EFFECT OF TEMPERATURE CYCLING ON RETROGRADATION OF RICE STARCH IN THE PRESENCE OF DIFFERENT β-GLUCANS

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ABSTRACT

Rice starch (RS) gels with an addition of four different β-glucans, i.e. curdlan (CL), oat (OG), barley (BG), and yeast (YG) β-glucans were subjected to different storage time and temperature conditions (temperature cycling). The cycled temperatures of 4 and 40°C were chosen to favour two steps of starch retrogradation, i.e. nucleation and propagation, respectively. Differential scanning calorimetry (DSC) demonstrated that all starch gels stored under the 4/40°C cycled conditions exhibited significantly higher onset (T_o), peak (T_p), and conclusion (T_c) temperatures of melting of recrystallized starch with narrower phase transition temperature ranges (T_c-T_o) and smaller melting enthalpy (ΔH_2) than those corresponding gels stored at a constant temperature of 4°C. The addition of any of these β-glucans did not affect the T_o, T_p, T_c, and (T_c - T_o), but significantly enhanced the rate ($\Delta H_2/\Delta H_1$) and extent (ΔH_2) of retrogradation of the temperature-cycled RS gels in the following order: OG ≈ BG > CL > YG. After nucleation at 4°C for one day, the starch gels with and without β-glucan stored at 40°C exhibited a marked increase in the T_o, T_p, and T_c during the first week of storage and slightly increased during the rest of storage time, whereas the $\Delta H_2/\Delta H_1$ steadily increased with storage time. It was concluded that the presence of β-glucan in the RS gels promoted nucleation of starch crystallites, whereas the temperature cycling enhanced the stability and homogeneity of these starch crystallites.

KEY WORDS: RICE STARCH/ β-GLUCAN/ TEMPERATURE CYCLING/ RETROGRADATION

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ผลของการสลับเปลี่ยนอุณหภูมิต่อเรโทรเกรเดชั่นของแป้งข้าวเจ้าที่มีส่วนผสมของเบต้า-กลูแคนชนิดต่างๆ

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บทคัดย่อ

เจลจากแป้งข้าวเจ้าที่มีส่วนผสมของเบต้า-กลูแคนชนิดต่างๆ 4 ชนิดได้แก่เบต้า-กลูแคนจากแบคทีเรีย (เคิร์ดแลน, CL), โอ๊ต (OG), บาร์เลย์ (BG), และยีสต์ที่ใช้แล้วในอุตสาหกรรมการผลิตเบียร์ (YG) ได้นำมาเก็บภายใต้สภาวะที่มีการสลับเปลี่ยนอุณหภูมิ ระหว่าง 4 และ 40 องศาเซลเซียสเพื่อเร่งการเกิดเรโทรเกรเดชั่นของแป้งในขั้นตอนนิวคลีเอชั่นและโพรเพเกชั่นตามลำดับ ข้อมูลที่ได้จากการศึกษาคุณสมบัติทางความร้อนโดยใช้วิธี Differential Scanning Calorimetry (DSC) แสดงให้เห็นว่าเจลของผสมที่เก็บโดยการสลับเปลี่ยนอุณหภูมิมีการเพิ่มของอุณหภูมิที่ผลึกแป้งเริ่มหลอมละลาย (T_o) อุณหภูมิที่ผลึกส่วนใหญ่หลอมละลาย (T_p) และอุณหภูมิที่ผลึกแข็งแรงที่สุกหลอมละลาย (T_c) โดยมีช่วงอุณหภูมิของการหลอมละลาย (T_c-T_o) แคบลง และค่าพลังงานความร้อนที่ใช้ในการหลอมละลาย (ΔH_2) ลดลงจากเจลของของผสมที่เก็บที่อุณหภูมิ 4 องศาเซลเซียสอย่างมีนัยสำคัญ การเติมเบต้า-กลูแคนชนิดใดชนิดหนึ่งลงในแป้งข้าวเจ้าที่มีการสลับเปลี่ยนอุณหภูมิในระหว่างการเก็บรักษาไม่มีผลกระทบต่อค่า T_o, T_p, T_c, และ T_c-T_o แต่เพิ่มอัตราการเกิดเรโทรเกรเดชั่น ($\Delta H_2/\Delta H_1$) และค่า ΔH_2 อย่างมีนัยสำคัญตามลำดับต่อไปนี้: OG ≈ BG > CL > YG เจลของของผสมที่เก็บที่อุณหภูมิ 40 องศาเซลเซียสโดยผ่านการเก็บที่อุณหภูมิ 4 องศาเซลเซียสก่อนเป็นเวลา 1 วันได้แสดงให้เห็นถึงการเพิ่มขึ้นของค่า T_o, T_p, และ T_c อย่างชัดเจนในช่วงสัปดาห์แรกของการเก็บรักษาและเพิ่มขึ้นเล็กน้อยในช่วงที่เหลือของการเก็บรักษา ขณะที่ค่า $\Delta H_2/\Delta H_1$ เพิ่มขึ้นอย่างต่อเนื่องตลอดช่วงเวลาการเก็บรักษา ซึ่งสรุปได้ว่าเบต้า-กลูแคนส่งเสริมการเกิดนิวคลีเอชั่นของเจลจากแป้งข้าวเจ้าในขณะที่การสลับเปลี่ยนอุณหภูมิเพิ่มความเสถียรภาพและความเป็นเนื้อเดียวกันของเจลแป้ง

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LIST OF ABBREVIATIONS

AACC	American Associations of Cereal Chemists
ANOVA	Analysis of variance
&	And
η	Apparent viscosity, Pa·s
AOAC	Association of Official Analytical Chemists
BG	Barley β-glucan
β	Beta
CL	Curdlan
°C	Degree Celcius
DP	Degree of polymerization
DSC	Differential scanning calorimeter
db	Dry basis
ed.	Edition
Ed.(Eds.)	Editor, editors, edited by
ΔH	Enthalpy, J/g
et al.	Et alii (Latin), and others
g	Gram
h	Hour
i.e.	Id est (latin), that is
J	Joule
G''	Loss modulus, Pa
μm	Micrometer
mg	Milligram
ml	Milliliter
min	Minute
Mw	Molecular weight
nm	Nanometer

LIST OF ABBREVATIONS (cont.)

NMR	Nuclear Magnetic Resonance
OG	Oat β-glucan
T_o, T_p, T_c	Onset, peak, conclusion temperature (°C)
p.(pp.)	Page (pages)
Pa	Pascal
/	Per
%	Percent
T_o-T_c	Phase transition temperature range (°C)
RS	Rice starch
s	Second
YG	Spent brewer's yeast β-glucan
SD	Standard deviation
SPSS	Statistical Package for the Social Science
G'	Storage modulus, Pa
T	Temperature
wt	Weight
w/v	Weight by volume
w/w	Weight by weight
XG	Xanthan gum

CHAPTER 1
INTRODUCTION

Starch is the major carbohydrate reserve in plant tubers and seed endosperm. Starch is the common ingredient used in many applications in food industry such as thickening and gelling agents. 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). The gelatinization and retrogradation of starch are important properties since they profoundly affect quality, acceptability, and shelf life of starch-containing foods (Biliaderis, 1991). When starches are subjected to high temperature, typically higher than 50 °C in the presence of water, the granules swell and rupture due to disruption of double helices amylopectin, while amylose preferentially leached out from the swollen granules (Tester, 1989). These events known as gelatinization are accompanied by a dramatic increase in the viscosity as the granule structure is progressively ruptured (Yang & Rao, 1997). When the gelatinized starch is stored, particularly at low temperature (Jang & Pyun, 1997), it undergoes retrogradation caused by recrystallization of the polymer chains.

There are many investigations reported that hydrocolloids such as guar gum, xanthan gum, carragenan, konjac glucomannan, locust bean gum, xyloglucan, β-glucan, etc., could be used with starch mixture to alter apparent viscosity (Alloncle, Lefebvre, Llamas, & Doublier, 1989; Bahnassey & Breene, 1994; Chaisawang & Suphantharika, 2005, 2006; Christianson, Hodge, Osborne, & Detroy, 1981; Rojas, Rosell, & Benedito de Barber, 1999; Shi & Bemiller, 2002) and retard retrogradation rate of starch gel during storage (Ferrero, Martino, & Zaritzky, 1994; Lee, Baek, Cha, Park, & Lim, 2002; Sae-kang & Suphantharika, 2006). β-Glucan is a very interesting hydrocolloid because of its positive effects to human and animal health such as immune-stimulation, anti-inflammatory, anitimicrobial, antitumoral, hepatoprotective,

chloresterol-lowering as well as antifibrotic, antidiabetic, and hypoglycaemic activity (Zeković, Kwiatkowski, Vrvić, Jakovljević, & Moran, 2005), and because β-glucan is naturally occurred polysaccharides found in plants and microorganisms.

Cereal β-glucans are linear homopolysaccharide of D-glucopyranosyl residues linked via a mixture of β-(1→3) and β-(1→4) linkages, with blocks of consecutive (1→4)-linked residues separate by (1→3)-linkages (Lazaridou & Biliaderis, 2007). The richest commercial natural sources of cereal β-glucan are barley and oats with levels usually in the range 4-7% (Burkus & Temelli, 2000). Cereal β-glucans exhibit considerable diversity in their structure, including the ratio of tri- to tetramers, the amount of cellulosic oligomers and the ratio of β-(1→4):β-(1→3) linkages (Izydorczyk & Biliaderis, 2000). The potential use of cereal β-glucans as hydrocolloid is based mainly on their rheological characteristic such as their gelling capacity and ability to increase the viscosity of aqueous solutions. Thus, β-glucans can be utilised as thickening agents to modify the texture and appearance of food formations or may be used as fat mimetics in the development of calorie-reduced foods (Burkus & Temelli, 2000).

Curdlan is an extracellular microbial polysaccharide produced by fermentation of the bacterium *Alcaligenes faecalis* var. *myxogenes* and is composed of linear homopolymer of D-glucose with β-(1→3) linkages (Harada, Masada, Fujimori, & Maeda, 1966). Curdlan is insoluble in water, but is aqueous dispersion forms two-types of heat-induced gels depending on temperature termed low-set gel and high-set gel. The low-set gel, which is typically obtained by heating the aqueous dispersion to between 55 °C and 60 °C and then cooling to below 40 °C, is thermoreversible, whereas the high-set gel, which is obtained by heating the aqueous dispersion to above 80^{o}C, is irreversible (Harada, Sato, & Harada, 1987; Konno & Harada, 1991). Curdlan is tasteless, odorless, and colorless. In most food application, curdlan is used as a texture modifier and a fat replacer (Funami, Yada, & Nakao, 1988; Miwa, Nakao, & Nara, 1994).

β-Glucan can also be produced from fermentation of the brewer's yeast, *Saccharomyces cerevisiae*. Yeast β-glucan is mainly composed of β-(1→3)

backbone chain, with a few β-(1→6) branches. Yeast's cell wall contains about 55-65% of β-glucan (Klis, Mol, Hellingwerf, & Brul, 2002).

The kinetics of starch retrogradation is temperature dependence as each step of retrogradation can be increase or decrease by temperature. The rate of retrogradation can be controlled by varying the storage temperature favoring the step of retrogradation and the storage time.

The objective of this study was to investigate the effect of time and temperature condition, called temperature cycle test on retrogradation behavior to rice starch in the presence of different β-glucans.

CHAPTER 2
LITERATURE REVIEW

2.1 Starch

Starch is the major carbohydrate reserve in plant tubers and seed endosperm. Starches occur in a variety of botanical source such as wheat, tapioca, rice, or corn (maize), which is the largest source of starch. Starch is used in a wide range of products either as raw material, or in the food and non-food industry. 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. For certain applications, starch is added to give a desired product texture and quality such as a thickener, gelling agent, water retention agent, emulsion stabilizer, and bulking agent.

2.1.1 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 by 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).

Starch consists primarily of D-glucopyranose polymers linked together by α-1,4 and α-1,6 glycosidic bonds. Glucose polymerization in starch results in two types of polymers, amylose and amylopectin (Figure 2.2). The structural differences between these two polymers contribute to significant difference in properties and functionality (Table 2.1). 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 2.2 (Patindol & Wang, 2002; Thomas & Atwell, 1999).

Amylose is considered to be an essentially linear polymer composed almost entirely of α-1,4-linked D-glucopyranose with a few α-1,6 branches (0.3-0.5% of the total linkages). The branches are generally either very long or very short and separated by large distances, allowing the molecules to act essentially as linear molecules, forming strong film and fibers and retrograding easily. The average molecular weight of amylose molecules of different commercial starches is reported to be in the approximately range 1.3×10^5 (Degree of Polymerization (DP) ~ 800-3000). Amylose chain gives the molecules a right-handed spiral or helical shape. The hydroxyl groups are positioned on the exterior of the coil so that the interior of the helix is linked with the hydrogen atoms and is lipophilic. Most starches contain about 25% amylose (BeMiller, 2007).

Amylopectin is a very large, highly branched molecule composed of α-1,4-linked glucose segments connected by α-1,6-linked branch points. Amylopectin molecule consists of a chain, called the C chain, which carries the one reducing end- group and numerous branches, termed B chains, to which A chains are attached, as shown in Figure 2.3. Average structures, average molecular weights, molecular weight ranges, and perhaps shape of amylopectin molecules vary with the botanical source. Amylopectin is present in all starches, constituting about three-fourths of most normal starches. Some starches contain only amylopectin, commonly called waxy starches. Fine structures of the amylopectin molecules, especially the ratios of the longer (B) chains to the shorter (A) chains, are important determinants of the properties of starch and foods that contain starch. For example, hard-cooking rice has more long chains (DP 92-98) as compared to soft-cooking rice, which has more short chains (DP <25).

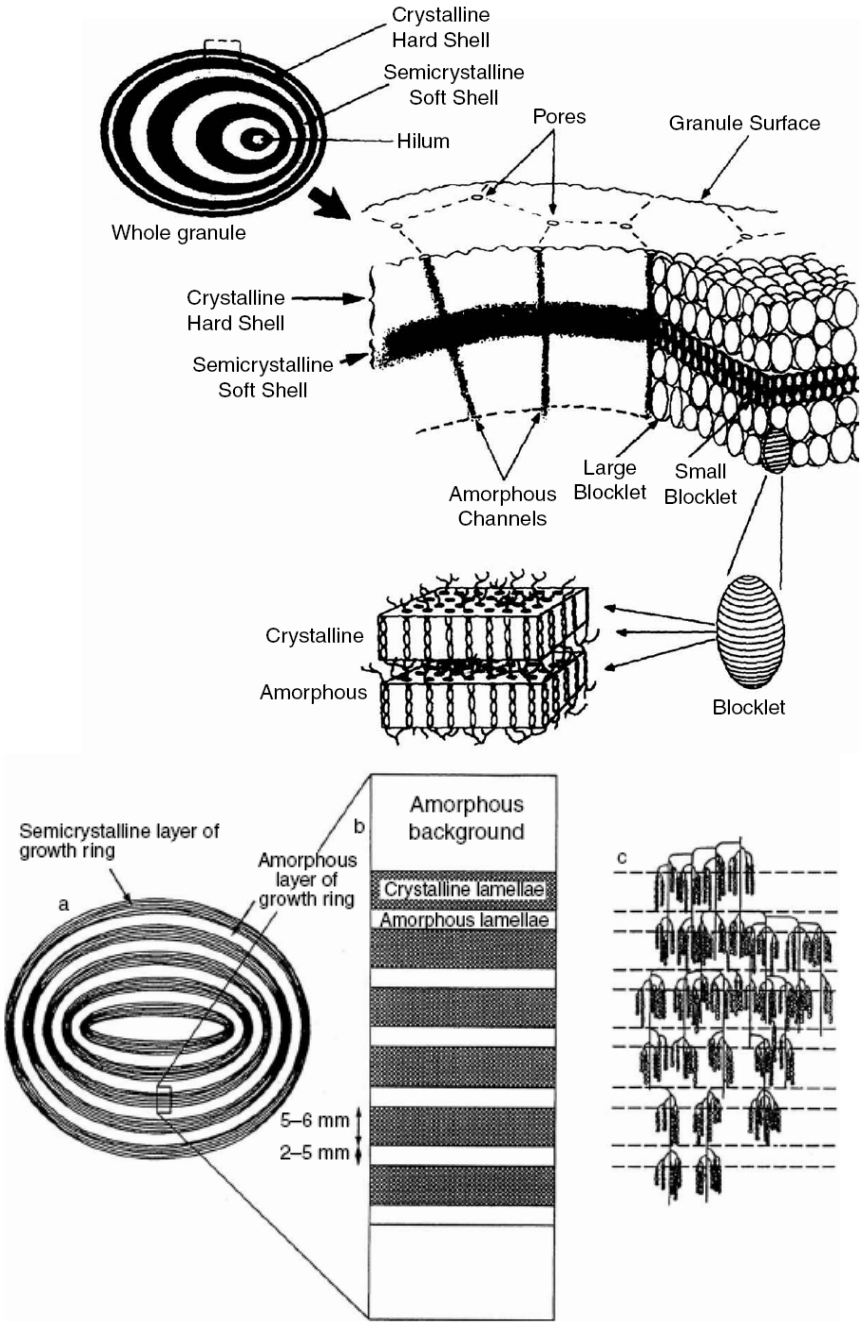

Figure 2.1 Overview of starch granule structure. (A) At the lowest level of granule organization (upper left), the alternating crystalline (hard) and semi-crystalline (soft) shells are shown. The shells are thinner towards the granule exterior and the hilum is shown off center. At a higher level of structure 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, and 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).

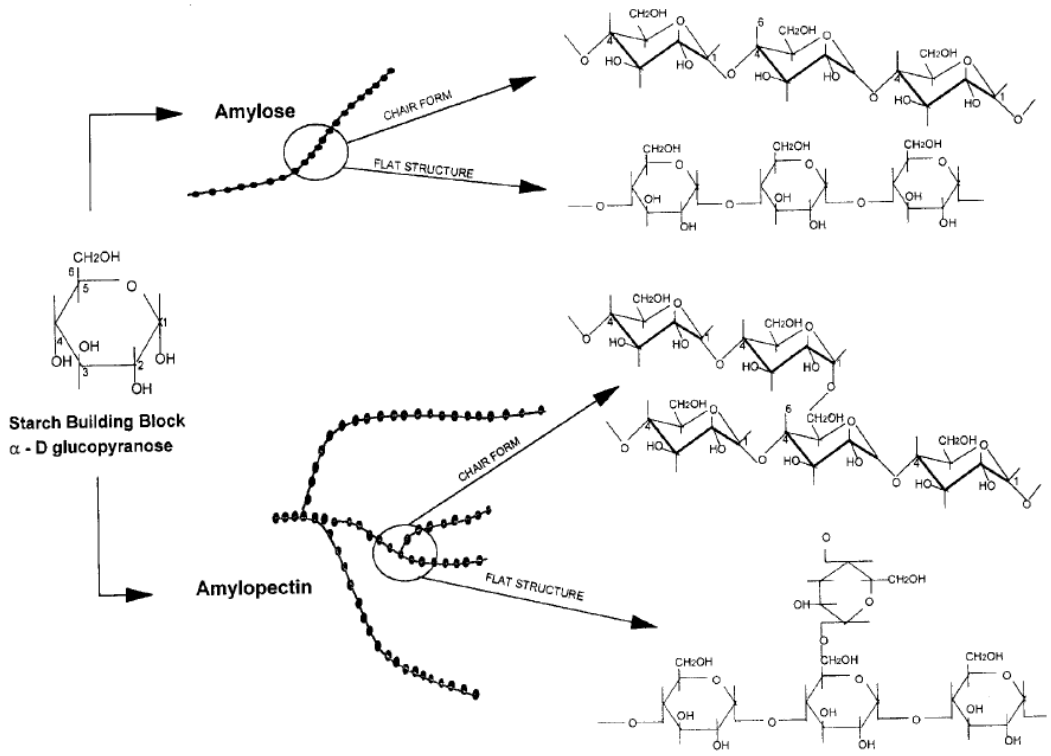

Figure 2.2 Linear and branched starch polymers (Taggart, 2004).

