-glucan on retrogradation of the RS. The retrogradation ratio ($\Delta H_2/\Delta H_1$) was

calculated by dividing the retrogradation enthalpy (ΔH_2) in the second-run heating by the gelatinization enthalpy (ΔH_1) in the first-run heating (Kohyama & Nishinari, 1992).

2.5 Rheological properties

2.5.1 Dynamic viscoelastic measurement

The freshly prepared RS alone and RS/β-glucan gels (3.5%, w/w) at a ratio of 5.5/0.5, obtained from the RVA were cooled to room temperature (25°C) and then kept at 4°C for 0, 1, 3, 7, 14, 21, 35, 49, and 63 days. At each storage time the samples were measured for viscoelastic properties by using a rheometer (Physica MCR 301, Anton Paar GmbH, Stuttgart, Germany). The samples were placed into the rheometer measuring system (cone and plate geometry sensor, 1° cone angle, 50 mm diameter, and 0.05 mm gap) which was equilibrated to 25°C. Two steps of rheological measurements were performed: the linear viscoelastic range was determined with a strain sweep (0.01-100%) at a fixed frequency of 10 rad/s. After that, a dynamic frequency sweep was conducted by applying a constant strain of 0.5% which was within the linear region, over a frequency range between 0.1 and 100 rad/s. The mechanical spectra were obtained recording the storage modulus (G'), loss modulus (G''), and loss tangent ($\tan \delta = G''/G'$) as a function of frequency (ω).

2.5.2 Steady flow test

Steady flow tests were performed immediately after the frequency sweep experiments to obtain shear rate versus shear stress (flow curves) data. The cone was programmed to increase the shear rate from 0 to 300 s^{-1} in 3 min (upward flow curve) followed immediately by the reduction from 300 to 0 s^{-1} in next 3 min (downward flow curve). Using the equipment software, the areas of the hysteresis loops were obtained and the experimental data of either upward or downward flow curve were well fitted by the Herschel-Bulkley rheological model using the following equation:

$$\sigma = \sigma_0 + K\dot{\gamma}^n$$

where σ is shear stress (Pa), σ_0 is yield stress (Pa), $\dot{\gamma}$ is shear rate (s^{-1}), K is consistency coefficient (Pa s^n), and n is the flow behavior index (dimensionless). The hysteresis loop area of all RS and RS/β-glucan gels were calculated by the equipment software, indicating their structural changes during storage time.

2.6 Texture analysis

Compression tests of the RS alone and RS/β-glucan gels were carried out with a TA.XT2i Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) equipped with a Texture Expert for Windows Version 1 equipment software and a 5 kg load was used for force calibration. One cycle was applied with a hemispherical probe (P/0.5HS), at a constant crosshead velocity of 1 mm/s, to a sample depth of 10 mm, followed by returned to the original position. Four different RS/β-glucan mixtures with 5.5/0.5 mixing ratio (6% w/w, dry basis) were prepared and then gelatinized in a Brabender Viscoamylograph Type E (Duisburg, Germany). Fresh RS/β-glucan mixed gels weighed 25 g were poured into cylindrical containers (3.5 cm internal diameter and 6.5 cm height) and kept at room temperature (~ 25°C) for 2 h prior to measurement. To investigate the retrogradation behavior of the gelatinized gels, fresh gels in cylindrical containers were kept at 4°C for 0, 1, 3, 7, 14, 21, 35, 49, and 63 days before measurements in the same manner. The gel hardness was measured according to definition of Pons and Fiszman (1996) as the peak force observed during the compression cycle.

2.7 Statistical analysis

For three replicates, results are expressed as mean ± standard deviations. A one-way analysis of variance (ANOVA) and Tukey's test were used to establish the significance of differences among the mean values at the 0.05 significance level. The statistical analyses were performed using SPSS version 15.0 for Windows program (SPSS Inc., Chicago, IL, USA).

CHAPTER IV
RESULTS

4.1 Chemical composition of rice starch and β-glucans

Table 4.1 shows the composition of rice starch (except amylose content) and various β-glucan preparations including their coding used in this study. Rice starch (RS) consisted of almost 100% carbohydrate and other minor constituents including proteins, fats, and ash. The amylose content of RS was 29.02%, indicating that RS is a high-amylose rice starch (Bao & Bergman, 2004). The main component of all β-glucan preparations was carbohydrates which in turn mainly consisted of β-glucan. Therefore, the other components (such as starch, protein, ash and fat) were described as the impurities of β-glucan. Curdlan (CL) had the highest β-glucan purity with the lowest contaminants such as protein and ash. Barley (BG), oat (OG), and yeast (YG) β-glucans contained lower β-glucan contents in a range of 58-72% (w/w) and higher impurities than CL, with different degrees. Therefore, the OG had the highest starch, protein, ash and fat contents, compared with the others. Both CL and YG contained almost 100% insoluble dietary fiber content which is much higher than BG and OG.

Table 4.1. Chemical composition (% w/w, dry basis) of rice starch and various β-glucan samples[1]

Sample[2]	Moisture[3]	Carbohydrate (by calculation)	β-Glucan (by calculation)	Starch	Crude protein (TN × 6.25)	Ash	Fat	Insoluble dietary fiber
Rice starch (RS)	11.23 ± 0.01[b]	97.97 ± 0.34[a]	nd[4]	nd	1.56 ± 0.34[b]	0.31 ± 0.01[e]	0.16 ± 0.00[c]	nd
Oat β-glucan (OG)	7.82 ± 0.02[c]	84.80 ± 0.21[e]	57.88 ± 2.36[b]	8.18 ± 0.19[a]	4.02 ± 0.21[a]	8.51 ± 0.00[a]	2.65 ± 0.06[a]	14.67 ± 0.42[c]
Barley β-glucan (BG)	6.29 ± 0.91[d]	91.22 ± 0.25[d]	71.58 ± 5.20[b]	2.42 ± 0.23[b]	3.24 ± 0.27[a]	4.95 ± 0.02[b]	0.59 ± 0.01[b]	7.64 ± 0.07[d]
Curdlan (CL)	7.03 ± 0.02[cd]	96.87 ± 0.09[b]	103.74 ± 8.85[a]	0.12 ± 0.06[d]	1.64 ± 0.10[b]	1.36 ± 0.03[c]	0.13 ± 0.02[c]	101.03 ± 0.73[a]
Yeast β-glucan (YG)	93.34 ± 0.02[a]	95.28 ± 0.48[c]	68.86 ± 2.20[b]	1.63 ± 0.05[c]	3.97 ± 0.50[a]	0.63 ± 0.00[d]	0.13 ± 0.02[c]	89.45 ± 0.00[b]

[1] Assays were performed in triplicate. Mean ± standard deviation values in the same column followed by the same superscripts are not significantly different ($p > 0.05$).

[2] The sample codes were denoted in parentheses.

[3] % w/w, wet basis.

[4] Not determined.

4.2 RVA pasting properties of RS/β-glucan mixtures

The typical pasting profile of different RS/β-glucan mixtures at the total solids content of 6%w/w (dry basis) with a 0.5/5.5 mixing ratio, determined by RVA analysis, are shown in Figure 4.1. Statistical analyses of all pasting parameters were also performed and are summarized in Table 4.2. β-Glucan dispersions without RS were also run under the same RVA conditions to establish a control viscosity curve and to determine whether the gums were completely dispersed before heating. The viscosities of all β-glucan dispersions remained almost constant at a very low values ranged from 1 to 4 RVU, indicating a complete dispersion of these gums.

Compared with RS alone, as a control, addition of all β-glucan preparations at a concentration tested resulted in significant ($p \leqslant 0.05$) increases in the peak, breakdown, setback, and final viscosities to different degrees, whereas the pasting temperatures tended to decrease and the peak times (time to reach the peak viscosity) were unaffected, excepted that of RS/CL sample.

The highest increment of peak viscosity was found in RS/OG, then RS/BG, RS/CL and RS/YG mixtures, respectively. All RS/β-glucan additions showed much higher breakdown viscosities than RS alone mixture. The pasting temperatures of RS alone could be decreased when all RS/β-glucans were added. The CL addition decreased peak time of RS alone whereas the other β-glucans had no significant effect on this parameter. The setback and final viscosities for RS/β-glucan mixtures were significantly larger than RS alone, except for RS/YG that showed no significant difference in setback. However, this result suggested that retrogradation of RS should be promoted at very early stage by all β-glucan additions.

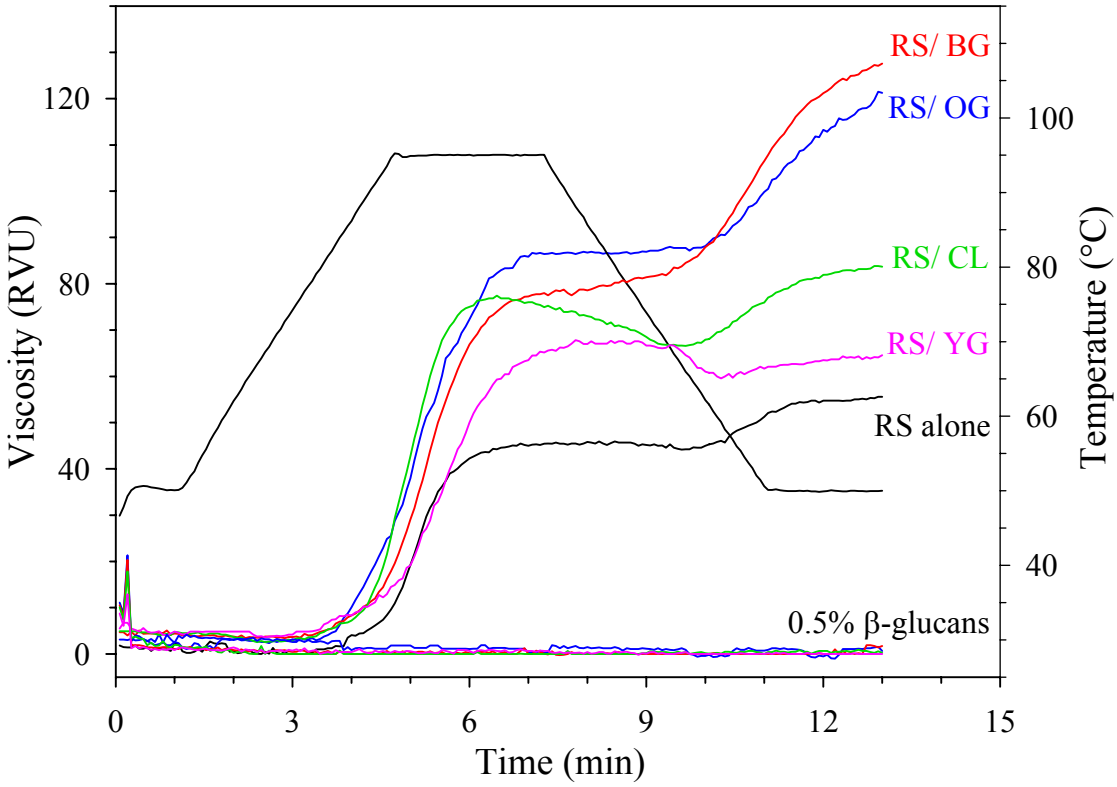


Figure 4.1 Typical RVA pasting profiles of 6%, w/w, RS alone, RS (5.5%), and 0.5% β-glucan alone suspensions. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

Table 4.2 Pasting properties of 6% (w/w) RS alone and RS (5.5%)/β-glucan (0.5%) suspensions measured by the rapid visco-analyzer (RVA)[1]

RS/β-glucan [2]	Peak viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)	Pasting temperature (°C)	Peak time (min)
RS alone	45.8 ± 0.4^{d}	3.3 ± 0.4^{b}	55.9 ± 1.3^{d}	12.5 ± 0.7^{d}	95.1 ± 0.1^{a}	7.0 ± 0.1^{a}
RS/OG	85.8 ± 1.2^{a}	10.1 ± 2.2^{a}	122.7 ± 1.5^{a}	47.0 ± 1.1^{b}	91.5 ± 1.2^{b}	7.0 ± 0.0^{a}
RS/BG	77.3 ± 0.5^{b}	10.0 ± 0.7^{a}	126.5 ± 1.8^{a}	59.2 ± 1.1^{a}	94.4 ± 0.0^{a}	7.0 ± 0.0^{a}
RS/CL	77.7 ± 0.8^{b}	11.6 ± 0.7^{a}	82.1 ± 1.7^{b}	16.0 ± 1.1^{c}	90.4 ± 0.7^{b}	6.6 ± 0.1^{b}
RS/YG	63.8 ± 0.5^{c}	13.4 ± 1.8^{a}	63.4 ± 1.1^{c}	13.1 ± 0.6^{d}	94.9 ± 0.1^{a}	7.0 ± 0.0^{a}

[1] Assays were performed in triplicate. Mean ± standard deviation values in the same column followed by the same superscripts are not significantly different ($p > 0.05$).

[2] Refer of Table 4.1 for the sample codes.

4.3 Thermal properties of RS/β-glucan mixtures

The gelatinization temperature, enthalpy and retrogradation ratio for 12%, w/w, RS alone and RS/β-glucan mixtures, determined by DSC, are summarized in Table 4.3. For the first run, the gelatinization temperatures (onset, T_o; peak, T_p; and conclusion, T_c) and gelatinization temperature ranges (T_c-T_o) seemed to be unaffected by addition of various β-glucan preparations at concentration tested, whereas the gelatinization enthalpies (ΔH_1) slightly decreased, except for RS/CL sample, compared to those of the control. The reheating DSC data of the RS/β-glucan retrograded gels stored for 21 days at 4°C are also presented in Table 4.3. The endothermic transition temperatures (T_o, T_p, and T_c) of retrogradation were found to be lower than the gelatinization temperatures. The melting enthalpies of retrograded gels (ΔH_2) were 36-47% smaller than the gelatinization enthalpies (ΔH_1). The retrogradation temperature ranges (T_c-T_o) were more than twofold broader than the gelatinization temperature ranges for a given sample. Addition of various β-glucan preparations resulted in a significant ($p \leq 0.05$) increase in the T_o, but no effect on the T_p and T_c, leading to a significant decrease in the T_c-T_o of retrograded RS gels. The retrogradation enthalpies (ΔH_2) and retrogradation ratios ($\Delta H_2/\Delta H_1$) of retrograded RS gels decreased by addition of all β-glucan preparations except for CL, indicating that β-glucans slowed the retrogradation rate of RS gels during storage.

Time dependence of the transition temperatures (T_o, T_p, and T_c) and the transition temperature ranges (T_c-T_o) as well as retrogradation ratios ($\Delta H_2/\Delta H_1$) of the retrograded RS alone and RS/β-glucan gels were plotted in Figure 4.2 and 4.3, respectively. The T_o, T_p, and T_c markedly decreased during the first week of storage and slightly decreased during the rest of storage time. The decreases in these transition temperatures were in the following order: $T_o > T_p > T_c$ (Figure 4.2), resulting in an increase in the T_c-T_o values with storage time (Figure 4.3a). The increase rates of $\Delta H_2/\Delta H_1$ (Figure 4.3b) for both RS alone and RS/β-glucan mixtures with storage time were observed as well. Addition of OG showed the greatest rate of retarding RS long term retrogradation, followed by BG, YG and CL, respectively. However, the rates of increments for the magnitude of both parameters could be reduced by addition of β-glucan preparations.

Table 4.3 Gelatinization temperature and enthalpy and retrogradation ratio for 12% (w/w) RS alone and RS/β-glucan mixtures at a ratio of 5.5/0.5 measured by the differential scanning calorimeter (DSC)[1,2]

RS/β-glucan[3]	First run					Second run (21 days at 4 °C)					
	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_1 (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_2 (J/g)	$\Delta H_2/\Delta H_1$
RS alone	72.3 ± 0.1^{a}	76.4 ± 0.2^{bc}	80.2 ± 0.2^{a}	7.9 ± 0.1^{a}	12.5 ± 0.1^{a}	40.4 ± 0.9^{b}	53.7 ± 0.7^{a}	62.6 ± 0.1^{a}	22.2 ± 0.9^{a}	8.0 ± 0.3^{a}	0.64 ± 0.03^{a}
RS/ OG	72.5 ± 0.3^{a}	76.5 ± 0.1^{abc}	80.4 ± 0.1^{a}	7.9 ± 0.4^{a}	12.2 ± 0.5^{ab}	43.7 ± 0.4^{a}	54.8 ± 0.1^{a}	62.2 ± 0.4^{a}	18.6 ± 0.4^{bc}	6.5 ± 0.1^{b}	0.53 ± 0.02^{b}
RS/ BG	72.7 ± 0.1^{a}	76.7 ± 0.1^{a}	80.6 ± 0.3^{a}	7.9 ± 0.3^{a}	11.5 ± 0.2^{b}	44.5 ± 0.5^{a}	54.8 ± 1.0^{a}	63.3 ± 1.2^{a}	16.3 ± 1.2^{c}	6.6 ± 0.2^{b}	0.58 ± 0.02^{ab}
RS/ CL	72.6 ± 0.2^{a}	76.5 ± 0.3^{ab}	80.4 ± 0.3^{a}	7.9 ± 0.1^{a}	12.7 ± 0.2^{a}	45.4 ± 1.3^{a}	53.0 ± 0.5^{a}	63.4 ± 1.1^{a}	17.4 ± 1.3^{bc}	8.0 ± 0.2^{a}	0.63 ± 0.03^{a}
RS/ YG	72.5 ± 0.1^{a}	76.2 ± 0.0^{cd}	80.8 ± 0.2^{a}	7.5 ± 0.3^{a}	9.3 ± 0.4^{c}	43.3 ± 0.9^{a}	54.9 ± 0.2^{a}	62.7 ± 0.2^{a}	19.4 ± 0.9^{b}	5.5 ± 0.2^{c}	0.59 ± 0.01^{ab}

[1] Assays were performed in triplicate. Mean ± standard deviation values in the same column followed by the same superscripts are not significantly different ($p > 0.05$).

[2] T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_1, gelatinization enthalpy; ΔH_2, re-gelatinization enthalpy; $\Delta H_2/\Delta H_1$, retrogradation ratio.

[3] Refer of Table 4.1 for the sample codes.

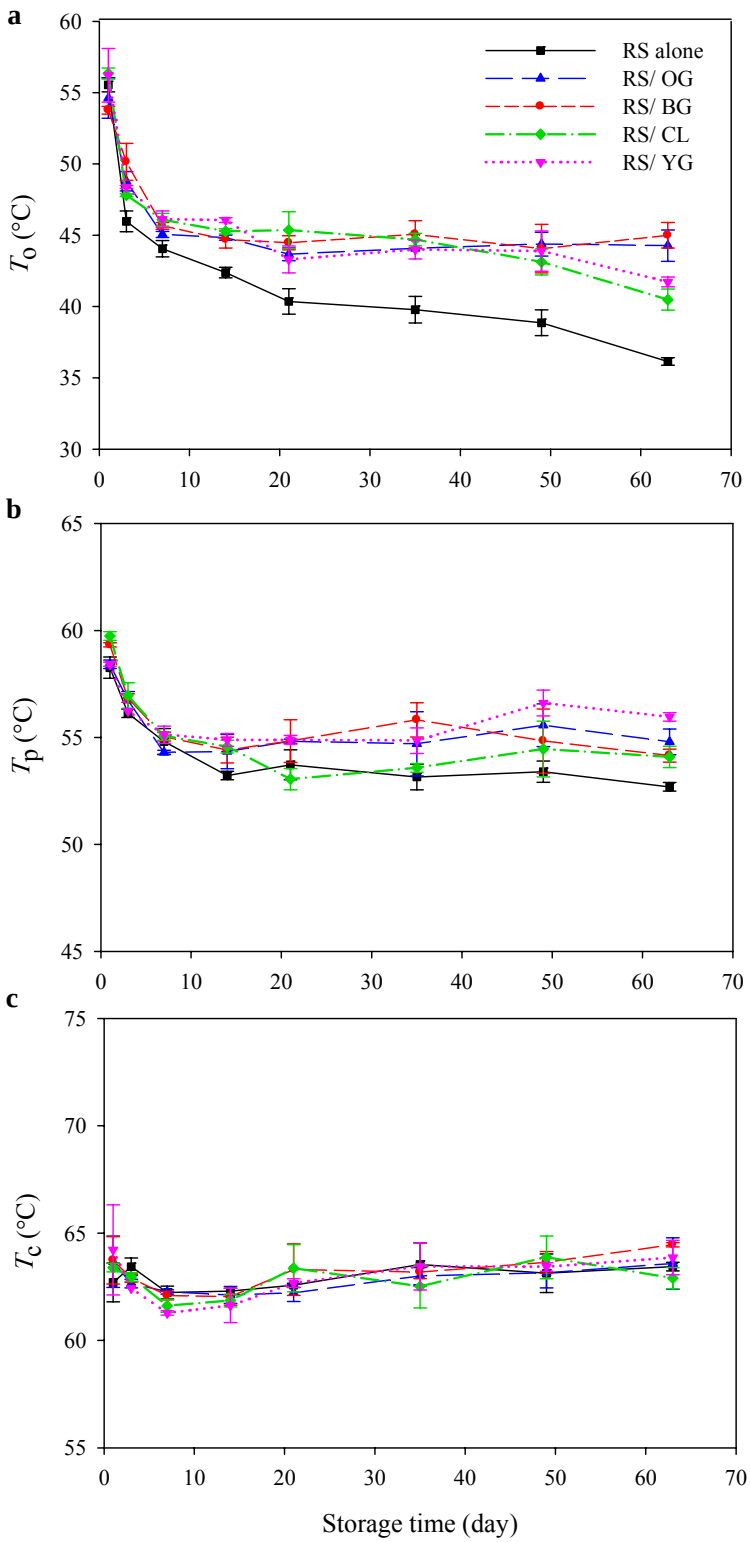


Figure 4.2 Changes in (a) onset temperature, T_o, (b) peak temperature, T_p, and (c) conclusion temperature, T_c, of 12%, w/w, RS alone and RS/β-glucan gels at a ratio of 5.5/0.5 as a function of storage time at 4°C. Error bars represent standard deviations. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

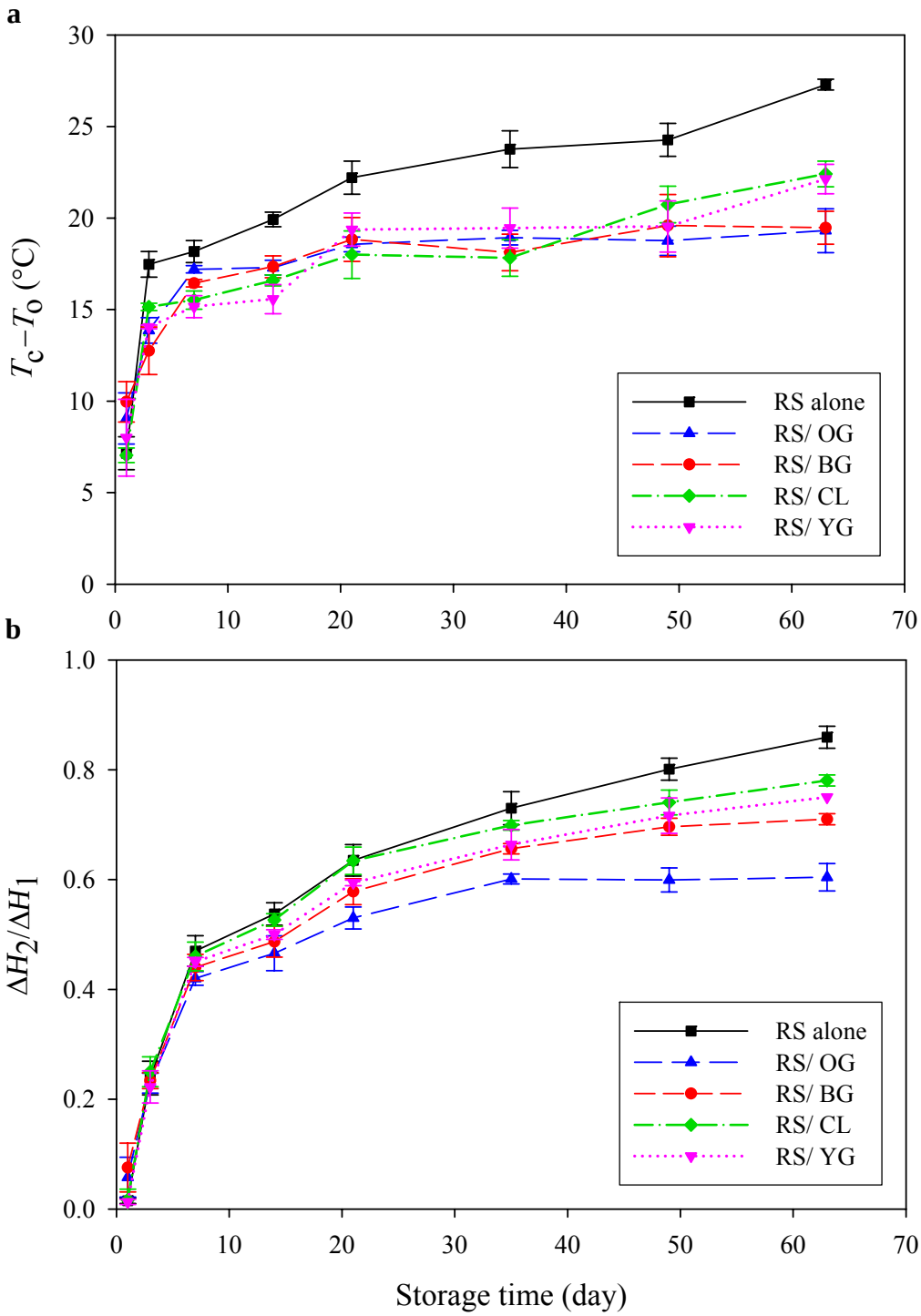


Figure 4.3 Changes in (a) transition temperature range (T_c-T_o) and (b) retrogradation ratio ($\Delta H_2/\Delta H_1$) of 12%, w/w, RS alone and RS/β-glucan gels at a ratio of 5.5/0.5 as a function of storage time at 4°C. Error bars represent standard deviations. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

4.4 Dynamic viscoelastic properties

Mechanical spectra of the RS alone and RS/β-glucan gels stored at 4°C for 0 (i.e. immediately after gelatinization and cooling to room temperature), 7, 21, 63 days are illustrated in Figure 4.4. These rheograms show that the storage modulus (G') was much larger than the loss modulus (G''), both moduli show only slight increases with increasing frequency (ω), and a cross over between these two moduli was not observed throughout the tested frequency range. This behavior may be classified rheologically as a typical weak gel structure, as proposed by Clark and Ross-Murphy (1987).

For the freshly prepared RS/β-glucan gels (Figure 4.4a), the addition of various β-glucan preparations to RS led to an increase in the G' and G'' values except for BG, which showed a slightly lower G' values than those of the control. The increases in G'' values were more pronounced in comparison with those in G'. The loss tangent (tan $\delta = G''/G'$) values of the RS/β-glucan gels were larger than that of the control (Figure 4.5b). Figure 4.4 also shows that stored sample gels at 4°C exhibited the opposite trend in which the G' and G'' values of the RS alone gels increased at much faster rates than those of the RS/β-glucan gels and became the highest values after 63 days of storage (Figure 4.4d).

The G' and tan δ values for the RS/β-glucan gels as a function of storage time are shown in Figure 4.5. The G' values increased steadily (Figure 4.5a), whereas the tan δ values decreased with storage time (Figure 4.5b), indicating the development of a more firm structure due to starch retrogradation (Figure 4.3). However, these effects could be reduced by different degrees by the addition of various β-glucan preparations. The G' values of all RS/β-glucan mixed gel were lower than RS alone, although RS/CL demonstrated a higher G' at the beginning of the experiment (Figure 4.5a). This result could consider a lower development of elastic behavior reflected to lower retrogradation rates in following degree: RS/OG > RS/BG > RS/YG > RS/CL > RS alone. Accordingly, the mechanical tan δ values for the RS/β-glucan gels at angular frequency 1 rad/s were between 0.03 and 0.17 (Figure 4.5b), all β-glucan additions had higher tan δ than RS alone gel.

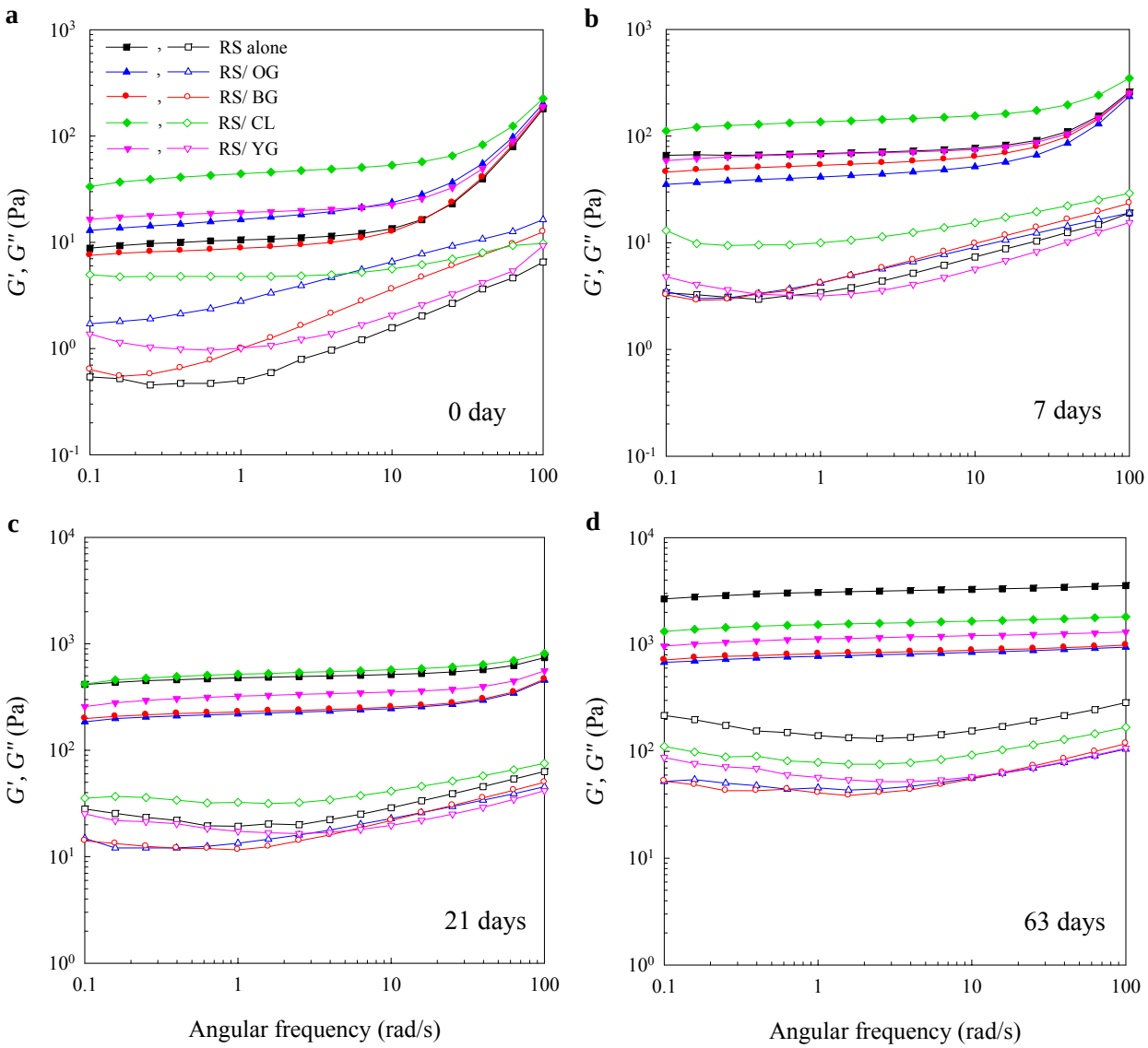


Figure 4.4 Frequency dependence of storage modulus, G' (closed symbol) and loss modulus, G'' (open symbol) of 3.5%, w/w, RS alone and RS/β-glucan gels at a ratio of 5.5/0.5 (a) immediately after gelatinization and (b) 7, (c) 21, and (d) 63 days after storage at 4°C. Measurements were made at 0.5% strain and 25°C. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

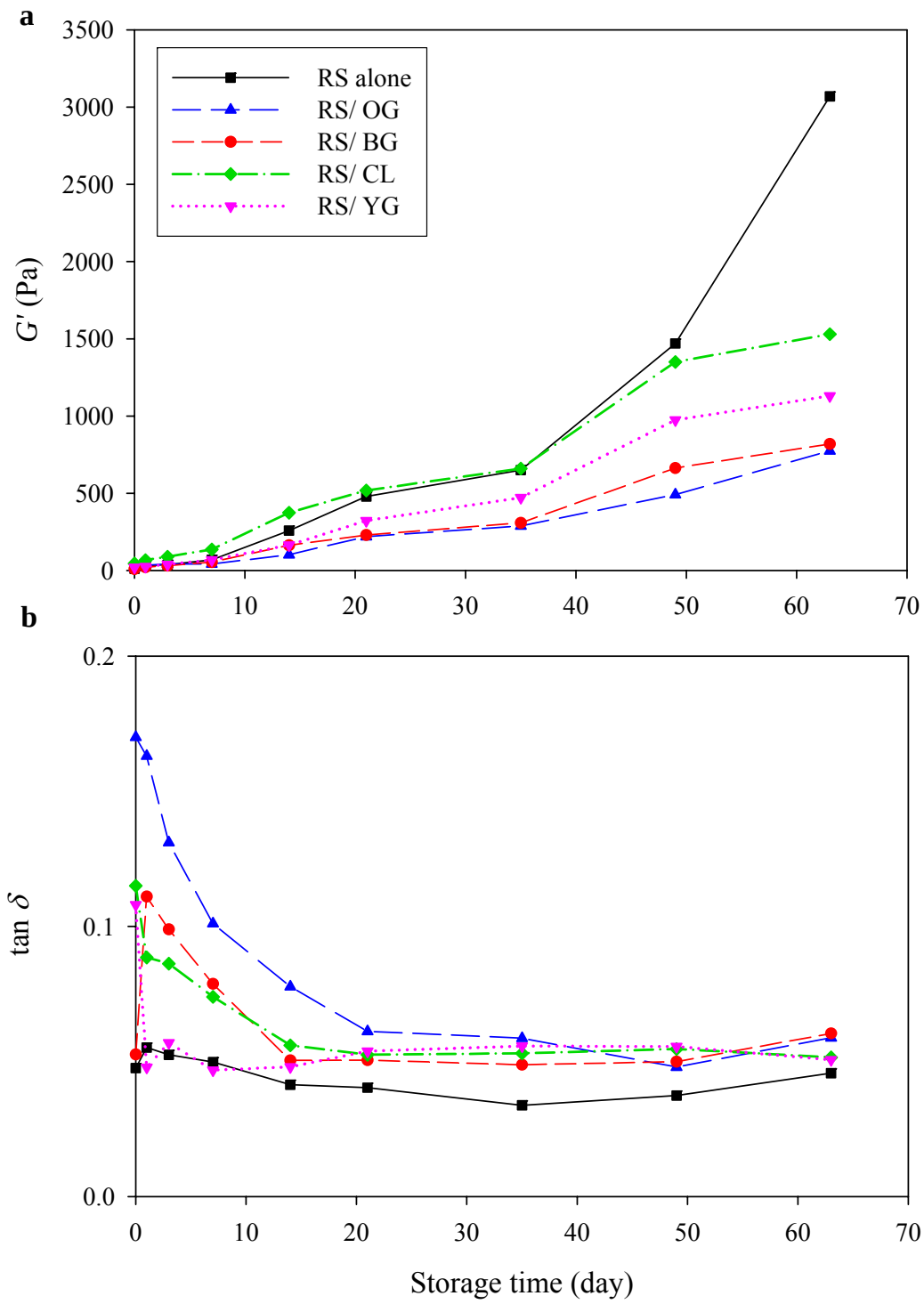


Figure 4.5 Changes in (a) storage modulus, G', and (b) loss tangent, $\tan\delta$, of 3.5%, w/w, RS alone and RS/β-glucan gels at a ratio of 5.5/0.5 as a function of storage time at 4°C. Measurements were made at an angular frequency of 1 rad/s, 0.5% strain and 25°C. Error bars represent standard deviations. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

4.5 Steady shear rheological properties

The steady flow characteristics of the 3.5% w/w RS alone and RS/β-glucan (5.5/0.5, w/w ratio) gels compared with RS alone gels stored for 0, 7, 21 and 63 day at 4°C are shown in Figure 4.6. For the range of shear rates from 0 to 300 s^{-1} (upward curve) and then back to 0 (downward curve), the Herschel – Bulkley model was applied. A hysteresis loop area was seen and calculated from this ascending and descending curve as well. All gels exhibited mainly time-dependent shear-thinning (thixotropic) with yield stress behavior. The Herschel-Bulkley model can only fit to the flow curves of freshly prepared gels except the upward flow curve of RS/CL gel (Figure 4.6a). All the upward flow curves of retrograded gels (Figure 4.6b-d) and the fresh RS/CL gel (Figure 4.6a) exhibited extremely thixotropic flow with gel strength having a spur forms in the hysteresis loop. However, the downward curves of all RS alone and RS/β-glucan gels as a function of storage time were also fitted to the Herschel-Bulkley model (Figure 4.8 a-c), resulting from their shear thinning charateristics without the zero-shear and/or infinite-shear viscosities of all gels (Figure 4.7). All the fitted curves illustrated the shear-thinning behavior classically, for which $n < 1$.