Table 2.1 Characteristics of amylose and amylopectin (Thomas & Atwell, 1999)

Characteristic	Amylose	Amylopectin
Shape	Essentially linear	Branched
Linkage	α-1,4 (some α-1,6)	α-1,4 and α-1,6
Molecular weight	Typically < 0.5 million	50-500 million
Films	Strong	Weak
Gel formation	Firm	Non-gelling to soft
Color with iodine	Blue	Reddish brown

Table 2.2 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.

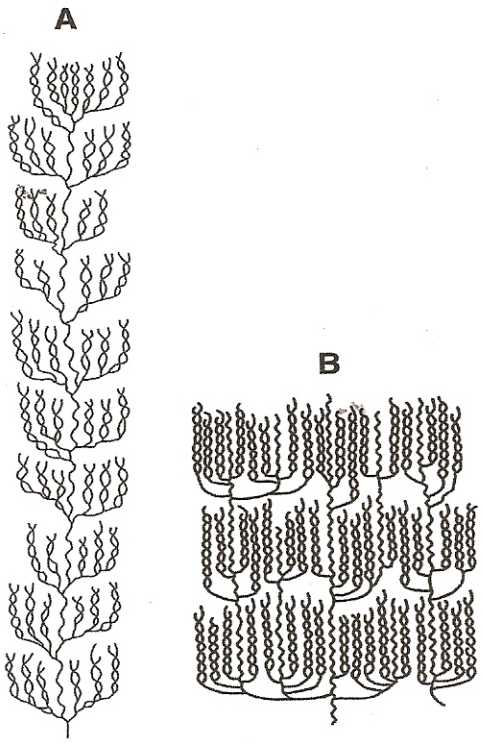

Figure 2.3 Diagrammatic representation of an amylopectin molecule, showing the C chain in the middle and numerous A and B chains. B, possible representation of packing clusters in a starch granule. Individual chains are helical; pairs of chains are double helical (Imberty, Buléon, Tran, & Perez, 1991).

2.1.2 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 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 goal for 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.

2.1.2.1 Constituents of rice starch

The difference between rice flour and starch is most of the native proteins and lipids have been removed from the starch. The protein content of milled rice in a germplasm collection reportedly ranged from 4.5 to 15.9% (Kennedy & Burlingame, 2003). Lipids exist in rice at much lower amounts. Therefore, isolation of starch from rice mainly involves techniques to remove proteins. The majority of rice protein is alkaline soluble, thus alkaline steeping methods are commonly used in industry and research to produce rice starch with good recovery and low residual protein content (Hogan, 1967; Lumdubwong & Seib, 2000). The goal for the protein content of isolated rice starch is generally 0.5% or less. Starch isolated by the alkaline steeping method (plus 0.1-0.2% sodium hydroxide) yielded 73-85% starch (dry basis), 0.07-0.24% residual protein, and 0.07-2.6% damaged starch. Beside proteins, other minor constituents including lipids, phosphorus, and trace elements, are commonly found in isolated starch (Champagne, 1996). Non-waxy rice contains 0.3-0.4% bound lipids; waxy rice starch reportedly contains less of this fraction (0.03%). In non-waxy starch, the composition of total starch lipids has an average of 32% free fatty acids and 68% lysophosphatidyl choline (Morrison, Milligan & Azudin 1984). Phosphorus plays an extremely important role in starch functional properties such as paste clarity, viscosity consistency, and paste stability. Phosphorus in starch is mainly in two forms; phosphate-monoesters, and phospholipids. In non-waxy rice starch, phosphorus is primarily in the form of phospholipids (0.013% dry basis for phosphate-monoesters and 0.048% for phospholipids), whereas in waxy rice starch mainly as starch phosphate monoesters-(0.003% for phosphate-monoesters and none detectable for phospholipids) (Lim, Kasemsuwan & Jane, 1994; Jane, Kasemsuwan & Chen, 1996). Starch phosphate-monoesters in native rice starches are primarily found in amylopectin, and only a trace is found in amylose.

2.1.2.2 Granular structure and crystallinity

Rice contains compound granules having 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 cell. 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 known to exist in cereal grains, with the size reported to range from 3 to 8 μm (Table 2.2). 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.

2.1.2.3 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% (w/w) of the amylose was linear and 33-60% (w/w) 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 the hydrophobic tube. Therefore, hydrophobic complexing agents can lie within the amylose helix stabilized by van der Waals forces between adjacent C-hydrogens and 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.

Rice amylopectin is highly branched molecule with the branch points being α-(1, 6) bonds. Multiangle laser light scattering with differential retractive index (MALLS-RI) detection has shown the molecular mass (Mw) of waxy rice amylopectin to be approximately 5.7 x 10^9 while non-waxy rice starch amylopectin is approximately 2.7 x 10^9 (Yoo & Jane, 2002). Fishman, Rodriguez, & Chao (1996) reported that the Mw of amylopectin decreases with the increase of maize amylose content. It has been postulated that space limitation in non-waxy starch granules caused by greater amylose content results in lower Mw amylopectin (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 2.4). The amylopectin structure is also affected by the growing environment. Umemoto, Nakamura, Satoh, & Terashima (1999) and Suzuki et al. (2003) reported that the temperature during grain-filling affected the chain length distribution of amylopectin in a similar way, i.e., grain filling at lower temperature leads to an increased proportion of short chains and decreased the percentage of long chains. Murugesan, Hizukuri, Fukuda, & Juliano (1992), however, reported that starch from a waxy rice cultivar harvested at various stages of maturity had similar amylopectin structure.

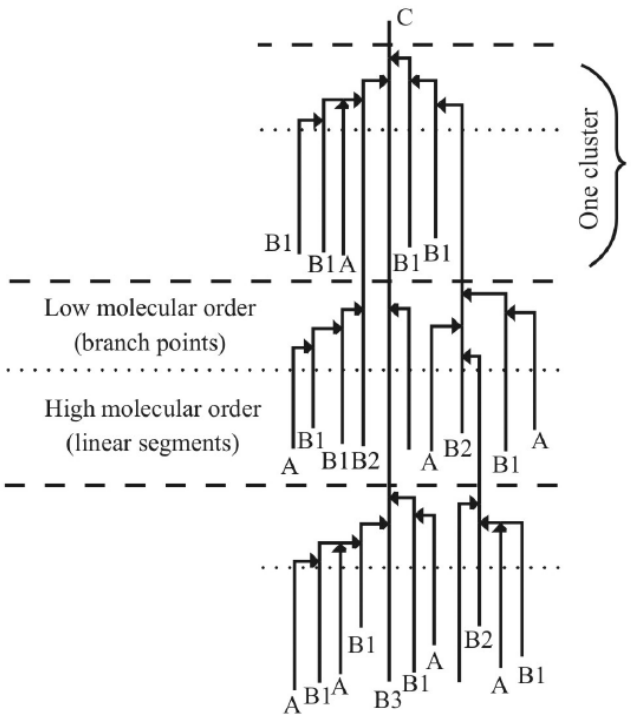

Figure 2.4 Schematic representation of the sub-chains within an amylopectin molecule. Where the branch points sit are regions of low order, and neighboring B chains can form double helices to make up the crystal structure.

2.1.2.4 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 the 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, 1990). The large DP molecules might have a lower tendency to leach out of the granule during heating or may trap other molecules. Lii, Tsai, & Tseng (1996)

observed that swelling power was inversely proportional to the rigidity of starch granules, thus higher swelling power suggests a less rigid granular structure exists.

2.1.2.5 Gelatinization and retrogradation

Gelatinization and retrogradation are the extremely important phenomenons occur during the food processing operations and storage. These two phenomena are also reflecting the quality of the starch-based foods.

1) Gelatinization

Gelatinization is the process that takes place when starch is heated in the presence of water, resulting in the irreversible disruption of molecular order within a starch granule. This loss of order can be seen by irreversible granule swelling, loss of birefringence, and loss of crystallinity. For gelatinization to occur the regions of amorphous starch must first melt or undergo grass transition (Slade & Levine, 1988). The heat energy required to completely gelatinize rice starch 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 can be measured by using 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 (1990) 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. The particle size of rice flour reportedly may or may not influence the enthalpy of gelatinization, as well as the gelatinization temperature. Soaking time is a significant factor in achieving reproducible DSC data (Chiang & Yeh, 2002). By increasing heating rate, the GT of rice flour increased, but the enthalpy decreased (Normand & Marshall, 1989). Also, the thermal properties of rice flour compared to starch prepared from the same sample are different. Starch reportedly exhibits lower T_o, T_p, and T_c, but higher ΔH compared to the flour prepared from the same sample (Teo, Karim, Cheah, Norziah, & Seow, 2000; Wang, Wang, Shephard, Wang, & Patindol, 2002). This reportedly is due to the heat-moisture treatment during sample preparation.

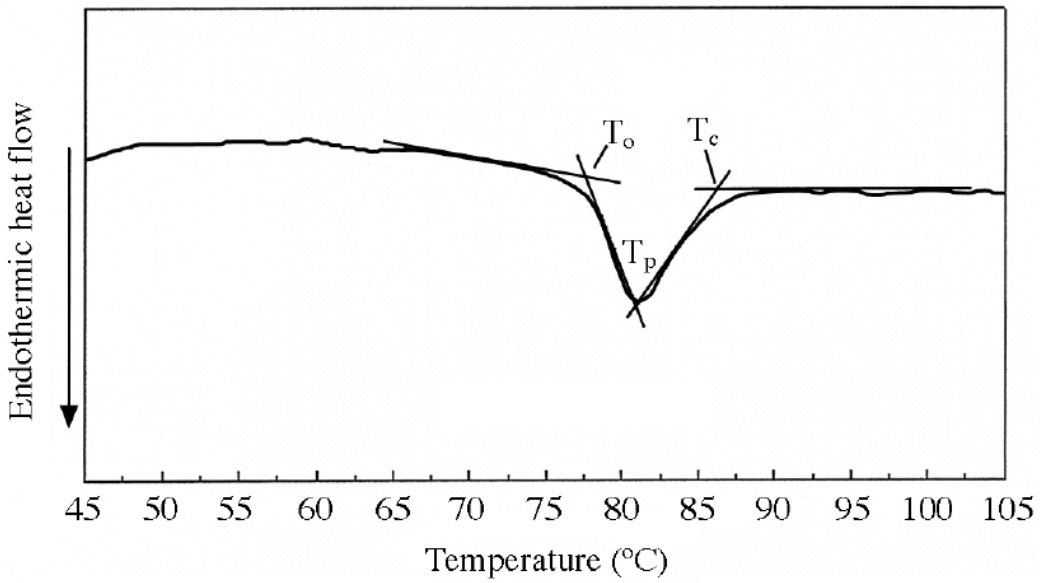

Figure 2.5 Thermal property of rice starch determined by differential scanning calorimetry (DSC). T_o: onset temperature, T_p: peak temperature, T_c: conclusion temperature (Bao & Bergman, 2004)

2) Retrogradation

Retrogradation describes the process in which a heated starch paste cools to below the melting temperature of starch crystallites, and the amylose and amylopectin reassociate and unite with the swollen starch granule in an ordered structure that results in viscosity increase, gel firming, and textural staling of predominantly starch-containing systems. The retrogradation properties can be measured by DSC (Qi, et al. 2003; Vandeputte, Vermeylenn, Geeroms, & Delcour, 2003b). This phenomenon is generally regarded as a crystallization or recrystalliztion (i.e. formation and subsequent aggregation of double helices) process of amylose and amylopectin. 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. Thus, amylose is responsible for short-term (less than one day) changes during retrogradation (Zhou, Robards, Helliwell, & Blanshard, 2002). 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. Thus, amylopectin proceeds slowly over several weeks of storage and contributes to the long term rheological and structural changes of starch systems (Lii, Lai, Lu, & Tsai, 1998; Zhou, et al. 2002).

The retrogradation kinetics of starch have received wide attention through the underlying mechanism of retrogradation has not been concluded. Lai, Lu, & Lii (2000) studied the retrogradation kinetics of pure amylopectin from 13 rice cultivars. Generally, the amylopectin systems showed two stages of retrogradation behavior during early ($\leq$ 7 days) and late ($\geq$ 7days) storage. Correlation analysis suggested that the kinetics of early stage retrogradation were more than the late stage retrogradation with the number-average molecular weight and chain lengths of the amylopectin molecules. Tako and Hizukuri (2000) proposed some mechanisms for rice starch retrogradation which is 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).

Amylose content, GT, amylopectin structure in relation to rice starch retrogradation has been studied. Yao, Zhang, & Ding (2002) indicated that amylose content after defatting and the peak value of amylopectin short-chain length has a significant positive association, while the amount of amylose-lipid complex has a negative relationship with the amylopectin crystallization rate constant. Qi, et al. (2003) found that retrogradation of the solubilized native high- and low-GT amylopectin molecules confirmed that the high-GT amylopectin molecules form higher dissociation temperatures and enthalpy crystalline domains. This confirms that the higher proportion of short and especially relatively longer chains promotes crystalline formation. Vandeputte et al. (2003b) indicated that both amylopectin retrogradation and gel textural characteristics were related to absolute, free, and lipid-complexed amylose contents and amylopectin chain length distributions. Other factors such as storage temperature, moisture content also have significant effects on the retrogradation properties of rice flour mentioned by Fan and Marks (1999).

2.2 Hydrocolloids

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. They are generally polysaccharides, but gelatin (a protein) is included because its functionality is very similar to that of the polysaccharide-based gums. The term 'hydrocolloid' refers to a contraction of hydrophilic colloid. Hydrocolloids are not really colloids, because they are truly water soluble and exhibiting certain of colloidal properties, such as the ability to remain suspended in water under the influence of gravity. Hydrocolloids can have linear or branched molecules (Figure 2.6). The linear type such as cellulose, amylose, algenate, and pectin are the most abundant in nature and have sugar units that repeat over the entire length of the polymer. They usually have side chains, which can be composed of single or multiple sugar units, or they can be as simple as carboxyl groups, sulfate groups, or methyl ether group. Generally, these side units greatly influence the properties of the hydrocolloid. Most hydrocolloid occurs naturally, but there are also several natural hydrocolloids that have been chemically modified such as carboxymethyl cellulose (CMC), also known as cellulose gum, and propylene glycol alginate. The commercially important hydrocolloids and their origin are given in Table 2.3.

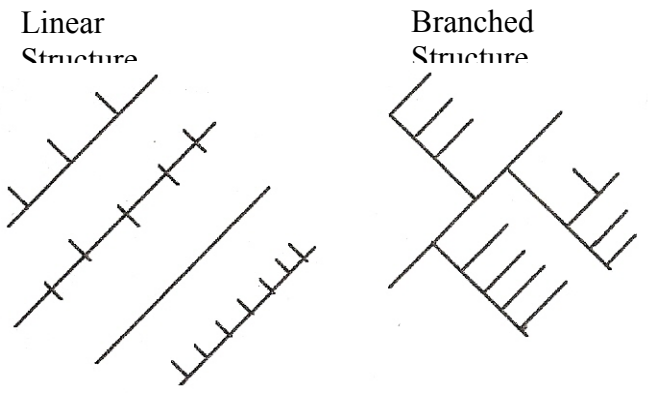

Figure 2.6 Comparison of linear and branch hydrocolloid molecules (Hoefler, 2004).

Table 2.3 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

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 group found on the individual sugar units of the hydrocolloid molecule, as shown in Figure 2.7. 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.

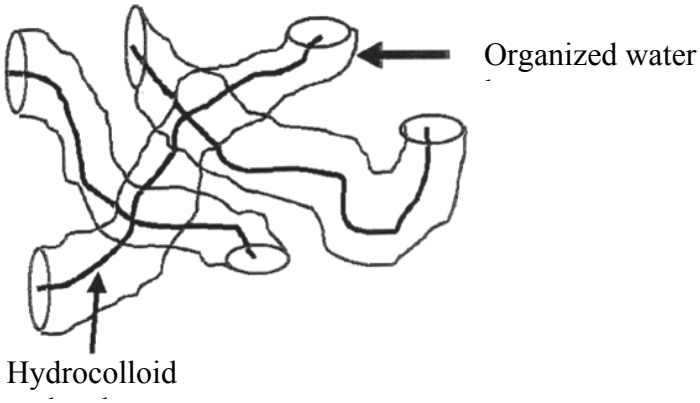

Figure 2.7 Hydrocolloid molecules surrounded by organized water (Hoefler, 2004).

Hydrocolloids divided roughly into two functional categories: the thickeners and the gelling agents. Thickeners consist of individual hydrated molecules that exhibit little interaction with each other, except for random collisions in solution. They move about randomly, with their layer of organized water following them. Some gums do not merely thicken, but cross-link or otherwise form associations using various types of bonds at junction zones to form a three-dimensional network called a gel (Figure 2.8). This forms a viscoelastic structure, often after cooling from applied heat. Some gels are thermally reversible; that is, the gel goes back in solution with reheating. Some gel such as low ester pectin or gellan requires divalent cations such as calcium to form a gel. Thixotropic gums are thought to form weak gels that are broken after applied shear reaches the yield point. Some of the common functions hydrocolloid performed in food systems are shown in Table 2.4. 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 groups along the macromolecular chain (Figure 2.9). Hydrodynamic volume and thickening properties are related to water–polysaccharide interactions. In contrast, gelation takes place as a result of a subtle equilibrium between polymer–polymer and polymer–solvent interactions. 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.

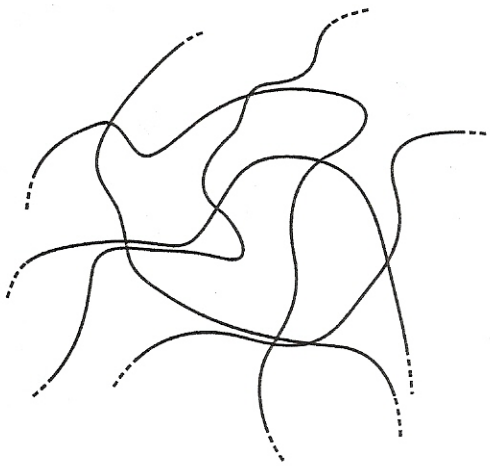

Figure 2.8 Network structure of a gel, known as fringed micelle structure. Molecules are joined at junction zones; the areas between the molecules contain an aqueous solution (Whistler & BeMiller, 1997).

Table 2.4 Function of hydrocolloids in food systems (Hoefler, 2004).