For the fresh gels, the consistency coefficients (K), flow behavior indices (n), and yield stresses (σ_0), along with the coefficients of determination (R^2) for each upward or downward flow curve, and hysteresis loop areas between these two curves are summarized in Table 4.4. A hysteresis loop was observed in all gels studied. The upward curve being above the downward curve which indicates that shearing at high shear rate modifies the gel structure by decreasing their viscosities. Shearing of the gels causes their structure to be broken down and this is the cause of the shear thinning behavior as evidenced by $n < 1.0$. On reducing the shear rate back to zero the structure builds up only in part within the duration of the experiment, resulting in the lower σ_0 values of the downward curves than those of the upward curves. Addition of the OG and BG markedly increased the K values, whereas addition of the CL and YG sharply enhanced the σ_0 values of RS gels. However, hysteresis loop area of fresh RS/OG gel was very small (Figure 4.6a); it means that shearing at high shear rate slightly modify

the structure of this system, indicating high shear resistant and structure recovery of RS in presence of OG.

For the retrograded gels, the σ_0, *K* and *n* values for the downward flow curves of all RS gels as a function of refrigerated storage time are shown in Figure 4.8. The σ_0 values of all RS alone and RS/β-glucan gels increased during refrigerated storage (Figure 4.8a). The RS alone gels exhibited the highest increase in this value, followed by RS/BG, RS/CL, RS/OG and RS/YG gels, respectively. The presence of CL and YG increased *K* value of RS gel, whereas OG and BG tended to decreased this value during storage time (Figure 4.8b). However, the opposite trend was observed for *n* values (Figure 4.8c).

The hysteresis loop areas for RS/β-glucan mixed gels as a function of refrigerated storage time are shown in Figure 4.9. The hysteresis loop areas of all RS gels increased steadily with storage time in the following order: RS alone > RS/CL > RS/YG > RS/BG > RS/OG.

The apparent viscosities at shear rate ($\dot{\gamma}$) of 100 s^{-1} ($\eta_{a,100}$) for the upward and downward flow curves of RS alone and RS/β-glucan mixed gels as a function of refrigerated storage time were plotted in Figure 4.10a and 4.10b, respectively. These diagrams presented the $\eta_{a,100}$ values of upward flow curves ranging from 0.09 – 3.31 Pa s, whereas the downward values were 0.08 – 1.26 Pa s. In general, these two $\eta_{a,100}$ values increased with storage time. Among the RS/β-glucan gels, the upward $\eta_{a,100}$ values of RS/CL gels was higher than those of RS alone gel after 14 day of storage time then became lower thereafter, whereas RS/YG, RS/BG and RS/OG exhibited the lower viscosities than RS alone gel throughout this experiment. During downward shearing, the $\eta_{a,100}$ values of RS alone and RS/β-glucan gels were decreased to different degrees.

Table 4.4 The Herschel-Bulkley parameters for 3.5% (w/w) RS alone and RS/ β-glucan gels at a ratio of 5.5/0.5 immediately after gelatinization and cooling to room temperature (25°C)[1]

RS/β-glucan[2]	Hysteresis loop area (Pa/s)	Upward curve				Downward curve			
		σ_0	K	n	R^2	σ_0	K	n	R^2
RS alone	252	2.94	0.47	0.56	0.998	0.90	0.50	0.58	0.999
RS/ OG	181	5.63	3.62	0.34	0.997	0.00	4.50	0.35	0.982
RS/ BG	378	0.85	2.64	0.49	0.999	0.00	1.81	0.56	0.998
RS/ CL	929	-	-	-	-	3.12	0.76	0.55	0.997
RS/ YG	436	6.71	0.18	0.69	0.997	1.23	0.63	0.55	0.999

[1] σ_0, yield stress (Pa); K, consistency coefficient (Pa s^n); n, flow behavior index (dimensionless).

Measurements were made at 25°C

[2] Refer of Table 4.1 for the sample codes.

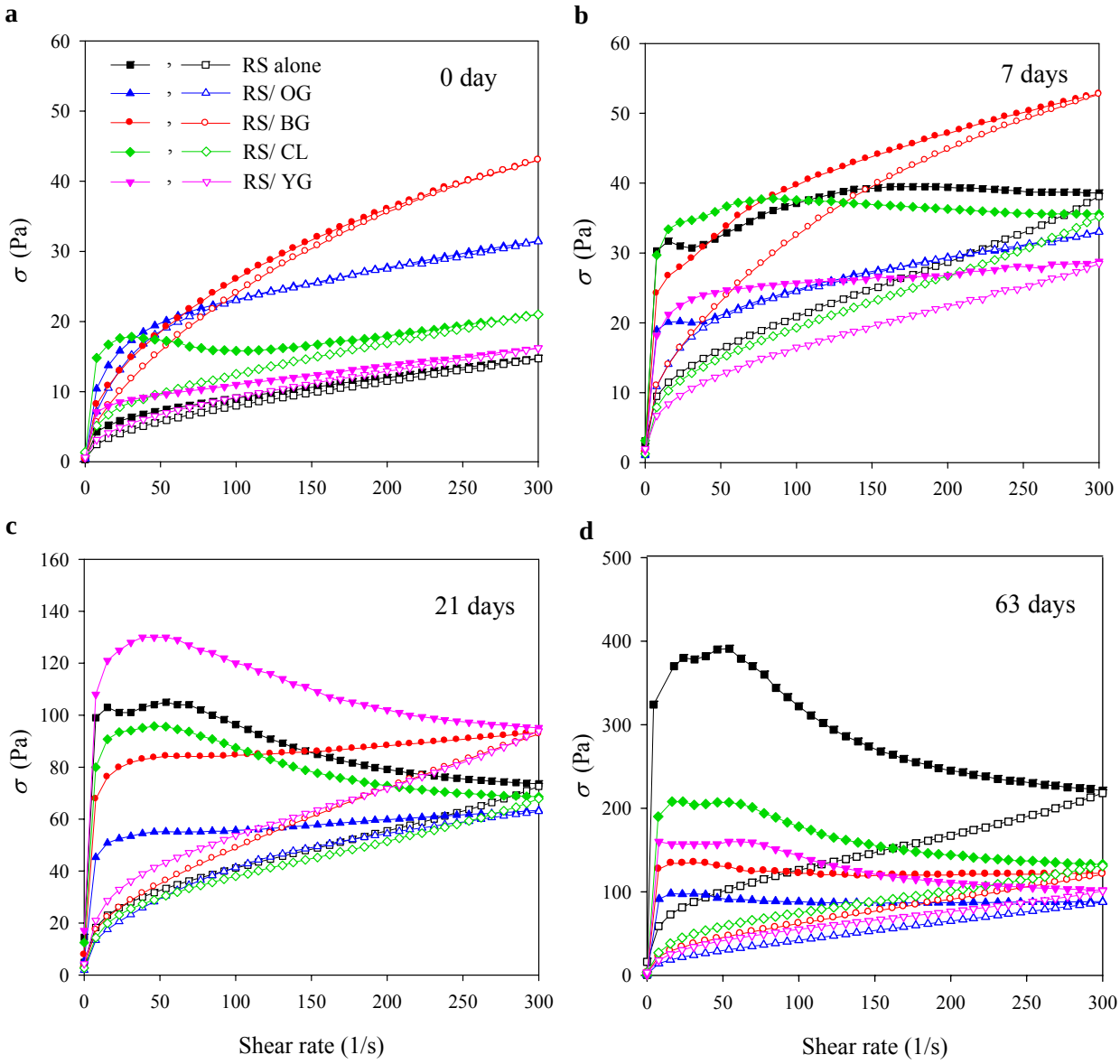


Figure 4.6 Flow curves of 3.5%, w/w, RS alone and RS/β-glucan gels at a ratio of 5.5/0.5 as a function of storage time; (a) immediately after gelatinization and (b) 7, (c) 21, and (d) 63 days after storage at 4°C. Measurements were made at 25°C. Closed symbols represent upward flow curves and open symbols represent downward flow curves. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

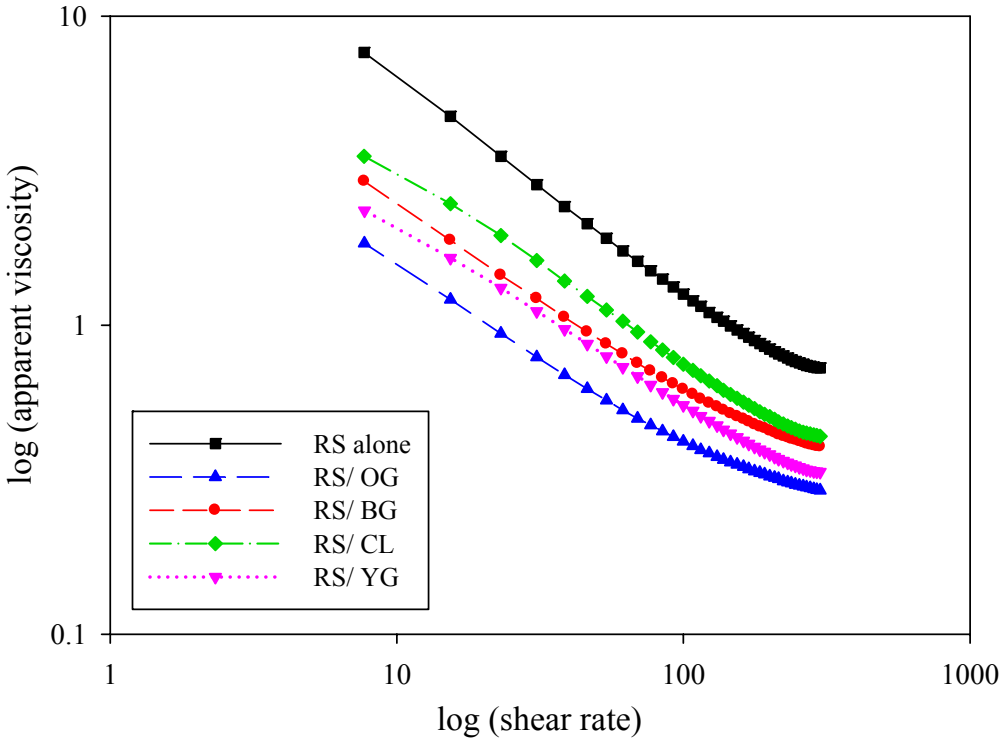


Figure 4.7 Plot of log shear rate ($\dot{\gamma}$) versus log apparent viscosity (η_a) for shear thinning of 3.5%, w/w, RS alone and RS/β-glucan gels at a ratio of 5.5/0.5 after storage at 4°C for 63 days. Measurements were made at 25°C. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

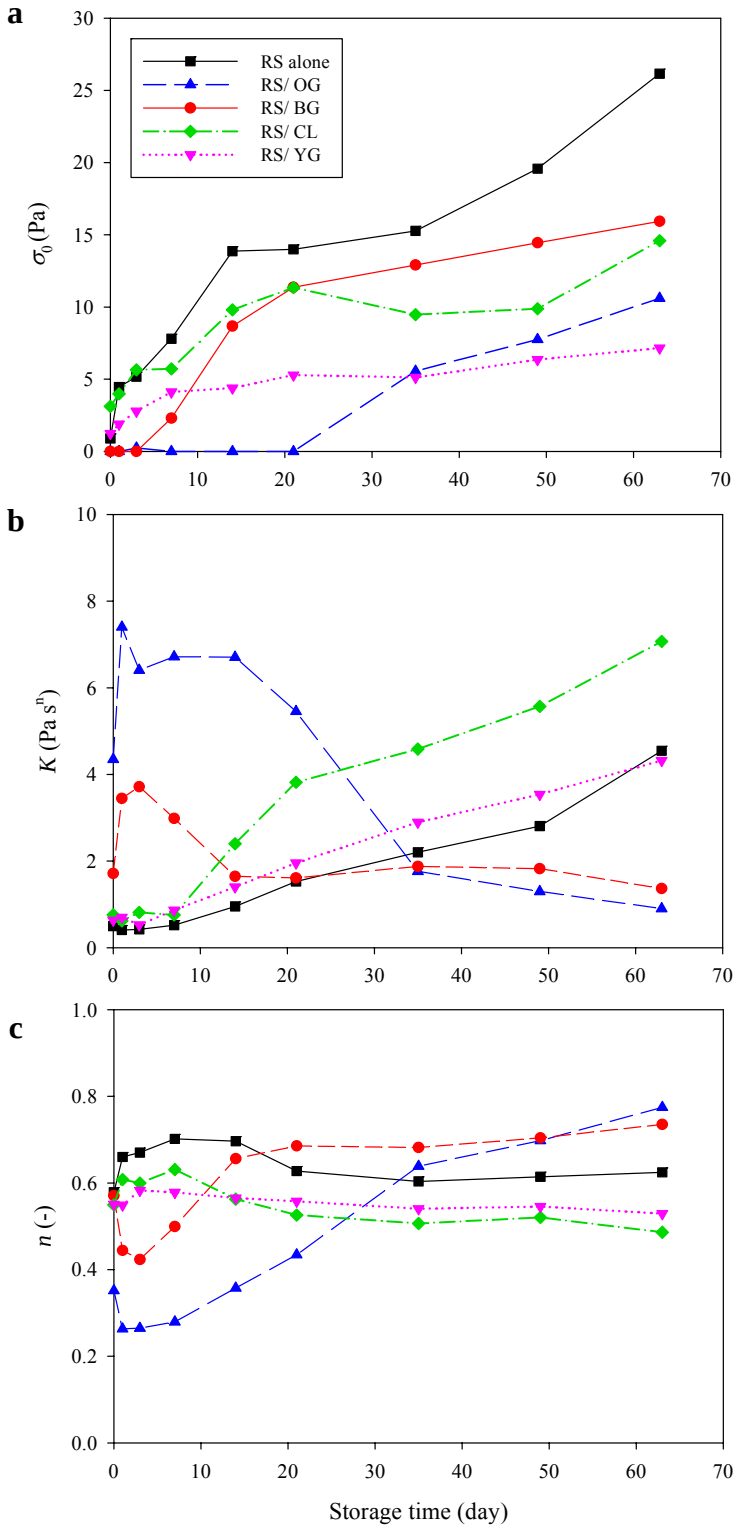


Figure 4.8 Changes in the Herschel – Bulkley parameters of downward flow curves; (a) yield stress (Pa), σ_0, (b) consistency coefficient (Pa s^n), *K*, and (c) flow behavior index (dimensionless), n, of 3.5%, w/w, RS alone and RS/β-glucan gels at a ratio of 5.5/0.5 as a function of storage time at 4°C. Measurements were made at 25°C. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

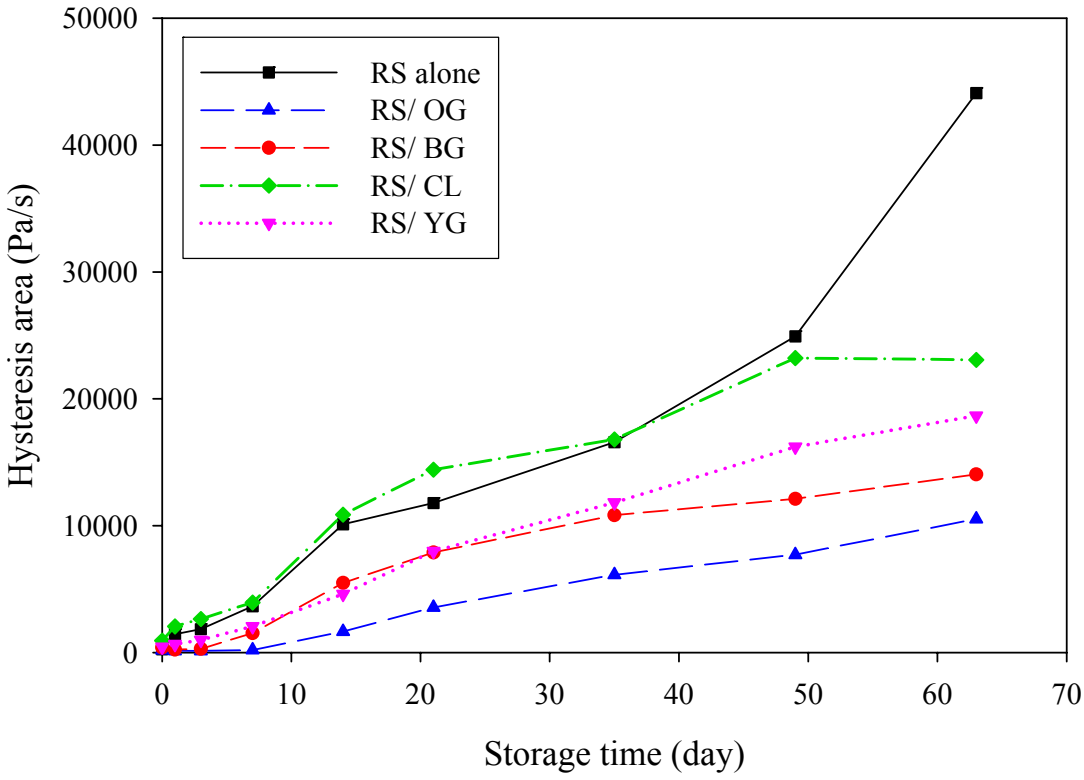


Figure 4.9 Changes in hysteresis loop area of 3.5%, w/w, RS alone and RS/β-glucan gels at a ratio of 5.5/0.5 as a function of storage time at 4°C. Measurements were made at 25°C. Error bars represent standard deviations. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

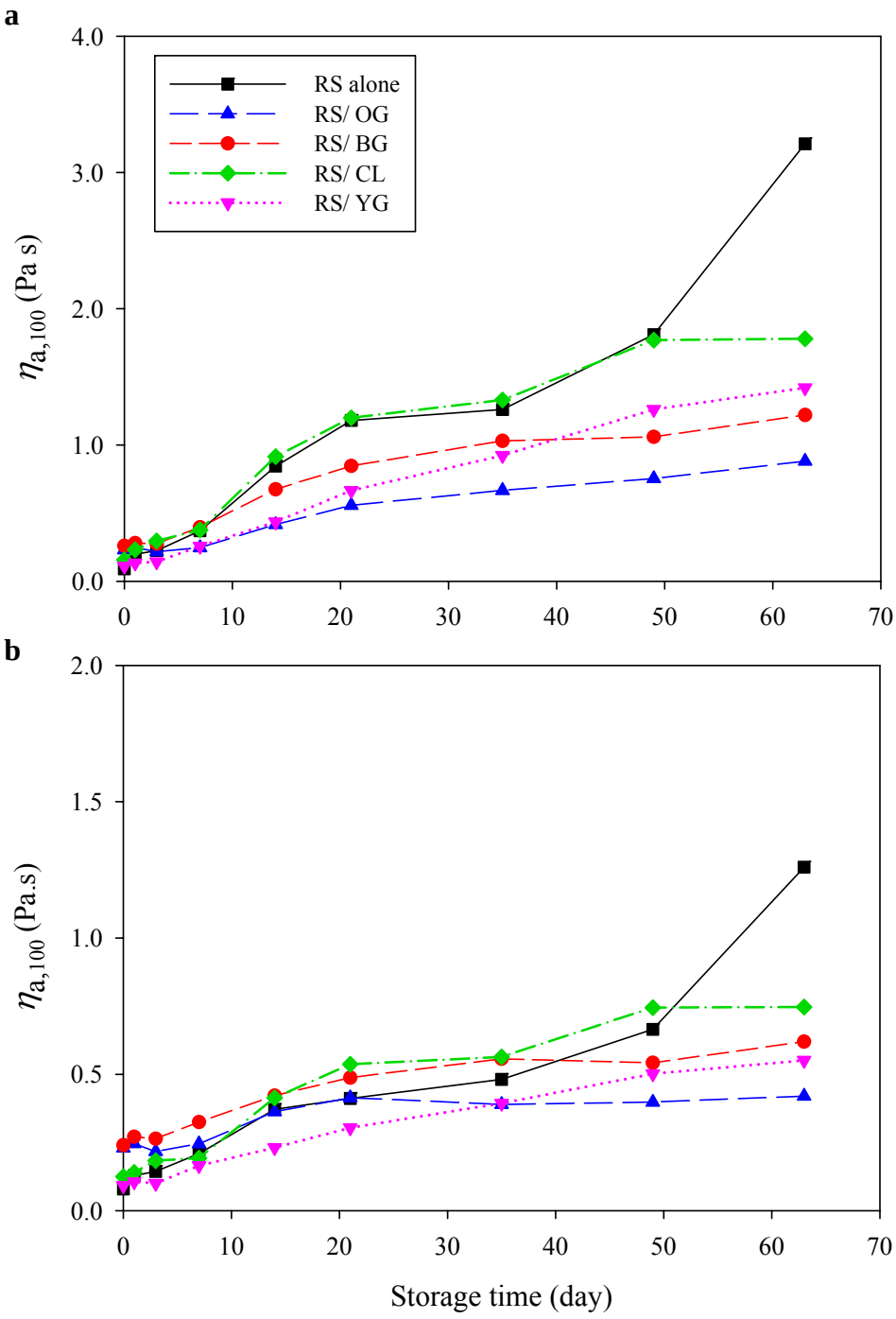


Figure 4.10 Changes in apparent viscosities at shear rate ($\dot{\gamma}$) = 100 s^{-1}, ($\eta_{a,100}$), for the (a) upward, and (b) downward flow curves, of 3.5%, w/w, RS alone and RS/β-glucan gels at a ratio of 5.5/0.5 as a function of storage time at 4°C. Measurements were made at 25°C. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

4.6 Textural properties

Textural changes in terms of gel hardness occurred during refrigerated storage of the RS/β-glucan mixed gels are presented in Figure 4.11. The RS alone gel showed the most pronounced increase in gel hardness with increasing storage times. All RS/β-glucan mixed gels appeared to result in a lower change in textural properties during storage as following: YG > OG ≈ BG > CL. From these observations, it could be concluded that the retrogradation of RS gels, was less affected by CL addition in comparison to the addition of other β-glucans tested.