Function	Systems
Adhesive agent	Glazes, icings, frostings
Binding agent	Pet foods
Bodying / mouthfeel agent	Reduced-calorie beverages
Clarifying agent	Beer, wine
Coating agent	Candies, confectionery, cereal, nuts
Crystallization inhibitor	Ice cream, frozen foods, confectionery
Emulsifier	Salad dressing
Emulsion stabilizer	Salad dressing, cream sauces
Encapsulating agent	Powdered flavors
Fat replacer	Salad dressing, Ice cream
Fiber, dietary	Cereals, beverages
Film former	Sausage casings
Foam stabilizer	Whipped toppings, beer
Gelling agent	Jams, jellies, preserves, dessert gels, canned pet foods, onion rings, confectionery
Instant viscosity / mouthfeel agent	Dry mix beverages
Protective colloid	Low-pH dairy beverages
Protein stabilizer	Yogurt drinks, acidic milk beverages, chocolate milk
Satiation agent	Reduced-calorie foods
Stabilizer	Frozen foods, salad dressings, sauces, ice cream
Suspending agent	Salad dressings, fruit juice drinks, chocolate milk
Syneresis inhibitor	Thick sauces, reduced-calorie preserves, pie filling, desserts, cheese, frozen foods
Thickener / viscosity agent	Canned goods, gravies, sauces, soups, dressings, reduced-calorie foods, reduced-calorie beverages, pie fillings, pancake syrup
Water binder	Baked goods, icings, candy
Whipping agent	Toppings, marshmallows

The chain length, or degree of polymerization (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 a straight one with the same molecular weight and therefore provides less viscosity. Longer hydrocolloids sweep out much greater volume as they randomly tumble in solution (Figure 2.10), leading to more collision with neighbors and resulting in an increase in viscosity. Longer hydrocolloids hydrate more slowly because they first need to untangle from the adjoining molecules. The number of side units per unit length of the monosaccharide chain is known as the degree of substitution (DS). Higher degrees of substitution prevent the backbone chains from forming hydrogen bonds together; therefore, they hydrate more quickly. The uniformity of this substitution also affects a gum's behavior.

Gums can help thicken products without the associated starchy mouthfeel or flavor-masking that starches sometimes create. Gelling agents such as a pectin, gellan, alginate, gelatin, and carrageenan tend to provide better flavor release and less flavor masking than an equally thick or gelled system based on starch. This is partly because gums are used at lower levels than starches. Gums are generally used in the range of 0.01 to 0.1%, while starches are usually used in the 0.75 to 10% range. Because starches are used at higher concentrations, they tend to encapsulate, or capture, flavor molecules more readily than gums, requiring that flavors be used at higher levels in order to achieve equal flavor impact.

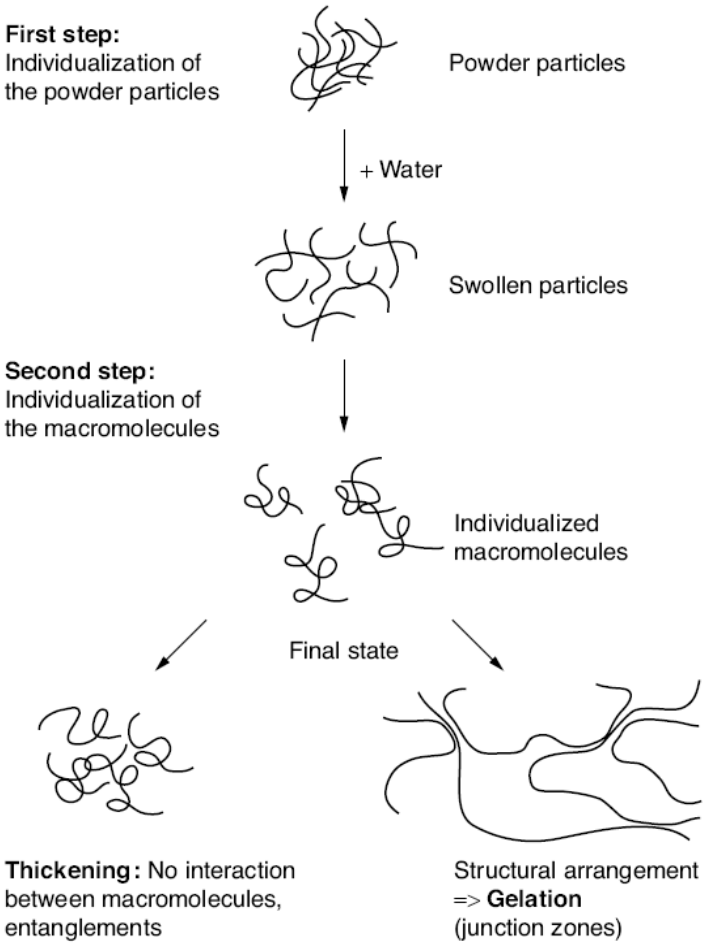

Figure 2.9 Schematic description of the different steps of the solubilization process of polysaccharides (Doublier & Cuvelier, 2006).

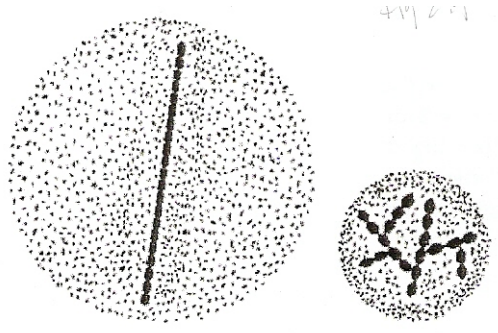

Figure 2.10 Linear hydrocolloid molecule (left) and branched molecule (right). Both have the same molecular weight, but the linear molecule sweeps a greater volume as it tumbles in solution (Hoefler, 2004).

2.3 β-glucans

β-glucans are polysaccharides occurring in the bran of cereal grains, cell wall of baker's yeast, certain types of fungi, mushrooms, and bacteria**.** They are attracting the attention of the pharmaceutical and functional food industry because of its positive effects on human and animal health, and because they are naturally produced from plants and microorganisms. Different source of β-glucan and fine structure are shown in the Table 2.5.

Table 2.5 Sources and fine structure of different β-glucans

Beta Glucan Type	**Structure**	**Description**
Bacterial		Linear β 1, 3-glucan (curdlan)
Fungal		Short β 1, 6 branched β 1, 3-glucan (i.e. Schizophyllan)
Yeast		Long β 1, 6 branched β 1, 3-glucan (WGP Beta Glucan, Betafectin™)
Cereal		Linear β 1, 3/ β 1, 4-glucan (i.e. oats, barley, rye)

2.3.1 Cereal β-glucans

Cereal β-glucans are linear polysaccharides that are composed of cello-oligomers separated by single (1→3)-β-linkages. Generally, those having high molecular weight form viscous solutions with the viscosity increasing with increasing molecular weight, while those of low molecular weight show gel-like behavior, while some types of β-glucans able to form thermoreversible gels. The major of use in foods to date has been as texturising agents, especially as the fat replacer in a wide range of dairy and bakery products. There is also interest in including into foods for health benefits such as lowering cholesterol, moderation of the glycaemic response. β-glucans are also the good source of soluble fiber because β-glucans cannot hydrolysed in the small intestine because human produce no β-glucan degrading enzymes. They are degraded by microbial fermentation in the large intestine, producing beneficial short-chain fatty acid particularly butyric acid.

2.3.1.1 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, 1996; Carpita & Gibeaut, 1993; Fincher, 1992; Fincher & Stone, 1986). 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 (Fincher & Stone, 1986; McCleary & Glennie-Holmes, 1985; Newman, Newman, & Graham, 1989; Wood, 1986, 1994). The variation in β-glucan content of the grain depends mainly on the cultivar type rather than environmental or agronomic factors during cropping. In oats, the bran contains from 5–10% β-glucan (Beer, Arrigoni, & Amado, 1996) and is enriched in β-glucan compared to the groats which contain from 3–7% β-glucan (Wood, 1994). In barley, the endosperm cell walls are composed of about 70% β-glucan (Fincher & Stone, 1986; Ballance & Manners, 1978), 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.

2.3.1.2 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, usually by centrifugation, 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 300,000 and 1,000,000. Variations exist on this basic process and include extraction under alkali conditions (Bhatty, 1993, 1995), and hydrolysis or precipitation of solubilised starch and protein after the extraction step, which increases the quantity of β-glucan in the gum.

2.3.1.3 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 2.11). 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 and cellotetraosyl 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 detail (Izydorczyk, Macri, & MacGregor, 1998a, 1998b; Wood, Weisz, & Blackwell, 1991; Woodward, Phillips, & Fincher, 1988; Yin & MacGregor, 1989). The enzyme, lichenase, solely cleaves the (1→4)-β-linkage immediately following a (1→3)-β-linkage (moving towards the reducing end of the polymer). 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 2.12), 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 2.13). 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 degree of polymerisation (DP) (Izydorczyk, et al. 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. Since adjacent (1→3)-β-linkages are not found in β-glucans and neither are single (1→4)-β-linkage flanked by two (1→3)-β-linkage, it is apparent that the glycosidic linkages are not completely random. Analysis of fragments from enzyme hydrolysis shows that the repeat sequence follows a second-order Markov chain rather than a random polymer model (Staudte, Woodward, Fincher & Stone, 1983; Henriksson et al., 1995), The linkage

sequence only depends on preceding linkages that are no further than two glucose residues away, although there is significant autocorrelation between glycosidic linkages 15 to 20 glucose units away. 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, et al. 1991).

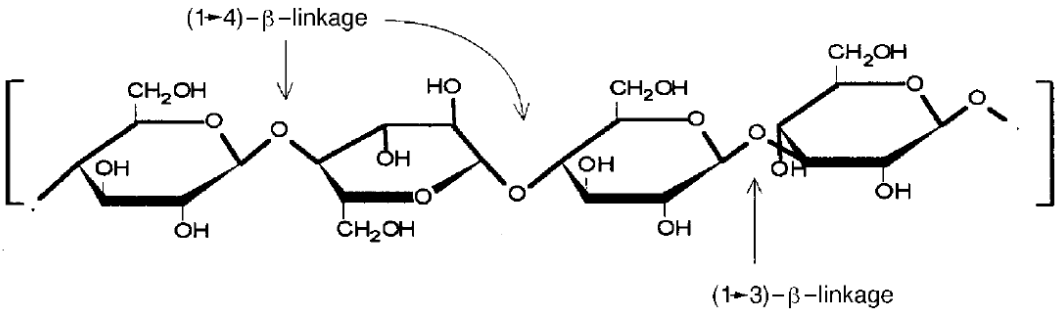

Figure 2.11 Molecular structure of a mixed linked (1→4),(1→3)-β-glucan (Morgan, 2000)

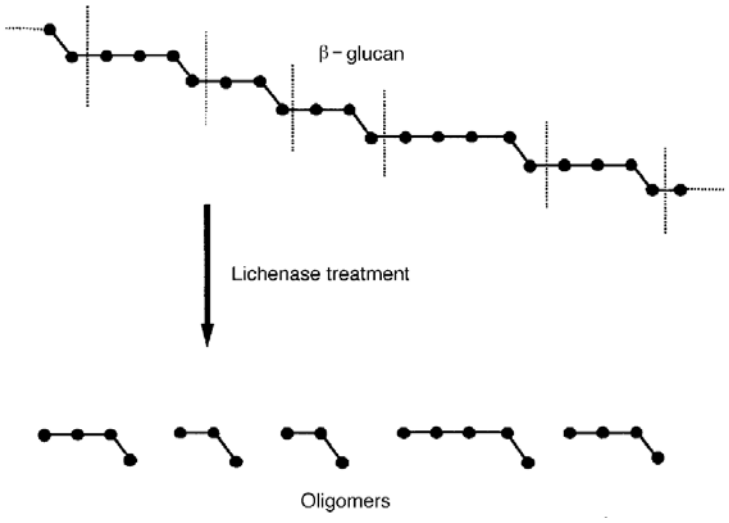

Figure 2.12 Lichenase treatment of a β-glucan (horizontal lines are (1→4)-β-linkages, angled lines (1→3)-β-linkages and vertical dashed lines are the site of lichenase hydrolysis (Izydorczyk, Macri, & MacGregor, 1998a).

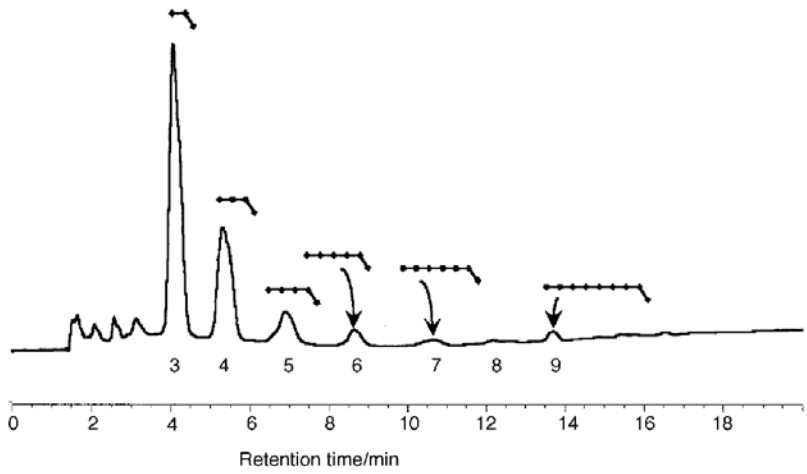

Figure 2.13 High-perfomance ion-chromatography of oligomers released from lichenase treatment of barley β-glucan (Izydorczyk, Macri, & MacGregor, 1998a).

2.3.1.4. 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. Lichenin, (1→3),(1→4)-β-glucan from Iceland moss (*Cetraria islandica*), has few blocks of adjacent (1→4) linkages in the polysaccharide 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). (1→3)-β-D-glucan forms a helix, which are a degree of polymerization comparable to barley β-glucan is insoluble in water (Rees & Scott, 1971).

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, et al. 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 (Beresford & Stone, 1983;

McCleary, 1988; Woodward, et al. 1988). Results describing the occurrence of consecutive (1→3)-linked units in barley and oat β-glucan are contradictory (Woodward et al. 1983). Insufficient enzymatic action during malting and mashing may possibly result in incomplete breakdown of the mixed-linkage β-glucans and produce molecules of similar linkage patterns, which have high tendency to associate and precipitate (Palmer, 1989).

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 units in the cereal β-glucan structure (approximately 4, 3, and 2 for wheat, barley, and oat β-glucan, respectively) and with molecular weight (Cui, Wood, Weisz, & Beer, 1999; Lazaridou, Biliaderis, & Izydorczyk, 2003). Drying of isolated barley cell walls and isolated β-glucan preparation reduces the solubility (Palmer & Bathgate, 1976). Nevertheless, Woodward et al. (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.

2.3.1.5 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. Table 2.6 shows 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). In addition, low molecular weight β-glucans in solution exhibit fairly rapid aggregation, which can occur during extraction (Lazaridou et al. 2003). β-glucan isolated from cold-water, wet-milled, fiber-rich oat bran is of higher molecular weight than that isolated from

ethanol–water, wet-milled oat bran (Mälkki et al. 1992). The β-glucans of oat have the highest molecular weights, followed by those of barley, malt, and rye.

Table 2.6 Molecular weight reported for mixed-linkage β-glucans (Autio, 2006).

Method	Source	Molecular Weight
Sedimentation velocity osmometry	Barley	200,000
Gel permeation chromatography	Oat	71,900
		49,000
Gel permeation chromatography	Oat bran extract	3 million
	Oat groat extract	2.9 million
	Pilot oat gum	1.2 million
	Bench oat gum	2.2 million
	Purified oat β-glucan	363,000
	Purified oat β-glucan[a]	180,000 - 850,000
	Purified oat β-glucan[b]	35,000 – 250,000
	Oat, Donald	2.9 million
	Oat, Marion	3.0 million
	Oat, Tibor	3.0 million
	Barley, Bruce	2.7 million
	Barley, Rodeo	1.9 million
	Barley, Birka	2.3 million
	Barley, Mingo	1.7 million
	Barley malts	1 – 1.5 million
	Barley β-glucan	195,000
	Barley β-glucan[b]	40,000 – 250,000
	Rye	1.1 million
	Oat[c]	1.5 million
	Oat[d]	1.1 million
	Oat[e]	370,000

[a] Water extraction at 47°C.

[b] Acid hydrolysis.

[c] Cold-water wet-milled.

[d] Ethanol-water wet-milled.

[e] Hydrolyzed, cold-water wet-milled.

2.3.1.6 Rheological properties

The chemical structure of the β-glucan is of great importance to the conformation the molecule adopts in solution. Schematic drawings of β-glucans with (1→3); (1→4); and (1→3),(1→4) substitution (Burton & Brant, 1983; Buliga & Brant, 1986) are shown in Figure 2.14. (1→3),(1→4)-β-Glucan exists in extended and flexible conformation in aqueous solution (Fincher & Stone, 1986).

The specific viscosity of oat β-glucan as a function of concentration gives a curve with three different concentration domains (Skendi, Biliaderis, Lazaridou, & Izydorczyk, (2003). The first transition is related to initial contact between the individual coils, the second to the intermediate line between the diluted and concentrated domains to rod-like conformation, and the third to interpenetration of the polymer coils. Differences in the critical concentrations among the samples could be explained in terms of molecular weight.

The flow properties of oat β-glucans have been studied with the help of rotational law constants and the power law equation (Wood, 1986; Autio, Myllymäki, & Mälkki, 1987; Wood, Braaten, Scott, Riedel, & Poste, 1990). Oat β-glucans exhibit strong shear thinning behavior in the shear rate range of 20 to 1600s^{-1} 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, 2004). Oat β-glucans are more stable than guar gum or carboxymethylcellulose (CMC) against the heat treatment (Autio, et al. 1987).

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, et al. 1987). 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%.

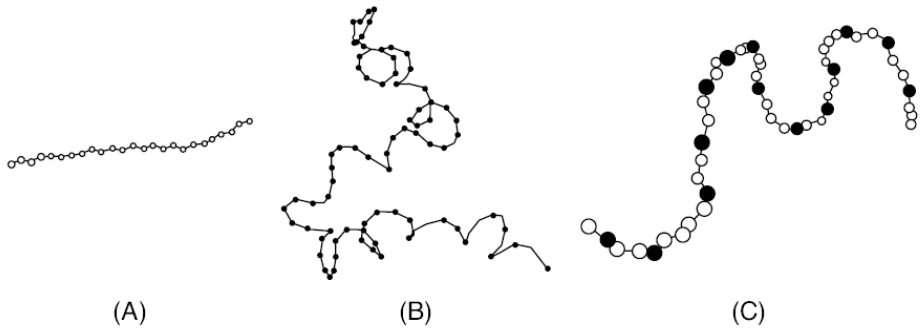

Figure 2.14 Schematic drawings of β-glucans. (A) (1→4)-β-glucans; (B) (1→3)-β-glucans; (C) mixed-linkage (1→3),(1→4)-β-glucans (Burton & Brant, 1983; Buliga & Brant, 1986).

2.3.1.7 Gel formation

Low-molecular-weight barley and oat β-glucans in solution aggregate rapidly, leading to a network structure (Böhm & Kulicke, 1999; Doublier & Wood, 1993; Lazaridou, et al. 2003; Skendi, et al. 2003). β-glucans gel formed by hydrogen bonds forming between β-glucan chains during gelation. The fact that shear forces induce gel formation has suggested the following mechanism for barley β-glucan gels: Shear forces cause the molecules to orient in such a way that intermolecular bonds can form between long sequences of (1→4) linkages in adjacent molecules. Gel formation can also occur at low temperatures (Igarashi & Amaha, 1969; Izawa, Kano, & Kamimura, 1990). An increase of gel strength (studied by compression tests) with increased concentration was observed for barley β-glucans with molecular weights of 50,000 to 300,000 (Böhm & Kulicke, 1999). The gelling rate increased with decreased molecular weight. A similar trend has been observed for low-molecular-weight oat β-glucan (Lazaridou, et al. 2003). The lower molecular weight suggested the higher the G' and the faster the gelling. Doublier and Wood (1995) suggested that the lower molecular weight molecules are more mobile, and cellulose-like blocks in the molecules achieve the orientation necessary for aggregation. 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.