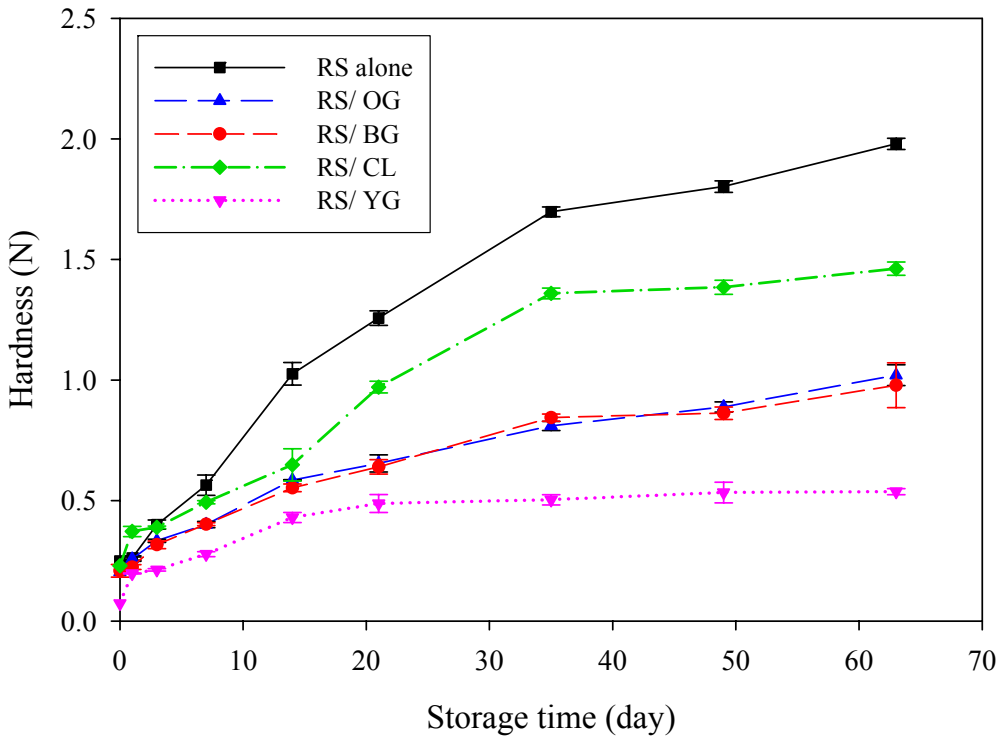


Figure 4.11 Developments in hardness of 6%, w/w, RS alone and RS/β-glucan gels at a ratio of 5.5/0.5 during storage at 4°C. Measurements were made at 25°C. Error bars represent standard deviations. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

CHAPTER V
DISCUSSION

5.1 Chemical composition

Rice starch can be classified into four group according to its amylose content: low-amylose (12–20%), intermediate amylose (20–25%), high-amylose (>25%), and waxy (little to no amylose) rice starches (Bao & Bergman, 2004). Therefore, RS used in this study was classified as a high-amylose rice starch, resulting from its amylose content was 29% (Table 4.1). Table 4.1 also shows the chemical composition of various β-glucan preparations. The CL had highest β-glucan purity as compared with other β-glucans. The contaminants of CL were attributed to the residual bacterial cell debris and proteins remained after the commercial manufacture of CL (Nishinari & Zhang, 2000). BG, OG, and YG contained lower β-glucan contents and higher impurities than CL. A slight difference in their compositions could be attributed to a difference in their origins and a variation in extraction conditions during their manufacture as pointed out by Temelli (1997) for BG, Wood et al. (1978) for OG, and Thammakiti et al. (2004) for YG. However, the high-purity β-glucan gums would be prohibitively expensive and may not be applicable or even not necessary to incorporate into commercial food products because real food systems are multicomponent and possible interactions or incompatibilities between β-glucan and other food components are not sufficiently known.

Additionally, the presence of impurities such as starch may enhance the rate of gelation as well as the gel strength of β-glucan gums and their presence may be beneficial (Burkus & Temelli, 2006). Both CL and YG contained almost 100% insoluble dietary fiber content of CL and YG which is much higher than BG and OG, indicating that CL and YG are insoluble in water whereas BG and OG are mostly water soluble. These results confirm the well known solubility characteristic of these polysaccharides reported in the literature (Lazaridou & Biliaderis, 2007; Nishinari & Zhang, 2000; Thammakiti et al., 2004).

The higher ash content of OG and BG gums could be defined as the inorganic compounds, such as calcium, sodium, iron and potassium, (as informed by the manufacturer) remained after extraction processes. The fiber fraction might help to explain the higher water holding capacity of the cereal β-glucan preparations (Symons & Brennan, 2004). The cereal β-glucans are uncharged linear polysaccharides; therefore, the mineral salts, such as calcium and sodium, might not affect their molecular conformation. The lipid contents of all OG and BG preparations were also higher than the others, which could form the amylose-lipid complexes. The DSC endothermic peaks of these complexes can be observed at higher temperatures rangeing from 94-120°C (Tester & Morrison, 1990) then those applied in this study (25-100°C), indicating no interference on the DSC result. However, these impurities might not/or negligible influence these RS/β-glucan systems because of their little amount present as compared with all contents in the systems.

5.2 Pasting characteristics

The pasting characteristics of RS in the presence or absence of various β-glucan preparations, determined by RVA analysis, are shown in Figure 4.1 and Table 4.2. Compared with RS alone, as a control, addition of all β-glucan preparations at a concentration tested resulted in significant increases in the peak, breakdown, setback, and final viscosities, whereas the pasting temperatures tended to decrease and the peak times were unaffected, excepted that of RS/CL sample.

The synergistic effect on the peak viscosity of RS/β-glucan systems was interpreted by assuming that the system in biphasic, with the β-glucan located entirely in the continuous phase. Its concentration would then increase as the volume of the phase accessible to the hydrocolloid was reduced due to swelling of the starch granules during pasting. This resulted in a pronounced increase in the viscosity of the continuous phase and in turn the overall viscosity of the suspension itself owing to the thickening properties of these hydrocolloids (Alloncle et al., 1989) added to the thickening produced by swollen starch granules (Techawipharat et al., 2008), leading to detection of pasting at lower temperature; meanwhile the interactions between hydrocolloid and leached amylose and low molecular weight amylopectin

(Christianson et al., 1981; Bahnessy & Breene, 1994) should be involved. The increase in viscosity would make the shear forces exerted on the swollen granules in the shear field much larger than those encountered in starch/water suspensions (Christianson, et al., 1981). It can be hypothesized that starch granules should become less resistant to thermal treatment and mechanical shearing (Lee et al., 2002), which means that morphological change of starch granules, involving a radial expansion of the granules to rupture, should be induced by addition of polysaccharide (Funami et al., 2005a). This results in the loss of granule integrity and subsequent disruption leading to a reduction in the paste viscosity, which is defined as breakdown viscosity, and a release of more solubilized starch, primarily amylose.

When the starch pastes are cooled, the mixture of swollen granules, granule fragments, and molecularly dispersed starch molecules exhibits a tendency to associate or retrograde, resulting in an increase viscosity, called setback viscosity. Subsequently, the interactions between certain leached starch molecules and gums could be responsible for the increases in final viscosities of the RS/β-glucan mixtures (Christianson et al., 1981; Shi & BeMiller, 2002). Moreover, the viscosity increased to a final viscosity at the end of RVA experiment, which could also be attributed to phase separation based on thermodynamic incompatibility between RS polysaccharide and β-glucan molecules, leading to mutual exclusion of each polymer. This mutual exclusion resulted in an acceleration of amylose (short term) retrogradation (Miles et al., 1985a,b) of the RS/β-glucan mixtures. These results are consistent with those found in our previous work on the RS/YG systems, in which the effect was observed to be enhanced by an increasing of YG concentrations from 0 to 1.0% (Satrapai & Suphantharika, 2007). A similar result was also reported for sweet potato starch/curdlan systems (Lee et al., 2002). However, at higher β-glucan concentrations (5%), Brennan and Cleary (2007) and Symons and Brennan (2004) found a reduction in the peak, breakdown, and final viscosities of wheat starch and flour substituted with 5% BG compared with the control. They postulated that the reduction in pasting characteristics was due to a result of soluble β-glucan competing for available water and thereby limiting starch granule swelling and associated gelatinization events. Relationships have also been established between β-glucan concentration and the pasting characteristics of oat flours. The increase in the pasting peak viscosity which

occurred with increasing β-glucan content of oat cultivars was explained by an increase in the water binding capacity of the flours (Colleoni-Sirghie et al., 2004; Zhou et al., 2000). In the present study, however, the magnitude of the effect was different among β-glucan preparations at the same concentration of 0.5%. This difference could be explained in terms of molecular weight and structure as well as purity of the β-glucans and the impurities present in the various β-glucan preparations.

Nevertheless, CL and YG addition illustrated less promotion of final viscosities than the others after cooled to 50°C. This effect may caused by CL and YG are insoluble in water whereas BG and OG are mostly water soluble. The insoluble dietary fiber content may provides less water holding capacity which lead to less increase in the effective concentration of starch, resulting less acceleration of amylose or short chain amylopectin retrogradation.

5.3 Thermal properties

The thermal properties of the RS/β-glucan mixtures and their corresponding retrograded gels are summarized in Table 4.3 DSC has been used to study not only the disordering behavior of starch during gelatinization but also the reordering behavior during aging of the retrograded gel. For the first run, the gelatinization temperatures (T_o , T_p and T_c) and gelatinization temperature ranges (T_c-T_o) seemed to be unaffected by addition of various β-glucan preparations at concentration tested. This demonstrates that there was enough water available for both starch and hydrocolloid, regardless of the type of β-glucans used (Khanna & Tester, 2006). Meanwhile, the gelatinization enthalpies (ΔH_1) slightly decreased, except for RS/CL sample, compared to those of the control. This slight decrease in enthalpy in the presence of hydrocolloid and excess water could be attributed to the lower heating rates and the decreased mobility of water molecules as pointed out by Krüger, Ferrero, and Zaritzky (2003). This observation suggests that the process of gelatinization involving destruction of starch crystallite and loss of helical conformation was not affected by the presence of β-glucans at the concentration tested. In general, it has been reported that β-glucans at the low concentrations (0.5-2.0%) used in several starches or flours markedly increased viscosity during pasting, but they seemed to slightly alter the DSC

gelatinization parameters (Biliaderis et al., 1997). However, these gelatinization parameters were significantly affected by the addition of β-glucans at higher levels (5-30%) (Brennan & Cleary, 2007; Kim & Setser, 1992; Symons & Brennan, 2004).

Within the assayed range of temperatures used in this study (25-100°C) only amylopectin retrogradation could be quantified by DSC. Retrogradation of amylopectin is a reversible process under 100°C, but amylose retrogradation needs more energy to revert the crystal formation (Miles et al., 1985a). The reheating of retrograded gels stored for 21 days at 4°C are also shown in Table 4.2. The endothermic transition temperatures (T_o, T_p, and T_c) of retrogradation were found to be lower than the gelatinization temperatures. The melting enthalpies of retrograded gels (ΔH_2) were smaller than the gelatinization enthalpies (ΔH_1). The retrogradation temperature ranges (T_c-T_o) were more than broader than the gelatinization temperature ranges for a given sample. These results suggest that retrogradation results in reassociation of the gelatinized starch molecules, but in less ordered and hence less perfect or stable forms and more heterogeneous in stability than those existing in the native starch granules (Karim et al., 2000).

The decreases in transition temperatures (T_o, T_p, T_c) of the retrograded RS alone and RS/β-glucan gels (Figure 4.2) resulted in an increase in the T_c-T_o values with storage time (Figure 4.3a). The rates of increments for the magnitude of T_c-T_o (Figure 4.3a) and $\Delta H_2/\Delta H_1$ (Figure 4.3b) for RS with and without β-glucans as a result of amylopectin retrogradation were highest during the first week of storage and then slowed down thereafter. The magnitude of both parameters was decreased by addition of all β-glucans tested, indicating that these β-glucans retarded the long-term retrogradation of RS gels. These results are in good agreement with our previous report in which it was found that the effect was more pronounced at higher β-glucan concentrations (Satrapai & Suphantharika, 2007). In this study, however, the soluble β-glucans (OG and BG) apparently exhibited more pronounced effect on retardation of amylopectin retrogradation compared to the insoluble ones (CL and YG) at the same concentration tested (Figure 4.3b).

Starch retrogradation is a non-equilibrium thermoreversible recrystallization process which is governed by a consecutive three-step mechanism of nucleation,

propagation, and maturation (Slade & Levine, 1987). However, the storage temperature applied in this study (4°C) favors the nucleation rather than the propagation of the crystallites which occurs at higher temperatures, i.e., 30–40°C (Silverio, Fredriksson, Andersson, Eliasson, & Åman, 2000; Slade & Levine, 1991). In general, the rate limiting step in the recrystallization process is nucleation (which is enhanced at lower temperatures) rather than propagation (which is enhanced at higher temperatures) (Slade & Levine, 1991). The T_o of the retrogradation endotherm concurs with the temperature where the least stable amylopectin crystallites formed during storage melt. Therefore, the marked decrease in T_o or even T_p means that there was a large amount of new and less stable amylopectin crystallites formed during storage, particularly during the first week (Figure 4.2a and b). A slight decrease in T_c (Figure 4.2c) which reflected the melting temperature of the most stable crystallites indicates that the propagation of amylopectin crystallites did not occur during storage.

The increase in T_c-T_o values during storage was observed (Figure 4.3a) and can be interpreted as a shift from a more homogeneous set of amylopectin crystallites with similar stability to a heterogeneous set with varying stability. As would be expected, the increase in $\Delta H_2/\Delta H_1$ values with storage time (Figure 4.3b) indicates that the more energy was required for melting a large amount of amylopectin crystallites formed during storage.

Because nucleation mechanism is a liquid state event which requires orientational mobility of the polymer chains in the amylopectin molecule (Slade & Levine, 1987), therefore addition of β-glucan, which absorbed water, decreased the mobility of the starch chains and in turn retarded the retrogradation of the starch gels. The evidence was clearly demonstrated by the higher T_o (Figure 4.2a and Table 4.3) and lower T_c-T_o (Figure 4.3a and Table 4.3) and $\Delta H_2/\Delta H_1$ (Figure 4.3b and Table 4.3) of the RS/β-glucan mixed gels as compared with the starch alone paste, indicating that a smaller amount of amylopectin crystallites formed in the pastes containing β-glucan to different degrees. The T_c-T_o and $\Delta H_2/\Delta H_1$ of all samples markedly increased during the first three weeks and then leveled off for the rest of the storage period. According to $\Delta H_2/\Delta H_1$ values, the decreases in development rates of amylopectin recrystallization were obtained as following degree: RS/OG > RS/BG > RS/YG > RS/CL.

4.4 Dynamic viscoelastic properties

For the freshly prepared RS/β-glucan gels at 0 day storage (Figure 4.4a), the addition of various β-glucan preparations to RS led to an increase in the G' and G'' values except for BG, which showed a slightly lower G' values than those of the control. The increases in G'' values were more pronounced in comparison with those in G'. This reflects the greater influence of β-glucans on viscous than on elastic properties of the RS/β-glucan systems. This result is in good agreement with those found in RS/galactomannan mixtures (Kim et al., 2006; Yoo et al., 2005).

After the junction zone has formed, only relatively slow structural alterations remain effective (crystallization process, long term retrogradation), in which the junction zones are expanded and reinforces. This structural development is essentially caused by amylopectin component of starch. Therefore, the sample gels were observed after various storage time (Figure 4.4b-d). These rheograms show that the G' and G'' values of the RS alone gels increased at much faster rates than those of the RS/β-glucan gels and became the highest values after 63 days of storage (Figure 4.4d). This result indicates that the added β-glucan retarded the formation of recrystallized amylopectin by reducing water availability and mobility of the starch chains as described earlier and in turn resulted in a weaker gel structure.

During storage times, the G' values increased steadily (Figure 4.5a), whereas the tan δ values decreased with storage time (Figure 4.5b), indicating the development of a more firm structure due to starch retrogradation (Figure 4.3) as described in DSC experiment. However, these effects could be reduced by different degrees by the addition of various β-glucan preparations. These rheological measurements indicated that the gels with added β-glucans are softer when fresh but become more rigid after storage but the gel strengths of the pure RS gels are never attained. Similar results were also reported in our previous work for the RS/YG gels (Satrapai & Suphantharika, 2007). Biliaderis et al. (1997) found that when β-glucan was incorporated in waxy maize starch, the gelation was retarded possibly as a result of interference in the intermolecular associations among amylopectin molecules by the β-glucan. In studies on waxy rice starch/guar gum mixtures, it was also shown that guar gum changes the nature of the starch network from viscoelastic to more viscous-like,

presumably by reducing the number of permanent cross-links between the amylopectin molecules (Kulicke et al., 1996).