2.3.1.8 Health benefits

The structure of β-glucan in solution is directly related to a number of its health benefits as a food ingredient. The hypocholesterolemic, that is cholesterol-lowering, ability of β-glucan is ascribed to the increase in viscosity of gut gastrointestinal contents as β-glucan dissolves, and that this reduces the reabsorption of bile acids. Studies on human subjects have shown that β-glucan lowers total cholesterol as well as LDL cholesterol ('bad' cholesterol), whereas HDL cholesterol levels remain the same (Braaten, et al. 1994; Hecker, Meier, Newman, & Newman, 1998; Lia, et al. 1995; Newman, et al. 1989), It was also noted in a number of these studies that low molecular weight β-glucans were ineffective. Similar results have been obtained for other viscous polysaccharides that are effective hypocholesterolemic agents. For instance, a study on guar gum noted that without high viscosity the gum was ineffective in lowering serum cholesterol levels (Davidson et al., 1998).

2.3.2 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, & Maeda, 1966; Maeda, 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). One of the unique features of curdlan is that aqueous suspensions can be thermally induced to produce high-set gels, which will not return to the liquid state upon reheating (Harada, Misaki, & Saito, 1968), and this has attracted the attention of the food industry. In addition to this, curdlan offers many health benefits, as the β-glucan family is well known among thescientific community to have immunestimulatory effects (Harada, Terasaki, & Harada, 1993). Curdlan is tasteless, odorless and colorless. Curdlan produces a retortable, freezable food gel. It can form a gel even while incorporating large amounts of fats and oils. Curdlan can modify the texture and improve the water-holding capacity of the meat products as well (Miwa, Nakao, & Nara, 1994). The applications of curdlan are shown in Table 2.7.

Table 2.7 Food applications of curdlan (Miwa, Nakao, & Nara, 1994)

Application	Function	Use level (%)
Noodle	Texture modifier	0.2–1
Kamaboko (boiled fish paste)	Texture modifier	0.2–1
Sausages, Hams	Texture modifier, water holding	0.2–1
Processed cooked foods	Binding agent, improvement in moisture retention and product yield	0.2–2
Processed rice cake	Retention of shape	4–6
Cakes	Retention of moisture	0.1–0.3
Ice cream	Retention of shape	0.1–0.3
Jellies	Gelling agent (stable against heating and freezing-thawing)	1–5
Fabricated foods Noodle-shaped tofu Processed tofu (frozen, retorted, freeze-dried) Thin-layered gel food (frozen) Konjac-like gel food (frozen) Heat-resistant cheese food	Gelling agent (stable against heating and freezing-thawing)	1–5
Edible films	Film formation	1–10
Dietetic foods	Low-energy ingredient	30–100

2.3.2.1 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, & Maeda, 1966; Maeda et al. 1967). Commercial curdlan may contain cellular debris, proteins and nucleic acids and other organic acids.

2.3.2.2 Chemical structure

Curdlan is one of the biopolymeric molecules known as (1→3)-β-D-glucans (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, a well-known natural biopolymer consisting of cellulose residues glycosidically linked in the (1→4)-β-D-configuration. The number-average degree of polymerization of curdlan is about 450 (Konno & 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 by ^{13}C NMR analysis. 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 2.15. 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**

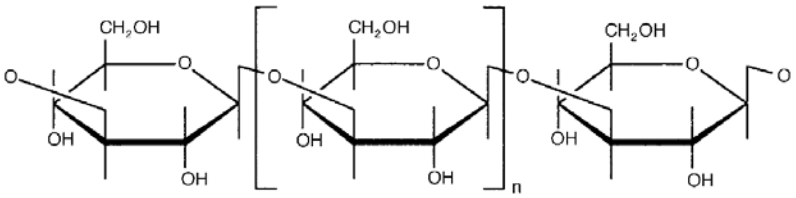

Figure 2.15 (a) Chemical structure of curdlan (Nishinari & Zhang, 2000); (b) Electron micrograph of curdlan granule (Kanzawa, Harada, Koreeda, & Harada, 1987)

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 et al. 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 55°C.

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. Curdlan aqueous gels can be formed by various methods such as heating, (Harada, et al. 1994; Konno, et al. 1978), neutralysing or dialysing against water an alkaline solution of curdlan (Kanzawa, Harada, Koreeda, & Harada, 1987; Harada, Okuyama, Konno, Koreeda, & Harada, 1994), or a solution of curdlan DMSO in a stationary state at ambient temperature. By heat treatment 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 55 °C then cooling, and the other thermo-irreversible gel termed as a high-set gel formed by heating at above 80 °C. This change is explained by the hypothesis that microfibrils dissociate at 60 °C 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 (Maeda 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 90 °C is much greater than that obtained by heating for 4 h at 70 °C. The high-set gel (Konna 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 and is not broken by frozen and thawed (Kanzawa & Harada, 1989). 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 and crystal structure of the anhydrous form of curdlan is composed of a triple standard 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). 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 2.16(a)). 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 2.16(b)) (Fulton & Atkins, 1980).

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 2.17. 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.

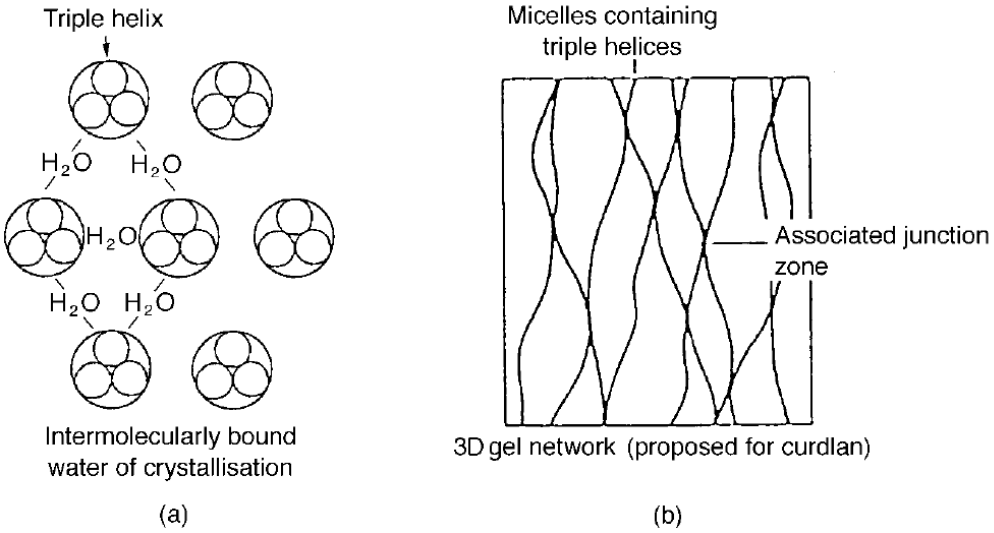

Figure 2.16 Schematic gel network of curdlan (Fulton & Atkins, 1980)

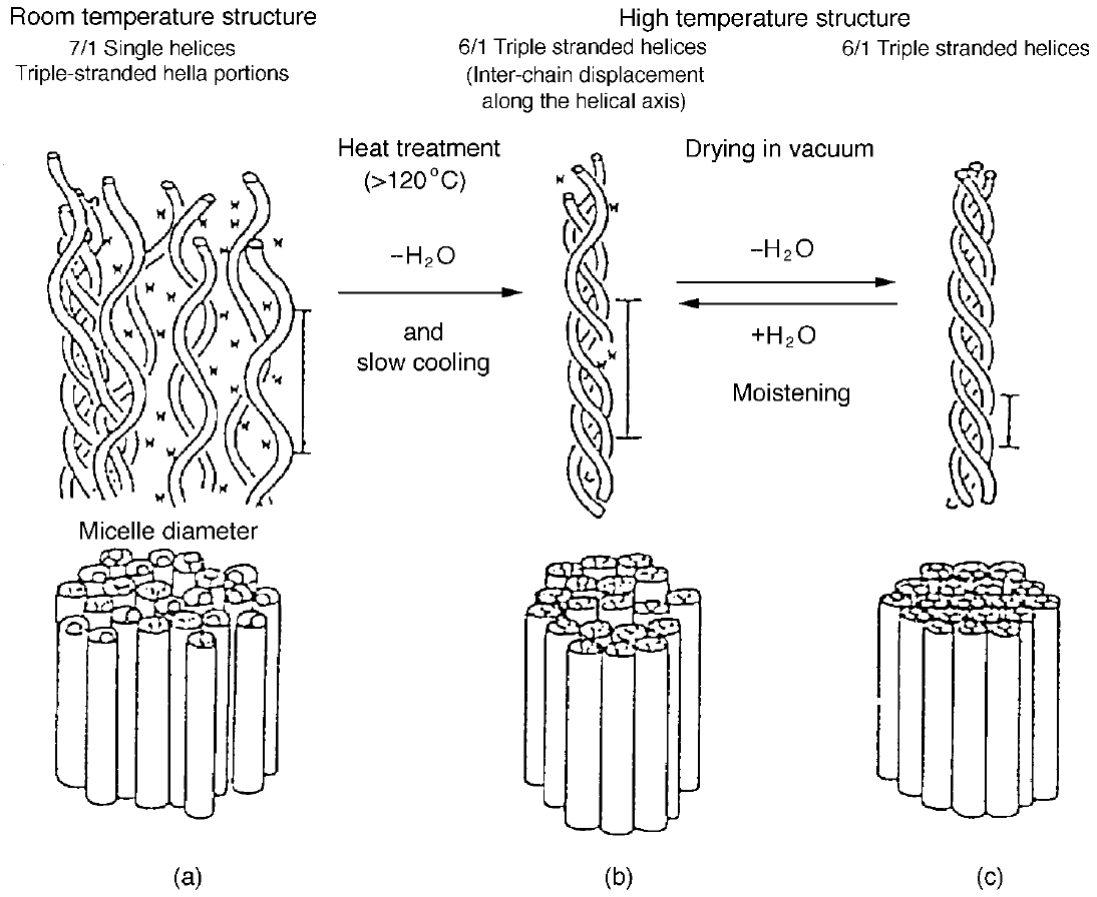

Figure 2.17 Schematic representation of structural change between three forms of curdlan (a) room temperature structure; (b) high temperature structure at high humidity; (c) high temperature structure at low humidity (Kasai & Harada, 1980).

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 2.18) (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.

When curdlan dispersions were heated to different temperatures then cooled, double exothermic peaks appeared at about 38 and 31 °C, which were attributed to the structure ordering due to the formation of hydrogen bonds (Hirashima, Takaya, & Nishinari, 1997). When the cooled gel was heated again, an endothermic peak appeared at around 60 °C. Figure 2.19 shows the second run heating DSC curves for 5% dispersions of curdlan which had been heated at various specified temperatures, *T*, for 60 min. and then quenched to 10 °C. Dispersions heated at temperatures higher than 60 °C showed an endothermic peak much broader than those at lower temperatures below 60 °C.

Though, curdlan may be considered as the best polymer to clarify the mechanism of gel formation due to its neutral characteristics, several conflicting gelation mechanisms of curdlan have been proposed. There is still a great deal of confusion concerning the exact structure of curdlan as well as DSC analysis, because curdlan takes various different conformations. 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.

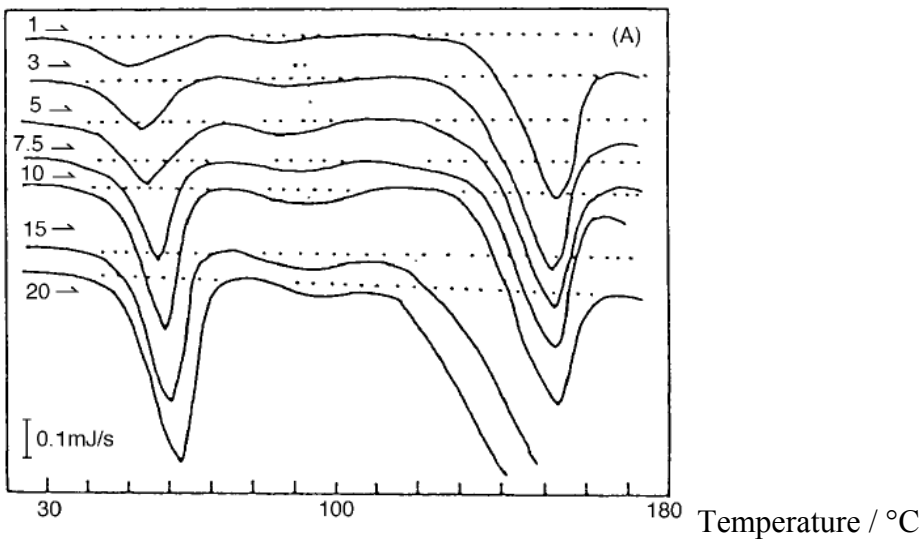

Figure 2.18 DSC heating curves of curdlan aqueous dispersions at various concentrations. Figures beside each curve represent the curdlan concentration in % (w/w) (Watase & Nishinari, 1994).

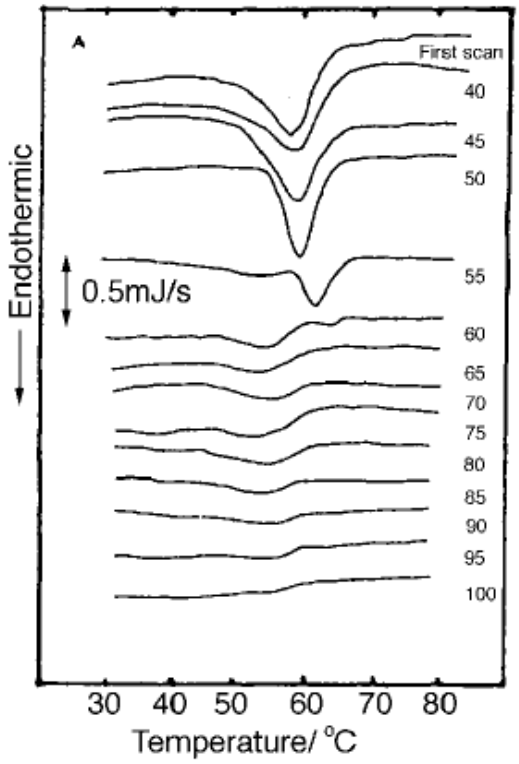

Figure 2.19 DSC heating curves of 5% aqueous dispersions of curdlan after heating at various temperatures for 60 min. Heating rate: 1 ºC min^{-1}. The numbers beside each curve represent the temperature in °C at which the dispersion was kept (Hirashima, Takaya, & Nishinari, 1997).

2.3.3 Yeast β-glucan

Among β-glucan sources, mushrooms and yeasts are also known to contain (1→3)-β-D-glucosidic linkages (Harada, 1992). One important source of β-glucan is the cell wall of yeast (*Saccharomyces cerevisiae*) which contains about 55-65% of β-glucan (Klis, Mol, Hellingwerf, & Brul, 2002). The composition and structure of the yeast cell wall is shown in Figure 2.20.

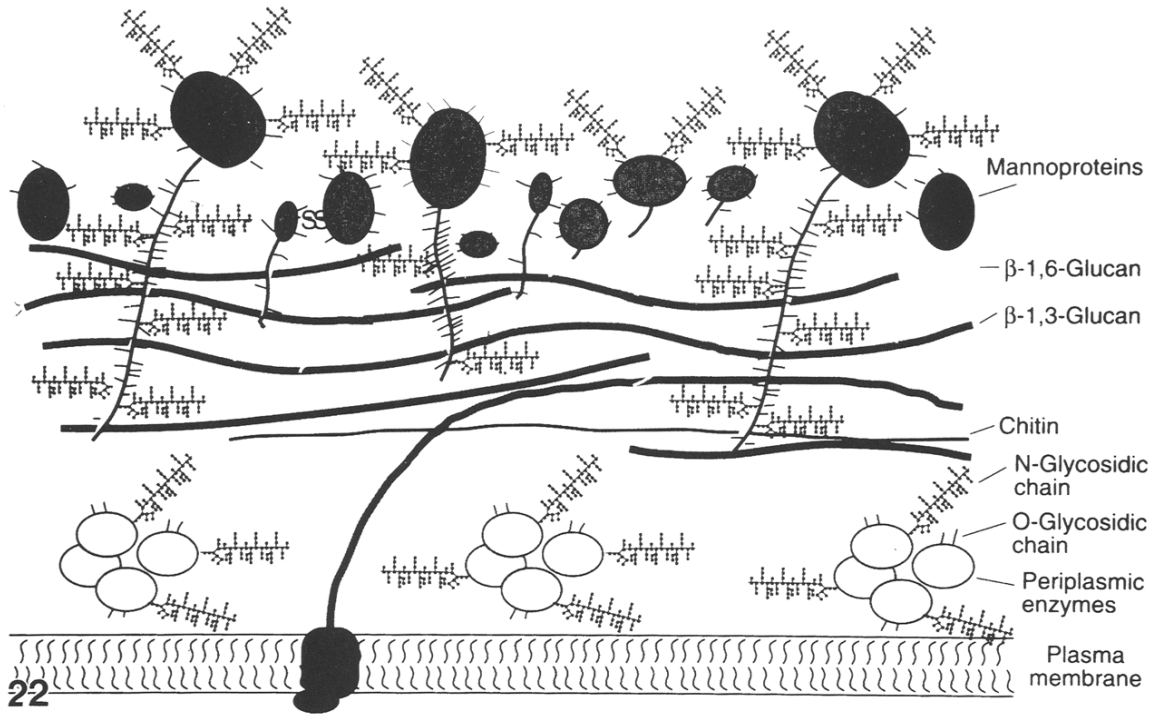

Figure 2.20 Composition and structure of the cell wall of *S. cerevisiae*. The cell wall, which is located outside the cell membrane, consists of two layers. The inner layer provides cell wall strength, and is made of β-1,3- and β-1,6-glucan that is complexed with chitin. The outer layer consists of mannoproteins and determines most of the surface properties of the cell. The majority of mannoproteins are covalently linked to the inner glucan layer. Periplasmic enzymes are trapped between the cell membrane and the inner skeletal layer (Klis, 1994).

2.3.3.1 Molecular structure

The β-glucan in the yeast cell wall consists approximately 85% of β-1,3-glucan, and 3% of β-1,6-glucan (Manners, Mason, & Patterson, 1973). Some products may be listed as β-(1,3/1,6) glucan in the case of yeast-derived products. Molecular structure of yeast β-glucan is shown below in Figure 2.21.

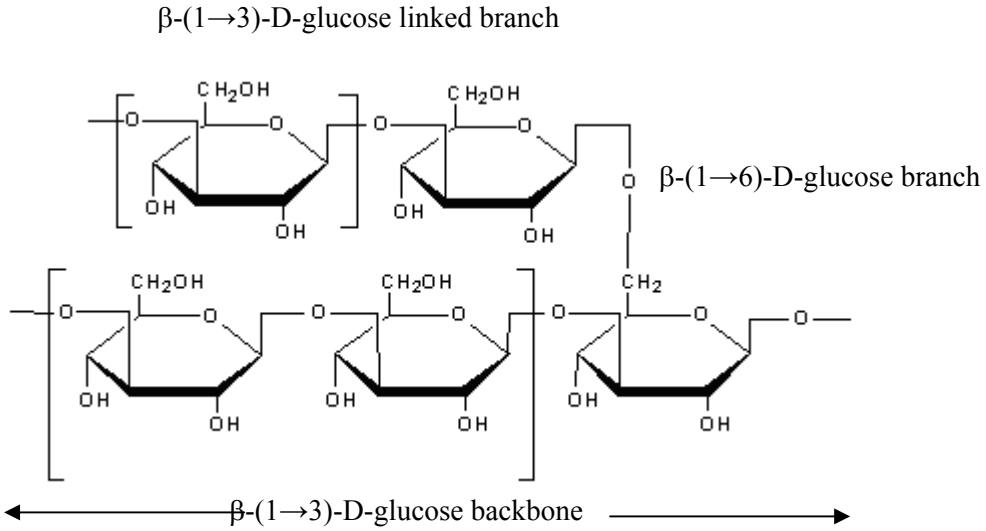

Figure 2.21 Molecular structure of yeast β-glucan (http://www.sigmaaldrich.com)

2.3.3.2 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, Suphantharika, Phaesuwan, & Verduyn 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 (Banchathanakij & Suphantharika, 2008; Satrapai & Suphantharika, 2007).