In Figure 4.5a, the G' values of all RS/β-glucan mixed gel were lower than RS alone. This could consider a lower development of elastic behavior reflected to lower retrogradation rates in following degree: RS/OG > RS/BG > RS/YG > RS/CL > RS alone, which corresponding to the rate of retrogradation ratio ($\Delta H_2/\Delta H_1$) in DSC data.

However, Lazaridou, Biliaderis, Micha-Screttas & Steele (2004) reported that the molar ratios of tri- to tetrasaccharides (DP3/DP4) of barley β-glucan (2.8–3.0) is larger than oat β-glucan (2.1), the critical concentration, viscosity, viscoelastic and shear thinning properties among the samples were influenced mainly from the molecular size of the polysaccharides, and were less dependent on fine structure. They also revealed that the rate of gel structure development increased with decreasing molecular size and increasing DP3/DP4 ratio, while increasing molecular size of β-glucans, revealed a decrease in the apparent storage modulus values (G'). The presence of impurities, such as starch, was also reported to enhance the rate of gelation as well as the gel strength of β-glucan gums (Burkus & Temelli, 2006). Furthermore, Ishida and Takeuchi (1981) reported the higher amount of β-glucan from bacterial (curdlan) results in increasing gel strength of various type of starch/curdlan mixed gel. As in our previous work, the RS/β-glucan mixtures with lower β-glucan content are more solid-like than those with higher β-glucan content prepared from spent brewer's yeast (Satrapai & Suphantharika, 2006).

4.5 Steady shear rheological properties

The steady flow characteristics of RS gels in the presence or absence of various β-glucan preparations are presented in Figure 4.6. For the range of shear rates used in this study, all gels exhibited mainly time-dependent shear-thinning (thixotropic) with yield stress behavior. The Herschel-Bulkley model can fit to the flow curves of freshly prepared gels except the upward flow curve of RS/CL gel (Figure 4.6a). All the upward curves of retrograded gels (Figure 4.6b-d) and the fresh RS/CL gel (Figure 4.6a) exhibited extremely thixotropic flow with gel strength having a spur forms in the hysteresis loop. The first peak in these upward flow curves was related to the stress needed to break the gel structure and caused the solution to revert to its normal viscous flow as reported for certain concentrated fluids (Halmos & Tiu, 1981) and concentrated (5%) aqueous suspension of CL (Funami, Yada, & Nakao, 1998). Therefore, these flow curves did not follow any of the typical viscous model behavior patterns.

Most shear-thinning biopolymer (hydrocolloid) dispersions exhibit similar three-stage viscous response when shear over the wide range; (1) at low shear rates, they show Newtonian properties with a constant zero-shear viscosity over a limited shear range, which is followed by (2) a shear-thinning range where solution viscosity decreases in accordance with the power law relationship–the reciprocal of the shear rate at which the transition from Newtonian to pseudoplastic behavior occurs is the characteristic time or the time constant–and (3) at high shear rates, they show a limiting and constant infinite-shear viscosity. The three regions may be thought of being due to rearrangement in the conformation of the biopolymer molecules in the dispersion due to shearing. In this study, the plot of a log shear rate ($\dot{\gamma}$) and log apparent viscosity (η_a) of RS alone and RS/β-glucan gels after stored for 63 day at 4°C (Figure 4.7) indicated that only shear-thinning region at intermediate shear rates (stage 2) was obtained inconsistent with those reported in the literature (Rao, 1999). This stage 2 also explained that the polymer chains undergo gradual arrangement with shear rate resulting in a power law behavior. Therefore, the Herschel-Bulkley model could be applied to fit the downward flow curves of all RS alone and RS/β-glucan gels as a function of storage time (Figure 4.8 a-c).

For the fresh gels, the consistency coefficients (*K*), flow behavior indices (*n*), and yield stresses (σ_0), along with the coefficients of determination (R^2) for each upward or downward flow curve, and hysteresis loop areas between these two curves are summarized in Table 4.4. A hysteresis loop area was observed in all gels studied, which can be interpreted as structural breakdown by the shear field to alter a structure or form a new structure, which then maintained a shear-thinning characteristic on following shear sweeps (Achayuthakan & Suphantharika, 2008). Shearing of the gels causes their structure to be broken down and this is the cause of the shear thinning behavior as evidenced by $n < 1.0$. On reducing the shear rate back to zero the structure builds up only in part within the duration of the experiment, resulting in the lower σ_0 values of the downward curves than those of upward curves. Addition of the soluble β-glucans (OG and BG) markedly increased the *K* values, whereas addition of the insoluble β-glucans (CL and YG) sharply enhanced the σ_0 values of RS gels. This result reflects that the soluble β-glucans mainly enhanced viscous properties, while the insoluble β-glucans promoted structure formation of RS gels.

For the retrograded RS gels, the dynamic yield stress (σ_0) values of all RS alone and RS/β-glucan gels increased during refrigerated storage. The RS alone gels exhibited the highest σ_0, indicating a high stability of their structure, followed by RS/BG, RS/CL, RS/OG and RS/YG gels, respectively (Figure 4.8a). Achayuthakan, Suphantharika and Rao (2006) also reported that yield stress of waxy maize starch and waxy maize starch-xanthan gum mixture, especially their bonding and network components, may explain the network formation. The addition of CL enhanced pseudoplasticity of RS gels to a greater degree than did addition of YG as evidenced by their higher *K* values and slightly lower *n* values, during refrigerated storage (Figure 4.8b and c). In contrast, the increase in *K* values and decrease in *n* values of RS/OG and RS/BG gels were also observed (Figure 4.8b and c), however the opposite trend of these two values was found after gel storage at 4°C for 21 and 35 days, respectively. This result showed that the RS/OG and RS/BG gels became less pseudoplastic after longer storage. It can be explained that OG and BG had higher water holding capacity in their gel structure than the others even after longer storage time. The RS/OG and RS/BG gels possibly had the high water content in their gel

structures, when placed into rheometer measuring system. After upward shearing, the gel structure was destroyed and more water subsequently released out, leading to detection of a high viscous properties.

The time-dependence of the hysteresis loop areas of RS gels in the presence or absence of various β-glucans during storage is shown in Figure 4.9. The hysteresis loop areas of all RS gels increased steadily with storage time in the following order: RS alone > RS/CL > RS/YG > RS/BG > RS/OG. The higher hysteresis loop areas, i.e. higher thixotropy, is attributed to the greater structural breakdown observed during shearing, reflecting a more structured of the gels. This result showed a trend similar to dynamic viscoelastic data (Figure 4.5a), which in turn reflected the extend of retrogradation of RS gels (Figure 4.3b). Up to this point, we can conclude that the various β-glucan preparations tested retarded long-term retrogradation of RS gels in the order OG > BG > YG > CL.

The apparent viscosities at shear rate ($\dot{\gamma}$) of 100 s^{-1} ($\eta_{a,100}$) for the upward and downward flow curves of RS gels in the presence or absence of various β-glucan preparations increased with storage time (Figure 4.10). The $\eta_{a,100}$ values of upward curves were higher than those of downward curves possibly due to a modification of the gel structure caused by shearing at high shear rate. Moreover, the $\eta_{a,100}$ of upward curves was used to describe the degree of starch retrogradation because it related to the stress needed to break the gel structure at this shear rate (Figure 4.10a), the degree of retrogradation during storage affected by the β-glucan addition in the order RS alone > CL > BG ≈ YG > OG.

4.6 Textural properties

Textural changes in terms of gel hardness occurred during refrigerated storage of RS gels in the presence or absence of various β-glucan preparations are presented in Figure 4.11. The RS alone gel showed the most pronounced increase in gel hardness with increasing storage times, which led to gel hardening and possibly unacceptable texture. Addition of the various β-glucans tested to RS gels appeared to result in a lower change in textural properties during storage in the order YG > OG ≈ BG > CL.

Consequently, it could be concluded that the retrogradation of RS gels, was less affected by CL addition in comparison to the addition of other β-glucans tested. This can be explained by the fact that CL gel itself can produce syneresis which is caused by gel shrinkage and a releasing of water and consequently results in an increase in gel strength with storage time. Syneresis and gel strength were reported to be decreased by addition of starch to an aqueous suspension of CL before heating. The swelling of starch granule during the formation of CL gel partially breaks the CL gel and inhibits the shrinkage and results in a repressing of the syneresis and gel strength (Ishida & Takeuchi, 1981; Nakao et al., 1991). Even though the RS/YG gel seemed to be more structured than the RS/OG or RS/BG gel (Figure 4.5), its gel hardness was lower (Figure 4.11), indicating that its gel structure is more fragile.

CHAPTER VI
CONCLUSION

The gelatinization and retrogradation characteristics of the rice starch (RS) were modified by OG, BG, CL or YG addition. Results of RVA indicated the increases in peak, breakdown, setback, and final viscosities of the RS/β-glucan dispersion during pasting, whereas the pasting temperatures tended to decrease and the peak times were unaffected. DSC data demonstrated that the gelatinization temperatures (T_o, T_p and T_c) and gelatinization temperature ranges (T_c-T_o) seemed to be unaffected by addition of various β-glucan preparations, while ΔH_1 slightly decreased. Addition of β-glucan preparations to RS resulted in a significant increase in the T_o, whereas a decrease in the T_c-T_o, ΔH_2 and $\Delta H_2/\Delta H_1$, indicating that β-glucans slowed the retrogradation rate of RS gels during storage. Dynamic viscoelastic measurements on the fresh RS/β-glucan gels indicated that the G' and G'' were increased by the addition various β-glucans. The RS/β-glucan gels had larger tan δ values than the RS alone gel indicating the greater influence of β-glucans on viscous than on elastic properties of the RS/β-glucan systems. Storage of RS gels showed that the G' values increased, whereas the tan δ values decreased with storage time, indicating the development of a more firm structure due to starch retrogradation; however, these effects could be reduced by different degrees by the addition of various β-glucan preparations. Steady flow tests showed that all gels exhibited mainly time-dependent shear-thinning (thixotropic) with yield stress behavior. The RS retrograded gels exhibited extremely thixotropic flow with gel strength having a spur forms in the hysteresis loop, however this value were reduced when various β-glucans were added. The addition of the various β-glucans tested to RS gels appeared to result in a lower change in textural properties during storage as well.

Moreover, the study on thermal and rheological properties showed a similar trend that the various β-glucan preparations tested retarded long-term retrogradation of RS gels in the order OG > BG > YG > CL.

This study illustrated that the incorporation of various β-glucan preparations in RS, under the present experimental conditions, significantly increased the paste viscosities of RS suspensions during pasting and retarded the retrogradation of RS gels during refrigerated storage in which the soluble β-glucans (OG and BG) were found to be more effective than the insoluble ones (CL and YG). The gelatinization behavior of RS, however, seemed to be unaffected by the addition of these β-glucan preparations. These results could be explained on the basis of the differences in molecular weight and structure as well as purity of β-glucans present in various preparations. These findings may provide some practical information on the role and potential usefulness of various β-glucans in RS-based products.

REFERENCES

AACC (2000). *Approved methods of the AACC* (10th ed.). St. Paul, MN, USA: American Association of Cereal Chemists.

AOAC (2000). *Official methods of analysis* (17th ed.). Gaithersburg, MD: AOAC International.

Achayuthakan, P., & Suphantharika, M. (2008). Pasting and rheological properties of waxy corn starch as affected by guar gum and xanthan gum. *Carbohydrate Polymers*, *71*, 9-17.

Achayuthakan, P., Suphantharika, M., & Rao M. A. (2006). Yield stress components of waxy corn starch–xanthan mixtures: Effect of xanthan concentration and different starches. *Carbohydrate Polymers*, 65, 469–478.

Alloncle, M., Lefebvre, J., Llamas, G., & Doublier, J. L. (1989). A rheological characterization of cereal starch-galactomannan mixtures. *Cereal Chemistry*, *66*, 90-93.

Anderson, J. W., & Bridges, S. R. (1993). Hypocholesterolemic effects of oat bran in humans. In P. J. Wood (Ed.), *Oat Bran* (pp. 139). Minnesota: American Association and Cereal Chemists.

Annable, P., Fitton, M. G., Harris, B., Phillips, G. O., & Williams, P. A. (1994). Phase behavior and rheology of mixed polymer systems containing starch. *Food Hydrocolloids*, *8*, 351-359.

Appelqvist, I. A. M., & Debet, M. R. M. (1997). Starch-biopolymer interactions–A review. *Food Reviews International, 13*, 163-224.

Atwell, W. A., Hood, L. F., Lineback, D. R., Varriano-Marston, E., & Zobel, H. F. (1988). The terminology and methodology associated with basic starch phenomena. *Cereal Foods World*, *33*, 306-311.

Autio, K. A. (1988). Rheological properties of solutions of oat β-glucans. In G. O. Phillips, D. J. Wedlock, & P. A. Williams (Eds.), *Gums and Stabilisers for the Food Industry* (pp. 483). Oxford: IRL Press.

Autio, K. (2006). Functional aspects of cereal cell-wall polysaccharides. In A.-C. Eliasson (Ed.), *Carbohydrates in food* (2nd ed., pp. 167–207). Boca Raton, FL: CRC Press.

Autio, K., Myllymäki, O., & Mälkki, Y. (1987). Flow properties of solutions of oat β-glucans. *Journal of Food Science*, *52*, 1364-1366.

Bacic, A. & Stone, B. A. (1981). Isolation and ultrastructure of aleurone cell walls from wheat and barley. *Australian Journal of Plant Physiology*, *8*, 453–474.

Bamforth, C. W., Martin, H. L., & Wainwright, T. A. (1979). A role for carboxypeptidase in the solubilization of barley β-glucan. *Journal of the Institute of Brewing*, *85*, 334.

Bahnassey, Y. A., & Breene, W. M. (1994). Rapid Visco-Analyzer (RVA) pasting profiles of wheat, corn, waxy corn, tapioca and amaranth starches (*A. hypochondriacus* and *A. cruentus*) in the presence of konjac flour, gellan, guar, xanthan and locust bean gums. *Starch/Stärke*, *46*, 134–141.

Baker, L. A., & Rayas-Duarte, P. (1998). Freeze-thaw stability of amaranth starch and the effects of salt and sugars. *Cereal Chemistry*, *75*, 301-303.

Bao, J., & Bergman, C. J. (2004). The functionality of rice starch. In A.-C. Eliasson (Ed.), *Starch in food: Structure, function and applications* (pp. 258-294). Cambridge, England: Woodhead Publishing Limited.

Bao, J. S., He, P., Xia, Y. W., Chen, Y., & Zhu, L. H. (1999). Starch RVA profile parameters of rice are mainly controlled by Wx gene. *Chinese Science Bulletin*, *44*, 2047-2051.

Beer, M. U., Arrigoni, E., & Amado, R. (1996). Extraction of oat gum from oat bran: effects of process on yield, molecular weight distribution, viscosity and (1→3)(1→4)-β-D-glucan content of the gum. *Cereal Chemistry, 73*, 58–62.

BeMiller, J. N. (2007). *Carbohydrate chemistry for food scientists* (2nd ed.). St. Paul, MN: AACC International Inc.

Bengtsson, S., Åman, P., & Graham, H. (1990). Chemical studies on mixed-linked β-glucans in hull-less barley cultivars giving different hypocholesterolaemic responses in chickens, *Journal of the Science of Food and Agriculture*, *52*, 435.

Bhatty, R. S. (1993). Extraction and enrichment of (1→3),(1→4)-β-D-glucan from barley and oat brans. *Cereal Chemistry*, *70*, 73–77.

Bhatty, R. S. (1995). Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat brans. *Journal of Cereal Science*, *22*, 163–170.

Biliaderis, C. G., & Zawistowski, J. (1990). Viscoelastic behaviour of aging starch gels: Effects of concentration, temperature, and starch hydrolysates on network properties. *Cereal Chemistry*, *67*, 240-246.

Biliaderis, C. G., Arvanitoyannis, I., Izydorczyk, M. S., & Prokopowich, D. J. (1997). Effect of hydrocolloids on gelatinization and structure formation in concentrated waxy maize and wheat starch gels. *Starch/Stärke*, *49*, 278-283.

Böhm, N., & Kulicke, W. M. (1999). Rheological studies of barley (1→3),(1→4)-β-D-glucan in concentrated solution: mechanistic and kinetic investigation of the gel formation. *Carbohydrate Research*, *315*, 302–311.

Bornet, F. (1993). Technological treatments of cereals. Repercussions on the physiological properties of starch. *Carbohydrate Polymers*, *21*, 195-203.

Brennan, C. S., & Cleary, L. J. (2007). Utilisation Glucagel® in the β-glucan enrichment of breads: A physicochemical and nutritional evaluation. *Food Research International*, *40*, 291–296.

Burkus, Z., & Temelli, F. (2006). Network formation by pilot plant and laboratory-extracted barley β-glucan and its rheological properties in aqueous solutions. *Cereal Chemistry*, *83*, 584-589.

Cao, Y., Dickinson, E., & Wedlock, D. J. (1990). Creaming and flocculation in emulsions containing polysaccharides, *Food Hydrocolloids*, *4*, 185–195.

Carpita, N. C., & Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *The Plant Journal*, *3*, 1–30.

Carpita, N. C. (1996). Structure and biogenesis of the cell walls of grasses. *Annual Review of Plant Physiology and Plant Molecular Biology*, *47*, 445–76.

Chaisawang, M., & Suphantharika, M. (2005). Effects of guar gum and xanthan gum additions on physical and rheological properties of cationic tapioca starch. *Carbohydrate Polymers*, *61*, 288–295.

Charalampopoulos, D., Wang, R., Pandiella, S. S., & Webb, C. (2002). Application of cereals and cereal components in functional foods: a review. *International Journal of Food Microbiology*, *79*, 131–141.

Champagne, E. T. (1996). Rice starch composition and characteristics. *Cereal Foods World*, *41*, 833-838.

Chen, C. R., & Ramaswamy, H. S. (1999). Rheology of tapioca starch. *Food Research International*, *32*, 319-325.

Chiang, P. Y., & Yeh, A. I. (2002). Effect of soaking on wet-milling of rice. *Journal of Cereal Science*, *35*, 85-94.

Christianson, D. D., Hodge, J. E., Osborne, D., & Detroy, R. W. (1981). Gelatinization of wheat starch as modified by xanthan gum, guar gum and cellulose gum. *Cereal Chemistry*, *58*, 513-517.

Chuah, C. T., Sarko, A., Deslandes, Y., & Marchessault, R. H. (1983). Packing analysis of carbohydrates and polysaccharides. Part 14. Triple-helical crystalline structure of curdlan and paramylon hydrates. *Macromolecules*, *16*, 1375–1382.

Clark, A. H., Gidley, M. J., Richardson, R. K., & Ross-Murphy, S. B. (1989). Rheological studies of aqueous amylose gels: the effects of chain length and concentration on gel modulus. *Macromolecules*, *22*, 346-351.

Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymer gels. *Advances in Polymer Science*, *83*, 57-192.

Clarke, A. E., & Stone, B. A. (1963). Chemistry and biochemistry of β-1,3-glucans. *Pure and Applied Chemistry*, *13*, 134.

Closs, C. B., Conde-Petit, B., Robert, I. D., Tolstoguzov, V. B., & Escher, F. (1999). Phase separation and rheology of aqueous starch/galactomannan system. *Carbohydrate Polymers*, *36*, 67-77.

Colleoni-Sirghie, M., Jannink, J. -L., & White, P. J. (2004). Pasting and thermal properties of flours from oat lines with high and typical amounts of β-glucan. *Cereal Chemistry*, *81*, 686-692.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinization. Origin of the enthalpic transition. *Carbohydrate Research*, *227*, 103-112.

Crawford, G. W., & Shen, C. (1998). The origins of rice agriculture: Recent progress in East Asia. *Antiquity*, *72*, 858–866.

Dais, P., & Perlin, A. S. (1982). High field ^{13}C-NMR spectroscopy of β-glucans, amylopectin, and glycogen. *Carbohydrate Research*, *252*, 6861.

Dallies, N., François, J., & Paquet, V. (1998). A new method for quantitative determination of polysaccharides in the yeast cell wall. Application to the cell wall defective mutants of *Saccharomyces cerevisiae*. *Yeast*, *14*, 1297–1306.

Deslandes, Y., Marchessault, R. H., & Sarko, A. (1980). Triple-Helical Structure of (1→3)-β-D-Glucan. *Macromolecules*, *13*, 1466–1471.

Donald, A. M. (2004). Understanding starch structure and functionality. In A.-C. Eliasson (Ed.), *Starch in food: Structure, function and applications* (pp. 156-184). Cambridge, England: Woodhead Publishing Limited.

Doublier, J. L., & Wood, P. J. (1993). Structure and rheological properties of hydrolyzed oat gums in aqueous solution. *Cereal Foods World*, *38*, 623.

Doublier, J. L., & Cuvelier, G. (2006). Gums and hydrocolloids: Functional aspects. In A.-C. Eliasson (Ed.), *Carbohydrates in food* (2nd ed., pp. 233–272). Boca Raton, FL: CRC Press.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Eerlingen, R. C., Jacobs, H., & Delcour, J. A. (1994). Enzyme-resistant starch. V. Effect of retrogradation of waxy maize starch on enzyme susceptibility. *Cereal Chemistry*, *71*, 351-355.

Eliasson, A. C., & Gudmundsson, M. (1996). Starch: physicochemical and functional aspects. In A. C. Eliasson (Ed.), *Carbohydrates in Foods* (pp. 431-503). New York: Marcel Dekker.

Ellis, H. S., Ring, S. G., & Whittam, M. A. (1989). A comparison of the viscous behaviour of wheat and maize starch pastes. *Journal of Cereal Science*, *10*, 33-44.

Evans, I. D., & Lips, A. (1992). Viscoelasticity of gelatinized starch dispersions. *Journal of Texture Studies*, *23*, 69–86.

Faraj, A., Vasanthan, T., & Hoover, R. (2006). The influence of α-amylase-hydrolysed barley starch fractions on the viscosity of low and high purity barley β-glucan concentrates. *Food Chemistry*, *96*, 56-65.

Fennema, O. R. (1985). Water and ice. In O. R. Fennema (Ed.), *Food Chemistry* (pp. 23-67). New York: Marcel Dekker.

Ferrero, C., Martino, M. N., & Zaritzky, N. E. (1994). Corn starch-xanthan gum interaction in its effect on the stability during storage of frozen gelatinized suspensions. *Starch/Stärke*, *46*, 300-308.

Ferry, J. D. (1980). *Viscoelastic properties of polymers* (3rd ed). New York: John Wiley.

Fincher, G. B. (1992). In P. R. Shewry, & O. Wallingford (Eds.), *Barley: genetics, biochemistry, molecular biology and biotechnology*. C.A.B. International.

Fincher, G. B., & Stone, B. A. (1986). Cell walls and their components in cereal grain technology. In Y. Pomeranz (Ed.), *Advances in Cereal Science and Technology* (pp. 207). Minnesota: American Association and Cereal Chemists.

Fitzgearld, M. A., Martin, M., Ward, R. M., Park, W. D., & Shead, H. J. (2003). Viscosity of rice flour: a rheological and biological study. *Journal of Agricultural and Food Chemistry*, *51*, 2295-2299.

Fulton, W. S., & Atkins, E. D. T. (1980). The gelling mechanism and relationship to molecular structure of microbial polysaccharide curdlan. In *Fibre Diffraction Methods* (pp.385-410). Washington, DC: American Chemical Society.

Funami, T., Yada, H., & Nakao, Y. (1998). Curdlan properties for application in fat mimetics for meat products. *Journal of Food Science*, *63*, 283-287.

Funami, T., Kataoka, Y., Omoto, T., Goto, Y., Asai, I., & Nishinari, K. (2005a). Effect of non-ionic polysaccharides on the gelatinization and retrogradation behavior of wheat starch. *Food Hydrocolloids*, *19*, 1-13.

Funami, T., Kataoka, Y., Omoto, T., Goto, Y., Asai, I., & Nishinari, K. (2005b). Food hydrocolloids control the gelatinization and retrogradation behavior of starch.2a. Functions of guar gum with different molecular weights on the gelatinization behavior of corn starch. *Food Hydrocolloids*, *19*, 15-24.

Gallant, D. J., Bouchet, B., & Baldwin P. M. (1997). Microscopy of starch: evidence of a new level of granule organization. *Carbohydrate Polymers*, *32*, 177–191.

Gao, A., Fukud, A., Katsuraya, K., Kaneko, Y., Mimura, T., Nakashima, H., & Uryu, T. (1997). Synthesis of Regioselective Substituted Curdlan Sulfates with Medium Molecular Weights and Their Specific Anti-HIV-1 Activities. *Macromolecules*, *30*, 3224–3228.

Generalized texture profile analysis curve. (http://www.texturetechnologies.com/texture_profile_analysis.html)

Gordon, D. T. (1989). Functional properties vs. physical action of total dietary fiber. *Cereal Foods World*, *34*, 517.

Gunning, P. A., Hibberd, D. J., Howe, A. M., & Robins, M. M. (1988). Gravitational destabilization of emulsions flocculated by non-adsorbed xanthan. *Food Hydrocolloids*, *2*, 119–129.

Halmos, A. L., & Tiu, C. (1981). Liquid foodstuffs exhibiting yield stress and shear degradability. *Journal of Texture Studies*, *12*, 39-46.

Harada, T. (1992). The story of research into curdlan and the bacteria producing it. *Trends in Glycoscience and Glycotechnology*, 4, 309.

Harada, T., Masada, M., Fujimori, K., Maeda, I. (1966). Production of a firmresilient gel-forming polysaccharide by a mutant of *Alcaligenes faecalis* var. *myxogenes* 10C3. *Agricultural and Biological Chemistry*, *30*, 196-198**.**

Harada, T., Misaki, A., & Saito, H. (1968). Curdlan: a bacterial gel-forming beta-1,3 glucan. *Archives of Biochemistry and Biophysics*, *124*, 292.

Harada, T., Terasaki, M., & Harada, A. (1993). *Industrial gums*. New York: Academic Press, 427.

Hoefler, A. C. (2004). *Hydrocolloids*. Minnesota: American Association and Cereal Chemists.

Hizukuri, S. (1986). Polymodal distribution of the chain lengths of amylopectin and its significance. *Carbohydrate Research*, *147*, 342-347.

Hizukuri, S. (1993). Towards an understanding of the fine structures of starch molecules. *Denpun Kagaku*, *40*, 133-147.

Hizukuri, S., Takeda, Y., & Yasuda, M. (1981). Multi-branched nature of amylose and the action of debranching enzymes. *Carbohydrate Research*, *94*, 205–213.

Imberty, A., Buléon, A., Tran, V., & Pérez, S. (1991). Recent advances in knowledge of starch structure. *Starch/Stärke*, *43*, 375–384.

Imeson, A. (1997). *Thickening and Gelling Agent for Food* (2nd ed.). UK: Blackie Academic & Professional.

Inaba, H., Hoshizawa, M., Adachi, T., Matsumura, Y., & Mori, T. (1994). Characterization of texture and mechanical properties of starch gels. *Food Hydrocolloids*, *8*, 33-44.

Ishida, K. & Takeuchi, T. (1981). Starch to repress syneresis of curdlan gel. *Agricultural and Biological Chemistry*, *45*, 1409-1412.

Izydorczyk, M. S., Macri, L. J., & MacGregor, A. W. (1998a). Structure and physicochemical properties of barley non-starch polysaccharides. I. Water-extractable β-glucans and arabinoxylans. *Carbohydrate Polymers*, *35*, 249–258.

Izydorczyk, M. S., Macri, L. J., & MacGregor, A. W. (1998b). Structure and physicochemical properties of barley non-starch polysaccharides. II. Alkali-extractable β-glucans and arabinoxylans. *Carbohydrate Polymers*, *35*, 259–69.

Jadhav, S. J., Lutz, S. E., Ghorpade, V. M., & Salunkhe, D. K. (1998). Barley: chemistry and value-added processing. *Critical Reviews in Food Science and Nutrition*, *38*, 123–171.

Jankowski, T. (1992). Influence of starch retrogradation on the texture of cooked potato tuber. *International Journal of Food Science and Technology*, *27*, 637-642.

Jenkins, P. J., & Donald, A. M. (1995). The influence of amylose on starch granule structure**.** *International Journal of Biological Macromolecules*, *17*, 315-321.

Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A., Wong, K. S., Radosavljevic, M., & Kasemsuwan, T. (1999). Effect of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry*, *76*, 629-637.

Juliano, B. O. (1984). Rice starch Production, Properties, and Uses. In R. L. Whistler, J. N., BeMiller, & E. F. Paschall, *Starch, Chemistry and Technology* (2nd ed.). New York: Academic Press, Inc.

Juliano, B. O. (1985). Polysaccharide, Protein, and Lipids of rice. In B. O. Juliano (Ed.), *Rice: Chemistry and Technology* (pp.1-2). St. Paul, Minnesota, U.S.A: The American Association of Cereal Chemists, Inc.

Juliano, B. O. (1998). Varietal impact on rice quality. *Cereal Food World*, *43*, 207-222.

Juliano, B. O., Villareal, R. M. (1987). Varietal differences in physicochemical properties of waxy rice starch. *Starch/Stärke*, *39*, 298-301.

Kanzawa, Y., Harada, T., Koreeda, A., & Harada, A. (1987). Curdlan Gel Formed by Neutralizing Its Alkaline Solution. *Agricultural and Biological Chemistry*, *51*, 1839–1843.

Karim, A. A., Norziah, M. H., & Seow, C. C. (2000). Methods for the study of starch retrogradation. *Food Chemistry*, *71*, 9-36.

Khanna, S., & Tester, R. F. (2006). Influence of purified konjac glucomannan on the gelatinisation and retrogradation properties of maize and potato starches. *Food Hydrocolloids*, *20*, 567-576.

Kim, S. S., & Setser, C. S. (1992). Wheat starch gelatinization in the presence of polydextrose or hydrolyzed barley β-glucan. *Cereal Chemistry*, *69*, 447–452.

Kim, C., & Yoo, B. (2006). Rheological properties of rice starch–xanthan gum mixtures. *Journal of Food Engineering*, *75*, 120–128.

Kishk, Y. F. M., & Al-Sayed, H. M. A. (2007). Free-radical scavenging and antioxidative activities of some polysaccharides in emulsions. *LWT-Food Science and Technology*, *40*, 270–277.

Klis, F. M., Mol, K., Hellingwerf, K. & Brul, S. (2002). Dynamics of cell wall structure in *Saccharomyces cerevisiae*, *FEMS Microbiology Reviews*, *26*, 1–8.

Kogan, G. (2000). 1→3, 1→6-β-D-Glucans of yeasts and fungi and their biological activity. *Studies in Natural Products Chemistry*, *23*, 107-152.

Kohyama, K., & Nishinari, K. (1992). Cellulose derivatives effects on gelatinization and retrogradation of sweet potato starch. *Journal of Food Science*, *57*, 128–131 and 137.

Konna, A., Okuyama, K., Koreeda, A., Harada, A., Kanzawa, Y., & Harada, T. (1994). Molecular association and disassociation in formation of curdlan gels. In K. Nishinari & E. Doi (Eds.), *Food Hydrocolloids: Structures, Properties and Functions* (pp. 113-118). New York: Plenum Press.

Konna, A., & Harada, T. (1991). Thermal properties of curdlan in aqueous suspension and curdlan gel. *Food Hydrocolloids*, *5*, 427–434.

Krüger, A., Ferrero, C., & Zaritzky, N. E. (2003). Modelling corn starch swelling in batch systems: Effect of sucrose and hydrocolloids. *Journal of Food Engineering*, *58*, 125–133.

Kugimiya, M. & Donovan, J. W. (1981). Calorimetric determination of the amyloselysolecithin complex. *Journal of Food Science*, *46*, 765.

Kulicke, W.-M., Eidam, D., Kath, F., Kix, M., & Kull, A. H. (1996). Hydrocolloids and rheology: Regulation of visco-elastic characteristics of waxy rice starch in mixtures with galactomannans. *Starch/Stärke*, *48*, 105–114.

Lazaridou, A., & Biliaderis, C. G. (2007). Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. *Journal of Cereal Science*, *46*, 101-118.

Lazaridou, A., Biliaderis, C. G., & Izydorczyk, M. S. (2003). Molecular size effects on rheological properties of oat β-glucans in solution and gels, *Food Hydrocolloids*, *17*, 693–712, 2003.

Lazaridou, A., Biliaderis ,C. G., Micha-Screttas, M., & Steele, B. R. (2004). A comparative study on structure–function relations of mixed-linkage (1 → 3), (1→ 4) linear β-D-glucans. *Food Hydrocolloids*, 18, 837–855.

Leao, A. M. A. C., Buchi, D. F., Lacomini, M., Gorin, P. A. J., & Oliverira, M. B. M. (1997). Cytotoxic Effects against HeLa Cells of Polysaccharides from the Lichen *Ramalina celastri*. *Journal of Submicroscopic Cytology and Pathology*, *29*, 503–509.

Lee, M. H., Baek, M. H., Cha, D. S., Park, H. J., & Lim, S. T. (2002). Freeze-thaw stabilization of sweet potato starch gel by polysaccharide gums. *Food Hydrocolloids*, *16*, 345–352.

Lehtonen, M., & Aikasalo, R. (1987). β-Glucan in two- and six-rowed barley. *Cereal Chemistry*, *64*, 191–193.

Leloup, V. M., Colonna, P., Ring, S. G., Roberts, K., & Wells, B. (1992). Microstructure of amylose gels. *Carbohydrate Polymers*, *18*, 189-197.

Lii, C. Y., Tsai, M. L., & Tseng, K. H. (1996a). Effect of amylose content on the rheological property of rice starch. *Cereal Chemistry*, *73*, 415-420.

Lii, C. Y., Lai, V. M. F., & Tsai, M. L. (1996b). Studies on starch gelatinization and retrogradation with dynamic rheometry- the influence of starch granular structure and composition. *Zywnosc Technologia Jakosc* (Poland), *2*, 27-53.

Lii, C. Y., Lai, V. M. F., Lu, S., & Tsai, M. L., (1998). Correlation between the physicl property, eating quality and the molecular structure of rice-starchy systems. *Zywnosc Technologia Jakosc* (Poland), *4*, 72-86.

Lipke, P. N. & Ovalle, R. (1998). Cell wall architecture in yeast: new structure and new challenges. *Journal of Bacteriology*, *180*, 3735–3740.

Liu, H., Eskin, N. A. M., & Cui, S. W. (2006). Effects of yellow mustard mucilage on functional and rheological properties of buckwheat and pea starches. *Food Chemistry*, *95*, 83–93.

Luchsinger, W. W., Chen, S. C., & Richards, A. W. (1965). Mechanism of action of malt beta-glucanases. The structure of barley beta-D-glucan and the specificity of A11-endo-beta-glucanase, *Archives of Biochemistry and Biophysics*, *112*, 531.

Lund, E. K., Gee, J. M., Brown, J. C., Wood, P. J., & Johnson, I. T. (1989). Effect of oat gum on the physical properties of the gastrointestinal contents and on the uptake of D-galactose and cholesterol by rat small intestine *in vitro*. *British Journal of Nutrition*, *62*, 91-101.

Manners, D. J., Masson, A. J., & Patterson, L. C. (1973). The structure of a β-(1 → 3) D-glucan from yeast cell walls. *Biochemical Journal*, 135, 19–30.

Martin, M., & Fitzgerald, M. A. (2002). Proteins in rice grains influence cooking properties. *Journal of Cereal Science*, *36*, 285-294.

McCleary, B. V. (1988). Purification of (1→3),(1→4)-β-D-glucan from barley flour. In W. A. Wood & S. T. Kellogg (Eds.), *Methods in Enzymology* (pp. 511). San Diego: Academic Press.

McCleary, B. V. & Glennie-Holmes, M. (1985). Enzymatic quantification of (1→3),(1→4)-β-glucan in barley and malt. *Journal of the Institute of Brewing*, *91*, 285–295.

Meada, I., Saito, H., Masada, M., Misaki, A., & Harada, T. (1967). Properties of gels formed by heat treatment of curdlan, a bacterial β-1, 3-glucan. *Agricultural and Biological Chemistry*, *31*, 1184–1188.

Mezger, T. (2002). Measuring systems. In T. Mezger, *The rheology handbook: For users of rotational and oscillation rheometers* (pp.55-68, pp.172). Vincentz Verlag, Hannover, Germany.

Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. G. (1985a). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research*, *135*, 271-281.

Miles, M. J., Morris, V. J., & Ring, S. G. (1985b). Gelation of amylose. *Carbohydrate Research*, *135*, 247-269.

Miwa, M., Nakao, Y., & Nara, K. (1994). Food application of curdlan. In K. Nishinari & E. Doi (Eds.), *Food Hydrocolloids: Structures, Properties and Functions* (pp. 119-124). New York: Plenum Press.

Molecular structure of yeast β-glucan from http://www.beta13dglucan.org

Morgan, K. (2000). Cereal β-glucans. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of hydrocolloids* (pp. 287–307). Cambridge, UK: CRC Press.

Morris, E. R. (1990). In P. Harris (Ed.), *Food gels* (pp. 291). London: Elsevier.

Morrison, W. R, & Azudin, M. N. (1987). Variation in the amylose and lipid contents and some physical properties of rice starches. *Journal of Cereal Science*, *5*, 35-39.

Morrison, W. R, Milligan, T. P., & Azudin, M. N. (1984). A relationship between the amylose and lipid contents of starches from diploid cereals. *Journal of Cereal Science*, *2*, 257-260.

Murphy, P. (2000). Starch. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of hydrocolloids* (pp. 41–66). Cambridge, UK: CRC Press.

Nakao, Y., Konno, A., Taguchi, T., Tawada, T., Kasai, H., Toda, J., & Terasaki, M. (1991). Curdlan: Properties and application to foods. *Journal of Food Science*, *56*, 769-772 and 776.

Newman, R. K., Newman, C. W., & Graham, H. (1989). The hypocholesterolemic function of barley β-glucans. *Cereal Foods World*, *34***,** 883-886.