2.3.3.3 Functional properties

β-Glucans have many reports about their positive 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 (Zeković, Kwiatkowski, Vrvić, Jakovljević, & Moran, 2005), and because it is a natural polysaccharide found

in microorganisms and plants. However, the mechanism of the effect of β-glucan is not yet fully understood and probably depends on the specific molecular structure.

2.4 Xanthan gum

Xanthan gum is an extracellular polysaccharide secreted from bacteria *Xanthomonas campestri*, which is commonly found on leaves of plants such as cabbage family. Xanthan gum is widely used in many food industries because of its unique and useful properties such as cold water soluble, excellent stability over a wide pH, and enzymatic degradation resistant. Commercially, xanthan gum is produced from a pure bacterium culture by an aerobic, submerged fermentation process. The characteristics of xanthan vary with the variations in the strain of the organism, the source of nitrogen and carbon, the degree of medium oxygenation, the temperature and pH of the fermentation, and the concentrations of various inorganic ions.

2.4.1 Molecular structure

The primary structure of xanthan is a linear (1→4) linked β-D-glucose backbone with a trisaccharide side chain on every other glucose at C-3, containing a glucuronic acid residue linked (1→4) to a terminal mannose unit and (1→2) to a second mannose that connects to a backbone (Jansson, Kenne, & Lindberg, 1975; Melton, Mindt, Rees, & Sanderson, 1976) (Figure 2.22). The structure, molecular weight, and physicochemical properties of xanthan vary with the strain of the microorganism and growth conditions. In addition, variations in structure, molecular size, and properties also result from changes in the types and concentrations of nirtrogen and carbon sources, concentrations of certain inorganic salts, and the degree of oxygenation, the pH, and the temperature of the growth medium. Xanthans of the highest pyruvic acid content have the highest viscosities and thermal stabilities.

2.4.2 Physiological properties

The structure and conformation of xanthan gum are responsible for its unique solution properties. The relationship between the structure of xanthan gum and its properties is summarized in Table 2.8. Xanthan is soluble in hot or cold water. Solution of xanthan has high viscosities in comparison with most other polysaccharide solutions and is pseudoplastic. These characteristics are exhibited over a wide range of pH values and a temperature range from 0 to 100°C. The high degree

of pseudoplasticity results in good flavor release and mouthfeel of final product. Xanthan solutions have a Newtonian plateau at low shear stress values (Figure 2.23). Its solutions also have a high elastic modulus (G'). Both properties make it excellent for generating and stabilizing emulsions and suspensions.

Xanthan is synergistically interacted with galactomannans, such as guar and locust bean gum, giving an increase in solution viscosity. The interaction with locust bean gum produces a heat-reversible gel. Locust bean gum molecules is effectively cross-link with the xanthan structures, whether the interaction is with single-helical portions, double-helical portions, or bundles of single- or double-xanthan molecules. Xanthan also interacts synergistically with konjac glucomannan.

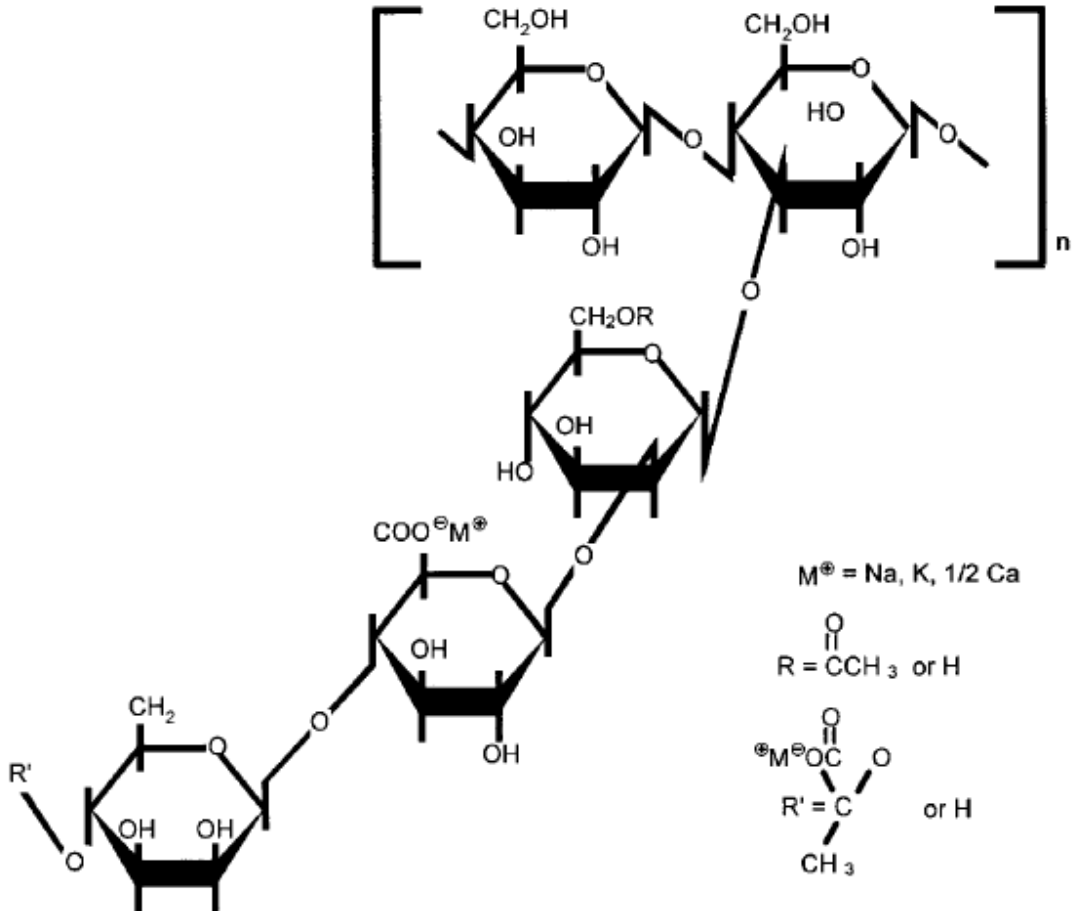

Figure 2.22 Primary structure of xanthan gum (Sworn & Monsanto, 2000)

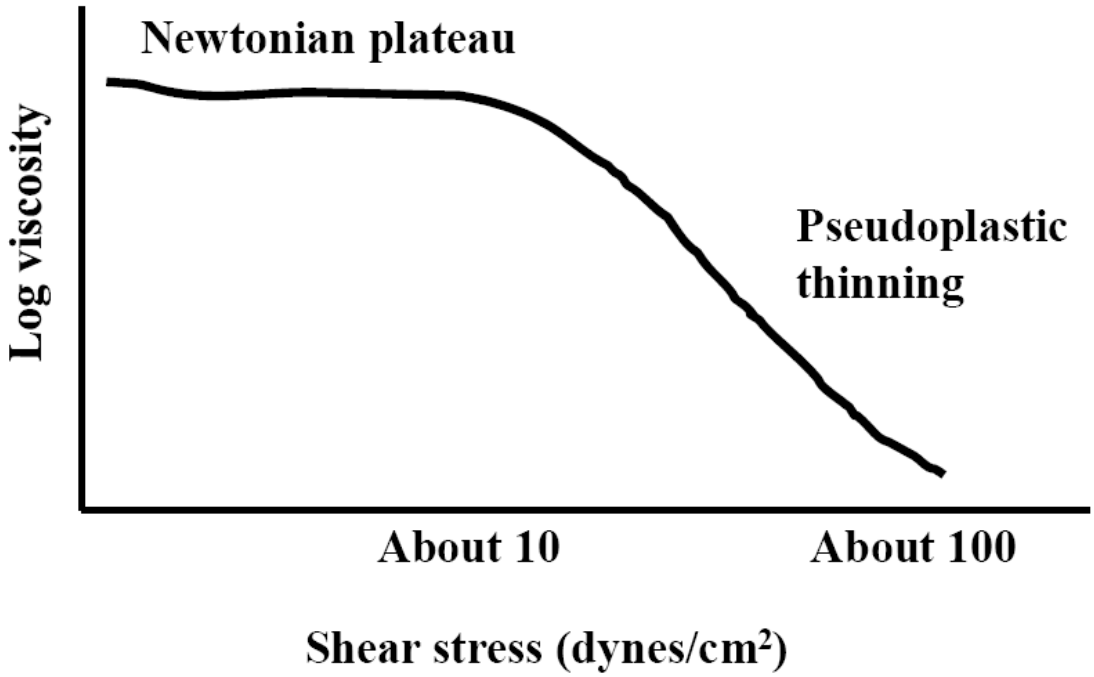

Figure 2.23 Am example to a response of a xanthan solution to shear rates, showing the Newtonian plateau and the pseudoplastic region (BeMiller, 2007)

Table 2.8 Structure/property relationship for xanthan gum (Sworn & Monsanto, 2000)

Structural features	Properties
Complex aggregates, with weak intermolecular forces	High viscosity at low shear rates (suspension stabilising properties) High viscosity at low concentrations High elastic modulus Pseudoplastic rheology
Rigid helical conformation, hydrogen bonded complexes, anionic charge on side chains	Temperature insensitivity and salt compatibility
Backbone protected by large overlapping side chains	Stability to acids, alkalis and enzymes

2.5 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, Arvanitoyannis, Izydorczyk, & Prokopowich, 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, An increase in starch peak viscosity in the presence of hydrocolloids has been reported previously (Alloncle et al., 1989; Sasaki, Yasui, & Matsuki, 2000; Satrapai & Suphantharika, 2007). 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, yielding an increase in gum concentration within the continuous phase, which was accompanied by a dramatic increase in viscosity. Yoshimura, Takaya, and Nishinari (1996) reported that addition of hydrocolloids increase the effective starch concentration by immobilizing water molecules. In contrast, the exclusion mechanism (Annable, Fitton, Harris, Phillips & Williams, 1994; Biliaderis et al., 1997; Morris, 1990) involves phase separation of biopolymer mixtures in aqueous solutions, which is a common phenomena due to incompatibility between unlike polymers, amylose and

hydrocolloids. That results in an increase of effective concentration of each component in its microdomain, causing a substantial enhancement in the viscosity of the mixed system with enhanced firmness of the mixed gel. Christianson et al. (1981) suggest that the presence of gum 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 gums, were responsible for the viscosity increase occurring, and these were dependent on the hydrocolloid structure so structural and rheological properties of the hydrocolloid are very relevant (Bahnassey, & Breene, 1994).

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 hydroplilic 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).

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. Satrapai and Suphantharika (2007) 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, Norziah, & Seow, 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.

2.6 Effect of time and temperature to starch retrogradation

2.6.1 Retrogradation kinetics

The classic theory of crystallization kinetics, applied to synthetic partially crystalline polymers is shown in Figure 2.24 (Levine & Slade, 1989). Figure 2.24 shows the dependence of crystallization rate on temperature within the range Tg < T < Tm, and emphasize the fact that gelation-via-crystallization can only occur in the rubbery (undercool liquid) state, between the temperature limits defined by Tg and Tm (Levine & Slade, 1988; March & Blanshard, 1988).

As shown in Figure 2.24, the nucleation rate is zero at Tm and increase rapidly with decreasing temperature to Tg. The rate of nucleation would be practically negligible at T < Tg because nucleation is a liquid state phenomenon (i.e., in part, a transport process through a viscous medium) that requires translational and orientational mobility, and such mobility is virtually disallowed in a mechanical solid of $\eta \geq 10^{12}$ Pa s (i.e., the temperature below Tg) (Slade & Levine, 1984; Blanshard, 1988; Marsh & Blanshard, 1988). In contrast, the propagation rate increases rapidly with increasing temperature, from a near-zero rate at Tg, and shows a large positive temperature coefficient over nearly the entire rubbery range, until it drops precipitously to a zero rate at Tm (Marsh & Blanshard, 1988; Wunderlich, 1976; Jolley, 1970). The rate of propagation goes essentially to zero below Tg, because propagation is a diffusion-limited process for which practical rates also require the liquid state. At T > Tm, the rate of overall crystallization also goes to zero because crystals can neither nucleate nor propagate at any temperature at which they would be melted instantaneously.

As shown by the symmetrical curve in Figure 2.24, the overall crystallization rate (i.e., the resultant rate of both the nucleation and propagation processes), at a single holding temperature, reaches a maximum at a temperature about midway between Tg and Tm, and approaches zero at Tg and Tm (Slade & Levine, 1984; Slade, 1984; Blanshard, 1988; Marsh & Blanshard, 1988; Wunderlich, 1976; Jolley, 1970).

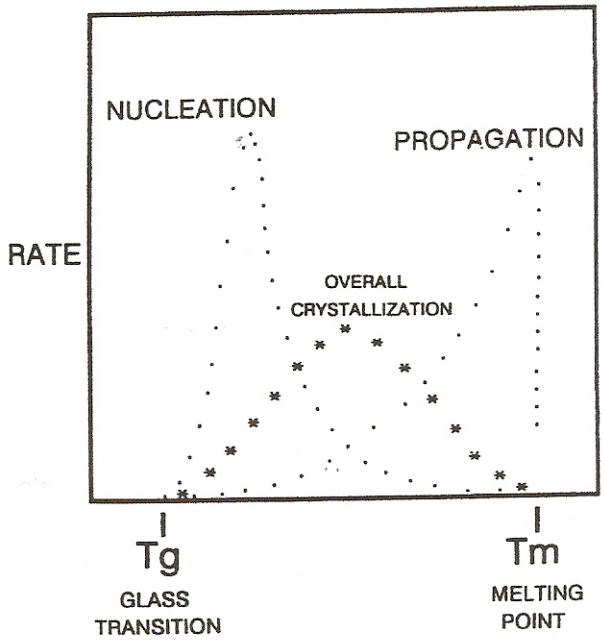

Figure 2.24 Crystallization kinetics of partially crystalline polymers, expressed in terms of crystallization rate as a function of temperature (Levine & Slade, 1989).

2.6.2 Temperature cycling

Temperature cycling, or Temperature Cycling Testing (TCT), normally determines the ability of polymers to resist extremely low or extremely high temperatures. As describe previously, the kinetics of starch retrogradation exhibit a strong temperature dependence because the nucleation and propagation rates are increased or decreased by increasing temperature up to Tm, or decreasing temperature down to Tg. Starch samples were temperature cycled by storing at different time and temperature conditions, favoring starch nucleation and propagation. There are many researches that use the temperature cycling to starch in order to obtain growth of the crystalline regions, perfection of crystallites and a change of crystalline to a more stable structure (Slade & Levine, 1991; Eerlingen, Crombez, & Delcour, 1993; Silverio, Fredriksson, Andersson, Eliasson, & Aman, 2000). Slade and Levine (1991) was concluded that temperature cycling give the highest rate of starch retrogradation, whereas the researches done by Eerlingen, et al. (1993) and Silverio et al. (2000) show the contrast results, i.e. temperature cycling lower the rate of starch retrogradation and give more stable crystallites.

2.6.3 Resistant starch

Resistant starch can be defined as the fraction of starch that escapes digestion in the small intestine, and may be digested in the large intestine (Englyst, Kingman & Cummings, 1992). Resistant starch is considered the third type of dietary fiber, as it can deliver some of the benefits of insoluble fiber and some of the benefits of soluble fiber. Potential forms of resistant starch in the diet are presented in Table 2.9, and the resistant starch contents of some food are listed in Table 2.10. Major mechanisms for the delivery of resistant starch to the large bowel in the case of heat-treated food items are retrogradation and botanical entrapment of the starch substrate. In the raw state, certain starches may also escape digestion and absorption in the upper gut (Englyst & Kingman, 1990). Enzyme resistant due to botanical entrapment is likely to be of particular importance in cereal products based on intact kernels and legumes, whereas retrogradation appears to be affected by the amylose/amylopectin ratio as well as by certain processing conditions. Processing conditions favoring resistant starch formation, by retrogradation of the amylose in particular, include repeated cycles of heating and cooling (Berry, 1986; Björck, Nyman, Pedersen, Asp & Eggum, 1987). In general, the more ordered the food form or the higher the crystallinity of the starch moiety, the higher the probability for the presence of resistant starch.

Table 2.9 Potential forms of resistant starch in the diet (Björck, 2006)

Potential Forms of Resistant Starch in the Diet
Raw starch granule (B-type)
Retrograded starch
Physically inaccessible starch (enclosed in cell or tissue structures)
Chemically modified starch
Amylose-lipid complexes

Table 2.10 Resistant starch content of some starchy products (Björck, 2006)

Product	**Percent (%) (Total Starch or Available Carbohydrate Basis)**
Ground rice	1
Flour-based bread	<3-8
Corn flour bread (arepas)	4
Corn flakes	3-4
Potatoes, freshly cooked	3
Rice	3-5
Pearled barley	6-13
Pumpernickel bread	9-11
Potatoes, cooled/reheated	8
Potatoes, cooled	12
Canned infant pea/potato product	18
Legume products	6-20
Bread made with high-amylose corn starch	24
High-amylose corn flour bread (arepas)	30

2.7 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, 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 Jg^{-1}) compared with gelatinization enthalpies (9-15 Jg^{-1}). However, the transition 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, Thompson, & Boyer, 1993), suggesting that retrogradation results in crystalline forms that are different in nature from those present in the native starch granules.

CHAPTER 3
MATERIALS AND METHODS

3.1 Materials

Rice starch (RS) was kindly supplied by Cho Heng Rice Vermicelli Factory Co. Ltd., Nakornpathom, Thailand. Hydrocolloids were purchased from commercial companies, i.e. xanthan gum (Jungbunzlauer Austria AG, Wulzeshofen, Austria), oat β-glucan and barley β-glucan (Viscofiber®, Cevena Bioproducts Inc., Canada), curdlan (Takeda-Kirin Foods Co. Ltd., Japan), and spent brewer's yeast slurry (a strain of *Saccharomyces uvarum*), a by-product from brewery was provided by Boonrawd Brewery Co. Ltd., Thailand.

3.2 Methods

3.2.1 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. Yeast cell walls were collected by centrifugation at 3565 g for 10 min. The suspension of yeast cell walls (15% solids content) was homogenized by using a high pressure homogenizer, extracted with alkali followed by acid and then washed with distilled water. The obtained β-glucan should be a light-tan colored paste.

3.2.2 Chemical analyses

Chemical analyses were performed in triplicate for each β-glucan preparation, except the determination of insoluble dietary fiber 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 an 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).

For the determination of β-glucan content, total glucose was determined by a hydrolysis method (Dallies, François, & Paquet, 1998) and then measured with a glucose oxidase kit (GOD-PAP method, Human Gesellschaft für Biochemica und Diagnostica mbH, Germany). 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) and then measured with the glucose oxidase kit. β-Glucan content was calculated by subtraction of the glucose obtained from glycogen or starch from the total glucose. Results are reported on a dry matter basis.