Newport Scientific Pty, Ltd. (1995). Operation manual for the Series 4 Rapid Visco Analyser (p. 23). Australia.

Nishinari, K., Hirashima, M., Miyoshi, E., & Takaya, T. (1998). Rheological and DSC studies of aqueous dispersions and gels of curdlan. In P. A. Williams & G. O. Phillips (Eds.), *Gum and Stabilizers for the Food Industry* (pp. 26-33).

Nishinari, K., & Zhang, H. (2000). Curdlan. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of hydrocolloids* (pp. 269–286). Cambridge, UK: CRC Press.

Noda, T., Takahata, Y., Sato, T., Ikoma, H., & Mochida, H. (1996). Physicochemical properties of starches from purple and orange fleshed sweet potato roots at two levels of fertilizer. *Starch/Stärke*, *48*, 395-399.

Noda, T., Nishiba, Y., Sato, T., & Suda, I. (2003). Properties of starches from several low-amylose rice cultivars. *Cereal Chemistry*, *80*, 193-197.

Noosuk, P., Hill, S. E., Pradipasena, P., & Mitchell, J. R. (2003). Structure-viscosity relationships for Thai rice starches. *Starch/Stärke*, *55*, 337–344.

Normand, F. L., & Marshall, W. E. (1989). Differential scanning calorimetry of whole grain milled rice and milled rice flour. *Cereal Chemistry*, *66*, 317-320.

Ogawa, K., & Tsurugi, J. (1973). The dependence of the conformation of a (1→3)-β-D-glucan on chain-length in alkaline solution. *Carbohydrate Research*, *29*, 397–403.

Okechukwu, P. E., & Rao, M. A. (1995). Influence of granule size on viscosity of cornstarch suspension. *Journal of Texture Studies*, *26*, 501-516.

Okuyama, K., Otsubo, A., Fukuzawa, Y., Ozawa, M., Harada, T., & Kasai, N. (1991). Single-helical structure of native curdlan and its aggregation state. *Journal of Carbohydrate Chemistry*, *10*, 645–656.

Ong, M. H., & Blanshard, J. M. V. (1995). Texture determinants in cooked, parboiled rice. I: rice starch amylose and the fine structure of amylopectin. *Journal of Cereal Science*, *21*, 251-260.

Palmer, G. H., & Bathgate, G. N. (1976). Malting and brewing. In Y. Pomeranz (Ed.), *Recent Advances in Cereal Science and Technology* (pp. 237). Mannesota: American Association of Cereal Chemists.

Parrou, J. L., & François, J. (1997). A simplified procedure for a rapid and reliable assay of both glycogen and trehalose in whole yeast cells. *Analytical Biochemistry*, *248*, 186–188.

Patindol, J., & Wang, Y. J. (2002). Fine structures of starches from long-grain rice cultivars with different functionality. *Cereal Chemistry*, *79*, 465-469.

Perez, E., Breene, W. M., & Bahnassey, Y. A. (1998). Variations in the gelatinization profiles of cassava, sagu and arrowroot native starches as measured with different thermal and mechanical methods. *Starch/Stärke*, *50*, 70-72.

Pons, M., & Fiszman, S. M. (1996). Instrumental texture profile analysis with particular reference to gelled systems. *Journal of Texture Studies*, *27*, 597-624.

Puchongkavarin, H., Varavinit, S., & Bergthaller, W. (2005). Comparative study of pilot scale rice starch production by an alkaline and an enzymatic process. *Starch/Stärke*, *57*, 134–144.

Qi, X., Tester, R. F., Snape, C. E., & Ansell, R. (2003). Molecular basis of the gelatinization and swelling characteristics of waxy rice starches grown in the same location during the same season. *Journal of Cereal Science*, *37*, 363-376.

Qiang, L. (2005). Understanding Starches and Their Role in Foods. In S. W. Cui (Ed.), *Food Carbohydrates: Chemistry, Physical Properties, and Applications*, (pp. 219-261). Boca Raton, Fl: CRC Press.

Rao, M. A. (1999). *Rheology of fluid and semisolid foods: Principles and applications*. Gaithersburg, MD: Aspen Publishers, Inc.

Reed, G., & Nagodawithana, T. W. (1991). Yeast-derived products. In G. Reed & T. W. Nagodawithana (Eds.), *Yeast technology* (2nd ed., pp. 369-440). New York: Van Nostrand Reinhold.

Reese, E. T., & Perlin, A. S. (1963). Enzymatic preparation of 3-O-β-cellobiosyl D-glucose. *Biochemical and Biophysical Research Communications*, *12*, 194-197.

Sajjan, S. U., & Rao, M. R. R. (1987). Effect of hydrocolloids on the rheological properties of wheat starch. *Carbohydrate Polymers*, *7*, 395–402.

Sanders, J. P. M. (1996). Starch manufacturing in the world. In: *Advanced Post Academic Course on Tapioca Starch Technology*. Jan. 22-26 & Feb. 19-23, 1996. AIT Center, Bangkok.

Sasaki, T., Abiko, N., Sugino, Y., & Nitta, K. (1978). Dependence on chain length of antitumour activity of (1,3)-β-D-glucan from *Alcaligenes faecalis* var. *myxogenes* IFO13140 and its acid-degraded products. *Cancer Research*, *38*, 379–383.

Sasaki, T., Yasui, T., & Matsuki, J. (2000b) Influence of non-starch polysaccharides isolated from wheat flour on the gelatinization and gelation of wheat starches. *Food Hydrocolloids*, *14*, 295-303.

Satrapai, S., & Suphantharika, M. (2007). Influence of spent brewer's yeast β-glucan on gelatinization and retrogradation of rice starch. *Carbohydrate Polymers*, *67*, 500-510.

Shi, X., & BeMiller, J. N. (2002). Effects of food gums on viscosities of starch suspensions during pasting. *Carbohydrate Polymers*, *50*, 7–18.

Sievert, D., & Pomeranz, Y. (1989). Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermomechanical, and microscopic methods. *Cereal Chemistry*, *66*, 342-347.

Silverio, J., Fredriksson, H., Andersson, R., Eliasson, A.-C., & Åman, P. (2000). The effect of temperature cycling on the amylopectin retrogradation of starches with different amylopectin unit-chain length distribution. *Carbohydrate Polymers*, *42*, 175–184.

Singh, V., Okadome, H., Toyoshima, H., Isobe, S., & Ohtsubo, K. (2000). Thermal and physicochemical properties of rice grain, flour and starch. *Journal of Agricultural and Food Chemistry*, *48*, 2639-2647.

Singh, N., Singh, J., Kaur, L., Sodhi, N. S., & Gill, B. S. (2003). Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry*, *81*, 219–231.

Slade, L., & Levine, H. (1987). Recent advances in starch retrogradation. In S. S. Stivala, V. Crescenzi, & I. C. M. Dea (Eds.), *Industrial polysaccharides—The impact of biotechnology and advanced methodologies* (pp. 387–430). New York: Gordon and Breach Science.

Slade, L., & Levine, H. (1988). Non-equilibrium melting of native granular starch. Part I. Temperature location of the glass transition associated with gelatinization of A-type cereal starches. *Carbohydrate Polymers*, *8*, 183-208.

Slade, L., & Levine, H. (1991). Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition*, *30*, 115–360.

Sodhi, N. S., & Singh, N. (2003). Morphological, thermal and rheological properties of starches separated from rice cultivars grown in India. *Food Chemistry*, *80*, 99-108.

Sources and fine structure of different β-glucans from http://www.immunebody.com/zArchive/pg_md_whatis.html

Spicer, E. J. F., Goldenthal, E. I., & Ikeda, T. (1999). A toxicological assessment of curdlan. *Food and Chemical Toxicology*, *37*, 455-479.

SPSS for Windows Evaluation Version (2006). *User's manual*, version 15.0, Chicago, IL: SPSS Inc.

Steffe, J. F. (1996). Introduction to rheology. In J. F. Steffe, *Rheological Methods in Food Process Engineering* (pp. 1-91). East Lansing, MI: Freeman Press.

Stone, B. A. & Clarke, A. E. (1992). *Chemistry and biology of (1→3)-β-D-glucans* (pp. 525-564). Australia: La Trobe University Press.

Sudhakar, V., Singhal, R. S., & Kulkarni, P. R. (1995). Studies on starch-hydrocolloid interactions: Effect of salts. *Food Chemistry*, *5*3, 405–408.

Sudhakar, V., Singhal, R. S., & Kulkarni, P. R. (1996). Starch-galactomannan interactions: functionality and rheological aspects. *Food Chemistry*, *55*, 259–264.

Swinkels, J. J. M. (1985). Composition and properties of commercial native starches. *Starch/Stärke*, *40*, 51–54.

Symons, L. J., & Brennan, C. S. (2004). The effect of barley β-glucan fiber fractions on starch gelatinization and pasting characteristics. *Journal of Food Science*, *69*, 257-261.

Taggart, P. (2004). Starch as an ingredient: manufacture and applications. In A. C. Eliasson (Ed.), *Starch in food: Structure, function and applications* (pp. 363-392). Cambridge, England: Woodhead Publishing Limited.

Takeda, Y., Hizukuri, S. & Juliano, B.O. (1987). Structures of rice amylopectins with low and high affinities for iodine. *Carbohydrate Research*, *168*, 79-88.

Tako, M., & Hizukuri, S. (2000). Retrogradation mechanism of rice starch. *Cereal Chemistry*, *77*, 473-477.

Temelli, F. (1997). Extraction and functional properties of barley β-glucan as affected by temperature and pH. *Journal of Food Science*, *62*, 1197-1201.

Temelli, F. & Burkus, Z. (2000). Stabilization of emulsions and foams using barley β-glucan. *Food Research International*, *33*, 27–33.

Tester, R. F. (1997). Starch: the polysaccharide fractions. In P. J. Frazier, P. Richmond & A. M. Donald (Eds.), *Starch: structure and functionality* (pp. 163-171). Royal Society of Chemistry.

Tester, R. F., Morrison, W. R. (1990a). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids. *Cereal Chemistry*, *67*, 551-557.

Tester, R. F., Morrison, W. R. (1990b). Swelling and gelatinization of cereal starches. II. Waxy rice starches. *Cereal Chemistry*, *67*, 558-563.

Thammakiti, S., Suphantharika, M., Phaesuwan, T., & Verduyn, C. (2004). Preparation of spent brewer's yeast β-glucans for potential applications in the food industry. *International Journal of Food Science and Technology*, *39*, 21-29.

Thomas, D. J., & Atwell, W. A. (1999). *Starches*. Minnesota: American Association and Cereal Chemists.

Umemoto, T., Nakamura, Y., Satoh, H., & Terashima, K. (1999). Differences in amylopectin structure between two rice varieties in relation to the effects of temperature during grain-filling. *Starch/Stärke*, *51*, 58-62.

Vaikousi, H., Biliaderis, C. G., & Izydorczyk, M. S. (2005). Solution flow behavior and gelling properties of water-soluble barley β-glucans varying in molecular size. *Journal of Cereal Science*, *39*, 119–137.

Vandeputte, G. E., Vermeylen, R., Geeroms, J., & Delcour, J. A. (2003a). Rice starches, I. Structural aspects provide insight into crystallinity characteristics and gelatinization behavior of granular starch. *Journal of Cereal Science*, *38*, 43-52.

Vandeputte, G. E., Vermeylen, R., Geeroms, J., & Delcour, J. A. (2003b). Rice starches, II. Structural aspects provide insight in swelling and pasting properties. *Journal of Cereal Science*, *38*, 53-59.

Vandeputte, G. E., Vermeylen, R., Geeroms, J., & Delcour, J. A. (2003c). Rice starches, III. Structural aspects provide insight in amylopectin retrogradation properties and gel texture. *Journal of Cereal Science*, *38*, 61-68.

Walstra, P. (1993). Introduction to aggregation phenomena in food colloids. In E. Dickinson, & P. Walstra (Eds.), *Food Colloids and Polymers: Stability and Mechanical Properties* (pp. 3–15). Cambridge: Royal Society of Chemistry.

Watase, M., & Nishinari, K. (1994). Rheology and DSC of curdlan-DMSO-water system. In K. Nishinari & E. Doi (Eds.), *Food Hydrocolloids: Structures, Properties and Functions* (pp. 125-129). New York: Plenum Press.

Whistler, R. L., BeMiller, J. N. & Paschall, E. F. (1984). *Starch, Chemistry and Technology* (2nd ed., pp.469-476, 688). New York: Academic Press, Inc.

White, P. J., Abbas, I. R., & Johnson, L. A. (1989). Freeze-thaw stability and refrigerated-storage retrogradation of starches. *Starch/Stärke*, *41*, 176-180.

Williams, G. O., & Phillips, G. O. (2000). Introduction to food hydrocolloids. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of hydrocolloids* (pp. 1–19). Cambridge, UK: CRC Press.

Wong, K. S., Kubo, A., Jane, J. L., Harada, K., Satoh, H., & Nakamura, Y. (2003). Structures and properties of amylopectin and phytoglycogen in the endosperm of sugary-1 mutant of rice. *Journal of Cereal Science*, *37*, 139-149.

Wood, P. J. (1986). Oat β-glucan: structure, location and properties. In F. H. Webster (Ed.), *Oats: Chemistry and Technology* (pp. 121). Minnesota: American Association and Cereal Chemists.

Wood, P. J. (1993). *Oat Bran*. Minnesota: American Association and Cereal Chemists.

Wood, P. J. (2007). Cereal β-glucans in diet and health. *Journal of Cereal Science*, *46*, 230–238.

Wood, P. J., Braaten, J. T., Scott, F. W., Riedel, K. D., Wolynetz M. S., & Collins, M. W. (1994). Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. *British Journal of Nutrition*, *72*, 731-744.

Wood, P. J., Siddiqui, I. R., & Paton, D. (1978). Extraction of high-viscosity gums from oats. *Cereal Chemistry*, *55*, 1038-1049.

Wood, P. J., Weisz, J., & Blackwell, B. A. (1991). Molecular characterization of cereal β-D-glucans: structural analysis of oat β-D-glucan and rapid structural evaluation of β-D-glucans from different sources by high-performance liquid

chromatography of oligosaccharides released by lichenase. *Cereal Chemistry*, *68*, 31-39.

Wood, P. J., Weisz, J., Fedec, P., & Burrows, V. D. (1989). Large-scale preparation and properties of oat fractions enriched in (1→3),(1→4)-β-D-glucan. *Cereal Chem*istry, *66*, 97–103.

Woodward, J. R., Fincher, G. B., & Stone, B. A. (1983). Water-soluble (1→3),(1→4)-β-D-glucans from barley (*Hordeum vulgare*) endosperm. II. Fine structure, *Carbohydrate Polymers*, *3*, 207-225.

Woodward, J. R., Phillips, D. R., & Fincher, G. B. (1988). Water-soluble 1→3),(1→4)-β-D-glucans from barley (*Hordeum vulgare*) endosperm. IV. Comparison of 40 ºC and 65 ºC soluble fractions. *Carbohydrate Polymers*, *8*, 85–97.

Worrasinchai, S., Suphantharika, M., Pinjai, S., & Jamnong, P. (2006). β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. *Food Hydrocolloids*, *20*, 68–78.

Yin, X. S., & MacGregor, A. W. (1989). Substrate specificity and nature of action of barley β-glucan solubilase. *Journal of the Institute of Brewing*, *95*, 105–109.

Yoo, S. H., & Jane, J. L. (2002) Molecular weights and gyration radii of amylopectins determined by high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and refractive index detectors. *Carbohydrate Polymers*, *49*, 307-414.

Yoshimura, M., Takaya, T., & Nishinari, K. (1996). Effects of konjac glucomannan on the gelatinization of corn starch as determined by rheology and differential scanning calorimetry. *Journal of Agricultural Food Chemistry*, *44*, 2970-297.

Yoshimura, M., Takaya, T., & Nishinari, K. (1998). Rheological studies on mixtures of corn starch and konjac–glucomannan. *Carbohydrate Polymers*, *35*, 71–79.

Yoshimura, M., Takaya, T., & Nishinari, K. (1999). Effects of xyloglucan on the gelatinization and retrogradation of corn starch as studied by rheology and differential scanning calorimetry. *Food Hydrocolloids*, *13*, 101-111.

Yuan, R. C., Thompson, D. B., & Boyer, C. D. (1993). Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from

three wx-containing genotypes in two inbred lines. *Cereal Chemistry*, *70*, 81-89.

Zeković, D. B., Kwiatkowski, S., Vrvić, M. M., Jakovljević, D., & Moran, C. A. (2005). Natural and modified (1→3)-β-D-glucans in health promotion and disease alleviation. *Critical Reviews in Biotechnology*, *25*, 205–230.

Zhou, Z. K., Robards, K., Helliwell, S., & Blanchard, C. (2002). Composition and functional properties of rice. *International Journal of Food Science and Technology*, *37*, 849-868.

APPENDICES

APPENDIX A

Apparent amylose content

The measurement of amylose content was based on followed AACC Method 61-03. Starch powder (100 mg) was put into 100 ml volumetric flask. Alcohol (95%) 1 ml was added in the volumetric flask in order to disperse starch. Sodium hydroxide (1N) 9 ml was added. The volumetric flask was put in a boiling water bath for 10 min and cooled to room temperature. After that, the sample was left at room temperature for at least 2 hr before the next step.

After waiting for 2 hr, distilled water was put into added to the volumetric flask to make the volume to 100 ml and mixed thoroughly. Five milliliters of the diluted sample was pipetted in to a new 100 ml volumetric flask that and filled with 50 ml distilled water were added. Acetic acid (1N) 1 ml and Iodine solution 2 ml were added, consecutively. Then, the sample was made up volume to 100 ml with distilled water and left for 20 min. The absorbance of the samples was measured at 620 nm.

Standard amylose and amylopectin from corn (Sigma company, USA) were used to prepare an amylose standard curve. The R-squared of the linear relationship was greater than 0.99, see figure…. The apparent amylose content of samples was calculated using that linear equation from an amylose standard curve.

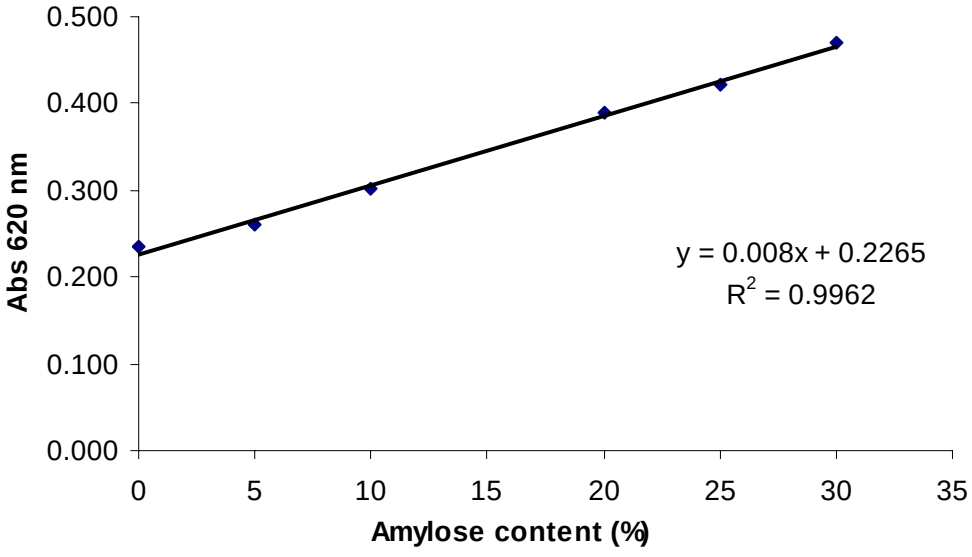


Figure A 1 Amylose standard curve

APPENDIX B

Thermal properties

Table A 1 Gelatinization temperature and enthalpy and retrogradation ratio for 12% (w/w) RS alone and RS/β-glucan mixtures at a ratio of 5.5/0.5 measured by the differential scanning calorimeter (DSC) at various storage time[1]

RS/β-glucan[2]	First run					Storage time (day)	Second run (stored at 4°C)					
	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_1 (J/g)		T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_2 (J/g)	$\Delta H_2/\Delta H_1$
RS alone	72.3 ± 0.1	76.4 ± 0.1	80.3 ± 0.1	8.0 ± 0.0	12.7 ± 0.1	1	55.6 ± 0.5	58.3 ± 0.5	62.7 ± 0.9	7.2 ± 0.9	0.2 ± 0.1	0.02 ± 0.00
RS/ OG	73.0 ± 0.1	76.9 ± 0.0	80.9 ± 0.1	8.0 ± 0.0	12.0 ± 0.1		54.6 ± 1.4	58.4 ± 0.2	63.7 ± 1.2	9.1 ± 1.4	0.7 ± 0.4	0.06 ± 0.04
RS/ BG	73.2 ± 0.0	77.0 ± 0.1	80.8 ± 0.1	7.7 ± 0.1	11.5 ± 0.2		53.8 ± 0.3	59.3 ± 0.1	63.7 ± 1.1	10.0 ± 1.1	0.9 ± 0.5	0.08 ± 0.04
RS/ CL	72.8 ± 0.1	76.8 ± 0.1	80.7 ± 0.1	7.8 ± 0.0	12.3 ± 0.3		56.3 ± 0.4	59.7 ± 0.2	63.4 ± 0.2	7.0 ± 0.4	0.2 ± 0.2	0.02 ± 0.02
RS/ YG	72.5 ± 0.0	76.3 ± 0.1	80.1 ± 0.1	7.6 ± 0.1	10.6 ± 0.1		56.2 ± 0.2	58.4 ± 0.1	64.2 ± 0.2	8.0± 0.2	0.1 ± 0.1	0.01 ± 0.00
RS alone	72.2 ± 0.0	76.4 ± 0.0	80.1 ± 0.3	7.8 ± 0.3	12.7 ± 0.6	3	46.0 ± 0.7	56.1 ± 0.2	63.4 ± 0.4	17.5 ± 0.7	3.0 ± 0.5	0.24 ± 0.03
RS/ OG	72.8 ± 0.1	76.8 ± 0.1	80.8 ± 0.1	8.0 ± 0.1	12.3 ± 0.3		48.8 ± 0.7	56.7 ± 0.4	62.7 ± 0.2	13.9 ± 0.7	2.8 ± 0.2	0.23 ± 0.02
RS/ BG	73.0 ± 0.1	77.0 ± 0.1	80.8 ± 0.2	7.8 ± 0.1	11.7 ± 0.1		50.2 ± 1.3	56.8 ± 0.2	62.9 ± 0.2	12.7 ± 1.3	2.7 ± 0.2	0.23 ± 0.01
RS/ CL	72.8 ± 0.0	76.8 ± 0.1	80.5 ± 0.3	7.7 ± 0.3	12.0 ± 0.5		47.8 ± 0.1	57.0 ± 0.6	63.0 ± 0.2	15.1 ± 0.2	3.0 ± 0.2	0.25 ± 0.03
RS/ YG	72.9 ± 0.4	76.8 ± 0.4	80.8 ± 0.5	7.9 ± 0.1	10.5 ± 0.7		48.4 ± 0.1	56.2 ± 0.0	62.4 ± 0.0	14.0 ± 0.1	2.4 ± 0.1	0.22 ± 0.03
RS alone	72.8 ± 0.2	76.6 ± 0.2	80.2 ± 0.3	7.4 ± 0.4	12.3 ± 0.1	7	44.1 ± 0.6	54.8 ± 0.6	62.2 ± 0.3	18.2 ± 0.6	5.8 ± 0.4	0.47 ± 0.03
RS/ OG	72.6 ± 0.0	76.5 ± 0.0	80.4 ± 0.0	7.8 ± 0.0	11.3 ± 0.1		45.1 ± 0.2	54.3 ± 0.1	62.3 ± 0.1	17.2 ± 0.2	4.8 ± 0.1	0.42 ± 0.01
RS/ BG	72.9 ± 0.0	76.8 ± 0.1	80.5 ± 0.2	7.6 ± 0.1	11.4 ± 0.4		45.7 ± 0.2	55.0 ± 0.2	62.1 ± 0.2	16.4 ± 0.3	5.0 ± 0.4	0.44 ± 0.02
RS/ CL	72.7 ± 0.0	76.5 ± 0.1	80.4 ± 0.2	7.8 ± 0.2	12.2 ± 0.1		46.1 ± 0.5	55.1 ± 0.2	61.6 ± 0.3	15.5 ± 0.5	5.6 ± 0.3	0.46 ± 0.03
RS/ YG	72.5 ± 0.1	76.4 ± 0.2	80.1 ± 0.1	7.6 ± 0.0	10.8 ± 0.1		46.1 ± 0.6	55.1 ± 0.4	61.3 ± 0.1	15.2 ± 0.6	4.9 ± 0.1	0.45 ± 0.01
RS alone	72.5 ± 0.1	76.6 ± 0.1	80.2 ± 0.0	7.7 ± 0.1	12.1 ± 0.5	14	42.4 ± 0.4	53.2 ± 0.2	62.3 ± 0.0	19.9 ± 0.4	6.5 ± 0.5	0.54 ± 0.02
RS/ OG	72.9 ± 0.1	76.8 ± 0.1	80.8 ± 0.1	7.9 ± 0.1	12.4 ± 0.1		44.8 ± 0.2	54.3 ± 0.8	62.1 ± 0.4	17.3 ± 0.4	5.8 ± 0.4	0.47 ± 0.03
RS/ BG	73.1 ± 0.1	76.9 ± 0.1	80.8 ± 0.2	7.7 ± 0.1	11.6 ± 0.2		44.7 ± 0.6	54.4 ± 0.6	62.0 ± 0.6	17.3 ± 0.6	5.7 ± 0.4	0.49 ± 0.03
RS/ CL	72.9 ± 0.2	76.9 ± 0.2	80.7 ± 0.2	7.8 ± 0.1	12.1 ± 0.6		45.3 ± 0.2	54.6 ± 0.3	61.9 ± 0.3	16.6 ± 0.3	6.4 ± 0.2	0.53 ± 0.01
RS/ YG	73.0 ± 0.0	77.0 ± 0.1	80.7 ± 0.0	7.7 ± 0.0	10.5 ± 0.2		46.1 ± 0.2	54.9 ± 0.3	61.6 ± 0.2	15.6 ± 0.2	5.3 ± 0.2	0.50 ± 0.01

Table A 1 Gelatinization temperature and enthalpy and retrogradation ratio for 12% (w/w) RS alone and RS/β-glucan mixtures at a ratio of 5.5/0.5 measured by the differential scanning calorimeter (DSC) at various storage time[1] (Continued)

RS/β-glucan[2]	First run					Storage time (day)	Second run (stored at 4°C)					
	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_1 (J/g)		T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_2 (J/g)	$\Delta H_2/\Delta H_1$
RS alone	72.3 ± 0.1	76.4 ± 0.2	80.2 ± 0.2	7.9 ± 0.1	12.5 ± 0.1	21	40.4 ± 0.9	53.7 ± 0.7	62.6 ± 0.1	22.2 ± 0.9	8.0 ± 0.3	0.64 ± 0.03
RS/ OG	72.5 ± 0.3	76.5 ± 0.1	80.4 ± 0.1	7.9 ± 0.4	12.2 ± 0.5		43.7 ± 0.4	54.8 ± 0.1	62.2 ± 0.4	18.6 ± 0.4	6.5 ± 0.1	0.53 ± 0.02
RS/ BG	72.7 ± 0.1	76.7 ± 0.1	80.6 ± 0.3	7.9 ± 0.3	11.5 ± 0.2		44.5 ± 0.5	54.8 ± 1.0	63.3 ± 1.2	16.3 ± 1.2	6.6 ± 0.2	0.58 ± 0.02
RS/ CL	72.6 ± 0.2	76.5 ± 0.3	80.4 ± 0.3	7.9 ± 0.1	12.7 ± 0.2		45.4 ± 1.3	53.0 ± 0.5	63.4 ± 1.1	17.4 ± 1.3	8.0 ± 0.2	0.63 ± 0.03
RS/ YG	72.5 ± 0.1	76.2 ± 0.0	80.8 ± 0.2	7.5 ± 0.3	9.3 ± 0.4		43.3 ± 0.9	54.9 ± 0.2	62.7 ± 0.2	19.4 ± 0.9	5.5 ± 0.2	0.59 ± 0.01
RS alone	72.2 ± 0.3	76.4 ± 0.2	80.2 ± 0.0	8.0 ± 0.3	11.8 ± 0.9	35	39.8 ± 0.9	53.2 ± 0.6	63.5 ± 1.0	23.8 ± 1.0	8.8 ± 0.1	0.74 ± 0.05
RS/ OG	72.7 ± 0.2	76.7 ± 0.1	80.6 ± 0.2	7.9 ± 0.1	12.3 ± 0.2		44.1 ± 0.0	54.7 ± 1.5	63.0 ± 0.4	18.9 ± 0.4	7.3 ± 0.1	0.60 ± 0.01
RS/ BG	72.9 ± 0.2	76.7 ± 0.2	80.7 ± 0.3	7.8 ± 0.2	11.5 ± 0.1		45.1 ± 1.0	55.8 ± 0.8	63.2 ± 0.3	18.1 ± 1.0	7.6 ± 0.1	0.66 ± 0.01
RS/ CL	72.8 ± 0.0	76.8 ± 0.1	80.7 ± 0.1	7.9 ± 0.1	12.4 ± 0.3		44.7 ± 0.4	53.6 ± 0.2	62.5 ± 1.0	17.8 ± 1.0	8.6 ± 0.0	0.70 ± 0.01
RS/ YG	72.5 ± 0.0	76.4 ± 0.0	80.0 ± 0.1	7.6 ± 0.1	10.0 ± 0.5		44.0 ± 0.7	54.9 ± 0.6	63.4 ± 1.1	19.5 ± 1.1	6.5 ± 0.1	0.66 ± 0.03
RS alone	72.3 ± 0.3	76.4 ± 0.2	80.3 ± 0.3	8.0 ± 0.1	12.3 ± 0.6	49	38.9 ± 0.9	53.4 ± 0.5	63.1 ± 0.9	24.3 ± 0.9	10.2 ± 0.3	0.80 ± 0.02
RS/ OG	72.7 ± 0.1	76.6 ± 0.1	80.7 ± 0.3	8.0 ± 0.2	12.3 ± 0.4		44.4 ± 0.8	55.6 ± 1.0	63.1 ± 0.7	18.8 ± 0.8	7.3 ± 0.1	0.60 ± 0.02
RS/ BG	72.7 ± 0.3	76.6 ± 0.2	80.4 ± 0.2	7.7 ± 0.1	11.3 ± 0.2		44.1 ± 1.7	54.8 ± 1.5	63.6 ± 0.5	19.6 ± 1.7	7.9 ± 0.1	0.70 ± 0.02
RS/ CL	72.5 ± 0.0	76.5 ± 0.2	80.3 ± 0.3	7.9 ± 0.3	12.6 ± 0.3		43.1 ± 0.9	54.5 ± 1.3	63.9 ± 1.0	20.7 ± 1.0	9.2 ± 0.2	0.74 ± 0.02
RS/ YG	72.5 ± 0.2	76.4 ± 0.0	80.2 ± 0.1	7.7 ± 0.1	10.8 ± 0.6		43.9 ± 1.4	56.6 ± 0.6	63.4 ± 0.2	19.5 ± 1.4	7.6 ± 0.1	0.72 ± 0.03
RS alone	72.5 ± 0.0	76.5 ± 0.0	80.5 ± 0.1	8.0 ± 0.1	14.4 ± 0.1	63	36.1 ± 0.3	52.7 ± 0.2	63.4 ± 0.2	27.3 ± 0.3	12.4 ± 0.4	0.86 ± 0.02
RS/ OG	72.8 ± 0.1	76.7 ± 0.1	80.7 ± 0.1	7.9 ± 0.1	12.2 ± 0.5		44.3 ± 1.1	54.8 ± 0.6	63.6 ± 1.2	19.3 ± 1.2	7.4 ± 0.1	0.60 ± 0.03
RS/ BG	73.0 ± 0.1	77.0 ± 0.1	80.7 ± 0.1	7.7 ± 0.1	11.4 ± 0.2		45.0 ± 0.9	54.2 ± 0.3	64.5 ± 0.1	19.5 ± 0.9	8.1 ± 0.5	0.71 ± 0.01
RS/ CL	72.8 ± 0.1	76.7 ± 0.2	80.6 ± 0.0	7.8 ± 0.0	12.4 ± 0.2		40.5 ± 0.7	54.1 ± 0.5	62.9 ± 0.5	22.4 ± 0.7	9.7 ± 0.4	0.78 ± 0.01
RS/ YG	72.5 ± 0.1	76.4 ± 0.0	80.1 ± 0.1	7.6 ± 0.1	11.0 ± 0.4		41.7 ± 0.3	56.0 ± 0.2	63.9 ± 0.8	22.1 ± 0.8	8.3 ± 0.1	0.75 ± 0.00

[1] T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_1, gelatinization enthalpy; ΔH_2, re-gelatinization enthalpy; $\Delta H_2/\Delta H_1$, retrogradation ratio.

[2] Refer of Table 4.1 for the sample codes.

APPENDIX C

Steady shear rheological properties

Table A 2 The Herschel-Bulkley parameters for 3.5% (w/w) RS alone and RS/ β-glucan gels at a ratio of 5.5/0.5 immediately after gelatinization and cooling to room temperature (25°C) and after stored at 4°C for various storage time[1]

Storage time (day)	RS/β-glucan[2]	Hysteresis loop area (Pa/s)	Up curve				Down curve			
			σ_0	K	n	R^2	σ_0	K	n	R^2
0	RS alone	252	2.94	0.47	0.56	0.998	0.90	0.50	0.58	0.999
	RS/ OG	181	5.63	3.62	0.34	0.997	0.00	4.50	0.35	0.982
	RS/ BG	378	0.85	2.64	0.49	0.999	0.00	1.81	0.56	0.998
	RS/ CL	929	-	-	-	-	3.12	0.76	0.55	0.997
	RS/ YG	436	6.71	0.18	0.69	0.997	1.23	0.63	0.55	0.999
1	RS alone	1438	-	-	-	-	4.46	0.41	0.66	0.998
	RS/ OG	116	-	-	-	-	0.00	7.40	0.26	0.996
	RS/ BG	227	-	-	-	-	0.00	3.45	0.44	0.998
	RS/ CL	2047	-	-	-	-	3.99	0.62	0.61	0.997
	RS/ YG	657	-	-	-	-	1.89	0.70	0.55	0.999
3	RS alone	1841	-	-	-	-	5.17	0.42	0.67	0.996
	RS/ OG	119	-	-	-	-	0.23	6.41	0.26	0.997
	RS/ BG	273	-	-	-	-	0.00	3.72	0.42	0.998
	RS/ CL	2633	-	-	-	-	5.65	0.82	0.60	0.996
	RS/ YG	981	-	-	-	-	2.79	0.53	0.58	0.999
7	RS alone	3644	-	-	-	-	7.80	0.52	0.70	0.995
	RS/ OG	197	-	-	-	-	0.00	6.72	0.28	0.996
	RS/ BG	1519	-	-	-	-	2.30	2.99	0.50	0.998
	RS/ CL	3937	-	-	-	-	5.71	0.76	0.63	0.994
	RS/ YG	2048	-	-	-	-	4.11	0.86	0.58	0.998
14	RS alone	10103	-	-	-	-	13.87	0.95	0.70	0.992
	RS/ OG	1653	-	-	-	-	0.00	6.71	0.36	0.986
	RS/ BG	5480	-	-	-	-	8.68	1.65	0.66	1.000
	RS/ CL	10864	-	-	-	-	9.80	2.40	0.56	0.990
	RS/ YG	4594	-	-	-	-	4.38	1.40	0.57	0.995
21	RS alone	11780	-	-	-	-	14.00	1.53	0.63	0.993
	RS/ OG	3545	-	-	-	-	0.00	5.45	0.43	0.995
	RS/ BG	7883	-	-	-	-	11.37	1.61	0.69	1.000
	RS/ CL	14406	-	-	-	-	11.34	3.82	0.53	0.989
	RS/ YG	7980	-	-	-	-	5.28	1.96	0.56	0.993
35	RS alone	16574	-	-	-	-	15.27	2.20	0.60	0.987
	RS/ OG	6123	-	-	-	-	5.56	1.76	0.64	0.999
	RS/ BG	10822	-	-	-	-	12.91	1.88	0.68	0.998
	RS/ CL	16,792	-	-	-	-	9.47	4.59	0.51	0.988
	RS/ YG	11,811	-	-	-	-	5.11	2.89	0.54	0.991

Table A 2 The Herschel-Bulkley parameters for 3.5% (w/w) RS alone and RS/ β-glucan gels at a ratio of 5.5/0.5 immediately after gelatinization and cooling to room temperature (25°C) and after stored at 4°C for various storage time[1] (Continued)

Storage time (day)	RS/β-glucan[2]	Hysteresis loop area (Pa/s)	Up curve				Down curve			
			σ_0	K	n	R^2	σ_0	K	n	R^2
49	RS alone	24,916	-	-	-	-	19.58	2.81	0.61	0.991
	RS/ OG	7,706	-	-	-	-	7.75	1.30	0.70	0.999
	RS/ BG	12,101	-	-	-	-	14.45	1.82	0.70	0.998
	RS/ CL	23,206	-	-	-	-	9.88	5.57	0.52	0.989
	RS/ YG	16,209	-	-	-	-	6.36	3.54	0.55	0.993
63	RS alone	44,100	-	-	-	-	26.16	4.55	0.62	0.992
	RS/ OG	10,523	-	-	-	-	10.61	0.90	0.77	0.999
	RS/ BG	14,035	-	-	-	-	15.93	1.37	0.74	0.997
	RS/ CL	23,058	-	-	-	-	14.59	7.07	0.49	0.989
	RS/ YG	18,650	-	-	-	-	7.15	4.32	0.53	0.993

[1] σ_0, yield stress (Pa); K, consistency coefficient (Pa s^n); n, flow behavior index (dimensionless).

[2] Refer of Table 4.1 for the sample codes.

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