3.2.3 Differential scanning calorimetry measurement

Gelatinization temperatures and enthalpy of the RS with and without addition of four different types of β-glucans were measured by a differential scanning calorimeter (DSC1, Mettler Toledo, Schwerzenbach, Switzerland). The total solids content of samples was selected to be 24% with 23/1 w/w ratio, w/w (dry basis), due to the sensitivity of the instrument. Distilled water in all samples was added with 0.02% (w/w) sodium azide to prevent microbial spoilage of the stored samples. The β-glucan solution was prepared by dispersing the calculated amounts of each β-glucan powder, i.e., oat (OG), barley (BG), or curdlan (CL) into distilled water with mild stirring for 15 minutes, followed by heating at 80°C for 10 minutes and then cooled to room temperature (25°C) with mild stirring to prevent aggregation of β-glucans. For the yeast β-glucan (YG), the YG paste was dispersed into distilled water and stirred for 15 minutes. Subsequently, RS was slurried into β-glucan solution and stirred for 15 minutes at room temperature to avoid lump formation. After hydration for 1 h at room temperature, 10–15 mg of the well stirred RS/BG dispersions was exactly weighed into 40 μl aluminum crucibles and hermetically sealed immediately to prevent

moisture loss. Scans were performed from 20 to 100°C at a constant rate of 10°C/min. A sealed empty pan was used as a reference and the DSC was calibrated using indium. The enthalpy and transition temperatures; the onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) were determined based on the first-run heating DSC curves. The gelatinization enthalpy 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 and kept at different temperature-cycled storage conditions modified from many researches (Slade & Levine, 1991; Silverio et al., 2000; Jayakody, Hoover, Liu & Webber, 2005). The low temperature (4°C) was chosen to flavour nucleation and the higher temperature (40°C) to promote propagation. The temperature cycling conditions in this study were short-term 1 and 2 round temperature cycling, short-term 4 and 7 days temperature cycling with varying nucleation and propagation period, and long-term temperature cycling with 1 day nucleation. Non-cycled storage at 4°C was applied to compare with temperature-cycled conditions. After this temperature cycling, the stored samples were heated again to study the effect of storage time and temperature on retrogradation properties of RS/β-glucan mixture. The retrogradation ratio was calculated by dividing the re-gelatinization enthalpy (ΔH_2) in the second-run heating by the gelatinization enthalpy (ΔH_1) in the first-run heating (Kohyama & Nishinari, 1992).

3.2.4 Statistical analysis

For three replicates, the data were subjected to statistical analysis using SPSS 15.0 for Windows Evaluation Version (SPSS Inc., Chicago, IL, USA). Mean and standard deviations for each treatment were calculated. Tukey's test was used to compare differences among the mean values of 0.05 level of confidence.

CHAPTER 4
RESULTS

4.1 Chemical composition of rice starch and β-glucans

The chemical composition of rice starch and various β-glucans were shown in Table 4.1. Rice starch (RS) consists of almost 100% carbohydrate with other minor constituent such as proteins, ash, and fats. The amylose content of RS was 29.02%, indicated that RS is a high amylose starch (Bao & Bergman, 2004). Main component of all β-glucans was carbohydrates which mainly consisted of β-glucan. Other components such as proteins, ash, and fats were expressed as impurities of β-glucans. Curdlan (CL) had the highest β-glucan purity with the lowest contaminants. Barley (BG), oat (OG), and yeast (YG) β-glucans had lower β-glucan purity in a range of 58-72% (w/w) with higher contaminants than CL. OG had the highest starch, protein, ash, and fat content compared with other β-glucans, where CL and YG shows a significant ($p \leq 0.05$) higher insoluble dietary fiber than OG and BG.

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 Effect of β-glucans on the gelatinization properties of rice starch

The DSC gelatinization data of both rice starch alone and rice starch/β-glucan mixture, i.e. the gelatinization temperatures (onset, T_o; peak, T_p; and conclusion, T_c), gelatinization temperature range (T_c-T_o), and enthalpy of gelatinization (ΔH_1) are shown in Table 4.2. The onset temperature (T_o) of rice starch with addition of β-glucans was significantly ($p \leq 0.05$) increased, and except for the RS/YG sample, the peak (T_p) and conclusion (T_c) temperatures, and gelatinization temperature range (T_c-T_o) of rice starch with addition of β-glucans were significantly ($p \leq 0.05$) increase from those of RS alone in the following order: OG > BG > CL. Gelatinization enthalpy (ΔH_1) of rice starch with addition of β-glucans except YG was significantly ($p \leq 0.05$) increased.

Table 4.2 Gelatinization temperature and enthalpy for 24% (w/w) rice starch (RS) alone and RS/β-glucans (23/1 (w/w) ratio) mixture measured by the differential scanning calorimeter (DSC)[1, 2]

RS/β-glucan[3]	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_1 (J/g)
RS	73.3 ± 0.1^c	77.2 ± 0.1^b	81.5 ± 0.2^d	8.2 ± 0.2^d	10.4 ± 0.2^d
RS/ OG	74.1 ± 0.2^a	77.9 ± 0.5^a	85.6 ± 0.5^a	11.5 ± 0.4^a	14.2 ± 0.3^a
RS/ BG	74.0 ± 0.3^a	78.1 ± 0.2^a	84.3 ± 0.3^b	10.3 ± 0.3^b	13.6 ± 0.2^b
RS/ CL	73.9 ± 0.1^a	77.9 ± 0.2^a	83.3 ± 0.3^c	9.4 ± 0.2^c	12.4 ± 0.3^c
RS/ YG	73.6 ± 0.2^b	77.5 ± 0.2^b	81.8 ± 0.3^d	8.1 ± 0.2^d	10.1 ± 0.6^d

[1] Assays were performed in triplicate. Mean ± SD values in the same column followed by different superscripts are significantly different ($p \leq 0.05$)

[2] T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_1, gelatinization enthalpy.

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

4.3 Effect of different short-term time-temperature cycling on RS/β-glucan retrograded gels

The DSC retrogradation data of both rice starch alone and rice starch/ β -glucan mixtures stored at different short-term time-temperature cycling, i.e. the retrogradation temperatures (onset, T_o; peak, T_p; and conclusion, T_c), retrogradation temperature range (T_c-T_o), and enthalpy of retrogradation (ΔH_2) are shown in Tables 4.3, 4.4, 4.5, and 4.6. For the starch gels stored for 12 h (Table 4.3), the retrogradation temperatures (T_o, T_p, and T_c) of all temperature-cycled starch gels were significantly higher than those of the non-cycled gels, whereas the retrogradation enthalpy (ΔH_2) and rate of retrogradation ($\Delta H_2/\Delta H_1$) of temperature-cycled gels were significantly ($p \leq 0.05$) smaller than the non-cycled gels. Addition of gums resulted in an increase in ΔH_2, and $\Delta H_2/\Delta H_1$ in both temperature-cycled and non-cycled conditions. In addition, a significant increase in onset temperature (T_o) was observed in the temperature-cycled starch gels with gum addition. For the 4 day storage conditions (Table 4.4), the T_o, T_p, and T_c of both one round (4/40°C) and two round (4/40/4/40°C) temperature cycled starch gels were significantly ($p \leq 0.05$) higher, whereas the (T_c-T_o), ΔH_2, and $\Delta H_2/\Delta H_1$ were significantly ($p \leq 0.05$) lower than those of the non-cycled gels (4/4). In addition, the 4/40/4/40°C starch gels showed a significant increase in ΔH_2, and $\Delta H_2/\Delta H_1$ compared to the 4/40°C gels. The ΔH_2, and $\Delta H_2/\Delta H_1$ of 4/40/4/40°C gels were not significantly different from those of the 4/4°C gels. For the 4 and 7 days temperature cycling conditions with varying nucleation time (Tables 4.5 and 4.6), the temperature-cycled starch gels exhibited a significant ($p \leq 0.05$) increase in T_o, T_p, and T_c compared with the non-cycled gels. Moreover, the samples stored at 4°C for two days, followed by 40°C for two days (4/4/40/40°C) showed a significantly ($p \leq 0.05$) higher in T_o, T_p, and T_c than the samples stored at 4°C for three days, followed by 40°C for one day (4/4/4/40°C). However, the ΔH_2, and $\Delta H_2/\Delta H_1$ of the samples stored at both conditions were not significantly ($p \leq 0.05$) different. For the 7 days treatment, the samples stored at longer propagation (40°C) time, i.e. 4°C for two days, followed by 40°C for five days (4/4/40/40/40/40/40°C) also showed a significantly ($p \leq 0.05$) higher in T_o, T_p, and T_c compared with the starch gels stored at shorter propagation time, i.e. 4°C for three days, followed by 40°C for four days (4/4/4/4/40/40/40°C). The

ΔH_2 and $\Delta H_2/\Delta H_1$ of the gels stored at both temperature-cycled conditions were not significantly ($p \leq 0.05$) different, which is similar to those observed in the 4 days storage conditions. In general, addition of β-glucan in both 4 and 7 days temperature-cycling did not significantly ($p \leq 0.05$) affect T_o, T_p, and T_c of the RS gels. However, ΔH_2 and $\Delta H_2/\Delta H_1$ of all RS retrograded gels were significantly ($p \leq 0.05$) increased by addition of OG and BG. Moreover, addition of CL showed a significant ($p \leq 0.05$) increase in $\Delta H_2/\Delta H_1$ in one and two round temperarture cyclings (4/40°C and 4/40/4/40°C) and two day non-cycled condition (4/4°C), whereas addition of YG did not significantly affect on both ΔH_2 and $\Delta H_2/\Delta H_1$ in all cases. In conclusion, addition of different β-glucans resulted in a significant incease in the retrogradation rates of RS gels in the following decreasing order OG ≈ BG > CL > YG.

Table 4.3 Transition temperature and enthalpy for 24% (w/w) RS alone and RS/β-glucan (23/1 w/w ratio) gels stored for 12 h measured by the differential scanning calorimeter (DSC)[1,2]

Temperature cycle (°C)	Sample	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_2 (J/g)	$\Delta H_2/\Delta H_1$
4 °C, 12h	RS	42.9 ± 0.3^{b}	55.9 ± 0.7^{b}	64.5 ± 0.1^{b}	21.5 ± 0.4^{b}	1.4 ± 0.1^{b}	0.13 ± 0.01^{b}
	RS/XG	44.2 ± 0.3^{a}	58.6 ± 0.4^{a}	66.3 ± 0.3^{a}	22.2 ± 0.5^{ab}	4.0 ± 0.9^{a}	0.30 ± 0.06^{a}
	RS/OG	42.3 ± 0.2^{c}	54.5 ± 0.2^{c}	62.5 ± 0.1^{c}	20.2 ± 0.2^{c}	4.3 ± 0.7^{a}	0.35 ± 0.05^{a}
	RS/BG	43.0 ± 0.2^{b}	55.7 ± 0.3^{bc}	65.9 ± 0.1^{a}	22.9 ± 0.1^{a}	4.3 ± 0.6^{a}	0.33 ± 0.05^{a}
4 °C, 6h-40 °C, 6h	RS	55.2 ± 0.1^{b}	62.4 ± 0.2^{a}	67.6 ± 0.3^{b}	12.3 ± 0.3^{a}	0.9 ± 0.1^{c}	0.07 ± 0.01^{c}
	RS/XG	56.2 ± 0.4^{a}	63.0 ± 0.4^{a}	68.1 ± 0.4^{ab}	11.9 ± 0.1^{a}	3.6 ± 0.2^{a}	0.26 ± 0.01^{a}
	RS/OG	56.1 ± 0.4^{a}	62.9 ± 0.4^{a}	67.9 ± 0.3^{ab}	11.9 ± 0.2^{a}	2.6 ± 0.1^{b}	0.21 ± 0.01^{b}
	RS/BG	56.2 ± 0.2^{a}	63.2 ± 0.2^{a}	68.5 ± 0.1^{a}	12.3 ± 0.1^{a}	3.6 ± 0.2^{a}	0.28 ± 0.02^{a}

[1] Assays were performed in triplicate. Mean ± SD values in the same column for each treatment followed by different superscripts are significantly different ($p \leq 0.05$).

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

[3] XG are xanthan gum, OG and BG refer of Table 4.1 for the sample codes.

Table 4.4 Transition temperature and enthalpy for 24% (w/w) RS alone and RS/β-glucan (23/1 w/w ratio) gels stored for 2 to 4 days measured by the differential scanning calorimeter (DSC)[1, 2]

Temperature cycle (°C)	Sample	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_2 (J/g)	$\Delta H_2/\Delta H_1$
4/4	RS	40.5 ± 0.5^{a}	55.8 ± 0.5^{a}	64.6 ± 0.0^{d}	24.2 ± 0.5^{c}	3.8 ± 0.0^{d}	0.36 ± 0.00^{c}
	RS/OG	38.4 ± 0.2^{b}	53.3 ± 0.5^{b}	68.0 ± 0.1^{a}	29.6 ± 0.3^{a}	10.6 ± 0.6^{a}	0.74 ± 0.03^{a}
	RS/BG	38.0 ± 0.7^{b}	52.8 ± 0.3^{b}	67.1 ± 0.1^{b}	29.0 ± 0.8^{a}	9.4 ± 0.1^{b}	0.69 ± 0.01^{a}
	RS/CL	39.6 ± 0.2^{a}	53.4 ± 0.1^{b}	65.6 ± 0.2^{c}	26.0 ± 0.3^{b}	6.8 ± 0.5^{c}	0.55 ± 0.03^{b}
	RS/YG	40.4 ± 0.3^{a}	55.8 ± 0.3^{a}	64.4 ± 0.2^{d}	24.0 ± 0.2^{c}	4.0 ± 0.1^{d}	0.40 ± 0.04^{c}
4/40	RS	61.5 ± 0.9^{a}	66.6 ± 0.7^{a}	70.7 ± 0.6^{a}	9.2 ± 0.3^{bc}	1.8 ± 0.4^{c}	0.18 ± 0.04^{c}
	RS/OG	60.6 ± 0.5^{a}	66.5 ± 0.3^{a}	71.5 ± 0.3^{a}	10.9 ± 0.2^{a}	8.2 ± 0.5^{a}	0.57 ± 0.03^{a}
	RS/BG	58.6 ± 0.2^{b}	64.8 ± 0.3^{b}	70.0 ± 0.5^{a}	11.5 ± 0.3^{a}	7.2 ± 0.3^{a}	0.53 ± 0.02^{a}
	RS/CL	61.5 ± 0.8^{a}	66.5 ± 0.9^{a}	70.8 ± 0.7^{a}	9.4 ± 0.2^{b}	3.9 ± 0.4^{b}	0.32 ± 0.03^{b}
	RS/YG	62.0 ± 0.8^{a}	66.6 ± 0.7^{a}	70.4 ± 0.6^{a}	8.5 ± 0.2^{c}	1.7 ± 0.5^{c}	0.17 ± 0.04^{c}
4/40/4/40	RS	59.3 ± 0.3^{b}	66.3 ± 0.6^{ab}	70.4 ± 0.6^{b}	11.1 ± 1.0^{a}	3.6 ± 0.2^{c}	0.34 ± 0.02^{c}
	RS/OG	61.0 ± 0.2^{a}	67.4 ± 0.4^{a}	72.2 ± 0.4^{a}	11.1 ± 0.2^{a}	9.5 ± 0.4^{a}	0.68 ± 0.00^{a}
	RS/BG	59.1 ± 0.1^{b}	65.4 ± 0.1^{b}	70.4 ± 0.1^{b}	11.3 ± 0.1^{a}	9.1 ± 0.3^{a}	0.66 ± 0.02^{a}
	RS/CL	61.0 ± 0.1^{a}	67.4 ± 0.7^{a}	71.5 ± 0.6^{ab}	10.5 ± 0.8^{a}	5.3 ± 0.5^{b}	0.43 ± 0.03^{b}
	RS/YG	58.7 ± 0.9^{b}	66.5 ± 0.7^{ab}	70.6 ± 0.9^{ab}	11.9 ± 1.7^{a}	3.6 ± 0.3^{c}	0.35 ± 0.02^{c}

[1] Assays were performed in triplicate. Mean ± SD values in the same column for each treatment followed by different superscripts are significantly different ($p \leq 0.05$).

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

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

Table 4.5 Transition temperature and enthalpy for 24% (w/w) RS alone and RS/β-glucan (23/1 w/w ratio) gels stored for 4 days measured by the differential scanning calorimeter (DSC)[1, 2]

Temperature cycle (°C)	Sample	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_2 (J/g)	$\Delta H_2/\Delta H_1$
4/4/4/4	RS	40.4 ± 0.2^{ab}	50.0 ± 0.6^{b}	61.4 ± 0.3^{b}	20.9 ± 0.4^{bc}	4.4 ± 0.1^{b}	0.38 ± 0.01^{c}
	RS/OG	39.3 ± 0.7^{c}	53.7 ± 0.4^{a}	62.5 ± 0.2^{a}	23.2 ± 0.8^{a}	6.0 ± 0.3^{a}	0.51 ± 0.02^{a}
	RS/BG	40.1 ± 0.3^{abc}	52.8 ± 0.2^{a}	61.8 ± 0.4^{ab}	21.6 ± 0.1^{b}	5.6 ± 0.4^{a}	0.45 ± 0.03^{b}
	RS/CL	41.2 ± 0.4^{a}	51.0 ± 0.4^{b}	61.2 ± 0.0^{b}	20.0 ± 0.4^{c}	4.5 ± 0.2^{b}	0.40 ± 0.02^{bc}
	RS/YG	39.5 ± 0.2^{bc}	50.2 ± 0.1^{b}	61.5 ± 0.4^{b}	22.0 ± 0.4^{ab}	4.6 ± 0.1^{b}	0.42 ± 0.01^{bc}
4/4/40/40	RS	57.7 ± 0.2^{a}	63.3 ± 0.2^{a}	67.8 ± 0.1^{a}	10.1 ± 0.3^{a}	3.6 ± 0.2^{c}	0.33 ± 0.01^{c}
	RS/OG	57.8 ± 0.4^{a}	63.5 ± 0.4^{a}	68.2 ± 0.3^{a}	10.3 ± 0.1^{a}	5.4 ± 0.6^{a}	0.46 ± 0.05^{a}
	RS/BG	58.3 ± 0.2^{a}	63.7 ± 0.3^{a}	68.1 ± 0.1^{a}	9.8 ± 0.3^{ab}	5.0 ± 0.4^{ab}	0.42 ± 0.04^{ab}
	RS/CL	58.3 ± 0.3^{a}	63.6 ± 0.4^{a}	68.4 ± 0.1^{a}	10.1 ± 0.5^{a}	4.1 ± 0.1^{bc}	0.38 ± 0.01^{abc}
	RS/YG	58.4 ± 0.4^{a}	63.3 ± 0.3^{a}	67.6 ± 0.6^{a}	9.2 ± 0.3^{b}	3.6 ± 0.2^{c}	0.37 ± 0.02^{bc}
4/4/4/40	RS	56.7 ± 0.5^{a}	62.0 ± 0.5^{a}	66.8 ± 0.2^{a}	10.1 ± 0.3^{a}	3.9 ± 0.2^{b}	0.37 ± 0.01^{b}
	RS/OG	56.5 ± 0.5^{a}	62.4 ± 0.7^{a}	66.8 ± 0.3^{a}	10.3 ± 0.2^{a}	5.3 ± 0.3^{a}	0.45 ± 0.03^{a}
	RS/BG	56.3 ± 0.3^{a}	62.3 ± 0.3^{a}	66.7 ± 0.1^{a}	10.4 ± 0.3^{a}	5.2 ± 0.3^{a}	0.44 ± 0.02^{a}
	RS/CL	56.8 ± 0.3^{a}	62.3 ± 0.4^{a}	66.4 ± 0.2^{a}	9.6 ± 0.4^{a}	4.2 ± 0.3^{b}	0.38 ± 0.02^{b}
	RS/YG	56.9 ± 0.2^{a}	62.6 ± 0.8^{a}	66.6 ± 0.8^{a}	9.7 ± 0.7^{a}	3.6 ± 0.1^{b}	0.37 ± 0.01^{b}

[1] Assays were performed in triplicate. Mean ± SD values in the same column for each treatment followed by different superscripts are significantly different ($p \leq 0.05$).

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

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

Table 4.6 Transition temperature and enthalpy for 24% (w/w) RS alone and RS/β-glucan (23/1 w/w ratio) gels stored for 7 days measured by the differential scanning calorimeter (DSC)[1, 2]

Temperature cycle (°C)	Sample	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_2 (J/g)	$\Delta H_2/\Delta H_1$
4/4/4/4/4/4/4	RS	40.7 ± 0.2^{a}	50.9 ± 0.1^{b}	61.8 ± 0.4^{b}	22.1 ± 1.8^{a}	5.5 ± 0.3^{d}	0.49 ± 0.01^{c}
	RS/OG	40.4 ± 0.5^{ab}	53.1 ± 0.6^{a}	63.9 ± 0.5^{a}	23.4 ± 0.8^{a}	7.6 ± 0.4^{a}	0.63 ± 0.03^{a}
	RS/BG	40.3 ± 0.4^{ab}	52.3 ± 0.3^{a}	62.0 ± 0.4^{b}	21.7 ± 0.7^{a}	6.9 ± 0.3^{ab}	0.57 ± 0.03^{ab}
	RS/CL	39.8 ± 0.4^{ab}	50.6 ± 0.7^{b}	61.9 ± 0.5^{b}	22.2 ± 0.9^{a}	6.4 ± 0.3^{bc}	0.58 ± 0.04^{ab}
	RS/YG	39.6 ± 0.3^{b}	52.9 ± 0.3^{a}	61.9 ± 0.2^{b}	22.3 ± 0.4^{a}	5.8 ± 0.2^{cd}	0.54 ± 0.03^{bc}
4/4/40/40 /40/40/40	RS	61.3 ± 0.6^{a}	66.4 ± 0.3^{a}	70.9 ± 0.3^{a}	9.7 ± 0.5^{ab}	4.1 ± 0.5^{b}	0.37 ± 0.04^{b}
	RS/OG	60.5 ± 0.4^{a}	66.3 ± 0.5^{a}	70.8 ± 0.3^{a}	10.3 ± 0.3^{a}	5.6 ± 0.5^{a}	0.47 ± 0.04^{a}
	RS/BG	58.8 ± 0.3^{b}	64.1 ± 0.1^{b}	68.7 ± 0.1^{b}	9.8 ± 0.2^{ab}	5.7 ± 0.3^{a}	0.46 ± 0.02^{a}
	RS/CL	60.8 ± 0.5^{a}	66.4 ± 0.7^{a}	71.1 ± 0.3^{a}	10.2 ± 0.2^{a}	5.3 ± 0.2^{a}	0.45 ± 0.02^{ab}
	RS/YG	59.1 ± 0.4^{b}	63.9 ± 0.2^{b}	68.1 ± 0.2^{b}	9.0 ± 0.4^{b}	4.2 ± 0.3^{b}	0.40 ± 0.03^{ab}
4/4/4/40 /40/40/40	RS	59.9 ± 0.4^{a}	65.7 ± 0.2^{a}	69.8 ± 0.4^{a}	9.8 ± 0.1^{a}	4.7 ± 0.4^{bc}	0.39 ± 0.03^{c}
	RS/OG	60.2 ± 0.2^{a}	65.4 ± 0.4^{a}	69.8 ± 0.5^{a}	9.7 ± 0.5^{a}	6.3 ± 0.6^{a}	0.52 ± 0.04^{a}
	RS/BG	57.9 ± 0.4^{b}	63.3 ± 0.1^{b}	67.7 ± 0.1^{b}	9.8 ± 0.4^{a}	5.8 ± 0.2^{a}	0.49 ± 0.01^{ab}
	RS/CL	60.3 ± 0.4^{a}	65.3 ± 0.4^{a}	69.9 ± 0.4^{a}	9.6 ± 0.2^{a}	5.5 ± 0.1^{ab}	0.48 ± 0.01^{ab}
	RS/YG	58.0 ± 0.2^{b}	63.0 ± 0.0^{b}	67.1 ± 0.2^{b}	9.1 ± 0.1^{a}	4.0 ± 0.4^{c}	0.41 ± 0.04^{bc}

[1] Assays were performed in triplicate. Mean ± SD values in the same column for each treatment followed by different superscripts are significantly different ($p \leq 0.05$).

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

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

4.4 Effect of different long-term time-temperature cycling on RS/β-glucan retrograded gels

The DSC retrogradation data of both rice starch alone and rice starch/β-glucan gels stored at different long-term time-temperature cycling, i.e. the retrogradation temperatures (onset, T_o; peak, T_p; and conclusion, T_c), retrogradation temperature range (T_c-T_o), and enthalpy of retrogradation (ΔH_2) are shown in Tables 4.7 and 4.8 and Figures 4.1 and 4.2. For the starch gels stored at 4^{o}C for 1 day, followed by a long-term storage at 40°C, the T_o, T_p, and T_c significantly ($p \leq 0.05$) increased in the following order: $T_o > T_p > T_c$ (Figure 4.1), resulting in a decrease in the transition temperature ranges (T_c-T_o) with storage time (Figure 4.2a). This effect was more pronounced in the first week of storage. The ΔH_2 (Table 4.7) and $\Delta H_2/\Delta H_1$ (Figture 4.2b) steadily increased with storage time. In addition, the starch gels stored at 4°C for three weeks (21 days) showed a significantly ($p \leq 0.05$) decrease in T_o, T_p, and an increase in T_c, leading to a significantly increase in (T_c-T_o) (Table 4.8). Moreover, the ΔH_2 and $\Delta H_2/\Delta H_1$ of the starch gels stored for three week at 4°C (Table 4.8) appeared to be significantly ($p \leq 0.05$) greater than those of the four-week temperature-cycled gels (Table 4.7). Addition of β-glucans in the long-term temperature-cycled conditions did not show a significant ($p \leq 0.05$) effect on the T_o, T_p, and T_c of rice starch as compared with those observed in the short-term storage conditions. Addition of various β-glucans resulted in a significant ($p \leq 0.05$) increase in ΔH_2 and $\Delta H_2/\Delta H_1$ of rice starch in the long-term storage at 40°C in the following order: BG > OG > CL > YG. The T_o, T_p, and T_c, of samples stored at 4°C for 3 weeks did not significantly ($p \leq 0.05$) affect by addition of β-glucans. Except for the RS/YG samples, the ΔH_2 and $\Delta H_2/\Delta H_1$ were not significantly ($p \leq 0.05$) different from those of RS alone sample.

Table 4.7 Transition temperature and enthalpy for 24% (w/w) RS alone and RS/β-glucan (23/1 w/w ratio) gels stored at 4°C for 1 day, followed by 40°C measured by the differential scanning calorimeter (DSC)[1, 2]

Storage time (day)	RS/β-glucan[3]	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_1 (J/g)	$\Delta H_2/\Delta H_1$
7	RS	61.4 ± 0.2^{c}	66.7 ± 0.1^{a}	71.1 ± 0.1^{b}	9.7 ± 0.2^{a}	3.1 ± 0.3^{d}	0.31 ± 0.03^{c}
	RS/OG	62.1 ± 0.2^{ab}	67.1 ± 0.1^{a}	71.8 ± 0.2^{a}	9.7 ± 0.4^{a}	5.2 ± 0.2^{b}	0.37 ± 0.02^{ab}
	RS/BG	62.4 ± 0.2^{a}	67.5 ± 0.2^{a}	72.1 ± 0.4^{a}	9.6 ± 0.2^{a}	5.8 ± 0.1^{a}	0.38 ± 0.01^{a}
	RS/CL	62.5 ± 0.2^{a}	67.1 ± 0.6^{a}	72.0 ± 0.2^{a}	9.5 ± 0.1^{a}	4.4 ± 0.1^{c}	0.32 ± 0.03^{bc}
	RS/YG	61.6 ± 0.2^{bc}	66.8 ± 0.4^{a}	70.8 ± 0.2^{b}	9.2 ± 0.0^{a}	3.3 ± 0.1^{d}	0.31 ± 0.01^{c}
14	RS	62.9 ± 0.1^{a}	67.6 ± 0.4^{a}	71.6 ± 0.4^{a}	8.7 ± 0.3^{a}	3.4 ± 0.1^{c}	0.31 ± 0.01^{b}
	RS/OG	63.1 ± 0.3^{a}	68.0 ± 0.6^{a}	72.4 ± 0.6^{a}	9.3 ± 0.3^{a}	5.7 ± 0.0^{ab}	0.39 ± 0.02^{ab}
	RS/BG	62.9 ± 0.2^{a}	67.6 ± 0.2^{a}	71.8 ± 0.4^{a}	8.9 ± 0.4^{a}	6.5 ± 0.3^{a}	0.43 ± 0.03^{a}
	RS/CL	63.2 ± 0.2^{a}	68.0 ± 0.0^{a}	72.4 ± 0.1^{a}	9.2 ± 0.1^{a}	5.1 ± 0.6^{b}	0.37 ± 0.04^{ab}
	RS/YG	63.2 ± 0.3^{a}	67.7 ± 0.2^{a}	71.9 ± 0.2^{a}	8.7 ± 0.2^{a}	3.6 ± 0.7^{c}	0.33 ± 0.05^{b}
21	RS	63.8 ± 0.1^{b}	68.5 ± 0.3^{a}	72.7 ± 0.3^{a}	8.9 ± 0.3^{a}	3.5 ± 0.4^{c}	0.32 ± 0.02^{c}
	RS/OG	64.1 ± 0.2^{ab}	68.7 ± 0.2^{a}	72.9 ± 0.2^{a}	8.9 ± 0.1^{a}	7.0 ± 0.1^{a}	0.45 ± 0.02^{ab}
	RS/BG	64.4 ± 0.2^{a}	69.1 ± 0.3^{a}	73.1 ± 0.3^{a}	8.7 ± 0.1^{a}	7.1 ± 0.3^{a}	0.48 ± 0.04^{a}
	RS/CL	64.0 ± 0.1^{ab}	68.7 ± 0.3^{a}	73.0 ± 0.2^{a}	9.0 ± 0.2^{a}	5.5 ± 0.3^{b}	0.40 ± 0.01^{abc}
	RS/YG	63.9 ± 0.4^{ab}	68.6 ± 0.4^{a}	72.5 ± 0.5^{a}	8.5 ± 0.7^{a}	3.7 ± 0.2^{c}	0.37 ± 0.05^{bc}
28	RS	64.8 ± 0.3^{a}	69.1 ± 0.3^{a}	73.4 ± 0.5^{a}	8.7 ± 0.2^{ab}	4.3 ± 0.3^{b}	0.39 ± 0.02^{b}
	RS/OG	63.9 ± 0.3^{a}	68.7 ± 0.1^{a}	73.2 ± 0.1^{a}	9.3 ± 0.3^{a}	7.2 ± 0.5^{a}	0.49 ± 0.04^{a}
	RS/BG	64.9 ± 0.4^{a}	69.4 ± 0.4^{a}	73.4 ± 0.4^{a}	8.5 ± 0.2^{b}	7.6 ± 0.9^{a}	0.49 ± 0.03^{a}
	RS/CL	64.4 ± 0.5^{a}	69.3 ± 0.5^{a}	73.5 ± 0.7^{a}	9.1 ± 0.2^{ab}	6.4 ± 0.9^{a}	0.44 ± 0.03^{ab}
	RS/YG	64.3 ± 0.4^{a}	69.1 ± 0.4^{a}	73.1 ± 0.6^{a}	8.8 ± 0.3^{ab}	4.7 ± 0.3^{b}	0.42 ± 0.05^{ab}

[1] Assays were performed in triplicate. Mean ± SD values in the same column for each treatment followed by different superscripts are significantly different ($p \leq 0.05$).

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

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

Table 4.8 Transition temperature and enthalpy for 24% (w/w) RS alone and RS/β-glucan (23/1 w/w ratio) gels stored at 4°C measured by the differential scanning calorimeter (DSC)[1,2]

Storage time (day)	RS/β-glucan[3]	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_1 (J/g)	$\Delta H_2/\Delta H_1$
1	RS	43.6 ± 0.4^{b}	55.8 ± 0.1^{a}	62.8 ± 0.2^{c}	19.2 ± 0.2^{c}	2.4 ± 0.1^{d}	0.24 ± 0.00^{c}
	RS/OG	45.4 ± 0.4^{a}	55.8 ± 0.4^{a}	64.4 ± 0.6^{ab}	18.9 ± 0.2^{c}	4.1 ± 0.4^{b}	0.31 ± 0.04^{a}
	RS/BG	42.8 ± 0.3^{b}	55.8 ± 0.3^{a}	64.7 ± 0.2^{a}	21.9 ± 0.5^{a}	4.6 ± 0.2^{a}	0.31 ± 0.03^{a}
	RS/CL	43.2 ± 0.4^{b}	56.0 ± 0.5^{a}	64.1 ± 0.2^{ab}	20.9 ± 0.3^{b}	3.7 ± 0.2^{c}	0.28 ± 0.01^{ab}
	RS/YG	42.9 ± 0.3^{b}	55.1 ± 0.6^{a}	63.7 ± 0.3^{b}	20.8 ± 0.0^{b}	2.8 ± 0.7^{d}	0.26 ± 0.04^{bc}
21	RS	40.5 ± 0.7^{ab}	51.9 ± 0.2^{a}	64.1 ± 0.3^{bc}	23.6 ± 0.6^{a}	10.6 ± 0.3^{ab}	0.94 ± 0.01^{a}
	RS/OG	41.6 ± 0.4^{a}	51.5 ± 0.3^{ab}	65.4 ± 0.3^{a}	23.9 ± 0.3^{a}	10.8 ± 0.8^{ab}	0.91 ± 0.03^{ab}
	RS/BG	40.7 ± 0.2^{ab}	51.5 ± 0.2^{ab}	64.8 ± 0.4^{ab}	24.1 ± 0.3^{a}	10.7 ± 0.4^{ab}	0.89 ± 0.02^{ab}
	RS/CL	40.2 ± 0.2^{b}	51.2 ± 0.3^{b}	64.8 ± 0.1^{ab}	24.6 ± 0.3^{a}	11.3 ± 0.8^{a}	0.90 ± 0.03^{ab}
	RS/YG	40.2 ± 0.7^{b}	52.2 ± 0.3^{a}	63.6 ± 0.3^{c}	23.4 ± 0.9^{a}	8.9 ± 1.0^{b}	0.85 ± 0.06^{b}

[1] Assays were performed in triplicate. Mean ± SD values in the same column for each treatment followed by different superscripts are significantly different ($p \leq 0.05$).

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

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

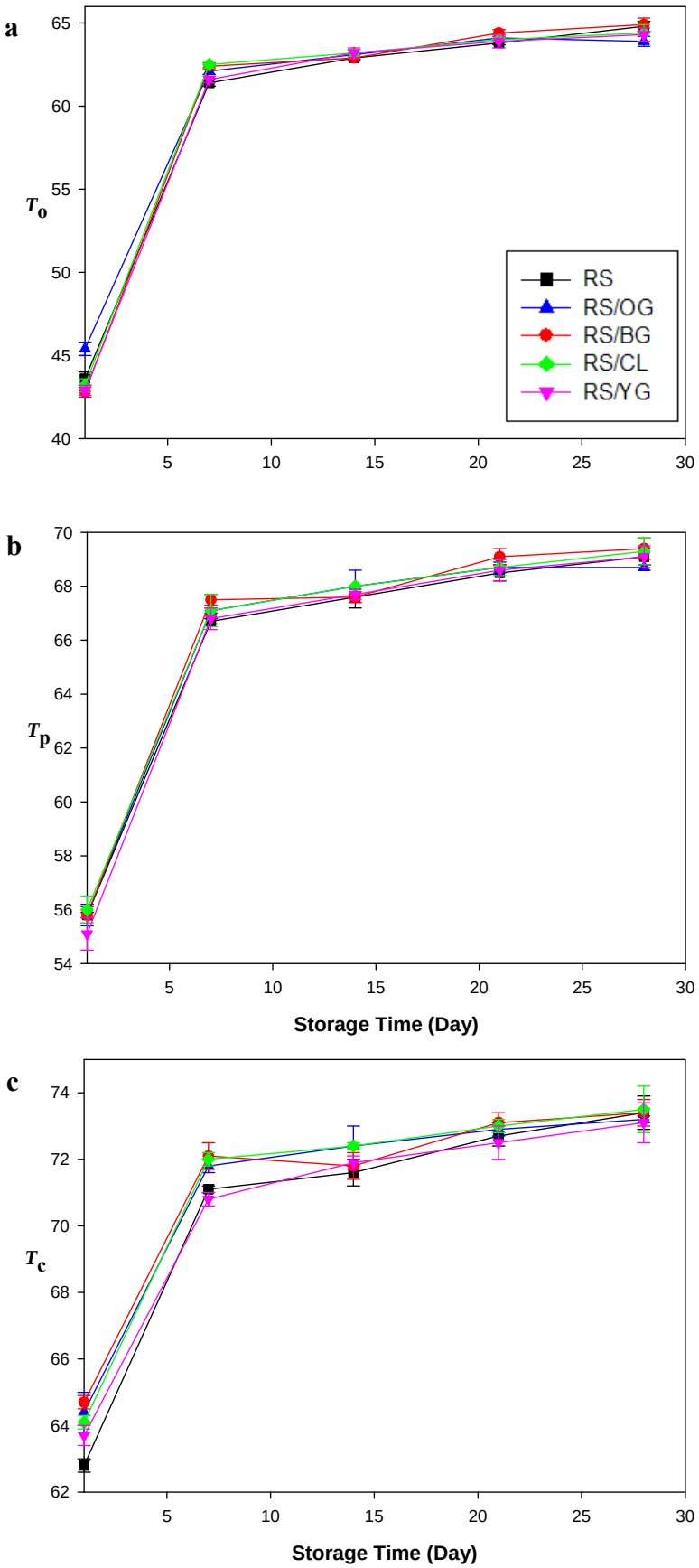

Figure 4.1 Changes in (a) onset temperature, T_o, (b) peak temperature, T_p, and (c) conclusion temperature, T_c, of 24%, w/w, RS alone and RS/β-glucan gels at a ratio of 23/1 as a function of storage time at 40°C with initial storage at 4°C for 1 day. Error bars represent standard deviations. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

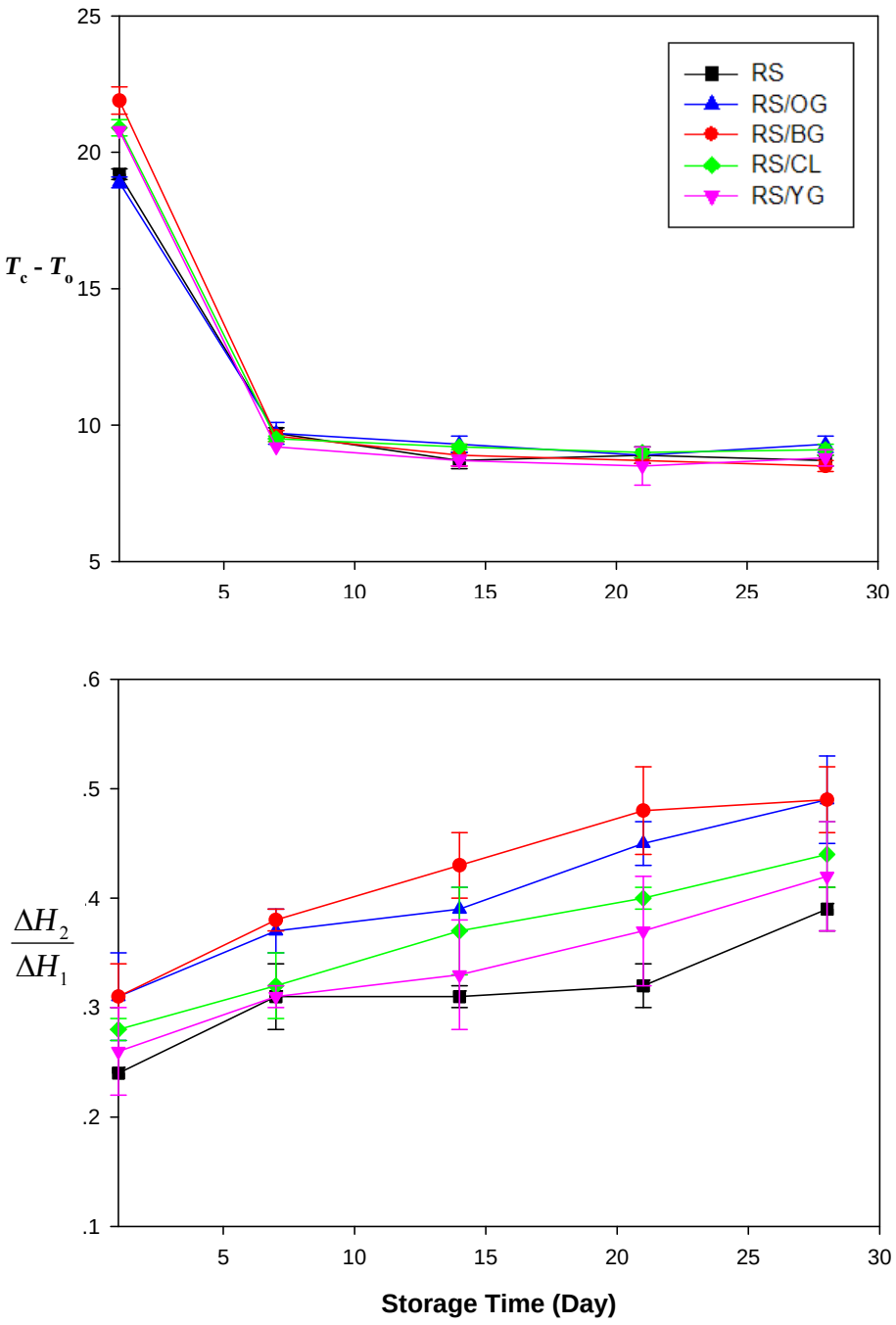

Figure 4.2 Changes in (a) transition temperature range (T_c-T_o) and (b) retrogradation ratio ($\Delta H_2/\Delta H_1$) of 24%, w/w, RS alone and RS/β-glucan gels at a ratio of 23/1 as a function of storage time at 40°C with initial storage at 4°C for 1 day. Error bars represent standard deviations. Refer to Table 4.1 for the sample codes of various β-glucan preparations.

CHAPTER 5
DISCUSSIONS

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).

5.2. Effect of β-glucans on the gelatinization properties of rice starch

The thermal properties of the gelatinized RS/β-glucans are listed in Table 4.2. 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. From Table 4.2, except for the RS/YG sample, the gelatinization temperature (T_o, T_p, and T_c), gelatinization temperature range (T_c–T_o), and the gelatinization enthalpy (ΔH_1) of RS with addition of β-glucans sample were significantly ($p \leq 0.05$) increased. This could probably be the effect of β-glucans to act as a stabilizer to the granular structure during heating, as mentioned by Biliaderis et al. (1997).

5.3 Effect of storage temperature to short-term starch retrogradation

Tables 4.3, 4.4, 4.5, and 4.6 show the reheating DSC data for RS/β-glucan retrograded gels stored for 12 h to 7 days at different time and temperature conditions (temperature cycling). Within the assayed range of temperatures used in this study (20-100°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., 1985). For the non-cycled RS/β-glucan gels, the retrogradation temperatures (T_o, T_p, and T_c) was 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 broader than the gelatinization temperature ranges for a given sample. This result suggests 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 retrogradation temperatures (T_o, T_p, and T_c) of the temperature-cycled RS alone and RS/β-glucan gels were found to be significantly ($p \leq 0.05$) higher than those of the non-cycled gels. The extent of these temperature changes were in the following decreasing order: $T_o > T_p > T_c$, leading to narrower temperature ranges (T_c-T_o) compared to those of the non-cycled gels. Moreover, the temperature-cycled gels exhibited significantly ($p \leq 0.05$) lower ΔH_2 and $\Delta H_2/\Delta H_1$ than the non-cycled gels. These results can be interpreted that a heterogeneous set of amylopectin crystals with varying stability obtained under storage at the nucleation flavored temperature (4°C) was shifted to a more homogenous and more stable set with similar stability under the propagation step (40°C) of starch retrogradation. This was an indication that, even though the overall crytallinity was reduced during the temperature cycling, the remaining amylopectin crystals were of a better quality, and that the storage temperature during the propagation step influenced the melting temperature of the least stable amylopectin crystals, resulting in a lower amount of amylopectin crystals

(Silverio et al., 2000). The two-cycled condition (4/40/4/40°C) did not affect the T_o, T_p, T_c, and T_c-T_o of the starch gels, but significantly increased the ΔH_2 and $\Delta H_2/\Delta H_1$ compared to the one-cycled treatment (4/40°C). It thus seems that the the second cycle with an additional storage period on the third day at 4°C did not result in new nucleation seeds, but the growth of the remaining crytals was still in progress during the fourth day storage at 40°C, as suggested by Silverio et al. (2000). The 4 and 7 days RS/β-glucan retrogradaed gels treated by temperature cycling with various nucleation favored temperature (4°C) periods are also listed in Tables 4.4, 4.5, and 4.6. The T_o, T_p, and T_c values significantly ($p \leq 0.05$) decreased with increasing the 4°C period. In addition, temperature-cycled starch gels were exhibit a lower ΔH_2 than non-cycled starch gels with no significantly ($p \leq 0.05$) difference between temperature-cycled conditions. These results reflected formation of new and less stable amylopectin crystallites during storage at 4°C, and melted during storage at 40°C, lead to a very little or no difference in T_c-T_o and ΔH_2 values between temperature-cycled starch gels.

5.4 Effect of β-glucans to short-term starch retrogradation

In the study of the effect of β-glucans on the retrogradation of RS gels stored under the constant 4°C for two days, all β-glucans tested, except for YG, promoted nucleation of starch crytallites with a wider range of stability than those of the control RS gel. This result can be attributed to phase separation effects, in which water was preferentially absorbed by b-glucans, the amylopectin phase would be more concentrated and would therefore exhibit a greater tendency for crystallization (Biliaderis et al., 1997). These results were concurrent with the precedent studies that addition of β-glucans promoted starch retrogradation during the short-term storage, and retarded it during longer storage time at 4°C (Satrapai & Suphantharika, 2007). In the case of YG addition at the concentration tested, there seemed to be no significant effect on these DSC data. This demonstrates that YG can absorb much less water than the other β-glucans, therefore there was enough water available for both starch and β-glucan. For the gels stored under the 4/40°C cycled conditions, addition of any of these β-glucans did not show a significant ($p \leq 0.05$) effect in the retrogradation temperatures (T_o, T_p, and T_c), but significantly enchanced the enthalpies (ΔH_2) and the rate of retrogradation ($\Delta H_2/\Delta H_1$) of the retrograded gels as compared with those of the control, except for the YG addition. These results can be interpreted that the temperature cycling transformed a set of the weak starch nuclei obtained during nucleation at 4°C to become a more stable and more homogenous set of starch cystals during propagation at 40°C regardless of the presence of β-glucans. However, an increase in melting enthalpies of the RS/β-glucan gels, except for the RS/YG sample, could be due to a larger amount of starch crystals formed, which in turn due to a larger amount of nuclei obtained during nucleation. The melting temperatures obtained from the second temperature cycling (4/40/4/40°C) were similar to those of the first cycling, indicating no formation of new nuclei. A steady increase in the melting enthalpies was attributed to a further propagation of the existing starch crytals on the fourth day at 40°C. In this study, it can be concluded that the soluble β-glucans (OG and BG) exhibited more pronounce effects on the thermal properties of the retrograded RS gels stored either isothermally at 4°C or at cycles of 4/40°C than the insoluble ones (CL and YG). This could be inferred that the soluble β-glucans enhanced nucleation of starch crytallites better than the insoluble ones. The chemical compositions, physical,

and rheological properties of these β-glucans were presented in our previous study (Santipanichwong & Suphantharika, 2009).

5.5 Effect of storage temperature to long-term starch retrogradation

Tables 4.7 and 4.8, and Figures 4.1 and 4.2 show the time dependence of reheating DSC data for RS/β-glucan retrograded gels stored at 4°C for 1 day, followed by 40°C for 7 to 28 days. Samples were stored at 4°C for 1 day, which refers to the short-term starch retrogradation period (Funami et al., 2008), to promote the nucleation of starch crytallites. These retrograded samples were then kept at 40°C to favor the propagation of the starch crytallites for another 28 days during which their themal properties were determinded. The increase in T_o, T_p, T_c, ΔH_2 and $\Delta H_2/\Delta H_1$ values and decrease in the T_c-T_o values of the temperature-cycled starch gels indicated a continuous propagation of starch crystallites, i.e. the growth of more stable crytallites at the expense of the less stable ones, during storage at 40°C. However, the formation of amylopectin crystallites ($\Delta H_2/\Delta H_1$) from the long-term temperature-cycled treatments was much lower than those obtained from long-term storage at 4°C. This result indicated that the existing amylopectin crystallites continue to grow with very little or no formation of new amylopectin crystallites during storage at 40°C. The smaller rate of retrogradation was pointed by Mohamed, Peterson, Grant, & Rayas-Duarte (2006) that amylose and some extent of amylopectin must be mobile enough to re-crystalize, but not so mobile that those weak linear associations are overcome by thermal energy, and it is possible that storage temperature of 4°C provided the most favorable of molecular mobility according to Ribotta, León, & Aňón (2003).

5.6 Effect of β-glucans to long-term starch retrogradation

The DSC data of the starch gels obtained from the long-term temperature cycling show that addition of β-glucans slightly enhanced retrogradation of the RS gels during the entire 4 weeks of storage**.** On the contrary, addition of β-glucans exhibited a slight retardation of retrogadation of the RS gels stored at 4 C for 3 weeks. However, a significant effect of these b-glucans on retardation of retrogradation of the RS gels was reported in our previous study when these gels were stored at 4°C up to 2 months (Banchathanakij & Suphantharika, 2009). Moreover, addition of OG and BG showed greater amylopectin retrogradation in 21 and 28 days of storage. This result can be concluded that addition of β-glucans did not retard starch retrogradation at 40°C, which is favorably for starch propagation step. According to Mohammed et al. (2008), it can be inferred that the movement of amylose and amylopectin at the propagation temperature was so mobile that β-glucans could not prevent the reformation of amylopectin crystallites.

CHAPTER 6
CONCLUSION

This work demonstrated that the retrogradation characteristics of rice starch (RS) with an addition of OG, BG, CL, and YG were affected by storage at different time-temperature conditions. Temperature cycling of the RS alone and RS/β-glucan gels resulted in a shift of retrograded amylopectin crystallites to a more stable and homogeneous sets. Addition of β-glucans did not retard the retrogradation of RS gels during temperature-cycled storage indicating that β-glucans did not retard the retrogradation of RS gels at 40°C as in low temperature storage (4°C). This could be due to the phase separation between RS and β-glucans at the concentration tested. These findings may provide some information and usefulness for various uses of β-glucans in RS-based products.

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APPENDIX

APPENDIX A
CHEMICAL ANALYSIS OF RICE STARCH AND β-GLUCANS

1. Nitrogen determination by Kjeldahl method

The measurement of nitrogen content was applied from AACC Method 920.87 and AOAC method 981.10. Firstly, 0.7-2.2 g of samples was weight and put into digestion flask. Add 0.8 g anhydrous $CuSO_4$, 15 g powder K_2SO_4 or anhydrous Na_2SO_4, and 25 ml H_2SO_4 (increase by 10 ml for each g of sample higher than 2.2g). Place flask in inclined position and heat gently until frothing ceases, boil until solution clears and then 30 min longer. After that, 200 ml distilled water was added into flask and cool to room temperature. Add 35 ml of Sulfide or thiosulfate solution and mix to precipitate Hg. Add few Zn granules to prevent bumping, tilt flask, and add layer of NaOH without agitation (Add 15 g solid NaOH for each 10 ml H_2SO_4 used to make contents strongly alkaline). Then connect flask to distillation bulb on condenser, immerse tip of condenser into standard acid, and put a flask with 25 ml of H_3BO_3 solution and mixed indicator into the receiver. Flask then rotated to mix contents and heated until all NH_3 had distilled. Remove receiver, wash tip of condenser, and titrate absorbing solution with 0.2 N HCl. Nitrogen percentage can be calculated as the followings:

$$\%N = 1.4007 \times [(\text{ml HCl with sample}) - (\text{ml HCl with blank})] \times \text{concentration of HCl/g sample}$$

%N was multiplied by 6.25 to obtain %crude protein of each sample.

2. Crude fat determination by Soxhlet method

The measurement of crude fat content was applied from AOAC Method 960.39. Firstly, equipments were prepared by rinse all of the glassware with petroleum ether, drain, dry in oven at 102°C for 30 mins, and cooled in a desiccators. Place a piece of cotton wool in the bottom of 100 ml beaker. Put a plug of cotton wool in the bottom of extraction thimble and stand the thimble in the beaker.

Next, samples were accurately weight for 5 g into the thimble. Add 1 - 1.5 g of sand or glass bead and mix the sand and sample with a glass rod. Wipe the glass rod with a piece of cotton wool and place cotton wool in the top of the thimble. Dry sample at 105°C for 5 h in oven and cooled in a desiccator. Then take the piece of cotton wool from the bottom of the beaker, place in the top of thimble, and insert the thimble into a soxhlet liquid/solid extractor. A clean and dry 150 ml round bottom flask was exactly weighted and about 90 ml of petroleum ether was added into the flask. Put the extraction unit over a water bath and heat solvent in the flask until it boils. Solvent drips from condenser into sample chamber were adjusted at the rate of 6 drops per second. Remove the extraction unit from water bath and detach the extractor and condenser after 6 h of extraction, replace the flask, and evaporate the solvent. The flask then dried at 105°C in an oven until reach constant weight, cooled in a desiccator and weighted. Crude fat percentage can be calculated as the followings:

$$\%\text{Crude fat} = (W_2 - W_1) \times \frac{100}{S}$$

Where W_1 = Weight of empty flask (g)

W_2 = Weight of flask with extracted fat (g)

S = Weight of sample (g)

3. Moisture determination by direct heating method

The measurement of moisture content was applied from AOAC Method 925.10. The aluminum dish was heated in an oven at 105°C for 1 h, cooled in a desiccator, and weight after reaching room temperature. Then 2 g of sample was added into the aluminum dish. The aluminum dish with a sample then heated at 105°C in an oven for 1 h, cooled, and weighted after reaching room temperature. Heat the moisture can in an oven again until constant weight is reached. Moisture content can be calculated as the followings:

$$\text{Moisture content (\%)} = \frac{(W_1 - W_2) \times 100}{W}$$

Where W_1 = Weight of aluminum dish with sample before heated (g)

W_2 = Weight of aluminum dish with sample after heated (g)

W = Weight of sample (g)

4. Ash determination by direct heating (incinerating) method

The measurement of ash content was applied from AOAC Method 923.03. The crucible was firstly heated in a furnace at 650°C for 6 h, cooled in a desiccator, and weighted after reaching room temperature. Then 2-4 g of sample was added into the crucible and heated in a hot plate for 1 h. After that, ignite the crucible at 600°C for 6 h or until sample become light gray ash. Cool the crucible in a desiccator, and weight after reaching room temperature. Ash content can be calculated as the followings:

$$\text{Ash content (\%)} = \frac{W - (W_1 - W_2) \times 100}{W}$$

Where W_1 = Weight of crucible with sample before incinerated (g)

W_2 = Weight of crucible with sample after incinerated (g)

W = Weight of sample (g)

5. Starch determination by glucoamylase method

The measurement of moisture content was based on followed from AOAC Method 979.10. Sample was weighed about 0.5 g into weighted Erlenmeyer flask. 25 ml distilled water was added into the flask and adjusted pH to 5-7. Boil the flak for 3 min and autoclave at 135°C for 1 h. Next, the flask was cooled to 55°C, 2.5 ml acetate buffer and sufficient distilled water was added to a total weight of solution 0f 45±1 g. Then the flask was immersed into 55°C water bath shaker, 5 ml glucoamylase solution was added, and hydrolyzed for 2 h with continuous shaking. After that, the solution was filtered through folded paper, washed, and diluted to volume. Add 1 ml of aliquots containing 20-60 μg D-glucose into test tube, add 2 ml enzyme-buffer-chromogen mixture, shake, and place in dark at 37°C for exactly 30 min to develop color. The reaction was stopped by addition of 2 ml H_2SO_4. The absorbance was measured at 540 nm. Standard curve was prepared from 0-60 μg D-glucose/ml and blank. Include control of sample containing starch of known purity fro source similar to material being examined. Starch content can be calculated as the followings:

$$\%\text{Starch} = 2.25 \times \text{M} \times \text{E} \times \text{MS} \times \frac{V_1}{V_0}$$

Where M = D-glucose from standard curve (μg)

E = Weight of sample (g)

MS = %solid of sample

$\frac{V_1}{V_0}$ = dilution factor (if diluted)

6. Insoluble dietary fiber determination by enzymatic-gravimetric method

The measurement of insoluble dietary fiber content was based on followed from AOAC Method 991.42. Duplicate of 1 g sample was accurately weighted to 0.1 ml into 400 ml tall-form beakers (duplicate sample weights should not differ > 20 mg). Each beaker was added with 50 ml phosphate buffer and adjusted pH to 6.0 ± 0.2 by 0.275 N NaOH and 0.325 N HCl.

Add 0.1 ml termamyl solution into each beaker, cover beakers with A1 foil, and incubate by shake gently in boiling water bath for 5 min. When thermometer indicates beaker contents have reached 100°C, continue incubating for 15 min. After that, solutions were cooled to room temperature and adjusted to pH 7.5 ± 0.1 by NaOH solution. Add 5 mg protease to each solution, cover beakers with A1 foil, and incubate at 60°C for 30 min with continuous agitation. Solutions were cooled to room temperature and adjusted pH to 4.0-4.6 by HCl solution. Add 0.3 ml amyloglucosidase to each solution, cover beakers with A1 foil, and incubate at 60°C for 30 min with continuous agitation.

Weight crucible containing Celite to nearest 0.1 mg, wet and redistribute bed of Celite into crucible (using stream of water from wash bottle). Apply suction to draw Celite onto fritted glass as even mat. Apply enzyme mixture from beaker into crucible, filtering into suction flask. Residue was washed 2 times with 10 ml water to removing soluble fiber, 2 times with 10 ml 95% alcohol, and 2 times with 10 ml acetone. Break surface film that develops after addition of sample to Celite with spatula to improve filtration. Careful intermittent suction throughout filtration and back-bubbling with air, if available, will speed up filtrations. Normal suction can be applied as washing.

Crucible containing residue as dried in hot air oven at 105°C for 5 h, cooled in a desiccator, and weighted to nearest 0.1 mg (subtract crucible and Celite weights to determin residue weight). Use 1 of the duplicate to analyze protein, and another duplicate to analyze ash. Insoluble dietary fiber can be calculated as the followings:

$$\text{Insoluble dietary fiber (\%)} = [(\text{residue weight} - \text{P} - \text{A} - \text{blank})\,\text{sample weight}] \times 100$$

Where residue weight = average weight for duplicate blank determination (mg)

sample weight = average weight of 2 sample weights taken (mg)

P = weight of protein (mg)

A = weight of ash (mg)

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